Novel One-Pot Methods for the Synthesis of Fluorescent Amino Acids

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A thesis submitted in part fulfilment of the requirements of the degree of Doctor of Philosophy

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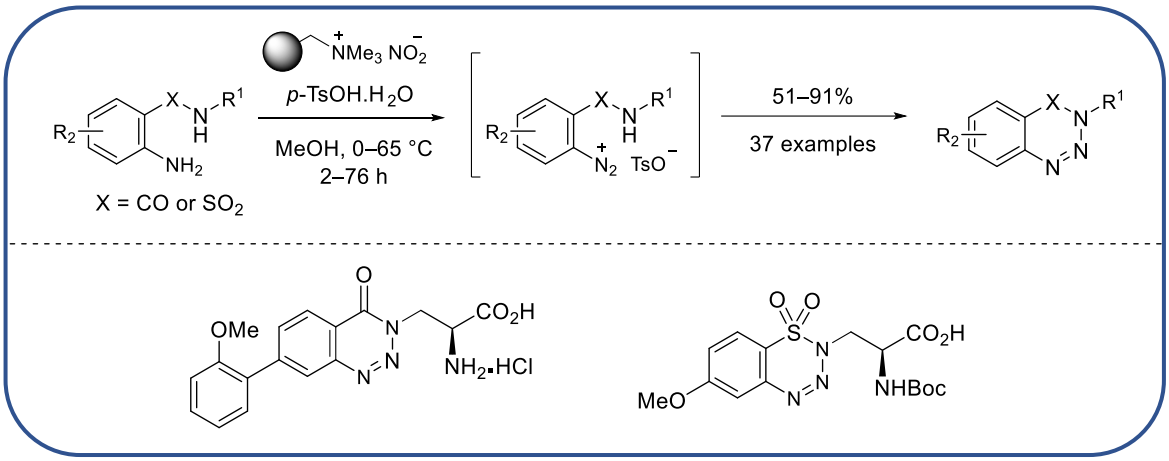
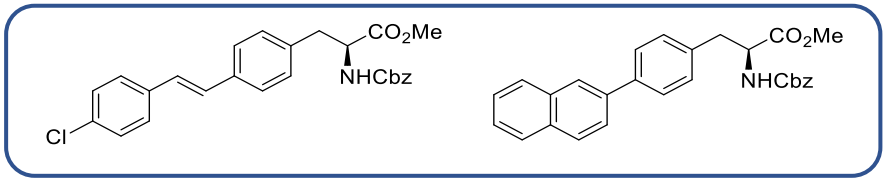
May 2023

Abstract

The aim of this PhD was to utilise novel methodology for the synthesis of fluorescent amino acids. The first section describes the development of a mild, one-pot diazotisation and cyclisation procedure for the synthesis of benzotriazinones and benzothiatriazinedioxides *via* stable aryl diazonium tosylates. The synthetic utility of the procedure was highlighted by the generation of various biologically active compounds containing these heterocycles. The second section discusses incorporation of these heterocyclic scaffolds into the side-chains of amino acids. This generated a range of novel fluorescent amino acids with dual emission properties. The amino acid with optimal fluorescent properties was incorporated into a cell-penetrating peptide using solid phase peptide synthesis and investigated for use as a biological imaging agent.



The third project focused on the synthesis of stilbene and biphenyl amino acids *via* short synthetic routes. Synthesis of the stilbene series was achieved *via* diazotisation of a protected 4-aminophenyalanine analogue followed by a Heck-Matsuda cross-coupling reaction. The biphenyl amino acids were synthesised utilising a novel one-pot nonaflate formation and Suzuki-Miyaura cross coupling reaction. The optical properties of both series were investigated.



The final section discusses functionalisation of tyrosine *via* a mild Suzuki-Miyaura cross-coupling reaction. This generated a library of novel amino acids with interesting fluorescent properties. It was found that a significant improvement in the brightness of this series could be achieved *via* a C–H bond activated cyclisation to give the corresponding dibenzofuran.

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Acknowledgements

First and foremost, I would like to thank my supervisor Professor Andrew Sutherland for giving me the opportunity to undertake this PhD. Thank you so much for your continued support and guidance the last 4 years. You have been patient with all my questions and your insightful knowledge has been invaluable. Most importantly, thank you for my Rod Stewart photo which sits pride of place on my desk. Thank you also to Professor Richard Hartley for providing excellent feedback at annual progression reviews and to the University of Glasgow for providing my college scholarship which has funded my research.

I'd like to thank all the technical staff in the Joseph Black Building including Karen and Finlay (stores), Andy, Jess and Gangi (MS), Dave and Alec (NMR) and Stuart and Arlene (IT). Thanks also to Dr William Peveler for training and use of the fluorimeter as well as Professor Andrew Jamieson and Dr Danielle Morgan for their indispensable guidance on peptide synthesis. Thanks to Dr Michael Capper for his aid in generating the cell cultures required for cell imaging and to Dr John Pediani for helping us capture the images.

Thank you to all the Sutherland group members, past and present, for making this such an enjoyable experience. Martyn, Rebecca, Euan, Holly, Liyao, Leanne, Nina, Lachlan, Valeria, Olivia, thank you. Special thanks to my side-kick Amy, I couldn't have asked for a better, more supportive friend to have experienced my PhD with! I'd like to extend my thanks to the Hartley group, especially Joe and Becca for all your great chat. Thanks also to my students throughout the years; Robyn, Katie and Ryan.

Finally, I would like to give a special thank you to my Mum, Dad, Danielle and Tony for being so supportive throughout my entire university experience. Thank you all for always listening to all my practice section talks and smiling and nodding when appropriate (even if it felt against your will). You all kept me calm and told me when to chill. Thank you so much to my fiancé Patrick who has been amazing throughout. Thanks for listening to all my boring chemistry fails and looking excited when I tell you something cool happened with one of my reactions. Thanks also for making me excellent dinners for the last 4 years as food is my true motivation. I'd like to extend my thanks to the rest of the Harrigan clan for their continued support over the years. Finally, thanks to my Ship Bank family for all the great memories the last five years.

"*Don't walk before you can run*" - Ellen Johnston

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis represents the original work of Rochelle McGrory and has not been submitted for any other degree at the University of Glasgow or any other institution. The research was carried out at the University of Glasgow in the Loudon Laboratory under the supervision of Professor Andrew Sutherland between October 2019 to March 2023. Aspects of the work described herein have been published elsewhere as listed below.

R. McGrory, Reka J. Faggyas and A. Sutherland, One-pot synthesis of *N*-substituted benzannulated triazoles *via* stable arene diazonium salts, *Org. Biomol. Chem*., 2021, **19**, 6127–6140

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Signature:

Abbreviations

°C	Degrees centigrade
Ac	Acetyl
A_{hx}	6-(Fmoc-amino)caproic acid
ANAP	3-(6-acetylnaphthalen-2-ylamino)-2-aminopropanoic acid
Ar	Aromatic
A. U.	Arbitrary units
[BMIM]NTf_2	1-Butyl-3-methyl-imidazolium bis(trifluoromethylsulfonyl)imide
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BODIPY	Boron-dipyrromethene
bpy	2,2'-Bipyridine
br	Broad
Bt	Benzotriazole
Bu	Butyl
Bz	Benzyl
Cbz	Carboxybenzyl
CDI	*N*,*N*'-Carbonyldiimidazole
CI	Chemical ionisation
COD	Cyclooctadiene
COSY	Correlated spectroscopy
CPP	Cell-penetrating peptide
d	Doublet
DABCO	1,4-Diazabicyclo[2.2.2]octane
DAP	2,3-Diaminopropanoic acid
dba	Dibenzylideneacetone
DCE	Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless enhancement polarisation transfer
DIAD	Diisopropyl azodicarboxylate
DIC	*N*,*N'*-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMABN	4-Dimethylaminobenzonitrile
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's modified eagle's medium

DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
dppf	1,1’-Bis(diphenylphosphino)ferrocene
EC_{50}	Half maximal effective concentration
EDC.HCl	Ethylcarbodiimide hydrochloride
ee	Enantiomeric excess
EI	Electron impact
ESI	Electrospray ionisation
Fmoc	Fluorenylmethyloxycarbonyl
Fmoc-Osu	Fmoc *N*-hydroxysuccinimide ester
FRET	Fluorescence resonance energy transfer
g	Grams
GFP	Green fluorescent protein
h	Hour
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N’,N’*-tetramethyluronium
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation spectroscopy
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
ICT	Intramolecular charge transfer
IR	Infrared
ISC	Intersystem crossing
J	NMR spectra coupling constant
m-	*Meta*
m	Multiplet
M	Molar
m/*z*	Mass to charge
Me	Methyl
mg	Milligrams
MHz	Megahertz
mL	Millilitres
mM	Millimolar
µM	Micromolar
mmol	Millimole

mol	Mole
MOM	Methoxymethyl
Mp	Melting point
MRI	Magnetic Resonance Imaging
MW	Microwave
NAT	*N*-Acetyltransferase
NBS	*N*-Bromosuccinimide
Nf	Nonafluorobutanesulfonate
NIS	*N*-Iodosuccinimide
nm	Nanometers
NMR	Nuclear magnetic resonance
Ns	Nosyl
o-	*Ortho*
p-	*Para*
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PBS	Phosphate-buffered saline
PET	Positron emission tomography
Ph	Phenyl
ppm	Parts per million
q	Quartet
R_f	Retention factor
rt	Room temperature
S_0	Ground state
S_1	First excited state
S_2	Second excited state
S_NAr	Nucleophilic aromatic substitution
S_N2	Nucleophilic substitution biomolecular
SPECT	Single-photon emission computed tomography
SPhos	2-Dicyclohexylphosphino-2’,6’-dimethoxybiphenyl
SPPS	Solid phase peptide synthesis
t	Triplet
T_1	Triplet excited state
TBAI	*tert*-Butyl ammonium iodide
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

TICT	Twisted intramolecular charge transfer
TIS	Triisopropylsilane
TLC	Thin layer chromatography
TMEDA	*N*,*N*,*N*',*N*'-Tetramethylethylenediamine
Tr	Trityl
Ts	Tosyl
UV	Ultraviolet
Vis	Visible
XPhos	2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl
ε	Molar attenuation coefficient
λ_{abs}	Absorbance maximum
λ_{em}	Emission maximum
Φ	Fluorescence quantum yield

1.0 Introduction

1.1 Benzannulated Triazoles

The benzotriazinone scaffold consists of a bicyclic system containing three consecutive nitrogen atoms and a carbonyl at the 4-position (Figure 1). The benzothiatriazinedioxide scaffold differs only in nomenclature and replacement of a carbonyl for a sulfur dioxide motif at the 1-position.

Figure 1: 1,2,3-Benzotriazin-(4*H*)-one (**1a**) and 1,2,3,4-benzothiatriazine-1,1(2*H*)-dioxide (**2a**).

A wide range of biological and chemical applications of these heterocycles has been discovered (Figure 2).[1-4] For example, Nucci and co-workers reported the synthesis of various N_3-substituted benzotriazinones which possess local anaesthetic activity, with several having comparable duration times and IC_{50} values to lidocaine.[1] Work by Mahmoud and co-workers in 2020 has shown promising results for the use of pyridinium benzotriazinones as potential anti-Alzheimer's agents *via* cholinesterase inhibition.[5] Furthermore, Ding *et al.*[6] have reported the synthesis of a benzotriazinone-derived antifungal agent with comparable activity against *Aspergillus fumigatus* as known antifungal agent, albaconazole. Finally, discovery of a benzotriazinone-based orally bioavailable GPR139 receptor agonist with an EC_{50} of 22 nM has been reported by Hitchcock and co-workers.[7] This analogue is currently in clinical studies for treatment of negative symptoms associated with Schizophrenia. The herbicide, insecticide and nematicide activity of various benzotriazinones has also been reported.[8-10] Benzothiatriazinedioxides were originally patented as azo dyes by Dickey and McNally in 1946.[11] The diuretic activity of these analogues was later reported by Childress in 1960.[12] Since these initial discoveries, benzothiatriazinedioxides have found a wide array of applications including heat sensitive recording materials, polymerisation catalysts and rubber components.[13-15] More recently, their use as nematicidal agents was patented in 2018.[16]

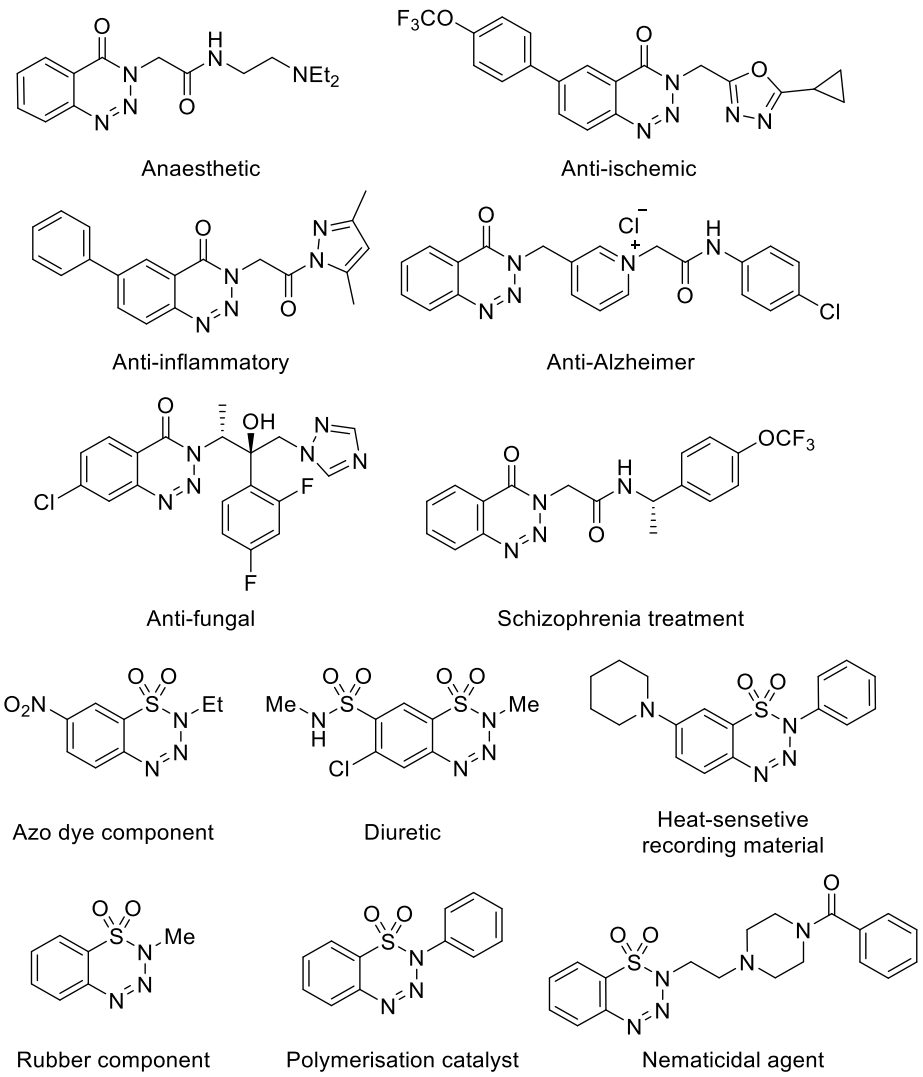
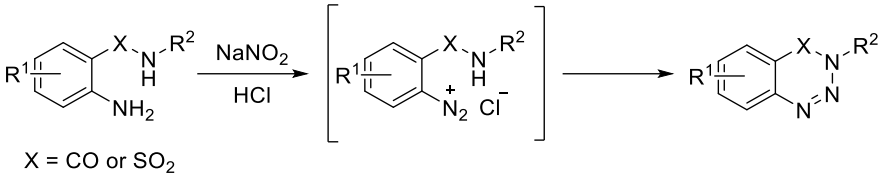


Figure 2: Applications of benzotriazinones and benzothiatriazinedioxides.

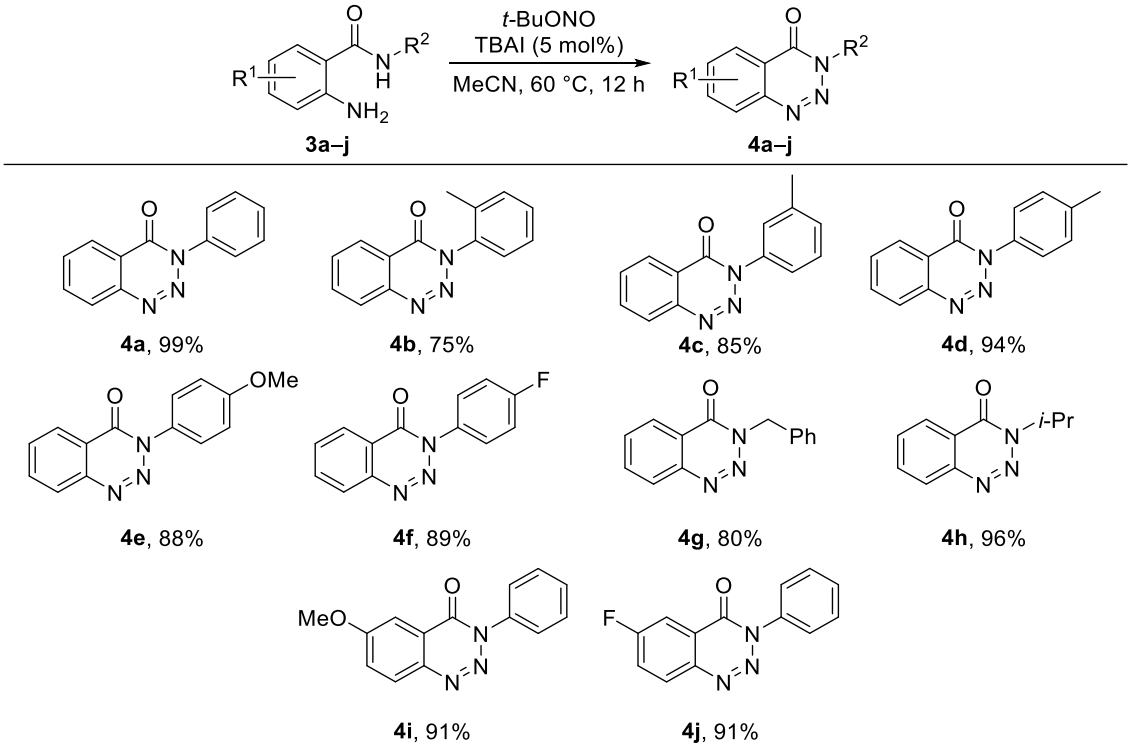
1.1.1 Synthesis of Benzannulated Triazoles

Traditional synthesis of these heterocycles involves conversion of 2-aminobenzamides or 2-aminobenzenesulfonamides to diazonium salt intermediates using sodium nitrite and hydrochloric acid (Scheme 1). The diazonium salts then undergo intramolecular cyclisation to give the desired heterocycles.[11,17] This method has several disadvantages. Firstly, generation of the diazonium chloride intermediates must be carried out *in situ* due to their unstable and potentially explosive nature.[18] Furthermore, the requirement of strong acid renders this procedure incompatible with starting materials bearing acid sensitive functionalities. Finally, the use of sodium nitrite and hydrochloric acid can result in the release of toxic nitrous oxides, resulting in an asphyxiation risk.



Scheme 1: Synthesis of benzannulated triazoles *via* diazonium chloride intermediates.

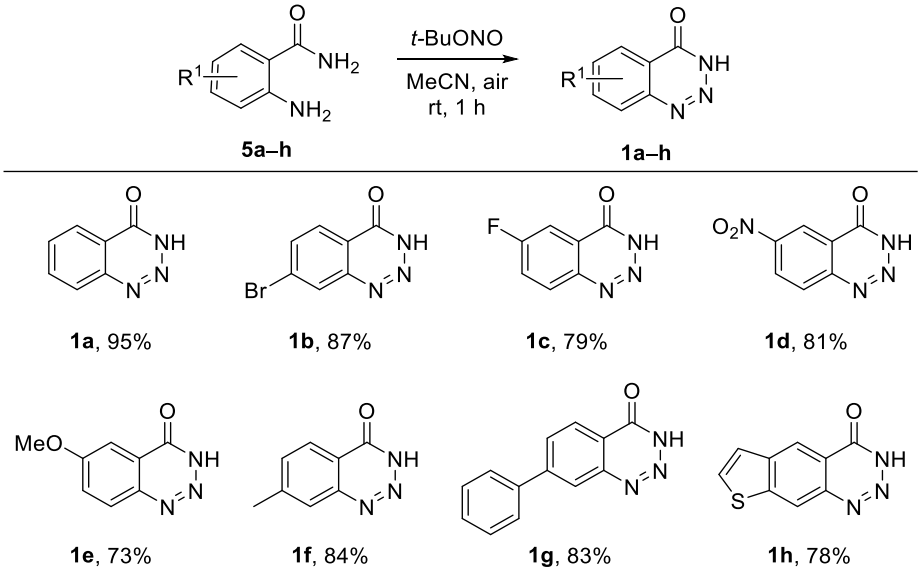
Consequently, substantial research has focused on the development of new methods for the synthesis of these heterocycles. For example, work by Yan *et al.*[19] has shown benzotriazinones can be synthesised *via* diazotisation and *in situ* cyclisation of 2-aminobenzamides using *tert*-butyl nitrite in the presence of a *tert*-butyl ammonium iodide (TBAI) catalyst (Scheme 2). The procedure was conducted in the absence of acid hence increasing the scope of this reaction. It is proposed *tert*-butyl ammonium iodide acts as a catalyst by allowing for anion exchange to form the more reactive diazonium iodide. The reaction was successful for the synthesis of N_3-substitued benzotriazinones bearing *ortho*-, *meta*- and *para*-substituted aryl rings (**4b**–**d**). The yields were unaffected by the electronic nature of the substituent at the N_3-position (**4e** vs. **4f**). N_3-Alkyl substitution was also tolerated (**4g**–**h**). The scope of R^1 substitution was also investigated, generating the desired compounds in excellent yields (**4i** and **4j**). Interestingly, generation of N_3-unsubstituted benzotriazinones is not discussed, suggesting this procedure is not compatible with their synthesis. Furthermore, the reaction requires an elevated temperature and long reaction times, limiting its utility on an industrial scale.



Scheme 2: Substrate scope of benzotriazinones synthesised *via* diazotisation and *in situ* cyclisation using *tert*-butyl nitrite with a *tert*-butyl ammonium iodide catalyst.[19]

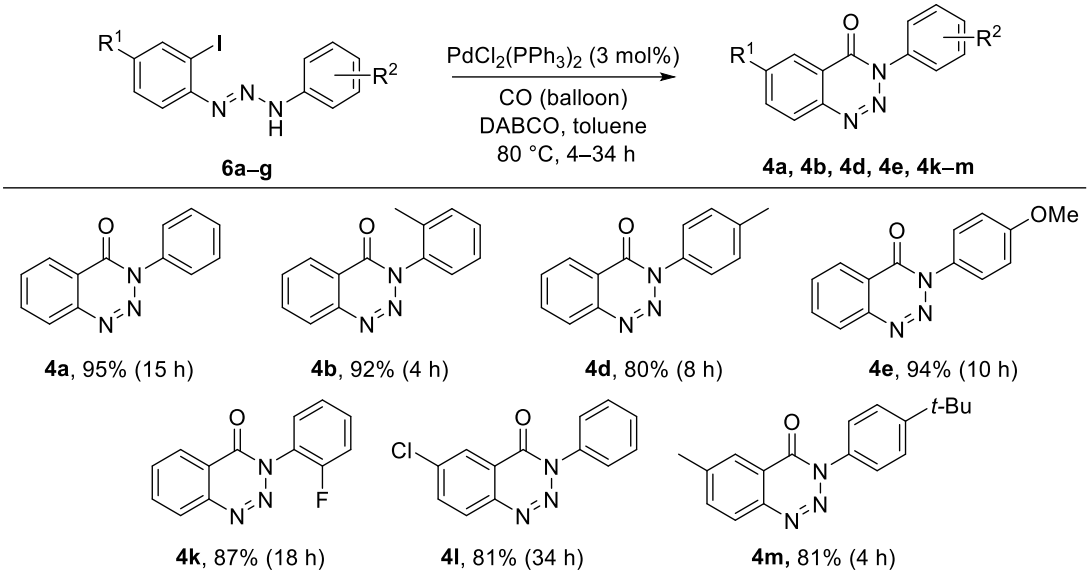
It should be noted that the team previously reported a synthesis for benzotriazinones from 2-aminobenzamides using potassium iodide and *tert*-butyl hydroperoxide in the presence of nitromethane.[20] However, this procedure involves *in situ* generation of nitrous acid, making it incompatible with various acid-sensitive substrates. Furthermore, the reaction required an elevated temperature of 120 °C. Similar issues were encountered when evaluating the procedure developed by Batra and co-workers using sodium nitrite in the presence of iodine.[21]

More recently, Wu and co-workers demonstrated that 2-aminobenzamides can be successfully converted to benzotriazinones in the absence of a catalyst or proton source. Synthesis was achieved using *tert*-butyl nitrite as both an oxidant and nitrogen source at room temperature in air (Scheme 3).[22] The reaction was high yielding for compounds bearing both electron withdrawing and electron donating substituents (**1b**–**f**). The reaction was also successful for biaryl benzamide **1g**. Heterocyclic scaffold **1h** was generated in a 78% yield. However, it should be highlighted that no examples of N_3-substituted benzotriazinones were presented in the substrate scope.



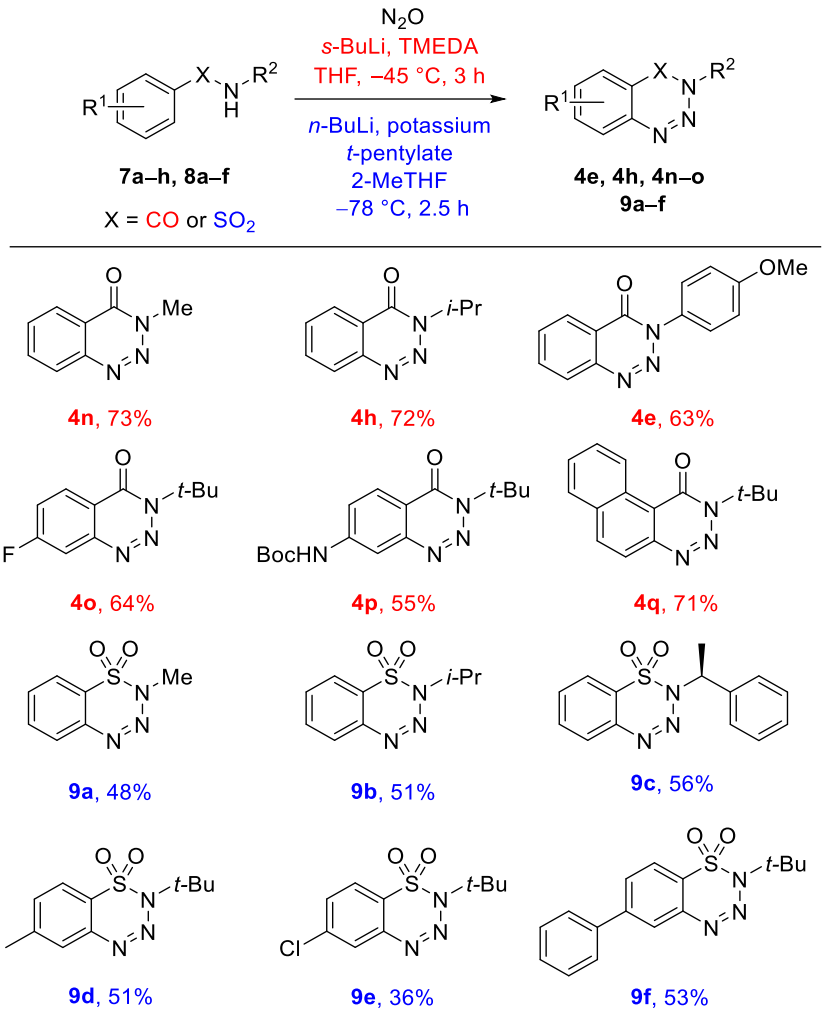
Scheme 3: Synthesis of N_3-unsubstituted benzotriazinones from 2-aminobenzamides using *tert*-butyl nitrite.[22]

Alternatively, work by Sankararaman and co-workers has shown N_3-substituted benzotriazinones can be generated from 1,3-diaryltriazenes *via* a palladium catalysed carbonylative annulation reaction (Scheme 4).[23] Utilising $PdCl_2(PPh_3)_2$ as the catalyst (3 mol%), in the presence of 1,4-diazobicyclo[2.2.2]octane (DABCO), the reaction generated benzotriazinones containing both *ortho*- and *para*-substituted R^2-aryl systems. Interestingly, *ortho*-substituted analogues were found to react faster than the corresponding *para*-derivatives (**4b**, 4 h vs. **4d**, 8 h). This is proposed to be due to steric repulsion which accelerates the reductive elimination step of the catalytic cycle. The reaction was also successful for analogues bearing R^2-aryl rings with electron withdrawing or electron donating substituents (**4e** and **4k**). The electronic nature of R^1-aryl substituents did not affect the yield (**4l** and **4m**). However, electron-withdrawing groups required extensive reaction times. In order to expand the scope of this methodology, the team developed a synthesis from 2-bromophenyl triazenes. However, this required an increased catalyst loading of 10 mol% and was generally found to give the desired benzotriazinones in lower yields. In addition to this, the reaction suffers from long reaction times and the use of carbon monoxide as a reagent is undesirable from a safety perspective.



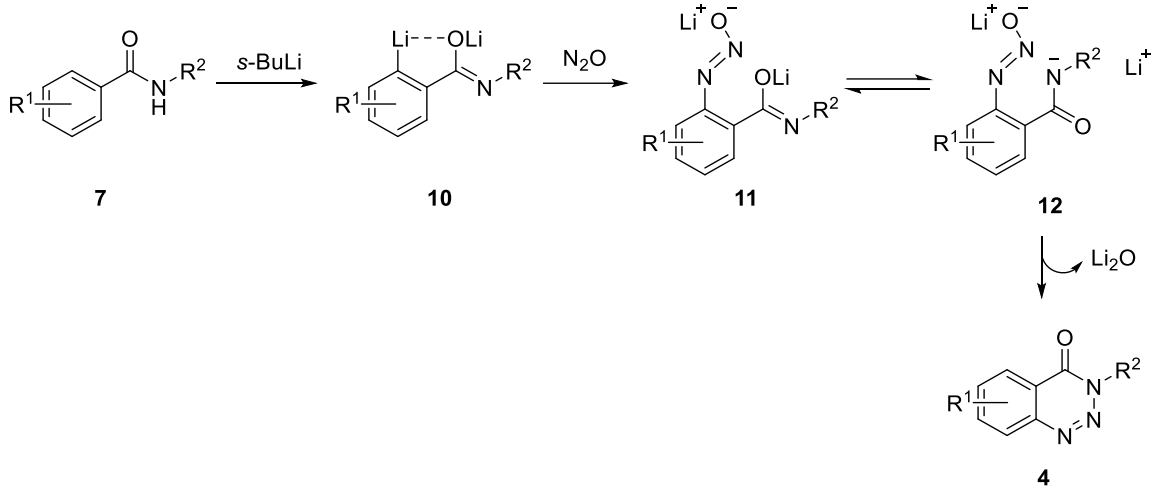
Scheme 4: Synthesis of N_3-substituted benzotriazinones *via* palladium-catalysed carbonylative annulation reaction.[23]

Methods for the synthesis of benzothiatriazinedioxides remain less well explored. Work by Cui and co-workers reported the development of a directed *ortho*-metalation/redox cyclisation of amides and sulfonamides for the synthesis of benzannulated triazoles.[24] Utilising *s*-BuLi or *n*-BuLi in the presence of nitrous oxide, the desired benzotriazinones and benzothiatriazinedioxides were generated in moderate to good yields (Scheme 5). Substitution at the N_3-position was tolerated for both series generating N_3-alkyl and N_3-aryl substituted heterocycles in good to excellent yields (**4e**, **4h**, **4n** and **9a**–**c**). A 2-aminobenzenesulfonamide containing a stereocentre was also examined, generating **9c** in a 56% yield. *para*-Substitution of both scaffolds was tolerated, producing the desired compounds in moderate to good yields for electron-rich and electron-deficient substrates (**4o** and **4p**). By negating the use of acid, the reaction was successful for the synthesis of Boc-protected amino derivative **4p**, which cannot be generated by conventional synthetic routes. Furthermore, *N*-(*tert*-butyl)-1-naphthamide-derived benzotriazinone (**4q**) was also successfully synthesised in a 71% yield. However, this methodology has several disadvantages. For example, the substrate scope of both heterocycles displayed few examples of *ortho*- or *meta*-substitution. This is likely due to generation of regioisomers. Furthermore, the reaction required the use of strong bases, *s*-BuLi and *n*-BuLi. When the reaction was attempted using weaker bases, no conversion to desired product was observed. Finally, the requirement of inert conditions increases the operational complexity of this procedure.



Scheme 5: Scope of benzannulated triazoles generated *via* redox cyclisation with nitrous oxide.[24]

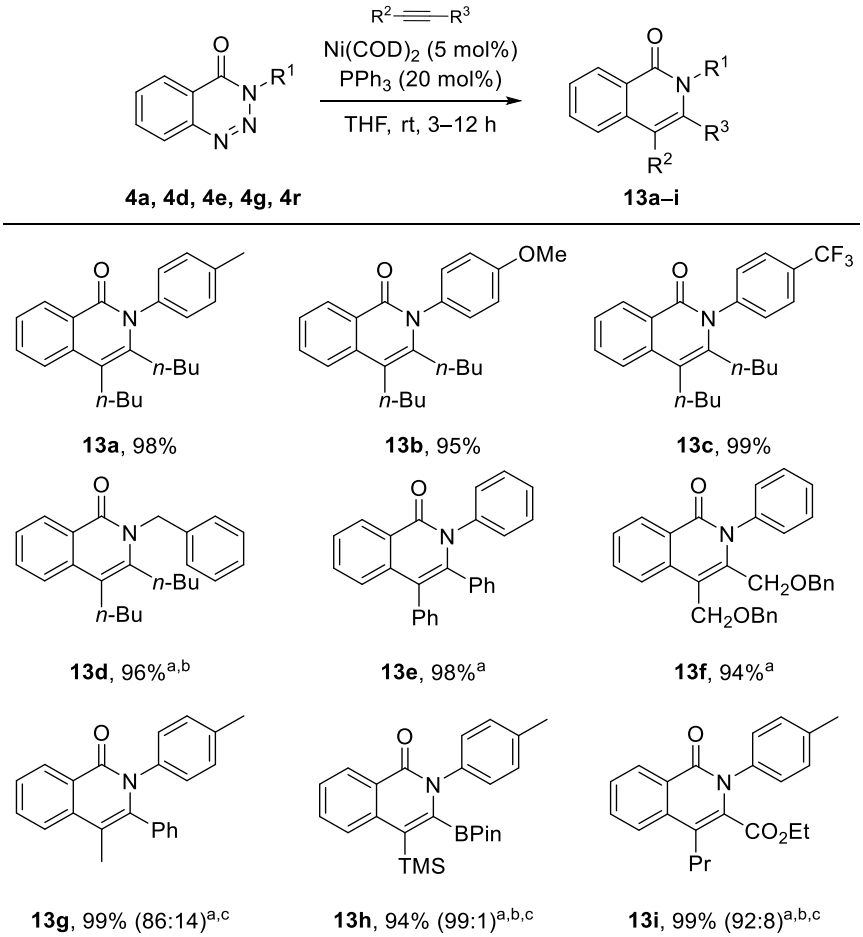
The proposed mechanism for the synthesis of benzotriazinones using this methodology is shown in Scheme 6. Firstly, directed *ortho*-metalation occurs using *s*-BuLi to generate aryl lithium **10**. Nitrous oxide (N_2O) is then bubbled through the reaction mixture, serving as both the oxidant and nitrogen source to generate **11**. Formation of imide **12** then occurs. This is followed by intramolecular cyclisation to give benzotriazinone **4** as well as lithium oxide as a by-product.



Scheme 6: Proposed mechanism for the formation of benzotriazinones from 2-aminobenzamides.[24]

1.1.2 Chemical Transformations of Benzannulated Triazoles

As well as biological applications, benzannulated triazoles have been used extensively for the synthesis of other important heterocycles. Some of the most studied transformations of benzannulated triazoles involve nickel-catalysed denitrogenative annulation reactions. In 2008, Murakami and colleagues reported the synthesis of 1(2*H*)-isoquinolones *via* nickel-catalysed denitrogenative alkyne insertion of benzotriazinones (Scheme 7).[25] 1(2*H*)-Isoquinolones are important heterocyclic scaffolds found in various biologically active compounds including anti-cancers and anti-hypertensives.[26,27] Utilising the optimised reaction conditions, benzotriazinones bearing *N*-aryl groups with either electron donating or electron withdrawing substituents were successfully converted to the corresponding 1(2*H*)-isoquinolones in excellent yields (**13a**–**c**). An elevated reaction temperature of 60 °C was required for *N*-benzyl derivative **13d**. The reaction was also successful for various symmetrical alkynes (**13e**–**f**) and showed high regioselectivity for unsymmetrical alkynes (**13g**–**i**).

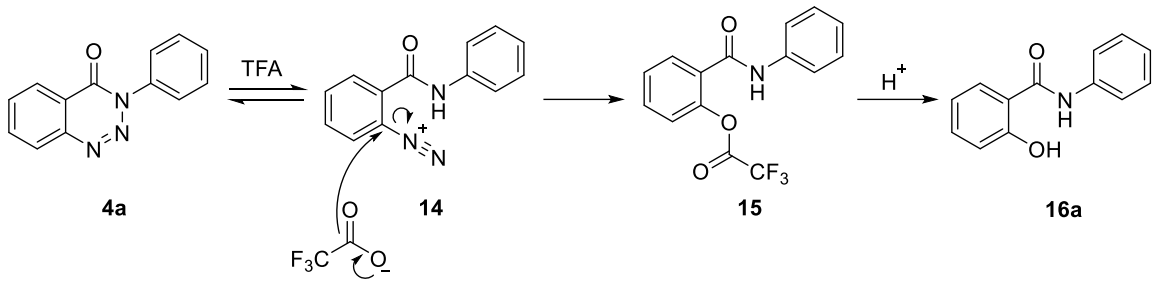


Scheme 7: Nickel-catalysed denitrogenative alkyne insertion of benzotriazinones. [a]PMe_3 (10 mol%) used as ligand; [b]Reaction carried out at 60 °C; [c]Combined yield of regioisomers, regioselectivity shown in parenthesis.[25]

Since their initial findings, the group have reported denitrogenative annulation reactions of benzotriazinones and benzothiatriazinedioxides with other small molecules including 1,3-dienes and alkenes. This has allowed the formation of various heterocycles including 3,4-dihydroisoquinolin-1(2*H*)-ones and 3,4-dihydro-1,2-benzothiazine-1,1-dioxides.[28-30] These heterocycles are found in various plant alkaloids and have a range of biological activities including *N*-acetyltransferase (NAT) inhibition to prevent drug resistance and, calpain I inhibition for treatment of neurodegenerative diseases.[31-33] A palladium-catalysed method incorporating isocyanides was later developed for the synthesis of 3-(imino)isoindolin-1-ones and 3-(imino)-thiaisoindoline-1,1-dioxides.[34] These heterocycles are of interest for their analgesic and anti-inflammatory properties as well as for the development of pesticides.[35,36]

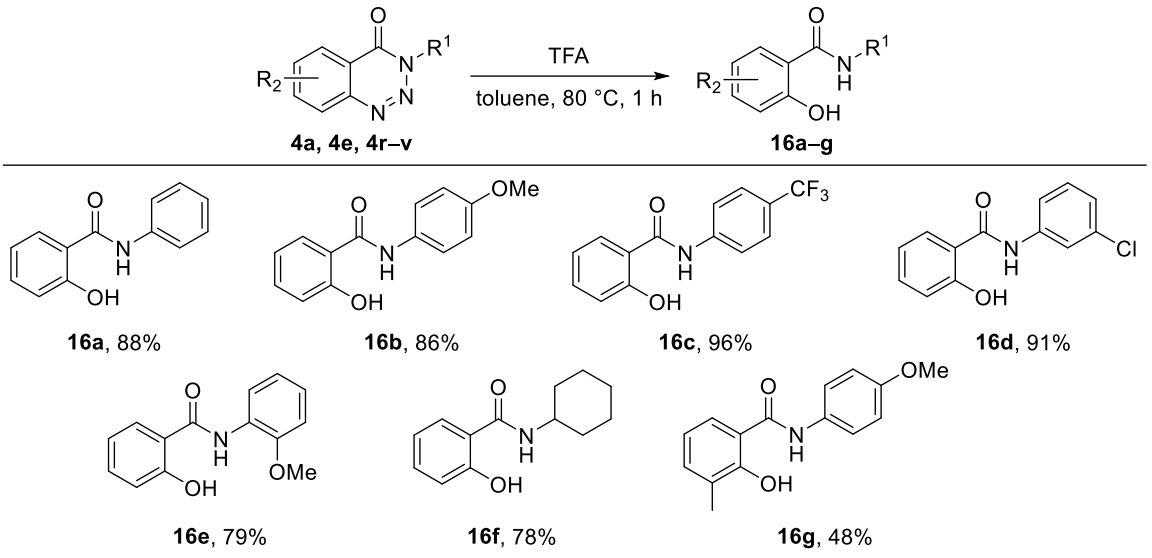
In 2022, Korivi and co-workers reported an acid-mediated denitrogenative hydroxylation of benzotriazinones.[37] Formation of the desired *ortho*-hydroxylated

benzamides is proposed to occur *via* reversible ring opening of the benzotriazinone scaffold in trifluoroacetic acid (TFA) (Scheme 8). The diazonium intermediate generated is then subjected to nucleophilic attack by the trifluoroacetate counterion to generate **15** with loss of dinitrogen. Hydrolysis then gives desired product **16a**.



Scheme 8: Proposed mechanism for formation of hydroxylated benzamide **16a**.[37]

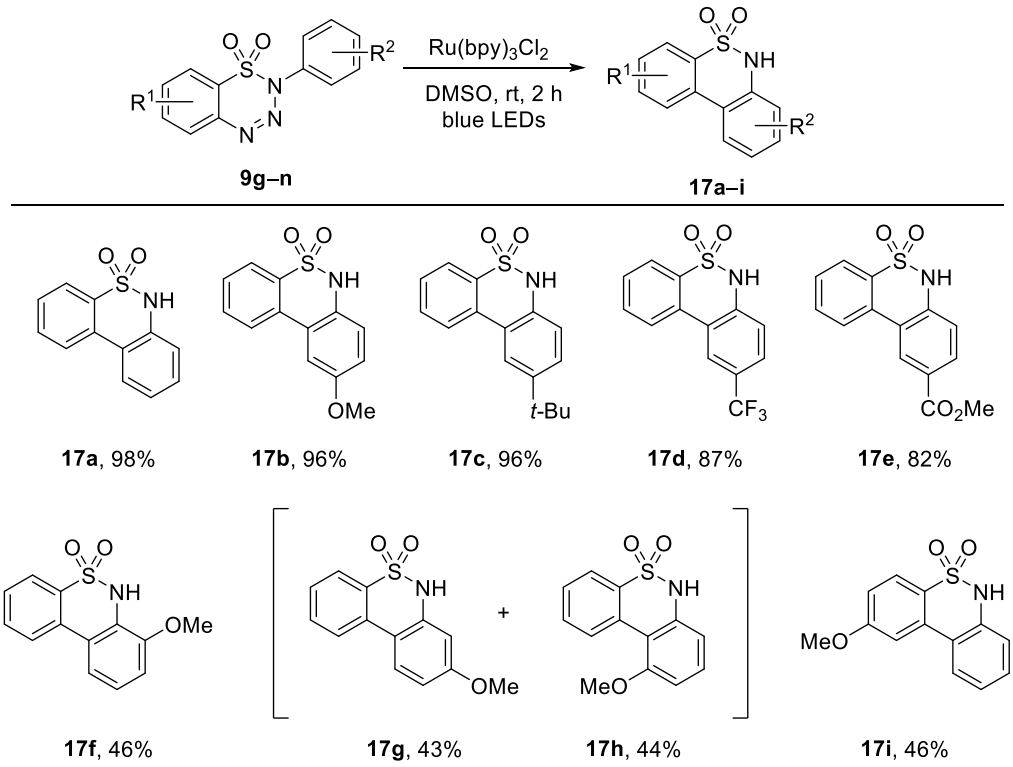
Following optimisation, the reaction was successful for a wide range of *N*-aryl benzotriazinones bearing both electron-donating and electron-withdrawing groups (**16a**–**c**, Scheme 9). High yields were obtained for *ortho*-, *meta*- and *para*-substituted rings (**16c**–**e**). *N*-Alkyl benzotriazinones also participated in the reaction (**16f**). The reaction was successful for the efficient (85%) gram scale synthesis of riparin C, a known anti-microbial. *ortho*-Substitution of the benzotriazinone scaffold resulted in a significant decrease in yield as indicated by **16g**.



Scheme 9: Substrate scope of hydroxylated benzamides generated *via* acid-mediated denitrogenative hydroxylation of benzotriazinones. [37]

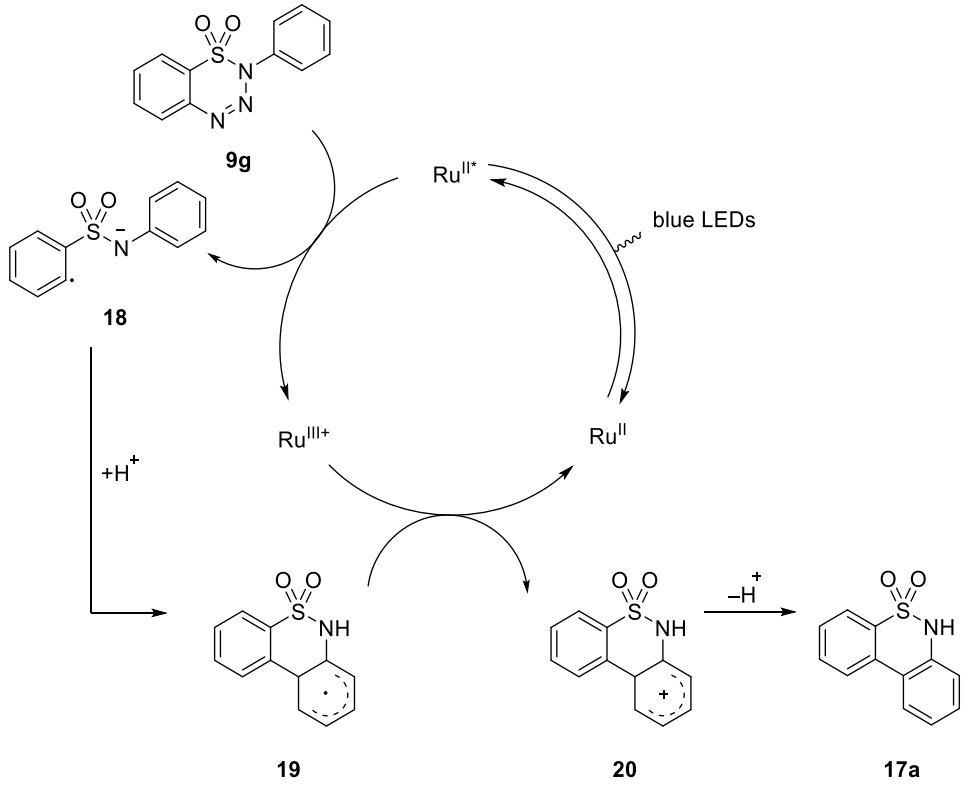
The photochemical transformations of benzothiatriazinedioxides have been reported as early as 1971 by Whitesides and Arhart.[38] In 2016, Yu and co-workers

reported the synthesis of biaryl sultams from benzothiatriazinedioxides *via* a visible-light promoted denitrogenative cyclisation reaction.[39] The anti-bacterial and anti-inflammatory properties of biaryl sultams have been reported.[40,41] By utilising a ruthenium photoredox catalyst, the desired cyclic systems were formed in high yields under mild conditions (Scheme 10). The reaction was successful for substrates with either electron rich or electron deficient *N*-aryl rings (**17b**–**e**). The efficiency of the reaction was subject to steric effects. This is highlighted by the difference in yields for *para*-substituted analogue **17b** (96%) compared to *ortho*-substituted analogue **17f** (46%). The m*eta*-substituted analogue was found to generate a 1:1 mixture of regioisomers **17g** and **17h**.



Scheme 10: Substrate scope of biaryl sultams generated *via* visible-light-promoted denitrogenative cyclisation of benzothiatriazinedioxides.[39]

The proposed mechanism for this transformation is shown in Scheme 11. Firstly, irradiation of the ruthenium photocatalyst results in formation of the excited state. This is then oxidatively quenched by **9g** generating nitrogen anion **18**. Intramolecular radical recombination and subsequent protonation gives species **19**. Oxidation of **19** by ruthenium(III) gives **20**, which rearomatises to give the desired biaryl sultam **17a**.



Scheme 11: Proposed mechanism for synthesis of biaryl sultams *via* visible-light-promoted denitrogenative cyclisation.[39]

The group later developed a one-pot procedure for the synthesis of biaryl sultams from 2-aminobenzenesulfonamides *via* a one-pot diazotisation and denitrogenative cyclisation process. A similar procedure has since been reported by Chen *et al*.[42] for the synthesis of *ortho*-phosphorylated benzamides from benzotriazinones.

1.2 Fluorescence Imaging

One aim of this PhD was to generate novel fluorescent amino acids incorporating the benzannulated triazoles discussed in Section 1.1. The following section will thus discuss the important features of fluorescence and fluorescence imaging.

Fluorescence imaging is a highly sensitive, non-invasive technique for imaging biomolecules.[43] The rapid detection time of this method allows for molecules to be visualised in real time and with high spatial resolution. There are a wide range of versatile fluorescent probes available including proteins, quantum dots and small molecules.[44] The ability to achieve single-molecule detection by chemically tuning the photoluminescent properties of probes has resulted in a wide range of both analytical and diagnostic applications.[45] For example, a Cy5-labelled fluorescent cell-penetrating peptide has been used to provide intraoperative visualisation of cancerous tissue, leading to improved tumour removal.[46] In addition, β-amyloid

plaques, associated with protein misfolding diseases such as Alzheimer's have been visualised *in vivo* using small molecule probe, NIAD-4.[47] Fluorescence imaging has several advantages over other imaging techniques. Both positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging require the use of short-lived radioisotopes.[48] Magnetic resonance imaging (MRI) suffers from low spatial resolution and long detection times.

1.2.1 Introduction to Fluorescence

Fluorescence occurs when a molecule emits a photon of light from an excited state.[49] The various processes which occur prior to this photon emission are represented using a Jabloński diagram (Figure 3).[50] Following absorption of a photon of light with the correct energy, an electron is promoted from the ground state (S_0) to an electronically excited state (S_1 or S_2).[43] Here, the electron can reside in any of the various vibrational energy levels (V_0–V_2). Generally, non-radiative relaxation from S_2 to S_1 occurs, this is known as internal conversion. Vibrational relaxation then occurs to populate the lowest vibrational energy level of S_1. Return to the ground state can then occur *via* emission of a photon. This is known as fluorescence. Alternatively, if a molecule in S_1 undergoes intersystem crossing, return to the ground state occurs from the triplet excited state (T_1).[49] This gives rise to phosphorescence. Phosphorescence is spin forbidden as the electron in the ground state has the same spin. Consequently, phosphorescence gives rise to longer excited-state lifetimes. Phosphorescence is often not observed for molecules in solution as return to the ground state can also occur *via* non-radiative pathways. These include bond rotation, collisional quenching and electron transfer.

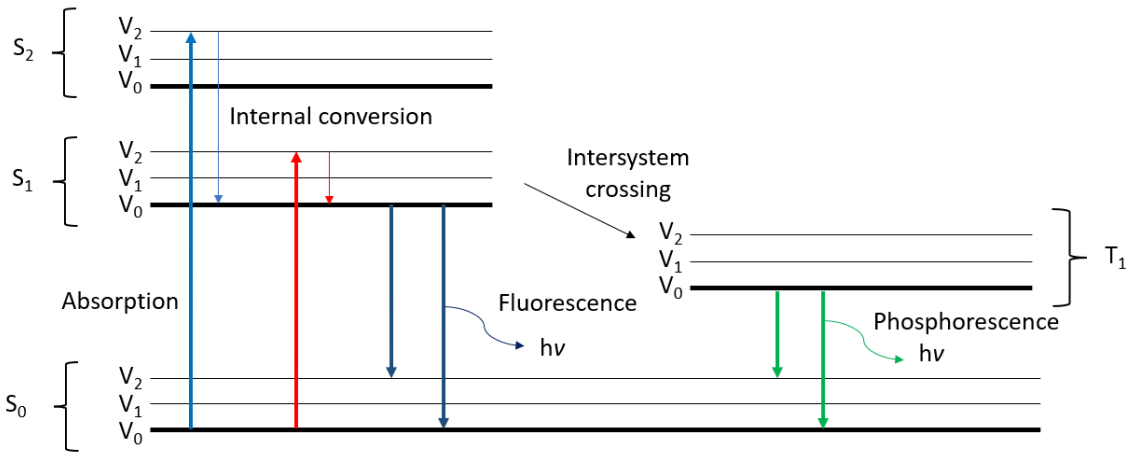


Figure 3: Jabloński diagram.

Fluorescence is generally reported as absorption and emission spectra (Figure 4). The absorbance maximum (λ_{abs}) represents the difference in energy between the ground state, S_0, and the electronically excited state, S_1. The emission maximum (λ_{em}) is dependant on the molecular structure of the fluorophore. Due to vibrational relaxation in the excited state as well as relaxation to higher vibrational levels of S_0, the energy gap of emission between S_1 and S_0 is smaller than for absorption. Consequently, the photon emitted is of a lower energy and longer wavelength. The difference in λ_{abs} and λ_{em} is known as the Stokes shift.[43] It is desirable for a fluorophore to have a large Stokes shift as this avoids measurement issues due to reabsorption of emitted light or self-quenching. Various other environmental factors such as solvent polarity and pH can also vary the Stokes shift.

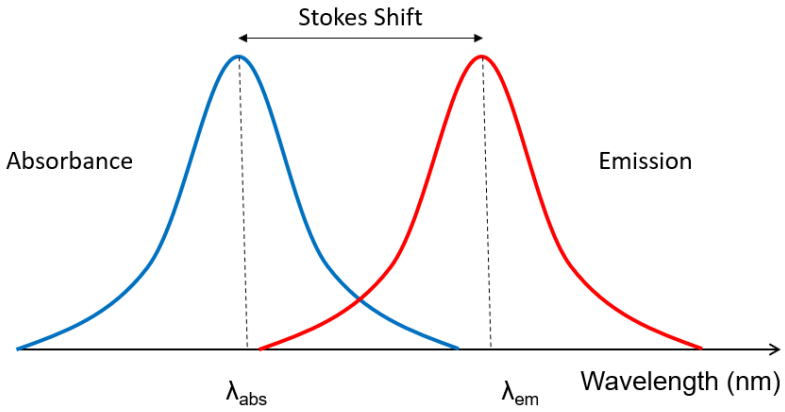


Figure 4: Absorption and emission spectrum.

Absorption and emission spectra for anthracene are shown in Figure 5. The distinct bands in both spectra represent transitions to/from the different vibrational energy levels of S_0 and S_1. As transitions between S_0 to S_1 typically occur in approximately 10^{-15} s, the nuclear geometry of a molecule is not altered and the spacing of the vibrational energy levels is maintained. This is known as the Franck-Condon principle.[49] As a result, the emission spectrum is often a mirror image of the absorption spectrum.[43] It should be noted that in molecules with less structural rigidity, broadening of the emission peaks occurs as a result of rotational relaxation.

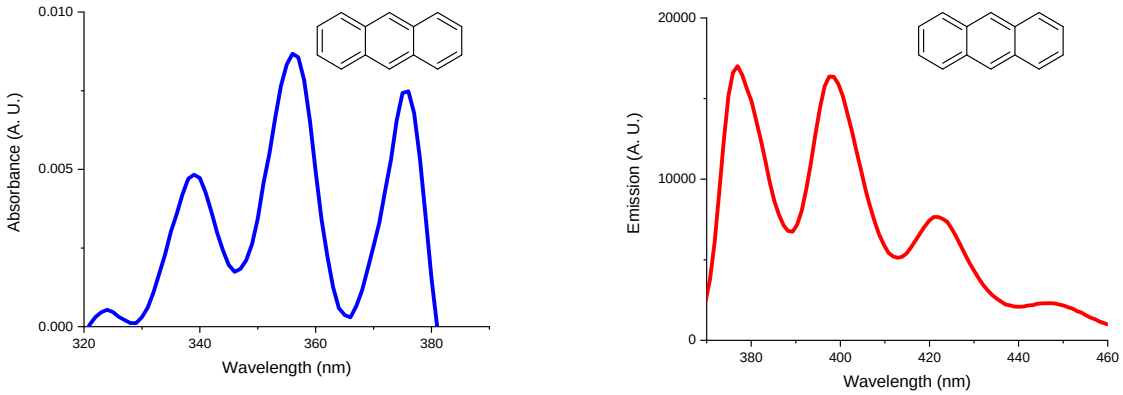


Figure 5: Absorption and emission data of anthracene (3 µM in ethanol, excited at 350 nm).

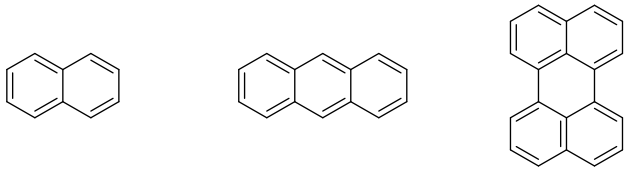
Various terms are often used to quantify fluorescence. The quantum yield (Φ) of a fluorophore is defined as the ratio of the number of photons emitted as fluorescence to the number of photons absorbed.[44] A quantum yield close to one is desirable as it indicates fluorescence is the primary decay pathway of the excited state. The molar attenuation coefficient (ε) is a measure of how strongly a molecule absorbs light at a given wavelength (generally λ_{abs}). The brightness of a fluorophore is defined as the product of its quantum yield and molar attenuation coefficient (Φ × ε).

Return to the ground state can also occur *via* a non-radiative decay mechanism known as quenching.[43] This includes collisional quenching, whereby interaction of the excited state with another molecule can result in return to the ground state without emission of a photon. The most common collisional quencher is molecular oxygen. It is proposed oxygen causes the fluorophore to undergo intersystem crossing to the triplet excited state. Various other small molecules such as ions, halogens and amines are known to act as collisional quenchers. Other quenching mechanisms include static quenching, where a non-fluorescent ground state is generated between the quencher and the fluorophore, and inclusion of heavy atoms which results in increased intersystem crossing.

1.2.2 Modifying the Photoluminescent Properties of Fluorophores

For most organic molecules, fluorescence occurs when an electron is excited to the π* orbital of a conjugated system and returns to S_0 with emission of a photon. Consequently, increasing the conjugation of a system can increase the emission wavelength of a photon by lowering the energy of the π–π* transitions.[51] For use in cellular imaging, it is desirable for fluorophores to have red-shifted absorption and

emission wavelengths as it avoids measurement errors due to cell auto-fluorescence from the naturally occurring amino acids, haemoglobin and melanin.[52] As highlighted by Yamaguchi *et al.,*[51] naphthalene (**21**) has an λ_{em} of 323 nm (Figure 6). Upon increasing the π-conjugation with addition of further aromatic rings, anthracene (**22**) has a red-shifted λ_{em} of 383 nm, while perylene (**23**) has a λ_{em} of 448 nm.



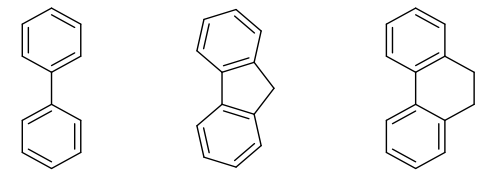
21
λ_{abs} = 286 nm
λ_{em} = 323 nm

22
λ_{abs} = 379 nm
λ_{em} = 383 nm

23
λ_{abs} = 438 nm
λ_{em} = 448 nm

Figure 6: Absorption and emission maxima for **21**, **22**, and **23**.[51]

A common method to improve the fluorescence quantum yield of a fluorophore is to restrict bond rotation.[53] This minimises loss of fluorescence through non-radiative decay methods by reducing the number of vibrational energy levels. Furthermore, restricting bond rotation often leads to more rigid structures which have improved conjugation due to better π-orbital overlap. Work by Nijegorodov and Downey has shown restricting bond rotation of biphenyl (**24**) results in a significant increase in fluorescence quantum yield (Figure 7).[54] Formation of fluorene (**25**) improves the quantum yield from 0.17 to 0.72. Formation of dihydrophenanthrene (**26**) also results in an improved quantum yield of 0.50. It should be noted that one disadvantage of restricting bond rotation is that it can often result in a smaller Stokes shift due to reduced relaxation of the excited state.



24
Φ = 0.17

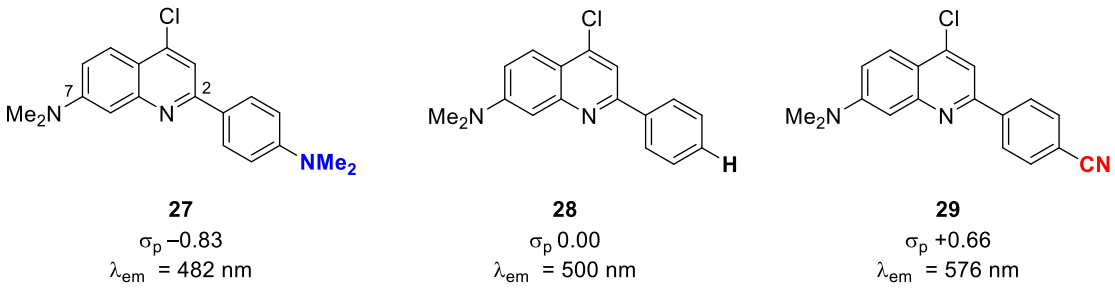
25
Φ = 0.72

26
Φ = 0.50

Figure 7: Quantum yields of **24**, **25** and **26**.[54]

It is well reported that the presence of both electron-withdrawing and electron-donating groups on a chromophore leads to improved photoluminescent properties

via generation of 'push-pull' systems.[55] In 'push-pull' systems, excitation increases the dipole moment of the fluorophore. If the dipole moment generated is sufficiently large, complete charge separation occurs between the donor and acceptor components. This is known as an intramolecular charge transfer (ICT). The charge transfer excited state is lower in energy than the locally excited state and thus a bathochromic shift in the emission wavelength is observed. An example of this phenomenon is highlighted by Chenoweth and co-workers who reported the synthesis of quinoline-based fluorophores with tuneable emission properties (Figure 8).[55] Due to the presence of the electron-donating dimethylamino moiety at the 7-position, it was found that increasing the electron-withdrawing nature of the substituent at the 2-position (indicated by a positive Hammett substituent constant) resulted in red-shifted emission by formation of a charge transfer state. For example, cyano analogue **29** was found to have a λ_{em} of 576 nm. In contrast, electron-donating substituents gave rise to a blue-shifted emission (**27**, λ_{em} 482 nm).



27
σ_p –0.83
λ_{em} = 482 nm

28
σ_p 0.00
λ_{em} = 500 nm

29
σ_p +0.66
λ_{em} = 576 nm

Figure 8: Emission wavelength of quinolines with varying C-2 substitution. σ_p = Hammett constant.[55]

In some instances, formation of a charge transfer state requires rotation of the donor and acceptor groups on the fluorophore (Figure 9).[49] This gives rise to a twisted-intramolecular charge transfer (TICT) state. As a result, two bands are often observed in the emission spectrum, one due to relaxation from the locally excited state, and a red-shifted band due to relaxation from the twisted charge transfer state. The first documentation of TICT fluorescence was highlighted by Grabowski and co-workers over fifty years ago where they reported the dual emission of 4-*N*,*N*-dimethylaminobenzonitrile (DMABN).[56] Upon initial excitation, DMABN forms a locally excited state. Due to steric repulsion of the dimethyl amino group and the phenyl ring, twisting occurs to place these groups in perpendicular geometry, generating a TICT state. Emission from the locally excited state gives rise to an

emission band with a λ_{em} at 354 nm in ethanol whereas emission from the TICT state generated an λ_{em} at 460 nm.[57]

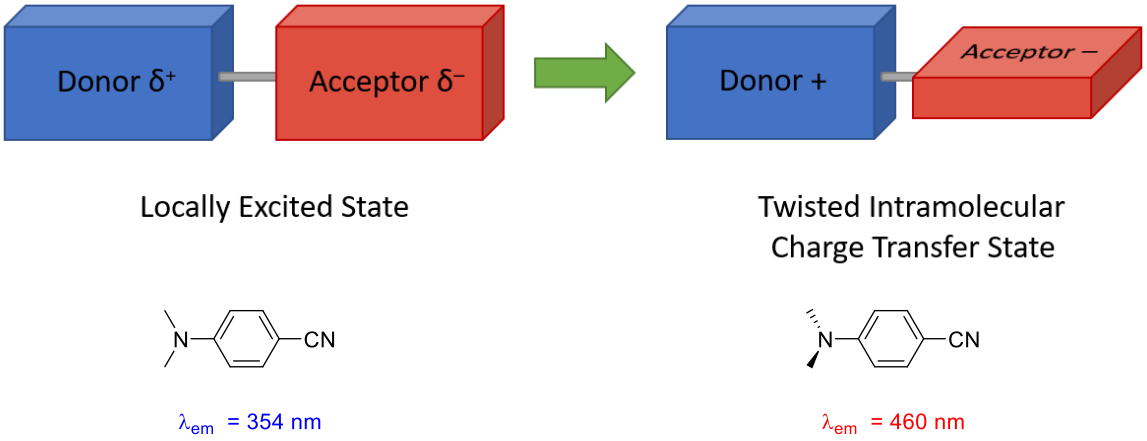
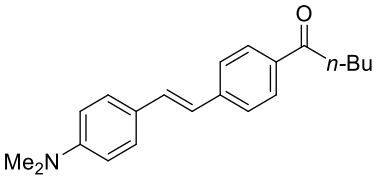


Figure 9: Emission maxima of DMABN from locally excited and TICT states.[56]

Changes in the λ_{abs} and λ_{em} of a chromophore due to changes in solvent polarity is known as solvatochromism.[49] As discussed, chromophores often experience an increase in dipole moment in the excited state. When this occurs, dipole moments of the surrounding solvent can reorientate to stabilise and lower the energy of S_1. Consequently, return to the ground state results in emission of a longer wavelength photon. Generally, the bathochromic shift is more pronounced in more polar solvents due to the increased dipole moment of the solvent. *Trans*-4-dimethylamino-4'-(1-oxobutyl) stilbene (**30**) is an example of a solvatochromic probe which shows a significant shift in λ_{em} when changing from non-polar to polar solvents (Table 1).[58] Due to the 'push-pull' system present in the fluorophore, a charge transfer state is formed upon excitation which is stabilised by increasingly polar solvents. In cyclohexane, the λ_{em} is 424 nm whereas in 1-butanol, the λ_{em} is 574 nm. Solvent sensitive probes are often used to determine the binding site polarity of macromolecules.[43]

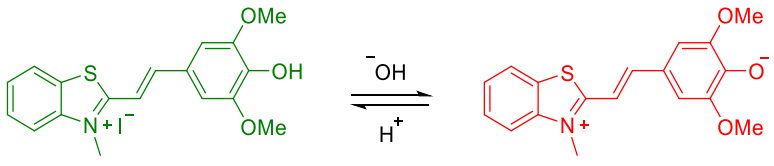


30

Solvent	λ_{em} (nm)
cyclohexane	424
toluene	475
2-MeTHF	505
acetonitrile	562
butanol	574

Table 1: Emission maxima of **30** in solvents of increasing polarity.[58]

Chromophores which exhibit pH-sensitive emission have applications as environmental probes. For example, Yu and co-workers have designed a single-molecule fluorescent probe for simultaneous two-colour visualisation of both the nuclei and mitochondria due to varying pH environments of different cell organelles (Figure 10).[59] Compound **31** has a pKa of 5.87. Consequently, at lower pH's, such as those found in the nuclei, emission occurs primarily from the protonated state, with a λ_{em} of 550 nm (**31**). However, as the environment becomes more basic, deprotonation of the phenol occurs. At pH 8, this results in a 350-fold enhancement of the red-shifted fluorescence emission at 605 nm (**32**) due to improved conjugation through the system. The pH dependency of **31** has been exploited to visualise cell apoptosis. It is proposed that upon treatment with paclitaxel, acidification of the mitochondria occurs, resulting in loss of the mitochondrial membrane potential and hence diminished emission at 605 nm.



31
λ_{em} = 550 nm

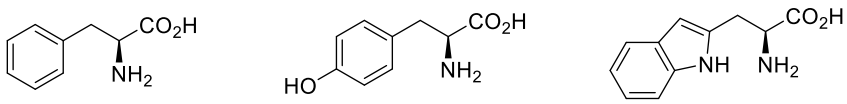
32
λ_{em} = 605 nm

Figure 10: Response of **31** to changes in pH.[59]

1.2.3 Fluorescent Amino Acids

Proteins play an essential role in almost all cellular functions. Consequently, there are a wide array of methods for their imaging.[60] One method involves attachment of

a fluorescent protein to the protein of interest. The most common fluorescent protein is green fluorescent protein (GFP) which has a quantum yield of 0.79 and λ_{em} at 504 nm.[61] However, the large size of fluorescent proteins can cause perturbations to the protein of interest, affecting its function. Furthermore, attachment of a fluorescent protein is generally only achieved at the C- or N-terminus of the protein of interest, drastically limiting the scope of this methodology.[62] An alternative imaging method which overcomes these issues is use of the naturally occurring fluorescent amino acids; L-phenylalanine, L-tyrosine and L-tryptophan (Figure 11).[63] This does however rely on the abundance of these amino acids in the protein of interest. Furthermore, the naturally occurring fluorescent amino acids are known to suffer from poor photoluminescent properties. The low quantum yield and molar attenuation coefficient of phenylalanine renders it only visible in the absence of both tyrosine and tryptophan.[64] Tyrosine, which has an improved quantum yield of 0.14, is prone to fluorescence quenching *via* energy transfer to tryptophan. Tryptophan, although the brightest of the naturally occurring amino acids, is particularly environmentally sensitive and this can lead to difficulty in obtaining consistent emission data. Furthermore, the UV-light required to excite these amino acids can cause damage to the cell or tissue of interest.



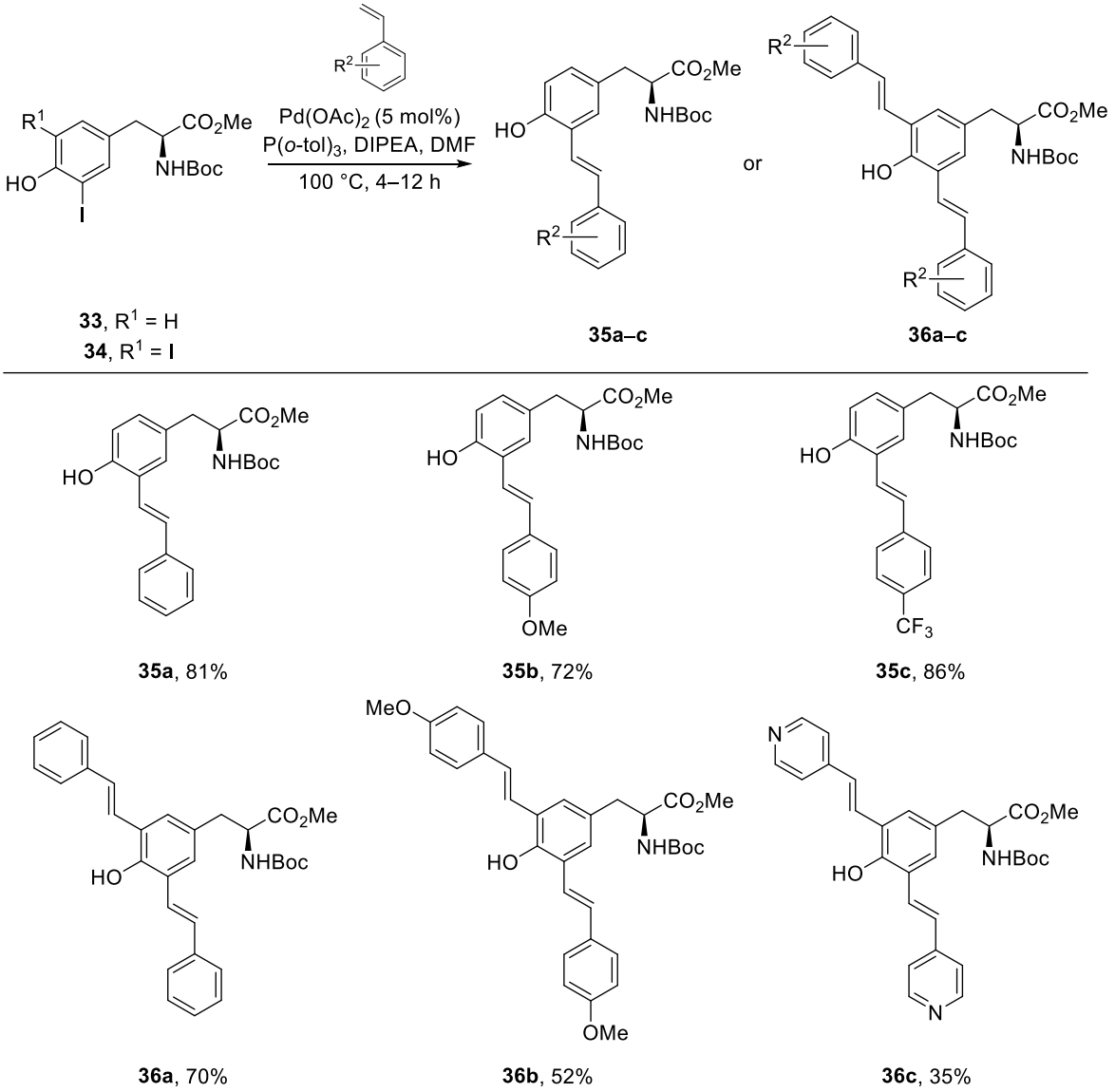
L-Phenylalanine	L-Tyrosine	L-Tryptophan
λ_{abs} = 258 nm	λ_{abs} = 275 nm	λ_{abs} = 279 nm
λ_{em} = 282 nm	λ_{em} = 310 nm	λ_{em} = 365 nm
Φ = 0.02	Φ = 0.1	Φ = 0.01–0.4
ε = 200 cm^{-1} M^{-1}	ε = 1410 cm^{-1} M^{-1}	ε = 5580 cm^{-1} M^{-1}

Figure 11: Structure of the naturally occurring fluorescent amino acids and their photoluminescent properties.

Consequently, the development of novel fluorescent amino acids with improved fluorescent properties has become of increased interest in recent years. Utilising structurally modified fluorescent amino acids for cellular imaging results in minimal perturbation to the protein of interest due to their steric size. Furthermore, novel fluorescent amino acids can be easily incorporated into the protein of interest using solid phase peptide synthesis (SPPS).

1.2.4 Synthesis of Novel Fluorescent Amino Acids

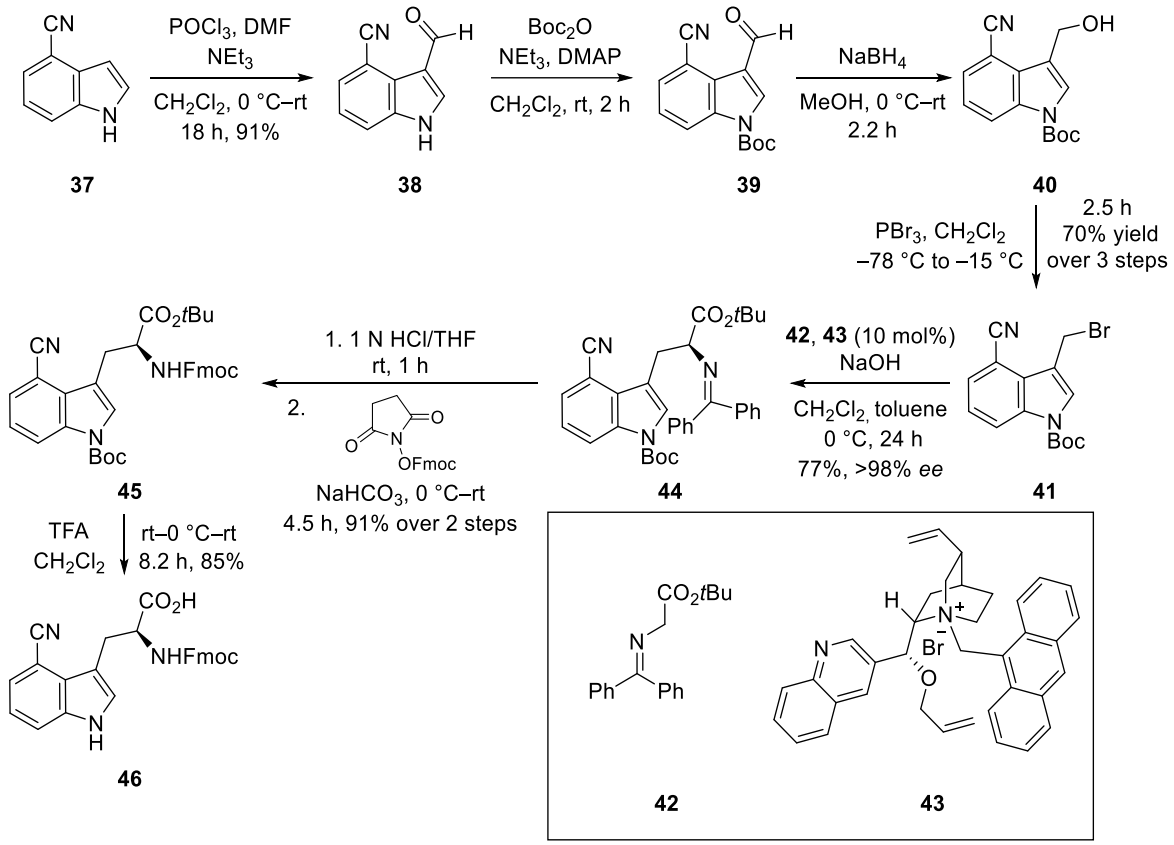
A common method for designing novel fluorescent amino acids involves chemical modification of the naturally occurring fluorescent amino acids. Work by Wang and co-workers reported the synthesis of styryl-functionalised tyrosine analogues with significantly improved photoluminescent properties *via* a Mizoroki-Heck cross coupling reaction (Scheme 12).[65] The desired mono- and bis-styryl analogues were generated in moderate to good yields utilising a $Pd(OAc)_2$ (5 mol%) catalyst and tri(*o*-tolyl)phosphine ($P(o\text{-tol})_3$) ligand. Unsurprisingly, mono-styryl analogues were found to have lower λ_{em} due to decreased π-conjugation compared to the bis-styryl analogues. For example, methoxy substituted analogue **35b** has a λ_{abs} at 292 nm and λ_{em} at 400 nm whereas bis-styryl **36b** has a λ_{abs} at 300 nm and 360 nm and λ_{em} at 420 nm. Analogues bearing electron-withdrawing groups on the aromatic ring were found to have longer λ_{em}, likely due to the generation of a 'push-pull' system with the electron-donating hydroxy group. The quantum yield of the mono-substituted analogues was generally maintained upon addition of a further styryl unit (0.87 for **35b** and 0.94 for **36b**). Interestingly, the group found that following addition of sodium hydroxide, a significant red-shift in the λ_{em} was observed for all mono-styryl analogues. This is proposed to be the result of formation of the phenolate anion which acts as an improved electron-donating source, thus lowering the energy of the excited state.



Scheme 12: Synthesis of mono/bis-stryly tyrosine analogues *via* a Mizoroki-Heck cross-coupling reaction.[65]

The synthesis of structurally modified tryptophan has been reported.[66,67] In particular, the synthesis of L-4-cyanotryptophan is of interest due to its red-shifted absorption which allows for selective excitation in the presence of the naturally occurring fluorescent amino acids.[67] In 2021, Micikas *et al*. reported a scalable route to protected 4-cyanotryptophan utilising an enantioselective phase transfer-catalysed alkylation as a key step in the synthesis.[68] Initially, 4-cyanoindole **37** underwent a Vilsmeier-Haack formylation to give aldehyde **38** in a 91% yield (Scheme 13). Boc-Protection of the amine was then achieved under standard conditions using di-*tert*-butyl dicarbonate (Boc_2O), triethylamine and 4-dimethylaminopyridine (DMAP) to give **39**. Reduction of aldehyde **39** was achieved using sodium borohydride to give alcohol **40**. Reaction of alcohol **40** with phosphorous tribromide generated alkyl

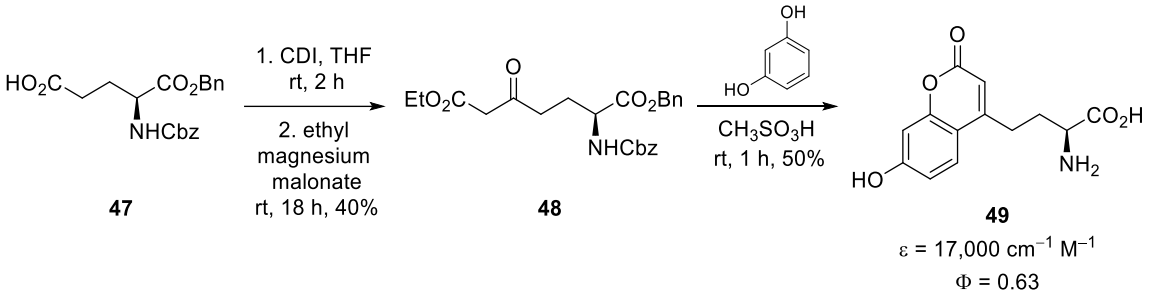
bromide **41**. Synthesis of alkyl bromide **41** was achieved in a 70% yield over the three steps without the requirement of column chromatography. Alkylation of imine **42** using alkyl bromide **41**, in the presence of phase-transfer catalyst **43** occurred in a 77% yield and >98% enantioselectivity. Selective hydrolysis of the imine was then achieved using 1 N hydrochloric acid. This allowed for installation of the Fmoc group to give **45** in a 91% yield over two steps. Finally, removal of the Boc-protecting group and hydrolysis of the ester was achieved using trifluoroacetic acid (TFA) in an 85% yield. The group went on to show that **46** could be successfully incorporated into the M2 protein of the influenza A virus and used as a hydration reporter due to changes in the emission spectrum upon changes in environment.



Scheme 13: Synthesis of L-N^{α}-Fmoc-4-cyanotryptophan (**46**).[68]

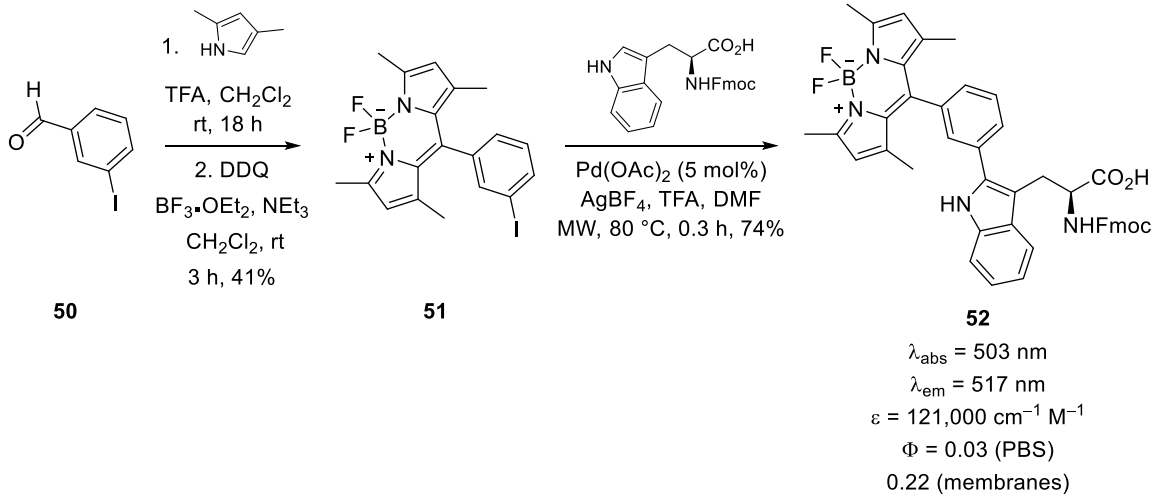
When designing novel fluorescent amino acids, it is common to incorporate a known chromophore into the side chain of an amino acid to exploit its photoluminescent properties. A wide range of fluorescent amino acids have been synthesised utilising this approach including BODIPY, rhodamine and xanthone derivatives.[69-71] Synthesis of coumarin-derived amino acid CouA (**49**) was reported by Wang *et al.* in 2006 (Scheme 14).[72] Firstly, formation of β-keto ester **48** from *N*-Cbz-L-glutamic acid α-benzyl ester (**47**) occurred in a 40% yield *via* a condensation reaction utilising

N,*N*'-carbonyldiimidazole (CDI) followed by treatment with ethyl magnesium malonate. A subsequent Pechmann reaction with resorcinol in methanesulfonic acid generated the coumarin scaffold whilst simultaneously removing the carboxylic acid and amine protecting groups to give **49** in a moderate 50% yield. CouA has a molar attenuation coefficient of 17,000 cm^{-1} M^{-1} and a quantum yield of 0.63. The level of protein unfolding caused by urea denaturation of holomyoglobin has been determined using **49** due to changes in its emission spectra upon exposure to increasingly polar environments.



Scheme 14: Synthesis of CouA (**49**).[72]

In 2016, Vendrell and co-workers reported the preparation of novel, BODIPY-derived fluorescent amino acid **52**.[73] Amino acid **52** was found to have an exceptional molar attenuation coefficient of 121,000 cm^{-1} M^{-1} and a small Stokes shift of 14 nm (Scheme 15). Both properties are characteristic of the BODIPY scaffold. Synthesis of **52** was achieved *via* condensation of 3-iodobenzaldehyde (**50**) with 2,4-dimethylpyrrole. Subsequent oxidation using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was followed by boron complexation to give **51**. Aryl iodide **51** then participated in a Pd-catalysed C–H bond activation of protected tryptophan.[74] Interestingly, the group noted that upon binding to phospholipid bilayer membranes, **52** underwent a significant increase in fluorescence quantum yield from 0.03 to 0.22.



Scheme 15: Synthesis of novel BODIPY-derived fluorescent amino acid **52** from 3-iodobenzaldehyde (**50**). PBS = phosphate-buffered saline.[73]

The group then demonstrated that **52** could be successfully incorporated into synthetic antimicrobial hexapeptide, AntiFungal 26 (PAF26), using solid phase peptide synthesis (SPPS) (Figure 12). Due to its relatively small size, **52** was incorporated into the cyclic peptide without a reduction in its selectivity or affinity for fungal cells. Consequently, **52** has found application in the multi-photon imaging of *Aspergillus fumigatus* fungal infections.

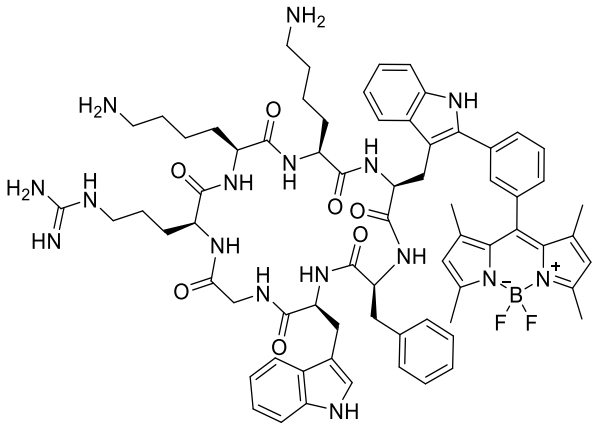
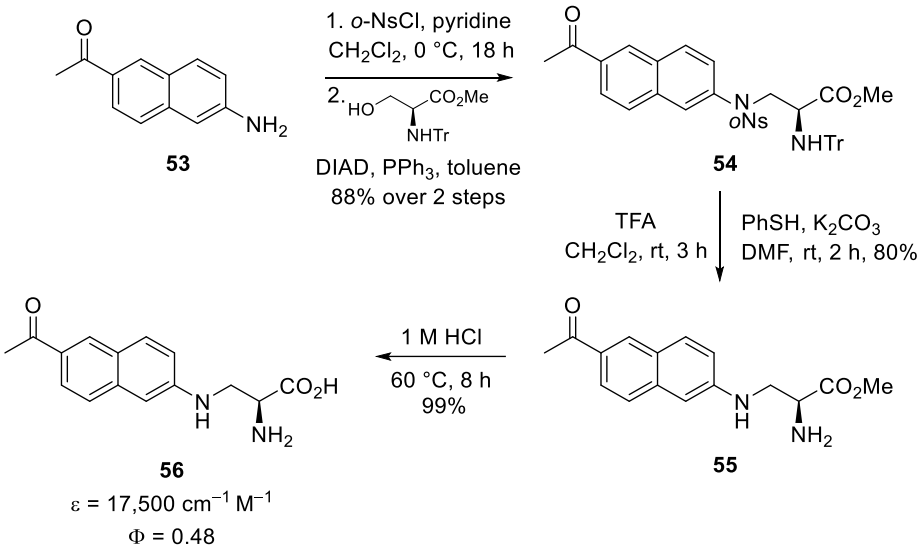


Figure 12: Cyclic PAF26 analogue containing novel BODIPY amino acid **52**.[73]

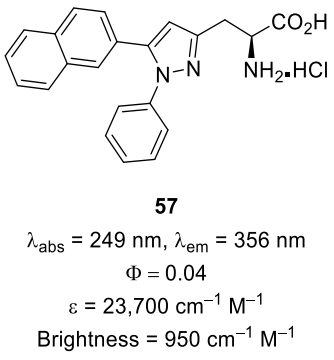
Another example of a fluorescent amino acid incorporating a known chromophore comes from Xiang *et al*. who have shown the successful enantioselective synthesis of L-3-(6-acetylnaphthalen-2-ylamino)-2-aminopropanoic acid (L-Anap, **56**) (Scheme 16).[75] L-Anap has a large molar attenuation coefficient of 17,500 cm^{-1} M^{-1} and quantum yield of 0.48, thus exhibiting an overall brightness of 8,400 cm^{-1} M^{-1}. Synthesis of **56** was achieved *via* a Fukuyama-Mitsunobu reaction of *o*-

nitrobenzensulfonyl protected amine **53** utilising diisopropyl azodicarboxylate (DIAD) and triphenylphosphine. Removal of the trityl group was achieved using TFA. This was followed by removal of the nosyl protecting group using thiophenol. Finally, hydrolysis of methyl ester **55** was achieved using 2 M hydrochloric acid. This gave parent amino acid **56** in a 99% yield. L-Anap has been utilised for imaging mammalian cells.[76]



Scheme 16: Synthesis of L-Anap (**56**).[75]

Sutherland and co-workers reported the synthesis of novel 5-arylpyrazole-derived fluorescent amino acids with interesting photoluminescent properties including large Stokes shifts and molar attenuation coefficients (Figure 13).[77] However, it was found that analogues in this series generally suffered from low quantum yields and hence low overall brightness.



57

λ_{abs} = 249 nm, λ_{em} = 356 nm

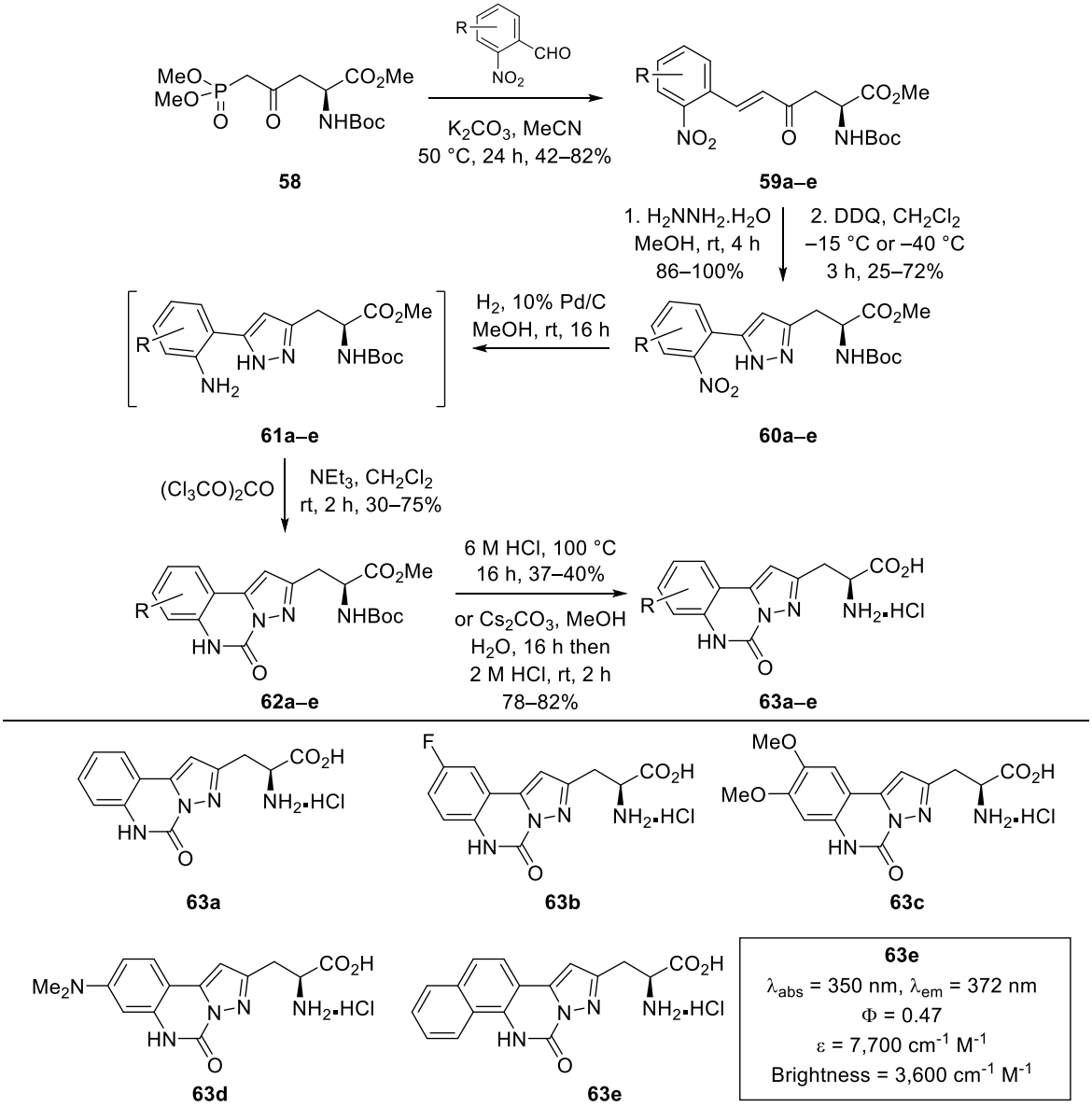
Φ = 0.04

ε = 23,700 cm^{-1} M^{-1}

Brightness = 950 cm^{-1} M^{-1}

Figure 13: Photoluminescent properties of 5-arylpyrazole-derived amino acid **57**.[77]

To improve the fluorescence quantum yield of these analogues, the group proposed that increasing the rigidity of the pyrazole scaffold would prevent alternative modes of relaxation. Consequently, the synthesis of conformationally rigid pyrazoloquinazoline-derived amino acids with large quantum yields has since been reported.[78] Synthesis of this series was achieved *via* a short synthetic route from phosphononorvaline **58** (Scheme 17). Firstly, **58** was subjected to a Horner-Wadsworth-Emmons reaction with various 2-nitrobenzaldehydes to give the corresponding enones **59a**–**e**. Formation of the pyrazole ring was then achieved *via* a one-pot condensation/aza-Michael process followed by oxidation with DDQ. Following reduction of the nitro group, carbonylation using triphosgene then gave the desired pyrazoloquinazolines (**62a**–**e**). Finally, deprotection to give parent amino acids **63a**–**e** was achieved either as a one-pot process using 6 M hydrochloric acid or stepwise, using caesium carbonate to hydrolyse the methyl ester followed by a milder procedure in 2 M hydrochloric acid to remove the Boc-protecting group. As shown in Scheme 17, naphthyl analogue **63e** was found to have a high quantum yield of 0.47, compared to 0.04 for the 5-naphthylpyrazole analogue **57**. In addition, dimethyl analogue **63d** was found to have a red-shifted λ_{em} of 414 nm. This is proposed to be the result of the 'push-pull' dimethylamine-pyrazole system which generates a charge transfer excited state. The existence of a charge transfer excited state is further implied by the solvatochromic characteristics of **63d**. It was found that in less polar solvents emission occurred at lower wavelengths (tetrahydrofuran, λ_{em} at 374 nm). In contrast, the use of phosphate-buffered saline as solvent resulted in a λ_{em} of 424 nm. More significantly, it was shown that **63d** could undergo two-photon excitation using near-IR excitation, without a change in the emission spectra. This is a significant advantage of this fluorophore as it avoids using wavelengths of light which could cause damage to the cell or tissue of interest.



Scheme 17: Synthesis of pyrazoloquinazoline-derived amino acids **63a**–**e**.[78]

In 2019, VanNieuwenhze *et al.* reported the synthesis of rotor-fluorogenic D-amino acids for the real-time monitoring of peptidoglycan synthesis in living cells.[79] The tetrahydroquinoline-based scaffolds were found to be environmentally sensitive, with incorporation into peptidoglycan causing enhanced fluorescence emission. This was proposed to be because of restricted bond rotation upon binding and hence reduced access to the non-radiative TICT excited state. To confirm the mode of action of these fluorescent amino acids, fluorescence intensity was measured as a function of solvent viscosity. Results showed approximately a 20-fold increase in fluorescence intensity in 80% glycerol-PBS compared to PBS, emphasising that fluorescence enhancement results from reduced access to the TICT excited state. Interestingly, control experiments utilising the L-enantiomer showed no fluorescence

under the same reaction conditions. This imaging technique could find application in high-throughput screening of potential anti-bacterials.

1.3 Summary

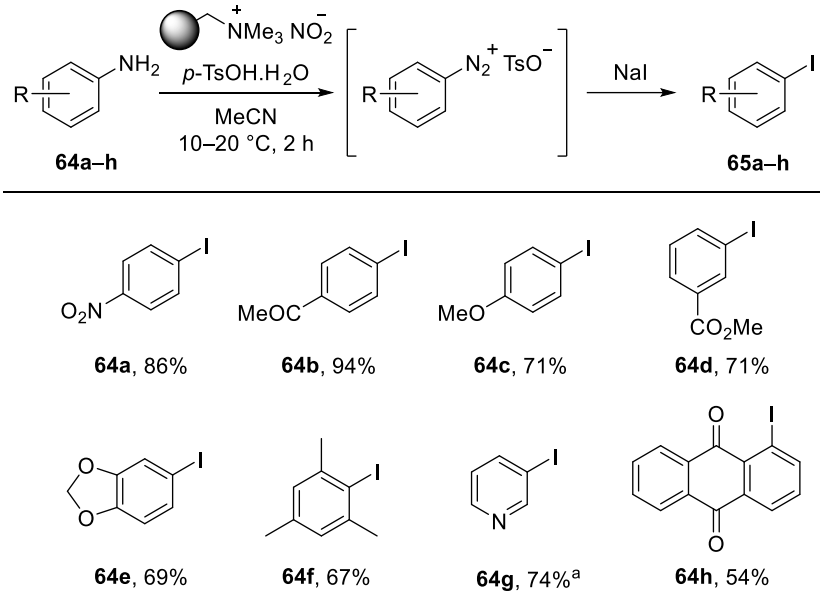
Benzannulated triazoles have a significant range of both chemical and biological applications.[1-3] Despite this, current methods for their synthesis show some deficiencies such as the use of strong acids, elevated temperatures or long reaction times.[19,23,24] Fluorescence imaging is a key technique for both analytical and diagnostic applications due to its rapid detection times and high resolution.[43,45] In particular, the use of fluorescent amino acids for cellular imaging has become of increased prevalence in recent years due to the lack of perturbation to the protein of interest.[60] Consequently, a key aim of this PhD is to develop a new method for the synthesis of benzannulated triazoles (Section 2.1). This methodology will then be utilised to generate novel fluorescent benzotriazinone and benzothiatriazinedioxide amino acids (Section 2.2).

2.0 Results and Discussion

2.1 One-Pot Process for the Synthesis of Benzannulated Triazoles

2.1.1 Previous Work in the Sutherland Group

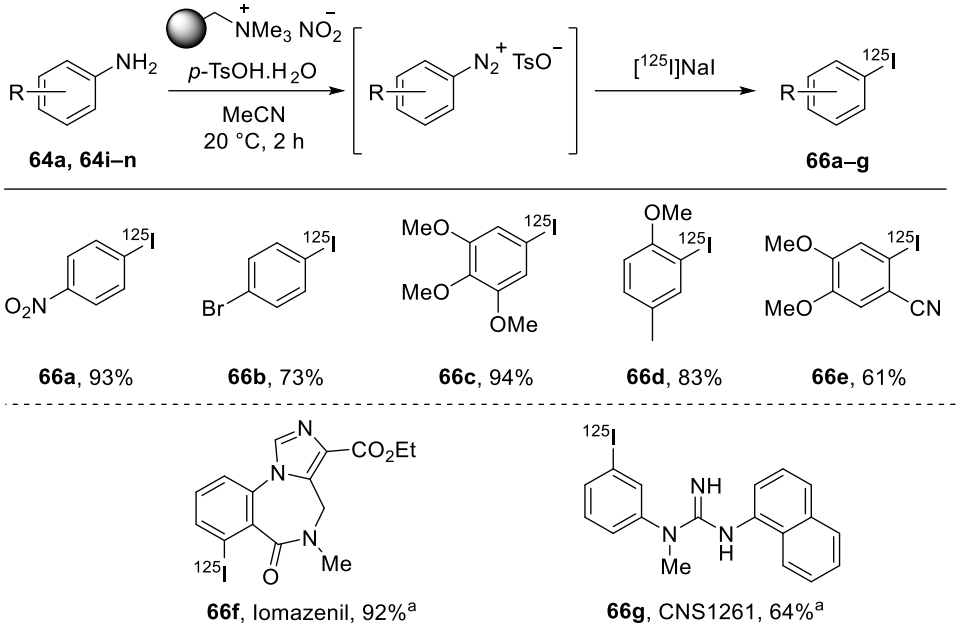
Although considered as highly versatile synthetic intermediates, diazonium salts are often avoided in organic synthesis due to their unstable and potentially explosive nature.[80] However, work by Filimonov *et al.* has shown that substantial improvements in stability can be achieved upon formation of tosylate diazonium salts.[80] Previous work in the Sutherland group has focused on utilising this method for formation of stable diazonium salts to carry out chemical transformations. By use of a polymer supported nitrite reagent in the presence of *p*-tosic acid, the group have developed a highly practical, scalable and mild one-pot procedure for the iodination of anilines.[81] The methodology generated a wide range of iodinated compounds in good to excellent yields (Scheme 18).



Scheme 18: One-pot, two-step iodination of anilines *via* stable diazonium salt intermediates.[81] [a]Reaction carried out at 60 °C for 16 h.

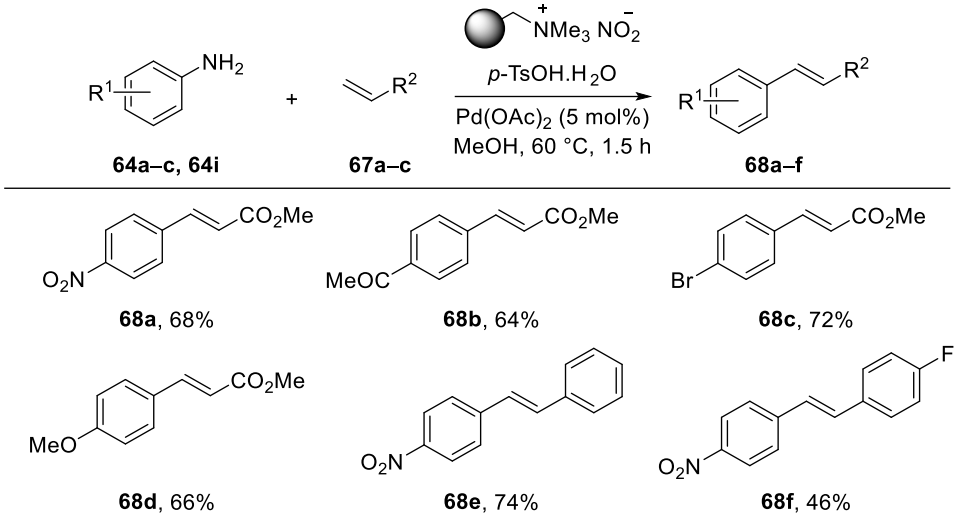
The scope of this methodology was then extended for the radioiodination of aryl amines, with the aim of accessing a range of SPECT imaging agents.[82] Prior methods for radioiodination generally involve conversion of aryl halides to organostannanes, followed by an iodo-destannylation reaction.[83] However, as well as posing toxicity concerns, organostannanes can be unstable. The Sutherland group's methodology allows for access to aryl radioiodinated products from commercially available starting materials *via* the one-pot, two-step method (Scheme

19). The reaction was successful for a range of electron-rich and electron-deficient anilines and generated various SPECT imaging agents in high radiochemical yields.



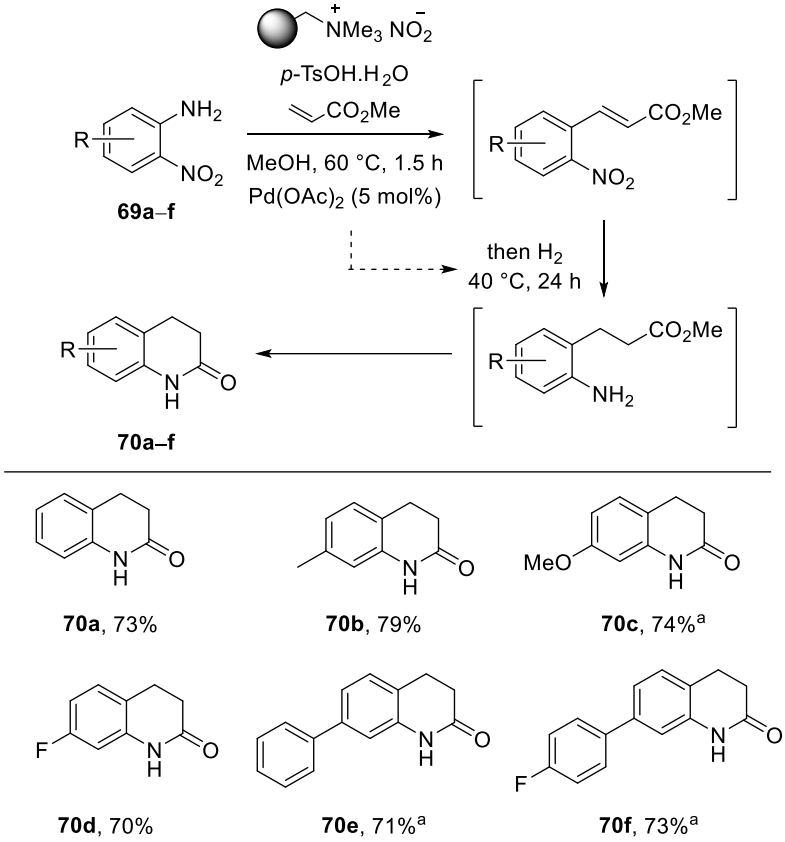
Scheme 19: One-pot, two-step radioiodination of anilines *via* stable diazonium salt intermediates.[82] [a]Reaction carried out at 60 °C.

The group also investigated the utility of the diazonium tosylates in palladium-catalysed cross-coupling reactions.[84] This led to the development of a mild, one-pot procedure for the synthesis of cinnamates and styrenes *via* diazotisation and subsequent Heck-Matsuda reactions (Scheme 20). Compared to the more popular Mizoroki-Heck reaction, Heck-Matsuda reactions can be carried out at lower temperatures and often negate the requirement of base due to the increased electrophilic nature of aryl diazonium salts.[85] Following optimisation, the reaction was successful for a range of anilines containing either electron-withdrawing or electron-donating substituents. Notably, on substrates containing halogens (**68c**), the reaction occurred chemoselectively, giving solely the Heck-Matsuda product with no trace of the Mizoroki-Heck derivatives. This chemoselectivity allowed for orthogonal functionalisation of 4-bromoaniline *via* a two-pot strategy to generate complex styryl cinnamates.



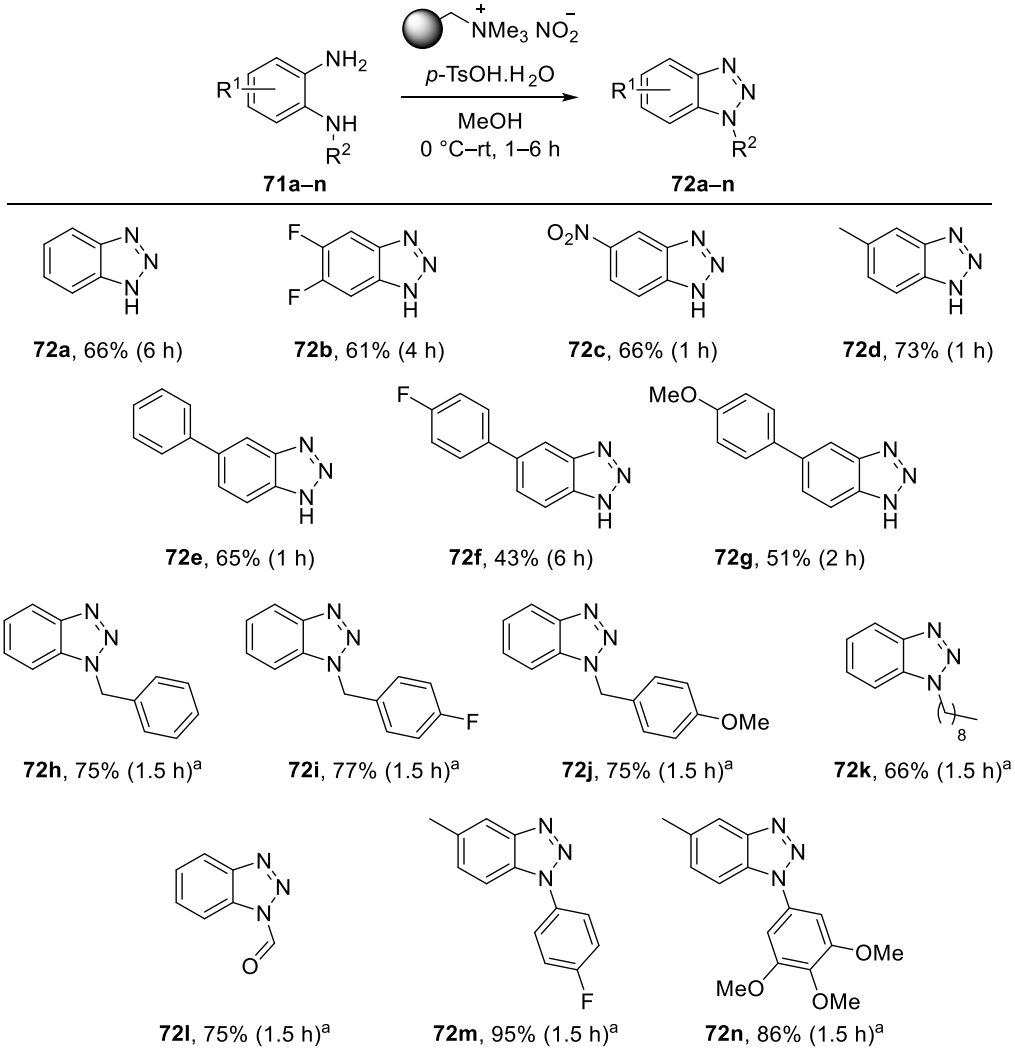
Scheme 20: One-pot tandem diazotisation and Heck-Matsuda reaction.[84]

The process was later extended to 2-nitroanilines, allowing for the synthesis of 3,4-dihydroquinolin-2-ones *via* a four-step, one-pot multibond forming process (Scheme 21). Following diazotisation and subsequent Heck-Matsuda cross-coupling of the 2-nitroanilines, simultaneous nitro-group reduction and alkene hydrogenation was achieved *via* generation of palladium black *in situ*. Finally, intramolecular cyclisation generated the desired products in excellent yields. The synthetic utility of the procedure was highlighted by the synthesis of a sodium ion channel modulator which was generated in 32% overall yield after eight steps.



Scheme 21: One-pot synthesis of 3,4-dihydroquinolin-2-ones from 2-nitroanilines.[85] [a]Reduction conducted under hydrogen atmosphere of 2.5 bar at room temperature.

The utility of diazonium tosylates for intramolecular reactions was also investigated. Benzotriazoles are known to have both chemical and biological applications.[86,87] Current methods for their synthesis generally require the use of elevated temperatures or long reaction times.[88,89] However, the Sutherland group have devised a new method for their synthesis from 1,2-aryldiamines utilising polymer supported nitrite resin in the presence of *p*-tosic acid.[86] The one-pot diazotisation and intramolecular cyclisation procedure was successful for a range of commercially available substrates (Scheme 22). Various 5-arylbenzotriazoles were successfully synthesised under standard conditions (**72e**–**g**). A major advantage of this methodology compared to 'click' style reactions is that it allows for the regioselective synthesis of a range of N_1-functionalised unsymmetrically substituted benzotriazoles (**72m** and **72n**).



Scheme 22: Synthesis of *N*-unsubstituted and N_1-substituted benzotriazoles from 1,2-aryldiamines.[86] [a]Reaction carried out in MeCN.

2.1.2 Proposed Research

The aim of this project was to extend the one-pot diazotisation and cyclisation procedure previously developed in the group for the synthesis of other heterocycles. In particular, the synthesis of benzotriazinones and benzothiatriazinedioxides was investigated. The first aim of this project was thus to optimise a one-pot procedure for the synthesis of 1,2,3-benzotriazin-4(3*H*)-one (**1a**). The optimised conditions would then be used to explore the scope of the reaction, synthesising N_3-unsubstituted and N_3-substituted benzotriazinones bearing both electron-donating and electron-withdrawing substituents (Figure 14). The methodology would then be extended to the synthesis of N_2-unsubstituted and N_2-substituted benzothiatriazinedioxides. The synthetic utility of both procedures would then be highlighted with the synthesis of biologically active compounds containing these heterocycles.

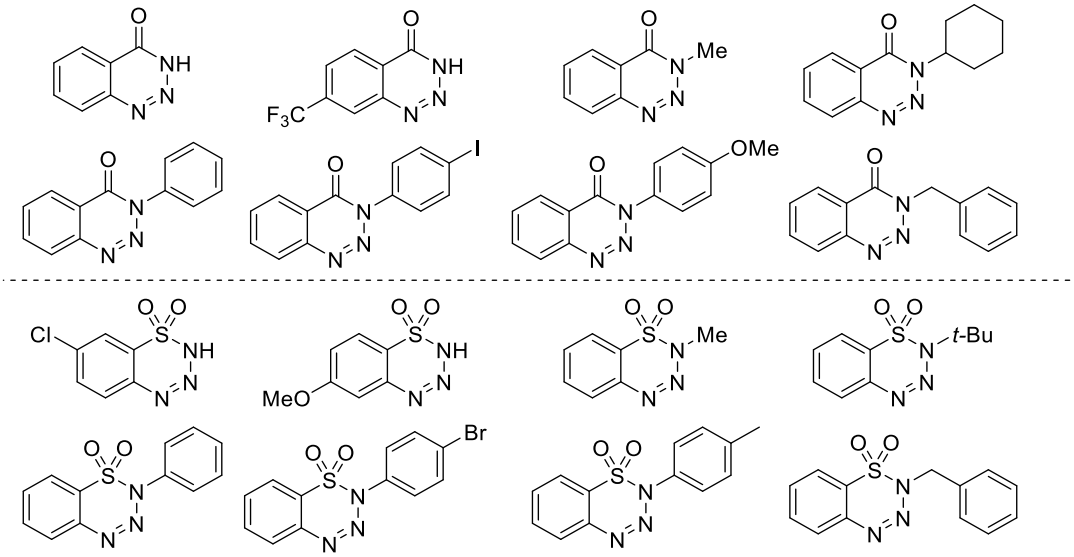
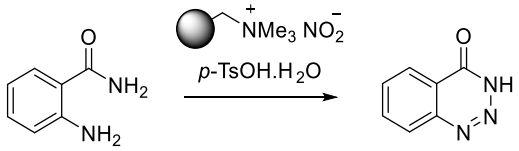


Figure 14: Library of N_3-unsubstituted and N_3-substituted benzotriazinones and N_2-unsubstituted and N_2-substituted benzothiatriazinedioxides.

2.1.3 Optimisation of the One-Pot Procedure for the Synthesis of Benzotriazinones

The first aim of this project was to optimise the one-pot diazotisation and cyclisation procedure for the synthesis of 1,2,3-benzotriazin-4(3*H*)-one (**1a**). The nitrite resin was easily prepared *via* ion exchange of tetraalkylammonium functionalised resin Amberlyst A-26 with aqueous sodium nitrite solution. The reaction was initially attempted using conditions found to be optimal for the synthesis of benzotriazoles based on previous group work.[90] These included polymer supported nitrite resin (3 equiv.) and *p*-tosic acid (3 equiv.) in methanol stirring at 0 °C for 0.5 h followed by room temperature for 1 h (Table 2, entry 1). This gave a 33% yield of desired product, Cyclisation to give **1a** was indicated by a significant downfield shift of the aromatic hydrogen atoms in the ^{1}H NMR spectrum. In order to improve the yield, the reaction times at both 0 °C and room temperature were extended (entry 2). This resulted in an increase in yield to 51%. It was found that altering the solvent used to wash the resin from ethyl acetate to methanol resulted in a further increase in yield to 65% (entry 3). The effects of reagent ratio on the reaction rate were also investigated. When the ratio of resin and *p*-tosic acid were reduced (1 equiv. each), the reaction mixture showed only 50% conversion to desired product by ^{1}H NMR spectroscopy after 18 h at room temperature. This resulted in a decreased yield of 39% (entry 4). When the reagent ratio was increased (6 equiv. each, entry 5), desired product was obtained in a 62% yield. A solvent screen was also carried out. When attempting the reaction in acetonitrile, the yield decreased from 65% to 55% (entry 6). There

was no difference in the yield when the solvent was changed from methanol to ethyl acetate (entry 7). Finally, the reaction was carried out using sodium nitrite rather than polymer supported nitrite resin. Although the reaction was complete within a similar time, the additional requirement of an aqueous work-up resulted in a lower yield of 56% (entry 8). The optimised conditions were therefore those described in entry 3.



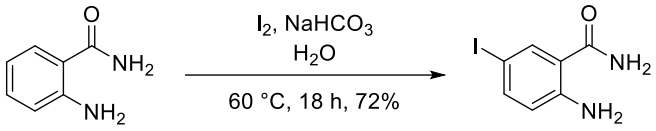
5a **1a**

Entry	Polymer supported nitrite resin (equiv.)	*p*-TsOH (equiv.)	Reaction time at 0 °C (h)	Reaction time at rt (h)	Solvent used for resin wash (10 mL/mmol)	Reaction Solvent	Yield[a]
1	3	3	0.5	1	EtOAc	MeOH	33%
2	3	3	1	2.5	EtOAc	MeOH	51%
3	3	3	1	1	MeOH	MeOH	65%
4	1	1	1	18	MeOH	MeOH	39%
5	6	6	1	1	MeOH	MeOH	62%
6	3	3	1	1	MeOH	MeCN	55%
7	3	3	1	1	MeOH	EtOAc	65%
8[b]	N/A	3	1	1.5	MeOH	MeOH	56%

Table 2: Optimisation of the one-pot procedure for the synthesis of 1,2,3-benzotriazin-4(3*H*)-one (**1a**). [a]Isolated yield, [b]Reaction performed using $NaNO_2$.

2.1.4 Substrate Scope: N_3-Unsubstituted Benzotriazinones

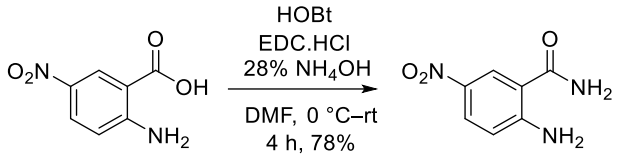
Once optimised, the next aim of the project was to explore the substrate scope of this methodology. Firstly, the synthesis of N_3-unsubstituted benzotriazinones were investigated. Various 2-aminobenzamides were commercially available. Synthesis of 2-amino-5-iodobenzamide (**5i**) was achieved *via* based mediated iodination as shown in Scheme 23.[91] Conversion to desired product was indicated by the presence of a doublet with a *meta*-coupling constant of 2.1 Hz in the ^{1}H NMR spectrum as well as the disappearance of an aromatic hydrogen atom.



5a **5i**

Scheme 23: Synthesis of 2-amino-5-iodobenzamide (**5i**).[91]

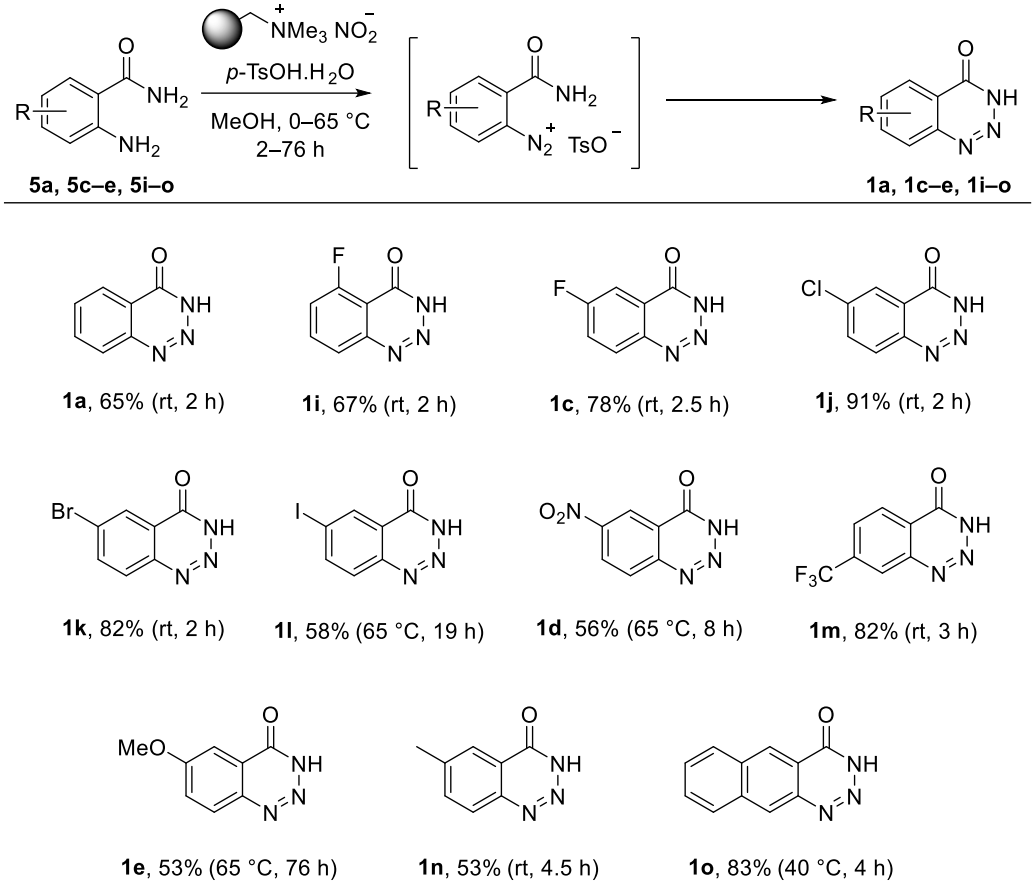
To generate 2-amino-5-nitrobenzamide (**5d**), 2-amino-5-nitrobenzoic acid (**73**) underwent an amide coupling reaction with ammonia in a good 78% yield (Scheme 24).[92]



73 **5d**

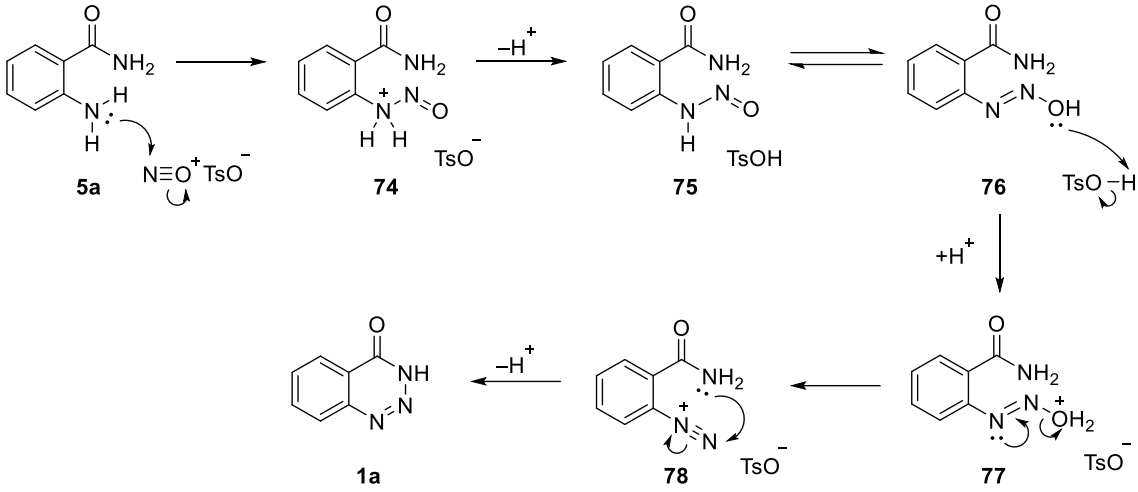
Scheme 24: Synthesis of 2-amino-5-nitrobenzamide (**5d**).[92]

The 2-aminobenzamide starting materials were then submitted to the one-pot procedure. This gave the desired compounds in moderate to excellent yield under mild conditions (Scheme 25). The reaction tolerated *ortho*, *meta* and *para*-substitution on the aromatic ring (**1i, 1j** and **1m**). Various halogenated analogues gave the desired compounds in high yields (**1c**, **1k**–**l**). It should be noted that an anomalous result was obtained for **1l**, which showed only 40% conversion to desired product by ^{1}H NMR spectroscopy after 3 h at room temperature. The reaction was therefore repeated by heating under reflux for 18 h which resulted in a 58% yield of the desired product. Similarly, synthesis of **1d** was initially attempted at room temperature for 1 h followed by heating to 40 °C for 4 h. However, this showed no conversion of starting material. The reaction was therefore repeated, by heating under reflux for 8 h. The reaction was successful for trifluoromethyl analogue **1m**, which is a privileged moiety within medicinal chemistry. The reaction also gave the desired compounds in moderate yields for analogues bearing electron-donating substituents (**1e** and **1n**). It is postulated these analogues required longer reaction times or slightly elevated temperatures due to the decreased electrophilicity of the diazonium salt and hence slower rates of intramolecular cyclisation. Finally, the reaction was also successful for naphthyl analogue **1o** in an excellent 83% yield.



Scheme 25: Scope of the one-pot diazotisation and cyclisation for the synthesis of N_3-unsubstituted benzotriazinones (all substrates stirred at 0 °C for 1 h prior to heating).

The mechanism of this reaction is shown in Scheme 26. Firstly, the combination of polymer supported nitrite resin and *p*-tosic acid generates the nitrosium cation *in situ*. This undergoes nucleophilic attack by 2-aminobenzamide (**5a**) to generate species **74**. Subsequent deprotonation and tautomerisation occurs to give **76**. This then undergoes protonation and dehydration to give the corresponding diazonium salt **78**. The increased stability of the diazonium salt can be attributed to the corresponding counterion. Tosylate diazonium salts are significantly more stable than their chloride counterparts due to the multiple close contacts made between the diazonium cation and the oxygen atom of the tosylate anion through charge-charge interactions.[80] Finally, intramolecular cyclisation gives the desired benzotriazinone scaffold which is then deprotonated to give **1a**.

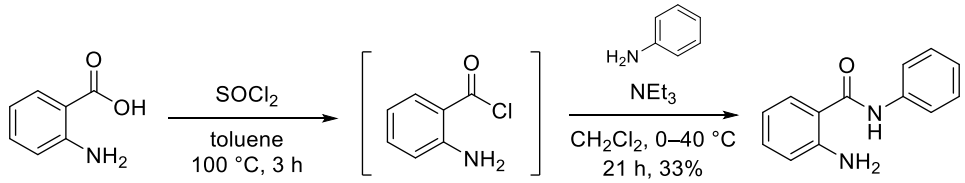


Scheme 26: Proposed mechanism for formation of **1a** from **5a**.

It should be noted that in cases where full conversion to the desired benzotriazinones was obtained, an easy and efficient work-up procedure was carried out. This involved filtration to remove the resin and subsequent base washing to remove residual *p*-tosic acid. This simple work-up and purification is one of the key advantages of using polymer supported reagents. For substrates which showed traces of starting material remained, following filtration to remove the resin, the mixture was purified directly by column chromatography. Another advantage of using polymer-supported resin is that it can be regenerated and re-used multiple times without reduction in efficiency.[18] Finally, the methodology is also applicable to larger scale synthesis, providing a 64% yield of benzotriazinone **1a** on a 1 gram scale.

2.1.5 Substrate Scope: N_3-Substituted Benzotriazinones

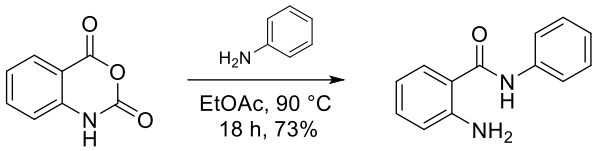
The next aim of this project was to extend the utility of the one-pot procedure for the synthesis of N_3-substituted benzotriazinones. Various *N*-alkyl 2-aminobenzamides were cheap and commercially available and therefore purchased directly. For synthesis of the *N*-phenyl analogues, this was originally investigated *via* conversion of anthranilic acid to the corresponding acid chloride followed by condensation with aniline (Scheme 27).[21] However, it was found that this gave a low yield of desired product **3a**. As well as formation of multiple by-products, it also proved difficult to separate the desired product from unreacted aniline starting material.



79 **3a**

Scheme 27: Synthesis of 2-amino-*N*-phenylbenzamide (**3a**) from anthranilic acid (**79**).

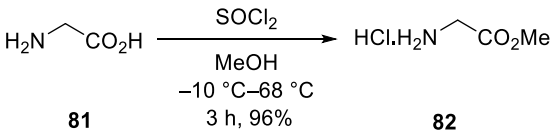
An alternative approach for the synthesis of the 2-aminobenzamides was therefore investigated. It was found that the ring opening reaction of isatoic anhydride with aniline gave 2-amino-*N*-phenylbenzamide (**3a**) in a high 73% yield (Scheme 28).[93]



80 **3a**

Scheme 28: Ring opening reaction of isatoic anhydride (**80**) with aniline.[93]

The anilines used for the ring opening reaction were commerically available, other than glycine methyl ester **82**, which was synthesised in a 96% yield from glycine under standard conditions using thionyl chloride and methanol (Scheme 29).[94]

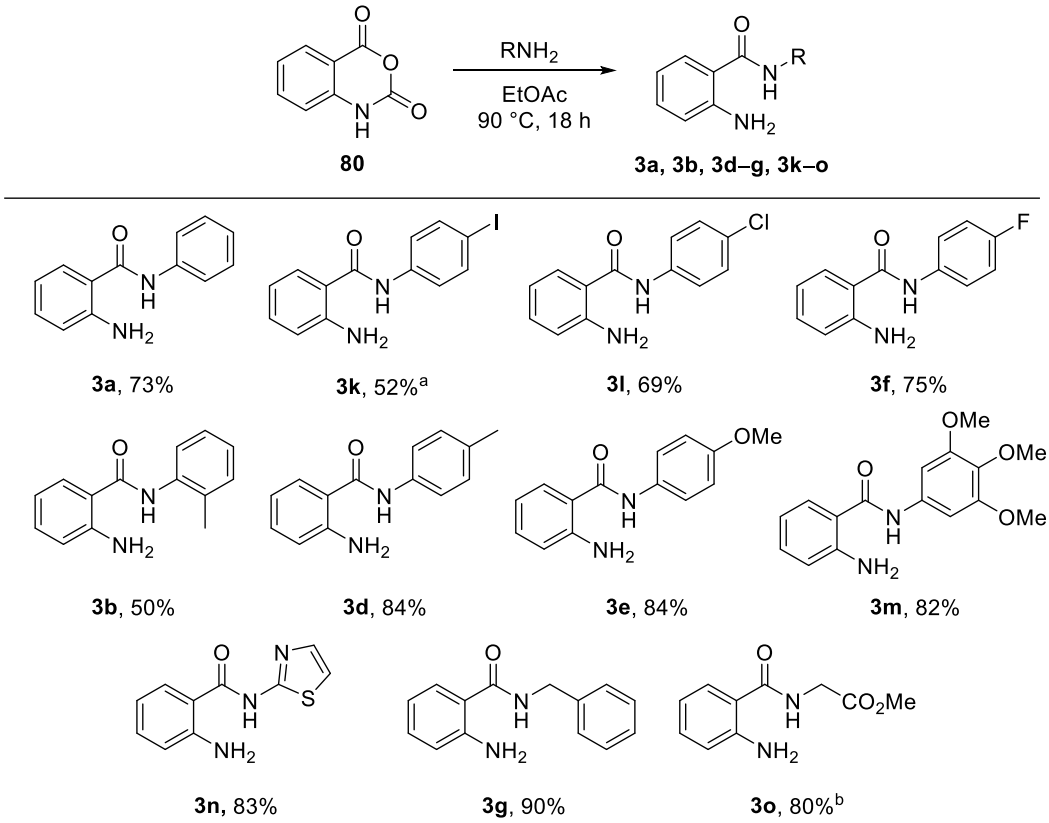


81 **82**

Scheme 29: Synthesis of glycine methyl ester hydrochloride (**82**).[94]

The ring opening reaction was applied to a wide range of substrates and gave moderate to high yields for both electron-withdrawing and electron-donating anilines (Scheme 30). The reaction was operationally simple with direct purifcation by column chromatography. *ortho*-Substituents were tolerated, as highlighted by the synthesis of **3b,** albiet in a lower yield. The reaction was succesful for 2-aminothiazole and benzylamine analogues, giving the desired products in 83% and 90% yields, respectively. Unsuccesful ring opening occurred when the reaction was attempted with 4-nitroaniline, which showed no conversion of the isatoic anhydride starting material by ^{1}H NMR spectroscopy after stirring at 90 °C for 18 h. A similar

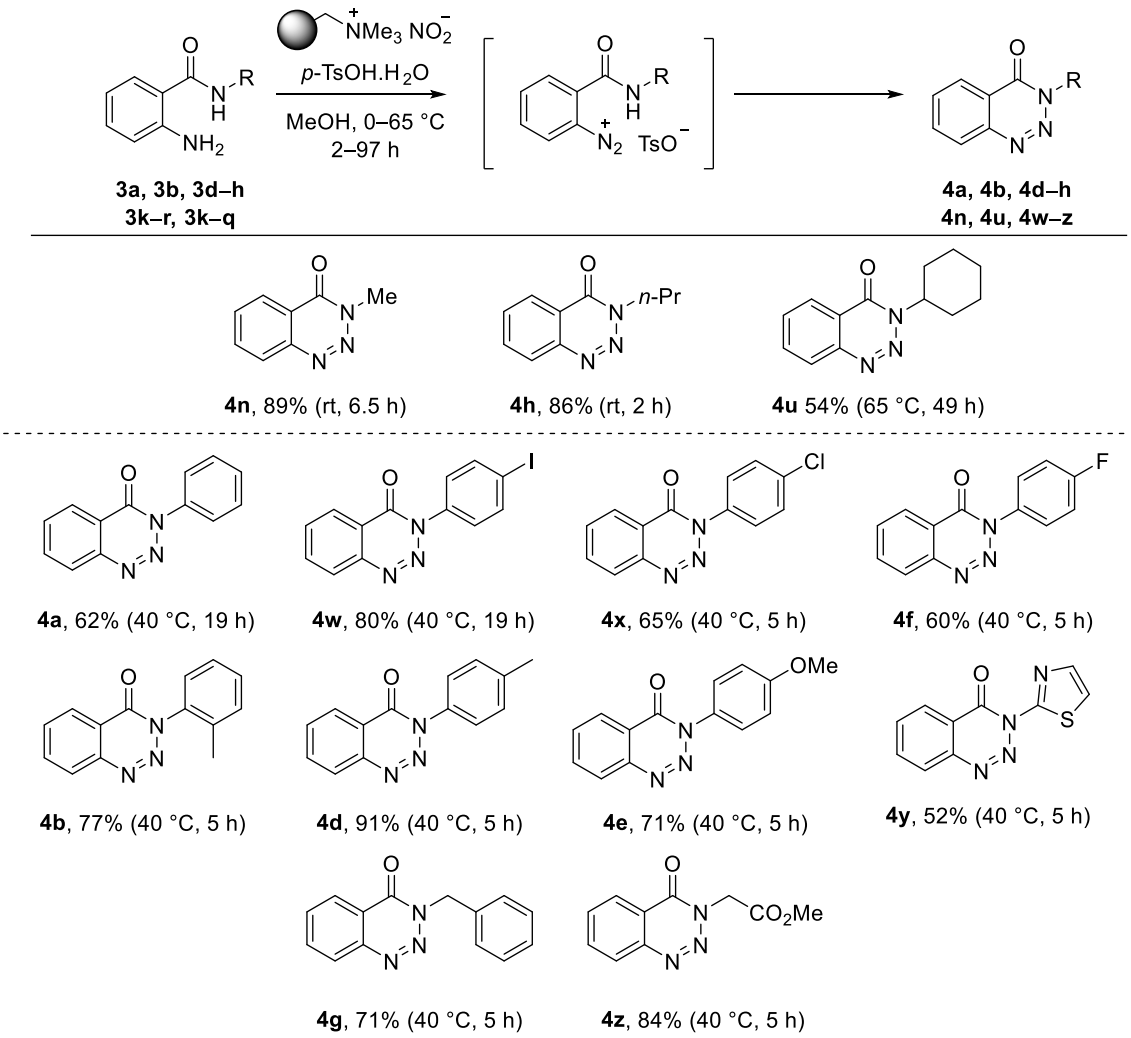
result was obtained for 4-aminobenzonitrile. It is postulated that these analogues did not participate in the ring opening reaction due to the decreased nucleophilicity of the amine as a result of the increased electron-withdrawing nature of the substituents. The reaction was also attempted using 2,6-dimethylaniline. However, this showed only trace amounts of desired product which is believed to be a result of steric hinderance at the amine functionality.



Scheme 30: Scope of 2-aminobenzamides synthesised *via* the ring opening reaction of isatoic anhydride (**80**). [a]Reaction carried out at 90 °C for 24 h; [b]1 equiv. NEt_3 of used as base.[93]

The one-pot diazotisation and cyclisation procedure gave the desired compounds in high yields under mild conditions for *N*-alkyl analogues **4n** and **4h** (Scheme 31). However, it became evident that steric factors affected the reaction rate. For cyclohexyl analogue **4u**, the ^{1}H NMR spectrum showed only approximately 55% conversion to desired product after 24 h heating under reflux. No further conversion to product was observed after an additional 24 h. Similarly, the *tert*-butyl derivative showed no evidence of product formation by ^{1}H NMR spectroscopy, despite being heated under reflux for 96 h. Reactivity of the *N*-phenyl compounds was initially investigated under the same conditions as the *N*-unsubstituted analogues. However,

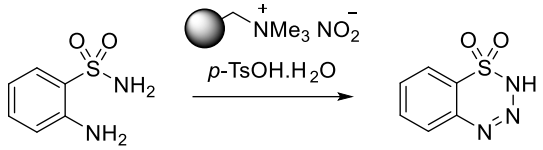
for *N*-phenyl analogue **4a**, only 35% conversion to desired product was observed by ^{1}H NMR spectroscopy after 18 h at room temperature and a low 5% yield of desired product was obtained. The reaction was therefore repeated, heating to 40 °C, which gave a 62% yield of desired product. 4-Iodophenyl analogue **4w** was also generated in an excellent 80% yield under the same conditions. *N*-Phenyl compounds bearing electron-donating groups were generated in high yields (**4b**, **4d** and **4e**). It is postulated this is a result of the increased nucleophilicity of the amide nitrogen. Interestingly, when the reaction was attempted using the trimethoxyphenyl analogue, a low conversion of starting material was observed by ^{1}H NMR spectroscopy and purification issues meant desired compound could not be isolated. The reaction was also succesful for thiazole and benzyl analogues and generated gylcine methyl ester analogue **4z** in an excellent 84% yield.



Scheme 31: Scope of the one-pot diazotisation and cyclisation for the synthesis of N_3-substituted benzotriazinones (all substrates stirred at 0 °C for 1 h prior to heating).

2.1.6 Optimisation of the One-Pot Procedure for the Synthesis of *N*$_2$-Unsubstituted Benzothiatriazinedioxides

Due to their interesting chemical and biological properties, the next aim of this project was to extend the utility of the one-pot procedure for the synthesis of benzothiatriazinedioxides. Firstly, optimisation of the one-pot procedure for the synthesis of 1,2,3,4-benzothiatriazine-1,1(2*H*)-dioxide (**2a**) was investigated. Initially the reaction was attempted under the conditions found to be optimal for the synthesis of 1,2,3-benzotriazin-4(3*H*)-one (**1a**). After stirring at room temperature for 1 h, ^{1}H NMR spectroscopy indicated 80% conversion of the 2-aminobenzenesulfonamide starting material (Table 3, entry 1). The reaction was therefore repeated, heating to 40 °C for 6 h which resulted in an 86% conversion of starting material (entry 2). However, following purification, it was found that rather than desired cyclised material, an alternative S$_N$Ar reaction had occurred with the reaction solvent to give 2-methoxybenzenesulfonamide. This was indicated by additional peaks at 3.68 ppm in the ^{1}H NMR spectrum and 56.8 ppm in the ^{13}C NMR spectrum. It is proposed that the reduced nucleophilicity of the sulfonamide nitrogen allows for the intermolecular S$_N$Ar reaction to occur faster than the desired intramolecular cyclisation. To overcome this issue, the reaction was repeated using acetonitrile as the solvent (entry 3). The ^{1}H NMR spectrum showed approximately 60% conversion of starting material after 2 h at room temperature. This was further improved by heating the reaction to 40 °C for 4 h (entry 4). However, desired product could not be isolated by column chromatography. It was proposed that the *p*-tosic acid may be causing protonation of what was believed to be desired product and hence its removal during the work-up. The reaction was therefore repeated, with adjustment to pH 8–10 using ammonium hydroxide solution (entry 5). However, conversion of starting material was slower and desired product was not isolated.

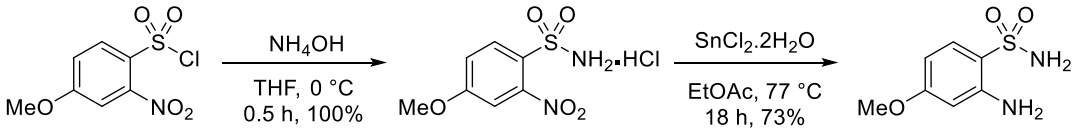


83 2a

Entry	Solvent	Reaction time at rt (h)	Reaction time at 40 °C (h)	Conversion of starting material (%)
1	MeOH	3	N/A	83%
2	MeOH	N/A	6	86%
3	MeCN	2	2	50%
4	MeCN	N/A	4	96%
5	MeCN	N/A	23	50%

Table 3: Optimisation of the one-pot procedure for the synthesis of 1,2,3,4-benzothiatriazine-1,1(2*H*)-dioxide (**2a**) (all reactions stirred at 0 °C for 1 h prior to heating).

In an attempt to better understand what occurred during the course of the reaction, cyclisation of 4-methoxy-2-aminobenzenesulfonamide (**86**) was attempted. It was proposed that the 1,2,4-substitution pattern of the aromatic ring would allow for better monitoring by ^{1}H NMR spectroscopy. To do this, 4-methoxy-2-aminobenzenesulfonamide (**86**) was synthesised in two steps. Firstly, 4-methoxy-2-nitrobenzenesulfonyl chloride (**84**) was converted to sulfonamide **85** in quantitative yield using ammonium hydroxide (Scheme 32). The nitro group was then reduced using tin(II) dichloride dihydrate to give the substituted 2-aminobenzenesulfonamide **86** in 73% yield.[95]



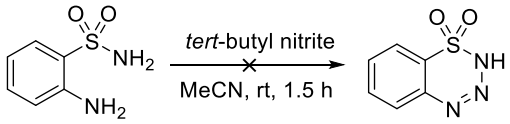
84 85 86

Scheme 32: Two-step synthesis of 2-amino-4-methoxybenzenesulfonamide (**86**) from 4-methoxy-2-nitrobenzenesulfonyl chloride (**84**).

When attempting the cyclisation of 4-methoxy-2-aminobenzenesulfonamide (**86**) in methanol, a complex mixture was observed in the ^{1}H NMR spectrum. As some S_NAr product was identified, the reaction was repeated in acetonitrile. However, the ^{1}H

NMR spectrum did not indicate conversion to desired product. It is therefore postulated that although the desired cyclised products may be formed during the reaction, they are not stable under the described reaction conditions and hence decomposition occurs. This conclusion is supported by the reduced prevalence of N_2-unsubstituted benzothiatriazinedioxides in the literature compared to N_2-substituted analogues.[24,96-98]

To further investigate the stability of the N_2-unsubstituted benzothiatriazinedioxide analogues, synthesis of **2a** was attempted under the conditions described in patent CN 108440447 A (Scheme 33).[96] The procedure utilises *tert*-butyl nitrite as the nitrogen source and reported conversion of starting material was observed by TLC analysis after 3 minutes. However, it was found these results could not be replicated and full conversion of starting material was only achieved after 1.5 h at room temperature. In addition to this, the ^{1}H NMR spectrum showed a complex mixture in the aromatic region and desired compound could not be isolated.

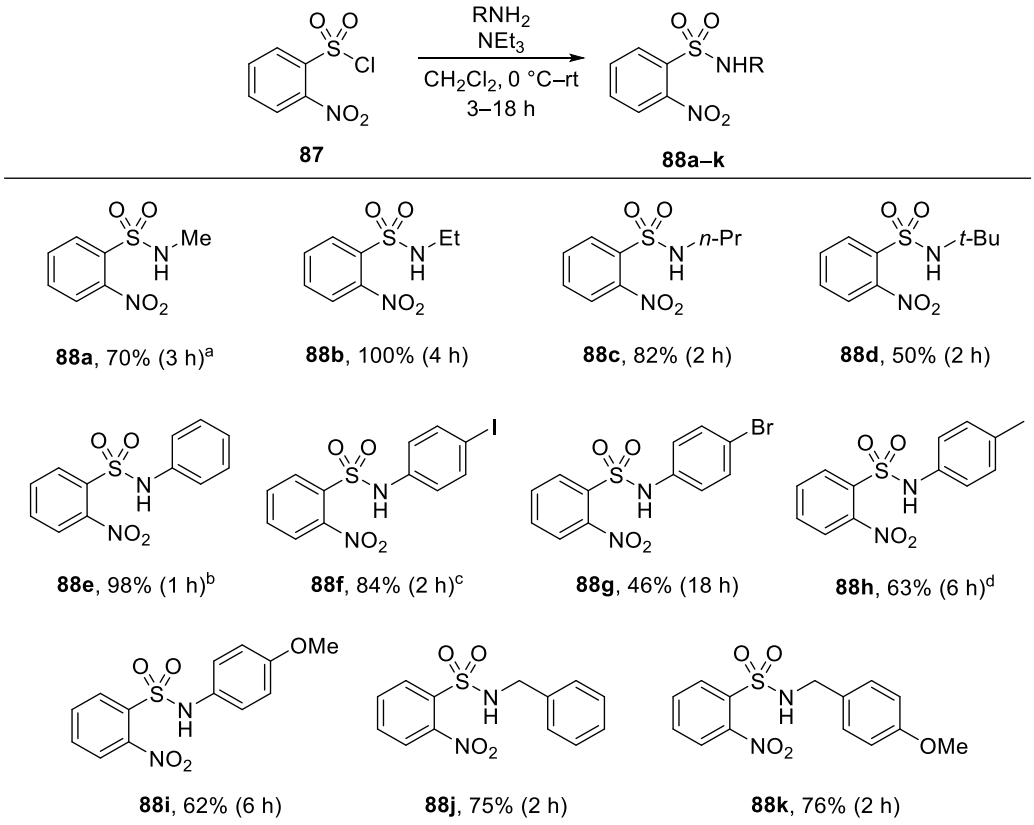


83 **2a**

Scheme 33: Attempted synthesis of 1,2,3,4-benzothiatriazine-1,1(2*H*)-dioxide (**2a**) from 2-aminobenzenesulfonamide (**83**) using *tert*-butyl nitrite.[96]

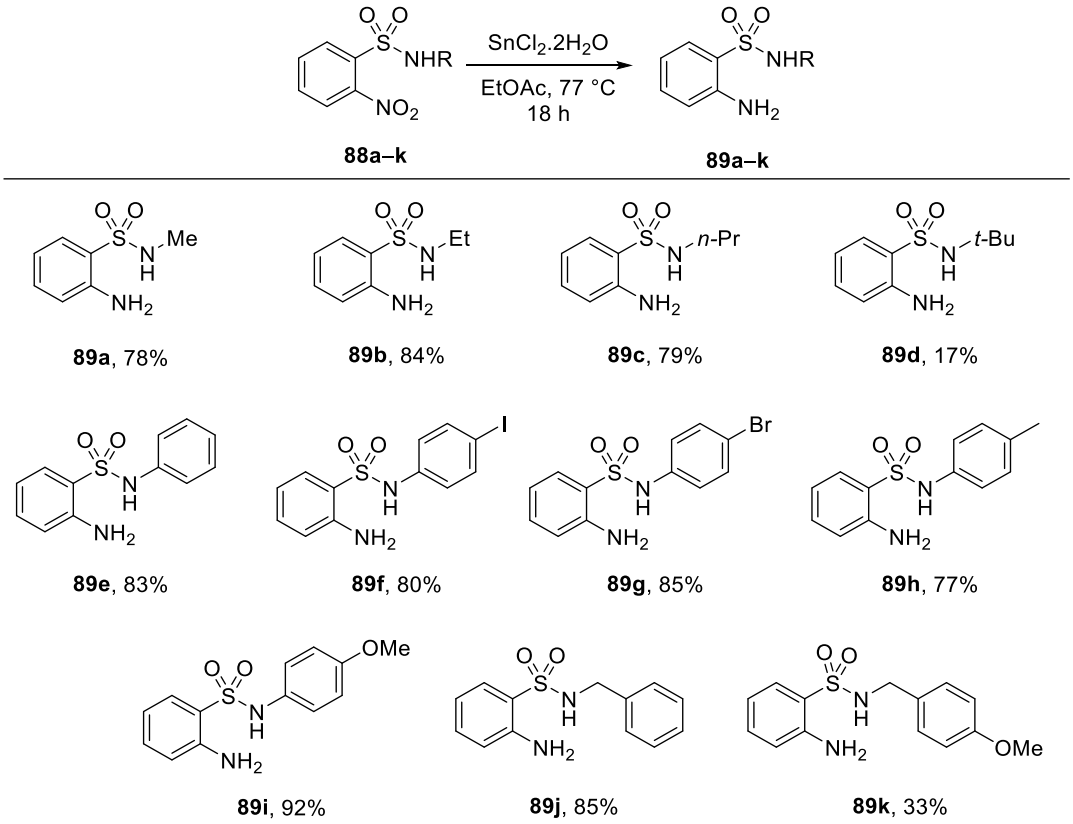
2.1.7 Substrate Scope: N_2-Substituted Benzothiatriazinedioxides

Despite the issues encountered when attempting to synthesise the N_2-unsubstituted benzothiatriazinedioxides, application of the one-pot procedure for the synthesis of N_2-substituted analogues was investigated. Due to the increased literature precedence, it was postulated that N_2-substitution increases the stability of these heterocycles, making them less susceptible to decomposition under the reaction conditions. To investigate this, various 2-aminobenzenesulfonamides were synthesised in two steps. Firstly, 2-nitrobenzenesulfonamides were generated *via* condensation of 2-nitrobenzenesulfonyl chloride (**87**) with amines (Scheme 34).[99] The reaction was operationally simple and successful for both electron-rich and electron-deficient anilines. For more electron-deficient analogues, such as **88g**, a lower yield was obtained. This was likely due to the decreased nucleophilicity of the amine. It was found that steric bulk hindered the reaction, as highlighted by the reduced yield of *tert*-butyl analogue **88d**.



Scheme 34: Synthesis of 2-nitrobenzenesulfonamides from 2-nitrobenzenesulfonyl chloride (**87**). [a]After 2 h at room temperature, 5 mL MeOH added and the reaction stirred at room temperature for a further 1 h; [b]Reaction solvent 50% aq. MeOH and NaOAc base, 60 °C; [c]Pyridine base; [d]Reaction stirred at 0 °C for 1 h followed by room temperature for 5 h.

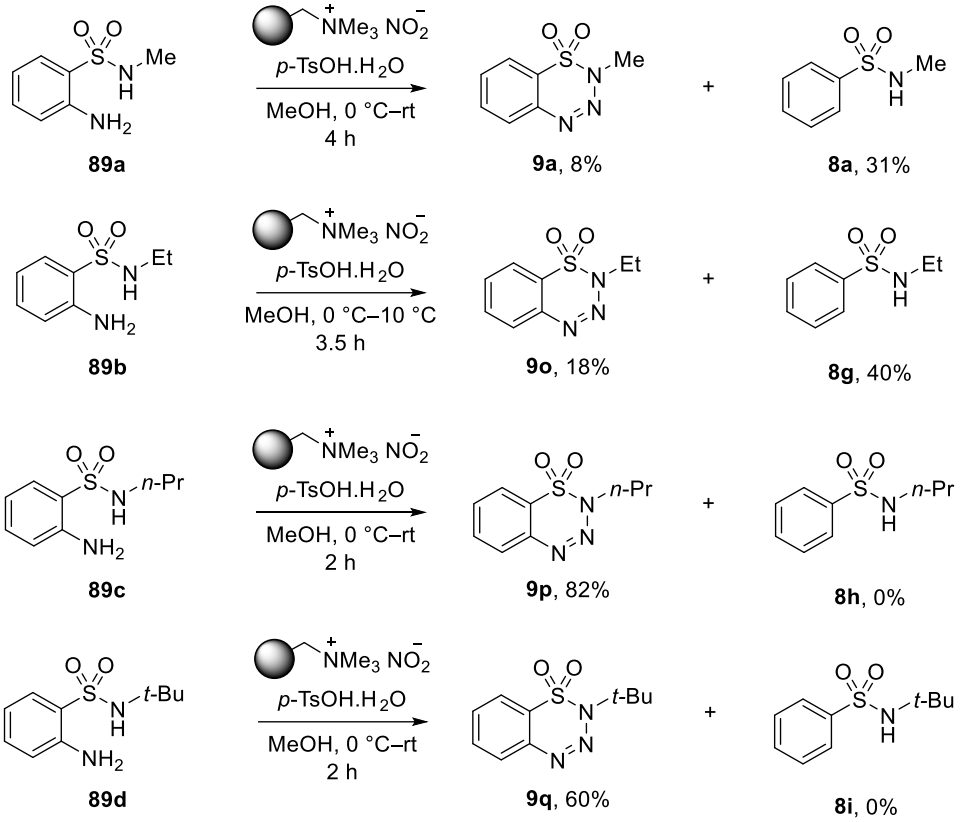
Nitro group reduction using tin(II) dichloride dihydrate was then carried out to give the corresponding 2-aminobenzenesulfonamides (Scheme 35).[95] This generally occurred in good to excellent yields for all substrates. The low yield obtained for **89d** is proposed to be the result of cleavage of the acid-labile *tert*-butyl group by hydrochloric acid generated during the course of the reaction.



Scheme 35: Synthesis of 2-aminobenzenesulfonamides from 2-nitrobenzenesulfonamides *via* reduction using tin(II) dichloride dihydrate.

The substrate scope of the *N*-alkyl benzothiatriazinedioxides was first investigated. The reaction proceeded well for bulky *tert*-butyl analogue **9q**, which was generated in 60% yield (Scheme 36). This was a suprising result as the corresponding *tert*-butyl benzotriazinone analogue could not be generated despite more forcing reaction conditions. *N*-Propyl analogue **9p** was also generated in an excellent 82% yield. However, both the *N*-methyl and *N*-ethyl substrates proved problematic. For the *N*-ethyl analogue, after 1 h at 10 °C, formation of desired product was observed by TLC analysis. However, traces of starting material were also present and so the reaction was stirred for a further 1.5 h. Although this resulted in full conversion of the starting material, an additional spot was also observed by TLC analysis. Following purification by column chromatography, only an 18% yield of desired product was obtained. The additional spot was later identified as dediazotised material **8g**, which was obtained in a 40% yield. Formation of **8g** was indicated by the presence of a monosubstituted benzene ring in the ^{1}H NMR spectrum. Dediazotised material was also isolated for *N*-methyl analogue **89a**. It is postulated that the dediazotisation occurs as a result of thermal decomposition due to the entropically favourable loss of dinitrogen. To overcome this, the reactions for both

the *N*-methyl and *N*-ethyl analogues were repeated at 0 °C. This resulted in 51% and 75% yields of the *N*-methyl and *N*-ethyl analogues, respectively.



Scheme 36: Initial results of the one-pot diazotisation and cyclisation procedure for the *N*-alkyl 2-aminobenzenesulfonamides (all substrates stirred at 0 °C for 1 h prior to heating).

For the *N*-phenyl analogues, initially some anomalous results were also obtained. For *N*-phenyl analogue **89e**, conversion to desired product was observed by TLC analysis. However, as with the *N*-methyl analogue, the reaction was stirred at room temperature for a further 1.5 h to allow for full consumption of starting material. Following the additional 1.5 h, TLC analysis showed the spot believed to be desired product had diminished and an additional spot was present. Similar reactivity was observed for the 4-bromophenyl and 4-iodophenyl analogues. Initially, it was believed dediazotisation had again occurred. However, the ^{1}H NMR spectrum of isolated material showed the presence of two 1,2-disubstituted aromatic rings, indicating formation of biaryl sultam **17a** (Figure 15).

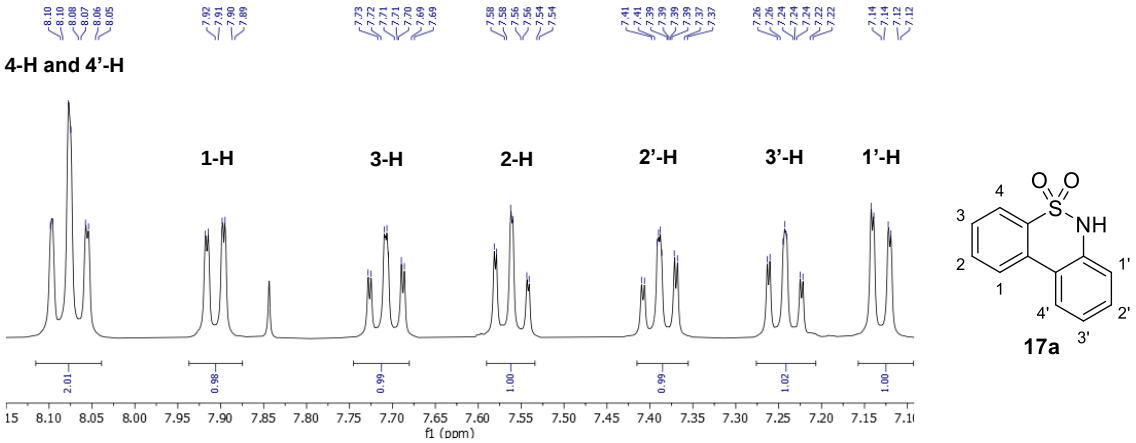
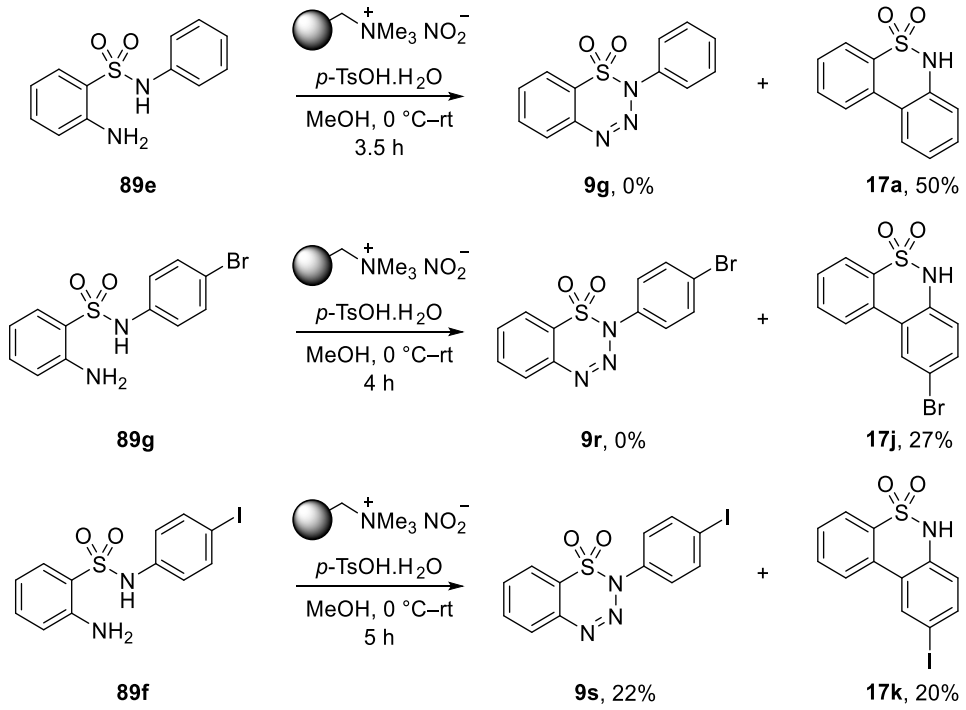


Figure 15: ^{1}H NMR spectrum of material isolated from attempted one-pot diazotisation and cyclisation of **89e** measured in CD_3OD (region from 7.10–8.15 ppm).

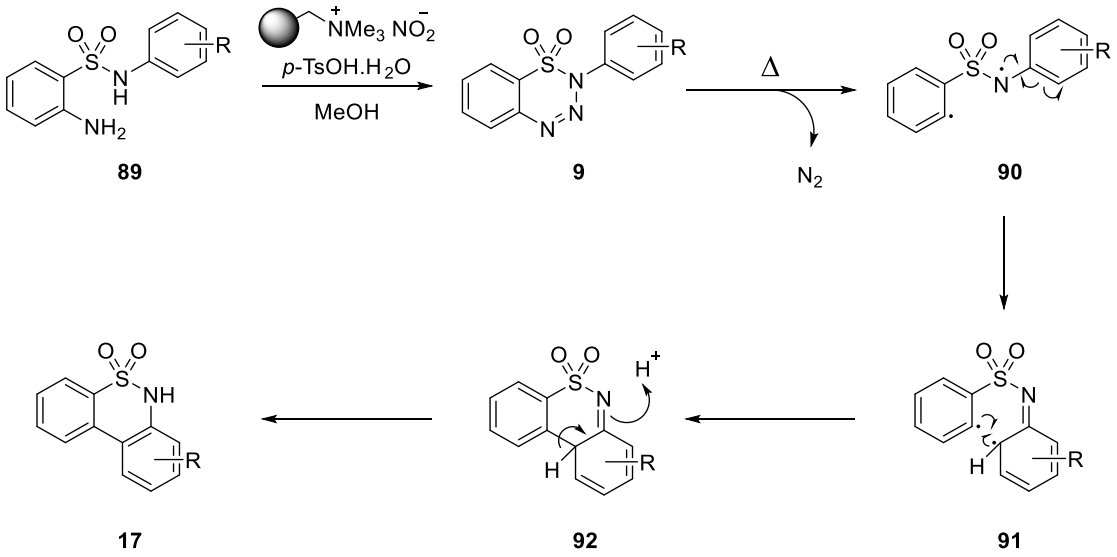
It was thus concluded that the corresponding biaryl sultams had also formed for *N*-phenyl analogues **89g** and **89f** (Scheme 37).



Scheme 37: Initial results of the one-pot diazotisation and cyclisation procedure for the *N*-phenyl-2-aminobenzenesulfonamides (all substrates stirred at 0 °C for 1 h prior to heating).

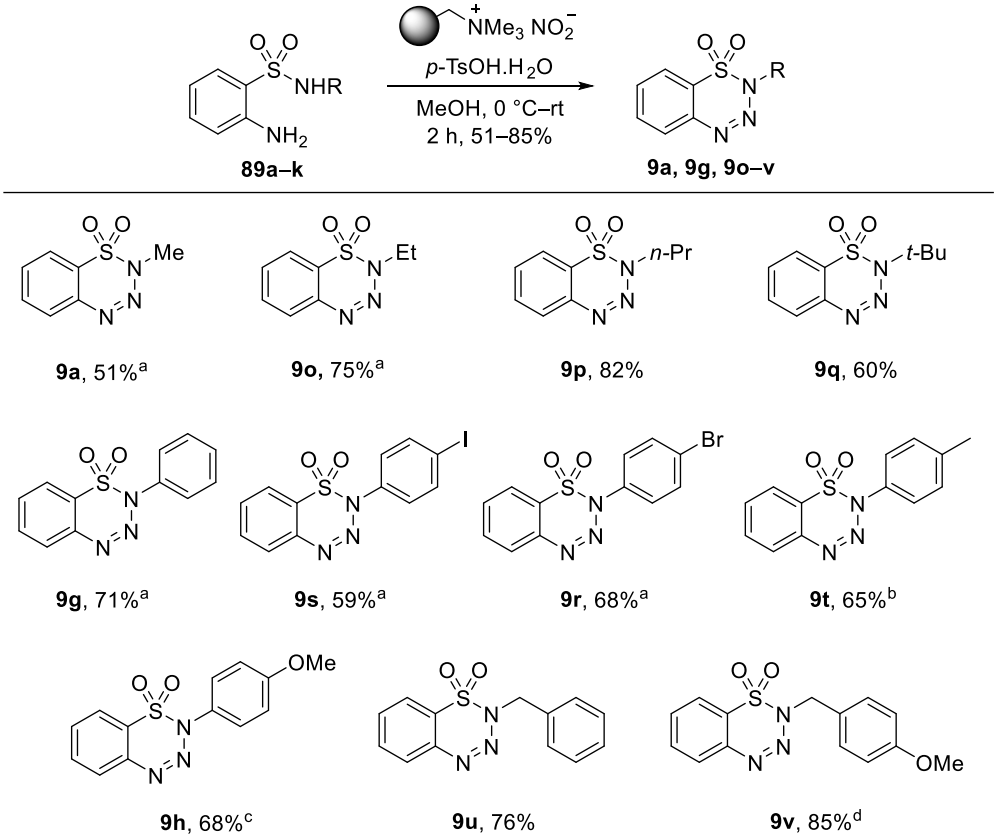
Thermal loss of dinitrogen from benzothiatriazinedioxides has been reported previously.[97,100] A proposed mechanism for formation of the biaryl sultams is shown in Scheme 38. Firstly, the desired benzothiatriazinedioxides form under the one-pot

diazotisation and cyclisation procedure conditions. These then undergo thermal loss of dinitrogen to generate diradical species **90**.[97] Subsequent rearrangement then gives second diradical **91**. Radical recombination then occurs due to the close proximity of the two radicals. Rearomatisation then gives biaryl sultam **17**.



Scheme 38: Proposed mechanism for formation of biaryl sultams from *N*-phenyl-2-aminobenzenesulfonamides *via* radical recombination.

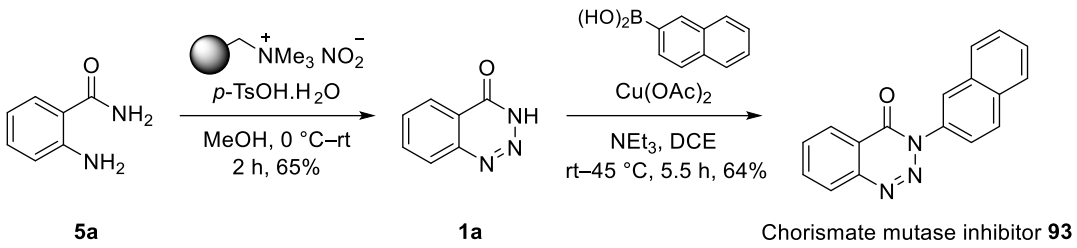
The substrate scope containing optimal yields for the N_2-substituted benzothiatriazinedioxides is shown in Scheme 39. Cooling the reaction allowed for the *N*-alkyl analogues to be obtained in good to excellent yields (**9a** and **9o**). Various *N*-phenyl analogues were also obtained in good yields under mild reaction conditions (**9g, 9h, 9r−t**). The reaction was succesful for *N*-benzyl analogues **9u** and **9v** in 76% and 85% yields, respectively.



Scheme 39: Substrate scope for the one-pot diazotisation and cyclisation procedure for the synthesis of N_2-substituted benzothiatriazinedioxides (all substrates stirred at 0 °C for 1 h prior to heating). [a]Reaction carried out at 0 °C for 2 h; [b]Reaction carried out at room temperature for 2.5 h; [c]Reaction carried out at room temperature for 1.3 h; [d]Reaction carried out at room temperature for 3.5 h.

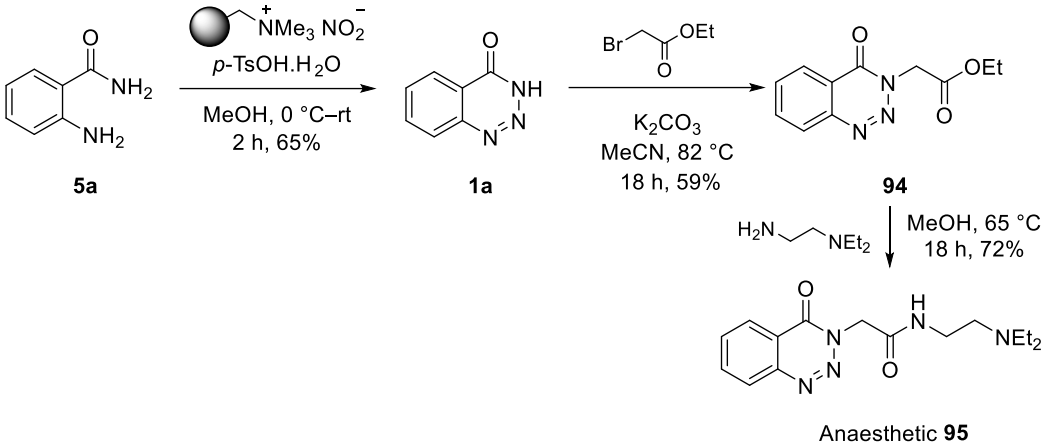
2.1.8 Synthesis of Biologically Active Compounds

As discussed in Section 1.1.1, benzotriazinone and benzothiatriazinedioxides scaffolds are prevalent in various biologically active compounds.[1,2,12,41,101] Therefore, to highlight the synthetic utility of the one-pot procedure, biological targets containing these heterocycles were synthesised. The first target was a chorismate mutase inhibitor. Chorismate mutase is essential for the synthesis of tyrosine and phenylalanine in bacteria.[102] It therefore provides an attractive target for small molecule inhibition for the treatment of bacterial diseases. Synthesis of **93** occurred in two steps. Firstly, the benzotriazinone scaffold was synthesised using the one-pot procedure as previously discussed (Scheme 40). The second step involved a Chan-Lam amination using 2-naphthalene boronic acid. This occurred in 64% yield to give desired product, **93**.



Scheme 40: Synthesis of chorismate mutase inhibitor (**93**).

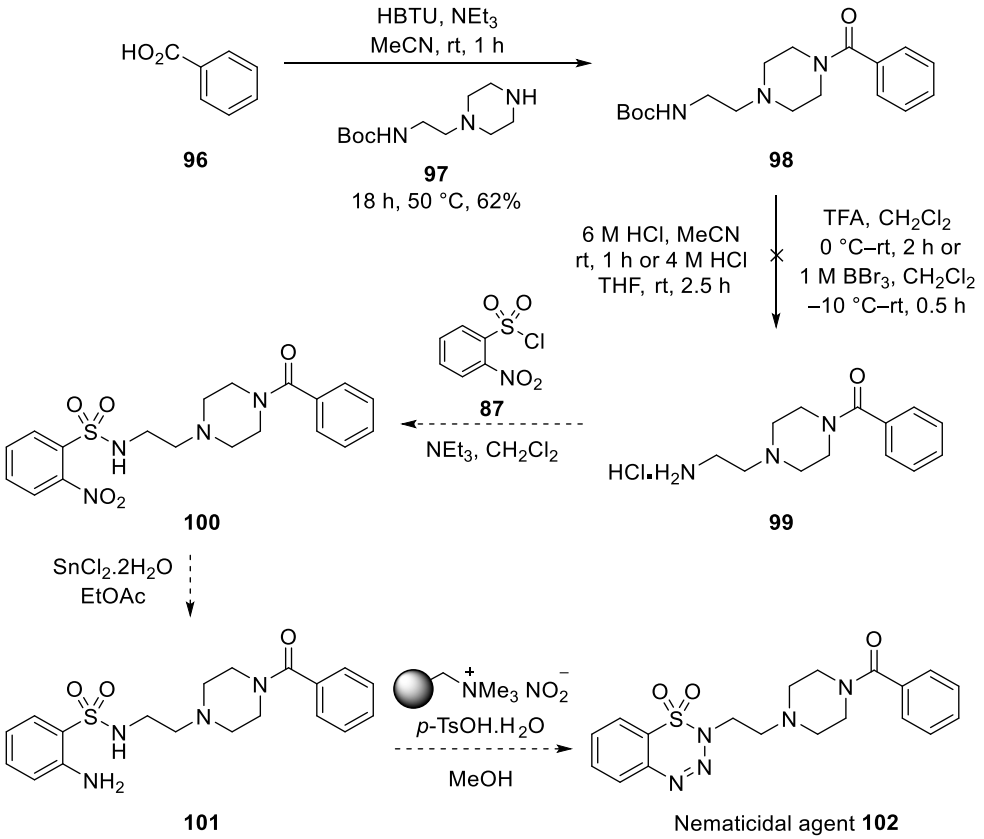
The second target was an anaesthetic with a comparable duration time and IC_{50} value to lidocaine.[1] Following formation of the benzotriazinone scaffold, an S_N2 reaction with ethyl bromoacetate gave **94** in a moderate 59% yield (Scheme 41). Condensation with *N,N*-diethylethylenediamine then gave anaesthetic **95** in a good 72% yield.



Scheme 41: Synthesis of anaesthetic **95**.

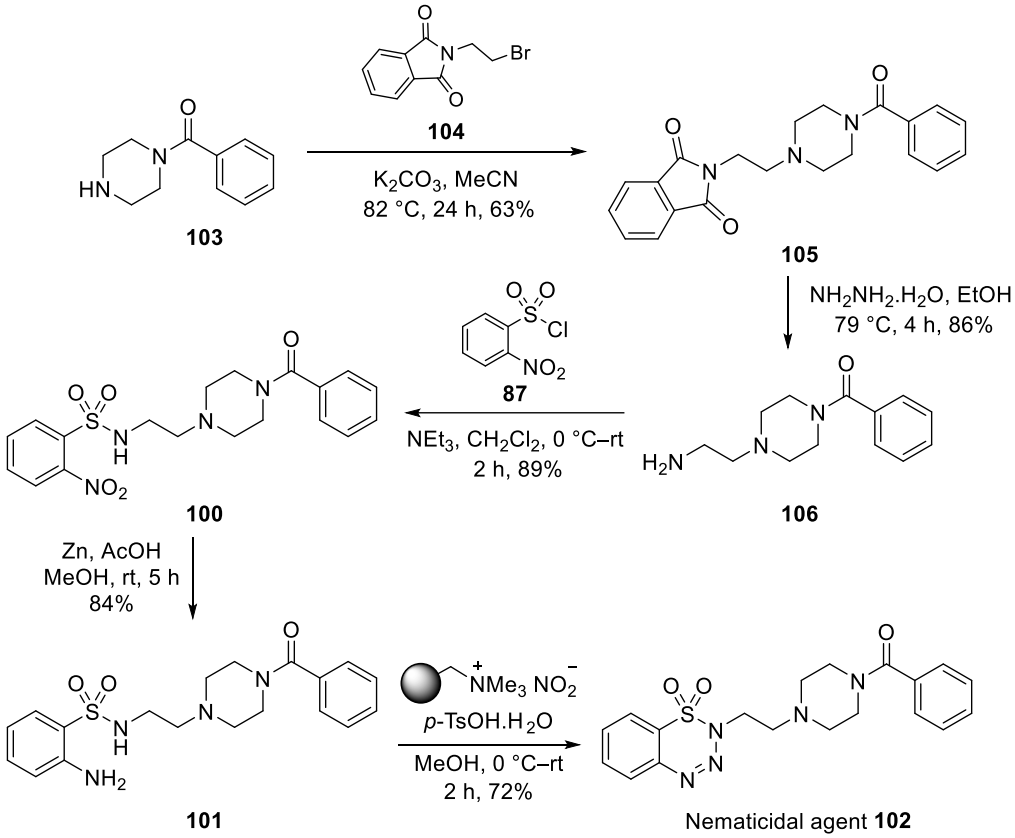
Finally, a nematicidal agent containing the benzothiatriazinedioxide scaffold was synthesised.[16] A proposed synthesis of nematicidal agent **102** is shown in Scheme 42. Firstly, benzoic acid (**96**) underwent an amide coupling reaction with monoalkylated piperazine **97** to give **98** in a 62% yield. The next step in the route was removal of the Boc-protecting group on the amine. This would allow for subsequent condensation with 2-nitrobenzenesulfonyl chloride (**87**) to give the desired 2-nitrobenzenesulfonamide. Reduction of the nitro group would then give the 2-aminobenzenesulfonamide required for the one-pot diazotisation and cyclisation procedure. However, synthesis of **99** proved problematic. Boc-group removal was attempted using various conditions including hydrochloric acid, trifluoroacetic acid and boron tribromide. These conditions proved either

unsuccessful or in cases when the Boc-group was removed, decomposition of the piperazine ring was also observed.



Scheme 42: Proposed synthesis of nematicidal agent **102**.

An alternative route to nematicidal agent **102** was therefore investigated. To avoid the decomposition issues assosiated with Boc-removal, *N*-benzoylpiperazine (**103**) underwent an alkylation reaction with *N*-2-bromoethylphthalimide (**104**) to give **105** in a 63% yield (Scheme 43). The phthalimide protecting group was removed using hydrazine hydrate in an excellent 86% yield. The free amine then underwent condensation with 2-nitrobenzenesulfonyl chloride (**87**) to give 2-nitrobenzenesulfonamide **100** in an excellent 89% yield. Subsequent nitro group reduction was then achieved using zinc and acetic acid to give **101**. Finally, the one-pot diazotisation and cyclisation procedure gave nematicidal agent **102** in a 72% yield.

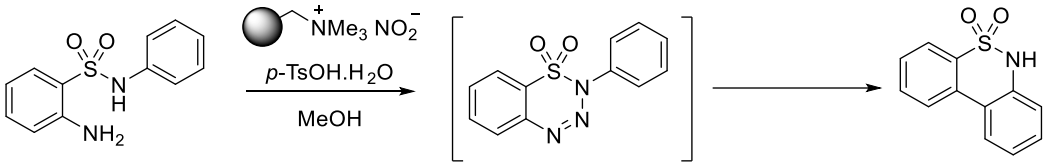


Scheme 43: Synthesis of nematicidal agent **102**.

2.1.9 Optimisation of the One-Pot Procedure for the Synthesis of Biaryl Sultams

Following the serendipitous discovery that the *N*-phenyl substituted benzothiatriazinedioxides undergo radical recombination to give biaryl sultams, optimisation of this reaction was investigated. Initially, the reaction was carried out at 0 °C for 1 h followed by room temperature for 2.5 h (Table 4, entry 1). This gave a 50% yield of biaryl sultam **17a**. To improve conversion of the benzothiatriazinedioxide intermediate, the room temperature phase of the reaction was extended to 3.5 h. The reaction was then stirred at 40 °C for 1 h. However, this resulted in no improvement in yield (entry 2). The reaction was also repeated by stirring at 0 °C for 2.5 h followed by room temperature for 5 h (entry 3). This showed full conversion of both the sulfonamide starting material and benzothiatriazinedioxide intermediate by TLC analysis. However, following purification by column chromatography, a 2:1 mixture of biaryl sultam product and dediazotised material was obtained. It is thus postulated dediazotisation may result from slowed formation of the benzothiatriazinedioxide intermediate. Finally, the reaction was repeated stirring at 0 °C for only 0.3 h followed by heating to 40 °C for 3 h (entry 4). This gave a 52% yield of desired biaryl sultam **17a**. It was thus

concluded that although the radical recombination is initiated by thermal loss of dinitrogen, as the rate of dediazotisation also increases with temperature, the procedure could not be optimised further.



89e 9g 17a

Entry	Reaction time at 0 °C (h)	Reaction time at rt (h)	Reaction time at 40 °C (h)	Reaction time at 68 °C (h)	Yield (%)
1	1	2.5	N/A	N/A	50%
2	1	3.5	1	N/A	48%
3	2.5	5	N/A	N/A	54% (2:1 product: dediazotised material)
4	0.3	N/A	3	N/A	52%

Table 4: Initial investigations into optimisation of the procedure for biaryl sultam synthesis.

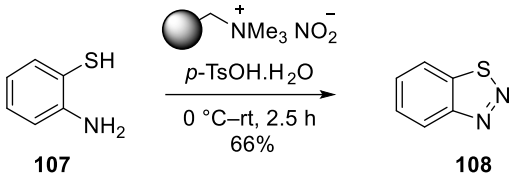
2.1.10 Conclusions

To conclude, a one-pot diazotisation and cyclisation procedure for the synthesis of benzotriazinones and benzothiatriazinedioxides has been developed. The methodology utilised a polymer-supported nitrite resin to generate stable diazonium salts in the presence of *p*-tosic acid. Using the mild and efficient procedure, N_3-unsubstituted benzotriazinones were synthesised from commercially available 2-aminobenzamides in good to excellent yields (53–91%). Where required, *N*-substituted 2-aminobenzamides were synthesised *via* the ring opening reaction of isatoic anhydride (**80**). Submission of the 2-aminobenzamides to the one-pot procedure generated N_3-substituted benzotriazinones in good to excellent yields (52–91%). In a similar manner, 2-aminobenzenesulfonamides were synthesised *via* the coupling of 2-nitrobenzenesulfonyl chloride (**87**) with various amines, followed by reduction of the nitro group using tin(II) dichloride dihydrate. The 2-aminobenzenesulfonamides were then submitted to the one-pot procedure to generate N_2-substituted benzothiatriazinedioxides in good to high yields (51–85%).

The synthetic utility of the one-pot procedure was highlighted by the synthesis of biologically active compounds containing these scaffolds.

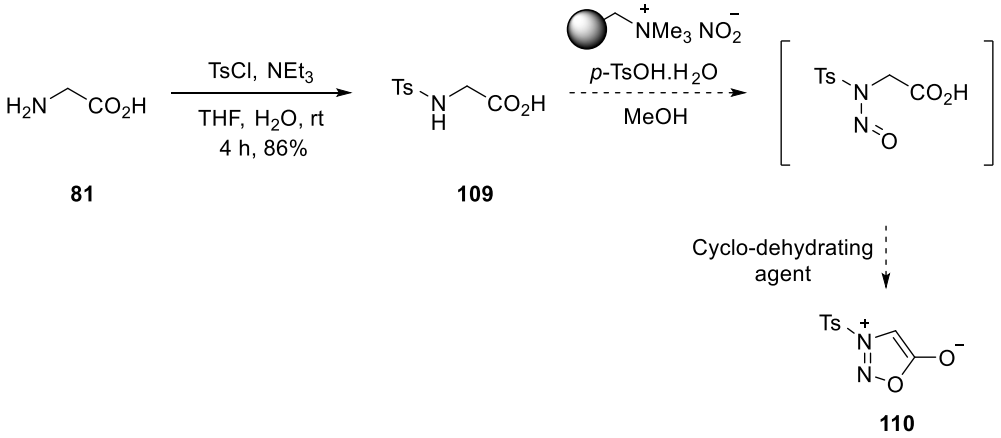
2.1.11 Future Work

Future work on this project will focus on expanding the scope of the one-pot procedure for the synthesis of other heterocycles. Benzothiadiazoles are valuable heterocyclic scaffolds found in various insecticides and nematicides.[103] Previous synthesis of these compounds utilised sodium nitrite and hydrochloric acid or required additives such as potassium iodide.[18,104] Work on this project has briefly shown the one-pot procedure can be utilised to synthesise benzothiadiazole **108** from 2-aminobenzenethiol (**107**) in a good 66% yield (Scheme 44). Investigations into the optimal reaction conditions for this cyclisation as well as the substrate scope will be carried out.



Scheme 44: Synthesis of benzothiadiazole **108** from 2-aminobenzenethiol (**107**).

Synthesis of sydnones utilising this methodology will also be investigated. Sydnones are heterocyclic compounds with a wide range of applications including indazole and pyrazole synthesis as well as anti-cancer properties.[105-107] Thus far, tosyl-protected glycine **109** has been synthesised in a 86% yield (Scheme 45). Future work will investigate the use of *N*-tosyl glycine **109** for the preparation of sydnones using the polymer supported process followed by cyclodehydration.

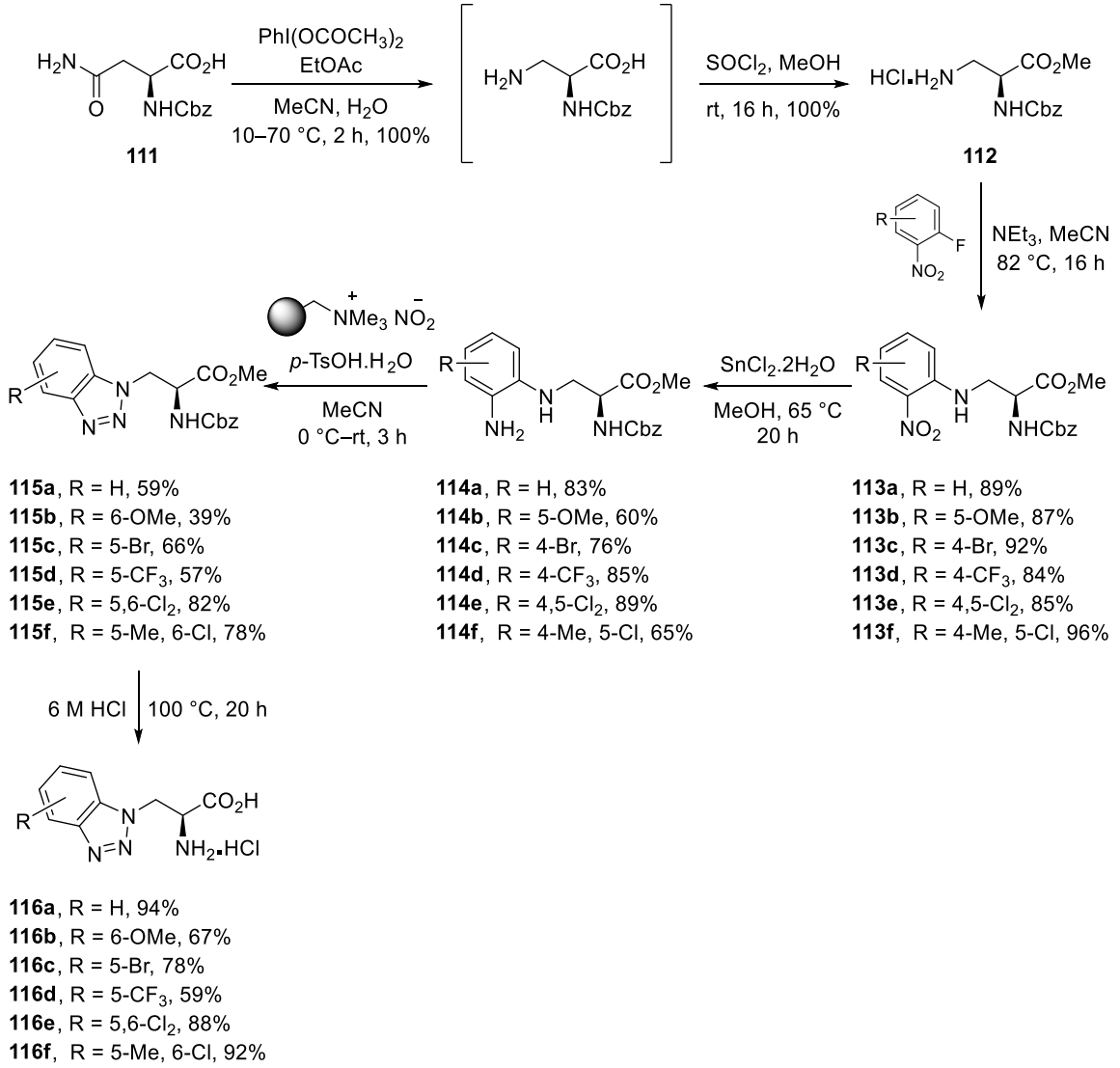


Scheme 45: Proposed synthesis of sydnone **110** from glycine (**81**).

2.2 Synthesis of Novel Fluorescent Benzotriazinone-Derived α-Amino Acids

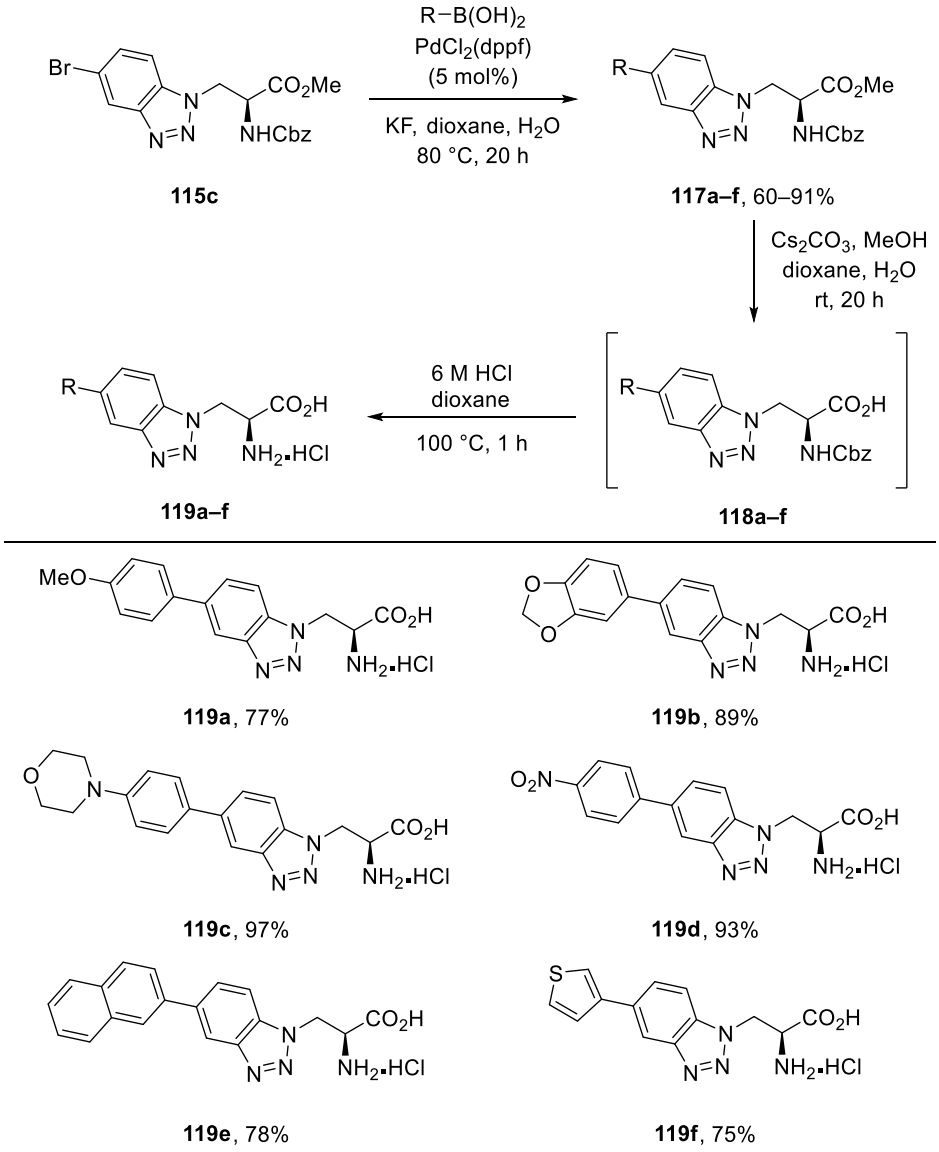
2.2.1 Previous Work in the Sutherland Group

A main programme of research within the Sutherland group focuses on the synthesis of novel imaging agents. This includes fluorescent α-amino acids, which are of great interest due to the lack of perturbation to the native protein compared to other methods.[60] Previous group work has resulted in the synthesis of fluorescent benzotriazole-derived unnatural α-amino acids.[90] The desired deprotected amino acids were synthesised in six steps from commercially available *N*-Cbz-L-asparagine (**111**) (Scheme 46). Initially, amide **111** underwent a Hofmann rearrangement using hypervalent iodine to generate the corresponding L-3-aminoalanine derivative, L-DAP. Formation of methyl ester **112** was then achieved under standard conditions using thionyl chloride and methanol. Protected L-3-aminoalanine **112** was subjected to an S_NAr reaction with functionalised *ortho*-fluoronitrobenzenes to give **113a**–**f**. Chemoselective reduction of the nitro group using tin(II) dichloride dihydrate allowed access to 1,2-aryldiamines **114a**–**f**. Submission of the 1,2-aryldiamines to the one-pot diazotisation and cyclisation procedure generated the corresponding benzotriazoles in yields ranging from 57% to 82%. Finally, deprotection under acidic conditions gave the desired amino acids.



Scheme 46: Synthesis of benzotriazole-derived α-amino acids.[90]

In order to improve the photoluminescent properties of this series, the group showed bromo analogue **115c** could be further functionalised *via* a Suzuki-Miyaura cross-coupling reaction (Scheme 47). This generated analogues **117a**–**f** in good to excellent yields. Hydrolysis of the methyl ester was achieved using caesium carbonate. This was followed by a fast reaction in 6 M hydrochloric acid to remove the Cbz-protecting group.



Scheme 47: Synthesis of 5-aryl benzotriazole-derived α-amino acids *via* Suzuki-Miyaura cross-coupling reaction of **115c** (yields over two steps).[90]

Photoluminescent data for the deprotected amino acids was then obtained. Methoxy analogue **119a** was found to have optimal fluorescent properties with a quantum yield of 0.17 and a MegaStokes shift of 162 nm. To assess its solvatochromic properties, absorption and emission data for **119a** were obtained in various solvents (Figure 16). It was found that as the polarity of the solvent increased, the λ_{em} became further red-shifted [Figure 16, (b)]. Consequently, due to their environmental sensitivity, these analogues have potential applications as probes for chemical biology.

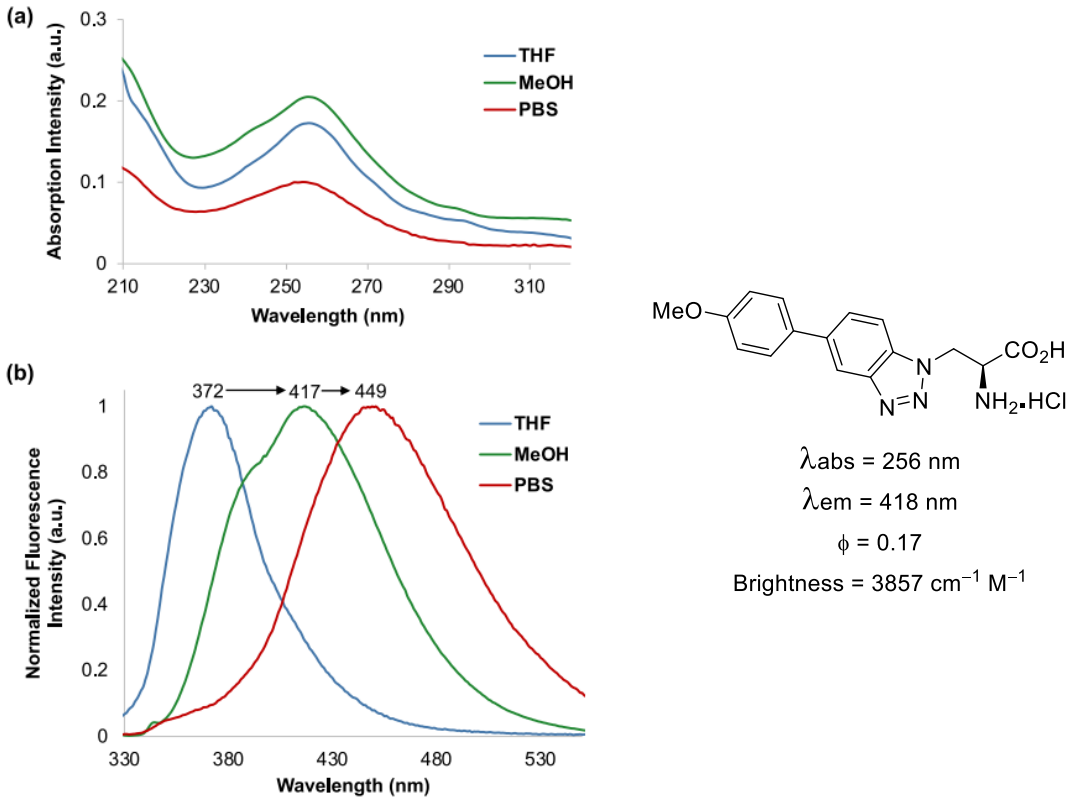
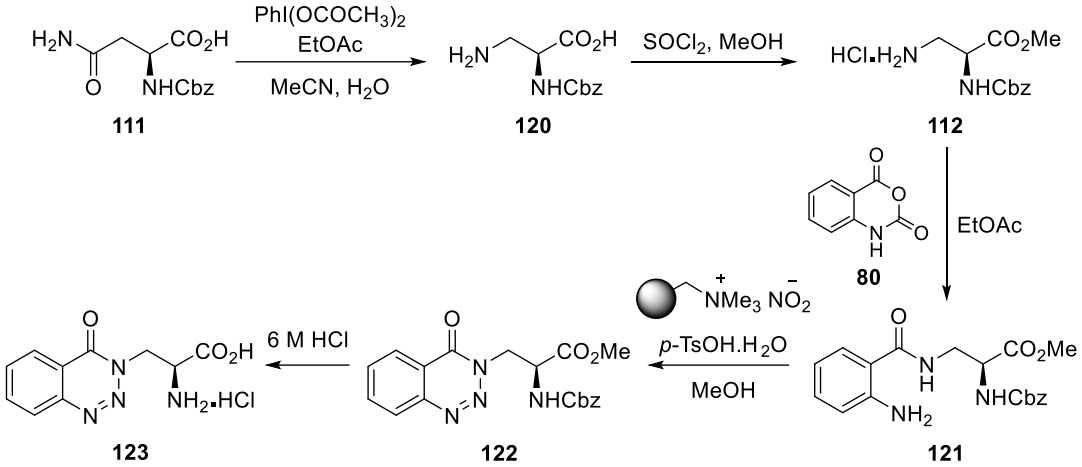


Figure 16: Absorption and emission spectra of **119a** in various solvents (10 µM).[90]

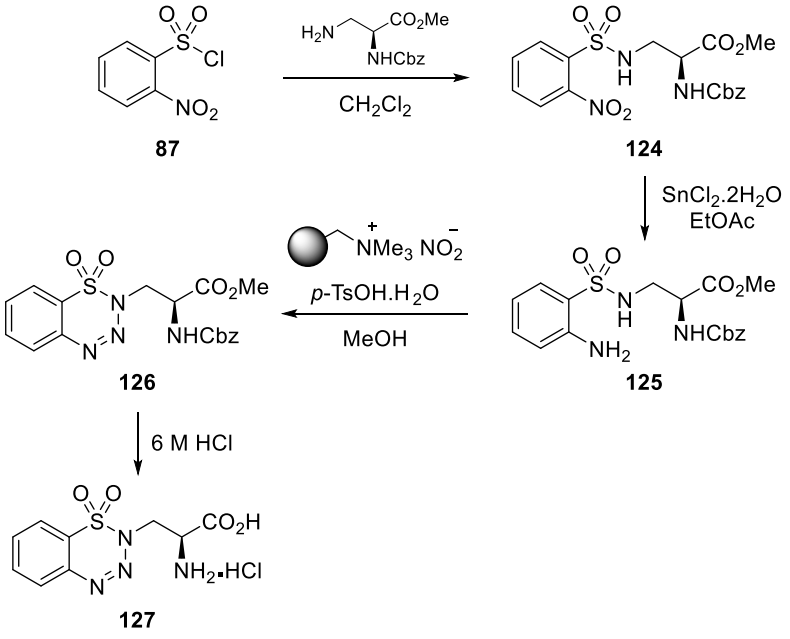
2.2.2 Proposed Research

While benzotriazole-derived amino acids such as **119a** were found to possess interesting photoluminescent properties, the absorption maxima for these analogues occurred at low wavelengths and coincided with the absorption properties of proteinogenic, fluorescent amino acids, such as tyrosine and tryptophan. For this reason, the aim of the project was to investigate whether benzotriazinone- and benzothiatriazinedioxide-derived amino acids which contain additional electron-withdrawing groups might result in red-shifted absorption maxima. Thus, the main objective was to utilise the methodology developed in Section 2.1 to incorporate the benzotriazinone and benzothiatriazinedioxide scaffolds into the side chains of amino acids. To achieve this, *N*-Cbz-L-asparagine (**111**) would be subjected to a Hofmann rearrangement to give amine **120** (Scheme 48). Following esterification, amine **112** would then participate in a ring opening reaction with isatoic anhydride (**80**) to give **121**. Submission of 2-aminobenzamide **121** to the one-pot diazotisation and cyclisation procedure would generate the benzotriazinone scaffold. Deprotection under acidic conditions would give novel α-amino acid **123**.



Scheme 48: Proposed synthesis of benzotriazinone-derived unnatural α-amino acid **123**.

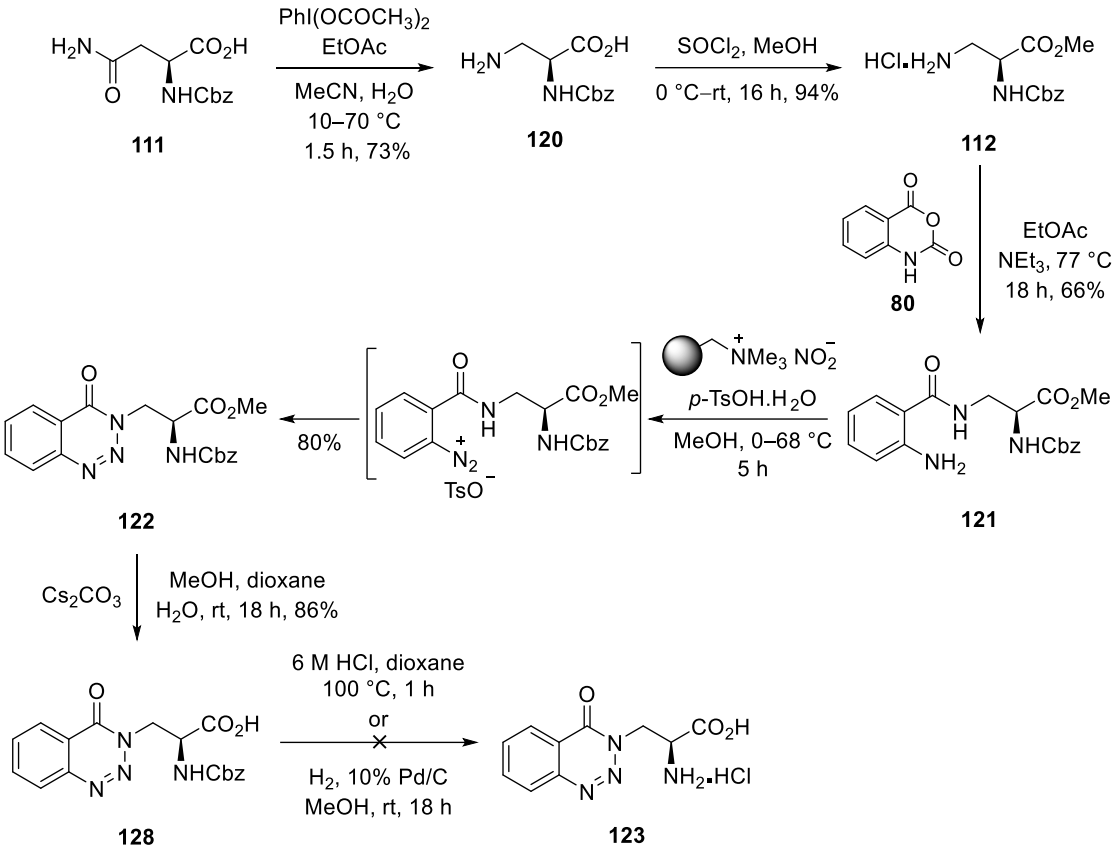
A similar route would be developed for the synthesis of benzothiatriazinedioxide derivative **127** (Scheme 49). In place of the ring opening reaction in the benzotriazinone route, primary amine **112** would instead undergo a condensation reaction with 2-nitrosbenzenesulfonyl chloride (**87**) to give **124**. Chemoselective reduction of the nitro group using tin(II) dichloride dihydrate would be followed by the one-pot diazotisation and cyclisation procedure to deliver the benzothiatriazinedioxide scaffold. Deprotection would then be carried out to give the desired amino acid. The fluorescent properties of these novel α-amino acids would then be investigated to evaluate their use as biological imaging agents.



Scheme 49: Proposed synthesis of benzothiatriazinedioxide-derived unnatural α-amino acid **127**.

2.2.3 Synthesis and Fluorescent Properties of Unsubstituted Benzotriazinone α-Amino Acid 123

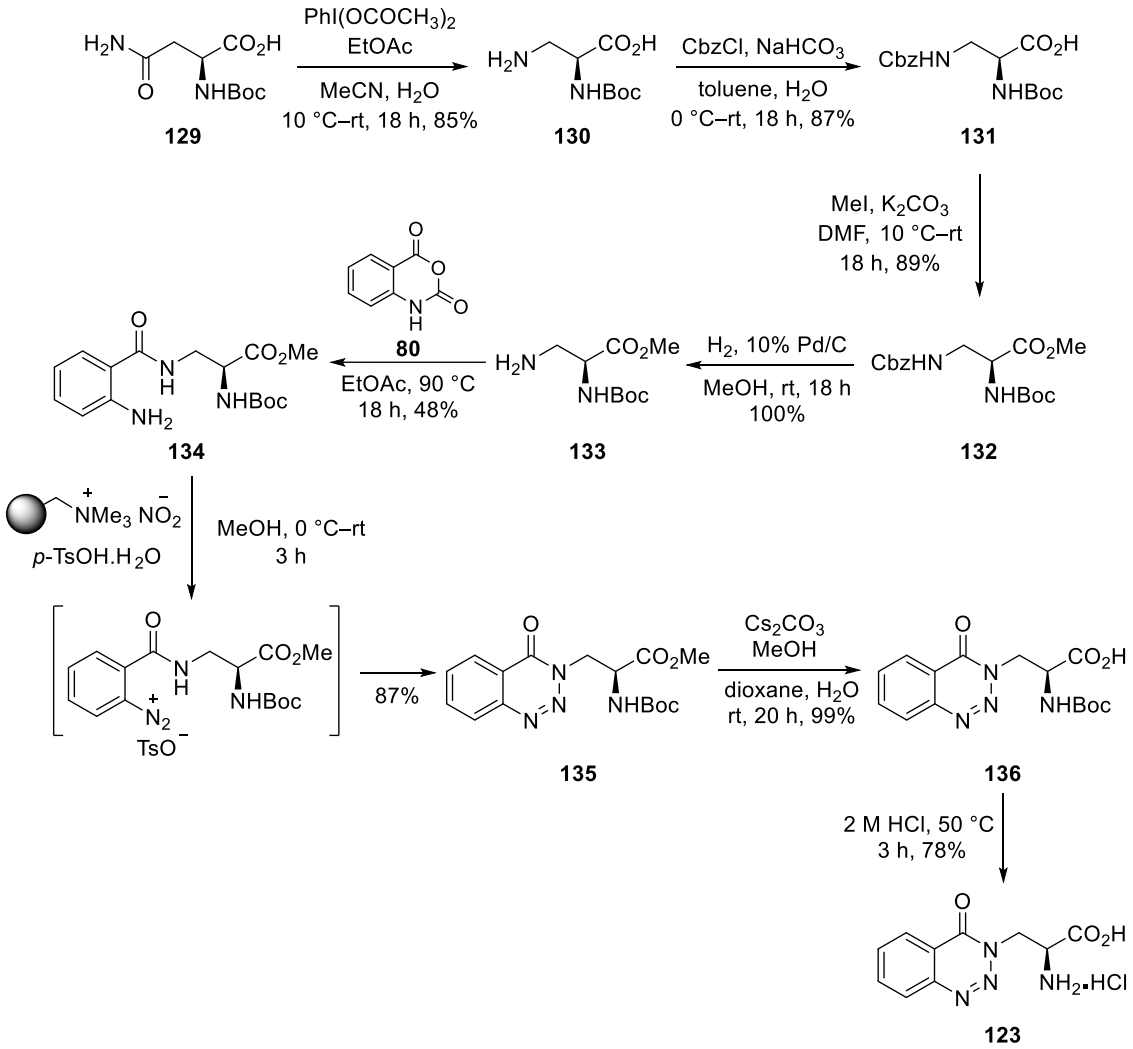
The first aim of this project was to synthesise unsubstituted benzotriazinone α-amino acid **123**. To do this, commercially available *N*-Cbz-L-asparagine (**111**) was converted to Cbz-protected 3-aminoalanine derivative **120** *via* a Hoffman rearrangement in a high 73% yield (Scheme 50).[108] Esterification was then carried out under standard conditions using thionyl chloride and methanol to give the corresponding methyl ester. This then participated in a ring opening reaction with isatoic anhydride (**80**) in a good 66% yield to give 2-aminobenzamide **121**.[93] Submission of 2-aminobenzamide **121** to the one-pot procedure, using polymer supported nitrite reagent and *p*-tosic acid, generated benzotriazinone **122** in an excellent 80% yield. Formation of cyclised product **122** was indicated by a significant downfield shift of the aromatic hydrogen atoms in the ^{1}H NMR spectrum. The final step in the synthesis required removal of the protecting groups to give the desired parent amino acid. This was initially attempted using 6 M hydrochloric acid heating under reflux for 18 h. However, the ^{1}H NMR spectrum of the crude reaction mixture indicated the harsh reaction conditions resulted in partial decomposition of desired product. A milder, step-wise procedure involving removal of the methyl ester followed by Cbz-group removal was therefore attempted. Conversion of methyl ester **122** to free acid **128** occurred in a high 86% yield. Removal of the Cbz-group was then attempted. In this instance, the reaction mixture was heated under reflux for only 1 h. However, this also resulted in decomposition. As it was proposed the strong acidic conditions may be contributing to the decomposition, removal of the Cbz-group was attempted *via* a hydrogenation reaction but this showed no conversion of starting material after 24 h.



Scheme 50: Synthesis of protected benzotriazinone amino acid **122** and attempted deprotection.

Due to the issues encountered with removal of the Cbz group, it was proposed that alternative Boc-protection of the amine would allow deprotection under milder conditions and therefore avoid decomposition. To do this, commercially available *N*-Boc-L-asparagine (**129**) was converted to Boc-protected 3-aminoalanine derivative **130** *via* a Hofmann rearrangement in a good 71% yield (Scheme 51). Protection of amine **130** was then carried out under standard conditions using benzyl chloroformate and sodium hydrogen carbonate in an excellent 84% yield. This allowed for subsequent esterification using methyl iodide which occurred in an 86% yield to give methyl ester **132**. Removal of the Cbz-group occurred quantitively *via* a hydrogenation reaction to give amine **133**.[109] Submission of **133** to the ring opening reaction gave desired 2-aminobenzamide **134** in 48% yield.[93] Although a moderate yield, this reaction was dual functional, generating both the amide and amine functionalities required for the one-pot procedure whilst simultaneously attaching the amino acid side chain. The one-pot diazotisation and cyclisation procedure then occurred in an excellent 87% yield to give benzotriazinone **135**. Finally, deprotection was carried out. Firstly, the methyl ester was removed using caesium carbonate in an excellent 99% yield. The Boc-group was then successfully

removed using 2 M hydrochloric acid under relatively mild conditions, generating amino acid **123** in a 78% yield.



Scheme 51: Synthesis of benzotriazinone-containing α-amino acid **123**.

The absorption and emission spectra for **123** are shown in Figures 17 and 18. The λ_{abs} was 288 nm and the λ_{em} was 372 nm. For use in biological imaging, fluorescent amino acids require longer absorption and emission wavelengths.[43] Excitation of fluorophores with high energy wavelengths below 300 nm can cause cell damage. Also, emission below 350 nm can cause measurement issues due to overlap of emission from the naturally occurring fluorescent amino acids.[110]

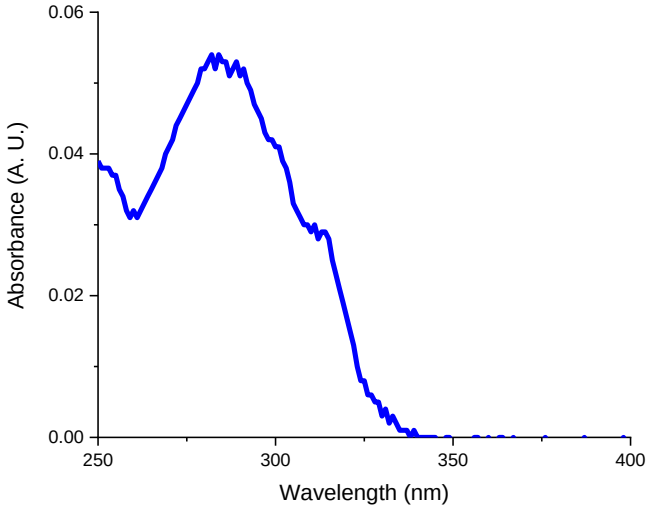


Figure 17: Absorption spectra of benzotriazinone α-amino acid **123** (10 μM in MeOH).

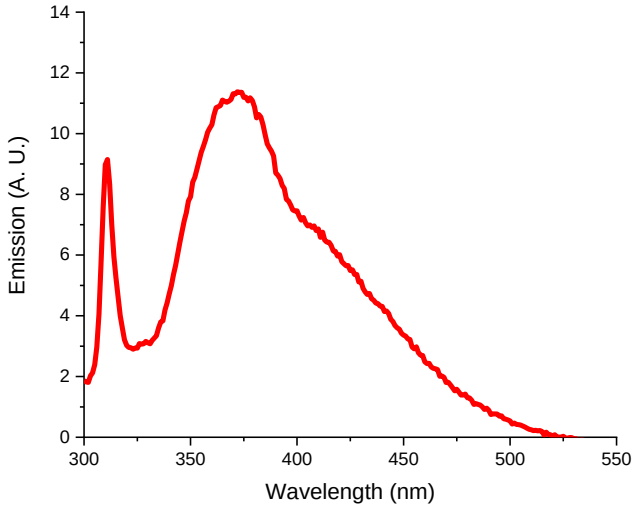
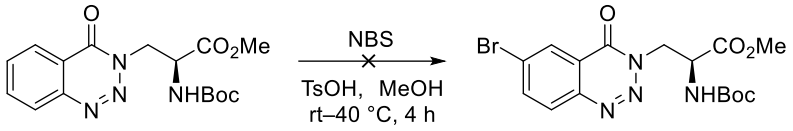


Figure 18: Emission spectra of benzotriazinone α-amino acid **123** (10 μM in MeOH, excited at 260 nm).

As highlighted by Figures 17 and 18, a bathochromic shift in both the absorption and emission spectrum is required. It was proposed that this could be achieved by bromination of the benzotriazinone system, resulting in an intermediate that could undergo cross-coupling reactions to generate more conjugated chromophores with improved photoluminescent properties.[51] This was attempted using *N*-bromosuccinimide and *p*-tosic acid (Scheme 52).[111] However, no conversion of starting material **135** was observed by ^{1}H NMR spectroscopy after 4 h at 40 °C.

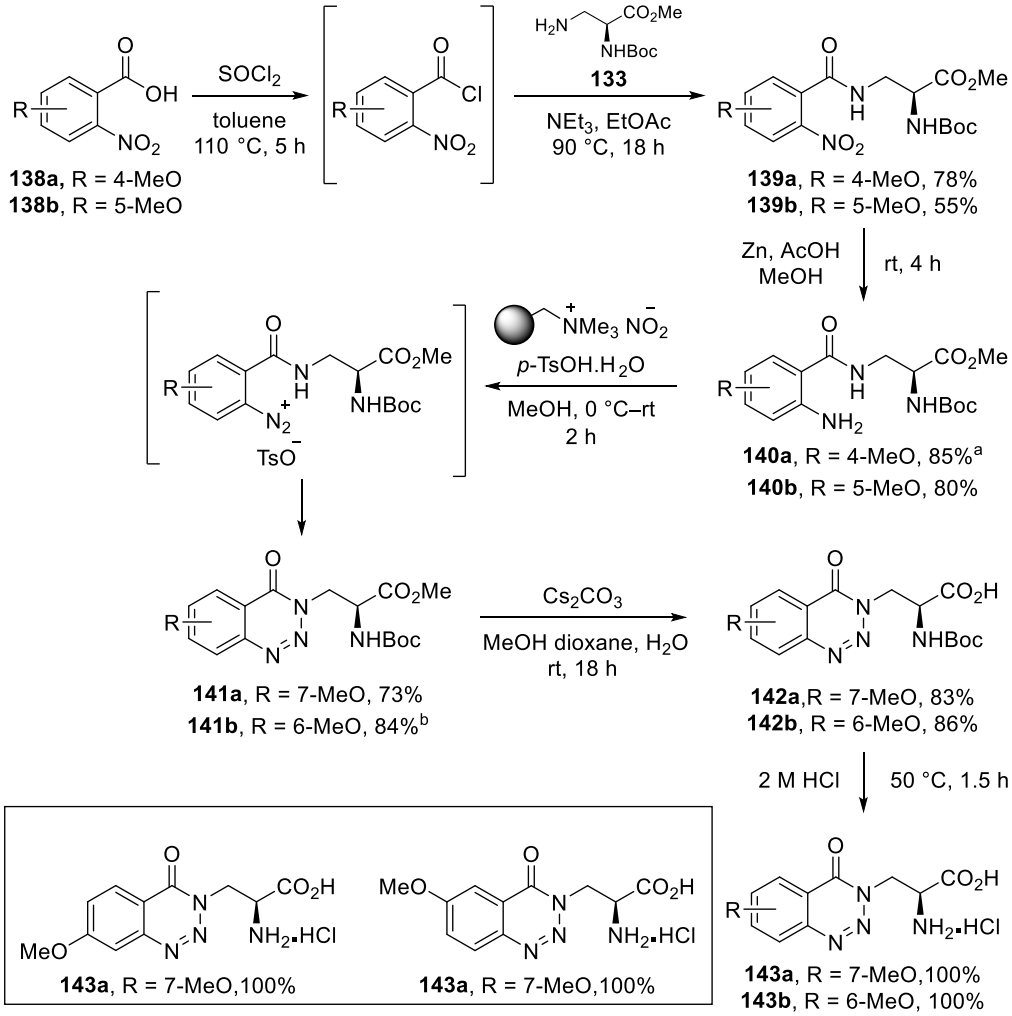


135 **137**

Scheme 52: Attempted bromination of benzotriazinone **135**.

2.2.4 Synthesis and Fluorescent Properties of Methoxy-Substituted Benzotriazinone α-Amino Acids

As discussed in Section 1.2.2, systems bearing both electron-withdrawing and electron-donating substituents are known to improve the fluorescent properties of molecules by ICT.[112] Due to the electron-withdrawing ability of the carbonyl moiety present in the benzotriazinone scaffold, it was proposed that incorporation of electron-donating substituents on the aromatic ring would generate a push-pull system and thus give rise to a bathochromic shift in the absorption and emission wavelengths. Therefore, the synthesis of 6'-methoxy and 7'-methoxy derivatives was investigated. The synthetic route to these analogues is shown in Scheme 53. Firstly, the substituted benzoic acids were converted to their corresponding acid chlorides under standard conditions using thionyl chloride. These were then successfully coupled to amine **133** (synthesis as shown in Scheme 51) to give 2-nitrobenzamides **139a** and **139b** in moderate to good yields. Nitro group reduction was achieved using zinc and acetic acid which gave 2-aminobenzamides **140a** and **140b** in excellent 85% and 80% yields for the 4-methoxy and 5-methoxy analogues, respectively. Submission of the 2-aminobenzamides to the one-pot procedure then gave the cyclised products **141** in excellent yields. As both the nitro reduction and cyclisation were carried out under mild acidic conditions, removal of the Boc-protecting group was not observed. Deprotection to give the desired amino acids was carried out as described for the unsubstituted analogue. Firstly, caesium carbonate was used to hydrolyse the methyl ester. Removal of the Boc-group then occurred quantitatively using 2 M hydrochloric acid to give amino acids **143a** and **143b**.



Scheme 53: Synthesis of methoxy-substituted benzotriazinone α-amino acids **143a** and **143b**. [a]Reaction carried out at room temperature for 18 h; [b]Reaction carried out at room temperature for 2.5 h.

The absorption data for the 6'-methoxy and 7'-methoxy analogues is shown in Figure 19. (2*S*)-2-Amino-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**143b**) has a λ_{abs} at 225 nm and (2*S*)-2-amino-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**143a**) has a λ_{abs} at 248 nm.

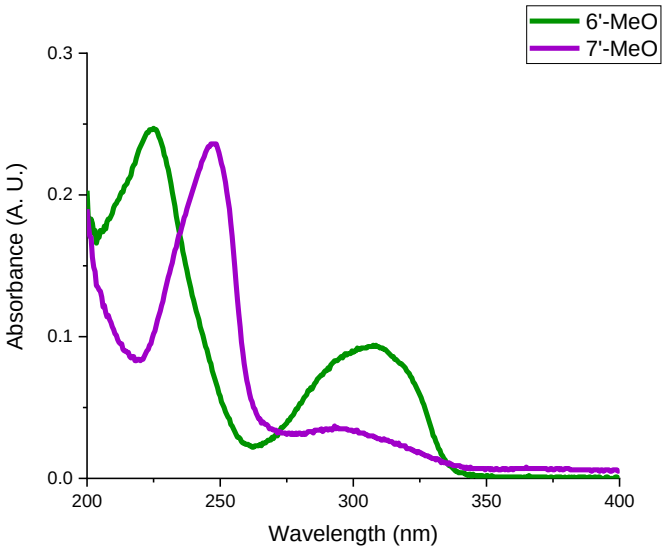


Figure 19: Absorption spectra of the methoxy-substituted benzotriazinone α-amino acids (10 μM in MeOH).

The emission data for the 6'-methoxy and 7'-methoxy analogues is shown in Figure 20. The λ_{em} are 275 nm and 271 nm for the 6'-methoxy and 7'-methoxy analogues, respectively (Figure 20).

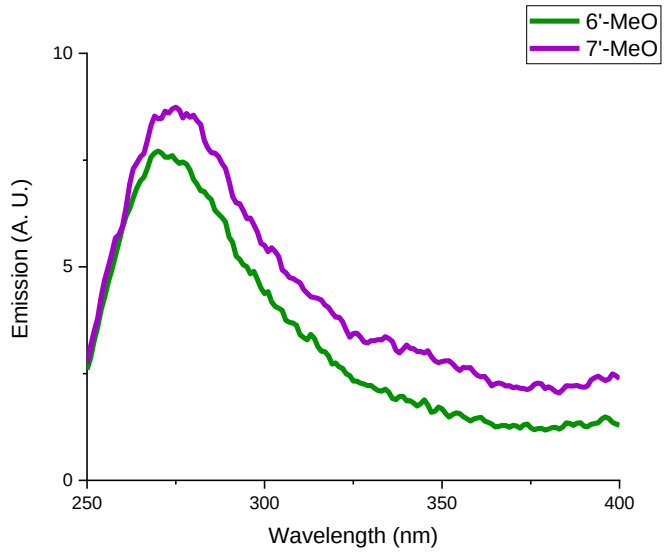
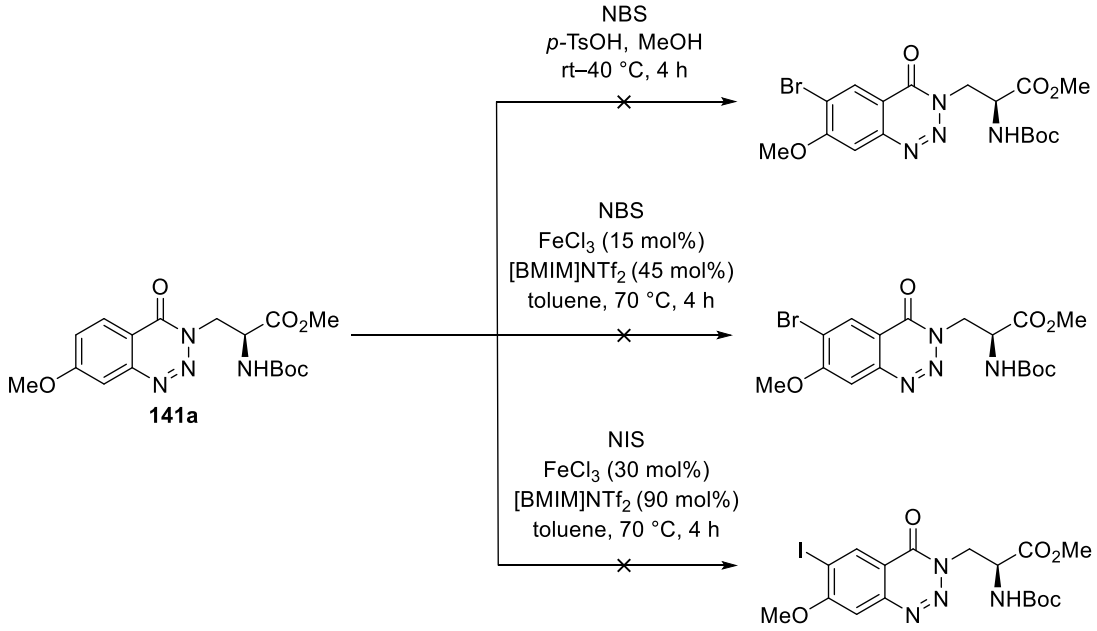


Figure 20: Emission spectra of the methoxy-substituted benzotriazinone α-amino acids (10 μM in MeOH, 6'-MeO excited at 225 nm and 7'-MeO excited at 248 nm).

It was proposed that the fluorescent properties of the methoxy-substituted benzotriazinone α-amino acids could be further improved by extending the conjugation of the system *via* a cross-coupling reaction. Although halogenation of

unsubstituted analogue **135** proved unsuccessful, it was postulated that the more activated aromatic ring of **141a** would allow for easier halogenation. Initially, bromination of **141a** was attempted using *N*-bromosuccinimide and *p*-tosic acid (Scheme 54).[111] However, this proved unsuccessful, showing no conversion of starting material by ^{1}H NMR spectroscopy after 4 h at 40 °C. The reaction was therefore attempted using iron(III) triflimide, a method previously developed within the Sutherland group for the regioselective bromination of aromatic rings.[113] However, this also proved unsuccessful. Finally, an iodination reaction was attempted using iron(III) triflimide and *N*-iodosuccinimide.[114] This again showed no conversion of starting material.

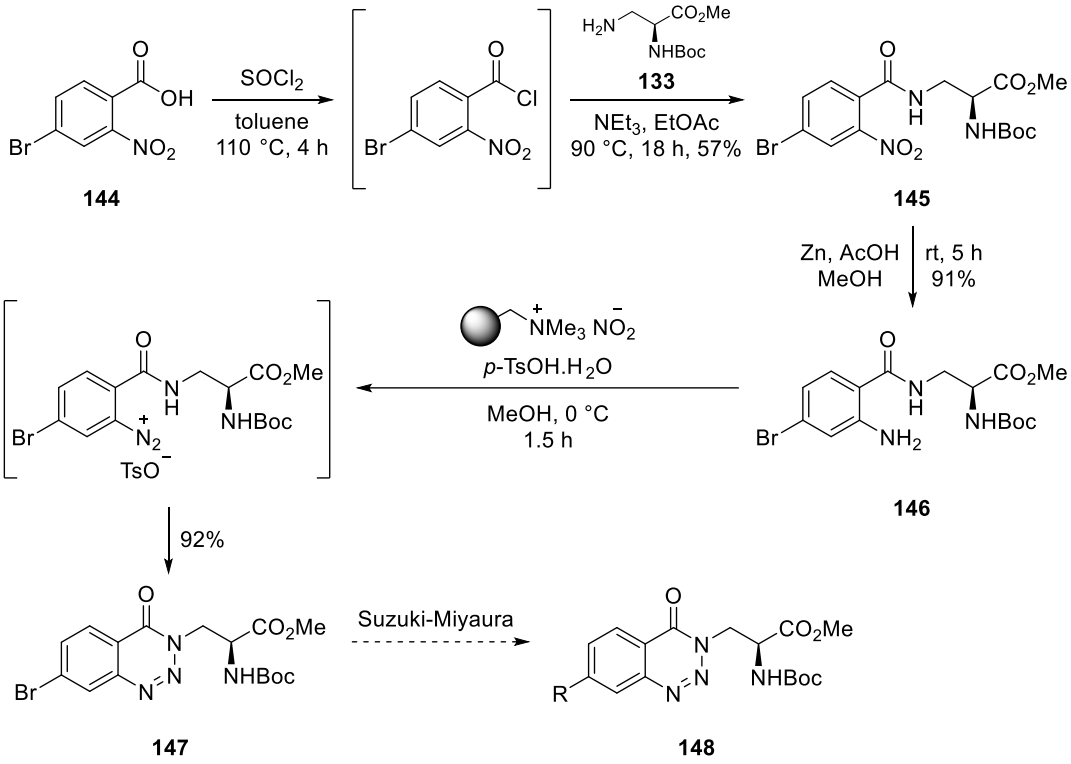


Scheme 54: Attempted bromination and iodination of 7'-methoxy substituted benzotriazinone **141a**.

2.2.5 Functionalisation of Benzotriazinone α-Amino Acids *via* a Suzuki-Miyaura Cross-Coupling Reaction

As late-stage halogenation of the benzotriazinone scaffold proved unsuccessful, an alternative route for extending the conjugation of these systems was investigated. Due to the electron-withdrawing nature of the carbonyl group present in the benzotriazinone scaffold, it was proposed that installation of a *para* carbon-bromine bond would be of great interest as it would allow for subsequent cross-coupling reactions, allowing for extended conjugation through the system. It was found that utilising 4-bromo-2-nitrobenzoic acid as the starting material allowed for easy incorporation of the halogen functionality into the benzotriazinone scaffold (Scheme

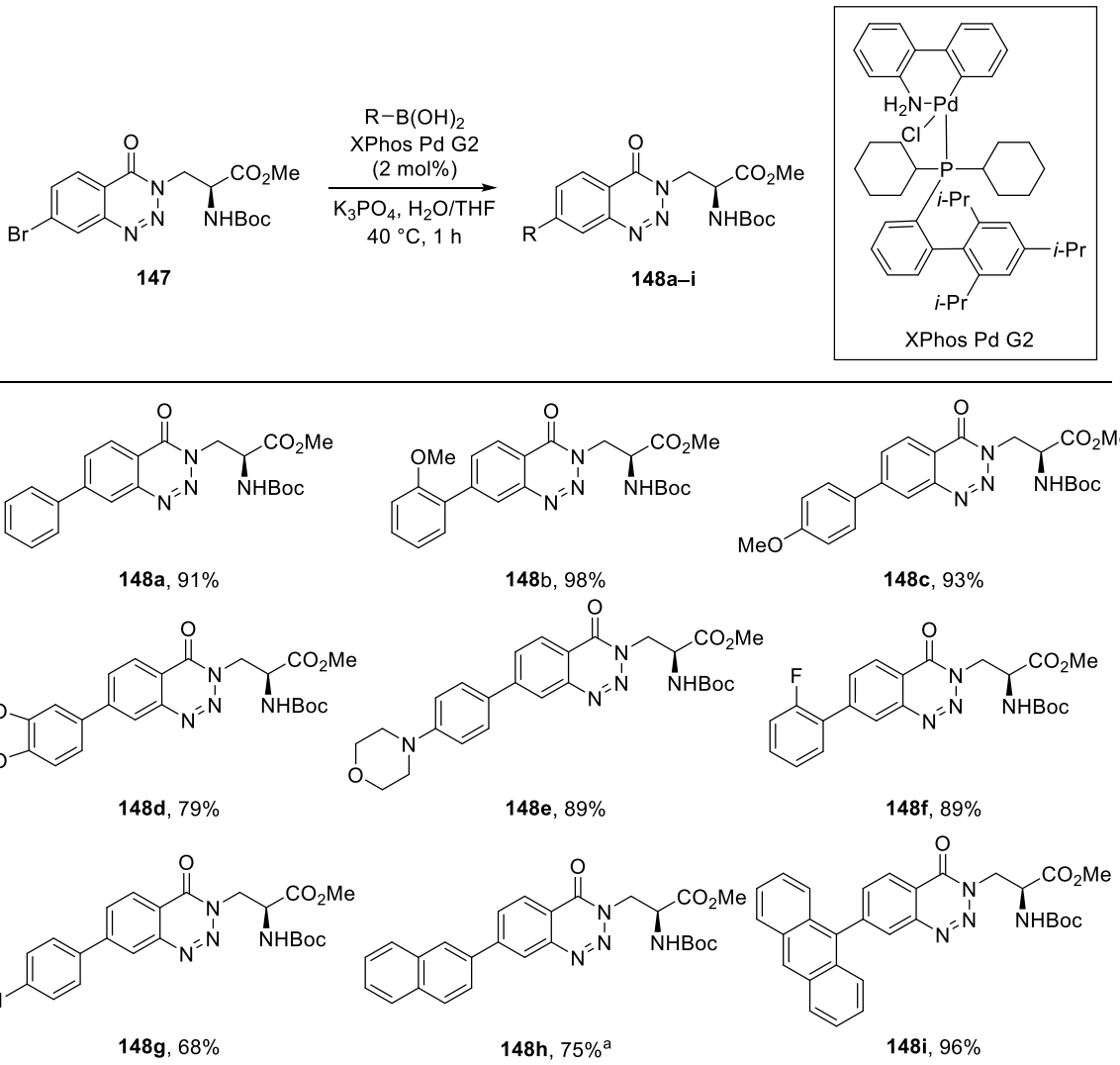
55). Formation of the acid chloride intermediate was achieved using thionyl chloride. This was then successfully coupled to methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (**133**) in a 57% yield. Nitro group reduction of **145** was achieved using zinc and acetic acid to generate 2-aminobenzamide **146** in a 91% yield. The one-pot diazotisation and intramolecular cyclisation was then performed and gave benzotriazinone **147** in an excellent 92% yield. It should be noted that despite the acidic conditions of these steps, the Boc-group was not affected under the relatively mild conditions.



Scheme 55: Synthesis of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**147**).

A Suzuki-Miyaura cross-coupling reaction of **147** was then attempted. Typical Suzuki-Miyaura cross-coupling conditions require the use of high temperatures above 80 °C.[115] However, the use of elevated temperatures in the presence of base can lead to racemisation of the stereogenic centre of amino acids. The reaction was thus attempted using XPhos Pd G2 as the catalyst as it is known to activate at low temperatures (Scheme 56). This is a result of the weak palladium-nitrogen bond present in the pre-catalyst which is easily broken *via* reductive elimination to generate the active palladium(0) species.[116] It was found that utilising a low catalyst loading (2 mol%), full conversion of the benzotriazinone starting material was

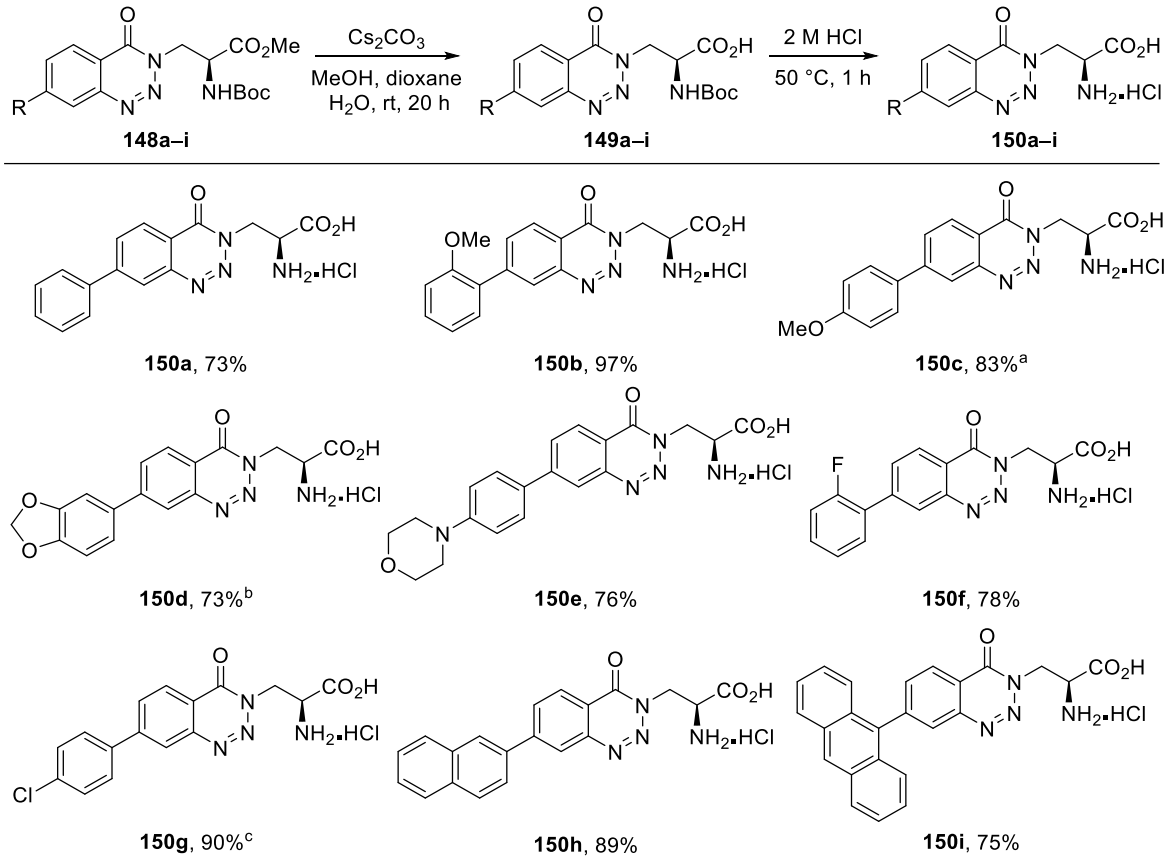
observed by ^{1}H NMR spectroscopy after only 1 h at 40 °C for all analogues (excluding naphthyl analogue **148h**). The reaction gave the desired products in excellent yields for boronic acids bearing both electron-withdrawing and electron-donating substituents. The reaction also occurred chemoselectively for 4-chlorophenylboronic acid, generating **148g** in a 68% yield. More complex derivatives were also successful. 4-Morpholinophenylboronic acid was coupled in an excellent 89% yield. The reaction was also successful for a dioxazole derivative in a good 70% yield. Naphthyl and anthracene analogues were generated cleanly in high yields (**148h** and **148i**).



Scheme 56: Suzuki-Miyaura cross-coupling reactions of 7'-bromo substituted benzotriazinone **147**. [a]Reaction carried out at 40 °C for 2 h.

Deprotection was then carried out as described for the unsubstituted analogue (Scheme 57). Ester hydrolysis using caesium carbonate at room temperature overnight gave the corresponding carboxylic acids. Removal of the Boc-protecting

group was then achieved using 2 M hydrochloric acid. This generated the desired deprotected compounds in high yields under mild conditions.



Scheme 57: Deprotection of functionalised α-amino acids (yields over two steps). [a]Reaction 2 carried out at 50 °C for 4 h; [b]Reaction 2 carried out at 50 °C for 18 h; [c]Reaction 2 carried out at 50 °C for 2 h.

2.2.6 Fluorescent Properties of Functionalised Benzotriazinone α-Amino Acids

Following successful synthesis of a novel library of functionalised benzotriazinone amino acids, the absorption and emission data for these compounds (**150a**–**i**) was recorded (Figures 21–29).

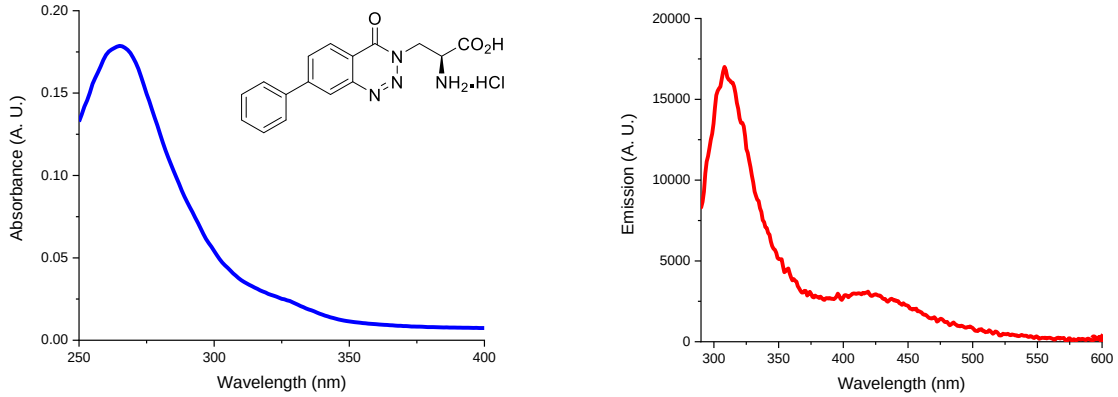


Figure 21: Absorption and emission spectra of **150a** (15 µM in MeOH, excitation at 266 nm).

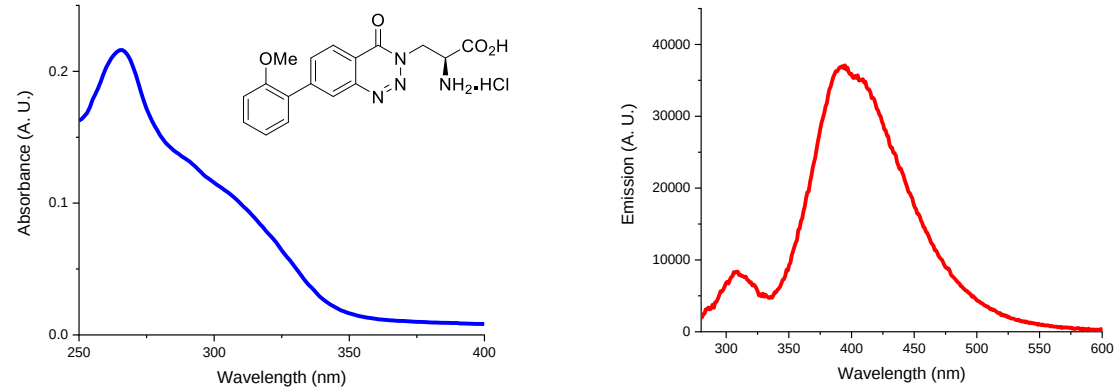


Figure 22: Absorption and emission spectra of **150b** (15 µM in MeOH, excitation at 266 nm).

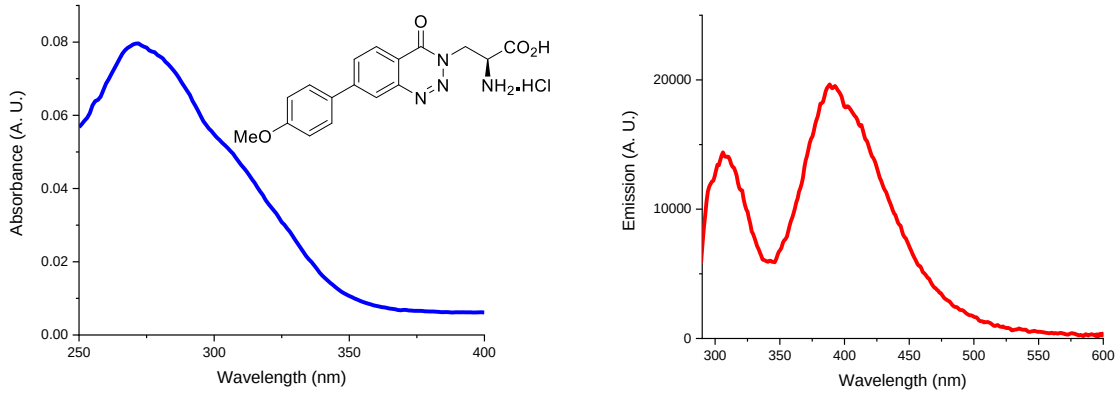


Figure 23: Absorption and emission spectra of **150c** (15 µM in MeOH, excitation at 269 nm).

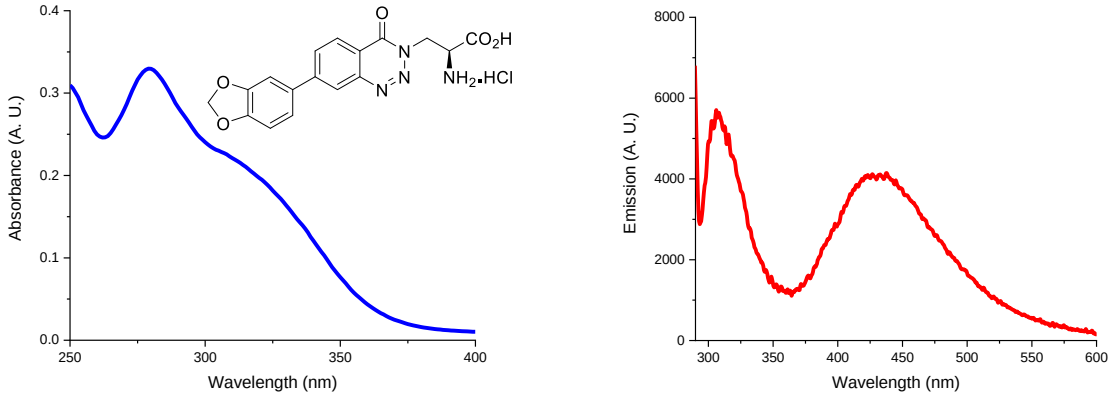


Figure 24: Absorption and emission spectra of **150d** (15 µM in MeOH, excitation at 279 nm).

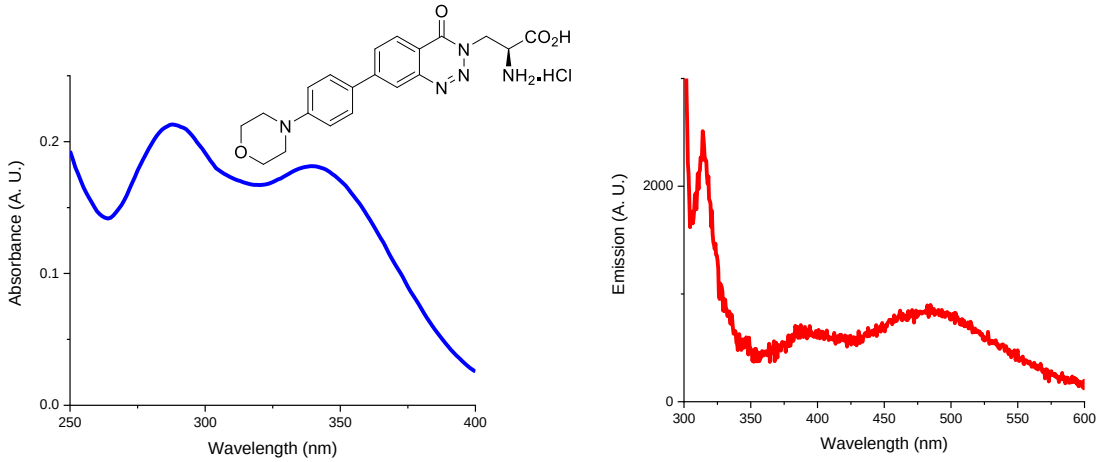


Figure 25: Absorption and emission spectra of **150e** (15 µM in MeOH, excitation at 290 nm).

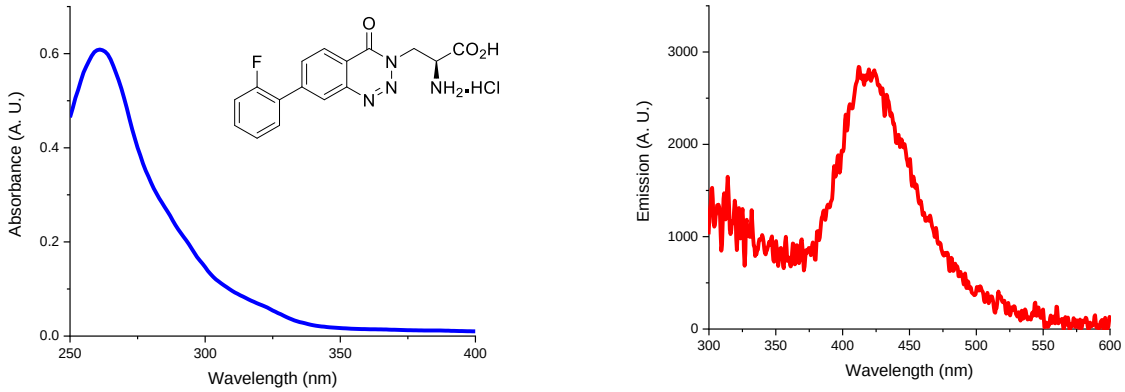


Figure 26: Absorption and emission spectra of **150f** (15 µM in MeOH, excitation at 262 nm).

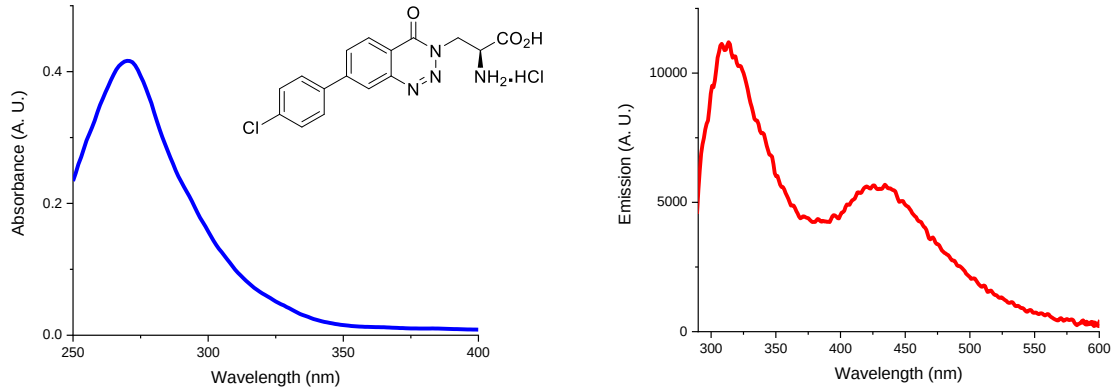


Figure 27: Absorption and emission spectra of **150g** (15 µM in MeOH, excitation at 270 nm).

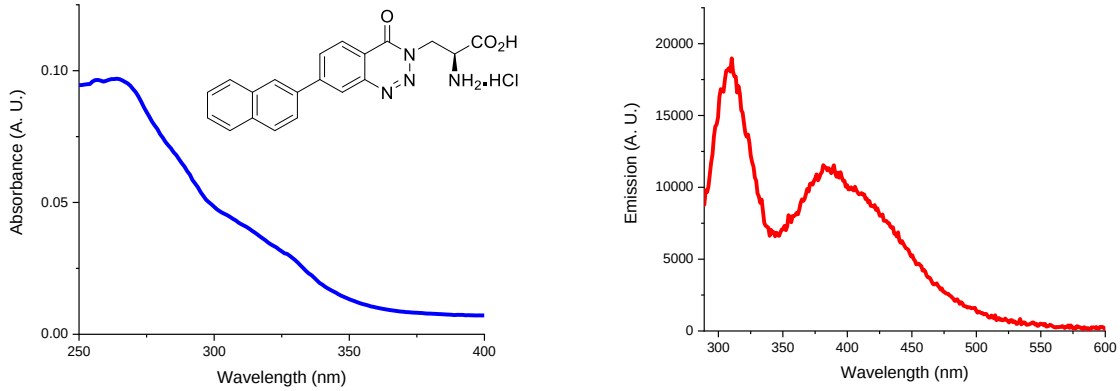


Figure 28: Absorption and emission spectra of **150h** (15 µM in MeOH, excitation at 267 nm).

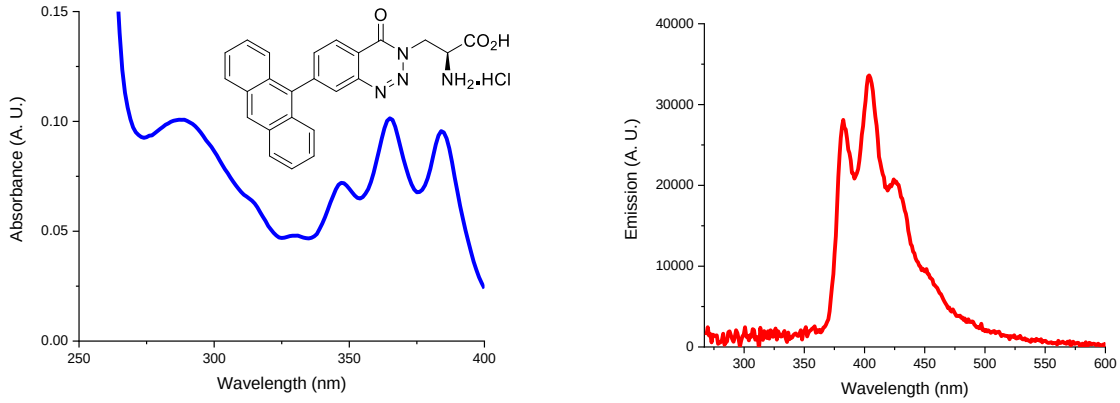


Figure 29: Absorption and emission spectra of **150i** (15 µM in MeOH, excitation at 252 nm).

Upon analysis of the absorption and emission data, the functionalised analogues were found to be dual emissive. This surprising observation, which violates Kasha's rule, is proposed to be the result of emission from both the locally excited (LE) and twisted-intramolecular charge transfer (TICT) states (Figure 30).[117,118] The ratio of emission from both states is largely dependent on the steric bulk of the aryl ring. For example, phenyl analogue **150a** was found to have a large emission band at 308 nm. This is proposed to be the result of emission from the LE state. A significantly weaker emission band was observed at 426 nm. In comparison, 2''-methoxyphenyl analogue **150b** shows weak emission at 312 nm and a significantly stronger band at 395 nm. It is postulated this is the result of emission from the TICT state which arises due to the steric interaction of the *ortho*-methoxy group which causes **150b** to adopt a twisted configuration. It is well documented that pre-twisting electron-donating groups in a fluorophore facilitates TICT as it lowers the barrier to the excited TICT state.[119,120]

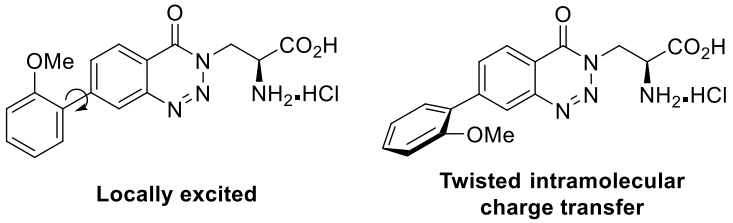


Figure 30: Proposed locally excited and TICT states for amino acid **150b**.

Full photoluminescent data was then obtained for the functionalised benzotriazinone amino acids and is summarised in Table 5. Due to formation of the TICT excited state, 2''-methoxyphenyl analogue **150b** was found to have a large quantum yield of 0.47 and thus a high brightness of 8540 cm^{-1} M^{-1}. This is an improved overall brightness than several amino acids currently used for imaging purposes (see L-Anap, Section 1.2.4). Facile rotation between the two aryl rings accounts for the lower quantum yield observed for *para*-methoxy analogue **150c** which was found to have a stronger LE band than **150b**. 2''-Fluorophenyl and 4''-chlorophenyl analogues **150f** and **150g** do not contain a push-pull system. Consequently, population of the charge transfer state is reduced, with more intense LE bands. Various analogues were found to have absorption >300 nm including the morpholine, dioxazole and anthracene derivatives. Morpholine analogue **150e** showed red-shifted emission of 486 nm. 2''-Fluorophenyl analogue **150f** showed a MegaStokes shift of 149 nm. This is desirable for imaging as it avoids measurement

issues due to reabsorption of emitted light.[43] Due to its rigid structure, anthracene derivative **150i** displays 3 distinct bands in the emission spectrum. Molar attenuation coefficients of 10,000–20,000 cm^{-1} M^{-1} were observed for most analogues.

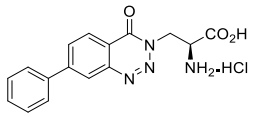
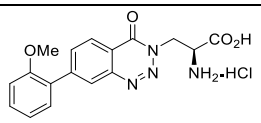
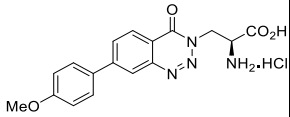
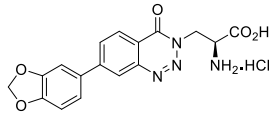
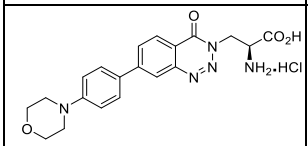
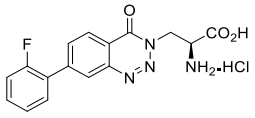
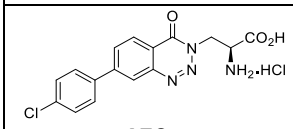
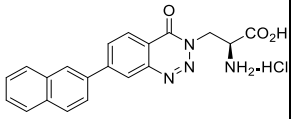
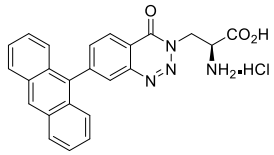
Compound	λ_{abs} (nm)	λ_{em} LE (nm)	λ_{em} CT (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Bright-ness (cm^{-1} M^{-1})
 **150a**	266	**308**	426	42	23900	0.005	110
 **150b**	266[a] 309	312	**395**	86	18200	0.47	8540
 **150c**	269	310	**388**	41	10600	0.13	1400
 **150d**	279[a] 317	**312**	435	33	15500	0.008	130
 **150e**	290[a] 340	**315**	486	25	20400	0.005	110
 **150f**	262	N/A	**411**	149	12400	0.002	20
 **150g**	270	**308**	435	38	21400	0.003	60
 **150h**	267	**310**	391	43	13100	0.02	300
 **150i**	252	N/A	382	152	9900	0.005	50

Table 5: Photoluminescence properties of Suzuki-coupled benzotriazinone α-amino acids. [a]Excitation wavelength. Major emission peak indicated in bold.

A solvatochromic study was carried out for amino acid **150b** by measuring absorption and emission maxima in various solvents (Figure 31). As expected, the λ_{abs} was found to be independent of solvent polarity. For the λ_{em}, it was found that when moving to more polar solvents, emission occurred at a longer wavelength. In tetrahydrofuran, the λ_{max} was found to be 310 nm. This shifted significantly to 432 nm in PBS buffer, a difference of 122 nm across the different solvents.

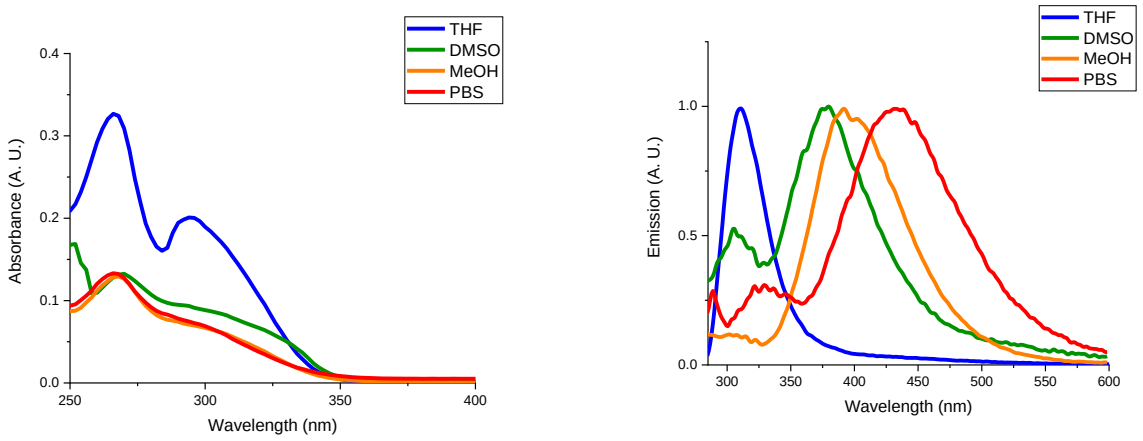


Figure 31: Absorption and emission spectra of **150b** (5 µM in MeOH, excitation at 267 nm). PBS = phosphate buffered saline.

The dependency of a fluorophore's Stokes shift on the polarity of the surrounding solvent can be observed by a Lippert-Mataga plot.[121,122] The Lippert-Mataga plot for amino acid **150b** is shown in Figure 32. The linear correlation between Stokes shift and solvent polarizability indicates **150b** undergoes an enlargement of dipole in the excited state due to electron migration, generating a charge-transfer state. Reorientation of solvent dipoles then occurs to stabilise the charge-transfer state.[43,123] As polarity increases, solvent dipoles become larger. Consequently, more polar solvents are better at stabilising the excited state, resulting in emission of a lower energy, longer wavelength photon. A bathochromic shift in the emission maxima is thus observed. Based on its environmental sensitivity, this amino acid could have potential applications as a solvatochromic probe.

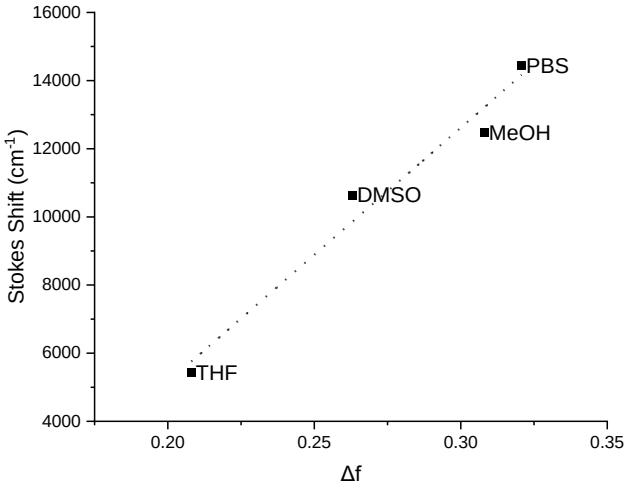


Figure 32: Lippert-Mataga Plot for amino acid **150b**.

The pH dependant properties of amino acid **150b** were also investigated (Figure 33). This was carried out by obtaining the absorption and emission spectra of **150b** in neutral methanol. Concentrated hydrochloric acid in methanol was then added to the stock solution to obtain solutions at pH 4 and pH 1. Compared to absorption and emission at pH 7, no change was observed at pH 4. However, it was found that adjusting the pH to 1 resulted in a significant change in the emission spectrum of **150b**. Although **150b** was still emissive at 395 nm, emission was significantly decreased. Interestingly, the emission at 312 nm remained unchanged.

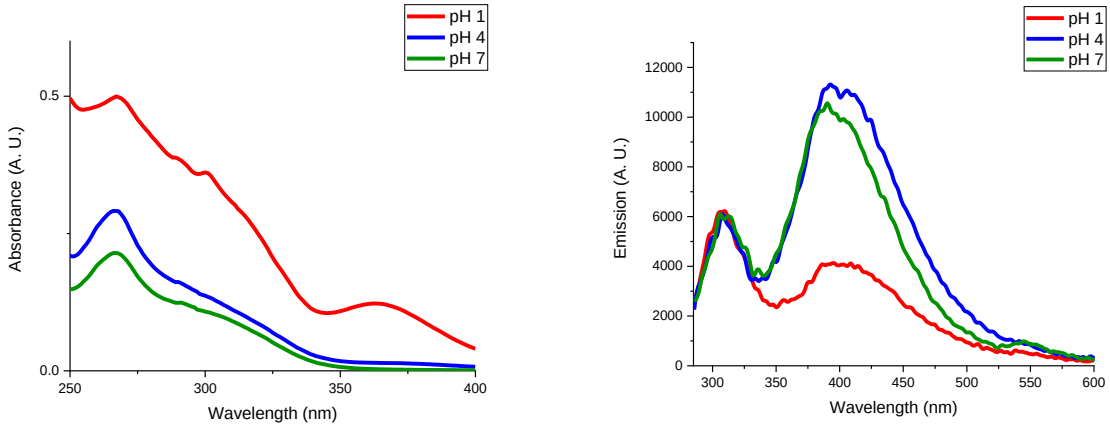


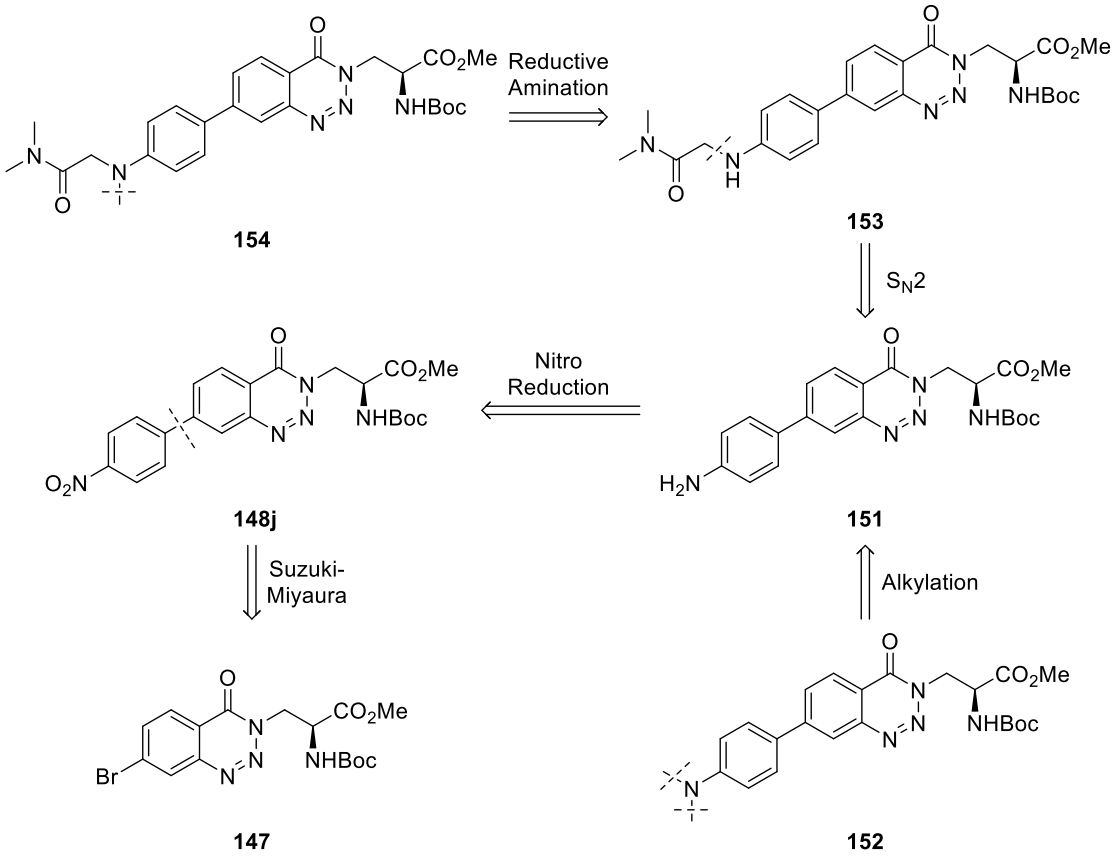
Figure 33: Absorption and emission spectra of **150b** (5 µM in MeOH, excitation at 267 nm).

As discussed in Section 1.1.2, protonation of the benzotriazinone scaffold can result in ring opening to give the corresponding diazonium intermediate. It is therefore postulated that protonation of amino acid **150b** at pH 1 quenches fluorescence

emission from the TICT state by reversible ring opening, allowing free rotation between the two ring systems. Consequently, dual emission is switched off and emission is observed mainly from the locally excited state. The interesting dual emissive properties displayed by amino acid **150b** suggest it could have applications as a pH probe, with changing acidity levels resulting in diminished fluorescence.

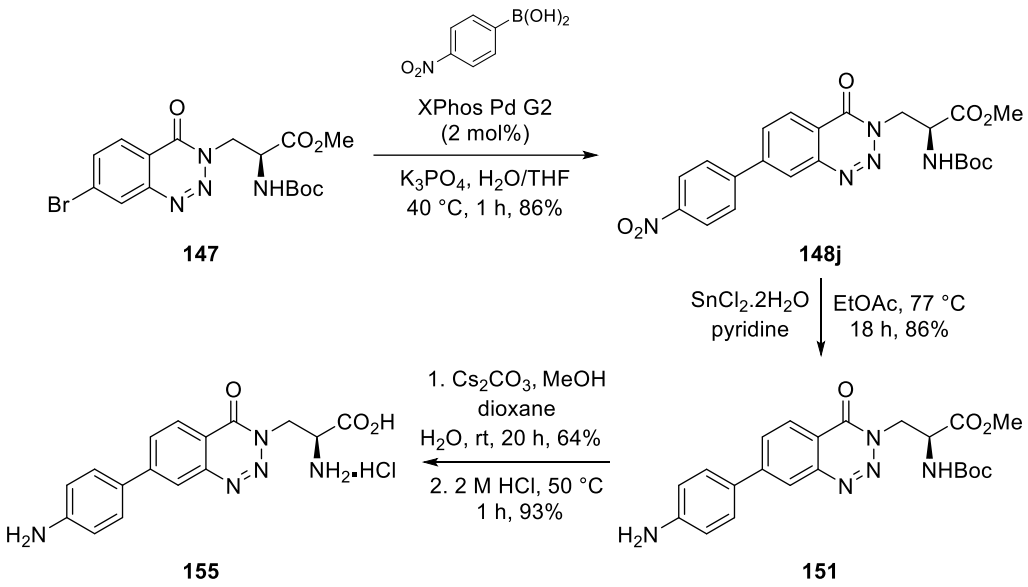
2.2.7 Attempted Improvement of the Fluorescent Properties of Benzotriazinone α-Amino Acids *via* Further Functionalisation

As discussed in Section 2.2.6, morpholine analogue **150e** shows excellent red-shifted absorption and emission, important properties for biological imaging. However, **150e** suffers from a low quantum yield. It is proposed the λ_{abs} and λ_{em} of **150e** arise from the electron-donating character of the 4''-morpholine ring. Consequently, analogues similar to compound **150e** which retain an electron-donating nitrogen in the 4''-position would be synthesised and their photoluminescent properties evaluated. Retrosynthetic analysis of these analogues is shown in Scheme 58. Firstly, to investigate if the low quantum yield of **150e** was a result of TICT quenching caused by the large steric bulk of the morpholine ring, unsubstituted analogue **151** would be generated. Synthesis of **151** would be achieved *via* a Suzuki-Miyaura cross-coupling reaction of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**147**) with 4-nitrobenzeneboronic acid as described in Section 2.2.5. Subsequent nitro group reduction would then generate amine **151**. Two analogues with differing amine functionality would then be generated. Firstly, alkylation of amine **151** would be carried out to form dimethyl analogue **152**. In addition, synthesis of β-carbonyl analogue **154** would be achieved *via* an S_N2 reaction of **151**, followed by reductive amination. A recent review by Wang and co-workers discusses the key parameters which contribute to fluorescence quenching *via* a TICT mechanism.[120] In this review, they highlight work by Zhang and co-workers which shows a significant improvement in fluorescence quantum yield as a result of installing a β-carbonyl group on the amine of interest.[124] The improved fluorescent properties are proposed to be a result of solvent extrusion by the β-carbonyl moiety.



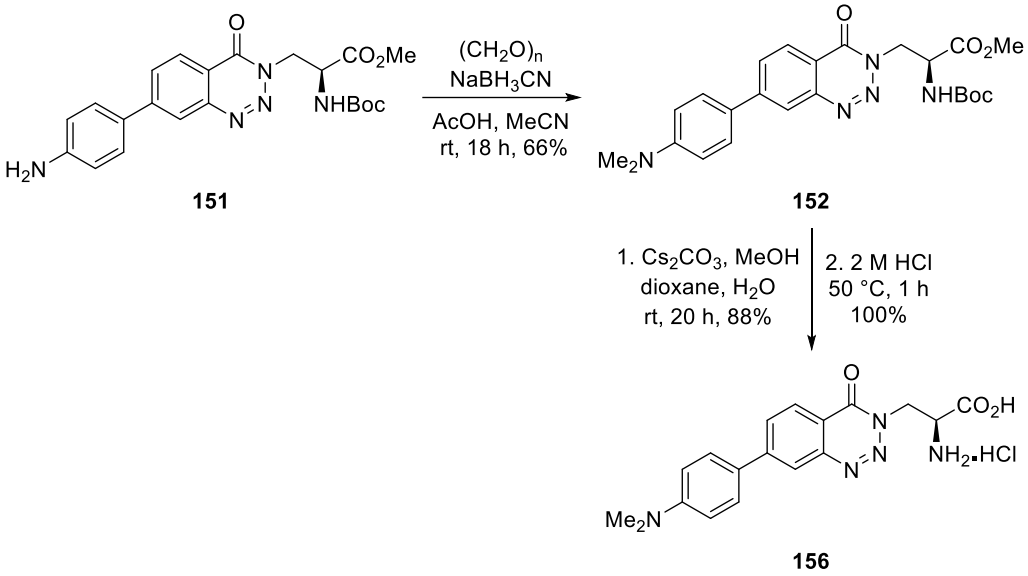
Scheme 58: Retrosynthetic analysis of 4''-amino-derived benzotriazinone α-amino acids.

The Suzuki-Miyaura cross-coupling reaction of benzotriazinone **147** with 4-nitrobenzeneboronic acid, using XPhos Pd G2 (2 mol%) as the catalyst, occurred in an excellent 86% yield (Scheme 59). Nitro group reduction was then achieved using tin(II) dichloride dihydrate in the presence of pyridine.[95] This procedure has been previously reported to be compatible with acid sensitive Boc-groups.[125] Formation of desired product **151** occurred in a 86% yield, indicated by an upfield shift of the aromatic hydrogen atoms. Ester deprotection was carried out under standard conditions in a 64% yield. This was followed by removal of the Boc-protecting group using 2 M hydrochloric acid stirring at 50 °C for 3 h to give deprotected amino acid **155** in an excellent 93% yield.



Scheme 59: Synthesis of 4''-amino analogue **155**.

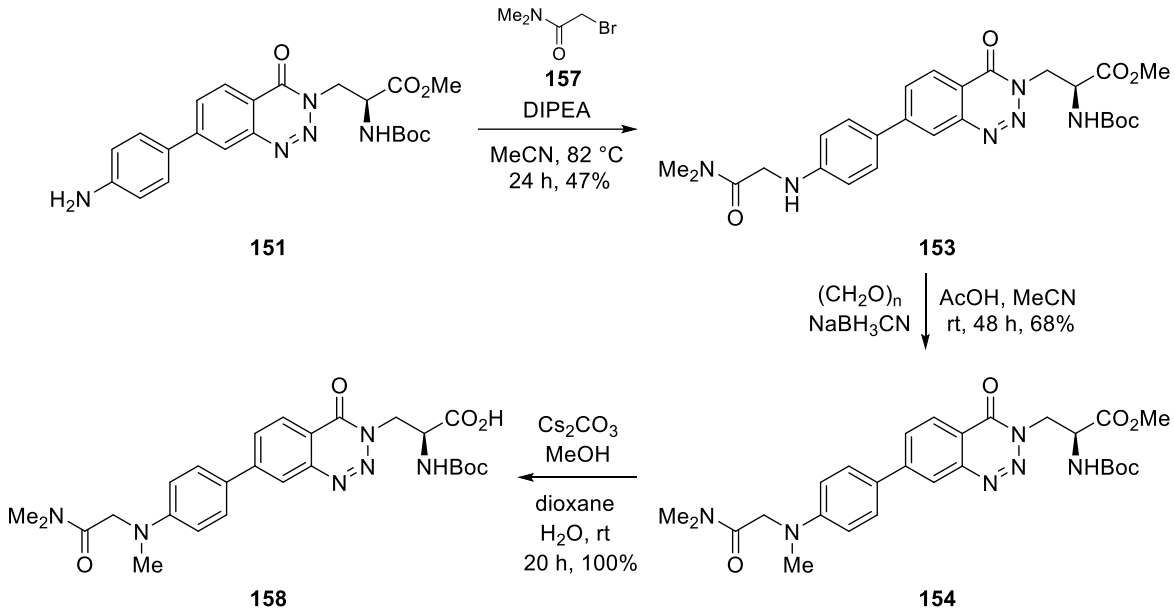
Synthesis of analogues **152** and **154** was then investigated. To synthesise dimethyl analogue **152**, amine **151** was subjected to reductive amination using paraformaldehyde and sodium cyanoborohydride (Scheme 60). This gave diamino analogue **152** in a 66% yield.[126] Subsequent ester hydrolysis using caesium carbonate and removal of the Boc-protecting group using 2 M hydrochloric acid gave amino acid **156** in excellent yield over the two steps.



Scheme 60: Synthesis of 4''-dimethylamino analogue **156**.

The synthesis of β-carbonyl analogue **154** was achieved *via* an S_N2 reaction of amine **151** with commercially available 2-bromo-*N,N*-dimethylethanamide (**157**) (Scheme 61). This gave secondary amine **153** in a 47% yield. This was then

subjected to a reductive amination to generate tertiary amine **154** in a 68% yield.[126] Finally, ester hydrolysis of **154** gave carboxylic acid **158** in a quantitative yield. Removal of the Boc-protecting group was not attempted due to time constrains and the limited quantity of starting material **151**.



Scheme 61: Synthesis of β-carbonyl analogue **158**.

Photoluminescent data for analogues **155**, **156** and **158** is presented in Figures 34–36. As shown, amine **155** showed a maximum at approximately 285 nm. Emission was detected at 435 nm, however, the intensity of the emission was too low to obtain quantum yield data. It is postulated that for **155**, fluorescence quenching occurs as a result of hydrogen bonding between the solvent and the free amine.[127] *N*,*N*-Dimethyl analogue **156** was also found to be poorly emissive. It is postulated this is due to fluorescence quenching by TICT between the dimethylamino moiety and the phenyl ring. This is highlighted by findings from Wang *et al*., in which they synthesise a *N*,*N*-dimethylamino analogue with a low quantum yield of 0.01.[120] This drastically improves to 0.19 upon formation of the corresponding azetidine derivative. Addition of the β-carbonyl group on the amine was found to extend the absorbance wavelength, with a λ_{abs} of 345 nm. Unfortunately, despite showing red-shifted emission at 460 nm, emission was weak suggesting fluorescence quenching occurs *via* an alternative mechanism.

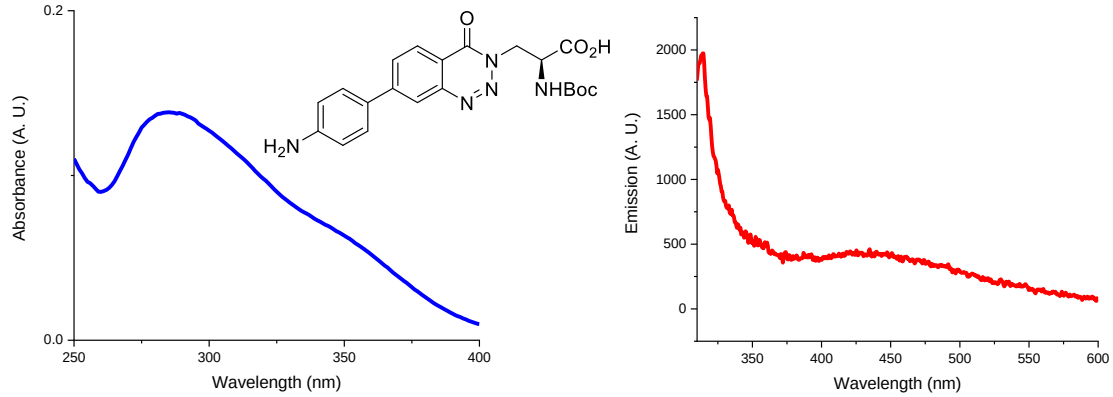


Figure 34: Absorption and emission spectra of **155** (10 µM in MeOH, excitation at 289 nm).

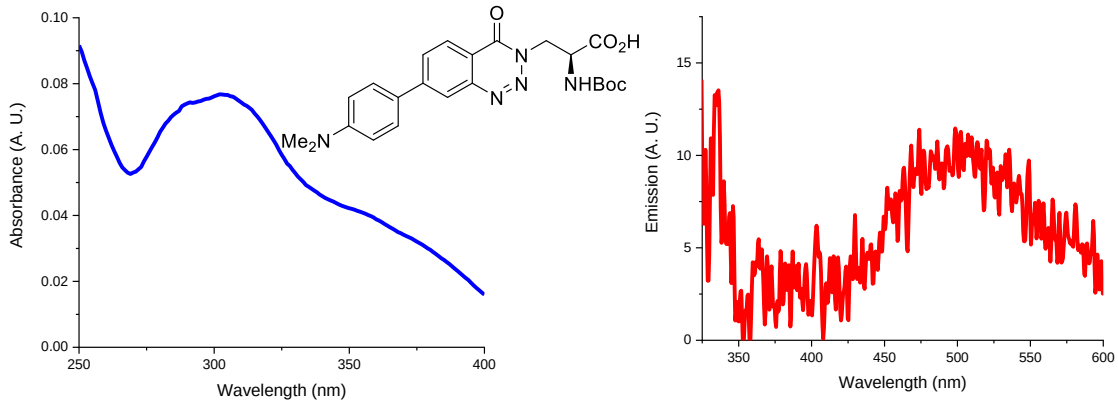


Figure 35: Absorption and emission spectra of **156** (10 µM in MeOH, excitation at 306 nm).

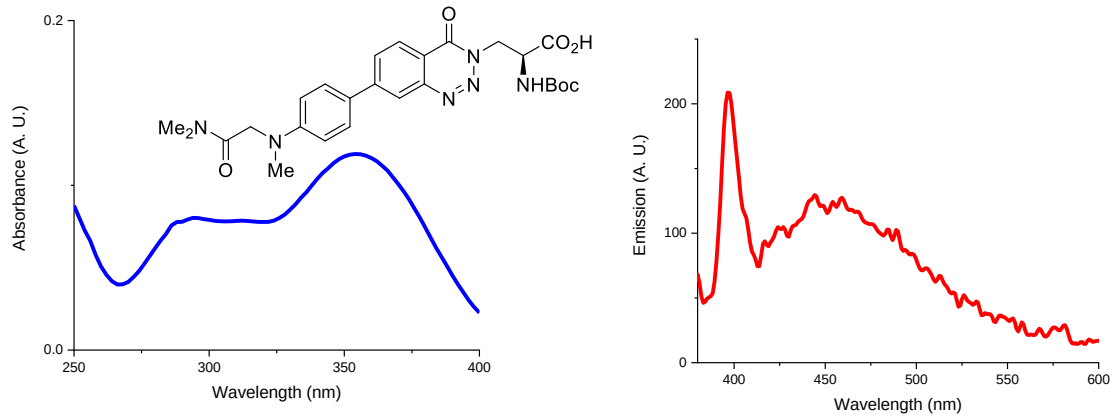
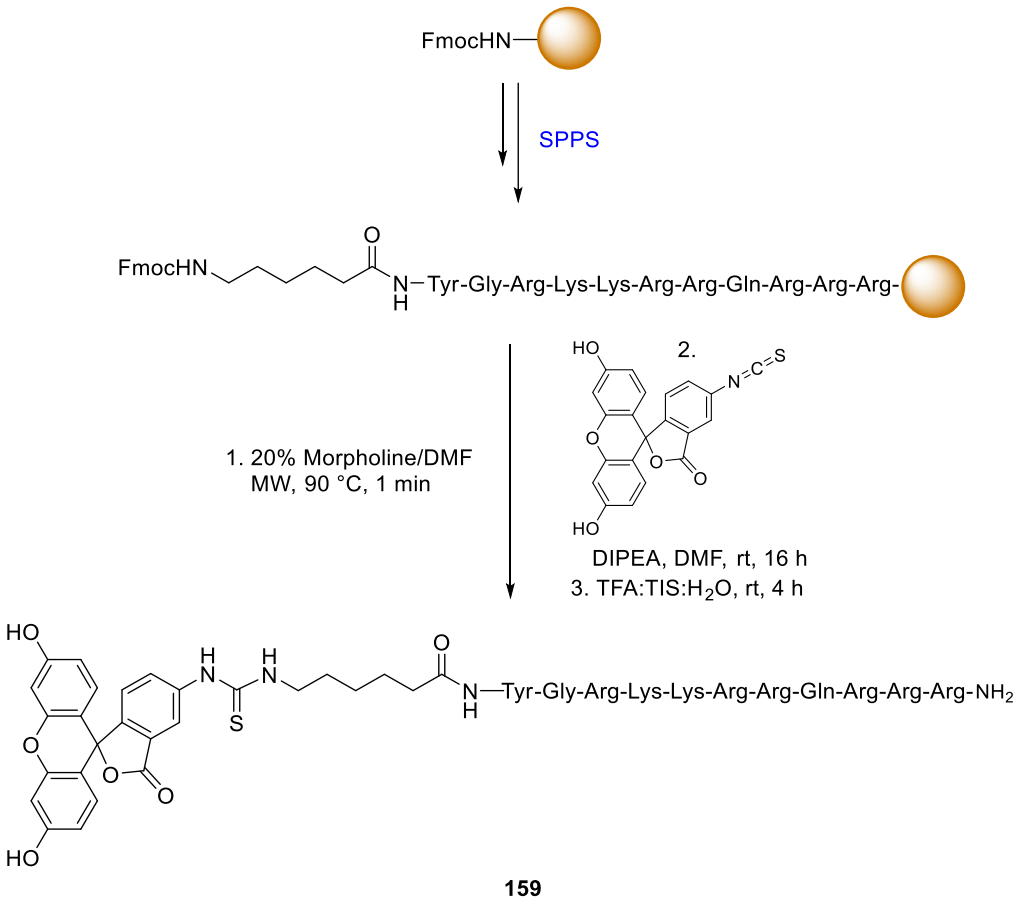


Figure 36: Absorption and emission spectra of **158** (5 µM in MeOH, excitation at 357 nm).

2.2.8 Incorporation of Functionalised Benzotriazinone α-Amino Acid into a Cell-Penetrating Peptide

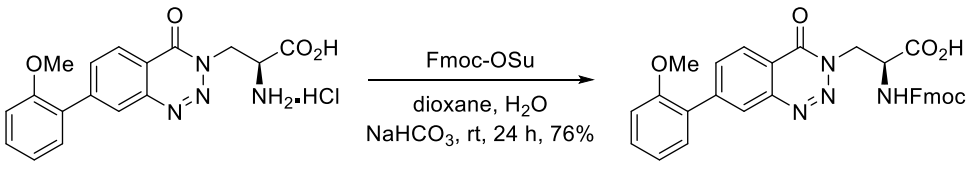
As synthesis of the 4''-amino analogues discussed in Section 2.2.7 led to no improvement of photoluminescent properties, 2''-methoxy analogue **150b** was chosen as a lead substrate due to its high quantum yield and large Stokes shift. To highlight the utility of this analogue for biological imaging, novel α-amino acid **150b** was incorporated into a cell-penetrating peptide (CPP) using solid-phase peptide synthesis (SPPS). TAT was chosen as the CPP sequence as it is frequently used in covalent binding to fluorophores with the aim of intracellular delivery *via* translocation across the plasma membrane.[128] Initially, a control peptide containing a fluorescein 5-isothiocyanate (FITC) fluorophore was synthesised (Scheme 62). The FITC fluorophore is commonly used in cellular microscopy as it can be easily incorporated at terminal amines *via* the isothiocyanate moiety.[129] It also has a large quantum yield of 0.71 and importantly, is fluorescent at physiological pH.[130] FITC has a λ_{abs} of 495 nm and a λ_{em} of 525 nm.[131] The cell-penetrating TAT-sequence was synthesised using a Rink Amide ChemMatrix® resin utilising an Fmoc/*tert*-butyl protection strategy. The resin was swollen using DMF before loading Fmoc-Arg(Pbf)-OH. Subsequent amino acids, including a 6-(Fmoc-amino)caproic acid (A_{hx}) linker, were introduced by Fmoc-deprotection of the terminal amine using 20% morpholine followed by treatment with *N*,*N'*-diisopropylcarbodiimide (DIC) and Oxyma Pure. The resin was washed with DMF between deprotection and coupling. The A_{hx} amino acid linker was required to prevent removal of the terminal amino acid *via* cyclisation of the FITC fluorophore.[129] Deprotection of the A_{hx} Fmoc-group followed by manual coupling allowed for installation of the FITC fluorophore label. Following a test cleave from the resin using trifluoroacetic acid, triisopropylsilane and water, full cleavage was carried out to achieve global deprotection of the side-chain protecting groups as well as remove the desired peptide from the resin. Synthesis of peptide **159** in a 6% overall yield was verified by HRMS. Preparative HPLC was used to isolate peptide **159** to >95% purity.



159

Scheme 62: Synthesis of control peptide **159**.

Following successful synthesis of the control peptide, synthesis of the TAT-sequence incorporating novel α-amino acid **150b** at the *N*-terminus was then investigated. Firstly, Fmoc-protection of amino acid **150b** was carried out using Fmoc-succinimide and sodium hydrogencarbonate. This generated **160** in a 76% yield under standard conditions (Scheme 63).[132]

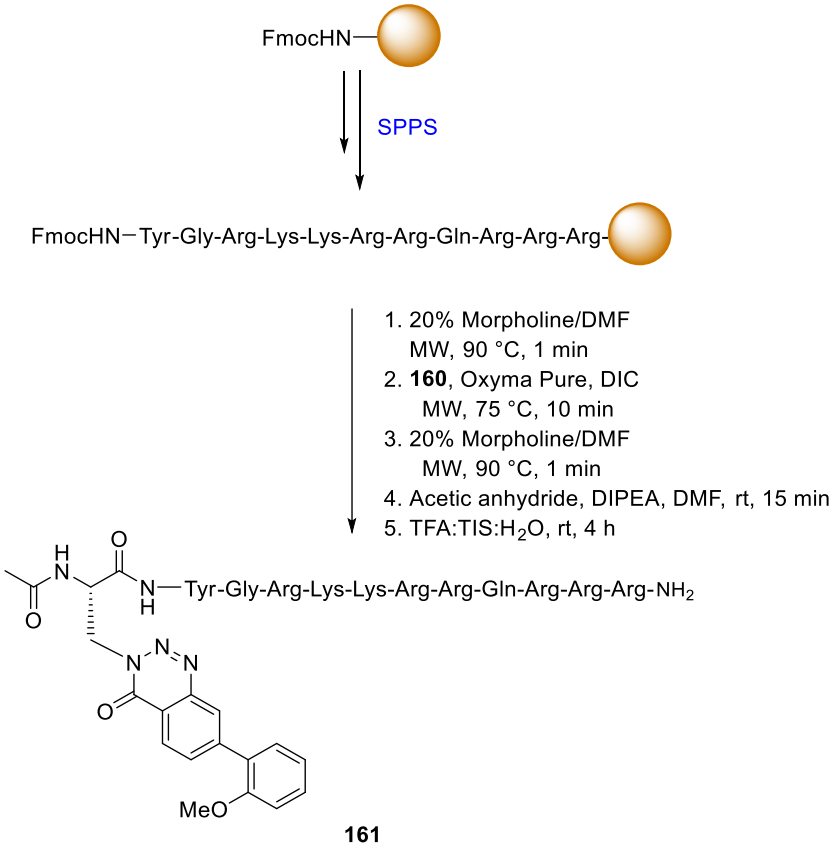


150b **160**

Scheme 63: Fmoc-protection of amino acid **150b**.

In accordance with control peptide **159**, the TAT-sequence was then synthesised (Scheme 64). It should be highlighted that addition of the A_{hx} linker was not required due to the absence of the FITC fluorophore. Manual coupling utilising DIC and Oxyma allowed for incorporation of novel amino acid **160**. Fmoc-deprotection of the

terminal amino acid was followed by *N*-acetylation using acetic anhydride. Finally, global deprotection and cleavage from the resin was achieved as per control peptide **159**. Synthesis of peptide **161** in a 3% overall yield was verified by HRMS. Preparative HPLC was used to isolate peptide **161** to >95% purity.



161

Scheme 64: Synthesis of TAT-peptide **161** with incorporation of novel fluorescent α-amino acid **160**.

Photoluminescent data of peptide **161** showed that the absorption and emission properties of amino acid **150b** were maintained upon incorporation into the TAT peptide (Figure 37).

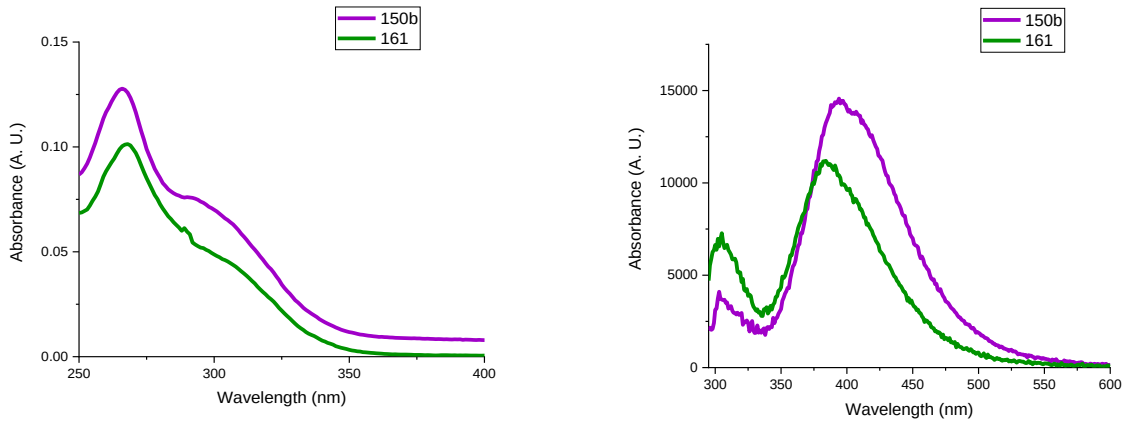


Figure 37: Absorption and emission spectra of amino acid **150b** and peptide **161** (7 µM in MeOH, excitation at 306 nm).

Surprisingly, despite similarities in the relative intensity of emission for amino acid **150b** and peptide **161**, peptide **161** was found to have a significantly lower quantum yield (Table 6). Cellular microscopy experiments were therefore undertaken to further evaluate this fluorescence data.

Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
150b	309	389	80	18200	0.47	8540
161	309	384	75	14000	0.02	280

Table 6: Photoluminescent data of novel amino acid **150b** and peptide **161**.

2.2.9 Cell Imaging Using Cell-Penetrating Peptide Containing Benzotriazinone α-Amino Acid

Following treatment with poly-D-lysine, HEK293 cells were seeded into 6-well plates on 30 mm cover glass slides and left to grow for two days. The cells were then incubated with fluorescently tagged peptides **159** and **161** in Dulbecco's Modified Eagle's Medium (DMEM). Following incubation, cells were fixed with aqueous formaldehyde.

Fluorescence microscopy was then used to confirm if the peptides successfully entered cells and fluoresced following excitation at 495 nm and 310 nm for peptides **159** and **161**, respectively. Images were acquired on a MetaMorph/Metafluor fluorescence imaging microscope system equipped with a 40 × Superfluor objective.

Image analysis and processing was performed using Fiji ImageJ. Images were obtained of the brightfield as well as with the corresponding fluorescence channel. Overlaid images indicated if the peptides had entered cells and fluoresced.

Photoluminescent data for peptide **159** is presented in Figure 38. The overlay of the brightfield and FITC channel suggests that the peptide was cell-penetrating due to the intracellular fluorescence generated when excited at 495 nm.

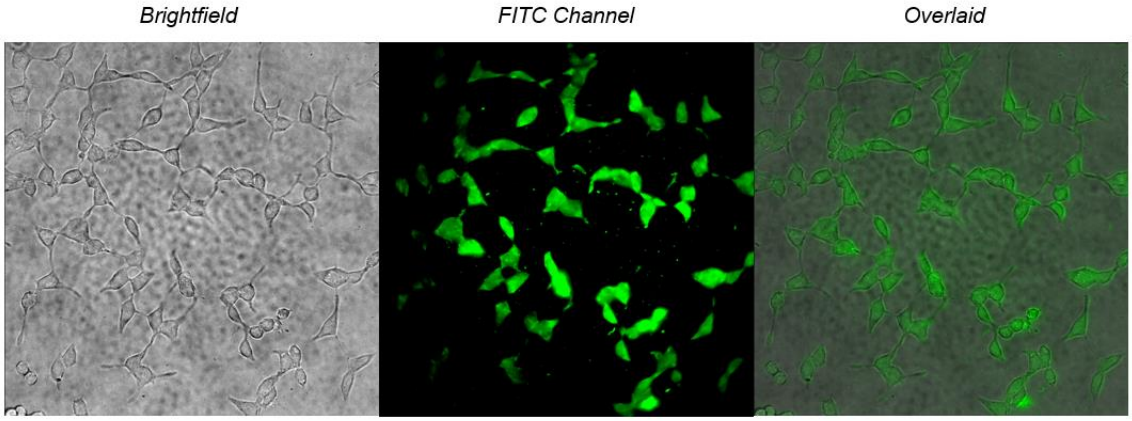


Figure 38: Positive control HEK293 cells incubated with peptide **159** (10 μM peptide).

Photoluminescent data for the negative control, DMSO, is presented in Figure 39. As expected, the overlay of the brightfield and FITC channel shows no fluorescence in the absence of the FITC peptide.

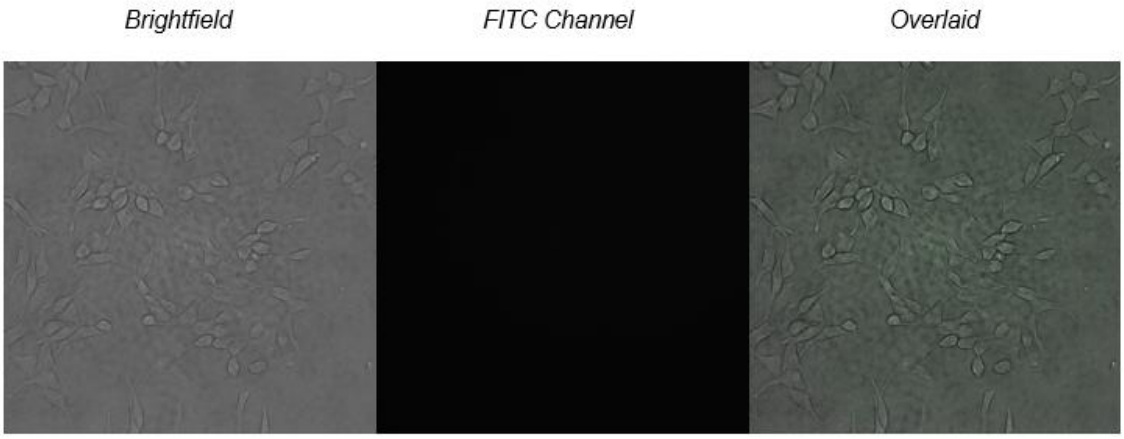


Figure 39: Negative control HEK293 cells incubated with DMSO (10 μM DMSO).

Photoluminescent data for peptide **161** is presented in Figure 40. The overlay of the brightfield and 310 channel generally does not show fluorescence occurring within

the cells, despite the higher concentration of peptide. Although some cells were visualised in the 310 channel, this is thought to be the result of a large accumulation of the peptide inside dead cells. It is postulated that attachment of novel amino acid **160** to the TAT peptide results in fluorescence quenching and thus peptide **161** could not be visualised when excited at 310 nm.

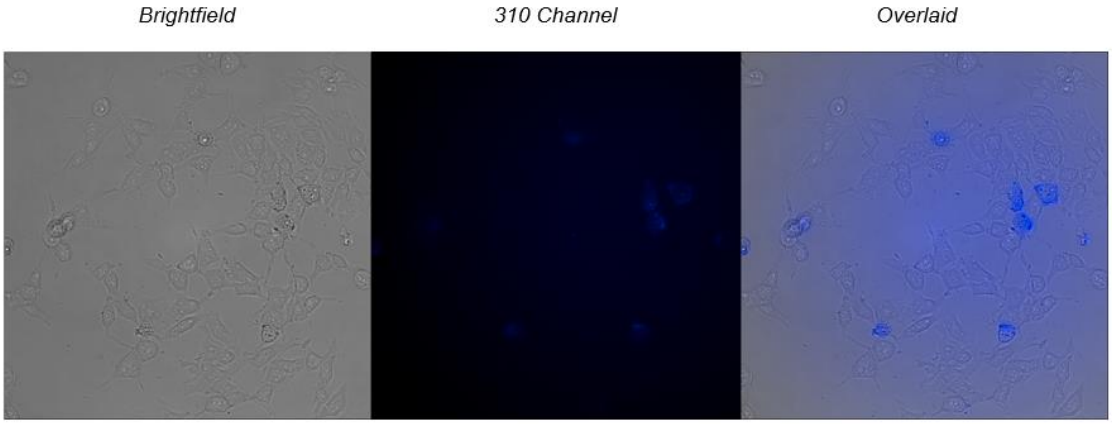
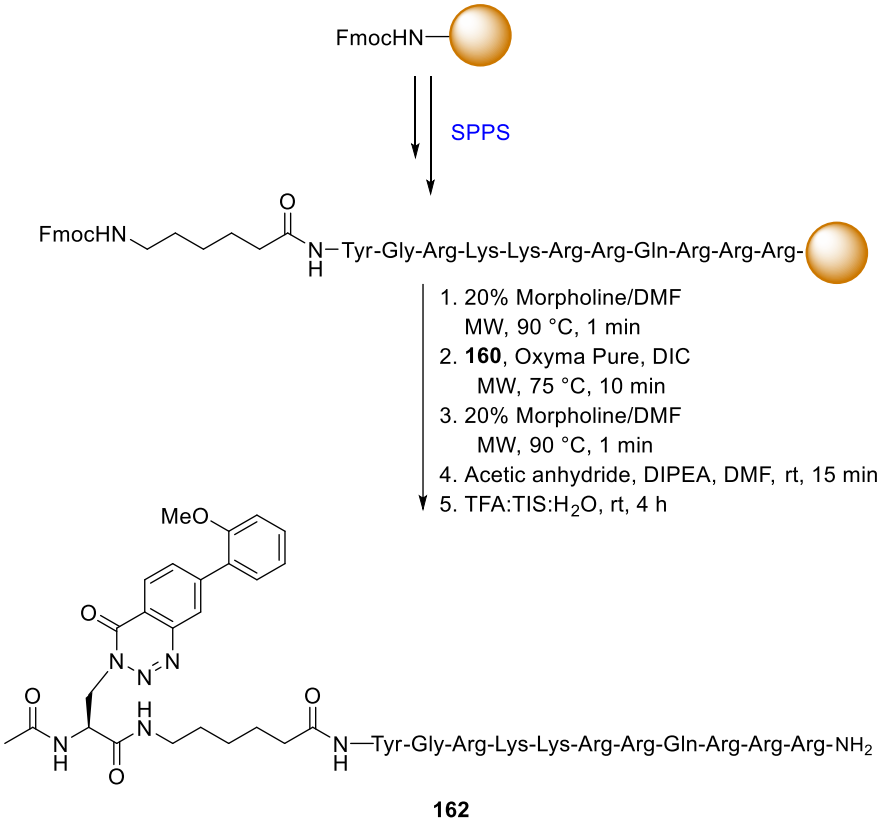


Figure 40: HEK293 cells incubated with peptide **161** (200 μM peptide).

Fluorescence quenching may be the result of distance-dependant energy transfer between novel amino acid **160** to the nearby tyrosine residue due to overlap of the emission of **160** with the absorbance of tyrosine. It was therefore proposed the addition of the A_{hx} linker would increase the distance between novel amino acid **160** and tyrosine, hence preventing energy transfer. The TAT-sequence was thus re-synthesised to include the A_{hx} linker prior to manual coupling of novel fluorescent amino acid **160** (Scheme 65). Successful synthesis of peptide **162** in 20% yield was confirmed by HRMS. Preparative HPLC was used to isolate peptide **162** to >99% purity.



162

Scheme 65: Synthesis of TAT-peptide **162** with incorporation of novel fluorescent α-amino acid **160** and A_{hx} linker.

Unfortunately, fluorescence data obtained for peptide **162** showed no improvement in fluorescence quantum yield following incorporation of the A_{hx} linker (Table 7). It is proposed that peptide folding may occur due to the flexibility of the hexyl linker which allows for close proximity of amino acid **160** and tyrosine and hence fluorescence quenching.

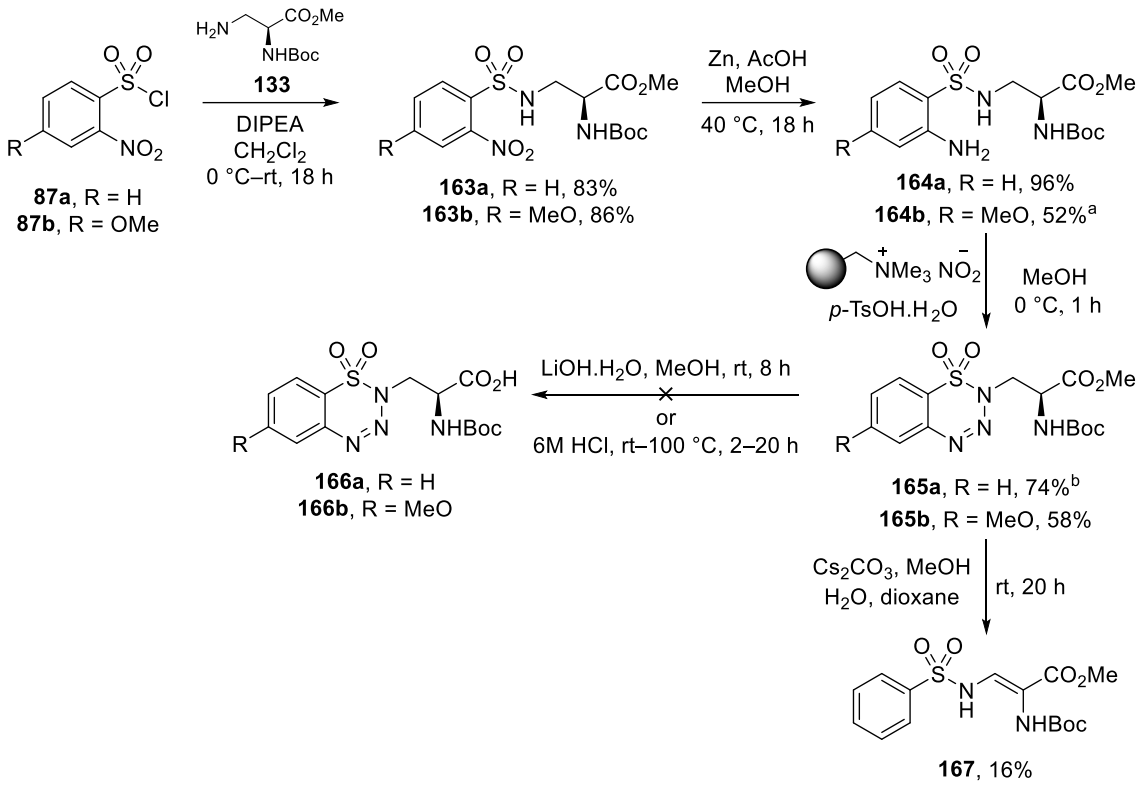
Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
162	309	372	63	17700	0.02	440

Table 7: Photoluminescent data of peptide **162** (data obtained in methanol).

2.2.10 Synthesis and Fluorescent Properties of Benzothiatriazinedioxide α-Amino Acids

In order to compare the fluorescent properties, benzothiatriazinedioxide α-amino acids **165a** and **165b** were synthesised by MSci placement student, Robyn Curran

(Scheme 66). Firstly, 2-nitrobenzenesulfonyl chlorides were submitted to a coupling reaction with amine **133** (synthesis described in Section 2.2.3).[99] This gave 2-nitrobenzenesulfonamides **163a** and **163b** in 83% and 86% yields, respectively. Nitro group reduction was then achieved using zinc and acetic acid. The lower yield of 4'-methoxy analogue **164b** was proposed to be a result of the reaction stalling at the partially reduced hydroxyl intermediate. The one-pot diazotisation and cyclisation procedure was then carried out under mild conditions to give the desired cyclised products. Deprotection of the carboxylic acid was initially attempted using caesium carbonate. However, this resulted in a complex mixture being observed in the aromatic region of the ^{1}H NMR spectrum. Following purification by column chromatography, analysis of the ^{1}H NMR spectrum showed a doublet with a coupling constant of 10.2 Hz at 7.13 ppm as well as a monosubstituted aromatic ring. This was later identified as alkene **167**. Formation of **167** is proposed to occur under basic conditions which leads to elimination of the acidic α-hydrogen adjacent to the carbonyl. As discussed in Section 2.1.7, benzothiatriazinedioxides are prone to dediazotisation *via* thermal loss of dinitrogen. It is postulated this does not occur for the corresponding benzotriazinone derivatives due to the decreased stability of the nitrogen radical generated by this process. The reaction was thus attempted using lithium hydroxide. However, decomposition was observed in the ^{1}H NMR spectrum. Deprotection of both the amine and carboxylic acid was attempted using 6 M hydrochloric acid. However, this also resulted in decomposition.



Scheme 66: Synthesis of unsubstituted benzothiatriazinedioxide-containing α-amino acid **165a** and 6'-methoxy derivative **165b**. [a]Reaction stirred at 30 °C for 18 h; [b]Reaction stirred at 0 °C for 1 h followed by room temperature for 1 h.

Due to the issues encountered when attempting to deprotect analogues **165a** and **165b**, the fluorescent properties of the protected analogues were investigated. The absorption and emission data for **165a** are shown in Figure 41. Unsubstituted analogue **165a** has a λ_{abs} at 305 nm and a λ_{em} at 366 nm, with a Stokes shift of 61 nm.

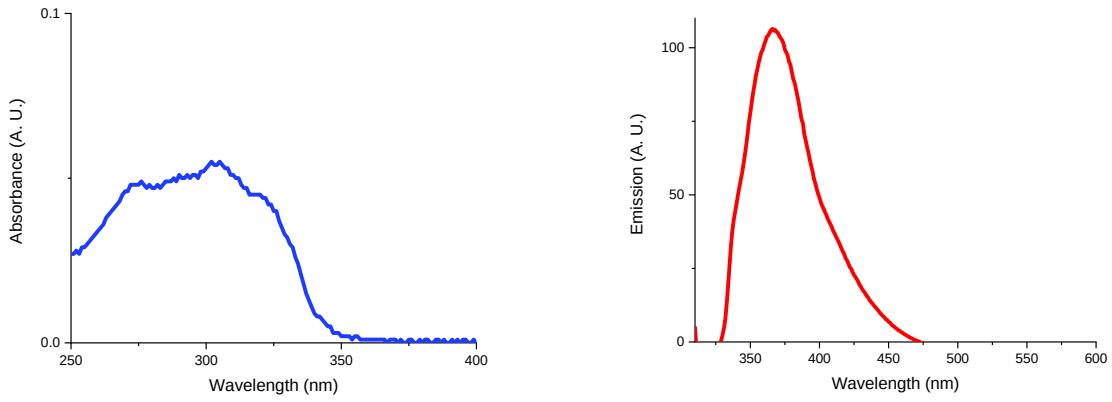


Figure 41: Absorption and emission spectra of **165a** (10 µM in MeOH, excitation at 305 nm).

The absorption and emission data for **165b** are shown in Figure 42. Methoxy-substituted analogue **165b** has a λ_{abs} at 293 nm and a λ_{em} at 441 nm. It is postulated that the additional electron-donating character of the methoxy substituent generates a push-pull system with the electron deficient benzothiatriazinedioxide ring, resulting in emission of a lower energy, longer wavelength photon. Consequently, **165b** displayed an excellent MegaStokes shift of 148 nm. The increased electron-withdrawing ability of the sulfonamide functionality is also highlighted by the red-shifted emission of analogue **165b** compared to the corresponding benzotriazinone derivative **143a** (emission λ_{em} 271 nm).

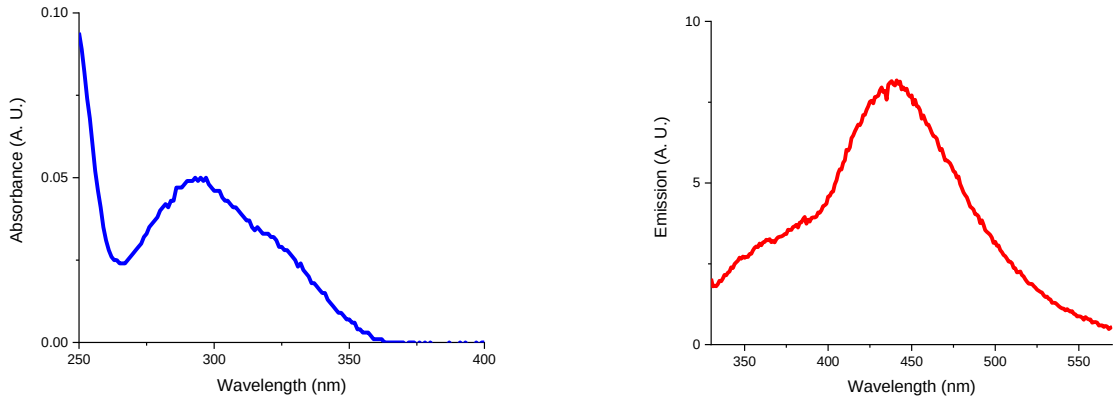


Figure 42: Absorption and emission spectra of **165b** (10 µM in MeOH, excitation at 293 nm).

2.2.11 Conclusions

To conclude, unsubstituted benzotriazinone-containing α-amino acid **123** was synthesised in eight steps from *N*-Boc-L-asparagine (**129**). The fluorescent properties of the benzotriazinone amino acid series was improved by extending the conjugation of the system *via* a palladium catalysed cross-coupling reaction of 7'-bromo substituted benzotriazinone **147**. This gave the desired cyclised products in high yields under mild conditions using the XPhos Pd G2 pre-catalyst. Photoluminescent data was obtained of the parent amino acids. All analogues displayed dual fluorescence, with emission occurring from both the locally excited and twisted intramolecular charge transfer states. 4''-Morpholine analogue **150e** showed red-shifted absorption and emission desirable for cellular imaging. However, it suffered from a low quantum yield. 4''-Amino analogues similar to **150e** were thus synthesised to investigate if the quenching mechanism could be overcome by a change in molecular structure. However, **155**, **156** and **158** also

showed weak emission. Based on these data, 2''-methoxy analogue **150b** was chosen as a lead compound due to its high quantum yield of 0.47 and large Stokes shift of 86 nm. To evaluate its potential applications for cellular imaging, amino acid **150b** was incorporated into a TAT cell-penetrating peptide. However, when excited at 310 nm, intracellular fluorescence was not observed for peptide **161**. It was postulated this may be the result of fluorescence quenching *via* energy transfer to nearby amino acid residues. The peptide was thus re-synthesised to include a A_{hx} linker as it was proposed the increased distance between the amino acid residues and the novel amino acid would prevent fluorescence quenching. However, it is proposed the flexibility of the hexyl linker allows fluorescence quenching to proceed. Novel amino acids containing the benzothiatriazinedioxide scaffold were also synthesised. However, these were found to be less stable than the corresponding carbon analogues and hence deprotection could not be achieved. Fluorescence data obtained for the protected analogues shows methoxy-substituted analogue **165b** has a λ_{abs} at 293 nm and a λ_{em} at 441 nm, generating an excellent MegaStokes shift of 148 nm.

2.2.12 Future Work

Despite the issues encountered with use of amino acid **150b** as a cell imaging agent, **150b** was found to possess other interesting properties and hence may find application as a biological probe. As shown in Figure 31, **150b** was found to exhibit excellent solvatochromic behaviour. In tetrahydrofuran, the λ_{max} was found to be 310 nm. This shifted significantly to 432 nm in PBS buffer. Consequently, **150b** could be used as an environmental probe, with changes in the λ_{max} correlating to real-time binding events or conformational changes of the protein of interest.[133]

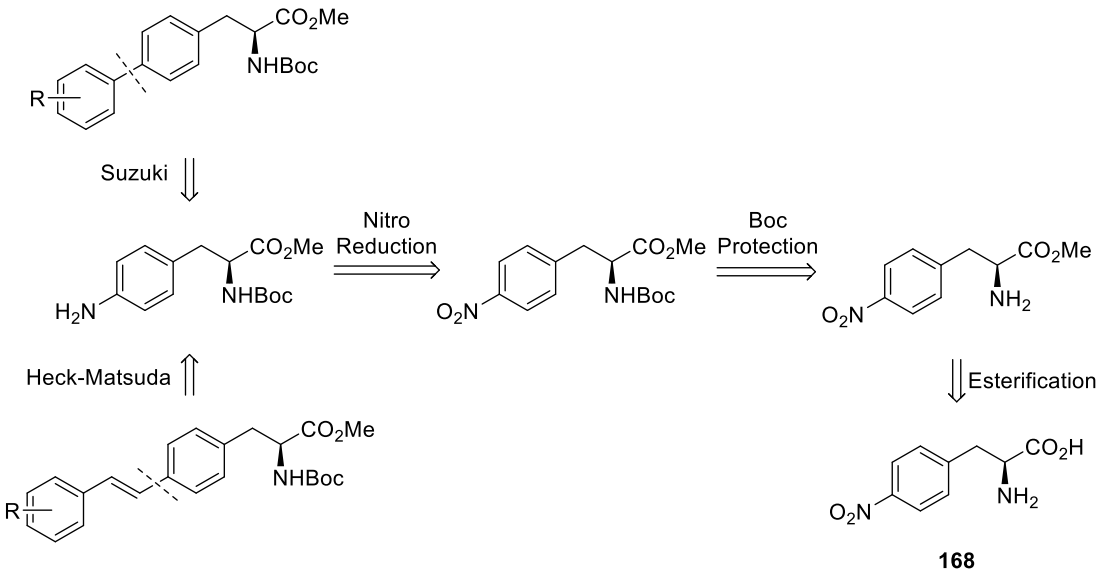
Furthermore, as highlighted in Figure 33, the dual emissive properties of **150b** were also found to show pH dependence. Under strongly acidic conditions, the λ_{max} for **150b** was found to occur at 312 nm, compared to 395 nm under neutral conditions. This environmental sensitivity is of particular interest in identification of cancers cells as tumour tissue generally exhibits a lower pH than healthy tissue.[134]

2.3 Synthesis of Biphenyl and Stilbene α-Amino Acids

2.3.1 Proposed Research

In designing fluorescent amino acids for cellular imaging, it is well documented that increasing the conjugation of a system can lead to improved fluorescent properties. (see work by Wang and co-workers, Section 1.2.4).[51,44,135] Diazonium salts present an excellent method for further diversification of biomolecules *via* late-stage cross-coupling reactions. A significant advantage of cross-coupling reactions utilising diazonium salts is that they often negate the requirement of base.[85] This is of particular interest in amino acid synthesis as the presence of base can result in racemisation of the stereogenic centre leading to reduced enantiopurity. With this in mind, it was proposed that synthesis of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-aminophenyl)propanoate would allow for access to both biphenyl and stilbene amino acids *via* diazotisation and subsequent Suzuki-Miyaura and Heck-Matsuda cross-coupling reactions. It was proposed that the extended conjugation of these systems could give rise to interesting photoluminescent properties.

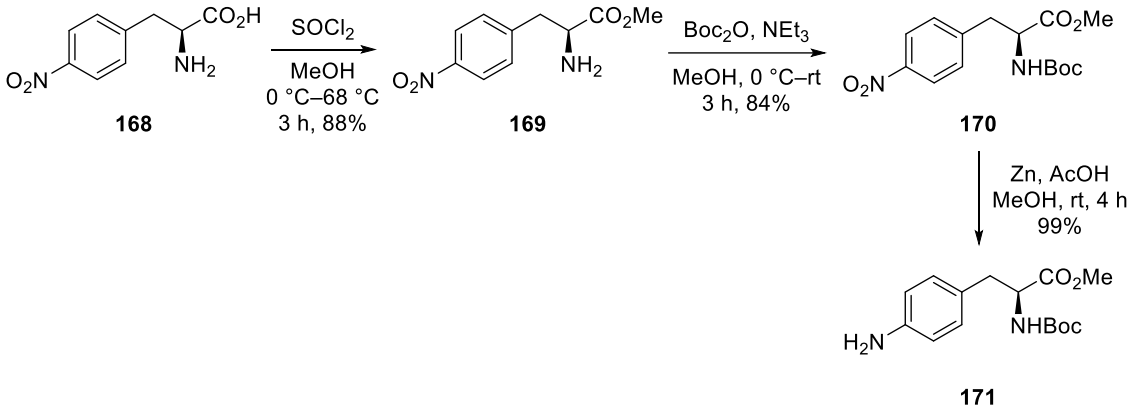
Retrosynthetic analysis of both series of compounds is shown in Scheme 67. It was proposed a protected 4-aminophenylalanine derivative could be accessed *via* a short synthetic route involving esterification and Boc-protection of commercially available 4-nitro-L-phenylalanine (**168**) followed by nitro group reduction. This could then be subjected to the relevant one-pot diazotisation and cross-coupling reactions to generate either biphenyl or stilbene amino acids.



Scheme 67: Retrosynthetic analysis of biphenyl and stilbene amino acids.

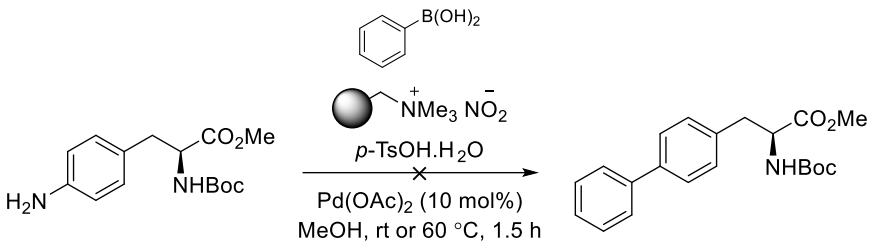
2.3.2 Attempted Synthesis of Biphenyl Amino Acids *via* a Suzuki-Miyaura Reaction

Synthesis of 4-aminophenylalanine derivative **171** began with conversion of commercially available 4-nitro-L-phenylalanine (**168**) to methyl ester **169**. This was done using the standard conditions of thionyl chloride and methanol and gave **169** in an 88% yield (Scheme 68). Subsequent Boc-protection using Boc_2O and triethylamine gave **170** in a 84% yield.[136] Nitro group reduction was then achieved using zinc and acetic acid. Despite the acid sensitive Boc-protecting group, this generated amine **171** under mild conditions and in an excellent 99% yield.



Scheme 68: Synthesis of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-aminophenyl)propanoate (**171**).[136]

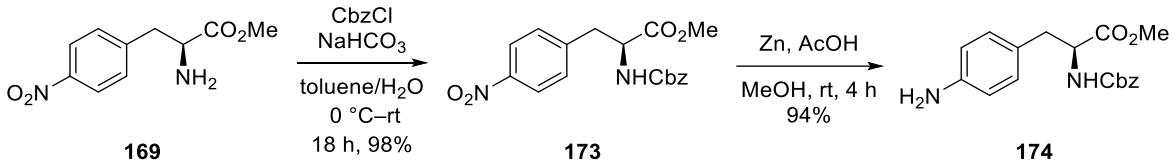
Synthesis of biphenyl amino acid **172** was then attempted *via* a one-pot diazotisation and Suzuki-Miyaura cross-coupling. Work by Kutonova *et al*.[137] has shown phenyl diazonium tosylates can successfully undergo Suzuki-Miyaura cross-coupling reactions under mild conditions. Consequently, a one-pot procedure involving diazotisation of **171** (as per Section 2.1) followed by an *in situ* Suzuki-Miyaura cross-coupling reaction was attempted (Scheme 69). However, utilising $Pd(OAc)_2$ as the catalyst in methanol at room temperature, resulted in no conversion to desired product, as observed by ^{1}H NMR spectroscopy. The reaction was therefore repeated, heating to 60 °C. However, ^{1}H NMR analysis after 1 h indicated removal of the Boc-protecting group had occurred due to the presence of *p*-tosic acid at the elevated reaction temperature.



171 172

Scheme 69: Attempted one-pot diazotization Suzuki-Miyaura cross-coupling reaction of **171**.

It was therefore concluded an alternative amine protecting group was required to allow the cross-coupling reaction to proceed at an elevated temperature. Consequently, Cbz-protected analogue **174** was synthesised in two steps from methyl (2*S*)-2-amino-3-(4'-nitrophenyl)propanoate (**169**) (Scheme 70). This involved protection of the amino group using benzyl chloroformate and sodium hydrogencarbonate, followed by nitro group reduction, again using zinc and acetic acid. This gave *N*-Cbz-protected 4'-aminophenylalanine **174** in high overall yield.

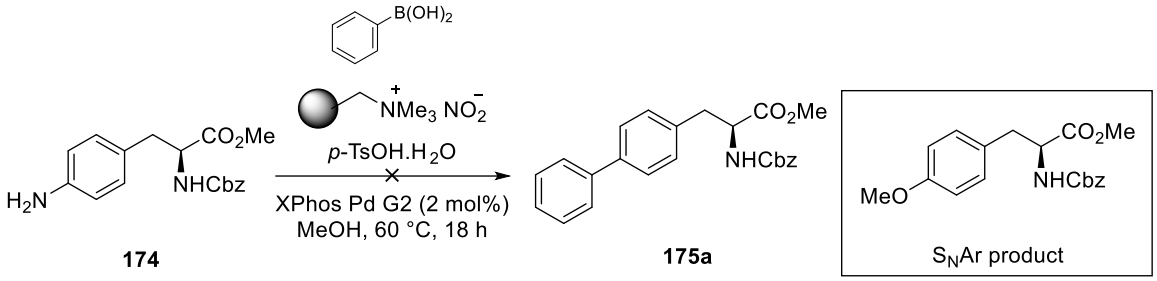


169 173 174

Scheme 70: Synthesis of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-aminophenyl)propanoate (**174**).

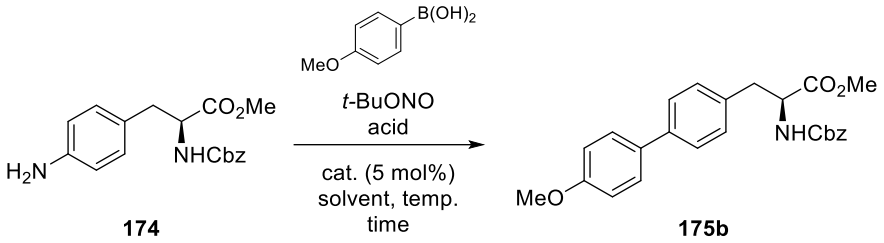
Synthesis of biphenyl α-amino acid **175a** *via* a one-pot diazotisation and Suzuki-Miyaura cross-coupling of **174** was then investigated. The reaction was initially attempted using XPhos Pd G2 as the catalyst due to its success in previous cross-coupling reactions (Section 2.2.5) (Scheme 71). Methanol was chosen as the solvent based on previous literature.[138,139] Following heating to 60 °C overnight, TLC analysis of the reaction mixture indicated conversion of starting material and formation of a new spot. However, following isolation by column chromatography, this was later identified as a side-product formed *via* an S_NAr reaction of the diazonium salt with the reaction solvent. This was indicated by the presence of an additional peak (3.48 ppm) representing three hydrogen atoms in the ^1H NMR spectrum. The reaction was therefore attempted in both acetonitrile and tetrahydrofuran. Formation of the diazonium salt was indicated in both reactions by

TLC analysis. However, no conversion to desired product was observed by ^{1}H NMR spectroscopy.



Scheme 71: Attempted one-pot diazotisation and Suzuki-Miyaura cross-coupling of **174**.

It was postulated that the diazonium tosylate salt generated, may be too stable to participate in the cross-coupling reaction. The reaction was therefore repeated utilising the corresponding diazonium acetate salt. This was generated using *tert*-butyl nitrite and acetic acid. The smaller size of the acetate counterion causes the diazonium salt to be less stable and thus more reactive. Initially, the reaction was attempted using $Pd(PPh_3)_4$ as the catalyst, based on work by Wang and co-workers (Table 8, entry 1).[140] However, this resulted in a 2:1 mixture of desired product and impurity which could not be separated. Based on analysis of the ^{1}H NMR spectrum, this impurity was proposed to be dediazotised material, indicated by the presence of a monosubstituted aromatic ring. In order to minimise dediazotisation, the reaction temperature was lowered from 90 °C to 50 °C (entry 2). This resulted in a 20% isolated yield of desired product **175b**. A further reduction in temperature to 30 °C gave no improvement in yield (entry 3). Various catalysts were then screened, including XPhos Pd G2, $Pd_2(dba)_3$ and $Pd(OAc)_2$ (entries 4–6). The desired product was generated in a 13% yield using $Pd(OAc)_2$. However, no conversion to desired product was observed for XPhos Pd G2 or $Pd_2(dba)_3$ by ^{1}H NMR spectroscopy.

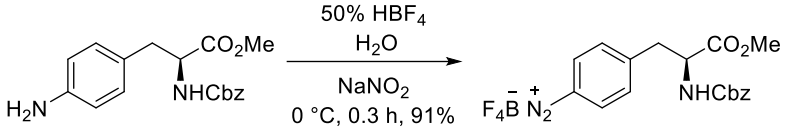


174 175b

Entry	Catalyst	Acid	Solvent	Temp. (°C)	Time (h)	Yield
1	$Pd(PPh_3)_4$	AcOH	DMF	90 °C	18	N/A
2	$Pd(PPh_3)_4$	AcOH	DMF	50 °C	18	20%
3	$Pd(PPh_3)_4$	AcOH	DMF	30 °C	18	20%
4	XPhos Pd G2	AcOH	DMF	90 °C	18	N/A
5	$Pd_2(dba)_3$[a]	AcOH	DMF	90 °C	18	N/A
6	$Pd(OAc)_2$	AcOH	DMF	90 °C	18	13%

Table 8: Optimisation of the one-pot diazotisation and Suzuki-Miyaura cross-coupling reaction for synthesis of biphenyl α-amino acids.

It was proposed that to improve the yield for the Suzuki-Miyaura cross-coupling reaction, the reactivity of the diazonium salt must be further increased. To do this, diazonium tetrafluoroborate **176** was generated in an excellent 91% yield using tetrafluoroboric acid and sodium nitrite as shown in Scheme 72.[141]

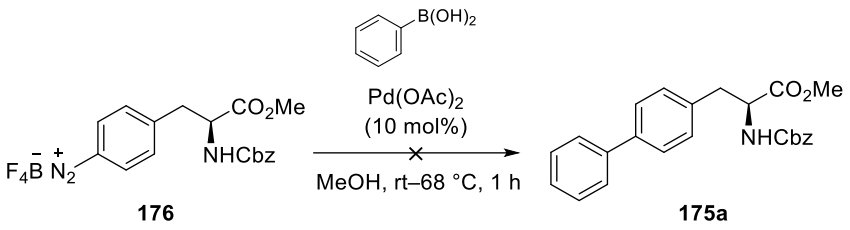


174 176

Scheme 72: Generation of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (**176**).[141]

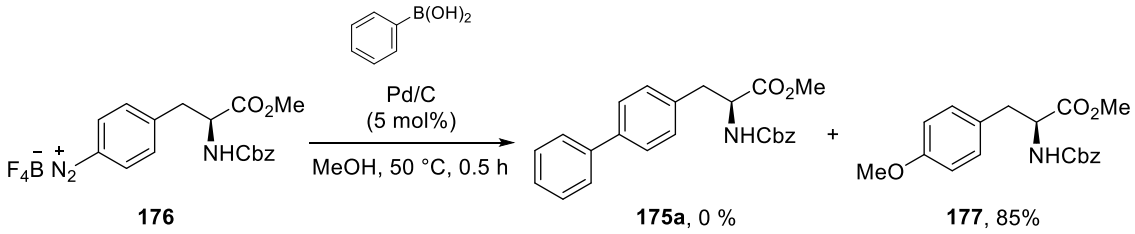
Diazonium salt **176** was then submitted to the Suzuki-Miyaura cross-coupling reaction under conditions described by Sengupta and Bhattacharyya (Scheme 73).[138] Following heating under reflux for 1 h, the ^{1}H NMR spectrum indicated a 2:1 mixture of desired product and dediazotised material. Interestingly, this issue was not reported by Bhattacharyya and co-workers, suggesting a reduction in reactivity of the diazonium salt due to stabilisation by the amino acid side chain or that the amino acid functionality may interact with the catalyst. To minimise side-product formation, the temperature was reduced to 40 °C. This improved the product and side-product ratio to 2.5:1. However, a further reduction in temperature resulted in

a 1:1 mixture of product and dediazotised material, suggesting the tetrafluoroborate diazonium salt will not undergo the cross-coupling reaction at lower temperatures.



Scheme 73: Attempted Suzuki-Miyaura cross-coupling reaction of **176** using $Pd(OAc)_2$ catalyst.

Work by Felpin and co-workers has shown biphenyls can be synthesised *via* a Suzuki-Miyaura reaction of tetrafluoroborates utilising a palladium on carbon catalyst.[142] The reaction of diazonium salt **176** with phenylboronic acid was therefore attempted under these conditions (Scheme 74). However, this resulted in an 85% yield of S_NAr product **177**. The reaction was consequently repeated using acetonitrile as the solvent but this resulted in no conversion to desired product. This was also the result when the reaction was attempted in dioxane. When attempted using *N*,*N*-dimethylformamide, a complex mixture was observed from the ^1H NMR spectrum. The desired product could not be isolated from this mixture.

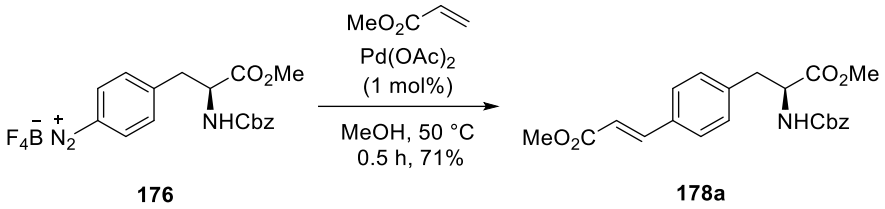


Scheme 74: Attempted Suzuki-Miyaura cross-coupling reaction of **176** using palladium on carbon catalyst.

2.3.3 Synthesis of Stilbene Amino Acids *via* a Heck-Matsuda Reaction

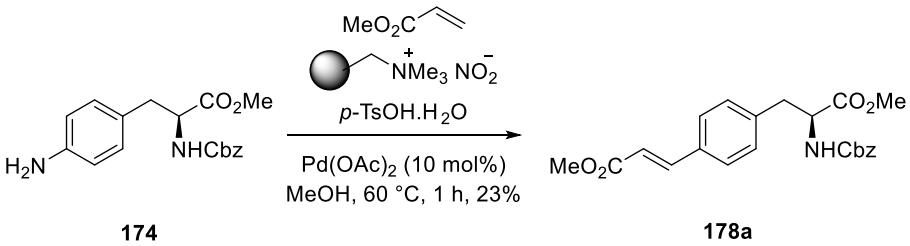
Despite the issues encountered with the Suzuki-Miyaura cross-coupling reaction, the ease of access to the tetrafluoroborate salts prompted an investigation into the applicability of these compounds for Heck-Matsuda reactions. This was of particular interest as it would allow for formation of novel α-amino acids *via* a base-free cross-coupling reaction. Initially, the reaction was attempted using $Pd(OAc)_2$ (1 mol%) and methyl acrylate as the coupling partner. This generated cinnamate **178a** in a good

71% yield after 0.5 h at 50 °C (Scheme 75).[143] Formation of desired product was confirmed by a pair of doublets at 6.40 ppm and 7.65 ppm in the ^{1}H NMR spectrum with a large coupling constant of 16.0 Hz, indicating the presence of an *E*-alkene.



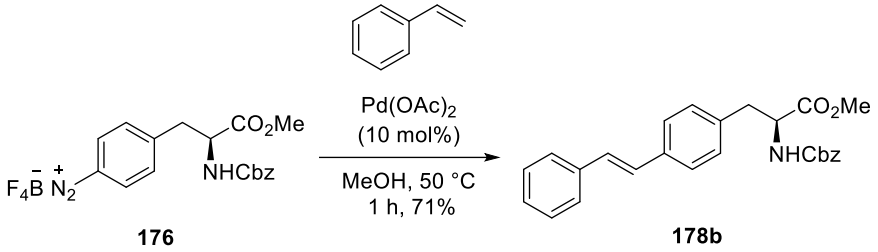
Scheme 75: Synthesis of **178a** *via* Heck-Matsuda cross-coupling reaction of **176** with methyl acrylate.

The same reaction was also attempted using polymer-supported nitrite resin and *p*-tosic acid to generate the diazonium tosylate intermediate (Scheme 76). Interestingly, after 1 h at 60 °C with 10 mol% of $Pd(OAc)_2$, this gave only a 23% yield of desired product despite a higher catalyst loading, emphasising the importance of the increased reactivity of the tetrafluoroborate salt.



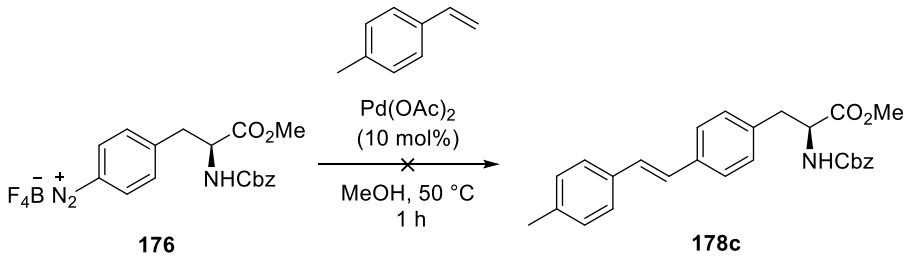
Scheme 76: Heck-Matsuda cross-coupling reaction *via* diazonium tosylates.

Following the successful reaction with methyl acrylate, the reaction was then attempted with styrene. Initially, utilising a 1 mol% catalyst loading resulted in a 1:1 inseparable mixture of desired product and S_NAr side product **177**. The reaction was therefore repeated in acetonitrile. However, this showed no presence of desired product by ^{1}H NMR spectroscopy, indicating the need for a polar protic solvent. Instead, the catalyst loading was increased. A catalyst loading of 5 mol% resulted in a 2:1 ratio of product and side-product. It was found that a further increase in catalyst loading to 10 mol% gave stilbene **178b** in a high 71% yield (Scheme 77).



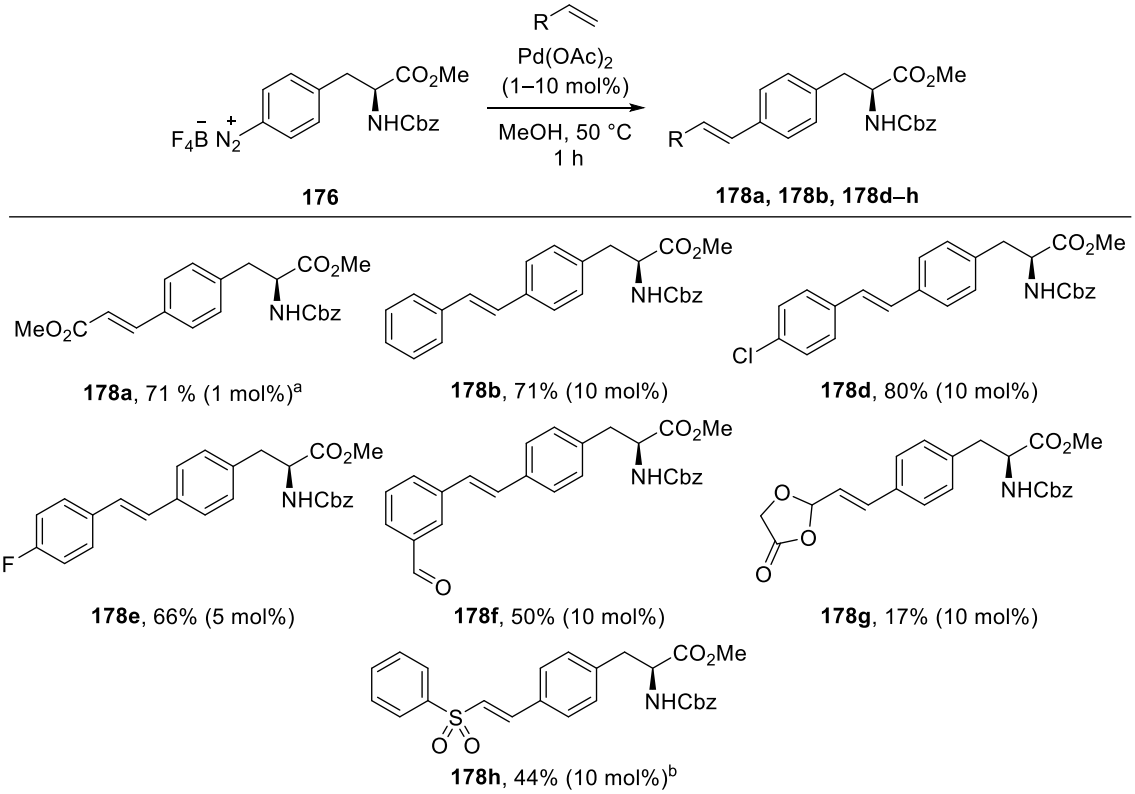
Scheme 77: Synthesis of **178b** *via* Heck-Matsuda cross-coupling reaction of **176** with styrene.

The cross-coupling reaction with 4-methylstyrene was also investigated (Scheme 78). However, utilising a 10 mol% loading of catalyst, a 2:1 product: side-product ratio was obtained. These could not be separated due to the similar R_f values of the two compounds. The decreased reactivity of 4-methylstyrene suggests the need for activated, electron-deficient alkenes as the coupling partner. Unsurprisingly, when the reaction was attempted using 4-methoxystyrene, only traces of desired product were observed by ^{1}H NMR spectroscopy.



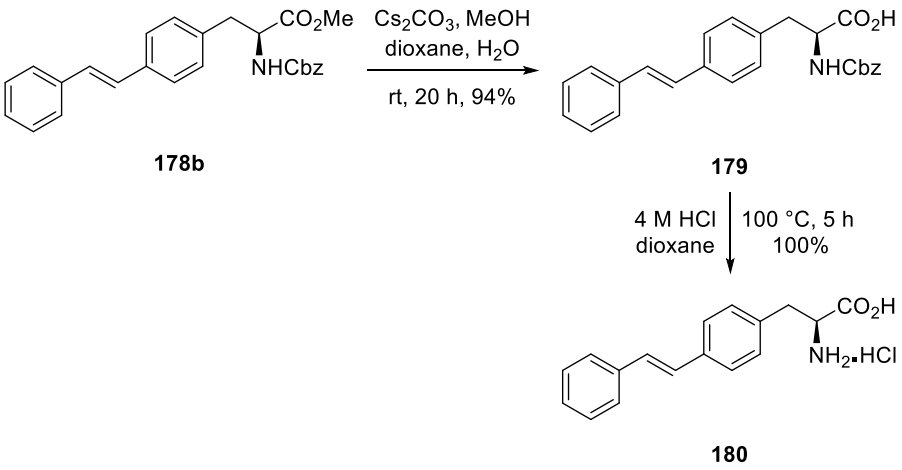
Scheme 78: Attempted Heck-Matsuda cross-coupling reaction of **176** with 4-methylstyrene.

A full substrate scope of the Heck-Matsuda cross-coupling reaction is shown in Scheme 79. As highlighted by the difference in catalyst loading required for synthesis of phenyl analogue **178a** compared to 4'''-fluorophenyl analogue **178e**, addition of aryl electron-withdrawing substituents increased the efficiency of the cross-coupling reaction. The reaction was also successful for heterocycle **178g**, albeit in a 17% yield. The decreased yield for this analogue is proposed to be a result of partial degradation of the heterocycle during the cross-coupling reaction. Finally, the reaction was also successful for the synthesis of phenylvinylsulfone **178h** in a 44% yield.



Scheme 79: Substrate scope of Heck-Matsuda reaction of **176** with alkenes. [a]Reaction carried out for 0.5 h; Reaction carried out for 5.5 h.

As a proof of principle experiment, compound **178b** was also successfully deprotected. Firstly, methyl ester **178b** was hydrolysed to carboxylic acid **179** under basic conditions using caesium carbonate and methanol in an excellent 94% yield (Scheme 80). Removal of the Cbz-protecting group was then achieved in quantitative yield using 4 M hydrochloric acid in dioxane, indicated by the absence of five aromatic hydrogen atoms in the ^{1}H NMR spectrum.



Scheme 80: Deprotection of **178b** to give parent amino acid **180**.

2.3.4 Fluorescent Properties of Stilbene Amino Acids

Following successful development of the Heck-Matsuda reaction of diazonium tetrafluoroborate **176**, the absorption and emission data for the coupled products **178a, 178b** and **178d–f, 178h** and **180** was recorded (Figures 43–49).

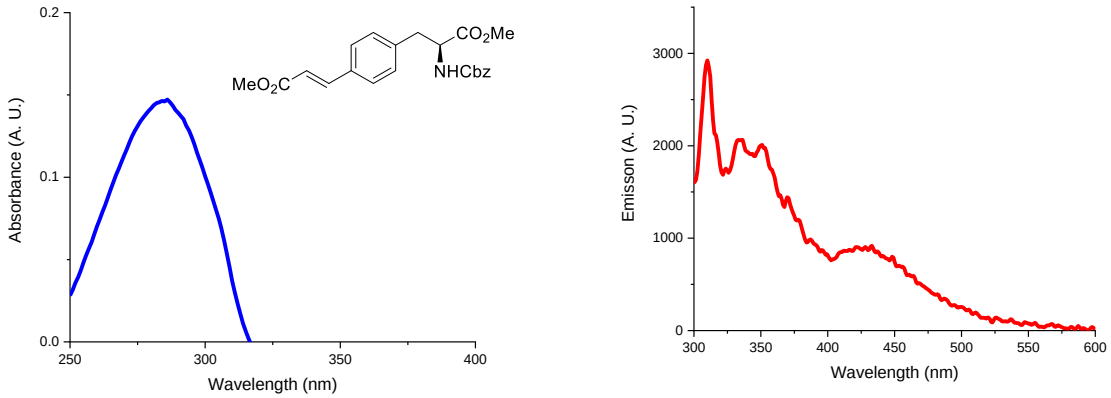


Figure 43: Absorption and emission spectra of **178a** (5 µM in MeOH, excitation at 285 nm).

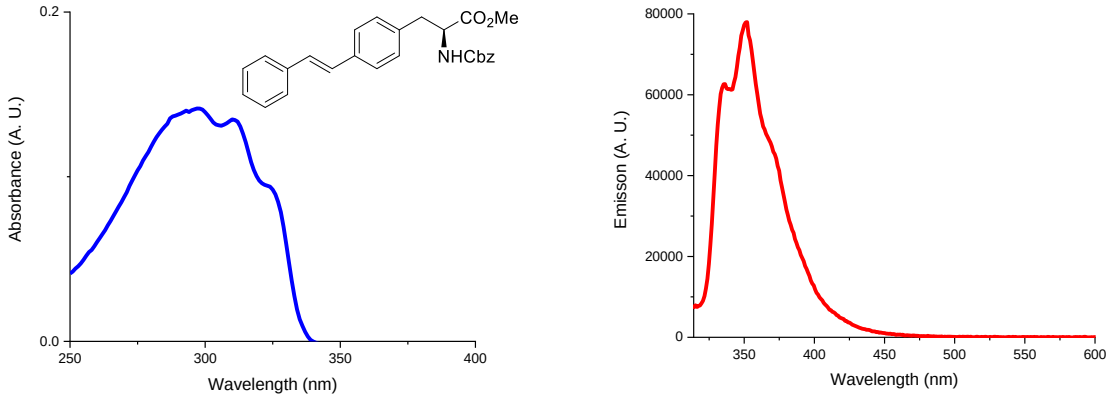


Figure 44: Absorption and emission spectra of **178b** (5 µM in MeOH, excitation at 275 nm).

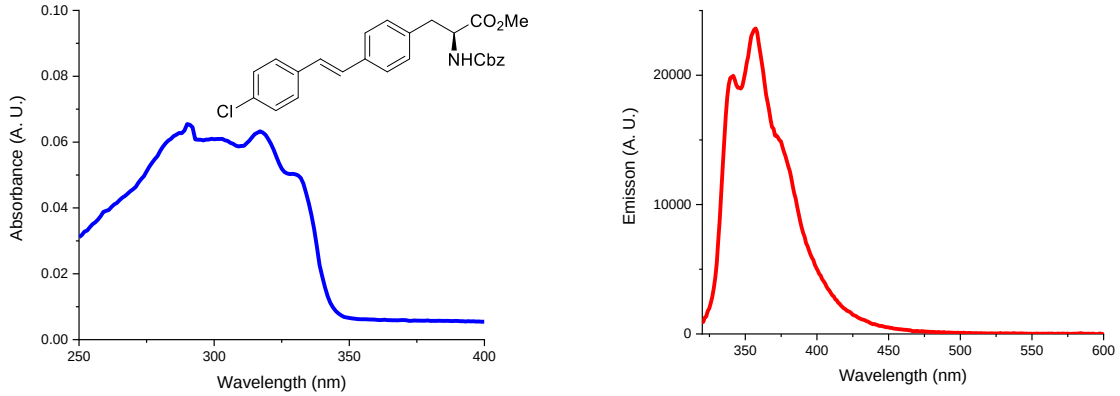


Figure 45: Absorption and emission spectra of **178d** (2 µM in MeOH, excitation at 316 nm).

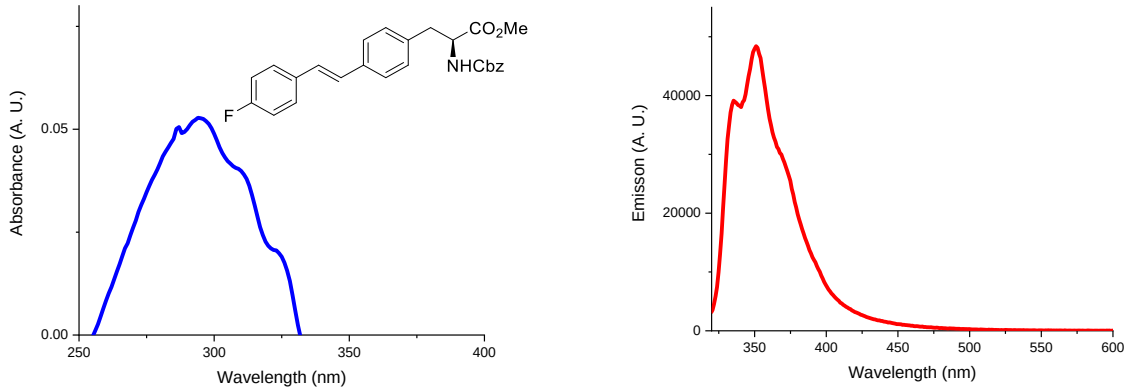


Figure 46: Absorption and emission spectra of **178e** (2 µM in MeOH, excitation at 294 nm).

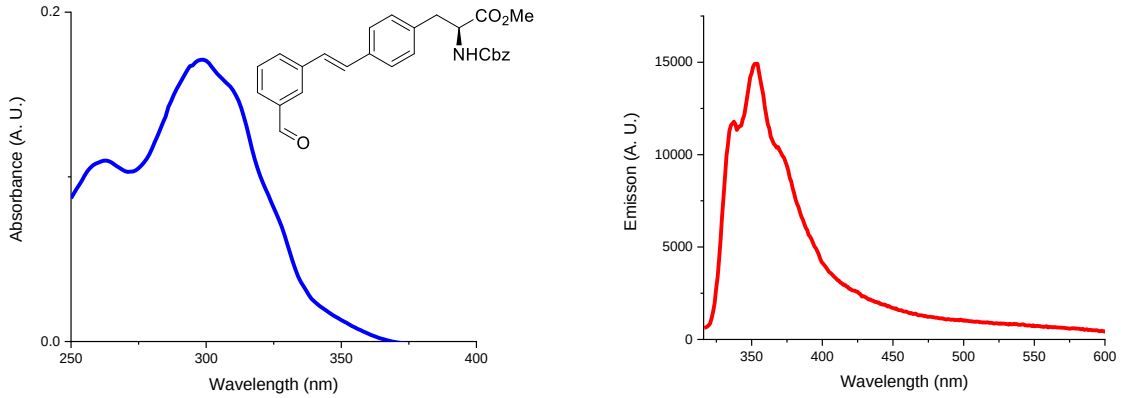


Figure 47: Absorption and emission spectra of **178f** (5 µM in MeOH, excitation at 299 nm).

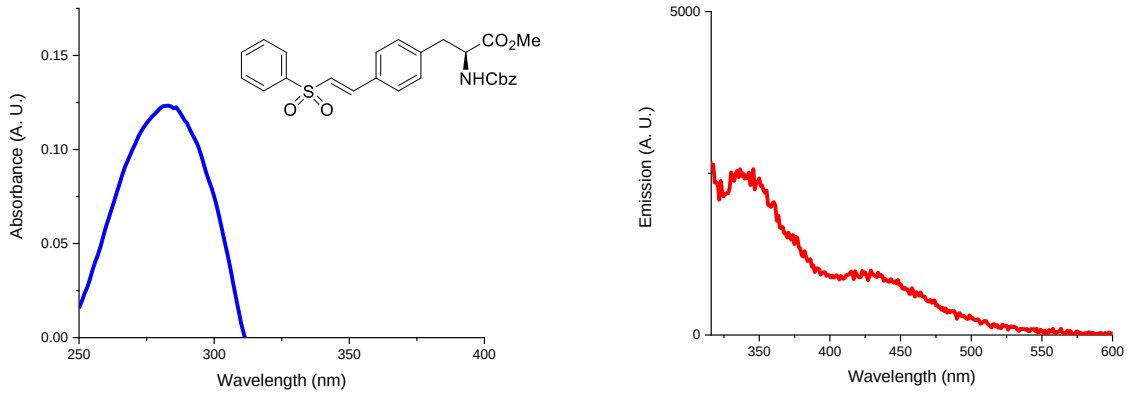


Figure 48: Absorption and emission spectra of **178h** (5 µM in MeOH, excitation at 284 nm).

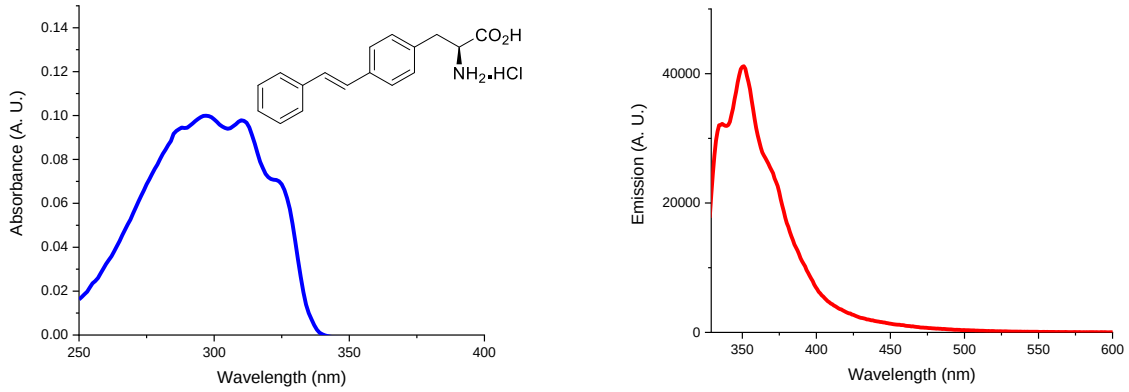


Figure 49: Absorption and emission spectra of **180** (5 µM in MeOH, excitation at 311 nm).

Full fluorescence data was obtained on analogues **178a, 178b, 178d–f, 178h** and **180** and is summarised in Table 9. The absorption and emission maximum for cinnamate **178a** were found to be 286 nm and 310 nm, respectively. However, **178a** showed very weak emission and hence quantum yield data could not be obtained. For the same reason, quantum yield data could also not be obtained for analogue **178h**. 4'''-Fluoro analogue **178e** showed the most promising fluorescent properties with a Stokes Shift of 57 nm and a large molar attenuation coefficient of 20,700 cm^{-1} M^{-1}. Compound **178e** also possessed the largest quantum yield of 0.07. Importantly, it was found that the fluorescent properties were maintained for styrene derivative **178b** following deprotection (analogue **180**).

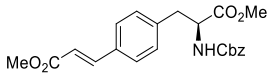
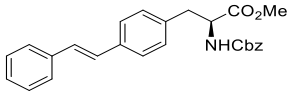
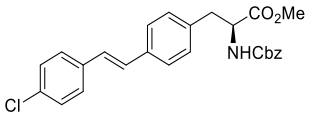
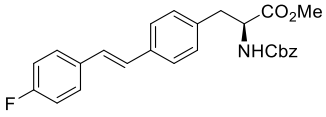
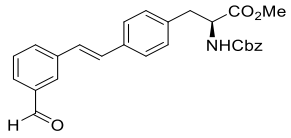
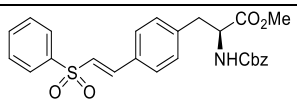
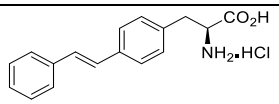
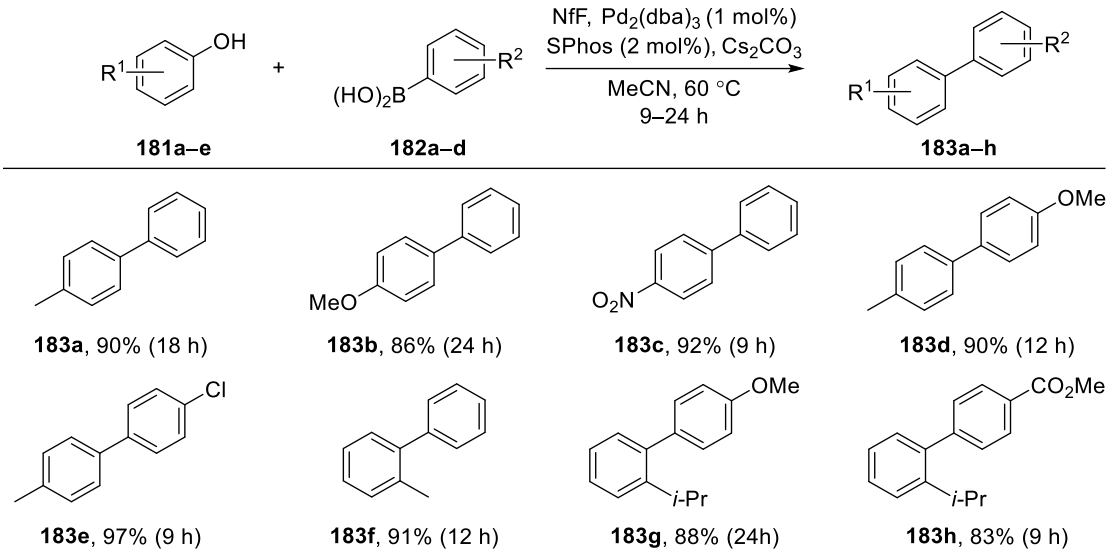
Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
 **178a**	286	310	24	N/A	N/A	N/A
 **178b**	314	349	35	20600	0.05	1070
 **178d**	317	357	40	22500	0.06	1430
 **178e**	295	352	57	20700	0.07	1380
 **178f**	300	351	51	24300	0.01	270
 **178h**	282	342	60	N/A	N/A	N/A
 **180**	310	351	41	19000	0.04	750

Table 9: Photoluminescence properties of stilbene α-amino acids.

2.3.5 Synthesis of Biphenyl Amino Acids *via* a One-Pot Nonaflate-Formation and Suzuki-Miyaura Cross-Coupling Reaction

Due to the issues encountered when attempting to generate biphenyl amino acids as described in Section 2.3.2, an alternative approach for their synthesis was investigated. Nonaflates present useful intermediates for palladium catalysed coupling reactions due to the excellent leaving group ability of the sulfonate ion. The electron-withdrawing ability of the perfluoroalkyl chain removes electron density from the adjacent carbon-oxygen bond and hence allows for efficient palladium insertion.[144] It was therefore proposed that biphenyl amino acids could be synthesised *via* formation of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(phenylnonafluorobutanesulfonate)-4'-yl]propanoate *in situ* followed by a subsequent Suzuki-Miyaura cross-coupling reaction. Previous work in this area has been carried out by Akai and co-workers, who have shown phenols can be

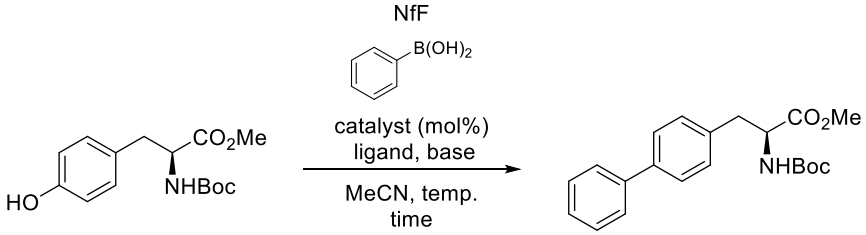
converted to the corresponding biphenyls using a one-pot nonaflate-formation and Suzuki-Miyaura cross-coupling reaction (Scheme 81).[145]



Scheme 81: Formation of biphenyls *via* one-pot nonaflate Suzuki-Miyaura cross-coupling reaction.[145]

Consequently, formation of biphenyl amino acid **175a** from Cbz-L-tyrosine methyl ester (**184**) was initially attempted under the same conditions described by Akai and co-workers.[145] Utilising $Pd_2(dba)_3$ (1 mol%) as the catalyst and SPhos (2 mol%) as the ligand, desired product **175a** was obtained in a 30% yield (Table 10, entry 1). When the reaction was attempted using XPhos Pd G2 (1 mol%) as the catalyst, no conversion to desired product was observed by ^{1}H NMR spectroscopy (entry 2). However, when the base was changed from caesium carbonate to potassium phosphate, **175a** was isolated in a 46% yield (entry 3). The reaction was then attempted using XPhos Pd G3 as a catalyst, however, no desired product was formed (entry 4). Interestingly, when the catalyst loading of XPhos Pd G2 was increased from 1 mol% to 3 mol%, this resulted in a decrease in yield to 30% (entry 5). The yield further decreased to 10% when the catalyst loading was increased to 5 mol% (entry 6). The presence of nonaflate intermediate in the ^{1}H NMR spectrum of the crude reaction mixture suggested the Suzuki-Miyaura cross-coupling was the limiting reaction. In order to improve conversion, water was added to solubilise the base. The reaction was therefore stirred at 60 °C for 2 h to allow for nonaflate formation, followed by addition of the boronic acid, catalyst and water before stirring at 60 °C overnight. This resulted in a 66% yield of desired product (entry 7). The yield increased to 71% upon addition of a further 1 mol% catalyst after 2 h (entry 8).

The reaction was repeated at 40 °C (entry 9). However, this resulted in a significant loss in yield to 18%. Finally, it was found that utilising three 1 mol% batches of catalyst 2 h apart resulted in an optimal yield of 91% (entry 10).



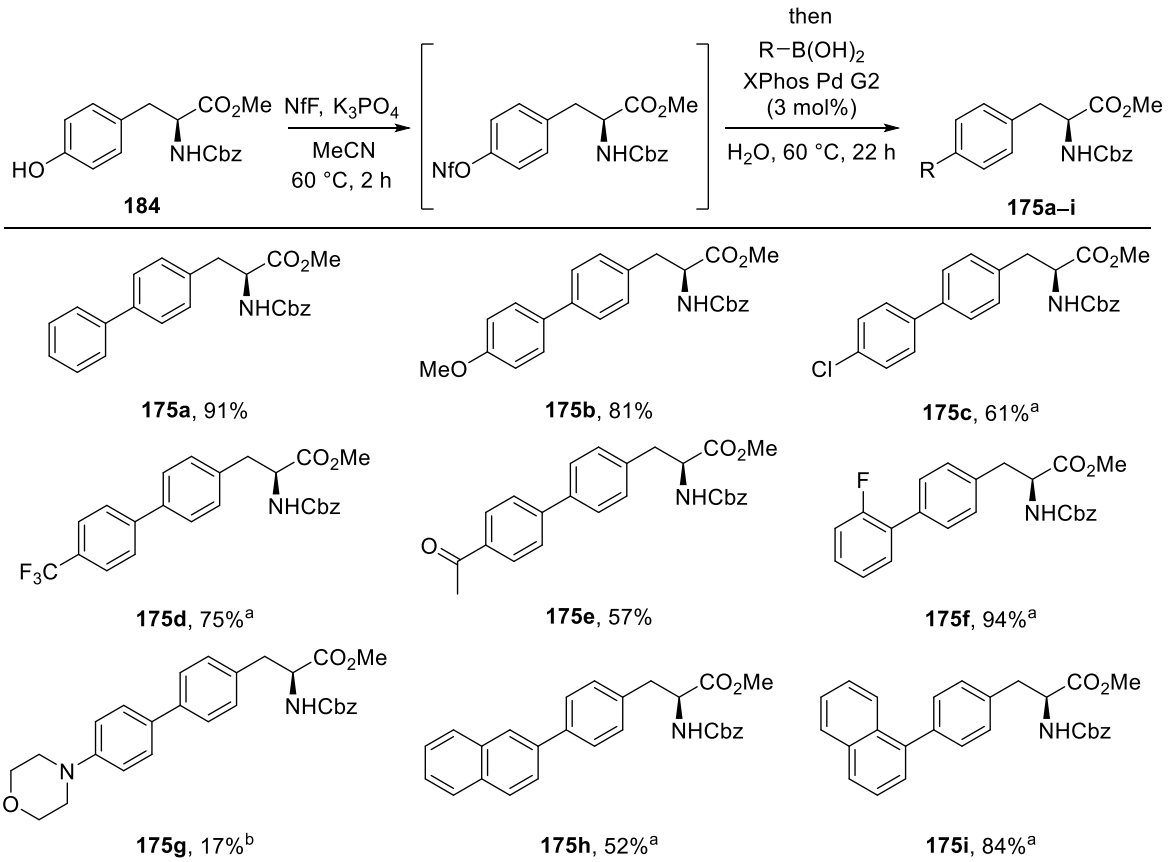
184 **175a**

Entry	Catalyst (mol%)/Ligand (mol%)	Base	Temperature (° C)	Time (h)	Yield (%)
1	$Pd_2(dba)_3$ (1 mol%)/SPhos (2 mol%)	Cs_2CO_3	60 °C	18 h	30%
2	XPhos Pd G2 (1 mol%)	Cs_2CO_3	60 °C	18 h	N/A
3	XPhos Pd G2 (1 mol%)	K_3PO_4	60 °C	18 h	46%
4	XPhos Pd G3 (1 mol%)	K_3PO_4	60 °C	18 h	N/A
5	XPhos Pd G2 (3 mol%)	K_3PO_4	60 °C	18 h	30%
6	XPhos Pd G2 (5 mol%)	K_3PO_4	60 °C	18 h	10%
7	XPhos Pd G2 (1 mol%)	K_3PO_4 With H_2O	60 °C	20 h	66%
8	XPhos Pd G2 (2 mol%)	K_3PO_4 With H_2O	60 °C	20 h	71%
9	XPhos Pd G2 (2 mol%)	K_3PO_4 With H_2O	40 °C	20 h	18%
10	XPhos Pd G2 (3 mol%)	K_3PO_4 With H_2O	60 °C	20 h	91%

Table 10: Optimisation of one-pot nonaflate Suzuki-Miyaura cross-coupling reaction of **184**.

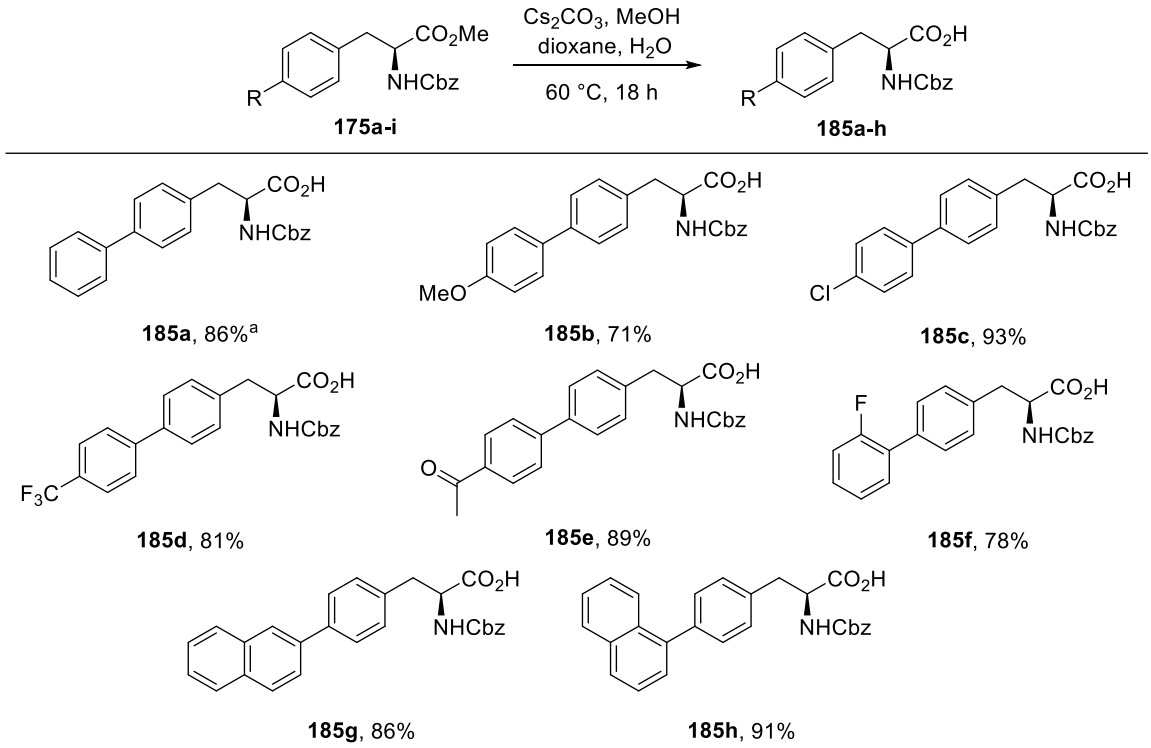
Following optimisation, the substrate scope of the one-pot procedure was explored. The reaction was successful for both electron-rich and electron-deficient boronic acids (**175b** and **175c**) (Scheme 82). For some analogues, it was found that an increased temperature of 80 °C was required to obtained full conversion of the nonaflate intermediate. These included **175c**, **175d**, **175f**, **175h** and **175i**. It is proposed the increase in temperature for **175d** was required due to the increased

electron-withdrawing nature of the trifluoromethyl substituent. For 2''-fluoro analogue **175f** and naphthyl analogues **175h** and **175i**, it is proposed the increased temperature was required due to steric effects. The reaction was attempted using 4-nitrobenzeneboronic acid. However, the ^{1}H NMR spectrum indicated no formation of the desired product despite full conversion to the nonaflate intermediate. Finally, the reaction was also attempted using 4-pyridylboronic acid. However, this is believed to have been unsuccessful due to solubility issues of the boronic acid. The reaction of 4''-morpholine derivate **175g** was initially attempted in acetonitrile. However, 4'-morpholinophenyl boronic acid was also found to be insoluble. The reaction was thus repeated in tetrahydrofuran which allowed for partial solubility of the boronic acid, generating desired coupled product **175g** in a 17% yield. It should be noted that compounds **175c**, **175e** and **175h** were synthesised by Sineenard Songsri.



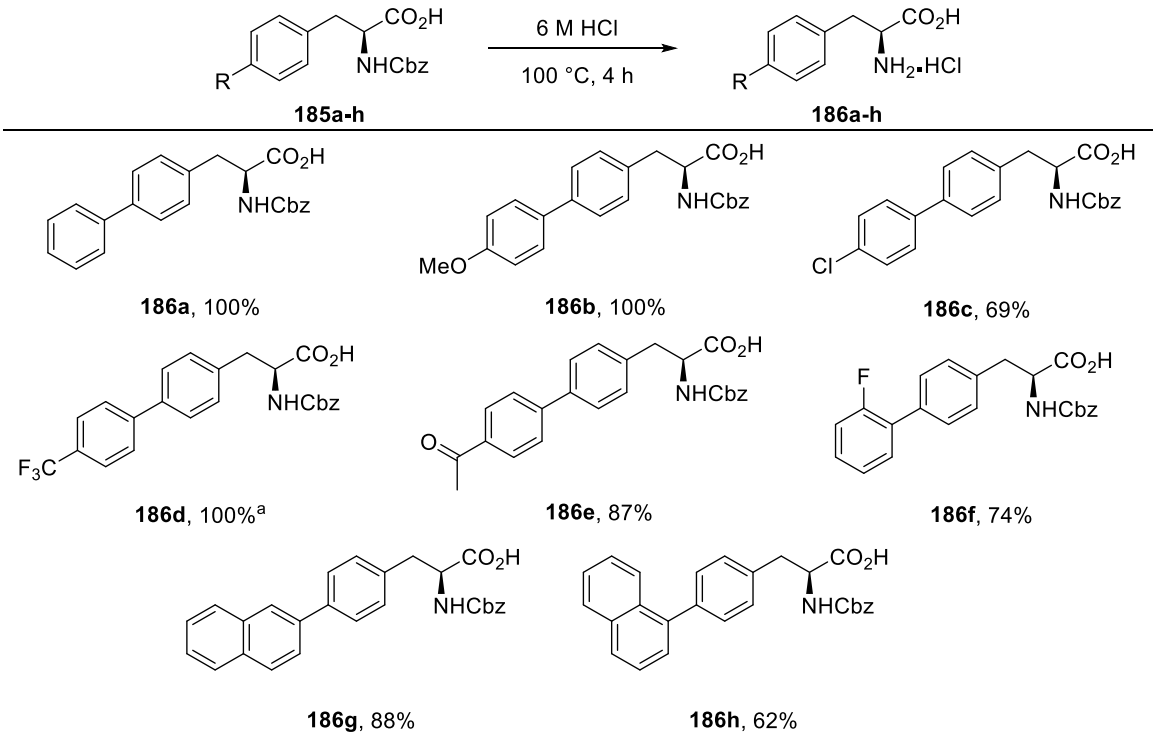
Scheme 82: Substrate scope of one-pot nonaflate Suzuki-Miyaura cross-coupling reaction of **184**. [a]Reaction carried out at 80 °C; [b]Reaction caried out in THF.

Methyl ester hydrolysis was achieved using caesium carbonate (Scheme 83). This gave the desired carboxylic acids in good to excellent yields.



Scheme 83: Methyl ester hydrolysis of biphenyl α-amino acids. [a]Reaction carried out at room temperature.

Finally, removal the Cbz-group was achieved using 6 M hydrochloric acid (Scheme 84). This gave the desired amino acids cleanly and in high yields.



Scheme 84: Cbz-Group removal of biphenyl α-amino acids. [a]Reaction stirred at 100 °C for 7 h.

2.3.6 Fluorescent Properties of Biphenyl Amino Acids

Following successful synthesis of a novel library of biphenyl amino acids, the absorption and emission data for these compounds, **186a–h and 175g** was recorded (Figures 50–58).

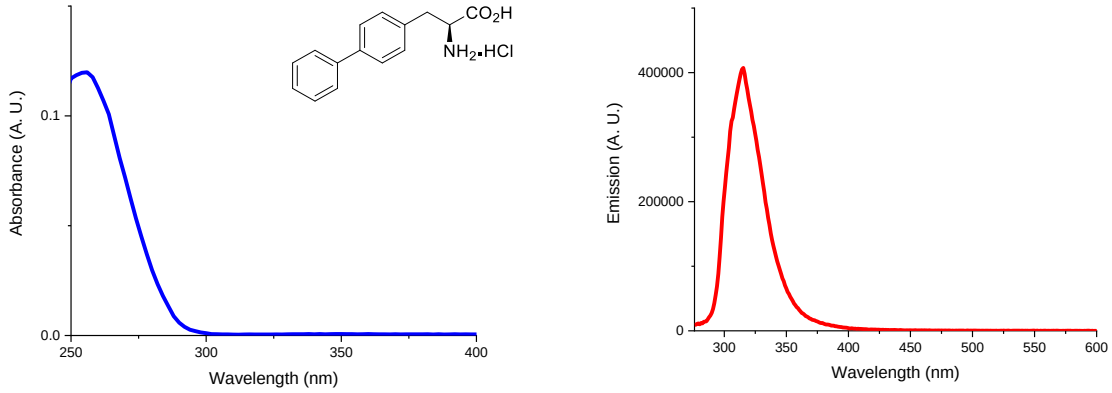


Figure 50: Absorption and emission spectra of **186a** (5 µM in MeOH, excitation at 256 nm).

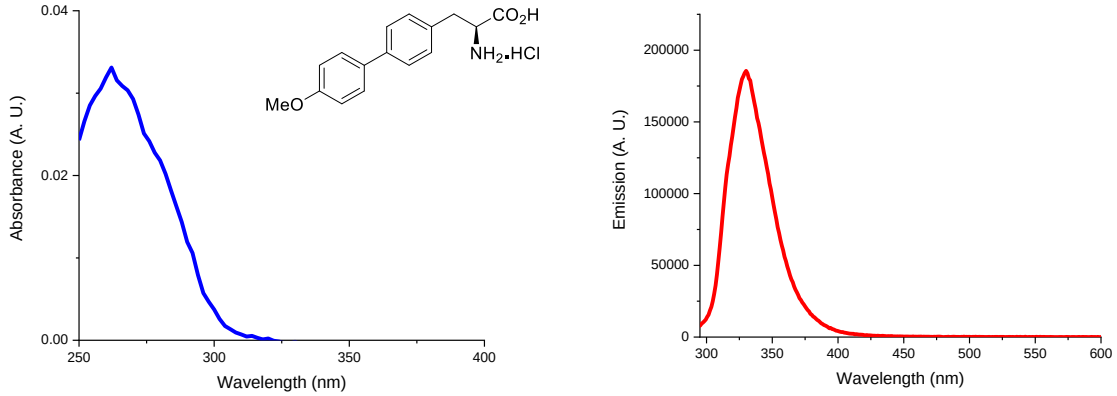


Figure 51: Absorption and emission spectra of **186b** (5 µM in MeOH, excitation at 275 nm).

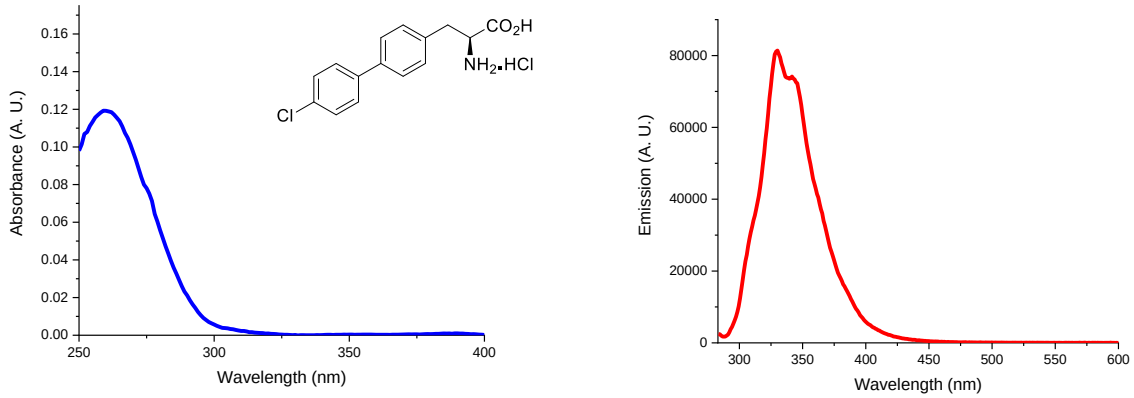


Figure 52: Absorption and emission spectra of **186c** (5 µM in MeOH, excitation at 275 nm).

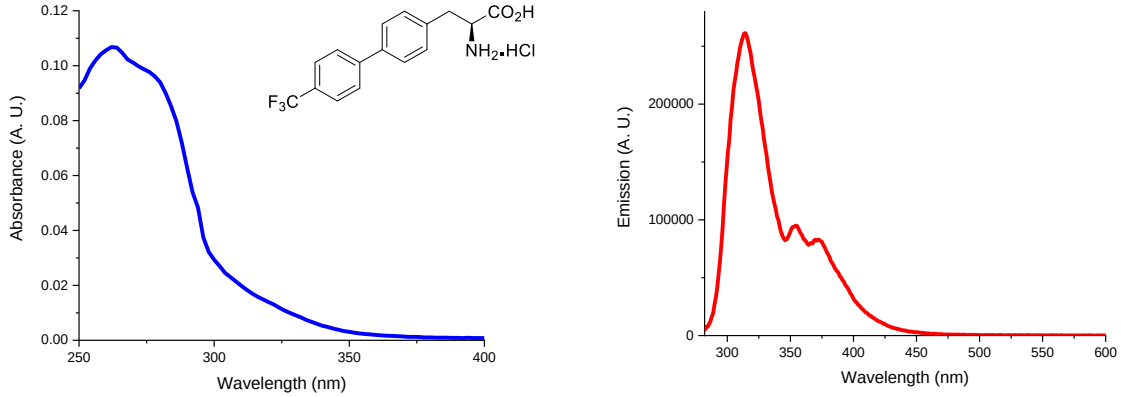


Figure 53: Absorption and emission spectra of **186d** (2 µM in MeOH, excitation at 262 nm).

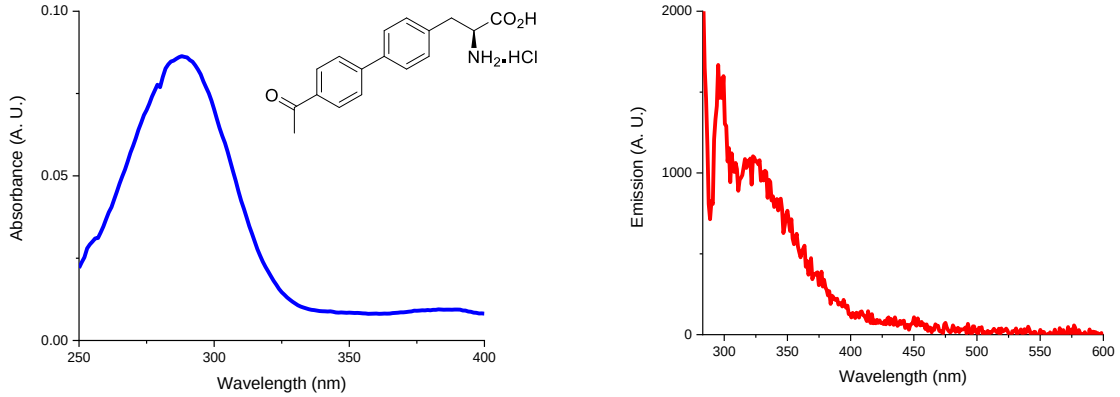


Figure 54: Absorption and emission spectra of **186e** (5 µM in MeOH, excitation at 275 nm).

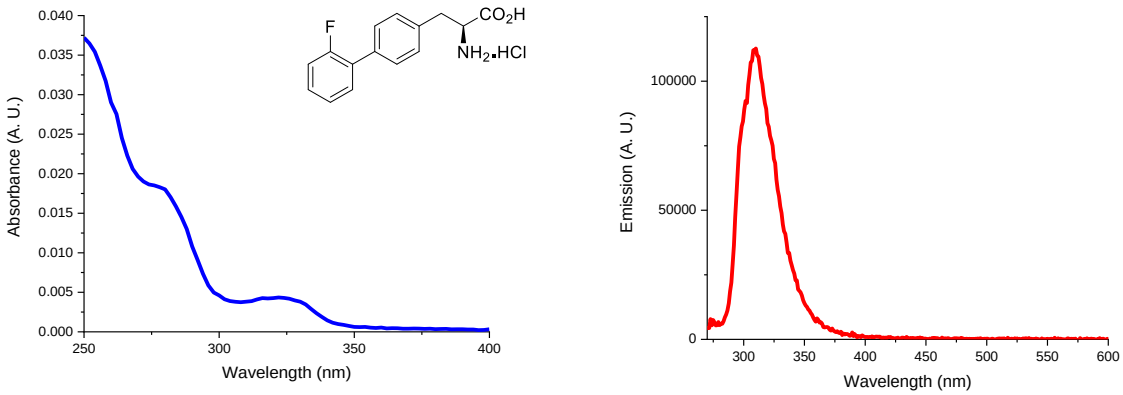


Figure 55: Absorption and emission spectra of **186f** (2 µM in MeOH, excitation at 260 nm).

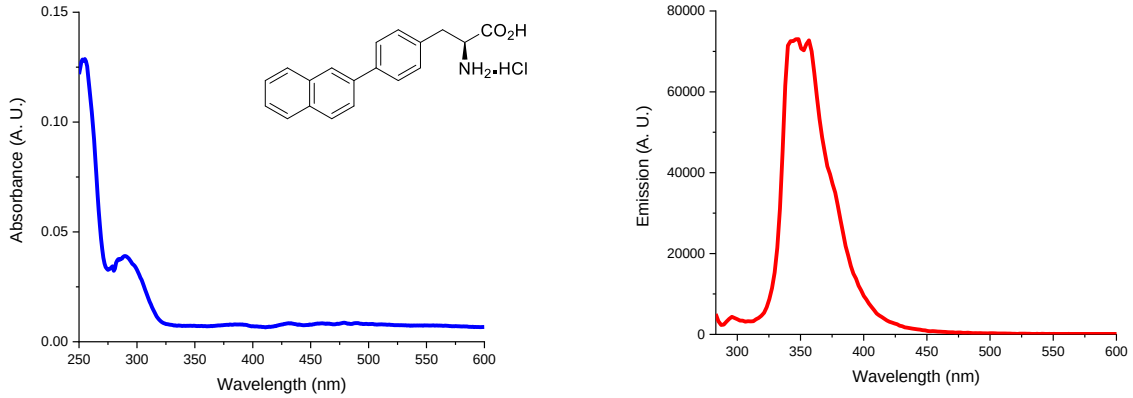


Figure 56: Absorption and emission spectra of **186g** (5 µM in MeOH, excitation at 275 nm).

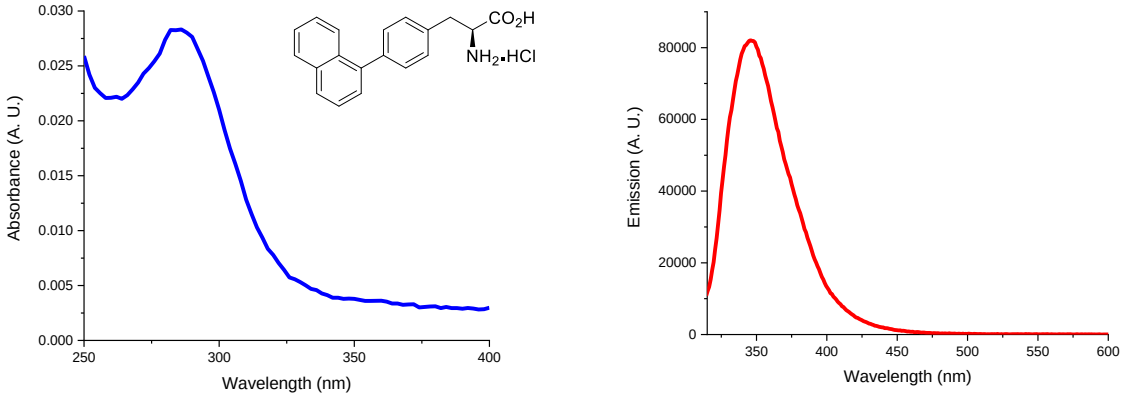


Figure 57: Absorption and emission spectra of **186h** (2 µM in MeOH, excitation at 286 nm).

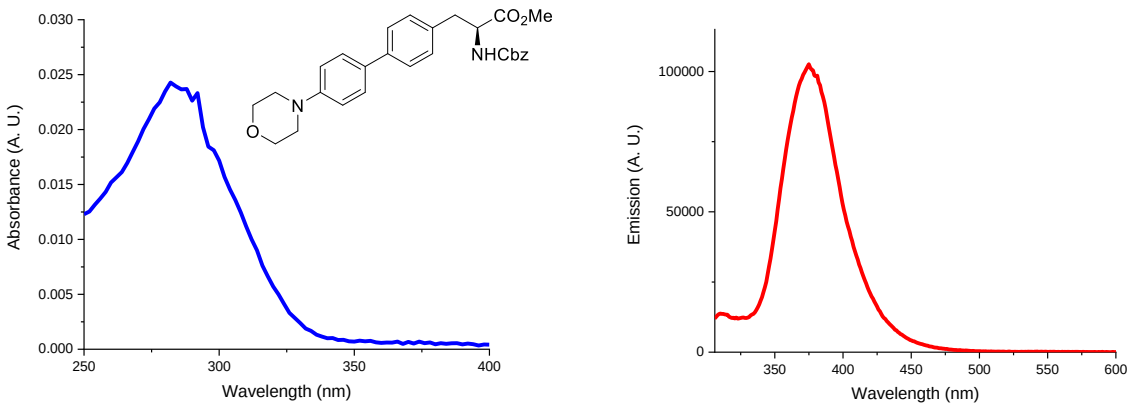


Figure 58: Absorption and emission spectra of **175g** (1.25 µM in MeOH, excitation at 286 nm).

The photoluminescent properties of the biphenyl amino acids were then investigated and the data is summarised in Table 11. The low yield of the Suzuki-Miyaura coupling for morpholine analogue **175g** meant that there was not enough material for the deprotection steps and so this was analysed in the protected form. There was found to be no correlation between the electronic nature of substituent groups and the wavelength of emission. All analogues also showed good Stokes shifts of at least 30 nm. In particular, trifluoromethyl derivative **186d** was found to have a MegaStokes shift of 109 nm. Analogues **186a, 186c**, **186d** and **186g** also showed excellent molar attenuation coefficients above 20,000 $cm^{-1}\,M^{-1}$. The quantum yields of all analogues were above 10% (excluding 4''-chloro analogue **186c** and 4''-acetyl analogue **186e**), with **186h** and **175g** showing good quantum yields of 0.27 and 0.31, respectively. 2''-Naphthyl derivative **186g** was found to have a large brightness of 5560 $cm^{-1}\,M^{-1}$. Interestingly, fully deprotected analogues **186a** and **186c** showed improved quantum yields and hence overall brightness compared to their protected stilbene derivative (Table 9, 2930 $cm^{-1}\,M^{-1}$ vs. 1070 $cm^{-1}\,M^{-1}$ for phenyl analogues **186a** and **178b**, 2570 $cm^{-1}\,M^{-1}$ vs. 1430 $cm^{-1}\,M^{-1}$ for 4''-chlorophenyl analogues **186c** and **178d**). The brightness of deprotected analogues is often lower due to removal of amine protecting groups which can contribute to fluorescence.

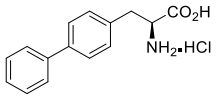
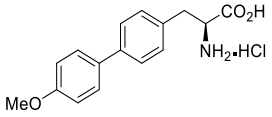
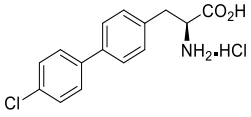
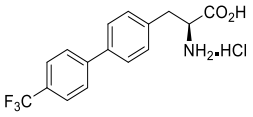
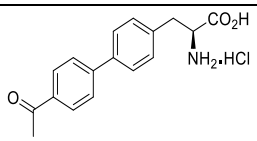
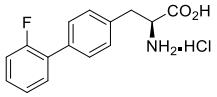
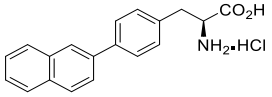
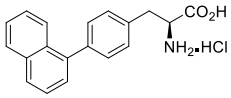
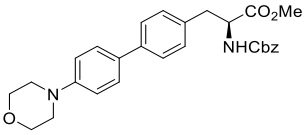
Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
 **186a**	254	314	60	24200	0.12	2930
 **186b**	262	328	66	14100	0.24	3430
 **186c**	261	330	69	32100	0.08	2570
 **186d**	262	312 371	50 109	23300	0.15	3490
 **186e**	289	323	34	19700	0.002	390
 **186f**	280	310	50	2900	0.19	540
 **186g**	256 289	356	87 100	30900	0.18	5560
 **186h**	292	342	50	10300	0.27	2800
 **175g**	292	373	81	17300	0.31	5370

Table 11: Photoluminescence properties of biphenyl α-amino acids.

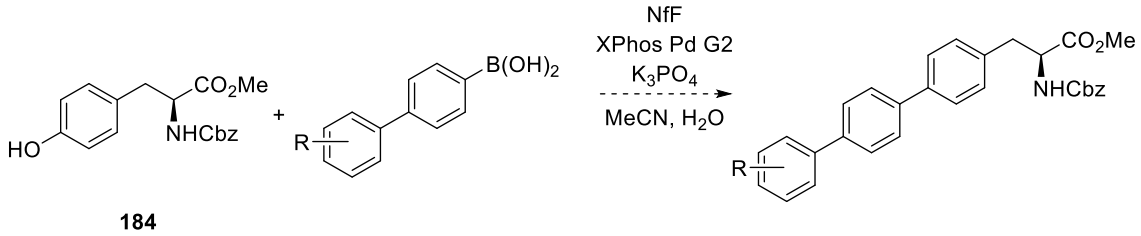
2.3.7 Conclusions

To conclude, protected 4-aminophenylalanine **174** has been successfully synthesised in three steps from 4-nitro-L-phenylalanine (**168**). Formation of diazonium tetrafluoroborate **176** from **174** was achieved *via* diazotisation using tetrafluoroboric acid and sodium nitrite. This allowed for the generation of a small library of novel stilbene-derived amino acids *via* a Heck-Matsuda reaction. A

significant advantage of this methodology is the ability to generate novel amino acids under base-free conditions. Fluorescence data was obtained of these analogues and 4'''-fluorophenyl analogue **178e** presented the most desirable fluorescent properties with a quantum yield of 0.07 and molar attenuation coefficient of 20,700 cm^{-1} M^{-1}. A Suzuki-Miyaura cross-coupling reaction was also attempted using diazonium salt **176** to generate biphenyl amino acids. However, this proved unsuccessful. An alternative strategy for the synthesis of these analogues was therefore devised utilising a one-pot nonaflate-formation and Suzuki-Miyaura cross-coupling reaction. This allowed for the synthesis of a diverse range of biphenyl amino acids in one step from commercially available starting material, Cbz-L-tyrosine methyl ester (**184**). Deprotection of the biphenyl amino acids was achieved using caesium carbonate mediated ester hydrolysis, followed by hydrochloric acid removal of the Cbz-protecting group. Analogues in biphenyl series were generally found to be brighter than the stilbene series, despite being fully deprotected.

2.3.8 Future Work

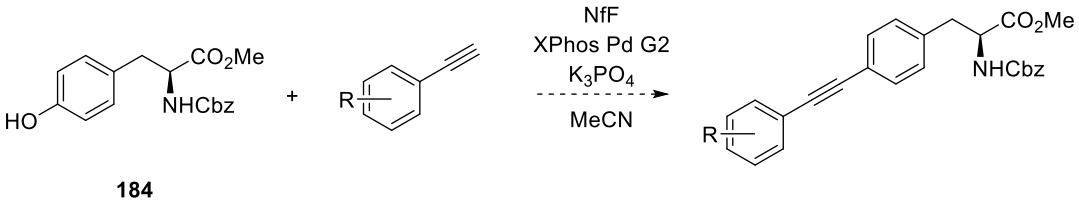
Future work on this project will involve synthesis of terphenyl α-amino acids using the one-pot nonaflate-formation and Suzuki-Miyaura cross-coupling reaction of Cbz-L-tyrosine methyl ester (**184**) with biphenyl boronic acids (Scheme 85). Terphenyl α-amino acids are reported to have quantum yields of approximately 0.7 and show red-shifted absorption compared to their corresponding biphenyl derivatives.[74] Also, 4-biphenyl-L-phenylalanine has been used to detect conformation changes of dihydrofolate reductase *via* FRET.[146]



Scheme 85: Proposed synthesis of terphenyl α-amino acids.

As well as synthesis of terphenyl amino acids, Cbz-L-tyrosine methyl ester (**184**) will also be subjected to Sonogashira cross-coupling conditions to generate the corresponding alkynyl amino acids (Scheme 86). Following optimisation, the substrate scope of alkynes will be explored to generate a small library of structurally diverse alkynyl amino acids. The fluorescent properties of these analogues will then

be investigated. Previous work in the Sutherland group on the synthesis of benzotriazole-derived amino acids has shown that inclusion of an alkyne moiety to generate a stretched chromophore with extended conjugation generally leads to compounds with improved fluorescent properties.[147] As well as this, these analogues could also be of interest for peptide modification/tagging *via* 'click' style reactions.

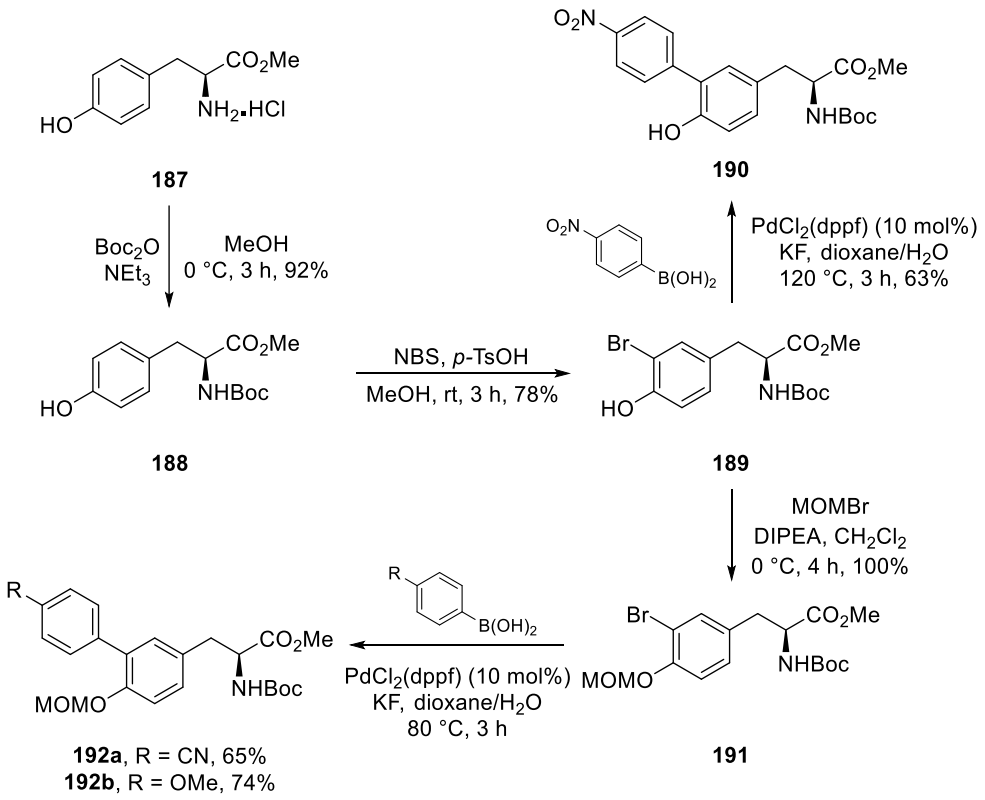


Scheme 86: Proposed synthesis of terphenyl α-amino acids.

2.4 Synthesis of Novel Fluorescent 3'-Aryl Tyrosine Analogues

2.4.1 Previous Work in the Sutherland Group

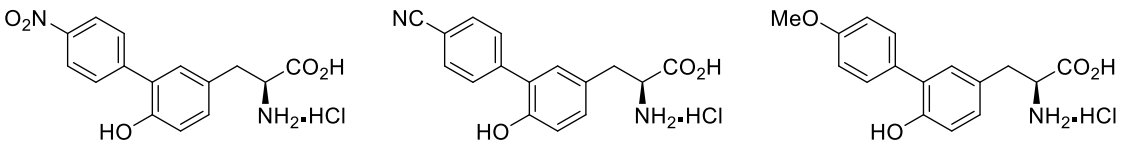
Inspired by work from Wang and co-workers, previous work in the Sutherland group has investigated the functionalisation of tyrosine with the aim of rapidly accessing a diverse range of novel fluorescent α-amino acids *via* a Suzuki-Miyaura cross-coupling reaction. Initially, L-tyrosine methyl ester (**187**) was Boc-protected under standard conditions using Boc_2O and triethylamine in a 92% yield (Scheme 87). *ortho*-Bromination of the aromatic ring was then achieved using *N*-bromosuccinimide in the presence of *p*-tosic acid. This allowed for a subsequent Suzuki-Miyaura cross-coupling reaction using $PdCl_2$(dppf) (10 mol%) to generate biphenyl **190** in a 63% yield. The free hydroxyl group was generally found to be problematic in the cross-coupling reaction and hence for the synthesis of 4''-cyanophenyl analogue **192a** and 4''-methoxyphenyl analogue **192b**, this was MOM-protected using bromomethyl methyl ether prior to the Suzuki-Miyaura reaction.



Scheme 87: Synthetic route to 3'-aryl tyrosine analogues.

Fluorescence data was obtained of the deprotected analogues and is summarised in Figure 59. It is proposed that analogues bearing electron-withdrawing groups in the 4''-position (**193a** and **193b**) generated red-shifted λ_{abs} and λ_{em} data due to

charge transfer through the chromophore from the electron-donating 4'-hydroxy group. All three analogues showed Stokes shifts above 50 nm.



193a
λ_{abs} = 332 nm
λ_{em} = 394 nm

193b
λ_{abs} = 307 nm
λ_{em} = 391 nm

193c
λ_{abs} = 290 nm
λ_{em} = 342 nm

Figure 59: Fluorescence data of deprotected 3'-aryl tyrosine analogues.

2.4.2 Proposed Research

Due to the interesting fluorescent properties displayed by the functionalised tyrosine derivatives (Figure 59), synthesis of a small library of structurally diverse analogues will be investigated (Figure 60). As it was proposed that the desirable red-shifted absorption and emission of analogues **193a** and **193b** arise from the electron-withdrawing nature of the 4''-substitutent, analogues bearing electron-withdrawing groups would be investigated. Analogues with extended conjugated systems as well as heterocyclic systems would also be synthesised.

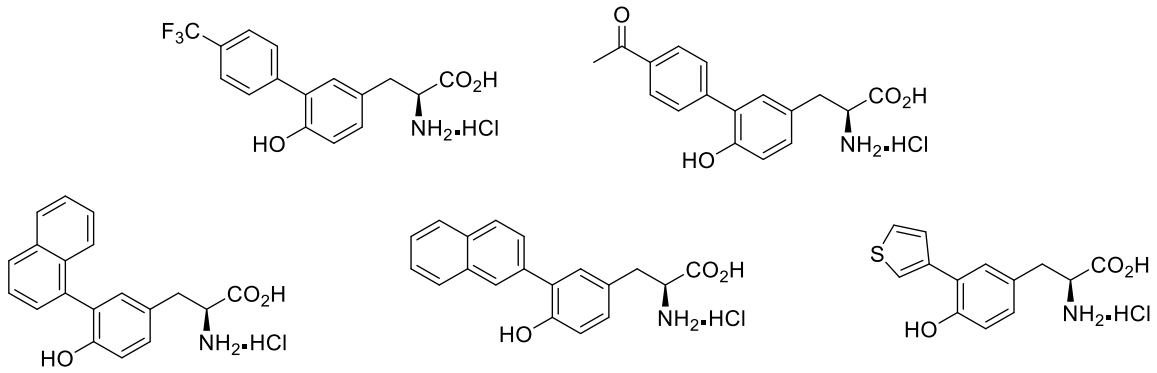


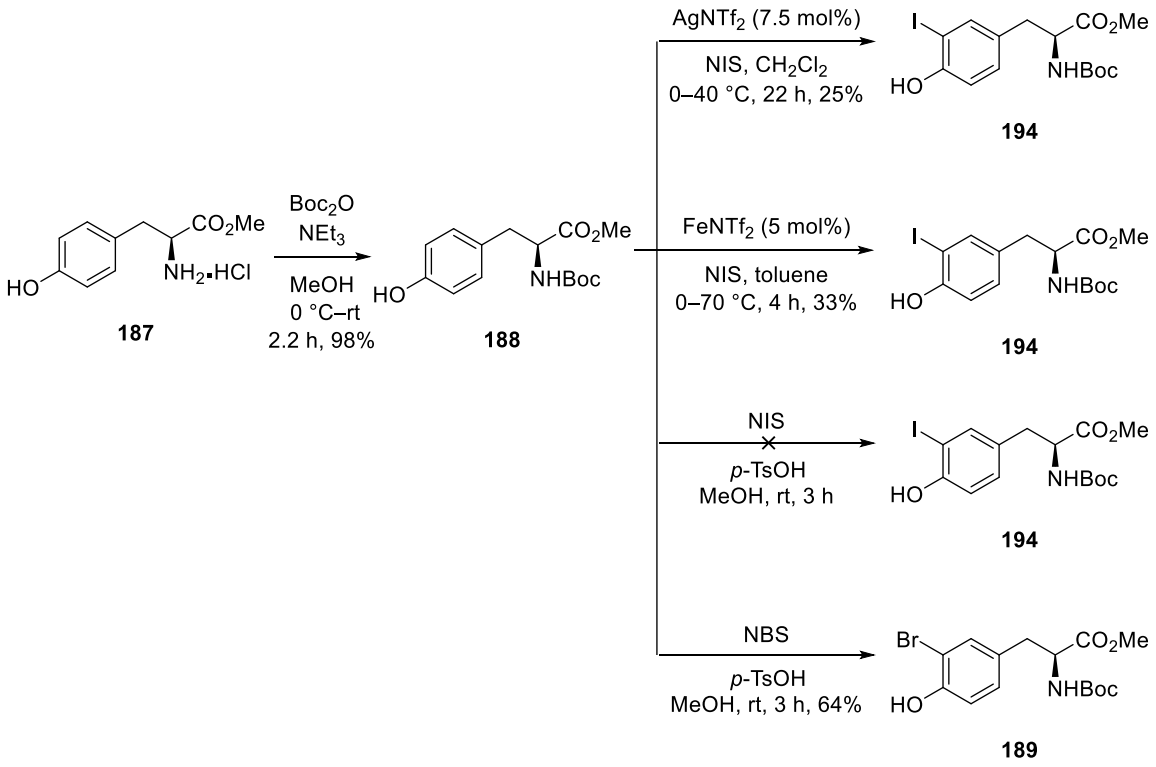
Figure 60: Proposed library of 3'-aryl tyrosine analogues.

A major drawback of the current synthesis is the harsh reaction conditions required for the Suzuki-Miyaura cross-coupling reaction. Reactions carried out at high temperatures under basic conditions can result in racemisation of the stereogenic centre and hence lower the enantiomeric purity of the desired amino acid. As shown in Section 2.2.5, the Suzuki-Miyaura conditions described by Buchwald and co-workers allowed for generation of a diverse benzotriazinone-based α-amino acid library under mild conditions. An investigation into utilising this cross-coupling

reaction as a key step for the synthesis of the tyrosine-derived amino acids was thus to be carried out.

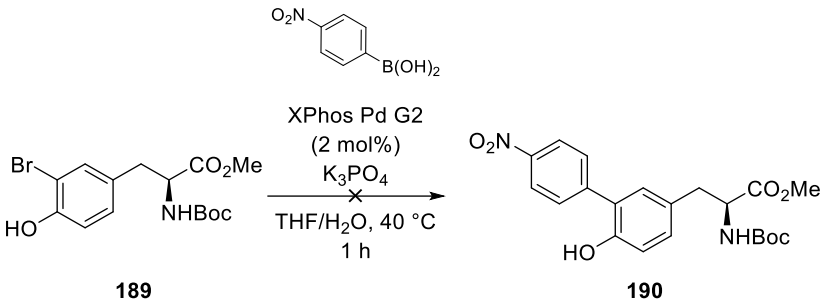
2.4.3 Route Optimisation

Firstly, Boc-protection of methyl (2*S*)-2-amino-3-(4'-hydroxyphenyl)propanoate hydrochloride (**187**) occurred in an excellent 98% yield under standard conditions using Boc_2O and triethylamine (Scheme 88). Iodination of the protected material was then attempted using *N*-iodosuccinimide and silver(I) trifilimide as it was proposed a more reactive carbon-iodine bond would allow for a milder cross-coupling reaction.[148] Formation of desired iodinated product **194** occurred in only 25% yield and was confirmed by ^{1}H NMR spectroscopy, which showed a coupling pattern consistent with the structure. When the reaction was attempted using iron(III) trifilimide, an improved yield of 33% was obtained.[114] The reaction was also attempted in the presence of catalytic amounts of *p*-tosic acid. However, the ^{1}H NMR spectrum of the crude material indicated this resulted in a complex mixture of starting material, product and di-iodinated material. The bromination strategy from the original synthesis was therefore employed, generating desired product **189** in a 64% yield after 3 h at room temperature.



Scheme 88: Halogenation reactions of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4'-hydroxyphenyl)propanoate (**188**).

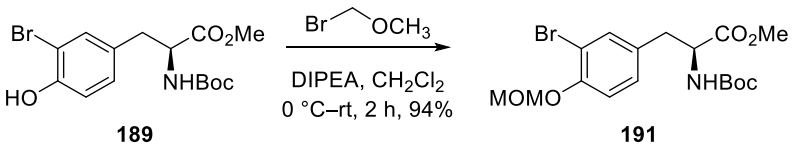
Initially, the Suzuki-Miyaura cross-coupling reaction was attempted under the conditions described in Section 2.2.5 using XPhos Pd G2 (2 mol%) (Scheme 89).[116] However, TLC analysis of the reaction mixture indicated no conversion of starting material after 1 h at 40 °C.



189 **190**

Scheme 89: Attempted Suzuki-Miyaura cross-coupling of **189** to generate biphenyl **190**.

As discussed in Section 2.4.1, issues were encountered with the cross-coupling reaction due to the presence of the free hydroxy group in the 4'-position. As it is proposed this was also the case in this instance, protection of the hydroxy group was carried out using bromomethyl methyl ether (Scheme 90). This occurred in an excellent 94% yield to give ether **191**.

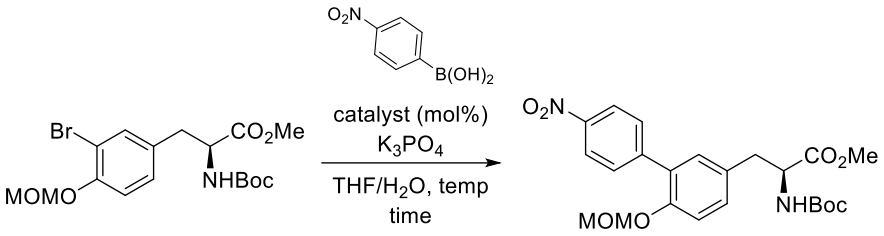


189 **191**

Scheme 90: MOM-Protection of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3'-bromo-4'-hydroxyphenyl)propanoate (**189**).

The Suzuki-Miyaura cross-coupling reaction was then re-attempted utilising the MOM-protected material and XPhos Pd G2 (2 mol%). However, no conversion to desired product was identified by ^{1}H NMR spectroscopy after 1 h at 40 °C (Table 12, entry 1). The catalyst loading was therefore increased from 2 mol% to 5 mol% (entry 2). After 1 h at 40 °C, the ^{1}H NMR spectrum again showed no conversion to desired product. The reaction was therefore heated to 70 °C and stirred for a further 3 h. This resulted in a 39% yield of biphenyl **192c**. It was found that heating at 60 °C from the beginning of the reaction generated **192c** in 70% yield (entry 3). The reaction was then repeated utilising XPhos Pd G3 as the catalyst (entry 4). XPhos Pd G3 replaces the chloride present in XPhos Pd G2 with a non-coordinating

methanesulfonate. The increased electron-withdrawing ability of the methanesulfonate allows for activation of the catalyst at lower temperatures by reduction of the electron density at the palladium centre.[149] This generated **192c** in a 73% yield after only 1 h at 40 °C. The reaction was also attempted at room temperature (entry 5). However, this showed a 1:1 mixture of product and starting material by ^{1}H NMR spectroscopy after 2 h. A reduction in the catalyst loading resulted in a decreased yield of 61% (entry 6). The optimised reaction conditions of entry 4 were therefore used for subsequent transformations.

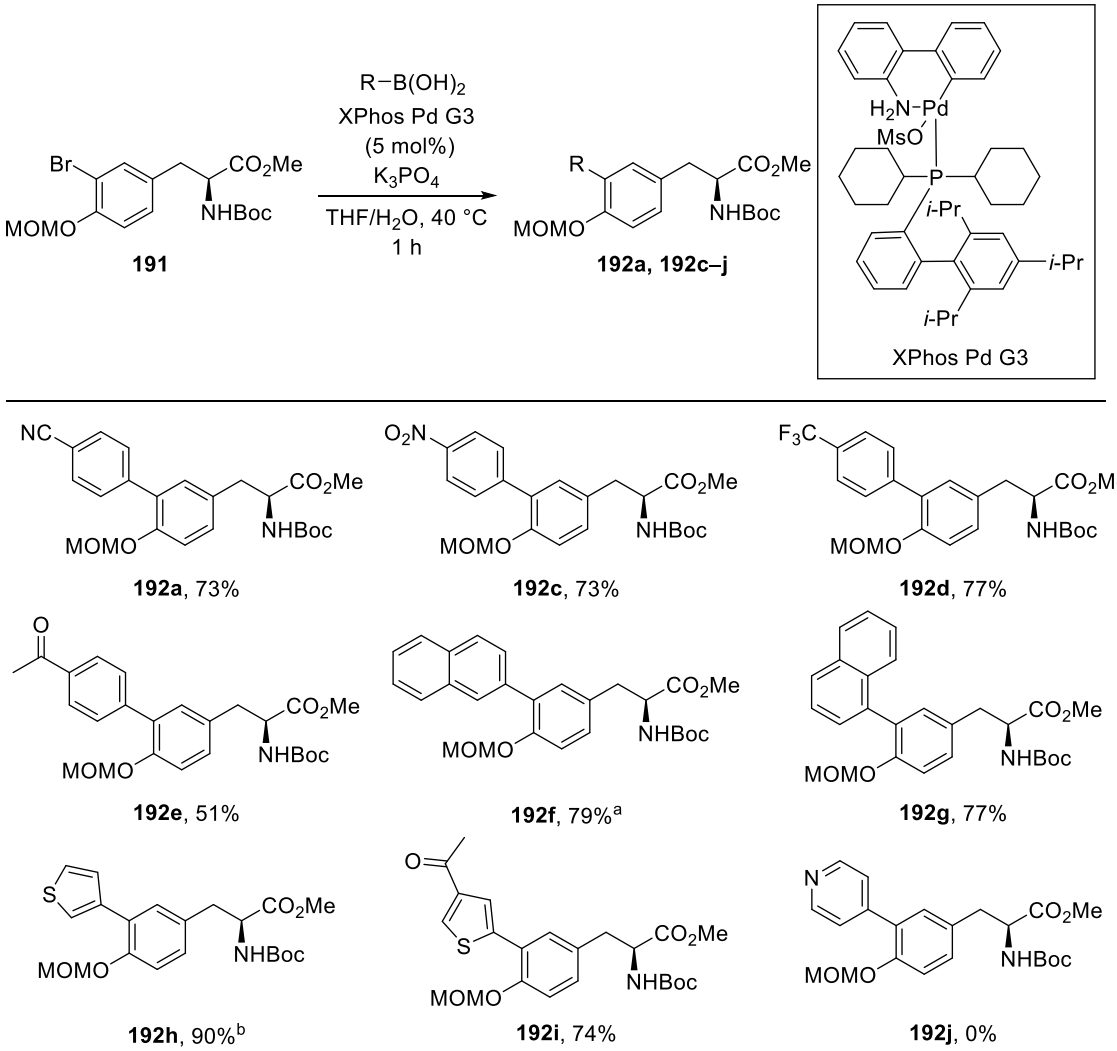


191 **192c**

Entry	Catalyst (mol%)	Temperature (° C)	Time (h)	Yield (%)
1	XPhos Pd G2 (2 mol%)	40 °C	1 h	N/A
2	XPhos Pd G2 (5 mol%)	40–70 °C	4 h	39%
3	XPhos Pd G2 (5 mol%)	60 °C	1 h	70%
4	XPhos Pd G3 (5 mol%)	40 °C	1 h	73%
5	XPhos Pd G3 (5 mol%)	rt	2 h	N/A
6	XPhos Pd G3 (2.5 mol%)	40 °C	1 h	61%

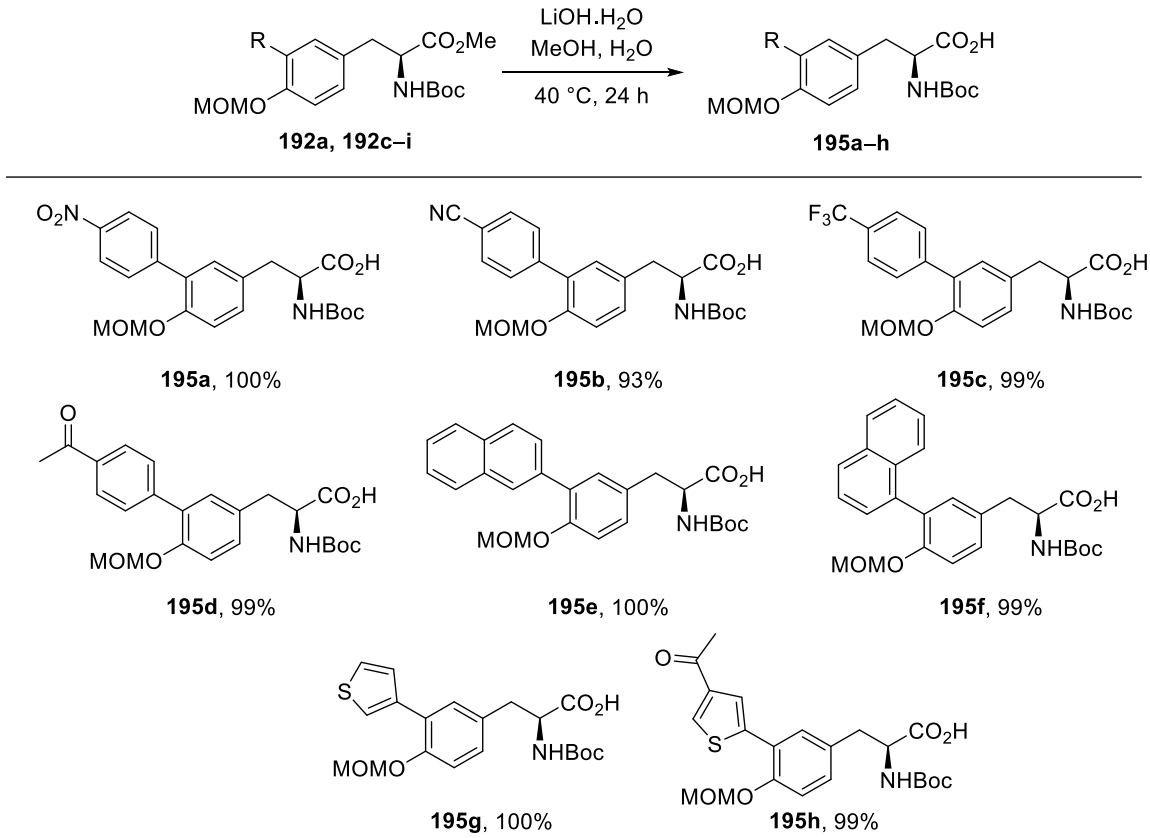
Table 12: Optimisation of Suzuki-Miyaura cross-coupling reaction of **191**.

Following optimisation, the substrate scope of the Suzuki-Miyaura cross-coupling was then explored (Scheme 91). Both the 4''-nitrophenyl and 4''-cyanophenyl analogues were generated in improved yields of 71% and 77%, respectively. Electron-deficient boronic acids **192d** and **192e** were found to undergo the cross-coupling reaction in high yields. The reaction also generated naphthyl analogue **192f** in a 79% yield *via* portion-wise addition of the catalyst (2 × 2.5 mol%). Thiophene analogues **192h** and **192i** were also found to be compatible with the procedure. The reaction was attempted using 4-pyridylboronic acid. However, this showed no conversion of starting material by ^{1}H NMR spectroscopy after 1 h at 40 °C.



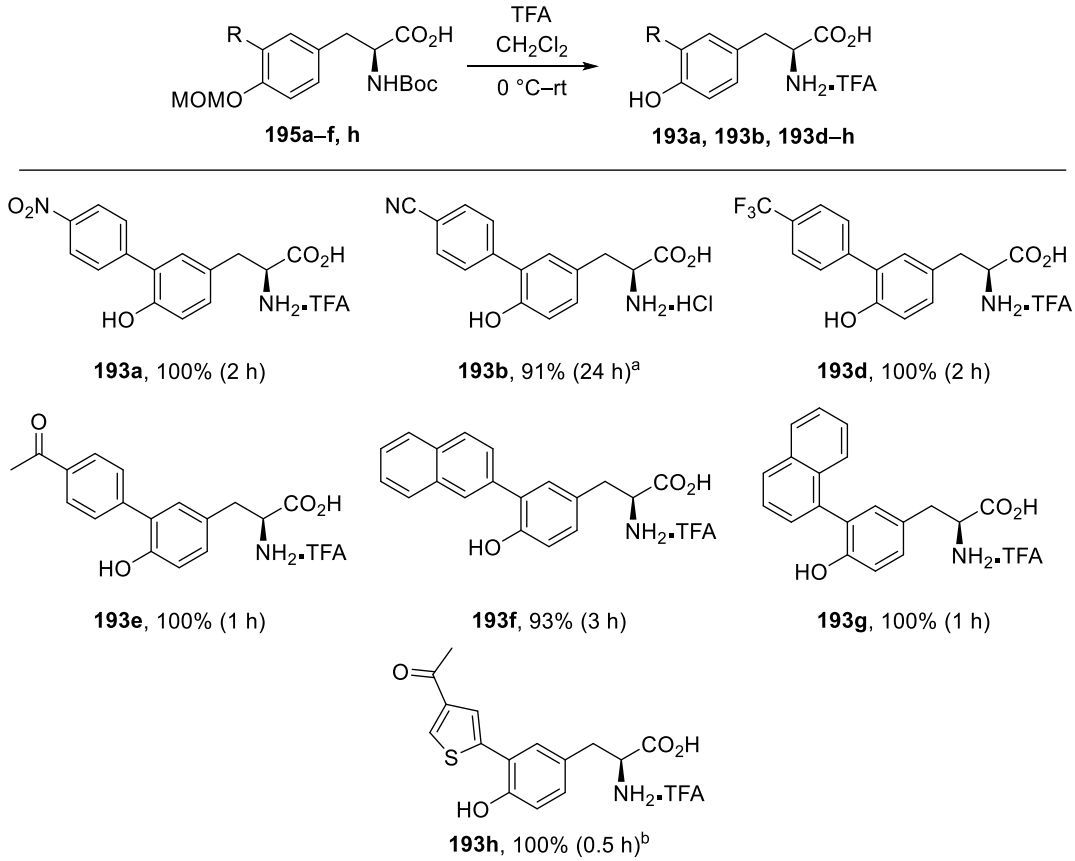
Scheme 91: Substrate scope of Suzuki-Miyaura cross-coupling reaction of **191**. [a]Catalyst added as 2 × 2.5 mol% batches; [b]XPhos Pd G2 used as catalyst, 60 °C for 1 h.

Subsequent ester hydrolysis was then carried out using lithium hydroxide (Scheme 92). This gave the corresponding carboxylic acids in excellent yields for all analogues.



Scheme 92: Ester hydrolysis of 3'-aryl tyrosine analogues.

Finally, simultaneous removal of the MOM and Boc-protecting groups was achieved stirring in trifluoroacetic acid and dichloromethane (Scheme 93). This gave the desired deprotected α-amino acids in high yields. It should be noted that partial decomposition was observed for 4''-cyanophenyl analogue **195b**. The reaction was therefore repeated using hydrochloric acid. This generated deprotected amino acid **193b** in an excellent 91% yield. It was found that deprotection of unsubstituted thiophene analogue **195g** was not possible. It is believed the acidic conditions resulted in partial decomposition of the desired product. This was also found to occur for 4''-acetylthiophenyl analogue **195h**. However, this was overcome by cooling the reaction to 0 °C and stirring for 3 h, before warming to room temperature.



Scheme 93: Generation 3'-aryl tyrosine analogues *via* MOM and Boc-group removal. [a]Reaction carried out using 2 M HCl; [b]Reaction stirred at 0 °C for 3 h prior to warming to room temperature.

2.4.4 Fluorescent Properties of 3'-Aryl Tyrosine Analogues

Following successful synthesis of a range of 3'-aryl tyrosine analogues, the absorption and emission data for these compounds (**193a, 193b, 193d–h**) was recorded (Figures 61–67).

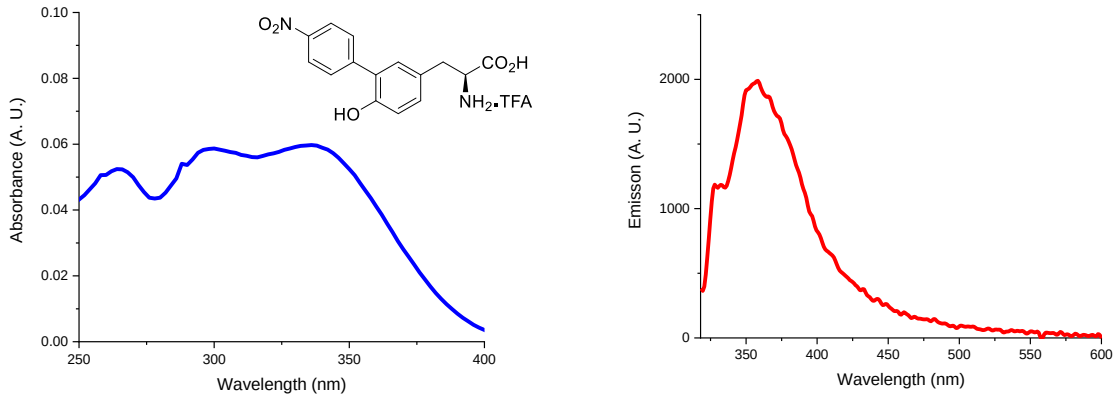


Figure 61: Absorption and emission spectra of **193a** (5 µM in MeOH, excitation at 300 nm).

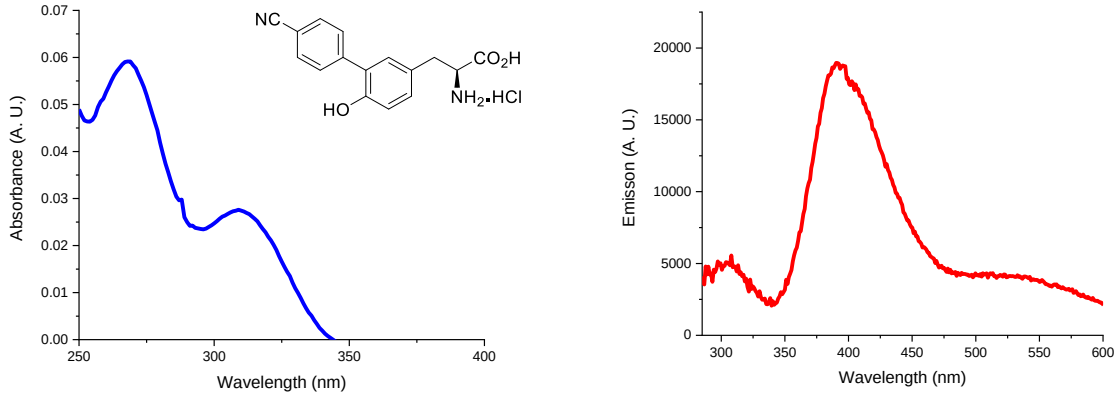


Figure 62: Absorption and emission spectra of **193b** (5 µM in MeOH, excitation at 264 nm).

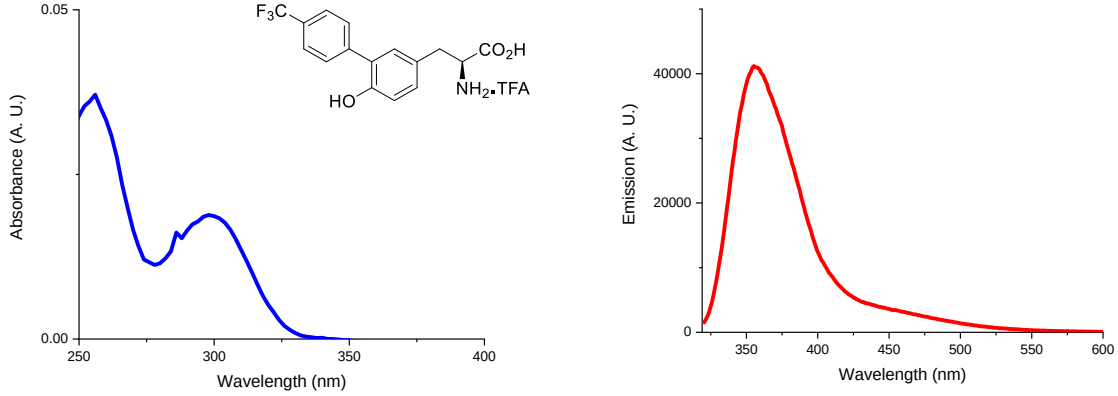


Figure 63: Absorption and emission spectra of **193d** (5 µM in MeOH, excitation at 301 nm).

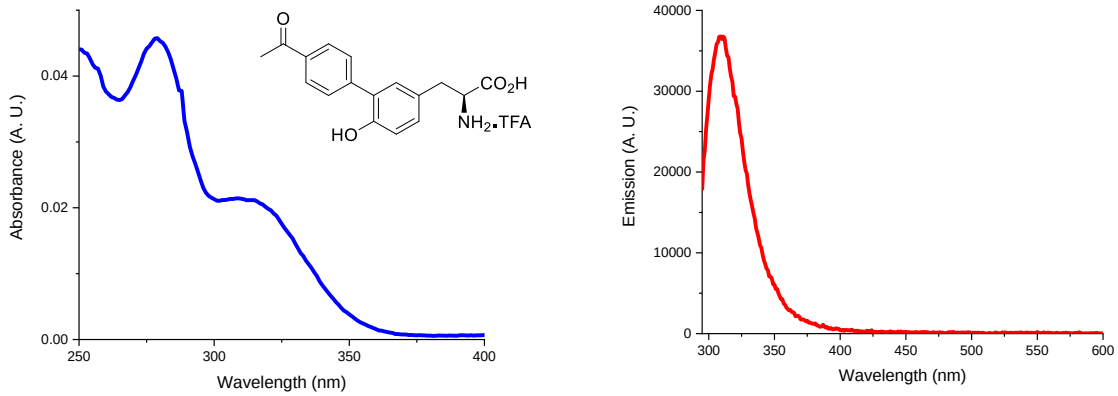


Figure 64: Absorption and emission spectra of **193e** (2.5 µM in MeOH, excitation at 275 nm).

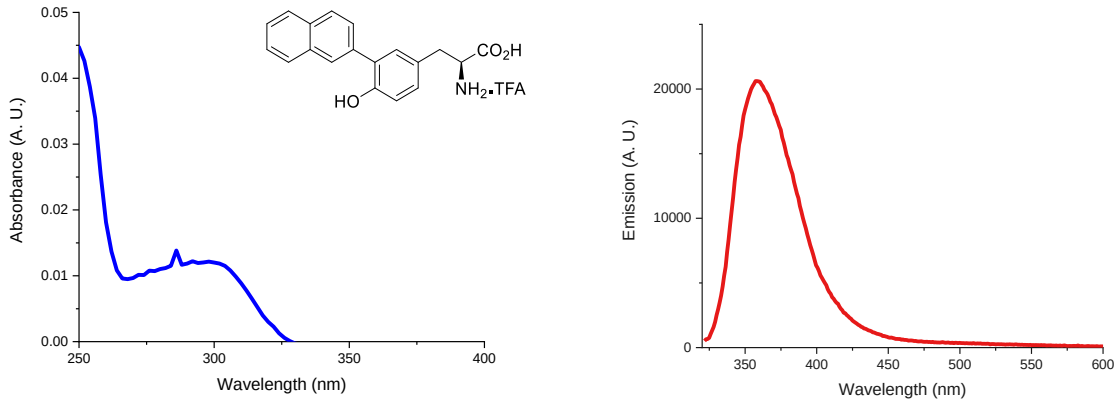


Figure 65: Absorption and emission spectra of **193f** (2 µM in MeOH, excitation at 303 nm).

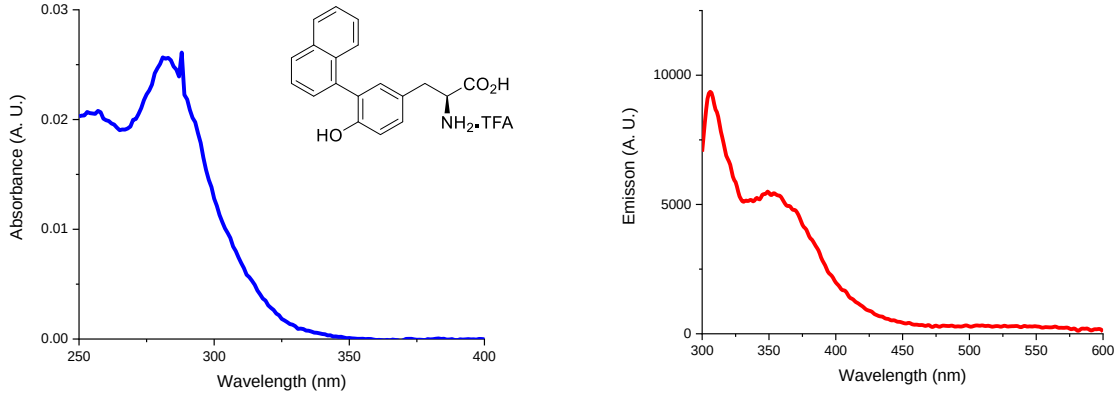


Figure 66: Absorption and emission spectra of **193g** (2 µM in MeOH, excitation at 282 nm).

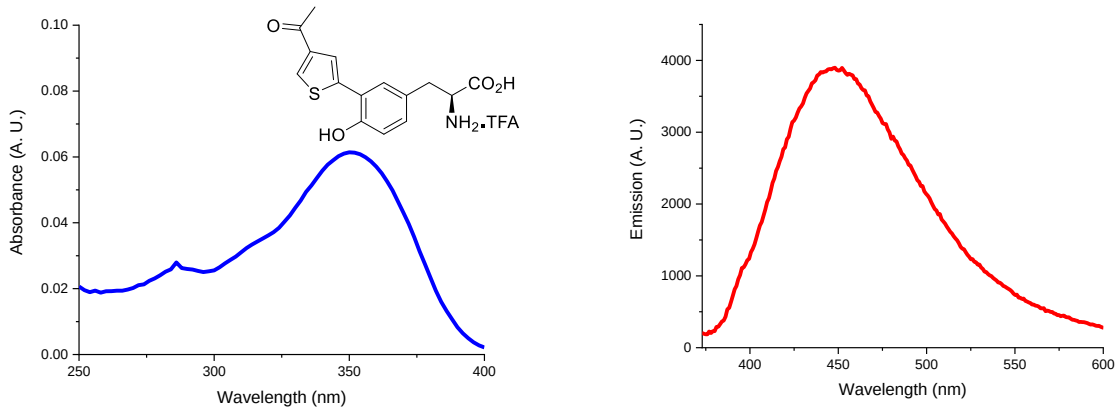


Figure 67: Absorption and emission spectra of **193h** (5 µM in MeOH, excitation at 354 nm).

The fluorescent properties of the amino acids were then further investigated and are summarised in Table 13. Nitro analogue **193a** was found to have a low quantum yield. Incorporation of nitro groups on aromatic rings is reported to quench fluorescence *via* intersystem crossing (ISC) due to the presence of multiple π-π* and n-π* electronic states close in energy.[150] Cyano analogue **193b** was found to have a slightly larger quantum yield as well as a desirable MegaStokes shift of 126 nm. Thiophene analogue **193h** showed red-shifted absorption and emission compared to the other analogues, suggesting strong charge transfer through the system from the 4'-hydroxy to the carbonyl on the thiophene ring. Naphthyl analogue **193f** showed the most promising photoluminescent data with a quantum yield of 0.15 and consequently a brightness of 1120 cm^{-1} M^{-1}. It was concluded that the brightness of these analogues would need to be improved to be considered for potential biological imaging applications.

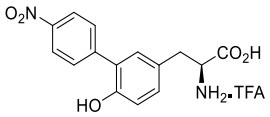
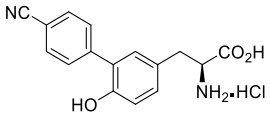
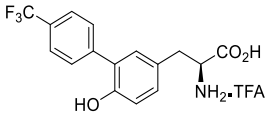
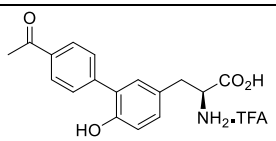
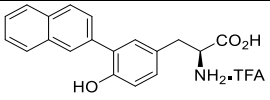
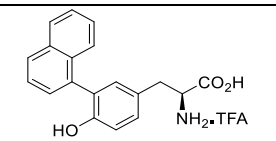
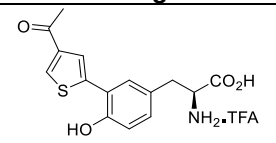
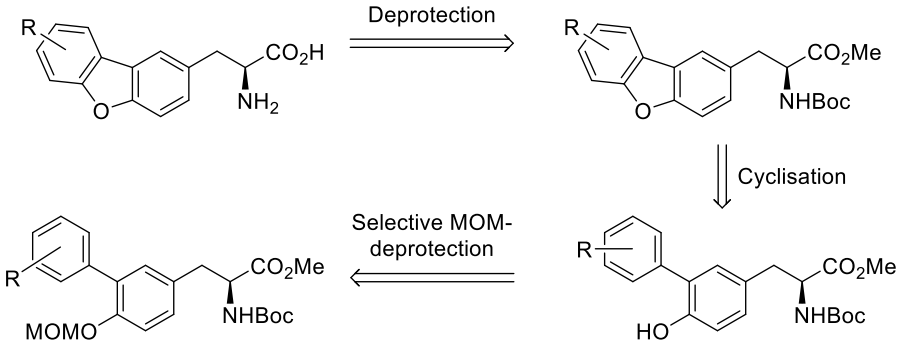
Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
 **193a**	300[a] 336	358	22 58	12200	0.00070	10
 **193b**	264[a] 309	390	81 126	10000	0.040	370
 **193d**	298	355	57	4300	0.047	200
 **193e**	275[a] 308	311	3 36	19600	0.054	1050
 **193f**	286	358	72	10600	0.15	1120
 **193g**	288	305 350	17 62	8600	0.040	340
 **193h**	350	448	98	12000	0.014	170

Table 13: Photoluminescence properties of 3'-aryl tyrosine analogues. [a]Excitation wavelength.

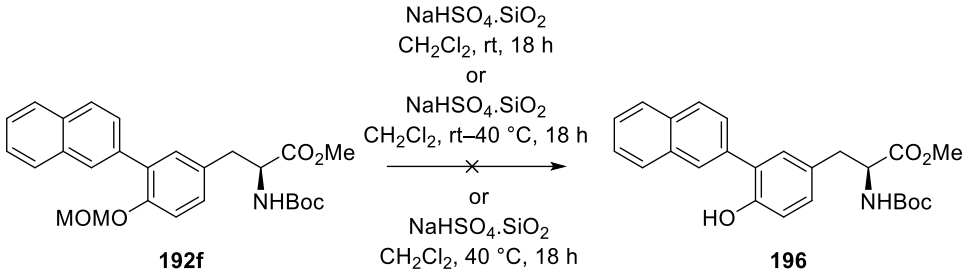
2.4.5 Cyclisation of 3'-Aryl Tyrosine Amino Acid 193f

It was proposed that increasing the rigidity of the tyrosine-based amino acids would result in an improved fluorescence quantum yield by preventing alternative, non-radiative, modes of relaxation.[44] This would thus improve the overall brightness. Retrosynthetic analysis of the proposed dibenzofuran heterocycle is shown in Scheme 94. Firstly, selective MOM-group removal would generate the free phenol. This could then participate in a C–H bond activated cyclisation to give the corresponding dibenzofuran. Finally, removal of the methyl ester and Boc-protecting groups would give the desired amino acids which could be evaluated for their photoluminescent properties.



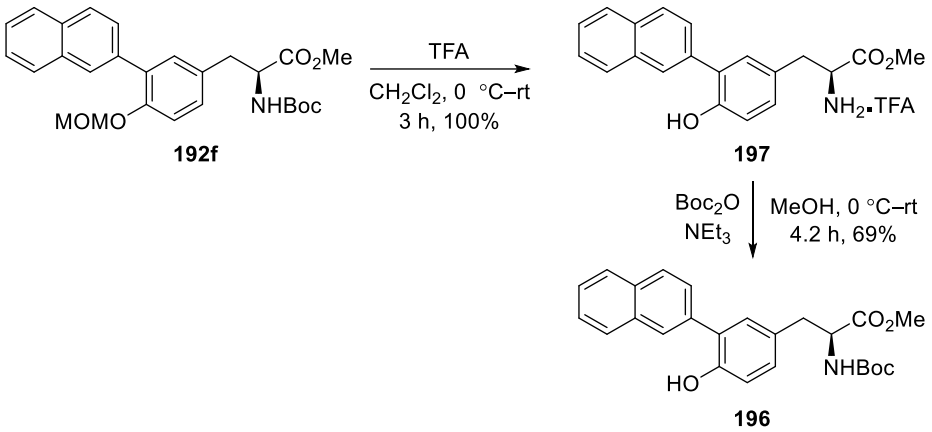
Scheme 94: Retrosynthetic analysis of proposed dibenzofuran amino acids.

Initially, selective removal of the MOM-group was investigated. Work by Das and co-workers has shown MOM-groups can be selectively deprotected in the presence of Boc-protecting groups using sodium hydrogen sulfate as a heterogeneous catalyst.[151] However, it was found that when utilising these conditions, no conversion to desired deprotected material was observed by ^{1}H NMR spectroscopy (Scheme 95). The reaction was thus repeated, stirring at room temperature for 2 h followed by heating to 40 °C for 18 h. However, this also resulted in no conversion of starting material. Finally, the reaction was repeated, heating to 40 °C from the beginning with an additional three equivalents of catalyst. In this instance, although the ^{1}H NMR spectrum indicated removal of the MOM-protecting group, removal of the Boc-group also occurred.



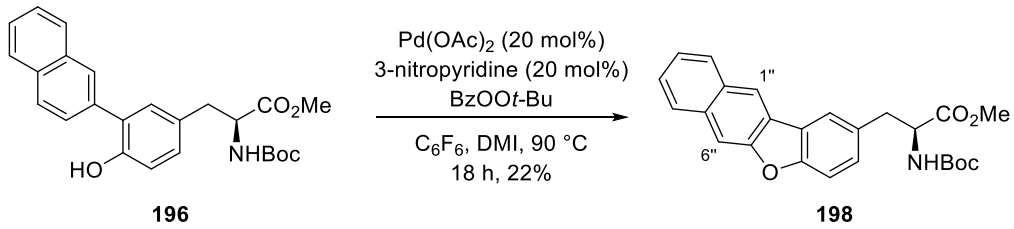
Scheme 95: Attempted selective MOM-group removal of **192f**.

Alternatively, the MOM and Boc-protecting groups were simultaneously removed using trifluoroacetic acid (Scheme 96). Free amine **197** was then re-protected using Boc_2O and triethylamine under standard conditions to give **196** in a 69% yield.



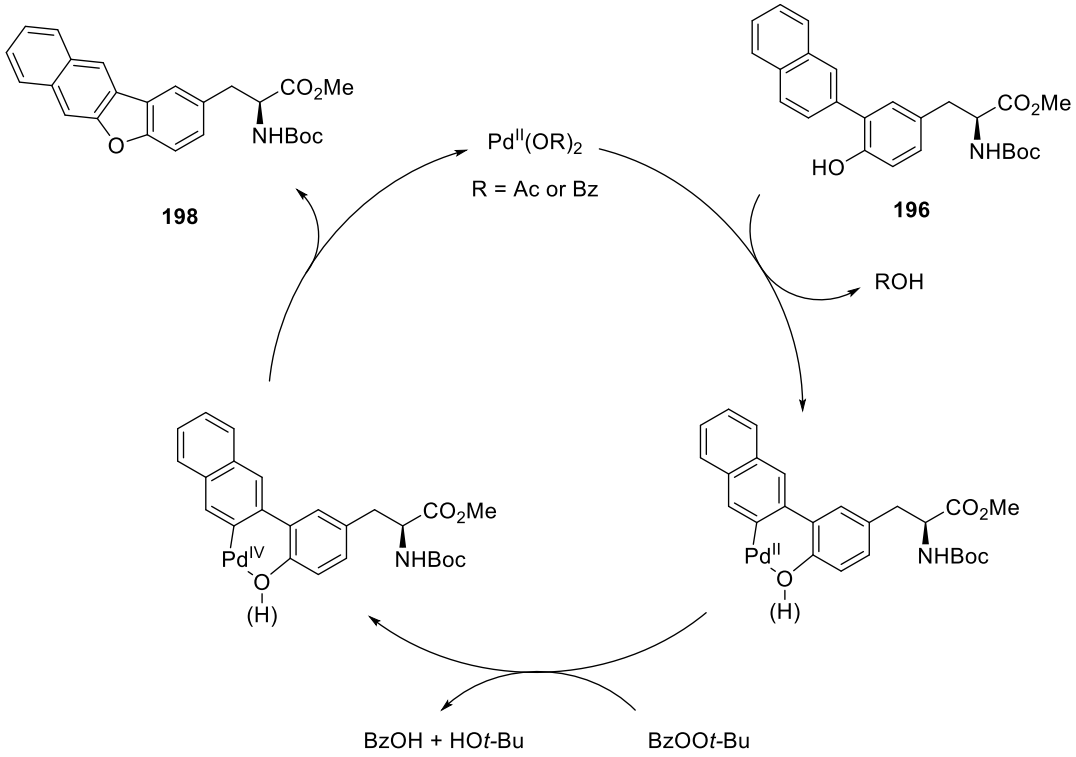
Scheme 96: Synthesis of **196** *via* MOM/Boc-group removal and subsequent Boc-protection.

Cyclisation of phenol **196** was then investigated using a Pd(II)/Pd(IV)-catalysed transformation, involving palladium acetate and *tert*-butyl peroxybenzoate as an oxidant, as described by Wei and Yoshikai, in a proof of concept experiment (Scheme 97).[152] Initial attempts showed little or no cyclisation but, increasing the catalyst loading to 20 mol% gave the corresponding naphthofuran **198** in a 22% yield. Formation of **198** as a single regioisomer was indicated by the presence of two singlets in the ^{1}H NMR spectrum at 7.90 ppm and 8.36 ppm for 6''-H and 1''-H, respectively.



Scheme 97: Cyclisation of **196** to give dibenzofuran α-amino acid **198**.

As highlighted above, the cyclisation is proposed to occur *via* a Pd(II)/Pd(IV) catalytic cycle (Scheme 98). Presence of the 4'-hydroxy group allows for phenol-directed C–H bond activation. It is proposed oxidation of the Pd(II) intermediate then occurs by *tert*-butyl peroxybenzoate to give the Pd(IV) species. This undergoes subsequent reductive elimination to give the desired dibenzofuran whilst simultaneously regenerating the palladium(II) catalyst. As noted, the C–H bond activation was found to occur regioselectively to give **198** as the sole product. This is likely due to C–H activation of the least sterically hindered *ortho*-position.



Scheme 98: Proposed mechanism for formation of amino acid **198**.

Following synthesis of naphthofuran **198**, absorption and emission spectra were recorded (Figure 68).

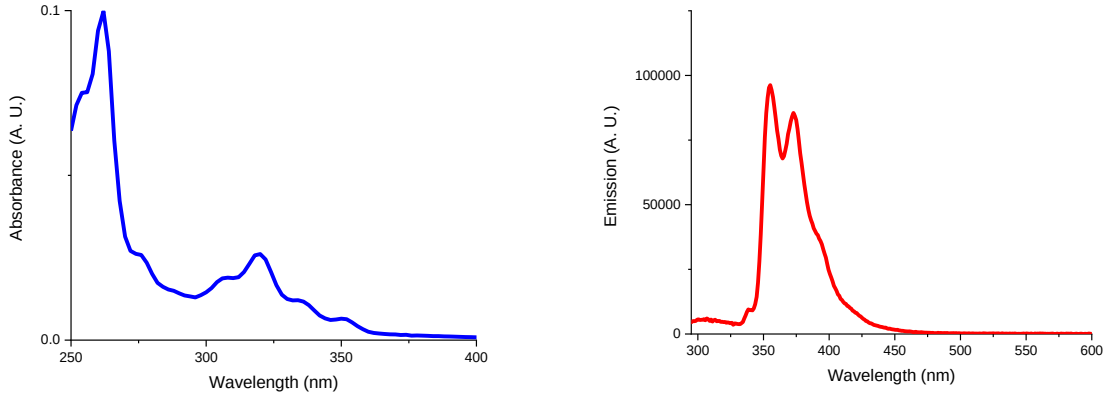


Figure 68: Absorption and emission spectra of **198** (1.25 µM in MeOH, excitation at 275 nm).

Photoluminescent data for **198** is summarised in Table 14. It was found that cyclisation of naphthyl analogue **196** resulted in red-shifted absorption and emission maxima, as well as a significant increase in the molar attenuation coefficient. Although naphthofuran **198** showed only a small improvement in the quantum yield

(from 0.15 to 0.16), the much stronger absorbance resulted in a ten-fold increase in brightness.

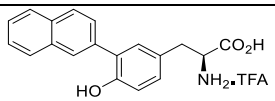
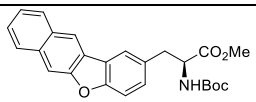
Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Molar Attenuation Coefficient (cm^{-1} M^{-1})	Quantum Yield (φ)	Brightness (cm^{-1} M^{-1})
 **193f**	286	358	72	10600	0.15	1120
 **198**	320	353 370	33, 50	78000	0.16	12100

Table 14: Photoluminescent data of cyclised analogue **198**.

2.4.6 Conclusions

To conclude, a range of tyrosine-derived novel fluorescent α-amino acids have been synthesised. The route originally developed within the Sutherland group has been optimised to give the desired amino acids under mild conditions. By employing XPhos Pd G3 as the catalyst for the Suzuki-Miyaura cross-coupling reaction, desired coupled products were generated in high yields after 1 h at 40 °C. Fluorescence data was obtained on all novel amino acids, with naphthyl derivative **193f** showing the most promising photoluminescent properties. In an attempt to improve the fluorescence quantum yield of these analogues, a palladium-catalysed oxidative cyclisation was attempted on hydroxy analogue **196**. This generated dibenzofuran **198** in a 22% yield. Fluorescence data for **198** showed a tenfold increase in overall brightness (1120 cm^{-1} M^{-1} to 12100 cm^{-1} M^{-1}) compared to deprotected-uncyclised derivative **193f**. This is a result of the significant improvement in molar attenuation coefficient. Cyclisation also resulted in red-shifted absorption and emission data.

2.4.7 Future Work

Future work on this project will involve optimisation of the cyclisation procedure for the synthesis of the dibenzofuran heterocycles (Figure 69). Once optimised, the procedure will be applied to other analogues to generate a small library of novel, fluorescent dibenzofuran α-amino acids. Following deprotection, the fluorescent properties of these analogues will be evaluated. Based on the findings, a lead compound may be chosen for further investigation as a biological imaging agent.

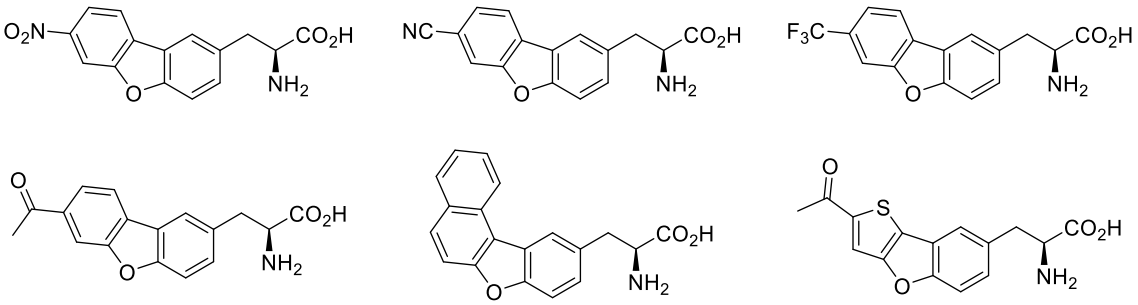


Figure 69: Proposed library of dibenzofuran α-amino acids.

3.0 Experimental

3.1 General Experimental

All reagents and starting materials were obtained from commercial sources and used as received. All dry solvents were purified using a PureSolv 500 MD solvent purification system. Brine refers to a saturated aqueous solution of sodium chloride. Flash column chromatography was performed using Merck Millipore matrix silicagel 60 (40–63 μM). Merck aluminium-backed plates pre-coated with silica gel 60F_{254} were used for thin layer chromatography and were visualised with a UV lamp or by staining with $KMnO_4$ or ninhydrin. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer or a Bruker DPX 500 spectrometer and data are reported as follows: chemical shift in ppm relative to tetramethylsilane (δ_H 0.00 and δC 0.00), or for ^{1}H NMR, relative to residual chloroform (δ_H 7.26), dimethylsulfoxide (δ_H 2.50) or methanol (δ_H 3.31) as standard. For ^{13}C NMR the chemical shifts are reported relative to the central resonance of $CDCl_3$ (δ_C 77.2), DMSO-d_6 (δ_C 39.5) or CD_3OD (δ_C 49.0) as standard. Assignments are based on two-dimensional COSY, HSQC, HMBC and DEPT experiments. Infrared spectra were recorded on a Shimadzu IR Prestige-21 spectrometer or a Shimadzu FTIR-84005 spectrometer; wavenumbers are indicated in cm^{-1}. Mass spectra were obtained either using a JEOL JMS-700 spectrometer for EI and CI, and Bruker Microtof-q or Agilent 6125B for ESI. Melting points were determined on a Reichert platform melting point apparatus or Stuart Scientific melting point apparatus. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using an Autopol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^{-1} g^{-1}.

Absorption and emission data were recorded on one of two instruments:

1. UV-Vis spectra were recorded on a Pekin Elmer Lamda 25 instrument. Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Emission data were measured using excitation and emission bandpass filters of 3 nm.

2. Both UV-Vis spectra and fluorescence spectra were recorded on a Horiba Duetta Fluorescence and Absorbance spectrometer. Absorbance spectra were recorded with an integration time of 0.05 s and a band pass of 5 nm. Fluorescence spectra were recorded with an excitation and emission band pass of 5 nm, an integration

time of 2.0 s, and with detector accumulations set to 1. Respective standard samples were recorded with the same parameters.

Quantum yields were determined using a comparative method against two standards. Anthracene (Φ = 0.27, in ethanol) and L-tryptophan (Φ = 0.14 in water) were used as standard references. The integrated fluorescence intensity of each compound was determined from the emission spectra given. Measurements were performed at a minimum of four different concentrations. Concentrations were chosen to ensure the absorption value was below 0.1 to avoid re-absorption effects. Integrated fluorescence intensity was plotted as a function of the measured absorbance and a linear fit was calculated. The resultant gradient was then used to calculate the quantum yield, using the equation below:

$$\phi_x = \phi_{ST}\left(\frac{Grad_{ST}}{Grad_x}\right)\left(\frac{\eta_x^2}{\eta_{ST}^2}\right)$$

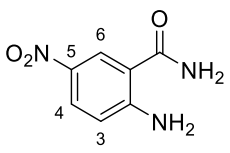
Subscript *ST* signifies the quantities associated with the quantum yield standard. Subscript *X* signifies the quantities associated with the novel compound. $Grad_{ST}$ is the determined gradient associated with the quantum yield standard. $Grad_x$ is the determined gradient associated with the novel compound. η is the refractive index of the solvent used in the fluorescence measurements. η = 1.333 for water, 1.361 for ethanol and 1.331 for methanol.

3.2 Benzotriazinones and Benzothiatriazinedioxides Experimental

General Procedure for Preparation of Polymer Supported Nitrite Resin

To a stirred solution of sodium nitrite (5.50 g, 80.0 mmol) in water (200 mL) was added Amberlyst A26 hydroxide form resin (10.0 g, 40.0 mmol). The reaction mixture was stirred at rt for 1 h. The polymer-supported resin was filtered and washed with water until the filtrate was of neutral pH.

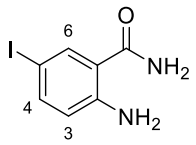
2-Amino-5-nitrobenzamide (5d)[5]



To a stirred solution of 2-amino-5-nitrobenzoic acid (0.150 g, 0.820 mmol) and hydroxybenzotriazole (0.122 g, 0.910 mmol) in *N*,*N*'-dimethylformamide (3 mL) was

added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.184 g, 0.910 mmol). The reaction mixture was stirred at room temperature for 2 h, cooled to 0 °C and 28% aqueous ammonia solution (83 µL) was added. The mixture was allowed to warm to room temperature and stirred for a further 2 h. The reaction mixture was diluted in ethyl acetate (30 mL) and washed with 5% aqueous sodium bicarbonate (30 mL). The organic layer was then washed with water (3 × 20 mL) and brine (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The reaction mixture was triturated with ethyl acetate:hexane (1:1) and the resulting solid was filtered to give 2-amino-5-nitrobenzamide (**5d**) (0.116 g, 78%) as a yellow solid. Mp 232–236 °C (lit.[153] 236 °C); δ_H (400 MHz, DMSO-d_6) 6.79 (1H, d, *J* 9.2 Hz, 3-H), 7.42 (1H, br s, NH), 7.91 (2H, br s, NH_2), 8.03 (1H, dd, *J* 9.2, 2.6 Hz, 4-H), 8.22 (1H, br s, NH), 8.55 (1H, d, *J* 2.6 Hz, 6-H); δ_C (101 MHz, DMSO-d_6) 112.1 (C), 116.0 (CH), 126.4 (CH), 127.6 (CH), 134.8 (C), 155.7 (C), 169.7 (C); *m*/*z* (EI) 181 (M^+. 82%), 164 (57), 133 (53), 90 (61), 78 (100), 63 (84).

2-Amino-5-iodobenzamide (5i)[154]



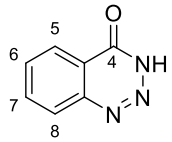
Iodine (0.373 g, 1.47 mmol) was added in portions over 1 h to a stirred solution of 2-aminobenzamide (0.200 g, 1.47 mmol) and sodium hydrogen carbonate (0.123 g, 1.47 mmol) in water (49 mL). The reaction mixture was then heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to room temperature, washed with 1 M aqueous sodium thiosulfate (10 mL) and then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation from water:methanol (10:1) gave 2-amino-5-iodobenzamide (**5i**) (0.277 g, 72%) as a brown solid. Mp 191–194 °C (lit.[154] 193–194 °C); δ_H (400 MHz, DMSO-d_6) 6.54 (1H, d, *J* 8.7 Hz, 3-H), 6.69 (2H, br s, NH_2), 7.13 (1H, br s, NH), 7.37 (1H, dd, *J* 8.7, 2.1 Hz, 4-H), 7.80 (1H, d, *J* 2.1 Hz, 6-H); δ_C (101 MHz, DMSO-d_6) 74.4 (C), 116.1 (C), 118.9 (CH), 136.5 (CH), 139.8 (CH), 149.7 (C), 169.9 (C); *m*/*z* (EI) 262 (M^+. 50%), 244 (100), 117 (48), 90 (33).

General Procedure for Synthesis of 1*H*-Benzotriazinones

To a stirred solution of the 2-aminobenzamides (1 equiv.) in methanol (10 mL/mmol) at 0 °C was added polymer-supported nitrite (containing 3.0 equiv. of NO_2) and *p*-toluenesulfonic acid monohydrate (3 equiv.). The reaction mixture was stirred for

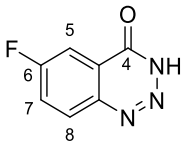
1 h at 0 °C. The reaction mixture was then warmed to room temperature and stirred until completion. The resin was filtered and washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography gave the 1*H*-benzotriazinones.

1,2,3-Benzotriazin-4(3*H*)-one (1a)[155]



The reaction was carried out as described in the general procedure using anthranilamide (0.303 g, 2.20 mmol), polymer-supported nitrite (1.89 g, containing 6.60 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (1.26 g, 6.60 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred 1 h. Purification by flash column chromatography, eluting with 35% ethyl acetate in hexane gave 1,2,3-benzotriazin-4(3*H*)-one (**1a**) (0.220 g, 65%) as a white solid. Mp 212–215 °C (lit.[155] 215–217 °C); δ_H (400 MHz, DMSO-d_6) 7.91 (1H, br t, *J* 7.7 Hz, 7-H), 8.08 (1H, br t, *J* 7.7 Hz, 6-H), 8.18 (1H, br d, *J* 7.7 Hz, 8-H), 8.22 (1H, br d, *J* 7.7 Hz, 5-H), 14.94 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 120.2 (C), 124.3 (CH), 127.8 (CH), 132.6 (CH), 135.5 (CH), 144.2 (C), 155.6 (C); *m*/*z* (EI) 147 (M^+. 100%), 104 (22), 92 (69), 76 (72), 63 (83).

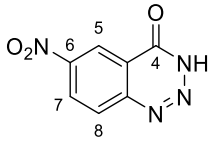
6-Fluoro-1,2,3-benzotriazin-4(3*H*)-one (1c)[156]



The reaction was carried out as described in the general procedure using 2-amino-5-fluorobenzamide (0.150 g, 0.970 mmol), polymer-supported nitrite (0.836 g, containing 2.92 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.556 g, 2.92 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 1.5 h. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave 6-fluoro-1,2,3-benzotriazin-4(3*H*)-one (**1c**) (0.125 g, 78%) as a white solid. Spectroscopic data were consistent with the literature.[156] Mp 206–208 °C; δ_H (400 MHz, DMSO-d_6) 7.91–8.00 (2H, m, 5-H and 7-H), 8.31 (1H, ddd, *J* 8.8, 5.0, 0.4 Hz, 8-H), 15.04 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 109.5 (CH, $^2J_{CF}$ 24.0 Hz), 122.4 (C, $^3J_{CF}$ 9.4 Hz), 123.9 (CH, d, $^2J_{CF}$ 24.4 Hz), 131.6 (CH, d, $^3J_{CF}$ 9.5 Hz), 141.5 (C, d, $^4J_{CF}$ 2.2 Hz), 155.0 (C, d, $^4J_{CF}$ 3.1 Hz),

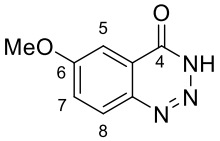
163.2 (C, d, $^{1}J_{CF}$ 253.4 Hz); *m*/*z* (EI) 165 (M^{+}. 20%), 122 (10), 94 (21), 78 (88), 63 (100).

6-Nitro-1,2,3-benzotriazin-4(3*H*)-one (1d)[156]



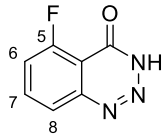
The reaction was carried out as described in the general procedure using 2-amino-5-nitrobenzamide (0.0800 g, 0.442 mmol), polymer-supported nitrite (0.378 g, containing 1.32 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.252 g, 1.32 mmol). The reaction mixture was stirred for 1 h at 0 °C and heated under reflux for 7 h. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 6-nitro-1,2,3-benzotriazin-4(3*H*)-one (**1d**) (0.0480 g, 56%) as a yellow solid. Mp 189–194 °C (lit.[157] 194 °C); δ_H (400 MHz, DMSO-d_6) 8.42 (1H, dd, *J* 8.9, 0.5 Hz, 8-H), 8.76 (1H, dd, *J* 8.9, 2.6 Hz, 7-H), 8.82 (1H, dd, *J* 2.6, 0.5 Hz, 5-H), 15.42 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 120.3 (CH), 121.1 (C), 129.4 (CH), 130.1 (CH), 146.4 (C), 148.5 (C), 154.8 (C); *m*/*z* (EI) 192 (M^{+}. 28%), 149 (28), 84 (82), 66 (100).

6-Methoxy-1,2,3-benzotriazin-4(3*H*)-one (1e)



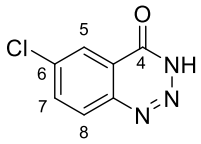
The reaction was carried out as described in the general procedure using 5-methoxy-2-aminobenzamide (0.100 g, 0.600 mmol), polymer-supported nitrite (0.515 g, containing 1.80 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.342 g, 1.80 mmol). The reaction mixture was stirred for 1 h at room temperature and then heated under reflux for 75 h. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave 6-methoxy-1,2,3-benzotriazin-4(3*H*)-one (**1e**) (0.0560 g, 53%) as a white solid. Mp 190–192 °C; ν_{max}/cm^{-1} (neat) 3159 (NH), 2959 (CH), 1662 (C=O), 1296, 1141, 895, 833; δ_H (400 MHz, DMSO-d_6) 3.96 (3H, s, 6-OCH_3), 7.55 (1H, d, *J* 2.8 Hz, 5-H), 7.62 (1H, dd, *J* 9.0, 2.8 Hz, 7-H), 8.12 (1H, d, *J* 9.0 Hz, 8-H), 14.79 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 56.2 (CH_3), 104.2 (CH), 122.0 (C), 124.6 (CH), 130.1 (CH), 139.4 (C), 155.6 (C), 161.9 (C); *m*/*z* (EI) 177.0534 (M^{+}. $C_8H_7N_3O_2$ requires 177.0538), 134 (12%), 106 (38), 78 (79), 63 (100).

5-Fluoro-1,2,3-benzotriazin-4(3*H*)-one (1i)



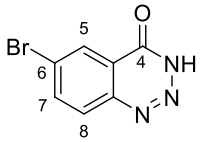
To a stirred solution of 6-fluoro-2-aminobenzamide (0.200 g, 1.30 mmol) in methanol (10 mL) at 0 °C was added polymer-supported nitrite (1.11 g, containing 3.89 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.740 g, 3.89 mmol). The reaction mixture was stirred at 0 °C for 1 h then room temperature for 1 h. The reaction mixture was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 70% diethyl ether in hexane gave 5-fluoro-1,2,3-benzotriazin-4(3*H*)-one (**1i**) (0.144 g, 67%) as a white solid. Mp 200–204 °C; ν_{max}/cm^{-1} (neat) 3161 (NH), 2548 (CH), 2160, 2012, 1700 (C=O), 1480, 1262, 815; δ_H (500 MHz, DMSO-d_6) 7.68 (1H, dd, *J* 10.5, 9.0 Hz, 6-H), 8.00 (1H, br d, *J* 8.0 Hz, 8-H), 8.07 (1H, ddd, *J* 10.5, 8.0, 5.0 Hz, 7-H), 14.97 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 109.9 (C, $^2J_{CF}$ = 8.8 Hz), 118.6 (CH, d, $^2J_{CF}$ = 20.2 Hz), 124.0 (CH, $^4J_{CF}$ = 3.8 Hz), 136.5 (CH, $^3J_{CF}$ = 10.1 Hz), 145.7 (C), 152.7 (C, $^3J_{CF}$ = 1.3 Hz), 158.7 (C, d, $^1J_{CF}$ = 264.6); *m*/*z* (ESI) 166.0410 (MH^+. $C_7H_5FN_3O$ requires 166.0411).

6-Chloro-1,2,3-benzotriazin-4(3*H*)-one (1j)[158]



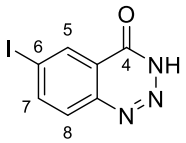
The reaction was carried out as described in the general procedure using 2-amino-5-chlorobenzamide (0.150 g, 0.880 mmol), polymer-supported nitrite (0.759 g, containing 2.65 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.504 g, 2.65 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 1 h. Purification by flash column chromatography, eluting with 35% ethyl acetate in hexane gave 6-chloro-1,2,3-benzotriazin-4(3*H*)-one (**1j**) (0.145 g, 91%) as a white solid. Mp 190–192 °C (lit.[158] 195–196 °C); δ_H (400 MHz, DMSO-d_6) 8.12 (1H, dd, *J* 8.7, 2.4 Hz, 7-H), 8.18 (1H, d, *J* 2.4 Hz, 5-H), 8.22 (1H, d, *J* 8.7 Hz, 8-H), 15.11 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 121.7 (C), 123.5 (CH), 130.2 (CH), 135.6 (CH), 136.9 (C), 142.8 (C), 154.6 (C); *m*/*z* (EI) 181 (M^+. 18%), 138 (7), 110 (11), 78 (88), 63 (100).

6-Bromo-1,2,3-benzotriazin-4(3*H*)-one (1k)[4]



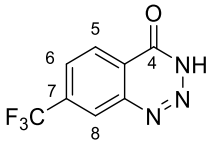
The reaction was carried out as described in the general procedure using 2-amino-5-bromobenzamide (0.150 g, 0.700 mmol), polymer-supported nitrite (0.601 g, containing 2.10 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.399 g, 2.10 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 1 h. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave 6-bromo-1,2,3-benzotriazin-4(3*H*)-one (**1k**) (0.129 g, 82%) as a white solid. Spectroscopic data were consistent with the literature.[4] Mp 207–209 °C; δ_H (400 MHz, DMSO-d_6) 8.12 (1H, d, *J* 8.6 Hz, 8-H), 8.24 (1H, dd, *J* 8.6, 2.2 Hz, 7-H), 8.31 (1H, d, *J* 2.2 Hz, 5-H), 15.10 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 121.7 (C), 125.6 (C), 126.5 (CH), 130.1 (CH), 138.3 (CH), 142.9 (C), 154.3 (C); *m*/*z* (EI) 227 (M^+. 23%), 225 (23), 184 (22), 182 (21), 156 (17), 154 (18), 78 (78), 63 (100).

6-Iodo-1,2,3-benzotriazin-4(3*H*)-one (1l)



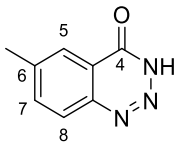
The reaction was carried out as described in the general procedure using 2-amino-5-iodobenzamide (0.150 g, 0.570 mmol), polymer-supported nitrite (0.497 g, containing 1.72 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.372 g, 1.72 mmol). The reaction mixture was stirred for 1 h at 0 °C and then heated under reflux for 18 h. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave 6-iodo-1,2,3-benzotriazin-4(3*H*)-one (**1l**) (0.0910 g, 58%) as a white solid. Mp 127–130 °C; ν_{max}/cm^{-1} (neat) 3071 (NH), 2945 (CH), 1672 (C=O), 1227, 1188; δ_H (400 MHz, CD_3OD) 7.90 (1H, d, *J* 8.6 Hz, 8-H), 8.37 (1H, dd, *J* 8.5, 2.0 Hz, 7-H), 8.64 (1H, d, *J* 2.0 Hz, 5-H); δ_C (101 MHz, CD_3OD) 99.5 (C), 122.8 (C), 130.5 (CH), 134.6 (CH), 145.2 (C), 145.6 (CH), 156.6 (C); *m*/*z* (ESI) 295.9288 (MNa^+. $C_7H_4IN_3NaO$ requires 295.9291).

7-Trifluoromethyl-1,2,3-benzotriazin-4(3*H*)-one (1m)



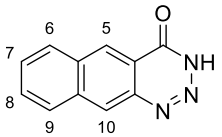
The reaction was carried out as described in the general procedure using 2-amino-4-trifluorobenzamide (0.150 g, 0.730 mmol), polymer-supported nitrite (0.630 g, containing 2.20 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.418 g, 2.20 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 2 h. Purification by flash column chromatography, eluting with 30% ethyl acetate in petroleum ether gave 7-trifluoromethyl-1,2,3-benzotriazin-4(3*H*)-one (**1m**) (0.130 g, 82%) as a white solid. Mp 185–187 °C; ν_{max}/cm^{-1} (neat) 3136 (NH), 1694 (C=O), 1651 (C=C), 1319, 1134, 864; δ_H (400 MHz, DMSO-d_6) 8.20 (1H, br d, *J* 8.3 Hz, 6-H), 8.40 (1H, br d, *J* 8.3 Hz, 5-H), 8.57 (1H, br s, 8-H), 15.25 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 123.1 (C, q, $^1J_{CF}$ 273.4 Hz), 123.1 (C), 125.2 (CH, q, $^3J_{CF}$ 3.2 Hz), 126.3 (CH), 128.1 (CH, q, $^3J_{CF}$ 3.2 Hz), 134.8 (C, d, $^2J_{CF}$ 33.1 Hz), 143.9 (C), 154.8 (C); *m*/*z* (EI) 215.0301 (M^+. $C_8H_4F_3N_3O$ requires 215.0306), 172 (6%), 160 (8), 144 (9), 78 (81), 63 (100).

6-Methyl-1,2,3-benzotriazin-4(3*H*)-one (1n)[159]



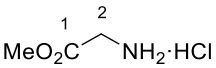
The reaction was carried out as described in the general procedure using 2-amino-5-methylbenzamide (0.100 g, 0.660 mmol), polymer-supported nitrite (0.573 g, containing 2.00 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.380 g, 2.00 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 3.5 h. Purification by flash column chromatography, eluting with 30% ethyl acetate in petroleum ether gave 6-methyl-1,2,3-benzotriazin-4(3*H*)-one (**1n**) (0.0570 g, 53%) as a white solid. Mp 219–220 °C (lit.[159] 216–219 °C); δ_H (400 MHz, DMSO-d_6) 2.54 (3H, s, 6-CH_3), 7.89 (1H, dd, *J* 8.2, 1.6 Hz, 7-H), 8.01 (1H, br s, 5-H), 8.07 (1H, d, *J* 8.2 Hz, 8-H), 14.86 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 21.3 (CH_3), 120.1 (C), 123.6 (CH), 127.8 (CH), 136.7 (CH), 142.6 (C), 143.4 (C), 155.6 (C); *m*/*z* (EI) 161 (M^+. 8%), 104 (8), 89 (6), 78 (82), 63 (100).

3*H*-Naphtho[2,3-*d*][1,2,3]triazin-4-one (1o)



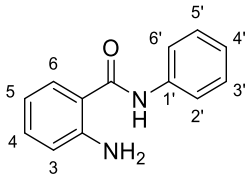
To a stirred solution of 2-aminonaphthalene-3-carboxamide (0.0330 g, 0.177 mmol) in methanol (2 mL) at 0 °C was added polymer-supported nitrite (0.152 g, containing 0.532 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.101 g, 0.532 mmol). The reaction mixture was stirred at 0 °C for 1 h then heated to 40 °C and stirred for 2 h. The reaction mixture was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 80% ethyl acetate in hexane with 1% triethylamine gave 3*H*-naphtho[2,3-*d*][1,2,3]triazin-4-one (**1o**) (0.0290, 83%) as a white solid. Mp 254–257 °C; ν_{max}/cm^{-1} (neat) 3006 (NH), 2850 (CH), 2325, 2072, 1823, 1667 (C=O), 1203, 747; δ_H (400 MHz, DMSO-d_6) 7.73–7.86 (2H, m, 7-H and 8-H), 8.27–8.38 (2H, m, 6-H and 9-H), 8.84 (1H, s, 10-H), 8.91 (1H, s, 5-H), 14.64 (1H, s, NH); δ_C (101 MHz, DMSO-d_6) 117.7 (C), 125.8 (CH), 127.9 (CH), 128.9 (CH), 129.1 (CH), 129.3 (CH), 129.4 (CH), 133.7 (C), 135.7 (C), 140.5 (C), 155.8 (C); *m*/*z* (ESI) 198.0662 (MH^+. $C_{11}H_8N_3O$ requires 198.0662).

Glycine methyl ester hydrochloride (82)[2]



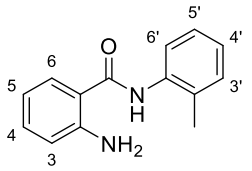
To a stirred solution of glycine (0.150 g, 2.00 mmol) in methanol (2 mL) at −10 °C was added dropwise thionyl chloride (175 µL, 2.40 mmol). The reaction mixture was then heated under reflux for 3 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give glycine methyl ester hydrochloride (**82**) (0.240 g, 96%) as a white solid. Mp 172–175 °C (lit.[160] 172–176 °C); δ_H (500 MHz, D_2O) 3.83 (3H, s, OCH_3), 3.92 (2H, s, 2-H_2); δ_C (126 MHz, D_2O) 40.0 (CH_3), 53.3 (CH_2), 168.8 (C); *m*/*z* (ESI) 112 (MNa^+. 100%).

2-Amino-*N*-phenylbenzamide (3a)[4]



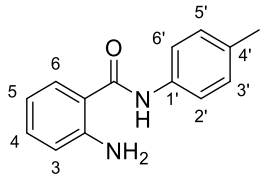
To a stirred solution of aniline (0.092 mL, 1.1 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.15 g, 0.92 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25–40% ethyl acetate in hexane gave 2-amino-*N*-phenylbenzamide (**3a**) (0.14 g, 73%) as a white solid. Mp 125–127 °C (lit.[4] 128–130 °C); δ_H (500 MHz, CD_3OD) 6.67–6.71 (1H, m, 5-H), 6.79 (1H, dd, *J* 8.2, 0.8 Hz, 3-H), 7.12 (1H, tt, *J* 7.4, 1.1 Hz, 4'-H), 7.20–7.25 (1H, m, 4-H), 7.31–7.36 (2H, m, 3'-H and 5'-H), 7.59 (1H, dd, *J* 7.9, 1.4 Hz, 6-H), 7.60–7.64 (2H, m, 2'-H and 6'-H); δ_C (126 MHz, CD_3OD) 117.4 (CH), 118.0 (C), 118.2 (CH), 122.5 (2 × CH), 125.3 (CH), 129.4 (CH), 129.7 (2 × CH), 133.4 (CH), 140.0 (C), 150.6 (C), 170.5 (C); *m*/*z* (ESI) 235 (MNa^+. 100%).

2-Amino-*N*-(2'-methylphenyl)benzamide (3b)[161]



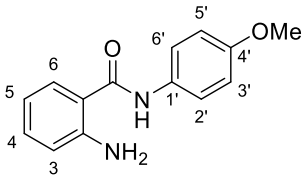
To a stirred solution of *o*-toluamide (0.236 mL, 2.21 mmol) in ethyl acetate (4 mL) was added isatoic anhydride (0.400 g, 2.45 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave 2-amino-*N*-(2'-methylphenyl)benzamide (**3b**) (0.252 g, 50%) as a white solid. Mp 115–120 °C (lit.[161] 113–115 °C); δ_H (400 MHz, $CDCl_3$) 2.33 (3H, s, CH_3), 5.54 (2H, s, NH_2), 6.69–6.78 (2H, m, 3-H and 5'-H), 7.13 (1H, td, *J* 7.6, 1.2 Hz, 4-H), 7.20–7.32 (3H, m, 5-H, 3'-H and 4'-H), 7.50 (1H, dd, *J* 8.4, 1.2 Hz, 6'-H), 7.60 (1H, s, NH), 7.83 (1H, d, *J* 8.4 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 18.1 (CH_3), 116.3 (C), 117.0 (CH), 117.8 (CH), 123.7 (CH), 125.6 (CH), 127.0 (CH), 127.3 (CH), 130.0 (C), 130.8 (CH), 132.9 (CH), 135.9 (C), 149.3 (C), 167.7 (C); *m*/*z* (ESI) 225 ($M–H^-$. 100%).

2-Amino-*N*-(4'-methylphenyl)benzamide (3d)[162]



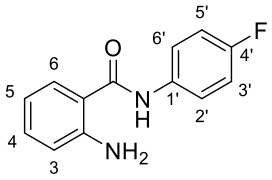
To a stirred solution of *p*-toluidine (0.089 mL, 0.83 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.15 g, 0.92 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 20–30% ethyl acetate in hexane gave 2-amino-*N*-(4'-methylphenyl)benzamide (**3d**) (0.16 g, 84%) as a white solid. Mp 149–152 °C (lit.[162] 148–150 °C); δ_H (500 MHz, $CDCl_3$) 2.34 (3H, s, 4'-CH_3), 5.49 (2H, br s, NH_2), 6.69–6.74 (2H, m, 3-H and 5-H), 7.17 (2H, br d, *J* 8.3 Hz, 3'-H and 5'-H), 7.23–7.28 (1H, m, 4-H), 7.42–7.48 (3H, m, 6-H, 2'-H and 6'-H), 7.67 (1H, br s, NH); δ_C (126 MHz, $CDCl_3$) 21.0 (CH_3), 116.5 (C), 116.9 (CH), 117.6 (CH), 120.8 (2 × CH), 127.3 (CH), 129.6 (2 × CH), 132.7 (CH), 134.3 (C), 135.4 (C), 149.0 (C), 167.7 (C); *m*/*z* (ESI) 249 (MNa^+. 100%).

2-Amino-*N*-(4'-methoxyphenyl)benzamide (3e)[162]



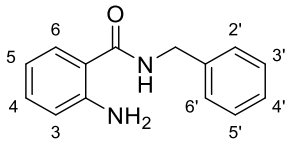
To a stirred solution of 4-methoxyaniline (0.103 g, 0.830 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.150 g, 0.910 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave 2-amino-*N*-(4'-methoxyphenyl)benzamide (**3e**) (0.170 g, 84%) as a brown solid. Mp 113–116 °C (lit.[162] 114–116 °C); δ_H (400 MHz, $CDCl_3$) 3.81 (3H, s, 4'-OCH_3), 5.49 (2H, br s, NH_2), 6.68–6.74 (2H, m, 3-H and 5-H), 6.88–6.94 (2H, m, 3'-H and 5'-H), 7.22–7.28 (1H, m, 4-H), 7.43–7.49 (3H, m, 6-H, 2'-H and 6'-H), 7.64 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 55.7 (CH_3), 114.4 (2 × CH), 116.5 (C), 117.0 (CH), 117.7 (CH), 122.7 (2 × CH), 127.2 (CH), 131.0 (C), 132.8 (CH), 149.1 (C), 156.9 (C), 167.7 (C); *m*/*z* (ESI) 265 (MNa^+. 100%).

2-Amino-*N*-(4'-fluorophenyl)benzamide (3f)[9]



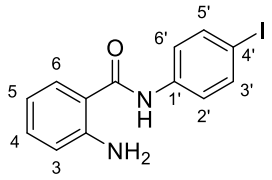
To a stirred solution of 4-fluoroaniline (0.080 mL, 0.83 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.15 g, 0.92 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave 2-amino-*N*-(4'-fluorophenyl)benzamide (**3f**) (0.14 g, 75%) as a white solid. Mp 122–125 °C (lit.[161] 125–127 °C); δ_H (400 MHz, DMSO-d_6) 6.31 (2H, br s, NH_2), 6.59 (1H, td, *J* 8.0, 1.0 Hz, 5-H), 6.75 (1H, dd, *J* 8.2, 1.0 Hz, 3-H), 7.12–7.23 (3H, m, 4-H, 2'-H and 6'-H), 7.61 (1H, dd, *J* 8.0, 1.3 Hz, 6-H), 7.68–7.76 (2H, m, 3'-H and 5'-H), 10.03 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 114.8 (2 × CH, d, $^2J_{CF}$ 23.8 Hz), 115.0 (C), 115.1 (CH), 116.4 (CH), 122.3 (2 × CH, d, $^3J_{CF}$ 7.7 Hz), 128.6 (CH), 132.1 (CH), 135.5 (C, d, $^4J_{CF}$ 2.6 Hz), 149.7 (C), 158.1 (C, d, $^1J_{CF}$ 240.1 Hz), 167.7 (C); *m*/*z* (ESI) 253 (MNa^+. 100%).

2-Amino-*N*-benzylbenzamide (3g)[162]



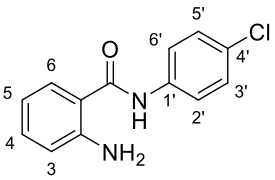
To a stirred solution of benzylamine (0.091 mL, 0.083 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.15 g, 0.91 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave 2-amino-*N*-benzylbenzamide (**3g**) (0.17 g, 90%) as a white solid. Mp 124–127 °C (lit.[162] 123–125 °C); δ_H (400 MHz, $CDCl_3$) 4.61 (2H, d, *J* 5.6 Hz, PhCH_2), 5.56 (2H, br s, NH_2), 6.32 (1H, br s, NH), 6.63 (1H, td, *J* 8.2, 0.9 Hz, 5-H), 6.69 (1H, dd, *J* 8.2, 0.9 Hz, 3-H), 7.21 (1H, td, *J* 8.2, 1.4 Hz, 4-H), 7.27–7.39 (6H, m, 6-H and Ph); δ_C (101 MHz, $CDCl_3$) 43.9 (CH_2), 116.3 (C), 117.1 (CH), 117.8 (CH), 127.2 (CH), 127.7 (CH), 128.0 (2 × CH), 128.9 (2 × CH), 132.6 (CH), 138.4 (C), 148.5 (C), 169.2 (C); *m*/*z* (ESI) 249 (MNa^+. 100%).

2-Amino-*N*-(4'-iodophenyl)benzamide (3k)



To a stirred solution of 4-iodoaniline (0.181 g, 0.830 mmol) in ethyl acetate (0.6 mL) was added isatoic anhydride (0.150 g, 0.920 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. An additional portion of isatoic anhydride (0.0300 g, 0.180 mmol) was then added. After 3 h, a final portion of isatoic anhydride (0.0300 g, 0.180 mmol) was added and the reaction mixture was stirred for 2.5 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave 2-amino-*N*-(4'-iodophenyl)benzamide (**3k**) (0.145 g, 52%) as a yellow solid. Mp 120–122 °C; ν_{max}/cm^{-1} (neat) 3285 (NH), 2529 (CH), 1622 (C=O), 1574 (C=C), 1522, 1481, 1385, 812; δ_H (500 MHz, CD_3OD) 6.64–6.70 (1H, m, 5-H), 6.78 (1H, br d, *J* 8.1 Hz, 3-H), 7.19–7.25 (1H, m, 4-H), 7.45–7.50 (2H, m, 2'-H and 6'-H), 7.58 (1H, dd, *J* 7.9, 1.3 Hz, 6-H), 7.63–7.68 (2H, m, 3'-H and 5'-H); δ_C (126 MHz, CD_3OD) 87.8 (C), 117.3 (CH), 117.6 (C), 118.2 (CH), 124.1 (2 × CH), 129.4 (CH), 133.6 (CH), 138.8 (2 × CH), 140.1 (C), 150.8 (C), 170.4 (C); *m*/*z* (ESI) 360.9809 (MNa^+. $C_{13}H_{11}IN_2NaO$ requires 360.9808).

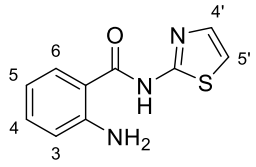
2-Amino-*N*-(4'-chlorophenyl)benzamide (3l)[163]



To a stirred solution of 4-chloroaniline (0.212 g, 1.64 mmol) in ethyl acetate (1.2 mL) was added isatoic anhydride (0.300 g, 1.84 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 20–40% ethyl acetate in hexane gave 2-amino-*N*-(4'-chlorophenyl)benzamide (**3l**) (0.277 g, 69%) as a white solid. Mp 197–200 °C (lit.[163] 198–200 °C); δ_H (500 MHz, DMSO-d_6) 6.32 (2H, br s, NH_2), 6.59 (1H, br t, *J* 7.9 Hz, 5-H), 6.75 (1H, br d, *J* 8.2 Hz, 3-H), 7.18–7.23 (1H, m, 4-H), 7.35–7.41 (2H, m, 2'-H and 6'-H), 7.61 (1H, dd, *J* 7.9, 1.1 Hz, 6-H), 7.72–7.78 (2H, m, 3'-H and 5'-H), 10.10 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 114.7 (CH), 114.9 (C), 116.4 (CH), 122.0 (2 × CH), 126.9 (C),

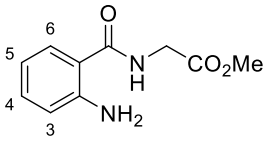
128.4 (2 × CH), 128.7 (CH), 132.3 (CH), 138.3 (C), 149.8 (C), 167.9 (C); *m*/*z* (ESI) 269 (MNa^+. 100%).

N-(2-Aminobenzoyl)-2'-aminothiazole (3n)[164]



To a stirred solution of 2-aminothiazole (0.166 g, 1.65 mmol) in ethyl acetate (1.2 mL) was added isatoic anhydride (0.300 g, 1.84 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 20–30% ethyl acetate in hexane gave *N*-(2-aminobenzoyl)-2'-aminothiazole (**3n**) (0.300 g, 83%) as an orange solid. Mp 157–159 °C (lit.[164] 151–153 °C); δ_H (500 MHz, $CDCl_3$) 5.68 (2H, br s, NH_2), 6.67–6.72 (1H, m, 5-H), 6.76 (1H, dd, *J* 8.2, 0.7 Hz, 3-H), 6.90 (1H, d, *J* 3.7 Hz, 5'-H), 7.12 (1H, d, *J* 3.7 Hz, 4'-H), 7.29–7.34 (1H, m, 4-H), 7.70 (1H, dd, *J* 8.0, 1.3 Hz, 6-H), 12.26 (1H, br s, NH); δ_C (126 MHz, $CDCl_3$) 113.1 (CH), 113.7 (C), 116.8 (CH), 117.5 (CH), 128.8 (CH), 133.8 (CH), 137.5 (CH), 149.9 (C), 160.1 (C), 167.4 (C); *m*/*z* (ESI) 242 (MNa^+. 100%).

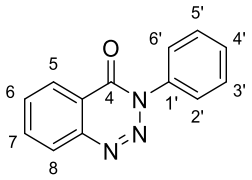
2-Amino-*N*-(methoxycarbonylmethyl)benzamide (3o)[13]



To a stirred solution of glycine methyl ester hydrochloride (0.240 g, 1.92 mmol) in ethyl acetate (4.2 mL) was added isatoic anhydride (0.344 g, 2.11 mmol) and triethylamine (0.227 mL, 1.92 mmol). The reaction mixture was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 20% ethyl acetate in dichloromethane gave 2-amino-*N*-(methoxycarbonylmethyl)benzamide (**3o**) (0.320 g, 80%) as a white solid. Mp 73–77 °C (lit.[165] 73–74 °C); δ_H (400 MHz, $CDCl_3$) 3.80 (3H, s OCH_3), 4.20 (2H, d, *J* 5.1 Hz, $CH_2CO_2CH_3$), 5.50 (2H, br s, NH_2), 6.59 (1H, br s, NH), 6.63–6.70 (2H, m, 3-H and 5-H), 7.19–7.25 (1H, m, 4-H), 7.40 (1H, dd, *J* 8.4, 1.6 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 41.6 (CH_2), 52.6 (CH_3), 115.2 (C), 116.8 (CH), 117.5 (CH), 127.6 (CH), 132.8

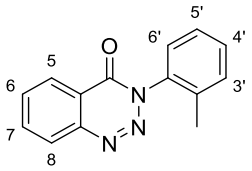
(CH), 149.0 (C), 169.4 (C), 170.8 (C); *m*/*z* (ESI) 231 (MNa^+. 100%).

N-Phenyl-1,2,3-benzotriazin-4(3*H*)-one (4a)[21]



The reaction was carried out as described in the general procedure using 2-amino-*N*-phenylbenzamide (0.0500 g, 0.236 mmol), polymer-supported nitrite (0.202 g, containing 0.707 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.135 g, 0.707 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 18 h. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave *N*-phenyl-1,2,3-benzotriazin-4(3*H*)-one (**4a**) (0.0330 g, 62%) as a white solid. Mp 150–153 °C (lit.[21] 150–152 °C); δ_H (400 MHz, $CDCl_3$) 7.49 (1H, tt, *J* 7.4, 1.3 Hz, 4'-H), 7.53–7.59 (2H, m, 3'-H and 5'-H), 7.63–7.68 (2H, m, 2'-H and 6'-H), 7.83–7.88 (1H, m, 6-H), 7.96–7.03 (1H, m, 7-H), 8.23 (1H, br d, *J* 8.1 Hz, 8-H), 8.45 (1H, dd, *J* 7.9, 1.1 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 120.6 (C), 125.8 (CH), 126.2 (2 × CH), 128.7 (CH), 129.1 (CH), 129.2 (2 × CH), 132.9 (CH), 135.2 (CH), 139.0 (C), 143.9 (C), 155.4 (C); *m*/*z* (ESI) 246 (MNa^+. 100%).

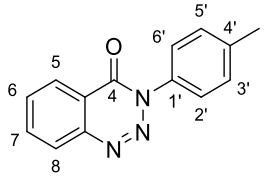
3-(2'-Methylphenyl)benzo-1,2,3-triazin-4(3*H*)-one (4b)[166]



To a stirred solution of 2-amino-*N*-(2'-methylphenyl)benzamide (0.100 g, 0.442 mmol) in methanol (4 mL) at 0 °C was added polymer-supported nitrite (0.379 g, containing 1.33 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.252 g, 1.33 mmol). The reaction mixture was stirred at 0 °C for 1 h then heated to 40 °C and stirred for 4 h. The reaction mixture was cooled to room temperature, filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 100% dichloromethane gave 3-(2'-methylphenyl)benzo-1,2,3-triazin-4(3*H*)-one (**4b**) (0.0810 g, 77%) as a white solid. Mp 158–160 °C (lit.[166] 153–155 °C); δ_H (500 MHz, $CDCl_3$) 2.21 (3H, s, CH_3), 7.33–7.49 (4H, m, 3'H, 4'H, 5'H and 6'-H), 7.86 (1H, br t, *J* 7.5 Hz, 6-H), 8.01 (1H, br t, *J* 7.5, 7-H), 8.25 (1H, br d, *J* 7.5 Hz, 8-H), 8.45 (1H, br d, *J* 7.5 Hz, 5-H); δ_C (126 MHz, $CDCl_3$) 17.8 (CH_3), 120.4 (C), 125.6

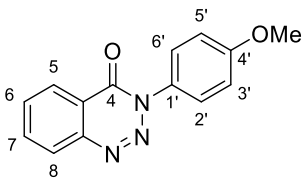
(CH), 127.1 (CH), 127.8 (CH), 128.7 (CH), 129.9 (CH), 131.2 (CH), 132.8 (CH), 135.2 (CH), 135.6 (C), 138.0 (C), 144.1 (C), 155.2 (C); *m*/*z* (ESI) 260 (MNa^+. 100%).

N-(4'-Methylphenyl)-1,2,3-benzotriazin-4(3*H*)-one (4d)[20]



The reaction was carried out as described in the general procedure using 2-amino-*N*-(4'-methylphenyl)benzamide (0.285 g, 1.26 mmol), polymer-supported nitrite (1.09 g, containing 3.78 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.719 g, 3.78 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in dichloromethane (20 mL) and washed with 1 M aqueous sodium hydroxide (6 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(4'-methylphenyl)-1,2,3-benzotriazin-4(3*H*)-one (**4d**) (0.272 g, 91%) as an orange solid. Mp 137–140 °C (lit.[20] 139–141°C); δ_H (400 MHz, $CDCl_3$) 2.45 (3H, s, 4'-CH_3), 7.33–7.39 (2H, m, 3'-H and 5'-H), 7.50–7.56 (2H, m, 2'-H and 6'-H), 7.82–7.87 (1H, m, 6-H), 7.96–8.01 (1H, m, 7-H), 8.22 (1H, br d, *J* 8.1 Hz, 8-H), 8.44 (1H, dd, *J* 7.9, 1.4 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 21.4 (CH_3), 120.6 (C), 125.8 (CH), 126.0 (2 × CH), 128.6 (CH), 129.8 (2 × CH), 132.8 (CH), 135.1 (CH), 136.5 (C), 139.2 (C), 143.9 (C), 155.5 (C); *m*/*z* (ESI) 260 (MNa^+. 100%).

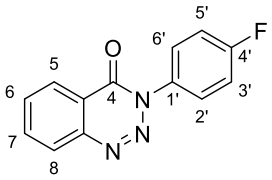
N-(4'-Methoxyphenyl)-1,2,3-benzotriazin-4(3*H*)-one (4e)[20]



The reaction was carried out as described in the general procedure using 2-amino-*N*-(4'-methoxyphenyl)benzamide (0.0500 g, 0.206 mmol), polymer-supported nitrite (0.177 g, containing 0.619 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.118 g, 0.619 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. Purification by flash column chromatography, eluting with 70% diethyl ether in hexane gave *N*-(4'-methoxyphenyl)-1,2,3-benzotriazin-4(3*H*)-one (**4e**) (0.0370 g, 71%) as a yellow solid. Mp 147–150 °C (lit.[20] 152–153 °C); δ_H

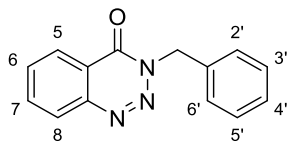
(400 MHz, $CDCl_3$) 3.88 (3H, s, 4'-OCH_3), 7.02–7.09 (2H, m, 3'-H and 5'-H), 7.52–7.60 (2H, m, 2'-H and 6'-H), 7.84 (1H, td, *J* 8.0, 1.0 Hz, 6-H), 7.94–8.01 (1H, m, 7-H), 8.21 (1H, br d, *J* 8.1 Hz, 8-H), 8.43 (1H, dd, *J* 8.0, 1.0 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 55.7 (CH_3), 114.4 (2 × CH), 120.5 (C), 125.7 (CH), 127.4 (2 × CH), 128.6 (CH), 131.9 (C), 132.8 (CH), 135.1 (CH), 143.9 (C), 155.5 (C), 160.0 (C); *m*/*z* (ESI) 276 (MNa^+. 100%).

N-(4'-Fluorophenyl)-1,2,3-benzotriazin-4(3*H*)-one (4f)[20]



The reaction was carried out as described in the general procedure using 2-amino-*N*-4'-fluorophenyl)benzamide (0.0500 g, 0.217 mmol), polymer-supported nitrite (0.187 g, containing 0.652 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.124 g, 0.652 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave *N*-(4'-fluorophenyl)-1,2,3-benzotriazin-4(3*H*)-one (**4f**) (0.0320 g, 60%) as a white solid. Mp 136–139 °C (lit.[20] 138–140 °C); δ_H (400 MHz, $CDCl_3$) 7.20–7.28 (2H, m, 2'-H and 6'-H), 7.61–7.68 (2H, m, 3'-H and 5'-H), 7.82–7.89 (1H, m, 6-H), 7.96–8.01 (1H, m, 7-H), 8.22 (1H, br d, *J* 8.0 Hz, 8-H), 8.43 (1H, dd, *J* 8.0, 1.1 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 116.1 (2 × CH, d, $^2J_{C\text{-}F}$ 23.0 Hz), 120.4 (C), 125.8 (CH), 128.0 (2 × CH, d, $^3J_{C\text{-}F}$ 8.8 Hz), 128.7 (CH), 133.0 (CH), 134.9 (C, d, $^4J_{C\text{-}F}$ 3.3 Hz), 135.3 (CH), 143.8 (C), 155.4 (C), 162.7 (C, d, $^1J_{C\text{-}F}$ 249.1 Hz); *m*/*z* (ESI) 264 (MNa^+. 100%).

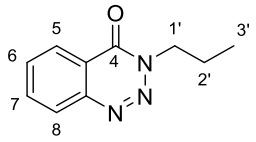
N-(Benzyl)-1,2,3-benzotriazin-4(3*H*)-one (4g)[20]



The reaction was carried out as described in the general procedure using 2-amino-*N*-(4-benzyl)benzamide (0.0500 g, 0.221 mmol), polymer-supported nitrite (0.190 g, containing 0.663 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.126 g, 0.663 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave *N*-(benzyl)-1,2,3-benzotriazin-4(3*H*)-one (**4g**) (0.0370 g,

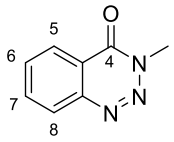
71%) as a white solid. Mp 115–119 °C (lit.[20] 118–120 °C); δ_H (400 MHz, $CDCl_3$) 5.63 (2H, s, PhCH_2), 7.26–7.37 (3H, m, 3'-H, 4'-H and 5'-H), 7.50–7.55 (2H, m, 2'-H and 6'-H), 7.74–7.80 (1H, m, 6-H), 7.89–7.95 (1H, m, 7-H), 8.14 (1H, br d, *J* 8.2 Hz, 8-H), 8.33 (1H, dd, *J* 8.0, 1.0 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 53.5 (CH_2), 120.2 (C), 125.3 (CH), 128.3 (CH), 128.5 (CH), 128.9 (2 × CH), 129.0 (2 × CH), 132.5 (CH), 134.9 (CH), 135.9 (C), 144.5 (C), 155.5 (C); *m*/*z* (ESI) 260 (MNa^+. 100%).

N-Propyl-1,2,3-benzotriazin-4(3*H*)-one (4h)[167]



The reaction was carried out as described in the general procedure using 2-amino-*N*-propylbenzamide (0.150 g, 0.840 mmol), polymer-supported nitrite (0.721 g, containing 2.52 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.479 g, 2.52 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 1 h. Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave *N*-propyl-1,2,3-benzotriazin-4(3*H*)-one (**4h**) (0.136 g, 86%) as a white solid. Mp 50–55 °C (lit.[167] 56–57 °C); δ_H (500 MHz, DMSO-d_6) 0.92 (3H, t, *J* 7.3 Hz, 3'-H_3), 1.83 (2H, sextet, *J* 7.3 Hz, 2'-H_2), 4.33 (2H, t, *J* 7.3 Hz, 1'-H_2), 7.88–7.93 (1H, m, 6-H), 8.04–8.09 (1H, m, 7-H), 8.16 (1H, br d, *J* 8.0 Hz, 8-H), 8.22 (1H, dd, *J* 8.0, 1.0 Hz, 5-H); δ_C (126 MHz, DMSO-d_6) 10.9 (CH_3), 22.7 (CH_2), 50.7 (CH_2), 119.2 (C), 124.5 (CH), 127.9 (CH), 132.8 (CH), 135.3 (CH), 143.6 (C), 154.7 (C); *m*/*z* (ESI) 212 (MNa^+. 100%).

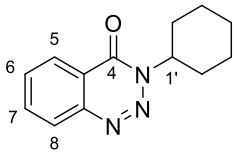
N-Methyl-1,2,3-benzotriazin-4(3*H*)-one (4n)[25]



The reaction was carried out as described in the general procedure using 2-amino-*N*-methylbenzamide (0.150 g, 1.00 mmol), polymer-supported nitrite (0.850 g, containing 3.00 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.571 g, 3.00 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 5.5 h. Purification by flash column chromatography, eluting with 25% ethyl acetate in petroleum ether gave *N*-methyl-1,2,3-benzotriazin-4(3*H*)-one (**4n**) (0.144 g, 89%) as a white solid. Mp 115–117 °C (lit.[24] 118–120 °C); δ_H (500 MHz, DMSO-d_6) 3.93 (3H, s, 3-CH_3), 7.89–7.95 (1H, m, 6-H), 8.05–8.10 (1H,

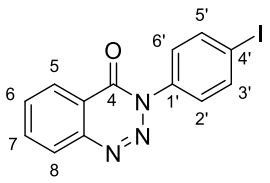
m, 7-H), 8.19 (1H, dd, *J* 8.1, 1.0 Hz, 8-H), 8.24 (1H, dd, *J* 8.1, 1.0 Hz, 5-H); δ_C (126 MHz, DMSO-d_6) 37.0 (CH_3), 119.2 (C), 124.3 (CH), 127.9 (CH), 132.8 (CH), 135.2 (CH), 143.9 (C), 155.1 (C); *m*/*z* (EI) 161 (M^+. 6%), 78 (82), 63 (100).

N-Cyclohexyl-1,2,3-benzotriazin-4(3*H*)-one (4u)[21]



The reaction was carried out as described in the general procedure using 2-amino-*N*-cyclohexylbenzamide (0.150 g, 0.690 mmol), polymer-supported nitrite (0.590 g, containing 2.06 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.392 g, 2.06 mmol). The reaction mixture was stirred at room temperature for 1 h and then heated under reflux for 48 h. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave *N*-cyclohexyl-1,2,3-benzotriazin-4(3*H*)-one (**4u**) (0.0850 g, 54%) as a yellow solid. Mp 130–133 °C (lit.[21] 129–131 °C); δ_H (500 MHz, CD_3OD) 1.30–1.42 (1H, m, C*H*H), 1.49–1.62 (2H, m, CH_2), 1.76–1.84 (1H, m, CH*H*), 1.92–2.07 (6H, m, 3 × CH_2), 4.95–5.06 (1H, m, 1'-H), 7.89 (1H, td, *J* 8.1, 1.0 Hz, 6-H), 8.02–8.07 (1H, m, 7-H), 8.15 (1H, br d, *J* 8.1 Hz, 8-H), 8.31 (1H, dd, *J* 8.1, 1.0 Hz, 5-H); δ_C (126 MHz, CD_3OD) 26.5 (CH_2), 26.9 (2 × CH_2), 32.9 (2 × CH_2), 58.2 (CH), 120.6 (C), 126.0 (CH), 129.0 (CH), 133.7 (CH), 136.4 (CH), 145.2 (C), 156.6 (C); *m*/*z* (EI) 229 (M^+. 68%), 172 (43), 158 (69), 148 (75), 130 (40), 105 (76), 78 (100), 63 (100).

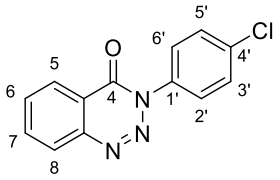
N-(4'-Iodophenyl)-1,2,3-benzotriazin-4(3*H*)-one (4w)[20]



The reaction was carried out as described in the general procedure using 2-amino-*N*-4'-iodophenyl)benzamide (0.0900 g, 0.266 mmol), polymer-supported nitrite (0.229 g, containing 0.799 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.152 g, 0.799 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 18 h. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave *N*-(4'-iodophenyl)-1,2,3-benzotriazin-4(3*H*)-one (**4w**) (0.0740 g, 80%) as a white solid. Mp 187–190 °C (lit.[20] 190–193 °C); δ_H (400 MHz, $CDCl_3$) 7.42–7.47 (2H, m, 2'-H and 6'-H), 7.83–7.92 (3H, m, 6-H, 3'-H and 5'-

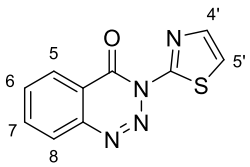
H), 7.97–8.04 (1H, m, 7-H), 8.23 (1H, br d, *J* 8.1 Hz, 8-H), 8.44 (1H, dd, *J* 7.9, 1.0 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 94.5 (C), 120.4 (C), 125.8 (CH), 127.8 (2 × CH), 128.8 (CH), 133.1 (CH), 135.4 (CH), 138.4 (2 × CH), 138.7 (C), 143.7 (C), 155.2 (C); *m*/*z* (ESI) 372 (MNa^+. 100%).

N-(4'-Chlorophenyl)-1,2,3-benzotriazin-4(3*H*)-one (4x)[168]



The reaction was carried out as described in the general procedure using 2-amino-*N*-4'-chlorophenyl)benzamide (0.150 g, 0.608 mmol), polymer-supported nitrite (0.522 g, containing 1.82 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.347 g, 1.82 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in ethyl acetate (20 mL) and washed with 1 M aqueous sodium hydroxide (6 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(4'-chlorophenyl)-1,2,3-benzotriazin-4(3*H*)-one (**4x**) (0.102 g, 65%) as a yellow solid. Mp 174–176 °C (lit.[166] 173–175 °C); δ_H (400 MHz, $CDCl_3$) 7.50–7.55 (2H, m, 2'-H and 6'-H), 7.61–7.67 (2H, m, 3'-H and 5'-H), 7.84–7.90 (1H, m, 6-H), 7.98–8.03 (1H, m, 7-H), 8.23 (1H, br d, *J* 8.2 Hz, 8-H), 8.44 (1H, dd, *J* 7.9, 1.2 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 120.4 (C), 125.8 (CH), 127.4 (2 × CH), 128.8 (CH), 129.4 (2 × CH), 133.1 (CH), 135.0 (C), 135.4 (CH), 137.4 (C), 143.7 (C), 155.3 (C); *m*/*z* (ESI) 280 (MNa^+. 100%).

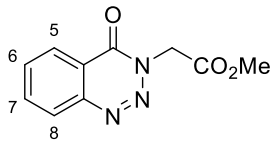
N-(Thiazol-2'-yl)-1,2,3-benzotriazin-4(3*H*)-one (4y)[21]



The reaction was carried out as described in the general procedure using *N*-(2-aminobenzoyl)-2-aminothiazole (0.147 g, 0.670 mmol), polymer-supported nitrite (0.578 g, containing 2.01 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.387 g, 2.01 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in ethyl acetate (20 mL) and washed with 1 M aqueous sodium hydroxide (6 × 20 mL). The organic layer

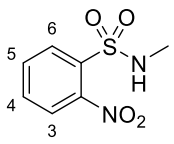
was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(thiazol-2'-yl)-1,2,3-benzotriazin-4(3*H*)-one (**4y**) (0.0800 g, 52%) as an orange solid. Mp 170–173 °C (lit.[21] 174–176 °C); δ_H (400 MHz, $CDCl_3$) 7.44 (1H, d, *J* 3.5 Hz, 5'-H), 7.89 (1H, d, *J* 3.5 Hz, 4'-H), 7.88–7.95 (1H, m, 6-H), 8.02–8.09 (1H, m, 7-H), 8.32 (1H, br d, *J* 8.1 Hz, 8-H), 8.51 (1H, dd, *J* 7.9, 1.4 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 119.1 (CH), 119.4 (C), 126.0 (CH), 129.5 (CH), 133.8 (CH), 135.9 (CH), 140.2 (CH), 142.6 (C), 154.3 (C), 156.3 (C); *m*/*z* (ESI) 253 (MNa^+. 100%).

N-(Methoxycarbonylmethyl)-1,2,3-benzotriazin-4(3*H*)-one (4z)[21]



To a stirred solution of 2-amino-*N*-(methoxycarbonylmethyl)benzamide (0.127 g, 0.601 mmol) in methanol (6 mL) was added polymer-supported nitrite (0.516 g, containing 1.80 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.342 g, 1.80 mmol). The reaction mixture was stirred at 0 °C for 1 h and then heated to 40 °C for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(methoxycarbonylmethyl)-1,2,3-benzotriazin-4(3*H*)-one (**4z**) (0.110 g, 84%) as a white solid. Mp 128–130 °C (lit.[21] 128–130 °C); δ_H (400 MHz, $CDCl_3$) 3.81 (3H, s OCH_3), 5.21 (2H, s, C*H*$_2$CO$_2$CH$_3$), 7.80–7.86 (1H, m, 6-H), 7.95–8.02 (1H, m, 7-H), 8.19 (1H, br d, *J* 8.1 Hz, 8-H), 8.37 (1H, dd, *J* 7.9, 1.1 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 50.9 (CH_2), 52.8 (CH_3), 119.9 (C), 125.4 (CH), 128.8 (CH), 132.8 (CH), 135.3 (CH), 144.5 (C), 155.7 (C), 167.7 (C); *m*/*z* (ESI) 242 (MNa^+. 100%).

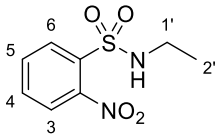
2-Nitro-*N*-methylbenzenesulfonamide (88a)[27]



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was added in portions to a mixture of methylamine hydrochloride (0.183 g, 2.71 mmol) and triethylamine (0.626 mL, 4.51 mmol) in dichloromethane (25 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. Methanol (5 mL) was then added and the reaction mixture stirred for a further 1 h. The reaction mixture was

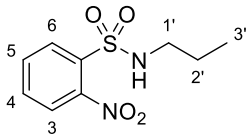
concentrated *in vacuo*, diluted with dichloromethane (25 mL) and washed with brine (2 × 25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave 2-nitro-*N*-methylbenzenesulfonamide (**88a**) (0.342 g, 70%) as a yellow solid. Mp 100–104 °C (lit.[169] 106 °C); δ_H (400 MHz, $CDCl_3$) 2.80 (3H, d, *J* 5.2 Hz, NCH_3), 5.22 (1H, br s, NH), 7.72–7.79 (2H, m, 4-H and 5-H), 7.84–7.90 (1H, m, 6-H), 8.11–8.17 (1H, m, 3-H); δ_C (101 MHz, $CDCl_3$) 29.9 (CH_3), 125.6 (CH), 131.7 (CH), 132.7 (C), 132.8 (CH), 133.8 (CH), 148.5 (C); *m*/*z* (ESI) 239 (MNa^+. 100%).

2-Nitro-*N*-ethylbenzenesulfonamide (88b)[29]



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of ethylamine (0.125 mL, 1.88 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was then concentrated *in vacuo*. Purification by flash column chromatography, eluting with 70% diethyl ether in hexane gave 2-nitro-*N*-ethylbenzenesulfonamide (**88b**) (0.433 g, 100%) as a white solid. Mp 96–98 °C (lit.[170] 98–100 °C); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, *J* 7.3 Hz, NCH_2CH_3), 3.16 (2H, qd, *J* 7.3, 6.0 Hz, NCH_2CH_3), 5.22 (1H, d, *J* 6.0 Hz, NH), 7.71–7.78 (2H, m, 4-H and 5-H), 7.83–7.89 (1H, m, 6-H), 8.11–8.17 (1H, m, 3-H); δ_C (101 MHz, $CDCl_3$) 15.3 (CH_3), 39.0 (CH_2), 125.5 (CH), 131.2 (CH), 132.9 (CH), 133.7 (CH), 133.9 (C), 148.2 (C); *m*/*z* (ESI) 253 (MNa^+. 100%).

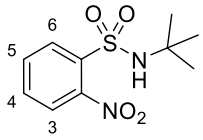
2-Nitro-*N*-propylbenzenesulfonamide (88c)[31]



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of *n*-propylamine (0.161 mL, 1.96 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was then concentrated *in vacuo*, diluted with dichloromethane (25 mL) and washed with water (3 × 30 mL), sodium bicarbonate

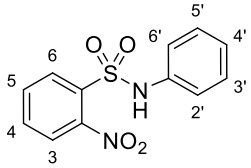
(2 × 20 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 2-nitro-*N*-propylbenzenesulfonamide (**88c**) (0.392 g, 82%) as a white solid. Mp 72–76 °C (lit.[171] 70 °C); δ_H (400 MHz, $CDCl_3$) 0.91 (3H, t, *J* 7.4 Hz, 3'-H_3), 1.55 (2H, sextet, *J* 7.4 Hz, 2'-H_2), 3.07 (2H, td, *J* 7.4, 6.2 Hz, 1'-H_2), 5.25 (1H, br t, *J* 6.3 Hz, NH), 7.70–7.77 (2H, m, 4-H and 5-H), 7.83–7.89 (1H, m, 6-H), 8.11–8.18 (1H, m, 3-H); δ_C (101 MHz, $CDCl_3$) 11.2 (CH_3), 23.1 (CH_2), 45.7 (CH_2), 125.5 (CH), 131.2 (CH), 132.9 (CH), 133.6 (CH), 134.0 (C), 148.3 (C); *m/z* (ESI) 267 (MNa^+. 100%).

2-Nitro-*N*-(*tert*-butyl)benzenesulfonamide (88d)



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of *tert*-butylamine (0.197 mL, 1.88 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 5 h. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (25 mL) and washed with water (3 × 30 mL), sodium bicarbonate (2 × 20 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 2-nitro-*N*-(*tert*-butyl)benzenesulfonamide (**88d**) (0.243 g, 50%) as a white solid. Mp 121–123 °C; ν_{max}/cm^{-1} (neat) 3264 (NH), 2974 (CH), 1541, 1368, 1323, 1153, 997; δ_H (400 MHz, $CDCl_3$) 1.32 (9H, s, NHC(CH_3)$_3$), 5.25 (1H, br s, NH), 7.66–7.76 (2H, m, 4-H and 5-H), 7.85 (1H, dd, *J* 7.3, 2.0 Hz, 6-H), 8.20 (1H, dd, *J* 7.3, 2.0 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 30.4 (3 × CH_3), 55.9 (C), 125.4 (CH), 130.6 (CH), 133.0 (CH), 133.2 (CH), 137.4 (C), 148.0 (C); *m/z* (ESI) 281.0566 (MNa^+. $C_{10}H_{14}N_2NaO_4S$ requires 281.0566).

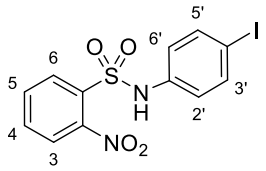
***N*-Phenyl-2-nitrobenzenesulfonamide (88e)[33]**



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was added in portions over 0.5 h to a stirred solution of aniline (0.247 mL, 2.71 mmol) in 50% aqueous methanol

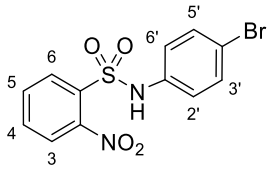
(5 mL) and sodium acetate (0.259 g, 3.16 mmol). The reaction mixture was then heated to 60 °C and stirred for 1 h. The reaction mixture was allowed to cool to room temperature, diluted in water (7 mL) and acidified to pH 2 using 1 M aqueous hydrochloric acid. The precipitate was filtered, washed with excess water and then recrystallised from 4:1 ethanol:water to give *N*-phenyl-2-nitrobenzenesulfonamide (**88e**) (0.620 g, 98%) as a white solid. Mp 106–110 °C (lit.[172] 109–110 °C); δ_H (400 MHz, $CDCl_3$) 7.15–7.31 (6H, m, Ph and NH), 7.57 (1H, td, *J* 7.9, 1.3 Hz, 5-H), 7.69 (1H, td, *J* 7.9, 1.3 Hz, 4-H), 7.82 (1H, dd, *J* 7.9, 1.3 Hz, 6-H), 7.86 (1H, dd, *J* 7.9, 1.3 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 123.5 (2 × CH), 125.4 (CH), 126.8 (CH), 129.6 (2 × CH), 132.0 (CH), 132.4 (C), 132.7 (CH), 134.1 (CH), 135.7 (C), 148.4 (C); *m*/*z* (ESI) 301 (MNa^+. 100%).

N-(4'-Iodophenyl)-2-nitrobenzenesulfonamide (88f)[34]



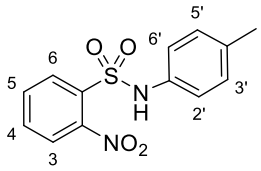
2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was added in portions to a stirred solution of 4-iodoaniline (0.495 g, 2.26 mmol) in dry pyridine (1.5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with 2 M aqueous hydrochloric acid (50 mL) and extracted with chloroform (2 × 50 mL). The organic layers were combined and washed with brine (2 × 50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave *N*-(4'-iodophenyl)-2-nitrobenzenesulfonamide (**88f**) (0.766 g, 84%) as an orange solid. Mp 95–100 °C (lit.[173] 92–94 °C); δ_H (400 MHz, $CDCl_3$) 6.93–7.00 (2H, m, 2'-H and 6'-H), 7.23 (1H, br s, NH), 7.56–7.65 (3H, m, 5-H, 3'-H and 5'-H), 7.71 (1H, td, *J* 7.8, 1.4 Hz, 4-H), 7.82–7.88 (2H, m, 3-H and 6-H); δ_C (101 MHz, $CDCl_3$) 91.2 (C), 125.1 (2 × CH), 125.6 (CH), 132.0 (CH), 132.2 (C), 132.9 (CH), 134.3 (CH), 135.5 (C), 138.7 (2 × CH), 148.4 (C); *m*/*z* (ESI) 427 (MNa^+. 100%).

***N*-(4'-Bromophenyl)-2-nitrobenzenesulfonamide (88g)[174]**



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of 4-bromoaniline (0.364 g, 2.12 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (20 mL) and washed with water (3 × 20 mL), sodium bicarbonate (2 × 10 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave *N*-(4'-bromophenyl)-2-nitrobenzenesulfonamide (**88g**) (0.348 g, 46%) as an off-white solid. Mp 106–110 °C (lit.[174] 105 °C); δ_H (400 MHz, $CDCl_3$) 7.06–7.12 (2H, m, 2'-H and 6'-H), 7.26 (1H, br s, NH), 7.36–7.42 (2H, m, 3'-H and 5'-H), 7.61 (1H, td, *J* 7.8, 1.3 Hz, 5-H), 7.71 (1H, td, *J* 7.8, 1.3 Hz, 4-H), 7.83 (1H, dd, *J* 7.8, 1.3 Hz, 6-H), 7.86 (1H, dd, *J* 7.8, 1.3 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 120.3 (C), 125.0 (2 × CH), 125.6 (CH), 132.0 (CH), 132.1 (C), 132.7 (2 × CH), 132.9 (CH), 134.3 (CH), 134.8 (C), 148.4 (C); *m*/*z* (ESI) 381 (MNa^+. 100%).

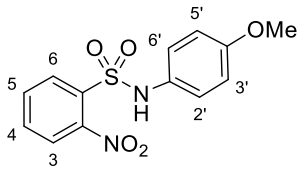
***N*-(4'-Methylphenyl)-2-nitrobenzenesulfonamide (88h)[174]**



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of *p*-toluidine (0.227 g, 2.12 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 5 h. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (20 mL) and washed with water (3 × 30 mL), sodium bicarbonate (2 × 20 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(4'-methylphenyl)-2-nitrobenzenesulfonamide (**88h**) (0.390 g, 63%) as a yellow solid. Mp 104–108 °C (lit.[174] 110 °C); δ_H (400 MHz, $CDCl_3$) 2.28

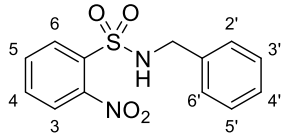
(3H, s, 4'-CH_3), 7.06 (4H, br s, 2'-H, 3'-H, 5'-H and 6'-H), 7.15 (1H, br s, NH), 7.56 (1H, td, *J* 7.9, 1.3 Hz, 5-H), 7.68 (1H, td, *J* 7.9, 1.3 Hz, 4-H), 7.80 (1H, dd, *J* 7.9, 1.3 Hz, 6-H), 7.85 (1H, dd, *J* 7.9, 1.3 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 21.1 (CH_3), 123.9 (2 × CH), 125.4 (CH), 130.2 (2 × CH), 132.1 (CH), 132.5 (C), 132.6 (CH), 132.9 (C), 133.9 (CH), 136.9 (C), 148.4 (C); *m*/*z* (ESI) 315 (MNa^+. 100%).

N-(4'-Methoxyphenyl)-2-nitrobenzenesulfonamide (88i)[37]



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of *p*-anisidine (0.261 g, 2.12 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 6 h. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (30 mL) and washed with water (3 × 50 mL), sodium bicarbonate (2 × 30 mL), 1 M aqueous hydrochloric acid (2 × 30 mL) and brine (2 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(4'-methoxyphenyl)-2-nitrobenzenesulfonamide (**88i**) (0.403 g, 62%) as a brown solid. Mp 106–108 °C (lit.[175] 106–107 °C); δ_H (400 MHz, $CDCl_3$) 3.76 (3H, s, OCH_3), 6.75–6.80 (2H, m, 3'-H and 5'-H), 7.06–7.12 (3H, m, NH, 2'-H and 6'-H), 7.56 (1H, td, *J* 7.8, 1.3 Hz, 5-H), 7.70 (1H, td, *J* 7.8, 1.4 Hz, 4-H), 7.74 (1H, dd, *J* 7.8, 1.4 Hz, 6-H), 7.86 (1H, dd, *J* 7.8, 1.3 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 55.6 (CH_3), 114.7 (2 × CH), 125.3 (CH), 126.4 (2 × CH), 128.0 (C), 132.1 (CH), 132.4 (C), 132.6 (CH), 133.9 (CH), 148.4 (C), 158.7 (C); *m*/*z* (ESI) 331 (MNa^+. 100%).

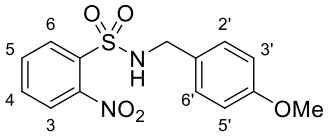
N-Benzyl-2-nitrobenzenesulfonamide (88j)[176]



2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of benzylamine (0.206 mL, 1.88 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was concentrated *in vacuo*, diluted with

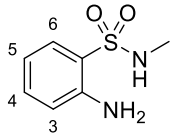
dichloromethane (20 mL) and washed with water (3 × 20 mL), sodium bicarbonate (2 × 10 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave *N*-benzyl-2-nitrobenzenesulfonamide (**88j**) (0.415 g, 75%) as a white solid. Mp 86–91 °C (lit.[176] 92 °C); δ_H (400 MHz, $CDCl_3$) 4.32 (2H, d, *J* 6.3 Hz, PhCH_2), 5.71 (1H, t, *J* 6.3 Hz, NH), 7.17–7.25 (5H, m, Ph), 7.63 (1H, td, *J* 7.8, 1.6 Hz, 5-H), 7.68 (1H, td, *J* 7.8, 1.5 Hz, 4-H), 7.82 (1H, dd, *J* 7.8, 1.5 Hz, 6-H), 8.01 (1H, dd, *J* 7.8, 1.6 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 48.0 (CH_2), 125.4 (CH), 128.0 (2 × CH), 128.2 (CH), 128.8 (2 × CH), 131.2 (CH), 132.8 (CH), 133.5 (CH), 134.2 (C), 135.8 (C), 148.0 (C); *m*/*z* (ESI) 315 (MNa^+. 100%).

N-(4'-Methoxybenzyl)-2-nitrobenzenesulfonamide (88k)[177]



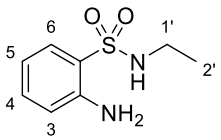
2-Nitrobenzenesulfonyl chloride (0.500 g, 2.26 mmol) was dissolved in dichloromethane (2 mL) and added dropwise to a stirred solution of 4-methoxybenzylamine (0.246 mL, 1.88 mmol) and triethylamine (0.315 mL, 2.26 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (20 mL) and washed with water (3 × 20 mL), sodium bicarbonate (2 × 10 mL), 1 M aqueous hydrochloric acid (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 50–70% dichloromethane in hexane gave *N*-(4'-methoxybenzyl)-2-nitrobenzenesulfonamide (**88k**) (0.458 g, 76%) as a white solid. Mp 112–116 °C (lit.[177] 117–119 °C); δ_H (400 MHz, $CDCl_3$) 3.75 (3H, s, OCH_3), 4.24 (2H, d, *J* 6.2 Hz, NHCH_2), 5.64 (1H, t, *J* 6.2 Hz, NH), 6.75 (2H, br d, *J* 8.4 Hz, 3'-H and 5'-H), 7.12 (2H, d, *J* 8.4 Hz, 2'-H and 6'-H), 7.64 (1H, td, *J* 7.6, 1.7 Hz, 5-H), 7.68 (1H, td, *J* 7.6, 1.5 Hz, 4-H), 7.82 (1H, dd, *J* 7.6, 1.5 Hz, 6-H), 8.01 (1H, dd, *J* 7.6, 1.7 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 47.6 (CH_2), 55.4 (CH_3), 114.2 (2 × CH), 125.4 (CH), 127.8 (C), 129.4 (2 × CH), 131.2 (CH), 132.8 (CH), 133.5 (CH), 134.2 (C), 148.0 (C), 159.5 (C); *m*/*z* (ESI) 345 (MNa^+. 100%).

2-Amino-*N*-methylbenzenesulfonamide (89a)[178]



To a stirred solution of 2-nitro-*N*-methylbenzenesulfonamide (0.126 g, 0.583 mmol) in ethyl acetate (6 mL) was added tin(II) dichloride dihydrate (0.658 g, 2.91 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (100 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (40 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 65% diethyl ether in hexane gave 2-amino-*N*-methylbenzenesulfonamide (**89a**) (0.0850 g, 78%) as a yellow oil. Spectroscopic data were consistent with the literature.[178] δ_H (400 MHz, $CDCl_3$) 2.58 (3H, d, *J* 5.5 Hz, NCH_3), 4.66 (1H, br s, NH), 4.85 (2H, br s, NH_2), 6.77 (1H, dd, *J* 8.1, 1.0 Hz, 3-H), 6.79–6.86 (1H, m, 5-H), 7.31–7.37 (1H, m, 4-H), 7.71 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 29.5 (CH_3), 117.9 (CH), 118.1 (CH), 120.7 (C), 130.2 (CH), 134.4 (CH), 145.2 (C); *m*/*z* (ESI) 209 (MNa^+. 100%).

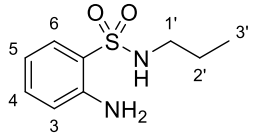
2-Amino-*N*-ethylbenzenesulfonamide (89b)



To a stirred solution of 2-nitro-*N*-ethylbenzenesulfonamide (0.142 g, 0.617 mmol) in ethyl acetate (6 mL) was added tin(II) dichloride dihydrate (0.696 g, 3.08 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (50 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-ethylbenzenesulfonamide (**89b**) (0.104 g, 84%) as a yellow oil. ν_{max}/cm^{-1} (neat) 3377 (NH), 2980 (CH), 1618 (C=C), 1483, 1454, 1315, 1140, 752; δ_H (400 MHz, $CDCl_3$) 1.07 (3H, t, *J* 7.3 Hz, 2'-H_3), 2.94 (2H, qd, *J* 7.3, 6.2 Hz, 1'-H_2), 4.61 (1H, br d, *J* 6.2 Hz, NH), 4.82 (2H, br s, NH_2), 6.77 (1H, dd, *J* 8.1, 0.9 Hz, 3-H), 6.79–6.84 (1H, m, 5-H), 7.30–7.36 (1H, m, 4-H), 7.71 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 15.1 (CH_3), 38.5 (CH_2),

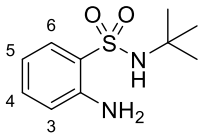
117.9 (CH), 118.1 (CH), 122.0 (C), 129.9 (CH), 134.2 (CH), 145.1 (C); *m/z* (ESI) 223.0509 (MNa^+. $C_8H_{12}N_2NaO_2S$ requires 223.0512).

2-Amino-*N*-propylbenzenesulfonamide (89c)



To a stirred solution of 2-nitro-*N*-propylbenzenesulfonamide (0.200 g, 0.819 mmol) in ethyl acetate (8 mL) was added tin(II) dichloride dihydrate (0.924 g, 4.09 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (60 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-propylbenzenesulfonamide (**89c**) (0.138 g, 79%) as a yellow oil. ν_{max}/cm^{-1} (neat) 3379 (NH), 2967 (CH), 1618 (C=C), 1481, 1454, 1315, 1134, 748; δ_H (400 MHz, $CDCl_3$) 0.83 (3H, t, *J* 7.4 Hz, 3'-H_3), 1.37–1.49 (2H, m, 2'-H_2), 2.82 (2H, q, *J* 6.9 Hz, 1'-H_2), 4.82–4.96 (3H, m, NH and NH_2), 6.72–6.81 (2H, m 3-H and 5-H), 7.27–7.33 (1H, m, 4-H), 7.69 (1H, dd, *J* 8.0, 1.4 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 11.2 (CH_3), 22.8 (CH_2), 45.1 (CH_2), 117.8 (CH), 117.8 (CH), 121.7 (C), 129.7 (CH), 134.1 (CH), 145.1 (C); *m/z* (ESI) 237.0664 (MNa^+. $C_9H_{14}N_2NaO_2S$ requires 237.0668).

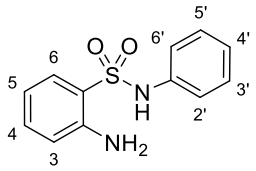
2-Amino-*N*-(*tert*-butyl)benzenesulfonamide (89d)



To a stirred solution of 2-nitro-*N*-(*tert*-butyl)benzenesulfonamide (0.222 g, 0.859 mmol) in ethyl acetate (9 mL) was added tin(II) dichloride dihydrate (0.970 g, 4.30 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (100 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave 2-amino-*N*-(*tert*-butyl)benzenesulfonamide (**89d**) (0.0330 g, 17%) as a beige solid. Mp 83–87 °C;

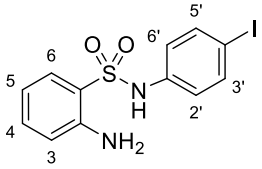
ν_{max}/cm^{-1} (neat) 3383 (NH), 2974 (CH), 1620 (C=C), 1483, 1315, 1136, 752; δ_H (400 MHz, $CDCl_3$) 1.18 (9H, s, NHC(CH_3)$_3$), 4.75–4.90 (3H, m, NH and NH_2), 6.73 (1H, br d, *J* 8.1 Hz, 3-H), 6.76–6.82 (1H, m, 5-H), 7.26–7.32 (1H, m, 4-H), 7.74 (1H, dd, *J* 8.0, 1.4 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 30.0 (3 × CH_3), 54.8 (C), 117.8 (CH), 118.0 (CH), 125.3 (C), 129.4 (CH), 133.8 (CH), 144.9 (C); *m*/*z* (ESI) 251.0824 (MNa^+. $C_{10}H_{16}N_2NaO_2S$ requires 251.0825).

2-Amino-*N*-phenylbenzenesulfonamide (89e)[41]



To a stirred solution of 2-nitro-*N*-phenylbenzenesulfonamide (0.265 g, 0.952 mmol) in ethyl acetate (10 mL) was added tin(II) dichloride dihydrate (1.07 g, 4.76 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (100 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-phenylbenzenesulfonamide (**89e**) (0.195 g, 83%) as a white solid. Mp 120–122 °C (lit.[179] 123–124 °C); δ_H (400 MHz, $CDCl_3$) 4.86 (2H, br s, NH_2), 6.63–6.70 (1H, m, 5-H), 6.74 (1H, dd, *J* 8.2, 1.0 Hz, 3-H), 6.77 (1H, br s, NH), 7.01–7.06 (2H, m, 2'-H and 6'-H), 7.12 (1H, tt, *J* 6.7, 1.2 Hz, 4'-H), 7.17–7.22 (2H, m, 3'-H and 5'-H), 7.24–7.28 (1H, m, 4-H), 7.48 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 117.8 (CH), 118.1 (CH), 121.2 (C), 123.0 (2 × CH), 126.1 (CH), 129.3 (2 × CH), 130.1 (CH), 134.6 (CH), 136.4 (C), 145.1 (C); *m*/*z* (ESI) 271 (MNa^+. 100%).

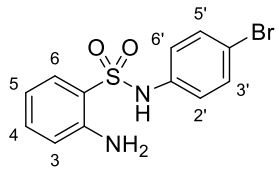
2-Amino-*N*-(4'-iodophenyl)benzenesulfonamide (89f)



To a stirred solution of *N*-(4'-iodophenyl)-2-nitrobenzenesulfonamide (0.200 g, 0.495 mmol) in ethyl acetate (5 mL) was added tin(II) dichloride dihydrate (0.558 g, 2.47 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The

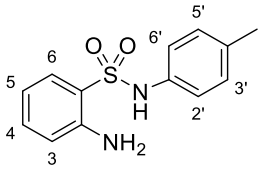
reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (60 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-(4'-iodophenyl)benzenesulfonamide (**89f**) (0.149 g, 80%) as a yellow solid. Mp 116–120 °C; ν_{max}/cm^{-1} (neat) 3383 (NH), 2916 (CH), 1620 (C=C), 1485, 1319, 1141; δ_H (400 MHz, $CDCl_3$) 4.84 (2H, br s, NH_2), 6.70 (1H, td, *J* 8.2, 1.0 Hz, 5-H), 6.73 (1H, dd, *J* 8.1, 1.0 Hz, 3-H), 6.77–6.84 (3H, m, NH, 2'-H and 6'-H), 7.26–7.32 (1H, m, 4-H), 7.47–7.53 (3H, m, 6-H, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 90.2 (C), 118.0 (CH), 118.4 (CH), 121.0 (C), 124.6 (2 × CH), 130.1 (CH), 134.8 (CH), 136.4 (C), 138.4 (2 × CH), 145.0 (C); *m*/*z* (ESI) 396.9481 (MNa^+. $C_{12}H_{11}IN_2NaO_2S$ requires 396.9478).

2-Amino-*N*-(4'-bromophenyl)benzenesulfonamide (89g)



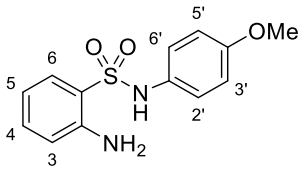
To a stirred solution of *N*-(4'-bromophenyl)-2-nitrobenzenesulfonamide (0.0940 g, 0.263 mmol) in ethyl acetate (3 mL) was added tin(II) dichloride dihydrate (0.297 g, 1.32 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (100 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (50 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-(4'-bromophenyl)benzenesulfonamide (**89g**) (0.0730 g, 85%) as a white solid. Mp 105–110 °C; ν_{max}/cm^{-1} (neat) 3225 (NH), 1616 (C=C), 1481, 1312, 1134, 910; δ_H (400 MHz, $CDCl_3$) 4.84 (2H, br s, NH_2), 6.67–6.72 (1H, m, 5-H), 6.75 (1H, dd, *J* 8.2, 0.8 Hz, 3-H), 6.83 (1H, br s, NH), 6.89–6.94 (2H, m, 2'-H and 6'-H), 7.26–7.34 (3H, m, 4-H, 3'-H and 5'-H), 7.48 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 118.0 (CH), 118.4 (CH), 119.4 (C), 121.0 (C), 124.6 (2 × CH), 130.1 (CH), 132.4 (2 × CH), 134.8 (CH), 135.6 (C), 145.0 (C); *m*/*z* (ESI) 348.9615 (MNa^+. $C_{12}H_{11}{}^{79}BrN_2NaO_2S$ requires 348.9617).

2-Amino-*N*-(4'-methylphenyl)benzenesulfonamide (89h)[43]



To a stirred solution of *N*-(4'-methylphenyl)-2-nitrobenzenesulfonamide (0.193 g, 0.660 mmol) in ethyl acetate (7 mL) was added tin(II) dichloride dihydrate (0.745 g, 3.30 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (100 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (30 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-(4'-methylphenyl)lbenzenesulfonamide (**89h**) (0.134 g, 77%) as a beige solid. Mp 123–125 °C (lit.[180] 125–126 °C); δ_H (400 MHz, $CDCl_3$) 2.25 (3H, s, 4'-CH_3), 4.85 (2H, br s, NH_2), 6.63–6.71 (2H, m, 5-H and NH), 6.74 (1H, dd, *J* 8.1, 0.8 Hz, 3-H), 6.88–6.94 (2H, m, 2'-H and 6'-H), 6.97–7.02 (2H, m, 3'-H and 5'-H), 7.23–7.29 (1H, m, 4-H), 7.46 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 21.0 (CH_3), 117.8 (CH), 118.1 (CH), 121.3 (C), 123.6 (2 × CH), 129.9 (2 × CH), 130.2 (CH), 133.7 (C), 134.5 (CH), 136.1 (C), 145.1 (C); *m*/*z* (ESI) 285 (MNa^+. 100%).

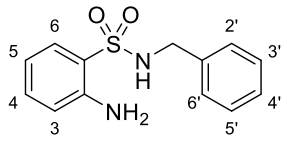
2-Amino-*N*-(4'-methoxyphenyl)benzenesulfonamide (89i)[181]



To a stirred solution of *N*-(4'-methoxyphenyl)-2-nitrobenzenesulfonamide (0.133 g, 0.431 mmol) in ethyl acetate (5 mL) was added tin(II) dichloride dihydrate (0.487 g, 2.16 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (100 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (50 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave 2-amino-*N*-(4'-methoxyphenyl)benzenesulfonamide (**89i**) (0.110 g, 92%) as an off-white solid. Mp 98–103 °C (lit.[181] 98–99 °C); δ_H (400 MHz, $CDCl_3$) 3.73 (3H, s, 4'-OCH_3), 4.84 (2H, br s, NH_2), 6.59 (1H, br s, NH), 6.63–6.77 (4H, m, 3-H, 5-H, 3'-H and 5'-H), 6.90–

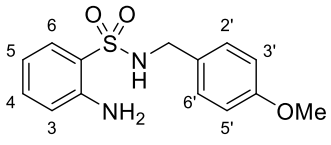
6.97 (2H, m, 2'-H and 6'-H), 7.26–7.31 (1H, m, 4-H), 7.40 (1H, dd, *J* 8.0, 1.4 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 55.5 (CH_3), 114.4 (2 × CH), 117.7 (CH), 118.1 (CH), 121.1 (C), 126.5 (2 × CH), 128.8 (C), 130.2 (CH), 134.5 (CH), 145.0 (C), 158.4 (C); *m*/*z* (ESI) 301 (MNa^+. 100%).

2-Amino-*N*-benzylbenzenesulfonamide (89j)



To a stirred solution of 2-nitro-*N*-benzylbenzenesulfonamide (0.176 g, 0.602 mmol) in ethyl acetate (6 mL) was added tin(II) dichloride dihydrate (0.679 g, 3.01 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (60 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (30 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave 2-amino-*N*-benzylbenzenesulfonamide (**89j**) (0.134 g, 85%) as a yellow oil; ν_{max}/cm^{-1} (neat) 3375 (NH), 1616 (C=C), 1481, 1454, 1315, 1138, 841; δ_H (400 MHz, $CDCl_3$) 4.05 (2H, d, *J* 6.2 Hz, PhCH_2), 4.84 (2H, br s, NH_2), 4.96 (1H, t, *J* 6.2 Hz, NH), 6.77 (1H, dd, *J* 8.1, 0.8 Hz, 3-H), 6.79–6.85 (1H, m, 5-H), 7.16–7.20 (2H, m, 2'-H and 6'-H), 7.24–7.37 (4H, m, 4-H, 3'-H, 4'-H and 5'-H), 7.74 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 47.5 (CH_2), 117.9 (CH), 118.2 (CH), 121.8 (C), 128.0 (2 × CH), 128.0 (CH), 128.8 (2 × CH), 129.9 (CH), 134.4 (CH), 136.4 (C), 145.2 (C); *m*/*z* (ESI) 285.0670 (MNa^+. $C_{13}H_{14}N_2NaO_2S$ requires 285.0668).

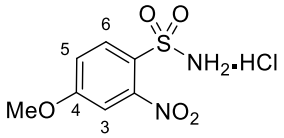
2-Amino-*N*-(4'-methoxybenzyl)benzenesulfonamide (89k)



To a stirred solution of 2-nitro-*N*-(4'-methoxybenzyl)benzenesulfonamide (0.250 g, 0.776 mmol) in ethyl acetate (7.5 mL) was added tin(II) dichloride dihydrate (0.875 g, 3.88 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (100 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (60 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried

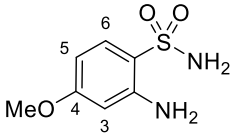
($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 65% diethyl ether in hexane gave 2-amino-*N*-(4'-methoxybenzyl)benzenesulfonamide (**89k**) (0.0760 g, 33%) as a colourless oil; ν_{max}/cm^{-1} (neat) 3372 (NH), 1612 (C=C), 1514, 1483, 1317, 1248, 1142, 752; δ_H (400 MHz, $CDCl_3$) 3.77 (3H, s, 4'-OCH_3), 3.98 (2H, d, *J* 6.1 Hz, NHCH_2), 4.83 (2H, br s, NH_2), 4.87 (2H, t, *J* 6.1 Hz, N*H*CH_2), 6.75–6.85 (4H, m, 3-H, 5-H, 3'-H and 5'-H), 7.06–7.12 (2H, m, 2'-H and 6'-H), 7.31–7.37 (1H, m, 4-H), 7.74 (1H, dd, *J* 8.0, 1.5 Hz, 6-H); δ_C (101 MHz, $CDCl_3$) 47.0 (CH_2), 55.4 (CH_3), 114.2 (2 × CH), 117.9 (CH), 118.1 (CH), 121.8 (C), 128.4 (C), 129.4 (2 × CH), 130.0 (CH), 134.4 (CH), 145.1 (C), 159.4 (C); *m/z* (ESI) 315.0770 (MNa^+. $C_{14}H_{16}N_2NaO_3S$ requires 315.0774).

4-Methoxy-2-nitrobenzenesulfonamide hydrochloride (85)



A solution of 4-methoxy-2-nitrobenzenesulfonyl chloride (0.0500 g, 0.199 mmol) in tetrahydrofuran (100 µL) and 28% aqueous ammonia solution (100 µL) was stirred at 0 °C for 0.5 h. The reaction mixture was diluted in water (5 mL), filtered and washed with water (20 mL) and diethyl ether (20 mL). Concentration *in vacuo* gave 4-methoxy-2-nitrobenzenesulfonamide hydrochloride (**85**) (0.0540 g, 100%) as a light brown solid. Mp 255–260 °C; ν_{max}/cm^{-1} (neat) 3282 (NH), 2342, 1604 (C=C), 1539, 1354, 1169; δ_H (400 MHz, CD_3OD) 3.94 (3H, s, OCH_3), 7.30 (1H, dd, *J* 8.9, 2.6 Hz, 5-H), 7.42 (1H, d, *J* 2.6 Hz, 3-H), 8.05 (1H, d, *J* 8.9 Hz, 6-H); δ_C (101 MHz, CD_3OD) 57.1 (CH_3), 111.7 (CH), 118.2 (CH), 129.3 (C), 132.5 (CH), 150.5 (C), 164.4 (C); *m/z* (ESI) 255.0048 (MNa^+. $C_7H_8N_2NaO_5S$ requires 255.0046).

2-Amino-4-methoxybenzenesulfonamide (86)



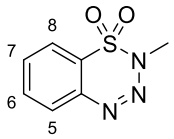
To a stirred solution of 2-nitro-4-methoxylbenzenesulfonamide hydrochloride (0.227 g, 0.845 mmol) in ethyl acetate (8.5 mL) was added tin(II) dichloride dihydrate (0.953 g, 4.22 mmol). The reaction was heated under reflux for 18 h. The reaction mixture was cooled to room temperature and sodium bicarbonate (150 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with

ethyl acetate (50 mL). The organic layer was washed with sodium bicarbonate (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 90% diethyl ether in hexane gave 2-amino-4-methoxybenzenesulfonamide (**86**) (0.125 g, 73%) as a white solid. Mp 124–130 °C; ν_{max}/cm^{-1} (neat) 3387 (NH), 3265, 1632 (C=C), 1566, 1445, 1296, 1152, 1130; δ_H (400 MHz, CD_3OD) 3.77 (3H, s, OCH_3), 6.27 (1H, dd, *J* 8.9, 2.4 Hz, 5-H), 6.35 (1H, d, *J* 2.4 Hz, 3-H), 7.56 (1H, d, *J* 8.9 Hz, 6-H); δ_C (101 MHz, CD_3OD) 55.7 (CH_3), 101.5 (CH), 104.7 (CH), 118.4 (C), 131.2 (CH), 149.0 (C), 165.4 (C); *m/z* (ESI) 255.0304 (MNa^+. $C_7H_{10}N_2NaO_3S$ requires 255.0304).

General Procedure for Synthesis of 2*H*-Benzothiatriazin-1,1-dioxides

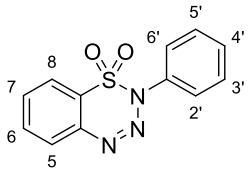
To a stirred solution of 2-aminobenzenesulfonamides (1 equiv.) in methanol (10 mL/mmol) at 0 °C was added polymer-supported nitrite (containing 3.0 equiv. of NO_2) and *p*-toluenesulfonic acid monohydrate (3 equiv.). The reaction mixture was stirred at 0 °C until completion or stirred at 0 °C for 1 h and then warmed to room temperature and stirred until completion. The resin was filtered and washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography gave the 2*H*-benzothiatriazin-1,1-dioxides

***N*-Methyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9a)[20]**



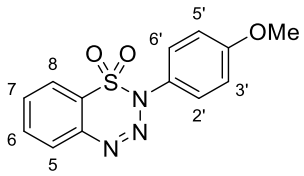
To a stirred solution of 2-amino-*N*-(methyl)benzenesulfonamide (0.0840 g, 0.451 mmol) in methanol (6 mL) was added polymer-supported nitrite (0.387 g, containing 1.35 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.257 g, 1.35 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with water (4 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% diethyl ether in hexane gave *N*-methyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9a**) (0.0450 g, 51%) as a yellow solid. Mp 70–72 °C (lit.[24] 68–72 °C); δ_H (400 MHz, $CDCl_3$) 3.91 (3H, s, NCH_3), 7.79 (1H, td, *J* 7.7, 1.2 Hz, 7-H), 7.87–7.92 (1H, m, 6-H), 8.02–8.07 (2H, m, 5-H and 8-H); δ_C (101 MHz, $CDCl_3$) 34.0 (CH_3), 120.6 (CH), 125.4 (C), 129.5 (CH), 132.8 (CH), 134.3 (CH), 141.8 (C); *m/z* (ESI) 220 (MNa^+. 100%).

_N_-Phenyl-1,2,3,4-benzothiatriazin-1,1(2_H_)-dioxide (9g)[47]



To a stirred solution of 2-amino-*N*-(phenyl)benzenesulfonamide (0.0390 g, 0.157 mmol) in methanol (1.5 mL) was added polymer-supported nitrite (0.135 g, containing 0.471 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.0900 g, 0.471 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% diethyl ether in hexane gave *N*-phenyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9g**) (0.0290 g, 71%) as an orange solid. Mp 111–115 °C (lit.[23] 111 °C); δ_H (400 MHz, $CDCl_3$) 7.51–7.60 (3H, m, 3'-H, 4'-H and 5'-H), 7.62–7.69 (2H, m, 2'H and 6'H), 7.84 (1H, td, *J* 7.7, 1.1 Hz, 7-H), 7.94 (1H, td, *J* 7.7, 1.4 Hz, 6-H), 8.10–8.15 (2H, m, 5-H and 8-H); δ_C (101 MHz, $CDCl_3$) 121.1 (CH), 126.6 (C), 128.1 (2 × CH), 129.7 (2 × CH), 129.8 (CH), 130.1 (CH), 133.2 (CH), 134.4 (CH), 135.0 (C), 141.4 (C); *m/z* (ESI) 282 (MNa^+. 100%).

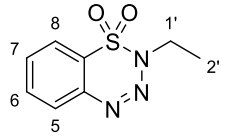
_N_-(4'-Methoxyphenyl)-1,2,3,4-benzothiatriazin-1,1(2_H_)-dioxide (9h)[182]



To a stirred solution of 2-amino-*N*-(4'-methoxyphenyl)benzenesulfonamide (0.0550 g, 0.197 mmol) in methanol (2 mL) was added polymer-supported nitrite (0.170 g, containing 0.592 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.113 g, 0.592 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 1.3 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% diethyl ether in hexane gave *N*-(4'-methoxyphenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9h**) (0.0390 g, 68%) as a yellow solid. Mp 77–82 °C (lit.[182] 83–84 °C); δ_H (400 MHz, $CDCl_3$) 3.88 (3H, s, 4'-OCH_3), 7.05 (2H, br d, *J* 8.8 Hz, 3'-H and 5'-H), 7.56 (2H, br d, *J* 8.8 Hz, 2'-H and 6'-H), 7.80–7.87 (1H, m, 6-H), 7.90–7.97 (1H, m, 7-H), 8.08–8.17 (2H, m, 5-H and 8-H); δ_C (101 MHz, $CDCl_3$) 55.8 (CH_3), 114.9 (2 × CH), 121.1 (CH), 126.5 (C), 127.1

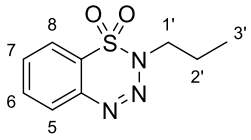
(C), 129.7 (CH), 130.0 (2 × CH), 133.1 (CH), 134.4 (CH), 141.4 (C), 161.1 (C); *m*/*z* (ESI) 284 (MNa^+–N_2. 100%).

N-Ethyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9o)[30]



To a stirred solution of 2-amino-*N*-(ethyl)benzenesulfonamide (0.0500 g, 0.250 mmol) in methanol (2.5 mL) was added polymer-supported nitrite (0.215 g, containing 0.749 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.142 g, 0.749 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with water (4 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave *N*-ethyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9o**) (0.0410 g, 75%) as a dark orange solid. Spectroscopic data were consistent with the literature.[30] Mp 75–78 °C; δ_H (400 MHz, $CDCl_3$) 1.59 (3H, t, *J* 7.2 Hz, 2'-H_3), 4.34 (2H, q, *J* 7.2 Hz, 1'-H_2), 7.77 (1H, td, *J* 7.8, 1.1 Hz, 6-H), 7.88 (1H, td, *J* 7.8, 1.4 Hz, 7-H), 8.01 (1H, dd, *J* 7.8, 1.4 Hz, 5-H), 8.03 (1H, dd, *J* 7.8, 1.1 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 16.0 (CH_3), 43.7 (CH_2), 120.4 (CH), 125.7 (C), 129.3 (CH), 132.6 (CH), 134.1 (CH), 141.8 (C); *m*/*z* (ESI) 234 (MNa^+. 100%).

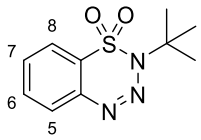
N-Propyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9p)



To a stirred solution of 2-amino-*N*-(propyl)benzenesulfonamide (0.0650 g, 0.303 mmol) in methanol (3 mL) was added polymer-supported nitrite (0.261 g, containing 0.910 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.173 g, 0.910 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 1 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-propyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9p**) (0.0560 g, 82%) as a red solid. Mp 83–86 °C; ν_{max}/cm^{-1} (neat) 2967 (CH), 1573 (C=C), 1471, 1446, 1329, 1313, 1185, 1160, 1111, 946, 766; δ_H (400 MHz, $CDCl_3$) 1.04 (3H, t, *J* 7.4 Hz, 3'-H_3), 2.01 (2H, sext, *J* 7.4 Hz, 2'-H_2), 4.23 (2H, t, *J* 7.4 Hz,

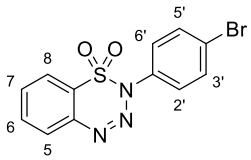
1'-H_2), 7.77 (1H, td, *J* 7.8, 1.1 Hz, 6-H), 7.88 (1H, td, *J* 7.8, 1.4 Hz, 7-H), 8.02 (1H, dd, *J* 7.8, 1.4 Hz, 5-H), 8.04 (1H, dd, *J* 7.8, 1.1 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 11.2 (CH_3), 23.7 (CH_2), 50.0 (CH_2), 120.5 (CH), 125.7 (C), 129.3 (CH), 132.6 (CH), 134.1 (CH), 141.8 (C); *m*/*z* (ESI) 248.0468 (MNa^+. $C_9H_{11}N_3NaO_2S$ requires 248.0464).

N-(*tert*-Butyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9q)[24]



To a stirred solution of 2-amino-*N*-(*tert*-butyl)benzenesulfonamide (0.0260 g, 0.114 mmol) in methanol (1.1 mL) was added polymer-supported nitrite (0.0980 g, containing 0.342 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.0650 g, 0.342 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 1 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (4 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave *N*-(*tert*-butyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9q**) (0.0160 g, 60%) as an orange solid. Mp 78–82 °C (lit.[24] 78–82 °C); δ_H (400 MHz, $CDCl_3$) 1.82 (9H, s, NC(CH_3)$_3$), 7.74 (1H, td, *J* 7.8, 1.2 Hz, 7-H), 7.85 (1H, td, *J* 7.8, 1.4 Hz, 6-H), 7.96–8.03 (2H, m, 5-H and 8-H); δ_C (101 MHz, $CDCl_3$) 30.5 (3 × CH_3), 67.9 (C), 120.4 (CH), 126.3 (C), 128.6 (CH), 132.3 (CH), 134.0 (CH), 141.2 (C); *m*/*z* (ESI) 262 (MNa^+. 100%).

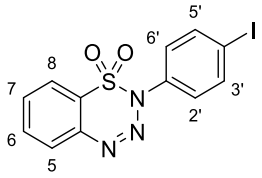
N-(4'-Bromophenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9r)



To a stirred solution of 2-amino-*N*-(4'-bromophenyl)benzenesulfonamide (0.100 g, 0.306 mmol) in methanol (3.1 mL) was added polymer-supported nitrite (0.263 g, containing 0.917 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.174 g, 0.917 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 40% diethyl ether in hexane gave *N*-(4'-bromophenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9r**) (0.0700 g, 68%) as an

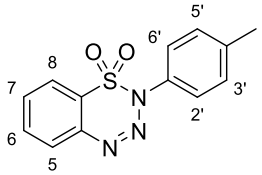
orange solid. Mp 107–112 °C; ν_{max}/cm^{-1} (neat) neat) 2981 (CH), 2326, 1572 (C=C), 1471, 1448, 1397, 1337, 1230, 1168, 1078, 904, 759; δ_H (400 MHz, $CDCl_3$) 7.50–7.56 (2H, m, 2'-H and 6'-H), 7.65–7.71 (2H, m, 3-'H and 5-'H), 7.85 (1H, td, *J* 7.7, 1.2 Hz, 6-H), 7.95 (1H, td, *J* 7.7, 1.3 Hz, 7-H), 8.12 (1H, dd, *J* 7.7, 1.3 Hz, 5-H), 8.13 (1H, dd, *J* 7.7, 1.2 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 121.1 (CH), 124.4 (C), 126.6 (C), 129.5 (2 × CH), 129.9 (CH), 132.9 (2 × CH), 133.4 (CH), 134.1 (C), 134.6 (CH), 141.3 (C); *m/z* (ESI) 359.9408 (MNa^+. $C_{12}H_8{}^{79}BrN_3NaO_2S$ requires 359.9413).

N-(4'-Iodophenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9s)



To a stirred solution of 2-amino-*N*-(4'-iodophenyl)benzenesulfonamide (0.119 g, 0.318 mmol) in methanol (3.2 mL) was added polymer-supported nitrite (0.273 g, containing 0.954 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.182 g, 0.954 mmol). The reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with water (4 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% diethyl ether in hexane gave *N*-(4'-iodophenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9s**) (0.0720 g, 59%) as an orange solid. Mp 115–118 °C; ν_{max}/cm^{-1} (neat) 1570 (C=C), 1472, 1454, 1338, 1182, 1159, 1128, 808, 762; δ_H (400 MHz, $CDCl_3$) 7.37–7.41 (2H, m, 2'-H and 6'-H), 7.83–7.91 (3H, m, 6-H, 3-'H and 5-'H), 7.96 (1H, td, *J* 7.8, 1.4 Hz, 7-H), 8.12 (1H, dd, *J* 7.8, 1.4 Hz, 5-H), 8.14 (1H, dd, *J* 7.8, 1.2 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 96.0 (C), 121.1 (CH), 126.6 (C), 129.6 (2 × CH), 130.0 (CH), 133.4 (CH), 134.6 (CH), 134.8 (C), 138.9 (2 × CH), 141.3 (C); *m/z* (ESI) 407.9274 (MNa^+. $C_{12}H_8IN_3NaO_2S$ requires 407.9274).

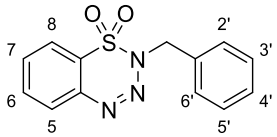
N-(4'-Methylphenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9t)[30]



To a stirred solution of 2-amino-*N*-(4'-methylphenyl)lbenzenesulfonamide (0.0650 g, 0.248 mmol) in methanol (2.5 mL) was added polymer-supported nitrite (0.213 g,

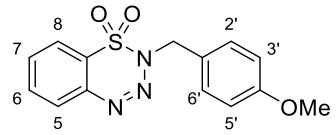
containing 0.743 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.141 g, 0.743 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 2.5 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave *N*-(4'-methylphenyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9t**) (0.0440 g, 65%) as an orange solid. Spectroscopic data were consistent with the literature.[30] Mp 100–102 °C; δ_H (400 MHz, $CDCl_3$) 2.45 (3H, s, 4'-CH_3), 7.35 (2H, br d, *J* 8.0 Hz, 3'-H and 5'-H), 7.50–7.55 (2H, m, 2'-H and 6'-H), 7.83 (1H, td, *J* 7.6, 1.2 Hz, 6-H), 7.93 (1H, td, *J* 7.6, 1.4 Hz, 7-H), 8.08–8.14 (2H, m, 5-H and 8-H); δ_C (101 MHz, $CDCl_3$) 21.4 (CH_3), 121.1 (CH), 126.7 (C), 128.1 (2 × CH), 129.7 (CH), 130.3 (2 × CH), 132.3 (C), 133.1 (CH), 134.4 (CH), 140.5 (C), 141.5 (C); *m*/*z* (ESI) 296 (MNa^+. 100%).

N-Benzyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (9u)[30]



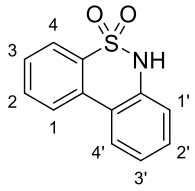
To a stirred solution of 2-amino-*N*-benzylbenzenesulfonamide (0.130 g, 0.496 mmol) in methanol (5 mL) was added polymer-supported nitrite (0.426 g, containing 1.49 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.283 g, 1.49 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 1 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-benzyl-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9u**) (0.104 g, 76%) as an orange solid. Spectroscopic data were consistent with the literature.[30] Mp 99–102 °C; δ_H (400 MHz, $CDCl_3$) 5.42 (2H, s, PhCH_2), 7.30–7.38 (3H, m, 3'-H, 4'-H and 5'-H), 7.46–7.51 (2H, m, 2'-H and 6'-H), 7.77 (1H, td, *J* 7.7, 1.2 Hz, 6-H), 7.87 (1H, td, *J* 7.7, 1.4 Hz, 7-H), 8.01 (1H, dd, *J* 7.7, 1.4 Hz, 5-H), 8.04 (1H, dd, *J* 7.7, 1.2 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 51.3 (CH_2), 120.6 (CH), 126.1 (C), 128.6 (CH), 128.9 (2 × CH), 129.0 (2 × CH), 129.5 (CH), 132.8 (CH), 134.2 (CH), 135.4 (C), 141.8 (C); *m*/*z* (ESI) 296 (MNa^+. 100%).

_N_-(4'-Methoxybenzyl)-1,2,3,4-benzothiatriazin-1,1(2_H_)-dioxide (9v)[30]



To a stirred solution of 2-amino-*N*-(4'-methoxybenzyl)benzenesulfonamide (0.0700 g, 0.293 mmol) in methanol (2 mL) was added polymer-supported nitrite (0.206 g, containing 0.718 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.137 g, 0.718 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 3.5 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-(4'-methoxybenzyl)-1,2,3,4-benzothiatriazin-1,1(2*H*)-dioxide (**9v**) (0.0610 g, 85%) as a red solid. Spectroscopic data were consistent with the literature.[30] Mp 92–95 °C; δ_H (400 MHz, $CDCl_3$) 3.79 (3H, s, 4'-OCH_3), 5.36 (2H, s, NCH_2), 6.85–6.91 (2H, m, 3'-H and 5'-H), 7.40–7.46 (2H, m, 2'-H and 6'-H), 7.76 (1H, td, *J* 7.8, 1.2 Hz, 7-H), 7.86 (1H, td, *J* 7.8, 1.4 Hz, 6-H), 8.01 (1H, dd, *J* 7.8, 1.2 Hz, 5-H), 8.03 (1H, dd, *J* 7.8, 1.4 Hz, 8-H); δ_C (101 MHz, $CDCl_3$) 51.0 (CH_2), 55.4 (CH_3), 114.3 (2 × CH), 120.6 (CH), 126.1 (C), 127.5 (C), 129.5 (CH), 130.6 (2 × CH), 132.7 (CH), 134.2 (CH), 141.8 (C), 159.9 (C); *m*/*z* (ESI) 326 (MNa^+. 100%).

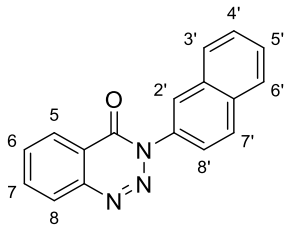
6_H_-Dibenzo[_c,e_][1,2]thiazine-5,5-dioxide (17a)[97]



To a stirred solution of 2-amino-*N*-phenylbenzenesulfonamide (0.0600 g, 0.242 mmol) in methanol (2.5 mL) was added polymer-supported nitrite (0.208 g, containing 0.725 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.138 g, 0.253 mmol). The reaction mixture was stirred at 0 °C for 0.2 h then heated to 40 °C for 3 h. The reaction mixture cooled to room temperature, diluted with ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 100% dichloromethane gave 6*H*-dibenzo[*c,e*][1,2]thiazine-5,5-dioxide (**17a**) (0.0290 g, 52%) as a light brown solid. Mp 195–198 °C (lit.[97] 196–197 °C); δ_H (400 MHz, CD_3OD) 7.17 (1H, dd, *J* 8.0, 1.1 Hz, 1'-H), 7.28 (1H, td, *J* 8.0, 1.1 Hz, 3'-

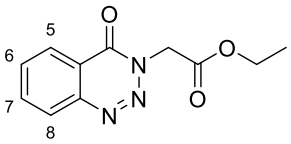
H), 7.43 (1H, td, *J* 8.0, 1.2 Hz, 2'-H), 7.60 (1H, td, *J* 7.7, 1.0 Hz, 2-H), 7.75 (1H, td, *J* 7.7, 1.4 Hz, 3-H), 7.95 (1H, dd, *J* 7.7, 1.4 Hz, 1-H), 8.08–8.15 (2H, m, 4-H and 4'-H); δ_C (101 MHz, CD_3OD) 120.9 (CH), 122.5 (CH), 123.5 (C), 125.2 (CH), 126.3 (CH), 126.5 (CH), 129.3 (CH), 131.4 (CH), 133.5 (CH), 133.9 (C), 136.3 (C), 138.0 (C); *m*/*z* (ESI) 254 (MNa^+. 100%).

N-Naphthyl-1,2,3-benzotriazin-4(3*H*)-one (93)[183]



To a stirred solution of 1,2,3-benzotriazin-4(3*H*)-one (0.100 g, 0.680 mmol), 2-naphthaleneboronic acid (0.175 g, 1.02 mmol) and copper acetate (0.123 g, 0.680 mmol) in dichloroethane (7 mL) was added triethylamine (188 µL, 1.36 mmol). The reaction was stirred at room temperature for 3 h and then heated to 45 °C for a further 2.5 h. The reaction mixture was cooled to room temperature and filtered through a pad of Celite®. The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave *N*-naphthyl-1,2,3-benzotriazin-4(3*H*)-one (**93**) (0.118 g, 64%) as a yellow solid. Mp 169–173 °C (lit.[183] 173–175 °C); δ_H (400 MHz, $CDCl_3$) 7.53–7.61 (2H, m, 4'-H and 5'-H), 7.76 (1H, dd, *J* 8.8, 2.1 Hz, 8'-H), 7.85–7.90 (1H, m, 6-H), 7.91–7.97 (2H, m, 3'-H and 6'-H), 7.98–8.04 (2H, m, 7-H and 7'-H), 8.18 (1H, d, *J* 2.1 Hz, 2'-H), 8.26 (1H, br d, *J* 8.1 Hz 8-H), 8.48 (1H, dd, *J* 8.0, 1.5 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 120.6 (C), 123.8 (CH), 125.1 (CH), 125.8 (CH), 126.9 (CH), 127.2 (CH), 127.9 (CH), 128.6 (CH), 128.7 (CH), 129.1 (CH), 133.0 (CH), 133.2 (C), 133.3 (C), 135.3 (CH), 136.4 (C), 143.9 (C), 155.6 (C); *m*/*z* (ESI) 296 (MNa^+. 100%).

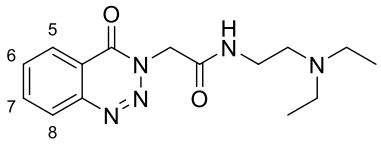
N-(Ethoxycarbonylmethyl)-1,2,3-benzotriazin-4(3*H*)-one (94)[184]



To a stirred solution of 1,2,3-benzotriazin-4(3*H*)-one (0.150 g, 1.02 mmol) in acetonitrile (10 mL) was added ethyl bromoacetate (0.113 mL, 1.02 mmol) and potassium carbonate (0.141 g, 1.02 mmol). The reaction mixture was heated under reflux for 18 h. The reaction mixture was cooled to room temperature, diluted in

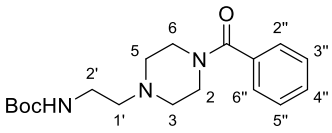
chloroform (30 mL) and washed with water (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave *N*-(ethoxycarbonylmethyl)-1,2,3-benzotriazin-4(3*H*)-one (**94**) (0.140 g, 59%) as an off-white solid. Mp 107–111 °C (lit.[184] 114 °C); δ_H (400 MHz, $CDCl_3$) 1.31 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.28 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 5.19 (2H, s, CH_2CO_2Et), 7.83 (1H, ddd, *J* 8.8, 7.9, 1.2 Hz, 6-H), 7.98 (1H, ddd, *J* 8.8, 8.2, 1.5 Hz, 7-H), 8.19 (1H, ddd, *J* 8.2, 1.2, 0.6 Hz, 8-H), 8.37 (1H, ddd, *J* 7.9, 1.5, 0.6 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 14.3 (CH_3), 51.0 (CH_2), 62.2 (CH_2), 119.9 (C), 125.4 (CH), 128.7 (CH), 132.8 (CH), 135.3 (CH), 144.5 (C), 155.7 (C), 167.2 (C); *m*/*z* (ESI) 256 (MNa^+. 100%).

N-(2'-Diethylaminoethyl)acetamide-1,2,3-benzotriazin-4(3*H*)-one (95)[184]



To a stirred solution of *N*-(ethoxycarbonylmethyl)-1,2,3-benzotriazin-4(3*H*)-one (0.10 g, 0.43 mmol) in anhydrous methanol (4 mL) was added dropwise *N,N*-diethylethylenediamine (0.061 mL, 0.43 mmol). The reaction mixture was heated under reflux for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 10% methanol in dichloromethane gave *N*-(2'-diethylaminoethyl)acetamide-1,2,3-benzotriazin-4(3*H*)-one (**95**) (0.094 g, 72%) as a white solid. Mp 147–150 °C (lit.[184] 155–156 °C); δ_H (400 MHz, $CDCl_3$) 0.97 (6H, t, *J* 7.1 Hz, 2 × NCH_2CH_3), 2.55 (4H, q, *J* 7.1 Hz, 2 × NCH_2CH_3), 2.61 (2H, t, *J* 5.7 Hz, Et_2NCH_2), 3.39 (2H, q, *J* 5.7 Hz, $CONHCH_2$), 5.13 (2H, s, CH_2CONH), 6.85 (1H, br s, NH), 7.79–7.85 (1H, m, 6-H), 7.94–8.00 (1H, m, 7-H), 8.19 (1H, dd, *J* 8.2, 0.5 Hz, 8-H), 8.36 (1H, dd, *J* 7.9, 1.5 Hz, 5-H); δ_C (101 MHz, $CDCl_3$) 11.4 (2 × CH_3), 36.9 (CH_2), 46.9 (2 × CH_2), 51.5 (CH_2), 52.8 (CH_2), 120.0 (C), 125.3 (CH), 128.7 (CH), 132.7 (CH), 135.2 (CH), 144.5 (C), 155.8 (C), 166.1 (C); *m*/*z* (ESI) 304 (MNa^+. 100%).

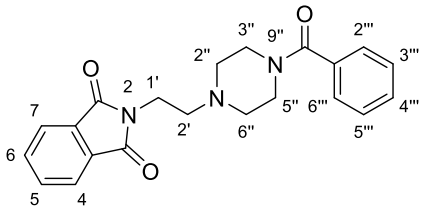
1-Benzoyl-4-[2'-(*N*-*tert*-butoxycarbonylamino)ethyl]piperazine (98)



To a stirred solution of benzoic acid (0.300 g, 2.46 mmol) and *O*-benzotriazole-*N,N,N',N'*- tetramethyluroniumhexafluorophosphate (1.03 g, 2.70 mmol) in

anhydrous acetonitrile (20 mL) was added triethylamine (0.491 mL, 3.69 mmol). The reaction mixture was stirred at room temperature for 3.5 h. 1-(2-*N*-Boc-aminoethyl)piperazine (0.563 g, 2.46 mmol) was added and the reaction mixture was heated to 50 °C for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The mixture was diluted with chloroform (50 mL) and washed with sodium bicarbonate (6 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 100% ethyl acetate gave 1-benzoyl-4-[2'-(*N*-*tert*-butoxycarbonylamino)ethyl]piperazine (**98**) (0.511 g, 62%) as a brown oil. ν_{max}/cm^{-1} (neat) 2938 (NH), 1698 (C=O), 1623, 1578 (C=C), 1495, 1434, 1391, 1266, 1162, 1013, 909, 710; δ_H (400 MHz, $CDCl_3$) 1.45 (9H, s, 3 × CH_3), 2.41 (4H, br s, 3-H_2 and 5-H_2), 2.49 (2H, br t, *J* 5.6 Hz, 1'-H_2), 3.24 (2H, br q, *J* 5.6 Hz, 2'-H_2), 3.43 (2H, br s, 2-H_2 or 6-H_2), 3.78 (2H, br s, 2-H_2 or 6-H_2), 4.91 (1H, br s, NH), 7.37–7.43 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.2 (CH_2), 42.2 (CH_2), 47.8 (CH_2), 53.3 (CH_2), 57.3 (2 × CH_2), 79.4 (C), 127.1 (2 × CH), 128.6 (2 × CH), 129.8 (CH), 135.9 (C), 156.0 (C), 170.4 (C); *m/z* (ESI) 334.2122 (MNa^+. $C_{18}H_{28}N_3NaO_3$ requires 334.2125).

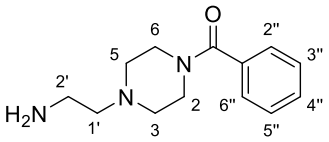
2-[2'-(4''-Benzoylpiperazin-1-yl)ethyl]isoindole-1,3-dione (105)



To an oven dried flask containing *N*-benzoylpiperazine (0.900 g, 4.73 mmol) in acetonitrile (30 mL) was added 2-(2-bromoethyl)isoindoline-1,3-dione (1.20 g, 4.73 mmol) and potassium carbonate (2.29 g, 16.6 mmol). The reaction mixture was heated under reflux for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted with water (60 mL) and extracted with ethyl acetate (5 × 60 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 70–80% ethyl acetate in hexane gave 2-[2'-(4''-benzoylpiperazin-1-yl)ethyl]isoindole-1,3-dione (**105**) (1.09 g, 63%) as a white solid. Mp 151–156 °C; ν_{max}/cm^{-1} (neat) 2813 (CH), 2359, 1765, 1702 (C=O), 1617, 1578 (C=C), 1432, 1399, 1288, 1009, 709; δ_H (500 MHz, CD_3OD) 2.50 (2H, br s, 2''-H_2), 2.63 (2H, br s, 6''-H_2), 2.69 (2H, br t, *J* 6.3 Hz, 2'-H_2), 3.38 (2H, br s, 3''-H_2), 3.70 (2H, br s, 5''-H_2),

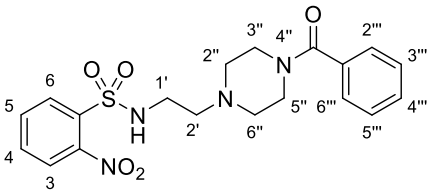
3.84 (2H, br t, *J* 6.3 Hz, 1'-H_2), 7.36–7.49 (5H, m, Ph), 7.77–7.88 (4H, m, 4-H, 5-H, 6-H and 7-H); δ_C (126 MHz, CD_3OD) 35.9 (CH_2), 43.2 (CH_2), 53.7 (CH_2), 54.2 (CH_2), 56.6 (2 × CH_2), 124.1 (2 × CH), 128.0 (2 × CH), 129.7 (2 × CH), 131.1 (CH), 133.5 (2 × C), 135.3 (2 × CH), 136.8 (C), 169.9 (2 × C), 172.4 (C); *m/z* (ESI) 386.1476 (MNa^+. $C_{21}H_{21}N_3NaO_3$ requires 386.1475).

1-Benzoyl-4-[2'-aminoethyl]piperazine (106)



To a stirred solution of 2-[2'-(4''-benzoylpiperazin-1-yl)ethyl]isoindole-1,3-dione (0.200 g, 0.550 mmol) in ethanol (5.5 mL) was added hydrazine monohydrate (0.149 mL, 0.605 mmol). The reaction was heated under reflux for 4 h. The reaction mixture was cooled to room temperature. The resulting white solid was filtered and washed with ethanol (50 mL). The filtrate was concentrated *in vacuo*. The solid was dissolved in 1 M aqueous sodium hydroxide (25 mL) and saturated with sodium chloride. The mixture was extracted with ethyl acetate (5 × 25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 1-benzoyl-4-[2'-aminoethyl]piperazine (**106**) (0.110 g, 86%) as a yellow oil. ν_{max}/cm^{-1} (neat) 3372 (NH), 2941 (CH), 1628 (C=O), 1576 (C=C), 1433, 1293, 1002, 761; δ_H (500 MHz, CD_3OD) 2.44 (2H, br s, 3-H_2), 2.50 (2H, br t, *J* 6.2 Hz, 1'-H_2), 2.57 (2H, br s, 5-H_2), 2.77 (2H, br t, *J* 6.2 Hz, 2'-H_2), 3.47 (2H, br s, 2-H_2), 3.79 (2H, br s, 6-H_2), 7.38–7.51 (5H, m, Ph); δ_C (126 MHz, CD_3OD) 38.8 (CH_2), 43.2 (CH_2), 53.9 (CH_2), 54.4 (CH_2), 60.7 (2 × CH_2), 128.0 (2 × CH), 129.7 (2 × CH), 131.1 (CH), 136.8 (C), 172.4 (C); *m/z* (ESI) 256.1418 (MNa^+. $C_{13}H_{19}N_3NaO$ requires 256.1420).

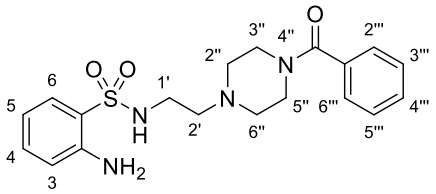
***N*-[2'-(4''-Benzoylpiperazin-1-yl)ethyl)-2-nitrobenzenesulfonamide (100)**



2-Nitrobenzenesulfonyl chloride (0.101 g, 0.454 mmol) was dissolved in dichloromethane (1 mL) and added dropwise to a stirred solution of 1-benzoyl-4-[2'-aminoethyl]piperazine (0.100 g, 0.429 mmol) and triethylamine (0.0630 mL, 0.454 mmol) in dichloromethane (0.2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was concentrated *in*

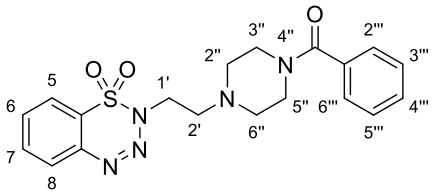
vacuo. Purification by flash column chromatography, eluting with 1% methanol in dichloromethane gave *N*-[2'-(4''-benzoylpiperazin-1-yl)ethyl)-2-nitrobenzenesulfonamide (**100**) (0.160 g, 89%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3335 (NH), 2941 (CH), 1627 (C=O), 1576 (C=C), 1539, 1437, 1364, 1166, 1012, 759; δ_H (500 MHz, CD_3OD) 2.39 (2H, br s, 2''-H_2), 2.49 (2H, br s, 6''-H_2), 2.54 (2H, br t, *J* 6.1 Hz, 2'-H_2), 3.21 (2H, br t, *J* 6.1 Hz, 1'-H_2), 3.37 (2H, br s, 3''-H_2), 3.68 (2H, br s, 5''-H_2), 7.35–7.51 (5H, m, Ph), 7.79–7.86 (2H, m, 4-H and 6-H), 7.90 (1H, br t, *J* 5.3 Hz, 5-H), 8.10–8.15 (1H, m, 3-H); δ_C (126 MHz, CD_3OD) 41.1 (CH_2), 43.0 (CH_2), 53.4 (CH_2), 54.0 (CH_2), 57.5 (2 × CH_2), 126.1 (CH), 128.0 (2 × CH), 129.7 (2 × CH), 131.2 (CH), 131.7 (CH), 133.7 (CH), 134.7 (C), 135.1 (CH), 136.6 (C), 149.6 (C), 172.4 (C); *m/z* (ESI) 441.1211 (MNa^+. $C_{19}H_{22}N_4NaO_5S$ requires 441.1203).

N-[2'-(4''-Benzoylpiperazin-1-yl)ethyl)-2-aminobenzenesulfonamide (101)



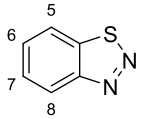
To a stirred solution of *N*-[2'-(4''-benzoylpiperazin-1-yl)ethyl)-2-nitrobenzenesulfonamide (0.0830 g, 0.198 mmol) in methanol (2 mL) was added zinc powder (0.129 g, 1.98 mmol) and acetic acid (0.113 mL, 1.98 mmol). The reaction was stirred at room temperature for 5 h. The reaction mixture was filtered through a pad of Celite® and washed with methanol (50 mL). The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 5% methanol in dichloromethane gave *N*-[2'-(4''-benzoylpiperazin-1-yl)ethyl)-2-aminobenzenesulfonamide (**101**) (0.0650 g, 84%) as a white solid. Mp 65–70 °C; ν_{max}/cm^{-1} (neat) 3373 (NH), 2944 (CH), 1617 (C=O), 1575 (C=C), 1454, 1305, 1146, 1012, 711; δ_H (500 MHz, CD_3OD) 2.29 (2H, br s, 2''-H_2), 2.35–2.47 (4H, m, 2'-H_2 and 6''-H_2), 2.96 (2H, br t, *J* 6.4 Hz, 1'-H_2), 3.39 (2H, br s, 3''-H_2), 3.71 (2H, br s, 5''-H_2), 6.70 (1H, br t, *J* 8.1 Hz, 5-H), 6.84 (1H, br d, *J* 8.1 Hz, 3-H), 7.29 (1H, td, *J* 8.1, 1.2 Hz, 4-H), 7.35–7.51 (5H, m, Ph), 7.62 (1H, dd, *J* 8.1, 1.2 Hz, 6-H); δ_C (126 MHz, CD_3OD) 40.6 (CH_2), 43.1 (CH_2), 53.4 (CH_2), 53.9 (CH_2), 57.3 (2 × CH_2), 117.2 (CH), 118.3 (CH), 121.7 (C), 128.0 (2 × CH), 129.7 (2 × CH), 130.6 (CH), 131.1 (CH), 135.0 (CH), 136.7 (C), 147.7 (C), 172.3 (C); *m/z* (ESI) 411.1463 (MNa^+. $C_{19}H_{24}N_4NaO_3S$ requires 411.1461).

_N_-[2'-(4''-Benzoylpiperazin-1-yl)ethyl)-1,2,3,4-benzothiatriazin-1,1(2_H_)-dioxide (102)



To a stirred solution of _N_-[2'-(4''-benzoylpiperazin-1-yl)ethyl)-2-aminobenzenesulfonamide (0.0760 g, 0.196 mmol) in methanol (2 mL) was added polymer-supported nitrite (0.164 g, containing 0.587 mmol of NO_2) and _p_-toluenesulfonic acid monohydrate (0.112 g, 0.587 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 1 h. The reaction mixture was filtered and the resulting resin was washed with methanol (10 mL). The reaction mixture was concentrated _in vacuo._ Purification by flash column chromatography, eluting with 60% ethyl acetate ether in hexane with 1% triethylamine gave _N_-[2'-(4''-benzoylpiperazin-1-yl)ethyl)-1,2,3,4-benzothiatriazin-1,1(2_H_)-dioxide (**102**) (0.0560 g, 72%) as a yellow oil. ν_{max}/cm^{-1} (neat) 2939 (CH), 1630 (C=O), 1575 (C=C), 1433, 1337, 1188, 1013, 761; δ_H (500 MHz, CD_3OD) 2.53 (2H, br s, 2''-H_2), 2.66 (2H, br s, 6''-H_2), 2.93 (2H, t, _J_ 6.5 Hz, 2'-H_2), 3.41 (2H, br s, 3''-H_2), 3.74 (2H, br s, 5''-H_2), 4.41 (2H, t, _J_ 6.5 Hz, 1'-H_2), 7.36–7.50 (5H, m, Ph), 7.91 (1H, td, _J_ 7.7, 1.4 Hz, 7-H), 8.01 (1H, td, _J_ 7.7, 1.2 Hz, 6-H), 8.06–8.11 (2H, m, 5-H and 8-H); δ_C (126 MHz, CD_3OD) 43.2 (CH_2), 46.2 (CH_2), 54.0 (CH_2), 54.2 (CH_2), 58.0 (2 × CH_2), 121.2 (CH), 127.3 (C), 128.0 (2 × CH), 129.7 (2 × CH), 130.3 (CH), 131.1 (CH), 134.4 (CH), 135.6 (CH), 136.8 (C), 142.9 (C), 172.4 (C); _m/z_ (ESI) 422.1253 (MNa^+. $C_{19}H_{21}N_5NaO_3S$ requires 422.1257).

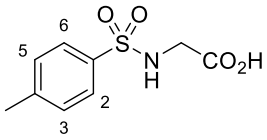
Benzo[_d_]-1,2,3-thiadiazole (108)[18]



To a stirred solution of 2-aminothiophenol (0.150 mL, 1.40 mmol) in methanol (10 mL) at 0 °C was added polymer-supported nitrite (1.20 g, containing 4.21 mmol of NO_2) and _p_-toluenesulfonic acid monohydrate (0.801 g, 4.21 mmol). The reaction mixture was stirred at 0 °C for 1 h and then room temperature for 1 h. The reaction mixture was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated _in vacuo._ Purification by flash column chromatography, eluting with 10% ethyl acetate in hexane gave benzo[_d_]-1,2,3-

thiadiazole (**108**) (0.127 g, 66%) as a yellow oil. Spectroscopic data were consistent with the literature.[18] δ_H (500 MHz, $CDCl_3$) 7.65 (1H, dd, *J* 8.0, 1.1 Hz, 6-H), 7.70 (1H, dd, *J* 8.0, 1.1 Hz, 7'-H), 8.11 (1H, br d, *J* 8.0 Hz, 8'-H), 8.65 (1H, br d, *J* 8.0 Hz, 5'-H); δ_C (126 MHz, $CDCl_3$) 119.2 (CH), 124.1 (CH), 127.0 (CH), 129.3 (CH), 141.1 (C), 158.5 (C); *m*/*z* (ESI) 159 (MNa^+. 100%).

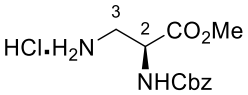
N-Tosylglycine (109)[185]



To a stirred solution of glycine (0.100 g, 1.33 mmol) in tetrahydrofuran:water (1:2) was added *p*-toluenesulfonly chloride (0.305 g, 1.60 mmol) and triethylamine (0.555 mL, 4.00 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted in water (15 mL) and extracted with diethyl ether (3 × 15 mL). The aqueous layer was acidified to pH 2 using 1 M aqueous hydrochloric acid. The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with brine (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *N*-tosylglycine (**109**) (0.261 g, 86%) as a white solid. Mp 150–155 °C (lit.[185] 149–151 °C); δ_H (500 MHz, DMSO-d_6) 2.37 (3H, s, CH_3), 3.54 (2H, d, *J* 4.7 Hz, CH_2), 7.37 (2H, d, *J* 8.0 Hz, 3-H and 5-H), 7.67 (2H, d, *J* 8.0 Hz, 2-H and 6-H), 7.95 (1H, t, *J* 4.7 Hz, NH), 12.68 (1H, br s, OH); δ_C (126 MHz, DMSO-d_6) 21.0 (CH_3), 43.8 (CH_2), 21.0(C), 126.6 (2 × CH), 129.5 (2 × CH), 137.8 (C), 142.6 (C), 170.3 (C); *m*/*z* (ESI) 252 (MNa^+. 100%).

3.3 Benzotriazinone Amino Acids Experimental

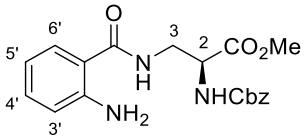
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-aminopropanoate hydrochloride (112)[186]



To a suspension of (2*S*)-2-(benzyloxycarbonylamino)-3-aminopropaonic acid (0.300 g, 1.26 mmol) in methanol (4.8 mL) at 0 °C under argon was added thionyl chloride (0.138 mL, 1.89 mmol) dropwise. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was concentrated *in vacuo* and azeotroped with toluene (3 × 50 mL) to remove excess thionyl chloride. The resulting residue was triturated with diethyl ether to afford methyl (2*S*)-2-(benzyloxycarbonylamino)-3-aminopropanoate hydrochloride (**112**) (0.341 g, 94%)

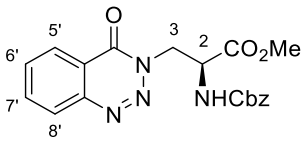
as a yellow solid. Mp 165–168 °C (lit.[187] 165–167 °C); $[\alpha]_D^{22}$ −41.4 (*c* 1.1, MeOH), lit.[187] $[\alpha]_D^{20}$ −42.5 (*c* 1.0, MeOH); δ_H (400 MHz, CD_3OD) 3.23 (1H, dd, *J* 13.2, 8.8 Hz, 3-*H*H), 3.45 (1H, dd, *J* 13.2, 5.1 Hz, 3-H*H*), 3.78 (3H, s, OCH_3), 4.51 (1H, dd, *J* 8.8, 5.1 Hz, 2-H), 5.14 (2H, s, OC*H*$_2$Ph), 7.30–7.40 (5H, m, PhH); δ_C (101 MHz, CD_3OD) 41.3 (CH_2), 53.1 (CH), 53.4 (CH_3), 68.2 (CH_2), 129.0 (2 × CH), 129.2 (CH), 129.5 (2 × CH), 137.8 (C), 158.7 (C), 170.9 (C); *m/z* (ESI) 275 (MNa^+. 100%).

Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (121)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-aminopropanoate hydrochloride (0.300 g, 1.31 mmol) in ethyl acetate (5 mL) was added isatoic anhydride (0.236 g, 1.45 mmol) and triethylamine (0.182 mL, 1.31 mmol). The reaction was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane with 2.5% triethylamine gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (**121**) (0.324 g, 66%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3348 (NH), 1717 (C=O), 1638, 1522 (C=C), 1302, 1260, 1163, 750; $[\alpha]_D^{20}$ –6.4 (*c* 0.5, MeOH); δ_H (400 MHz, $CDCl_3$) 3.70–3.85 (5H, m, OCH_3 and 3-H_2), 4.49–4.58 (1H, m, 2-H), 5.05–5.15 (2H, m, C*H*$_2$Ph), 5.93 (1H, d, *J* 6.6 Hz, 2-NH), 6.60–6.71 (3H, m, 3'-H, 5'-H and 3-NH), 7.16–7.23 (1H, m, 4'-H), 7.27–7.38 (6H, m, 6'-H and Ph); δ_C (101 MHz, $CDCl_3$) 42.0 (CH_2), 53.0 (CH_3), 54.6 (CH), 67.4 (CH_2), 115.5 (C), 117.0 (CH), 117.6 (CH), 127.5 (CH), 128.3 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 132.8 (CH), 136.2 (C), 148.8 (C), 156.5 (C), 170.1 (C), 171.0 (C); *m/z* (ESI) 394.1373 (MNa^+. $C_{19}H_{21}N_3NaO_5$ requires 394.1373).

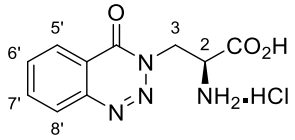
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (122)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (0.300 g, 0.808 mmol) in methanol (8 mL) at 0 °C

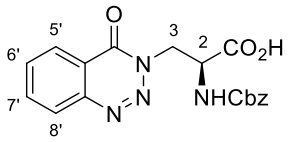
was added polymer-supported nitrite (0.693 g, containing 2.42 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.461 g, 2.42 mmol). The reaction mixture was stirred at 0 °C for 1 h then heated under reflux for 4 h. The reaction mixture was cooled to room temperature, filtered, and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (30 mL) and washed with water (4 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**122**) (0.247 g, 80%) as an orange solid. Mp 105–107 °C; ν_{max}/cm^{-1} (neat) 3304 (NH), 1749 (C=O), 1678 (C=O), 1529 (C=C), 1303, 1207, 1171, 779; $[\alpha]_D^{20}$ –95.7 (*c* 0.25, MeOH); δ_H (400 MHz, $CDCl_3$) 3.80 (3H, s, OCH_3), 4.76–4.86 (1H, m, 2-H), 4.90–5.00 (2H, m, 3-H_2), 5.04 (2H, s, CH_2Ph), 5.79 (1H, d, *J* 6.9 Hz, 2-NH), 7.24–7.37 (5H, m, Ph), 7.80 (1H, br t, *J* 7.8 Hz, 6'-H), 7.92–7.98 (1H, m, 7'-H), 8.14 (1H, br d, *J* 7.8 Hz, 8'-H), 8.33 (1H, br d, *J* 7.8 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 50.3 (CH_2), 53.1 (CH_3), 53.6 (CH), 67.3 (CH_2), 119.7 (C), 125.4 (CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 128.6 (CH), 132.8 (CH), 135.2 (CH), 136.2 (C), 144.1 (C), 155.9 (C), 156.2 (C), 170.2 (C); *m*/*z* (ESI) 405.1166 (MNa^+. $C_{19}H_{18}N_4NaO_5$ requires 405.1169).

(2*S*)-2-Amino-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (123)



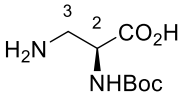
A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.040 g, 0.12 mmol) in 2 M aqueous hydrochloric acid (2 mL) was stirred at 50 °C for 3 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**123**) (0.026 g, 78%) as a white solid. Mp 175–180 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 3220 (CH), 1679 (C=O), 1651, 1122, 784; $[\alpha]_D^{19}$ –5.4 (*c* 0.2, MeOH); δ_H (400 MHz, CD_3OD) 4.63 (1H, dd, *J* 7.2, 4.4 Hz, 2-H), 4.94 (1H, dd, *J* 14.6, 7.2 Hz, 3-*H*H), 5.11 (1H, dd, *J* 14.6, 4.4 Hz, 3-H*H*), 7.95 (1H, td, *J* 8.1, 1.2 Hz, 6'-H), 8.10 (1H, td, *J* 8.1, 1.2 Hz, 7'-H), 8.21 (1H, br d, *J* 8.1 Hz, 8'-H), 8.35 (1H, dd, *J* 8.1, 1.2 Hz, 5'-H); δ_C (101 MHz, CD_3OD) 49.7 (CH_2), 53.3 (CH), 120.9 (C), 126.0 (CH), 129.6 (CH), 134.4 (CH), 136.8 (CH), 145.4 (C), 157.8 (C), 169.1 (C); *m*/*z* (ESI) 257.0643 ($[MNa–HCl]^+$. $C_{10}H_{10}N_4NaO_3$ requires 257.0645).

(2*S*)-2-(Benzyloxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (128)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.200 g, 0.523 mmol) in methanol (15 mL), dioxane (7.5 mL), and water (7.5 mL) was added caesium carbonate (0.222 g, 0.681 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (100 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**128**) (0.165 g, 86%) as a white solid. Mp 68–70 °C; ν_{max}/cm^{-1} (neat) 3332 (NH), 2928 (CH), 1682 (C=O), 1520 (C=C), 1300, 1215, 1053; $[\alpha]_D^{20}$ –52.7 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) 4.59–4.70 (1H, m, 3-*H*H), 4.72–4.81 (1H, m, 2-H), 4.89 (2H, s, C*H*$_2$Ph), 5.00–5.10 (1H, m, 3-H*H*), 7.01–7.28 (5H, m, Ph), 7.86 (1H, br t, *J* 7.8 Hz, 6'-H), 8.02 (1H, br t, *J* 7.8 Hz,7'-H), 8.12 (1H, br d, *J* 7.8 Hz, 8'-H), 8.28 (1H, br d, *J* 7.8 Hz, 5'-H); δ_C (101 MHz, CD_3OD) 52.9 (CH_2), 55.5 (CH), 67.4 (CH_2), 120.8 (C), 125.9 (CH), 128.6 (2 × CH), 128.8 (CH), 129.2 (CH), 129.3 (2 × CH), 133.8 (CH), 136.4 (CH), 138.0 (C), 145.4 (C), 157.5 (C), 158.3 (C), 175.3 (C); *m*/*z* (ESI) 391.1020 (MNa^+. $C_{18}H_{16}N_4NaO_5$ requires 391.1013).

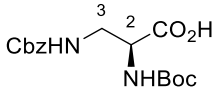
***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-3-amino-L-alanine (130)[109]**



A suspension of *N*$^{\alpha}$-(*tert*-butoxycarbonyl)-L-asparagine (5.00 g, 21.5 mmol) and (diacetoxyiodo)benzene (8.32 g, 25.8 mmol) in ethyl acetate (30 mL), acetonitrile (30 mL) and water (5 mL) was stirred at 10 °C for 18 h. The resulting white precipitate was filtered and washed with diethyl ether (200 mL) and ethyl acetate (200 mL). The solid was dried *in vacuo* to give *N*$^{\alpha}$-(*tert*-butoxycarbonyl)-3-amino-L-alanine (**130**) (3.75 g, 85%) as a white solid. Mp 202–204 °C (lit.[109] 206 °C); $[\alpha]_D^{21}$ +19.6 (*c* 1.0, MeOH), lit.[188] $[\alpha]_D^{25}$ +19.0 (*c* 0.3, MeOH); δ_H (400 MHz, DMSO-d_6) 1.38 (9H, s, 3 × CH_3), 2.75 (1H, dd, *J* 11.8, 9.2 Hz, 3-*H*H), 3.01 (1H, dd, *J* 11.8, 4.8 Hz, 3-H*H*), 3.60–3.69 (1H, m, 2-H), 6.20 (1H, d, *J* 4.9 Hz, NH); δ_C (101 MHz, DMSO-d_6)

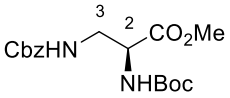
28.1 (3 × CH_3), 40.8 (CH_2), 51.2 (CH), 78.1 (C), 155.1 (C), 171.1 (C); *m*/*z* (ESI) 227 (MNa^+. 100%).

3-(Benzyloxycarbonylamino)-*N*-(*tert*-butoxycarbonyl)-L-alanine (131)[109]



To a stirred solution of N^{α}-(*tert*-butoxycarbonyl)-3-amino-L-alanine (6.95 g, 34.0 mmol) in water (50 mL) was added sodium bicarbonate (7.14 g, 85.1 mmol). A solution of benzyl chloroformate (7.76 mL, 40.8 mmol) dissolved in toluene (9 mL) was then added dropwise and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* and the residue was washed with diethyl ether (100 mL). The residue was dissolved in water (300 mL) and a concentrated citric acid solution was added until a white precipitate formed. This was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with water (6 × 300 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 3-(benzyloxycarbonylamino)-*N*-(*tert*-butoxycarbonyl)-L-alanine (**131**) (10.0 g, 87%) as a colourless oil. Spectroscopic data were consistent with the literature.[109] $[\alpha]_D^{19}$ –4.6 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 1.37 (9H, s, 3 × CH_3), 3.25–3.38 (2H, m, 3-H_2), 3.97–4.05 (1H, m, 2-H), 5.01 (2H, s, PhCH_2), 6.91 (1H, br d, *J* 7.8 Hz, 2-NH), 7.23–7.39 (6H, m, Ph and 3-NH); δ_C (101 MHz, DMSO-d_6) 28.6 (3 × CH_3), 42.2 (CH_2), 54.1 (CH), 65.8 (CH_2), 78.7 (C), 128.1 (2 × CH), 128.2 (CH), 128.8 (2 × CH), 137.5 (C), 155.8 (C), 156.7 (C), 172.7 (C); *m*/*z* (ESI) 361 (MNa^+. 100%).

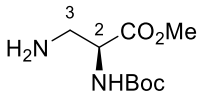
Methyl (2*S*)-3-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonylamino)propanoate (132)[109]



To a stirred solution of 3-(benzyloxycarbonylamino)-*N*-(*tert*-butoxycarbonyl)-L-alanine (1.27 g, 3.75 mmol) in *N*,*N*'-dimethylformamide (20 mL) at 10 °C was added potassium carbonate (1.04 g, 7.51 mmol). Methyl iodide (1.17 mL, 18.8 mmol) was then added dropwise. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was concentrated *in vacuo,* dissolved in ethyl acetate (50 mL) and extracted with water (3 × 50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column

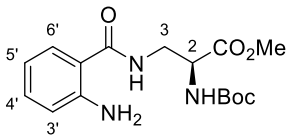
chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-3-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonylamino)propanoate (**132**) (1.18 g, 89%) as a colourless oil. Spectroscopic data were consistent with the literature.[109] $[\alpha]_D^{19}$ –5.7 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 1.37 (9H, s, 3 × CH_3), 3.33 (2H, d, *J* 5.8 Hz, 3-H_2), 3.58 (3H, s, OCH_3), 4.06–4.14 (1H, m, 2-H), 5.02 (2H, br s, PhCH_2), 7.10 (1H, d, *J* 8.0 Hz, 2-NH), 7.27–7.39 (6H, m, Ph and 3-NH); δ_C (101 MHz, DMSO-d_6) 28.1 (3 × CH_3), 41.5 (CH_2), 51.9 (CH_3) 53.7 (CH), 65.5 (CH_2), 78.6 (C), 127.7 (2 × CH), 127.8 (CH), 128.4 (2 × CH), 137.0 (C), 155.3 (C), 156.3 (C), 171.3 (C); *m*/*z* (ESI) 375 (MNa^+. 100%).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (133)[109]



To a stirred solution of methyl (2*S*)-3-(benzoxycarbonylamino)-2-(*tert*-butoxycarbonylamino)propanoate (4.51 g, 12.8 mmol) in methanol (60 mL) was added 10% palladium on carbon (0.450 g, 10%w/w). The reaction mixture was purged with hydrogen for 0.5 h and then stirred under a hydrogen atmosphere at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite® and washed with methanol (50 mL). The reaction mixture was concentrated *in vacuo* to give methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (**133**) (3.00 g, 100%) as a colourless oil. $[\alpha]_D^{20}$ –16.4 (*c* 1.0, MeOH), lit.[189] $[\alpha]_D^{20}$ –14.0 (*c* 1.0, MeOH); δ_H (400 MHz, DMSO-d_6) 1.39 (9H, s, 3 × CH_3), 2.93 (1H, dd, *J* 13.0, 8.5 Hz, 3-*H*H), 3.05 (1H, dd, *J* 13.0, 4.8 Hz, 3-H*H*), 3.65 (3H, s, OCH_3), 4.17–4.27 (1H, m, 2-H), 7.33 (1H, d, *J* 7.9 Hz, 2-NH); δ_C (101 MHz, DMSO-d_6) 28.1 (3 × CH_3), 40.4 (CH_2), 52.2 (CH), 53.3 (CH_3), 78.8 (C), 155.5 (C), 170.8 (C); *m*/*z* (ESI) 219 (MH^+. 100%).

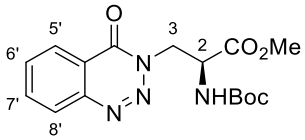
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (134)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (0.0900 g, 0.412 mmol) in ethyl acetate (1.5 mL) was added isatoic anhydride (0.0740 g, 0.454 mmol). The reaction was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was

concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane with 1% triethylamine gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (**134**) (0.0664 g, 48%) as a white solid. Mp 120–124 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2976 (CH), 2928 (CH), 1741 (C=O), 1705 (C=O), 1522, 1368, 1256, 1161; $[\alpha]_D^{19}$ +25.6 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 3.67–3.85 (5H, m, OCH_3 and 3-H_2), 4.45–4.55 (1H, m, 2-H), 5.51 (2H, br s, 2'-NH_2), 5.60 (1H, br d, *J* 4.8 Hz, 2-NH), 6.58–6.69 (2H, m, 3'-H and 5'-H), 6.78 (1H, br s, 3-NH), 7.19 (1H, br t, *J* 7.8 Hz, 4'-H), 7.33 (1H, br d, *J* 7.8 Hz, 6'-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 42.5 (CH_2), 52.9 (CH), 53.9 (CH_3), 80.6 (C), 115.5 (C), 116.8 (CH), 117.4 (CH), 127.5 (CH), 132.7 (CH), 148.8 (C), 156.1 (C), 169.9 (C), 171.3 (C); *m*/*z* (ESI) 360.1532 (MNa^+. $C_{16}H_{23}N_3NaO_5$ requires 360.1530).

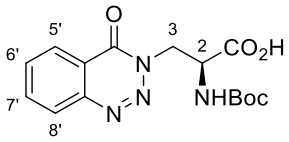
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (135)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-aminobenzoylamino)propanoate (0.100 g, 0.297 mmol) in methanol (3 mL) at 0 °C was added polymer-supported nitrite (0.256 g, containing 0.890 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.169 g, 0.890 mmol). The reaction mixture was stirred at 0 °C for 1 h and then 2 h at room temperature. The reaction mixture was filtered and the resulting resin was washed with methanol (10 mL). The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (20 mL) and washed with water (4 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% diethyl ether in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**135**) (0.0900 g, 87%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3333 (NH), 2976 (CH), 2928 (CH), 1746 (C=O), 1713 (C=O), 1688 (C=O), 1508, 1368, 1302, 1163; $[\alpha]_D^{18}$ –18.2 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.32 (9H, s, 3 × CH_3), 3.80 (3H, s, OCH_3), 4.72 (1H, dd, *J* 12.8, 6.8 Hz, 3-*H*H), 4.85–5.00 (2H, m, 2-H and 3-H*H*), 5.43 (1H, br d, *J* 6.4 Hz, 2-NH), 7.81 (1H, br t, *J* 8.0 Hz, 6'-H), 7.95 (1H, td, *J* 8.0, 0.9 Hz, 7'-H), 8.14 (1H, br d, *J* 8.0 Hz, 8'-H), 8.35 (1H, dd, *J* 8.0, 0.9 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 52.9 (CH), 53.0 (CH_3), 80.4 (C), 119.8 (C), 125.4 (CH), 128.5

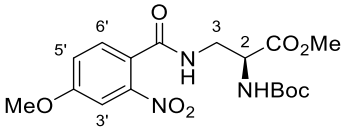
(CH), 132.7 (CH), 135.1 (CH), 144.2 (C), 155.2 (C), 156.2 (C), 170.5 (C); *m*/*z* (ESI) 371.1323 (MNa^+. $C_{16}H_{20}N_4NaO_5$ requires 371.1326).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (136)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.0700 g, 0.201 mmol) in methanol (3.5 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.0850 g, 0.261 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (30 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**136**) (0.0660 g, 99%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3351 (NH), 2973 (CH), 1689 (C=O), 1394, 1367, 1301, 1164, 779; $[\alpha]_D^{19}$ –52.5 (*c* 0.2, MeOH); δ_H (400 MHz, CD_3OD) 1.23 (9H, s, 3 × CH_3), 4.62 (1H, dd, *J* 13.2, 9.6 Hz, 3-*H*H), 4.79 (1H, dd, *J* 9.6, 4.3 Hz, 2-H), 5.02 (1H, dd, *J* 13.2, 4.3 Hz, 3-H*H*), 7.89 (1H, br t, *J* 7.8 Hz, 6'-H), 8.04 (1H, br t, *J* 7.8 Hz, 7'-H), 8.13 (1H, br d, *J* 7.8 Hz, 8'-H), 8.33 (1H, br d, *J* 7.8 Hz, 5'-H); δ_C (101 MHz, CD_3OD) 28.5 (3 × CH_3), 52.3 (CH_2), 54.8 (CH), 80.6 (C), 120.8 (C), 125.9 (CH), 129.1 (CH), 133.8 (CH), 136.4 (CH), 145.5 (C), 157.5 (C), 157.6 (C), 173.0 (C); *m*/*z* (ESI) 357.1164 (MNa^+. $C_{15}H_{18}N_4NaO_5$ requires 357.1169).

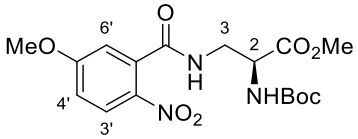
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-methoxybenzoylamino)propanoate (139a)



To a stirred solution of 2-nitro-4-methoxybenzoic acid (0.0900 g, 0.456 mmol) in toluene (3 mL) was added thionyl chloride (0.113 mL, 1.52 mmol). The reaction mixture was heated under reflux for 5 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and azeotroped with chloroform (5 × 10 mL). A solution of the acid chloride in ethyl acetate (2 mL) was added dropwise to a stirred

solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (0.0660 g, 0.304 mmol) and triethylamine (0.0880 mL, 0.608 mmol) in ethyl acetate (3 mL). The reaction mixture was heated to 90 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-methoxybenzoylamino)propanoate (**139a**) (0.0940 g, 78%) as a colourless oil. ν_{max}/cm^{-1} (neat) 2977 (CH), 2483, 1742 (C=O), 1692 (C=O), 1641, 1536 (C=C), 1413, 1352, 1241, 1166, 753; $[\alpha]_D^{21}$ –5.3 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 1.45 (9H, s, 3 × CH_3), 3.65 (1H, dd, *J* 13.8, 7.0 Hz, 3-*H*H), 3.75 (1H, dd, *J* 13.8, 5.1 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 3.92 (3H, s, 4'-OCH_3), 4.38–4.44 (1H, m, 2-H), 7.27 (1H, dd, *J* 8.5, 2.5 Hz, 5'-H), 7.51 (1H, d, *J* 8.5 Hz, 6'-H), 7.56 (1H, d, *J* 2.5 Hz, 3'-H); δ_C (101 MHz, CD_3OD) 28.7 (3 × CH_3), 42.0 (CH_2), 52.9 (CH_3), 54.9 (CH), 56.7 (CH_3), 80.9 (C), 110.8 (CH), 119.7 (CH), 125.5 (C), 131.4 (CH), 149.5 (C), 157.8 (C), 162.6 (C), 169.7 (C), 172.6 (C); *m/z* (ESI) 420.1379 (MNa^+. $C_{17}H_{23}N_3NaO_8$ requires 420.1377).

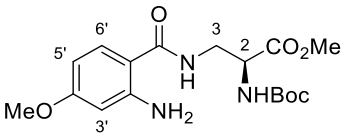
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-5'-methoxybenzoylamino)propanoate (139b)



To a stirred solution of 2-nitro-5-methoxybenzoic acid (0.542 g, 2.75 mmol) in toluene (9 mL) was added thionyl chloride (0.681 mL, 9.17 mmol). The reaction mixture was heated under reflux for 4 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and azeotroped with chloroform (5 × 15 mL). A solution of the acid chloride in ethyl acetate (4.5 mL) was added dropwise to a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (0.400 g, 1.83 mmol) and triethylamine (0.530 mL, 3.67 mmol) in ethyl acetate (4.5 mL). The reaction mixture was heated to 90 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 60% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-5'-methoxybenzoylamino)propanoate (**139b**) (0.399 g, 55%) as an off-white solid. Mp 115–120 °C, ν_{max}/cm^{-1} (neat) 3323 (NH), 2979 (CH), 1746 (C=O), 1704 (C=O), 1651 (C=O), 1581 (C=C), 1517, 1340, 1248, 1163, 1026, 733; $[\alpha]_D^{25}$ +26.3 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.73–3.95 (2H, m, 3-H_2), 3.79 (3H, s, OCH_3), 3.90 (3H, s, 5'-OCH_3), 4.41–

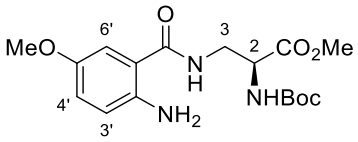
4.58 (1H, br s, 2-H), 5.62 (1H, d, *J* 5.8 Hz, 2-NH), 6.53 (1H, br s, 3-NH), 6.92 (1H, d, *J* 2.6 Hz, 6'-H), 6.96 (1H, dd, *J* 9.1, 2.6 Hz, 4'-H), 8.08 (1H, d, *J* 9.1 Hz, 3'-H); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 42.5 (CH_2), 53.0 (CH_3), 53.7 (CH), 56.3 (CH_3), 80.6 (C), 114.1 (CH), 115.0 (CH), 127.3 (CH), 135.3 (C), 138.8 (C), 156.0 (C), 163.9 (C), 167.3 (C), 171.1 (C); *m/z* (ESI) 420.1374 (MNa^+. $C_{17}H_{23}N_3NaO_8$ requires 420.1377).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-methoxybenzoylamino)propanoate (140a)



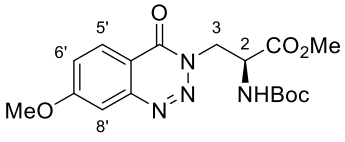
To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-methoxy-benzoylamino)propanoate (0.279 g, 0.702 mmol) in methanol (6 mL) was added zinc powder (0.459 g, 7.02 mmol) and acetic acid (0.402 mL, 7.02 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite® and washed with methanol (50 mL). The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (30 mL) and was washed with water (5 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-methoxybenzoylamino)propanoate (**140a**) (0.219 g, 85%) as a white solid. Mp 140–145 °C; ν_{max}/cm^{-1} (neat) 3343 (NH), 2976 (CH), 2499, 1714 (C=O), 1689 (C=O), 1623, 1412, 1366, 1255, 1216, 1165, 765; $[\alpha]_D^{17}$ –5.7 (*c* 0.2, MeOH); δ_H (400 MHz, CD_3OD) 1.42 (9H, s, 3 × CH_3), 3.62–3.70 (2H, m, 3-H_2), 3.73 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.32–4.39 (1H, m, 2-H), 6.18 (1H, dd, *J* 8.8, 2.5 Hz, 5'-H), 6.26 (1H, d, *J* 2.5 Hz, 3'-H), 7.36 (1H, d, *J* 8.8 Hz, 6'-H); δ_C (101 MHz, CD_3OD) 28.7 (3 × CH_3), 41.6 (CH_2), 52.8 (CH_3), 55.5 (CH), 55.5 (CH_3), 80.8 (C), 101.3 (CH), 104.8 (CH), 109.8 (C), 130.7 (CH), 152.8 (C), 157.9 (C), 164.6 (C), 172.1 (C), 173.1 (C); *m/z* (ESI) 390.1637 (MNa^+. $C_{17}H_{25}N_3NaO_6$ requires 390.1636).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-5'-methoxybenzoylamino)propanoate (140b)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-5'-methoxy-benzoylamino)propanoate (0.200 g, 0.503 mmol) in methanol (5 mL) was added zinc powder (0.329 g, 5.03 mmol) and acetic acid (0.288 mL, 5.03 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite® and washed with methanol (50 mL). The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (30 mL) and was washed with water (5 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-5'-methoxybenzoylamino)propanoate (**140b**) (0.148 g, 80%) as a brown solid. Mp 130–134 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2976 (CH), 1744 (C=O), 1698 (C=O), 1651 (C=O), 1581, 1515, 1367, 1339, 1238, 1161, 731; $[\alpha]_D^{26}$ –8.5 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, CD_3OD) 1.42 (9H, s, 3 × CH_3), 3.67–3.85 (2H, m, 3-H_2), 3.74 (3H, s, OCH_3), 3.77 (3H, s, 5'-OCH_3), 4.41–4.54 (1H, br s, 2-H), 4.99 (2H, br s, 2'-NH_2), 5.64 (1H, d, *J* 6.5 Hz, 2-NH), 6.64 (1H, d, *J* 8.8 Hz, 3'-H), 6.85 (1H, dd, *J* 8.8, 2.7 Hz, 4'-H), 6.92 (1H, br s, 6'-H), 7.00 (1H, br s, 3-NH); δ_C (126 MHz, CD_3OD) 28.4 (3 × CH_3), 42.6 (CH_2), 52.9 (CH_3), 54.0 (CH), 56.1 (CH_3), 80.6 (C), 112.1 (CH), 117.0 (C), 119.1 (CH), 119.9 (CH), 142.2 (C), 151.6 (C), 156.2 (C), 169.5 (C), 171.3 (C); *m/z* (ESI) 390.1633 (MNa^+. $C_{17}H_{25}N_3NaO_6$ requires 390.1636).

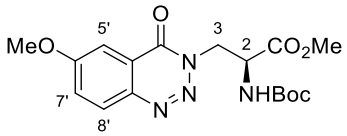
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (141a)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-methoxy-benzoylamino)propanoate (0.219 g, 0.596 mmol) in methanol (6 mL) at 0 °C was added polymer-supported nitrite (0.510 g, containing 1.79 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.340 g, 1.79 mmol). The reaction mixture was stirred at 0 °C for 1 h and then room temperature for 1 h. The reaction mixture

was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**141a**) (0.166 g, 73%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3365 (NH), 2976 (CH), 1748 (C=O), 1710 (C=O), 1682 (C=O), 1610, 1506, 1461, 1438, 1363, 1284, 1251, 1162, 757; $[\alpha]_D^{20}$ –11.1 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.79 (3H, s, OCH_3), 3.98 (3H, s, 7'-OCH_3), 4.72 (1H, dd, *J* 13.2, 7.2 Hz, 3-*H*H), 4.82–4.94 (2H, m, 2-H and 3-H*H*), 5.46 (1H, br d, *J* 7.2 Hz, 2-NH), 7.34 (1H, dd, *J* 8.8, 2.4 Hz, 6'-H), 7.47 (1H, d, *J* 2.4 Hz, 8'-H), 8.23 (1H, d, *J* 8.8 Hz, 5'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.6 (CH_2), 53.0 (CH), 53.1 (CH_3), 56.2 (CH_3), 80.3 (C), 108.7 (CH), 113.3 (C), 122.7 (CH), 127.0 (CH), 146.4 (C), 155.2 (C), 155.9 (C), 165.0 (C), 170.6 (C); *m*/*z* (ESI) 401.1429 (MNa^+. $C_{17}H_{22}N_4NaO_6$ requires 401.1432).

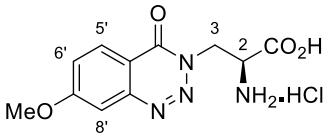
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (141b)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-5'-methoxy-benzoylamino)propanoate (0.270 g, 0.735 mmol) in methanol (7.5 mL) at 0 °C was added polymer-supported nitrite (0.629 g, containing 2.21 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (0.419 g, 2.21 mmol). The reaction mixture was stirred at 0 °C for 1 h and then room temperature for 2.5 h. The reaction mixture was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**141b**) (0.233 g, 84%) as a white solid. Mp 121–126 °C; ν_{max}/cm^{-1} (neat) 3363 (NH), 2979 (CH), 1747 (C=O), 1711 (C=O), 1679 (C=O), 1610, 1486, 1367, 1295, 1239, 1161, 735; $[\alpha]_D^{26}$ –16.9 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.80 (3H, s, OCH_3), 3.98 (3H, s, 6'-OCH_3), 4.71 (1H, dd, *J* 13.1, 6.9 Hz, 3-*H*H), 4.86–4.96 (2H, m, 2-H and 3-H*H*), 5.46 (1H, br d, *J* 7.4 Hz, 2-NH), 7.47 (1H, dd, *J* 8.9, 2.6 Hz, 7'-H), 7.66 (1H, d, *J* 2.6 Hz, 5'-H), 8.05 (1H, d, *J* 8.9 Hz, 8'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 52.9 (CH), 53.0 (CH_3), 56.4 (CH_3), 80.4 (C), 104.5 (CH), 113.3 (CH), 121.6 (C), 125.2 (CH), 130.5 (CH), 139.4 (C), 155.2 (C),

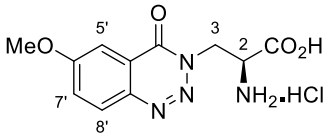
156.4 (C), 162.9 (C), 170.6 (C); *m*/*z* (ESI) 401.1430 (MNa^+. $C_{17}H_{22}N_4NaO_6$ requires 401.1432).

(2*S*)-2-Amino-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (143a)



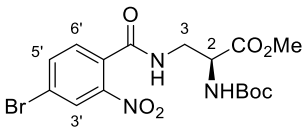
To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)- one]propanoate (0.166 g, 0.439 mmol) in methanol (7 mL), dioxane (3.5 mL) and water (3.5 mL) was added caesium carbonate (0.186 g, 0.571 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (20 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (5 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.133 g, 83%) as a white solid. This material was used immediately in the following step. A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.050 g, 0.14 mmol) in 2 M aqueous hydrochloric acid (3 mL) was stirred at 50 °C for 1.5 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**143a**) (0.043 g, 100%) as an orange solid. Mp 210–215 °C; ν_{max}/cm^{-1} (neat) 2902 (CH), 1762 (C=O), 1675 (C=O), 1214, 1038; $[\alpha]_D^{22}$ –2.1 (*c* 0.1, MeOH); δ_H (500 MHz, CD_3OD) 4.03 (3H, s, 7'-OCH_3), 4.60 (1H, dd, *J* 7.1, 4.4 Hz, 2-H), 4.93 (1H, dd, *J* 14.6, 7.1 Hz, 3-*H*H), 5.08 (1H, dd, *J* 14.6, 4.4 Hz, 3-H*H*), 7.50 (1H, dd, *J* 8.8, 2.1 Hz, 6'-H), 7.63 (1H, d, *J* 2.1 Hz, 8'-H), 8.26 (1H, d, *J* 8.8 Hz, 5'-H); δ_C (126 MHz, CD_3OD) 49.7 (CH_2), 53.4 (CH), 56.9 (CH_3), 110.2 (CH), 114.3 (C), 123.8 (CH), 127.7 (CH), 147.8 (C), 157.6 (C), 166.9 (C), 169.2 (C); *m*/*z* (ESI) 265.0931 ($[MH–HCl]^+$. $C_{11}H_{13}N_4O_4$ requires 265.0931).

(2*S*)-2-Amino-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (143b)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.060 g, 0.16 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.067 g, 0.21 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (20 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (5 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.050 g, 86%) as a white solid. This material was used immediately in the following step. A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.040 g, 0.10 mmol) in 2 M aqueous hydrochloric acid (2 mL) was stirred at 50 °C for 1.5 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[6'-methoxy-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**143b**) (0.038 g, 100%) as a yellow solid. Mp 195–200 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 2481 (CH), 2328, 2236, 2126, 2073, 1754 (C=O), 1667 (C=O), 1608, 1486, 1365, 1286, 1239; $[\alpha]_D^{22}$ –13.7 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 4.01 (3H, s, OCH_3), 4.62 (1H, dd, *J* 7.3, 4.5 Hz, 2-H), 4.92 (1H, dd, *J* 14.6, 7.3 Hz, 3-*H*H), 5.09 (1H, dd, *J* 14.6, 4.5 Hz, 3-H*H*), 7.62 (1H, dd, *J* 9.0, 2.8 Hz, 7'-H), 7.70 (1H, d, *J* 2.8 Hz, 5'-H), 8.13 (1H, d, *J* 9.0 Hz, 8'-H); δ_C (101 MHz, CD_3OD) 48.1 (CH_2), 53.3 (CH_3), 56.9 (CH), 106.0 (CH), 122.8 (C), 125.9 (CH), 131.8 (CH), 140.5 (C), 158.0 (C), 164.7 (C), 169.2 (C); *m*/*z* (ESI) 265.0927 ($[MH–HCl]^+$. $C_{11}H_{13}N_4O_4$ requires 265.0931).

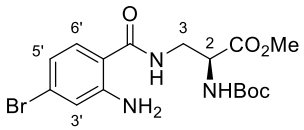
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-bromobenzoylamino)propanoate (145)



To a stirred solution of 2-nitro-4-bromobenzoic acid (0.447 g, 1.82 mmol) in toluene (9 mL) was added thionyl chloride (0.900 mL, 12.1 mmol). The reaction mixture was

heated under reflux for 4 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and azeotroped with chloroform (5 × 50 mL). A solution of the acid chloride in ethyl acetate (4.5 mL) was added dropwise at 0 °C to a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (0.264 g, 1.21 mmol) and triethylamine (0.350 mL, 2.42 mmol) in ethyl acetate (4.5 mL). The reaction mixture was heated to 90 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-bromobenzoylamino)propanoate (**145**) (0.306 g, 57%) as a white solid. Mp 165–170 °C; ν_{max}/cm^{-1} (neat) 3302 (NH), 2978 (CH), 1744 (C=O), 1698 (C=O), 1651, 1536 (C=C), 1348, 1160, 732; $[\alpha]_D^{18}$ +31.8 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 3.74–3.93 (5H, m, 3-H_2 and OCH_3), 4.43–4.53 (1H, m, 2-H), 5.58 (1H, d, *J* 7.0 Hz, 2-NH), 6.72 (1H, br s, 3-NH), 7.40 (1H, d, *J* 8.1 Hz, 6'-H), 7.77 (1H, d, *J* 8.1 Hz, 5'-H), 8.17 (1H, s, 3'-H); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 42.8 (CH_2), 53.1 (CH_3), 53.6 (CH), 80.8 (C), 124.2 (C), 127.8 (CH), 130.2 (CH), 131.3 (C), 136.8 (CH), 147.1 (C), 156.1 (C), 166.1 (C), 171.0 (C); *m/z* (ESI) 468.0377 (MNa^+. $C_{16}H_{20}N_3NaO_7{}^{79}Br$ requires 468.0377).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-bromobenzoylamino)propanoate (146)

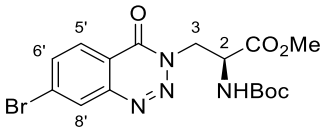


To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-nitro-4'-bromobenzoylamino)propanoate (0.306 g, 0.686 mmol) in methanol (2 mL) was added zinc powder (0.448 g, 6.86 mmol) and acetic acid (0.392 mL, 6.86 mmol). The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was filtered through a pad of Celite®, washed with methanol (60 mL) and concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (25 mL) and was washed with water (5 × 25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 35% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-bromobenzoylamino)propanoate (**146**) (0.259 g, 91%) as a white solid. Mp 115–120 °C; ν_{max}/cm^{-1} (neat) 3347 (NH), 2977 (CH), 1743 (C=O), 1698 (C=O), 1515, 1367, 1248, 1158, 909, 730; $[\alpha]_D^{18}$ +30.9 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 3.65–3.83 (5H, m, 3-H_2 and

OCH_3), 4.47–4.54 (1H, m, 2-H), 5.58 (1H, d, *J* 7.1 Hz, 2-NH), 6.74 (1H, br d, *J* 8.4 Hz, 5'-H), 6.83 (1H, s, 3'-H), 6.88 (1H, br s, 3-NH), 7.19 (1H, d, *J* 8.4 Hz, 6'-H); δ_C (126 MHz, $CDCl_3$) 28.4 (3 × CH_3), 43.0 (CH_2), 53.1 (CH_3), 53.6 (CH), 80.8 (C), 114.2 (C), 119.8 (2 × CH), 126.9 (C), 128.9 (CH), 149.9 (C), 156.3 (C), 169.2 (C), 171.1 (C); *m/z* (ESI) 438.0632 (MNa^+. $C_{16}H_{22}N_3NaO_5{}^{79}Br$ requires 438.0635).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-bromobenzoylamino)propanoate was also synthesised *via* a ring-opening reaction of 4-bromoisatoic anhydride. To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-aminopropanoate (0.200 g, 0.916 mmol) in ethyl acetate (4.5 mL) was added 4-bromoisatoic anhydride (0.222 g, 0.916 mmol) and triethylamine (0.127 mL, 0.916 mmol). The reaction was heated to 90 °C and stirred for 18 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-bromo-benzoylamino)propanoate (**146**) (0.174g, 46%) as a white solid.

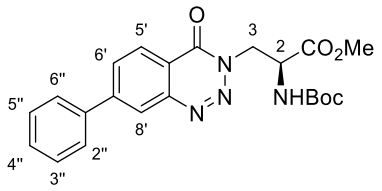
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (147)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(2'-amino-4'-bromo-benzoylamino)propanoate (1.23 g, 2.97 mmol) in methanol (20 mL) at 0 °C was added polymer-supported nitrite (2.53 g, containing 8.85 mmol of NO_2) and *p*-toluenesulfonic acid monohydrate (1.68 g, 8.85 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was filtered and the resulting resin was washed with methanol (20 mL/mmol). The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**147**) (1.16 g, 92%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3369 (NH), 2974 (CH), 1747 (C=O), 1693 (C=O), 1597, 1367, 1162, 758; $[\alpha]_D^{23}$ –1.7 (*c* 3.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.31 (9H, s, 3 × CH_3), 3.80 (3H, s, OCH_3), 4.67 (1H, dd, *J* 13.2, 7.4 Hz, 3-*H*H), 4.84–4.91 (1H, m, 2-H), 4.94 (1H, dd, *J* 13.2, 3.9 Hz, 3-H*H*), 5.40 (1H, d, *J* 7.6 Hz, 2-NH), 7.89 (1H, d, *J* 8.4 Hz, 6'-H), 8.20 (1H, d, *J* 8.4 Hz, 5'-H), 8.28 (1H, s, 8'-H); δ_C (126 MHz, $CDCl_3$) 28.2 (3 × CH_3), 51.1

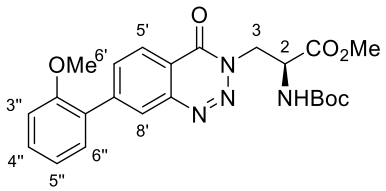
(CH_2), 52.6 (CH), 53.1 (CH_3), 80.5 (C), 118.5 (C), 127.0 (CH), 129.9 (C), 131.0 (CH), 136.0 (CH), 144.8 (C), 155.2 (C), 155.6 (C), 170.3 (C); *m*/*z* (ESI) 449.0433 (MNa^+. $C_{16}H_{19}N_4NaO_5{}^{79}Br$ requires 449.0431).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148a)



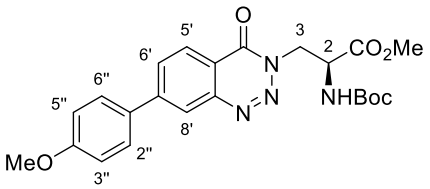
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.082 g, 0.19 mmol), phenylboronic acid (0.035 g, 0.29 mmol) and potassium phosphate (0.081 g, 0.38 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0030 g, 0.0038 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2.5–5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148a**) (0.075 g, 91%) as a white solid. Mp 125–130 °C; ν_{max}/cm^{-1} (neat) 3379 (NH), 2978 (CH), 1748, 1686 (C=O), 1616, 1505, 1304, 1161, 733; $[\alpha]_D^{22}$ –4.5 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 4.74 (1H, dd, *J* 12.9, 6.7 Hz, 3-*H*H), 4.87–5.00 (2H, m, 2-H and 3-H*H*), 5.45 (1H, d, *J* 7.2 Hz, 2-NH), 7.48 (1H, tt, *J* 9.0, 1.5 Hz, 4''-H), 7.51–7.58 (2H, m, 3''-H and 5''-H), 7.69–7.75 (2H, m, 2''-H and 6''-H), 8.04 (1H, dd, *J* 8.3, 1.3 Hz, 6'-H), 8.33 (1H, d, *J* 1.3 Hz, 8'-H), 8.41 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 53.0 (CH), 53.0 (CH_3), 80.4 (C), 118.4 (C), 126.0 (CH), 126.4 (CH), 127.7 (2 × CH), 129.3 (CH), 129.5 (2 × CH), 131.6 (CH), 138.7 (C), 144.8 (C), 148.3 (C), 155.2 (C), 156.1 (C), 170.5 (C); *m*/*z* (ESI) 425.1816 (MH^+. $C_{22}H_{25}N_4O_5$ requires 425.1819).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148b)



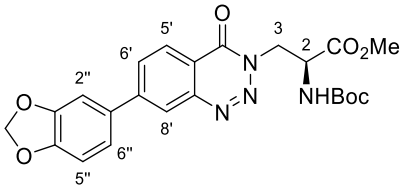
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.23 mmol), 2-methoxyphenylboronic acid (0.053 g, 0.35 mmol) and potassium phosphate (0.099 g, 0.47 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0040 g, 0.0051 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148b**) (0.10 g, 98%) as a white solid. Mp 190–195 °C; ν_{max}/cm^{-1} (neat) 3370 (NH), 2956 (CH), 2524, 2249, 1971, 1748 (C=O), 1682 (C=O), 1615, 1491, 1244, 1163, 911, 734; $[\alpha]_D^{25}$ –1.4 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.35 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 3.85 (3H, s, 2''-OCH_3), 4.76 (1H, dd, *J* 13.3, 6.8 Hz, 3-*H*H), 4.85–4.92 (1H, m, 2-H), 4.95 (1H, dd, *J* 13.3, 4.2 Hz, 3-H*H*), 5.47 (1H, d, *J* 7.7 Hz, 2-NH), 7.05 (1H, br d, *J* 8.3 Hz, 3''-H), 7.11 (1H, td, *J* 7.6, 1.0 Hz, 5''-H), 7.40 (1H, dd, *J* 7.6, 1.5 Hz, 6''-H), 7.42–7.46 (1H, m, 4''-H), 7.99 (1H, dd, *J* 8.2, 1.5 Hz, 6'-H), 8.31 (1H, br s, 8'-H), 8.35 (1H, d, *J* 8.2 Hz, 5'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.5 (CH_2), 53.0 (CH_3), 53.1 (CH), 55.7 (CH_3), 80.4 (C), 111.6 (CH), 118.0 (C), 121.4 (CH), 124.8 (CH), 128.2 (C), 129.0 (CH), 130.5 (CH), 131.0 (CH), 134.4 (CH), 144.3 (C), 146.1 (C), 155.2 (C), 156.2 (C), 156.6 (C), 170.6 (C); *m*/*z* (ESI) 477.1741 (MNa^+. $C_{23}H_{26}N_4NaO_6$ requires 477.1745).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148c)



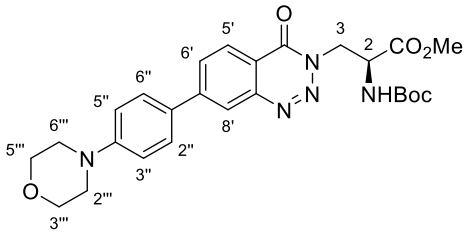
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.103 g, 0.241 mmol), 4-methoxyphenylboronic acid (0.055 g, 0.36 mmol) and potassium phosphate (0.10 g, 0.48 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0040 g, 0.0051 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148c**) (0.10 g, 93%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3366 (NH), 2976 (CH), 2541, 2250, 1973, 1748 (C=O), 1682 (C=O), 1605, 1524, 1251, 1162, 734; $[\alpha]_D^{22}$ –10.6 (*c* 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 3.89 (3H, s, 4''-OCH_3), 4.74 (1H, dd, *J* 13.1, 6.8 Hz, 3-*H*H), 4.85–4.98 (2H, m, 2-H and 3-H*H*), 5.47 (1H, d, *J* 7.6 Hz, 2-NH), 7.06 (2H, d, *J* 8.5 Hz, 3'-H and 5'-H), 7.67 (2H, d, *J* 8.5 Hz, 2'-H and 6'-H), 8.00 (1H, d, *J* 8.3 Hz, 6'-H), 8.28 (1H, s, 8'-H), 8.36 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (126 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 53.0 (CH), 53.1 (CH_3), 55.6 (CH_3), 80.4 (C), 114.9 (2 × CH), 117.7 (C), 125.4 (CH), 125.9 (CH), 128.8 (2 × CH), 131.0 (C), 131.1 (CH), 144.8 (C), 147.8 (C), 155.2 (C), 156.1 (C), 160.7 (C), 170.6 (C); *m*/*z* (ESI) 477.1744 (MNa^+. $C_{23}H_{26}N_4NaO_6$ requires 477.1745).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148d)



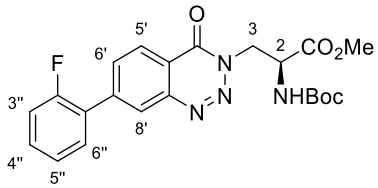
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.15 g, 0.35 mmol), 3,4-(methylenedioxy)phenylboronic acid (0.087 g, 0.53 mmol) and potassium phosphate (0.15 g, 0.70 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0060 g, 0.0076 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148d**) (0.13 g, 79%) as a white solid. Mp 76–80 °C; ν_{max}/cm^{-1} (neat) 3358 (NH), 2955 (CH), 2361, 2342, 1748 (C=O), 1686 (C=O), 1613, 1505, 1462, 1304, 1234, 1165, 1038, 733; $[\alpha]_D^{24}$ –16.9 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 4.74 (1H, dd, *J* 12.8, 6.6 Hz, 3-*H*H), 4.84–5.00 (2H, m, 2-H and 3-H*H*), 5.45 (1H, d, *J* 7.3 Hz, 2-NH), 6.06 (2H, s, OCH_2O), 6.96 (1H, d, *J* 8.0 Hz, 5''-H), 7.18 (1H, d, *J* 1.7 Hz, 2''-H), 7.20 (1H, dd, *J* 8.0, 1.7 Hz, 6''-H), 7.95 (1H, dd, *J* 8.3, 1.4 Hz, 6'-H), 8.24 (1H, d, *J* 1.4 Hz, 8'-H), 8.36 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 53.0 (CH), 53.0 (CH_3), 80.4 (C), 101.8 (CH_2), 107.8 (CH), 109.2 (CH), 118.0 (C), 121.8 (CH), 125.8 (CH), 125.9 (CH), 131.3 (CH), 132.9 (C), 144.8 (C), 147.9 (C), 148.8 (C), 148.9 (C), 155.2 (C), 156.1 (C), 170.5 (C); *m*/*z* (ESI) 491.1543 (MNa^+. $C_{23}H_{24}N_4NaO_7$ requires 491.1537).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148e)



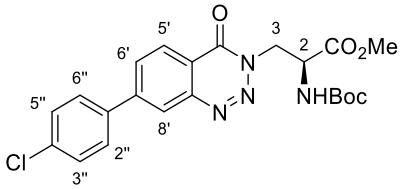
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.15 g, 0.35 mmol), 4-morpholinophenylboronic acid (0.11 g, 0.53 mmol) and potassium phosphate (0.15 g, 0.70 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0060 g, 0.0076 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148e**) (0.16 g, 89%) as a yellow solid. Mp 158–163 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2970 (CH), 2361, 1748 (C=O), 1709 (C=O), 1682 (C=O), 1601, 1304, 1234, 1165, 733; $[\alpha]_D^{23}$ –15.4 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.22–3.33 (4H, m, 2'''-H and 6'''-H), 3.80 (3H, s, OCH_3), 3.86–3.93 (4H, m, 3'''-H and 5'''-H), 4.74 (1H, dd, *J* 13.2, 6.8 Hz, 3-*H*H), 4.85–4.98 (2H, m, 2-H and 3-H*H*), 5.47 (1H, d, *J* 7.3 Hz, 2-NH), 7.03 (2H, d, *J* 8.8 Hz, 3''-H and 5''-H), 7.67 (2H, d, *J* 8.8 Hz, 2''-H and 6''-H), 8.00 (1H, dd, *J* 8.3, 1.4 Hz, 6'-H), 8.27 (1H, d, *J* 1.4 Hz, 8'-H), 8.34 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 48.7 (2 × CH_2), 50.6 (CH_2), 53.0 (CH_3), 53.1 (CH), 66.9 (2 × CH_2), 80.4 (C), 115.7 (2 × CH), 117.5 (C), 125.0 (CH), 125.8 (CH), 128.4 (2 × CH), 129.2 (C), 130.8 (CH), 144.9 (C), 147.8 (C), 152.0 (C), 155.2 (C), 156.1 (C), 170.6 (C); *m*/*z* (ESI) 532.2170 (MNa^+. $C_{26}H_{31}N_5NaO_6$ requires 532.2167).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148f)



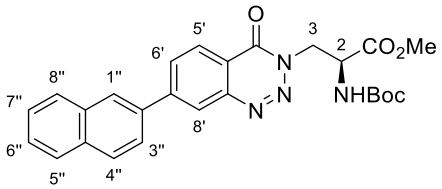
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.082 g, 0.19 mmol), 2-fluorophenylboronic acid (0.040 g, 0.29 mmol) and potassium phosphate (0.081 g, 0.38 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0030 g, 0.0038 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2.5% ethyl acetate in dichloromethane followed by 5% diethyl ether in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148f**) (0.076 g, 89%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3372 (NH), 2978 (CH), 2361, 2253, 1744 (C=O), 1682 (C=O), 1616, 1161, 729; $[\alpha]_D^{23}$ –2.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 4.74 (1H, dd, *J* 13.0, 6.8 Hz, 3-*H*H), 4.85–5.01 (2H, m, 2-H and 3-H*H*), 5.47 (1H, d, *J* 7.6 Hz, 2-NH), 7.22 (1H, ddd, *J* 10.7, 8.3, 1.0 Hz, 3''-H), 7.30 (1H, td, *J* 7.7, 1.0 Hz, 6''-H), 7.40–7.48 (1H, m, 4''-H), 7.53 (1H, td, *J* 7.7, 1.7 Hz, 5''-H), 7.98 (1H, d, *J* 8.3 Hz, 6'-H), 8.30 (1H, s, 8'-H), 8.39 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 52.9 (CH), 53.0 (CH_3), 80.4 (C), 116.7 (CH, $^2J_{CF}$ 22.4 Hz), 118.6 (C), 125.0 (CH, $^3J_{CF}$ 3.7 Hz), 125.5 (CH), 126.8 (C, $^2J_{CF}$ 12.8 Hz), 128.5 (CH, $^4J_{CF}$ 2.9 Hz), 130.8 (CH, $^4J_{CF}$ 2.7 Hz), 131.0 (CH, $^3J_{CF}$ 2.9 Hz), 133.4 (CH, $^4J_{CF}$ 2.9 Hz), 143.0 (C), 144.4 (C), 155.2 (C), 155.9 (C), 159.9 (C, $^1J_{CF}$ 249.9 Hz), 170.5 (C); *m*/*z* (ESI) 443.1723 (MH^+. $C_{22}H_{24}FN_4O_5$ requires 443.1725).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148g)



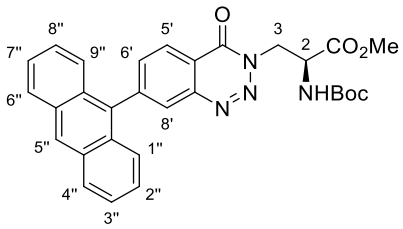
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.15 g, 0.35 mmol), 4-chlorophenylboronic acid (0.082 g, 0.53 mmol) and potassium phosphate (0.15 g, 0.70 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0060 g, 0.0076 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 60% diethyl ether in hexane followed by 50% diethyl ether in hexane with 1% triethylamine gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148g**) (0.11 g, 68%) as a white solid. Mp 126–130 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2978 (CH), 2361, 1748 (C=O), 1686 (C=O), 1616, 1505, 1366, 1308, 1161, 733; $[\alpha]_D^{24}$ –13.9 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.81 (3H, s, OCH_3), 4.73 (1H, dd, *J* 12.9, 6.8 Hz, 3-*H*H), 4.85–5.00 (2H, m, 2-H and 3-H*H*), 5.44 (1H, d, *J* 7.3 Hz, 2-NH), 7.49–7.54 (2H, m, 2''-H and 6''-H), 7.61–7.67 (2H, m, 3''-H and 5''-H), 7.99 (1H, dd, *J* 8.3, 1.4 Hz, 6'-H), 8.29 (1H, d, *J* 1.4 Hz, 8'-H), 8.40 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.8 (CH_2), 52.9 (CH), 53.1 (CH_3), 80.4 (C), 118.6 (C), 126.2 (CH), 126.2 (CH), 128.9 (2 × CH), 129.7 (2 × CH), 131.3 (CH), 135.7 (C), 137.1 (C), 144.7 (C), 147.0 (C), 155.2 (C), 155.9 (C), 170.5 (C); *m*/*z* (ESI) 481.1241 (MNa^+. $C_{22}H_{23}{}^{35}ClN_4NaO_5$ requires 481.1249).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148h)



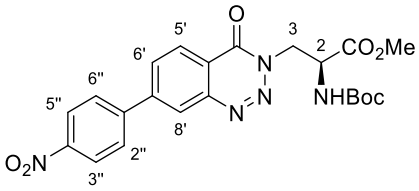
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.084 g, 0.20 mmol), 2-napthaleneboronoic acid (0.051 g, 0.30 mmol) and potassium phosphate (0.084 0.39 mmol) in tetrahydrofuran/water (1:2, 3mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0030 g, 0.0038 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 2 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 25–30% ethyl acetate in hexane followed by 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148h**) (0.070 g, 75%) as a white solid. Mp 164–168 °C; ν_{max}/cm^{-1} (neat) 2980 (CH), 2543, 2250, 1973, 1748 (C=O), 1683 (C=O), 1614, 1505, 1367, 1163, 910, 734; $[\alpha]_D^{24}$ –16.6 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.35 (9H, s, 3 × CH_3), 3.82 (3H, s, OCH_3), 4.75 (1H, dd, *J* 12.7, 6.6 Hz, 3-*H*H), 4.87–5.01 (2H, m, 2-H and 3-H*H*), 5.50 (1H, d, *J* 7.5 Hz, 2-NH), 7.53–7.60 (2H, m, 6''-H and 7''-H), 7.82 (1H, dd, *J* 8.5, 1.9 Hz, 3''-H), 7.88–7.98 (2H, m, 5''-H and 8''-H), 8.00 (1H, d, *J* 8.5 Hz, 4''-H), 8.16 (1H, dd, *J* 8.0, 1.6 Hz, 6'-H), 8.17 (1H, br s, 1''-H), 8.43 (1H, d, *J* 8.0 Hz, 5'-H), 8.44 (1H, br s, 8'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 53.0 (CH), 53.0 (CH_3), 80.4 (C), 118.3 (C), 125.0 (CH), 126.0 (CH), 126.5 (CH), 127.0 (CH), 127.2 (CH), 127.2 (CH), 127.9 (CH), 128.7 (CH), 129.3 (CH), 131.8 (CH), 133.5 (C), 133.6 (C), 135.9 (C), 144.8 (C), 148.2 (C), 155.2 (C), 156.1 (C), 170.5 (C); *m*/*z* (ESI) 497.1792 (MNa^+. $C_{26}H_{26}N_4NaO_5$ requires 497.1795).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148i)



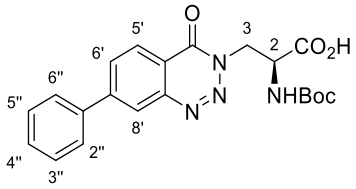
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.15 g, 0.35 mmol), 9-anthraceneboronic acid (0.117 g, 0.526 mmol) and potassium phosphate (0.15 g, 0.70 mmol) in tetrahydrofuran/water (1:2, 3 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0060 g, 0.0076 mmol, 2 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2.5% ethyl acetate in dichloromethane followed by 60% diethyl ether in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148i**) (0.18 g, 96%) as a yellow solid. Mp 120–125 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2978 (CH), 1748 (C=O), 1686 (C=O), 1616, 1505, 1366, 1304, 1161, 733; $[\alpha]_D^{23}$ –36.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.37 (9H, s, 3 × CH_3), 3.86 (3H, s, OCH_3), 4.79 (1H, dd, *J* 13.1, 7.2 Hz, 3-*H*H), 4.94–5.02 (1H, m, 2-H), 5.05 (1H, dd, *J* 13.1, 4.0 Hz, 3-H*H*), 5.50 (1H, d, *J* 7.8 Hz, 2-NH), 7.34–7.42 (2H, m, 2 × Ar-H), 7.45–7.55 (4H, m, 4 × Ar-H), 7.88 (1H, dd, *J* 8.0, 1.5 Hz, 6'-H), 8.07–8.12 (2H, m, 2 × Ar-H), 8.25 (1H, br s, 8'-H), 8.56 (1H, d, *J* 8.0 Hz, 5'-H), 8.60 (1H, s, 5''-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.9 (CH_2), 52.9 (CH), 53.1 (CH_3), 80.4 (C), 119.0 (C), 125.5 (CH), 125.5 (CH), 125.6 (CH), 125.8 (CH), 125.9 (CH), 126.4 (CH), 126.4 (CH), 128.1 (CH), 128.8 (2 × CH), 129.9 (C), 129.9 (C), 131.0 (CH), 131.4 (2 × C), 133.8 (C), 136.1 (CH), 144.4 (C), 146.9 (C), 155.3 (C), 156.1 (C), 170.5 (C); *m*/*z* (ESI) 547.1962 (MNa^+. $C_{30}H_{28}N_4NaO_5$ requires 547.1952).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-nitrophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (148j)



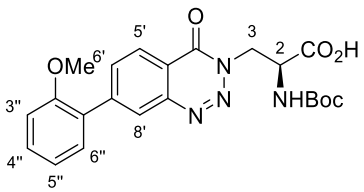
A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-bromo-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (1.00 g, 2.34 mmol), 4-nitrophenylboronic acid (0.587 g, 3.51 mmol) and potassium phosphate (0.992 g, 4.66 mmol) in tetrahydrofuran/water (1:2, 60 mL) was degassed under argon for 0.2 h. To this was added XPhos Pd G2 (0.0370 g, 0.0470 mmol, 2 mol%). The reaction mixture was heated to 40 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 4% ethyl acetate in dichloromethane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-nitrophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**148j**) (0.940 g, 86%) as a white solid. Mp 178–182 °C; ν_{max}/cm^{-1} (neat) 3370 (NH), 2977 (CH), 2255, 1745 (C=O), 1687 (C=O), 1519, 1345, 1161; $[\alpha]_D^{24}$ –8.5 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.83 (3H, s, OCH_3), 4.74 (1H, dd, *J* 13.2, 7.2 Hz, 3-*H*H), 4.88–4.96 (1H, m, 2-H), 4.99 (1H, dd, *J* 13.2, 4.0 Hz, 3-H*H*), 5.42 (1H, d, *J* 7.5 Hz, 2-NH), 7.84–7.90 (2H, m, 2''-H and 6''-H), 8.04 (1H, dd, *J* 8.3, 1.7 Hz, 6'-H), 8.37 (1H, d, *J* 1.7 Hz, 8'-H), 8.38–8.43 (2H, m, 3''-H and 5''-H), 8.47 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 51.0 (CH_2), 52.8 (CH), 53.1 (CH_3), 80.5 (C), 119.5 (C), 124.7 (2 × CH), 126.6 (CH), 127.1 (CH), 128.6 (2 × CH) 131.4 (CH), 144.7 (C), 144.9 (C), 145.6 (C), 148.4 (C), 155.2 (C), 155.7 (C), 170.4 (C); *m*/*z* (ESI) 470.1668 (MH^+. $C_{22}H_{24}N_5O_7$ requires 470.1670).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149a)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.072 g, 0.17 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.072 g, 0.22 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149a**) (0.055 g, 79%) as a white solid. Mp 155–160 °C; ν_{max}/cm^{-1} (neat) 3341 (NH), 2978 (CH), 2932, (CH), 1686 (C=O), 1616, 1512, 1304, 1161, 910, 729; $[\alpha]_D^{23}$ –5.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 4.82 (1H, dd, *J* 13.5, 7.1 Hz, 3-*H*H), 4.92–5.00 (1H, m, 2-H), 5.03 (1H, dd, *J* 13.5, 5.0 Hz, 3-H*H*), 5.61 (1H, d, *J* 7.3 Hz, 2-NH), 7.42–7.57 (3H, m, 3''-H, 4''-H and 5''-H), 7.67–7.74 (2H, m, 2''-H and 6''-H), 8.03 (1H, dd, *J* 8.3, 1.6 Hz, 6'-H), 8.33 (1H, br s, 8'-H), 8.39 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 52.9 (CH), 80.8 (C), 118.2 (C), 126.0 (CH), 126.2 (CH), 127.7 (2 × CH), 129.3 (CH), 129.4 (2 × CH), 131.7 (CH), 138.6 (C), 144.7 (C), 148.4 (C), 155.7 (C), 156.4 (C), 172.8 (C); *m*/*z* (ESI) 433.1473 (MNa^+. $C_{21}H_{22}N_4NaO_5$ requires 433.1482).

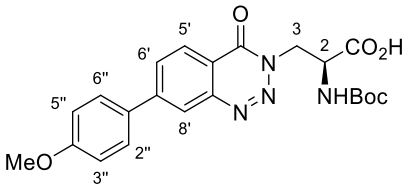
(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149b)



To a stirred solution methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.22 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.093 g, 0.29 mmol). The reaction mixture was stirred at room

temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149b**) (0.094 g, 97%) as an off-white solid. Mp 150–155 °C; ν_{max}/cm^{-1} (neat) 2966 (CH), 2247, 1987, 1682 (C=O), 1614, 1243, 1160, 909, 731; $[\alpha]_D^{23}$ –10.6 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.35 (9H, s, 3 × CH_3), 3.84 (3H, s, 2''-OCH_3), 4.84 (1H, dd, *J* 13.1, 7.0 Hz, 3-*H*H), 4.89–4.98 (1H, m, 2-H), 5.02 (1H, dd, *J* 13.1, 4.0 Hz, 3-H*H*), 5.60 (1H, d, *J* 7.1 Hz, 2-NH), 7.04 (1H, d, *J* 8.2 Hz, 3''-H), 7.09 (1H, t, *J* 7.5 Hz, 5''-H), 7.37–7.48 (2H, m, 4''-H and 6''-H), 7.99 (1H, dd, *J* 8.2, 1.3 Hz, 6'-H), 8.32 (1H, br s, 8'-H), 8.35 (1H, d, *J* 8.2 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.5 (CH_2), 53.0 (CH), 55.7 (CH_3), 80.8 (C), 111.6 (CH), 117.9 (C), 121.4 (CH), 124.8 (CH), 128.1 (C), 129.0 (CH), 130.6 (CH), 131.0 (CH), 134.6 (CH), 144.3 (C), 146.3 (C), 155.7 (C), 156.6 (C), 156.6 (C), 172.7 (C); *m*/*z* (ESI) 463.1586 (MNa^+. $C_{22}H_{24}N_4NaO_6$ requires 463.1588).

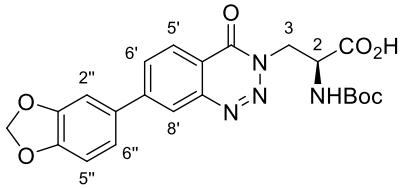
(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149c)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.22 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.094 g, 0.29 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (15 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149c**) (0.097 g, 99%) as a white solid. Mp 210–215 °C (decomposition); ν_{max}/cm^{-1} (neat) 3372 (NH), 2248, 1974, 1733 (C=O), 1705 (C=O), 1651 (C=O), 1521, 1159, 908, 728; $[\alpha]_D^{25}$ +1.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.35 (9H, s, 3 × CH_3), 3.89 (3H, s, 4''-OCH_3), 4.83 (1H, dd, *J* 13.2, 7.4 Hz, 3-*H*H),

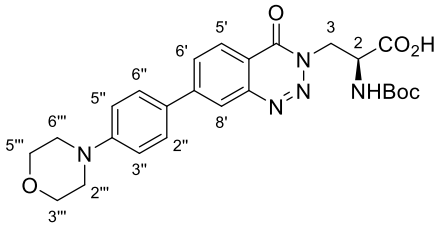
4.89–4.96 (1H, m, 2-H), 5.01 (1H, dd, *J* 13.2, 4.1 Hz, 3-H*H*), 5.60 (1H, d, *J* 7.4 Hz, 2-NH), 7.05 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 7.67 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.00 (1H, dd, *J* 8.3, 1.6 Hz, 6'-H), 8.29 (1H, d, *J* 1.6 Hz, 8'-H), 8.36 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.6 (CH_2), 52.9 (CH), 55.6 (CH_3), 80.8 (C), 114.9 (2 × CH), 117.6 (C), 125.4 (CH), 125.9 (CH), 128.8 (2 × CH), 130.9 (C), 131.2 (CH), 144.8 (C), 148.0 (C), 155.7 (C), 156.5 (C), 160.8 (C), 172.5 (C); *m*/*z* (ESI) 463.1591 (MNa^+. $C_{22}H_{24}N_4NaO_6$ requires 463.1588).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149d)



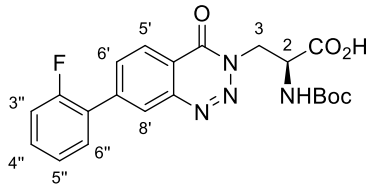
To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.170 g, 0.363 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.154 g, 0.472 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149d**) (0.121 g, 73%) as a white solid. Mp 229–234 °C; ν_{max}/cm^{-1} (neat) 3422 (NH), 2924 (CH), 2361, 1686 (C=O), 1613, 1238, 1161, 1034; $[\alpha]_D^{21}$ –43.8 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 1.22 (9H, s, 3 × CH_3), 4.44–4.57 (2H, m, 3-*H*H and 2-H), 4.86 (1H, dd, *J* 11.1, 2.6 Hz, 3-H*H*), 6.12 (2H, s, OCH_2O), 7.08 (1H, d, *J* 8.1 Hz, 5''-H), 7.28 (1H, d, *J* 7.9 Hz, 2-NH), 7.42 (1H, dd, *J* 8.1, 1.8 Hz, 6''-H), 7.53 (1H, d, *J* 1.8 Hz, 2''-H), 8.19 (1H, dd, *J* 8.4, 1.7 Hz, 6'-H), 8.25 (1H, d, *J* 8.4 Hz, 5'-H), 8.39 (1H, d, *J* 1.7 Hz, 8'-H); δ_C (101 MHz, DMSO-d_6) 28.0 (3 × CH_3), 50.3 (CH_2), 51.4 (CH), 78.4 (C), 101.6 (CH_2), 107.7 (CH), 109.0 (CH), 117.5 (C), 121.7 (CH), 124.6 (CH), 125.2 (CH), 131.0 (CH), 131.7 (C), 144.3 (C), 146.4 (C), 148.3 (C), 148.4 (C), 154.9 (C), 155.3 (C), 171.2 (C); *m*/*z* (ESI) 477.1380 (MNa^+. $C_{22}H_{22}N_4NaO_7$ requires 477.1381).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149e)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.20 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.083 g, 0.26 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149e**) (0.074 g, 76%) as a yellow solid. Mp 235–240 °C; ν_{max}/cm^{-1} (neat) 3349 (NH), 2963 (CH), 1744 (C=O), 1686 (C=O), 1601, 1308, 1234, 1161; $[\alpha]_D^{21}$ –68.8 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 1.23 (9H, s, 3 × CH_3), 3.19–3.27 (4H, m, 2'''-H and 6'''-H), 3.72–3.81 (4H, m, 3'''-H and 5'''-H), 4.44–4.58 (2H, m, 3-*H*H and 2-H), 4.86 (1H, dd, *J* 11.8, 3.5 Hz, 3-H*H*), 7.10 (2H, d, *J* 8.8 Hz, 3''-H and 5''-H), 7.30 (1H, d, *J* 8.2 Hz, 2-NH), 7.82 (2H, d, *J* 8.8 Hz, 2''-H and 6''-H), 8.18–8.28 (2H, m, 5'-H and 6'-H), 8.37 (1H, s, 8'-H); δ_C (101 MHz, DMSO-d_6) 28.0 (3 × CH_3), 47.6 (2 × CH_2), 50.1 (CH_2), 51.3 (CH), 66.0 (2 × CH_2), 78.3 (C), 115.1 (2 × CH), 116.9 (C), 123.4 (CH), 125.2 (CH), 127.3 (C), 128.1 (2 × CH), 130.2 (CH), 144.5 (C), 146.6 (C), 151.6 (C), 154.9 (C), 155.3 (C), 171.2 (C); *m*/*z* (ESI) 496.2201 (MH^+. $C_{25}H_{30}N_5O_6$ requires 496.2191).

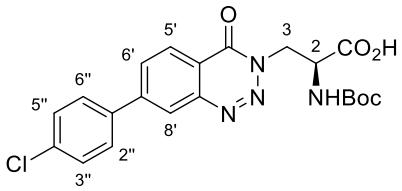
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149f)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.071 g, 0.16 mmol) in

methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.068 g, 0.21 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149f**) (0.056 g, 81%) as a white solid. Mp 83–87 °C; ν_{max}/cm^{-1} (neat) 3337 (NH), 2978 (CH), 1686 (C=O), 1620, 1408, 1254, 1215, 1161, 760, 733; $[\alpha]_D^{23}$ –10.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.32 (9H, s, 3 × CH_3), 4.81 (1H, dd, *J* 13.6, 7.2 Hz, 3-*H*H), 4.91–5.09 (2H, m, 2-H and 3-H*H*), 5.61 (1H, d, *J* 6.2 Hz, 2-NH), 7.17–7.27 (1H, m, 3''-H), 7.29 (1H, t, *J* 7.5 Hz, 6''-H), 7.39–7.49 (1H, m, 4''-H), 7.53 (1H, t, *J* 7.5 Hz, 5''-H), 7.99 (1H, d, *J* 8.3 Hz, 6'-H), 8.32 (1H, s, 8'-H), 8.39 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.7 (CH_2), 52.9 (CH), 80.8 (C), 116.7 (CH, $^2J_{CF}$ 22.3 Hz), 118.5 (C), 125.0 (CH, $^3J_{CF}$ 3.7 Hz), 125.6 (CH), 126.7 (C, $^2J_{CF}$ 12.8 Hz), 128.5 (CH, $^4J_{CF}$ 2.9 Hz), 130.9 (CH, $^4J_{CF}$ 2.3 Hz), 131.0 (CH, $^3J_{CF}$ 8.4 Hz), 133.6 (CH, $^4J_{CF}$ 3.3 Hz), 143.2 (C), 144.4 (C), 155.7 (C), 156.3 (C), 159.9 (C, $^1J_{CF}$ 250.0 Hz), 172.9 (C); *m*/*z* (ESI) 451.1397 (MNa^+. $C_{21}H_{21}FN_4NaO_5$ requires 451.1388).

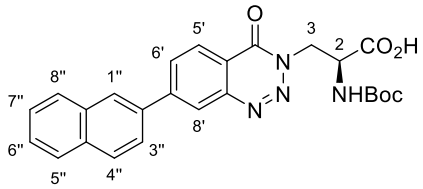
(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149g)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.073 g, 0.16 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.067 g, 0.21 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149g**) (0.064 g, 90%) as a white solid. Mp 195–200 °C;

ν_{max}/cm^{-1} (neat) 3345 (NH), 2978 (CH), 2928, 1740 (C=O), 1686 (C=O), 1616, 1520, 1308, 1161, 733; $[\alpha]_D^{21}$ –46.0 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 1.25 (9H, s, 3 × CH_3), 4.63 (1H, dd, *J* 13.3, 9.5 Hz, 3-*H*H), 4.80 (1H, dd, *J* 9.5, 4.3 Hz, 2-H), 5.00 (1H, dd, *J* 13.3, 4.3 Hz, 3-H*H*), 7.50–7.55 (2H, m, 2''-H and 6''-H), 7.77 (2H, br d, *J* 8.6 Hz, 3''-H and 5''-H), 8.12 (1H, dd, *J* 8.3, 1.3 Hz, 6'-H), 8.31 (1H, d, *J* 1.3 Hz, 8'-H), 8.34 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, CD_3OD) 28.5 (3 × CH_3), 52.2 (CH_2), 53.3 (CH), 80.7 (C), 119.7 (C), 126.7 (CH), 126.8 (CH), 130.1 (2 × CH), 130.5 (2 × CH), 132.3 (CH), 136.4 (C), 138.3 (C), 146.0 (C), 148.0 (C), 157.2 (C), 157.6 (C), 172.9 (C); *m*/*z* (ESI) 467.1099 (MNa^+. $C_{21}H_{21}{}^{35}ClN_4NaO_5$ requires 467.1093).

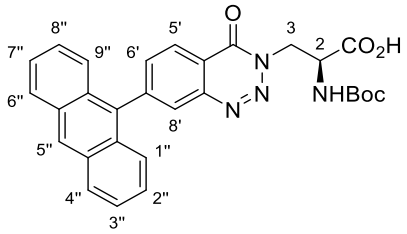
(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149h)



To a stirred solution methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.081 g, 0.17 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.072 g, 0.22 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (15 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149h**) (0.068 g, 86%) as an off-white solid. Mp 210–215 °C; ν_{max}/cm^{-1} (neat) 2974 (CH), 2250, 1974, 1682 (C=O), 1614, 1504, 1367, 1158, 908, 729; $[\alpha]_D^{19}$ +33.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.36 (9H, s, 3 × CH_3), 4.85 (1H, dd, *J* 13.5, 7.4 Hz, 3-*H*H), 4.92–4.99 (1H, m, 2-H), 5.03 (1H, dd, *J* 13.5, 3.8 Hz, 3-H*H*), 5.61 (1H, d, *J* 6.8 Hz, 2-NH), 7.52–7.61 (2H, m, 6''-H and 7''-H), 7.82 (1H, dd, *J* 8.5, 1.7 Hz, 3''-H), 7.87–7.97 (2H, m, 5''-H and 8''-H), 8.00 (1H, d, *J* 8.5 Hz, 4''-H), 8.14–8.21 (2H, m, 6'-H and 1''-H), 8.43 (1H, d, *J* 8.3 Hz, 5'-H), 8.47 (1H, s, 8'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.8 (CH_2), 52.8 (CH), 80.7 (C), 118.1 (C), 124.9 (CH), 126.0 (CH), 126.3 (CH), 127.0 (CH), 127.1 (2 × CH), 127.8 (CH), 128.7 (CH), 129.3 (CH), 131.8 (CH), 133.4 (C), 133.6 (C), 135.7 (C),

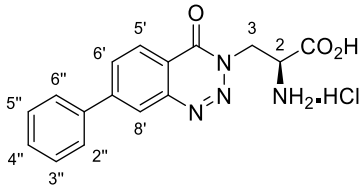
144.7 (C), 148.1 (C), 155.7 (C), 156.4 (C), 172.8 (C); *m*/*z* (ESI) 483.1646 (MNa^+. $C_{25}H_{24}N_4NaO_5$ requires 483.1639).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (149i)



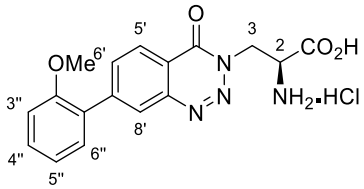
To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.093 g, 0.18 mmol) in methanol (3 mL), dioxane (1.8 mL) and water (1.8 mL) was added caesium carbonate (0.075 g, 0.23 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (10 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**149i**) (0.090 g, 100%) as a yellow solid. Mp 220–224 °C; ν_{max}/cm^{-1} (neat) 3368 (NH), 2978 (CH), 2253, 1686 (C=O), 1616, 1508, 1300, 1161, 907, 733; $[\alpha]_D^{22}$ –24.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.36 (9H, s, 3 × CH_3), 4.89 (1H, dd, *J* 13.1, 7.5 Hz, 3-*H*H), 5.01–5.18 (2H, m, 2-H and 3-H*H*), 5.66 (1H, d, *J* 6.7 Hz, 2-NH), 7.30–7.40 (2H, m, 2 × Ar-H), 7.42–7.56 (4H, m, 4 × Ar-H), 7.88 (1H, d, *J* 7.8 Hz, 6'-H), 8.02–8.11 (2H, m, 2 × Ar-H), 8.24 (1H, s, 8'-H), 8.52–8.62 (2H, m, 5'-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.8 (CH_2), 52.9 (CH), 80.8 (C), 118.9 (C), 125.5 (2 × CH), 125.7 (CH), 125.8 (CH), 125.8 (CH), 126.4 (CH), 126.5 (CH), 128.2 (CH), 128.8 (2 × CH), 129.9 (C), 129.9 (C), 131.0 (CH), 131.4 (2 × C), 133.7 (C), 136.3 (CH), 144.4 (C), 147.1 (C), 155.7 (C), 156.5 (C), 173.0 (C); *m*/*z* (ESI) 533.1804 (MNa^+. $C_{29}H_{26}N_4NaO_5$ requires 533.1795).

(2*S*)-2-Amino-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150a)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.030 g, 0.073 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[7'-phenyl-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150a**) (0.023 g, 92%) as white solid. Mp 223–228°C; ν_{max}/cm^{-1} (neat) 3422 (NH), 2920 (CH), 2153, 1960, 1732 (C=O), 1674 (C=O), 1616, 1497, 1204, 1034, 760; $[\alpha]_D^{22}$ –11.9 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 4.44 (1H, dd, *J* 7.7, 5.2 Hz, 2-H), 4.79 (1H, dd, *J* 14.3, 7.7 Hz, 3-*H*H), 4.95 (1H, dd, *J* 14.3, 5.2 Hz, 3-H*H*), 7.52 (1H, t, *J* 9.0 Hz, 4''-H), 7.58 (2H, t, *J* 9.0 Hz, 3''-H and 5''-H), 7.93 (2H, d, *J* 9.0 Hz, 2''-H and 6''-H), 8.29 (1H, dd, *J* 8.3, 1.7 Hz, 6'-H), 8.33 (1H, d, *J* 8.3 Hz, 5'-H), 8.52 (1H, d, *J* 1.7 Hz, 8'-H); δ_C (101 MHz, DMSO-d_6) 48.4 (CH_2), 50.8 (CH), 118.4 (C), 125.4 (CH), 125.4 (CH), 127.4 (2 × CH), 129.2 (CH), 129.3 (2 × CH), 131.5 (CH), 137.6 (C), 144.3 (C), 146.9 (C), 155.3 (C), 168.3 (C); *m*/*z* (ESI) 333.0955 (MNa^+. $C_{16}H_{14}N_4NaO_3$ requires 333.0958).

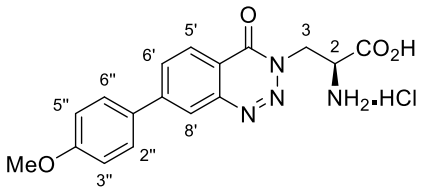
(2*S*)-2-Amino-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150b)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.020 g, 0.045 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150b**) (0.018 g, 100%) as a yellow solid. Mp 208–211 °C; ν_{max}/cm^{-1} (neat) 3430 (NH), 2836 (CH), 2496, 1745 (C=O), 1682 (C=O), 1614, 1513, 1242, 1122, 980, 757; $[\alpha]_D^{23}$ –7.1 (*c* 0.1, MeOH); δ_H (500 MHz, CD_3OD) 3.86 (3H, s, 2''-

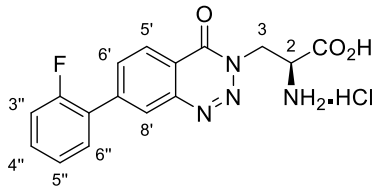
OCH_3), 4.64 (1H, dd, *J* 7.2, 4.5 Hz, 2-H), 4.95 (1H, dd, *J* 14.6, 7.2 Hz, 3-*H*H), 5.12 (1H, dd, *J* 14.6, 4.5 Hz, 3-H*H*), 7.11 (1H, t, *J* 7.4 Hz, 5''-H), 7.17 (1H, d, *J* 8.2 Hz, 3''-H), 7.42–7.49 (2H, m, 4''-H and 6''-H), 8.08 (1H, dd, *J* 8.2, 1.2 Hz, 6'-H), 8.31 (1H, d, *J* 1.2 Hz, 8'-H), 8.33 (1H, d, *J* 8.2 Hz, 5'-H); δ_C (126 MHz, CD_3OD) 49.8 (CH_2), 53.3 (CH), 56.1 (CH_3), 112.9 (CH), 119.1 (C), 122.3 (CH), 125.4 (CH), 129.0 (C), 129.8 (CH), 131.8 (CH), 131.8 (CH), 135.8 (CH), 145.4 (C), 147.9 (C), 157.8 (C), 157.9 (C), 169.2 (C); *m*/*z* (ESI) 363.1063 ($[MNa–HCl]^+$. $C_{17}H_{16}N_4NaO_4$ requires 363.1064).

(2*S*)-2-Amino-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150c)



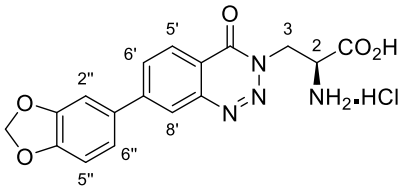
A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.044 g, 0.10 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[7'-(4''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150c**) (0.032 g, 84%) as a yellow solid. Mp 228–233 °C; ν_{max}/cm^{-1} (neat) 3403 (NH), 2922 (CH), 2219, 1765 (C=O), 1678 (C=O), 1601, 1251, 1034, 852; $[\alpha]_D^{22}$ +48.3 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 3.84 (3H, s, 4''-OCH_3), 4.36–4.45 (1H, m, 2-H), 4.76 (1H, dd, *J* 14.4, 7.7 Hz, 3-*H*H), 4.93 (1H, dd, *J* 14.4, 4.9 Hz, 3-H*H*), 7.12 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 7.91 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.22–8.32 (2H, m, 5'-H and 6'-H), 8.46 (1H, s, 8'-H); δ_C (101 MHz, DMSO-d_6) 48.5 (CH_2), 51.0 (CH), 55.4 (CH_3), 114.8 (2 × CH), 117.7 (C), 124.4 (CH), 125.3 (CH), 128.8 (2 × CH), 129.7 (C), 130.9 (CH), 144.4 (C), 146.6 (C), 155.3 (C), 160.3 (C), 168.3 (C); *m*/*z* (ESI) 339.1098 ($[(M–H)–HCl]^-$. $C_{17}H_{15}N_4O_4$ requires 339.1099).

(2*S*)-2-Amino-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150d)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.030 g, 0.070 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[7'-(2''-fluorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150d**) (0.024 g, 96%) as white solid. Mp 215–220 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 2924 (CH), 2361, 2153, 1979, 1717 (C=O), 1670 (C=O), 1616, 1497, 1207, 760; $[\alpha]_D^{22}$ –30.4 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 4.41 (1H, dd, *J* 7.9, 5.2 Hz, 2-H), 4.78 (1H, dd, *J* 14.3, 7.9 Hz, 3-*H*H), 4.95 (1H, dd, *J* 14.3, 5.2 Hz, 3-H*H*), 7.38–7.47 (2H, m, 3''-H and 6''-H), 7.53–7.61 (1H, m, 4''-H), 7.76 (1H, td, *J* 7.8, 1.4 Hz, 5''-H), 8.14 (1H, d, *J* 8.2 Hz, 6'-H), 8.35 (1H, d, *J* 8.2 Hz, 5'-H), 8.39 (1H, s, 8'-H); δ_C (101MHz, DMSO-d_6) 48.5 (CH_2), 50.9 (CH), 116.4 (CH, $^2J_{CF}$ 22.1 Hz), 118.8 (C), 125.1 (CH), 125.4 (CH, $^3J_{CF}$ 3.5 Hz), 126.0 (C, $^2J_{CF}$ 12.6 Hz), 127.9 (CH, $^4J_{CF}$ 3.1 Hz), 131.1 (CH, $^4J_{CF}$ 2.5 Hz), 131.3 (CH, $^3J_{CF}$ 8.3 Hz), 133.4 (CH, $^4J_{CF}$ 2.9 Hz), 142.0 (C), 143.8 (C), 155.3 (C), 159.1 (C, $^1J_{CF}$ 247.5 Hz), 168.3 (C); *m*/*z* (ESI) 351.0864 (MNa^+. $C_{16}H_{13}FN_4NaO_3$ requires 351.0864).

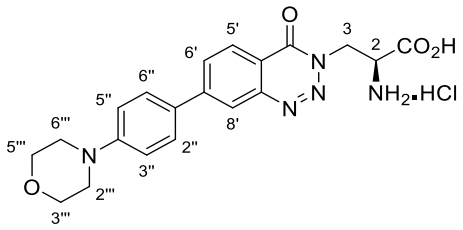
(2*S*)-2-Amino-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150e)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.025 g, 0.055 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-(3'',4''-methylenedioxybenzene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150e**) (0.021 g, 100%)

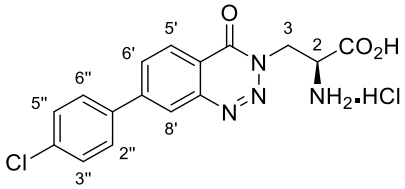
as a white solid. Mp 223–227 °C; ν_{max}/cm^{-1} (neat) 3140 (NH), 2909 (CH), 1767 (C=O), 1678 (C=O), 1609, 1501, 1034, 814; $[\alpha]_D^{21}$ +57.7 (*c* 0.1, DMSO); δ_H (400 MHz, DMSO-d_6) 4.42 (1H, dd, *J* 7.7, 5.1 Hz, 2-H), 4.76 (1H, dd, *J* 14.4, 7.7 Hz, 3-*H*H), 4.94 (1H, dd, *J* 14.4, 5.1 Hz, 3-H*H*), 6.13 (2H, s, OCH_2O), 7.11 (1H, d, *J* 8.1 Hz, 5''-H), 7.46 (1H, dd, *J* 8.1, 1.9 Hz, 6''-H), 7.58 (1H, d, *J* 1.9 Hz, 2''-H), 8.24 (1H, dd, *J* 8.4, 1.3 Hz, 6'-H), 8.28 (1H, d, *J* 8.4 Hz, 5'-H), 8.47 (1H, d, *J* 1.3 Hz, 8'-H); δ_C (101 MHz, DMSO-d_6) 48.5 (CH_2), 51.0 (CH), 101.6 (CH_2), 107.7 (CH), 109.0 (CH), 117.9 (C), 121.7 (CH), 124.8 (CH), 125.2 (CH), 131.2 (C), 131.6 (CH), 144.4 (C), 146.6 (C), 148.4 (C), 148.4 (C), 155.3 (C), 168.4 (C); *m*/*z* (ESI) 377.0850 ([MNa–HCl]$^+$. $C_{17}H_{14}NNa_4O_5$ requires 377.0856).

(2*S*)-2-Amino-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150f)



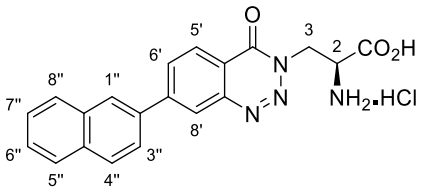
A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.040 g, 0.081 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[7'-(4''-morpholinophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150f**) (0.038 g, 100%) as a white solid. Mp 234–240 °C; ν_{max}/cm^{-1} (neat) 3433 (NH), 2920 (CH), 2457, 1998, 1767 (C=O), 1682 (C=O), 1601, 1505, 1404, 1300, 1219, 1119, 1045; $[\alpha]_D^{21}$ –6.6 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6), 3.19–3.29 (4H, m, 2'''-H and 6'''-H), 3.74–3.82 (4H, m, 3'''-H and 5'''-H), 4.40–4.50 (1H, m, 2-H), 4.78 (1H, dd, *J* 14.3, 7.5 Hz, 3-*H*H), 4.93 (1H, dd, *J* 14.3, 5.4 Hz, 3-H*H*), 7.08–7.23 (2H, m, 3''-H and 5''-H), 7.86 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.21–8.29 (2H, m, 6'-H and 8'-H), 8.41–8.47 (1H, m, 5'-H); δ_C (101 MHz, DMSO-d_6) 47.7 (2 × CH_2), 48.3 (CH_2), 50.9 (CH), 66.0 (2 × CH_2), 115.1 (2 × CH), 117.2 (C), 123.6 (CH), 125.2 (CH), 127.2 (C), 128.1 (2 × CH), 130.4 (CH), 144.5 (C), 146.8 (C), 151.7 (C), 155.4 (C), 168.5 (C); *m*/*z* (ESI) 396.1667 ([MH–HCl]$^+$. $C_{20}H_{22}N_5O_4$ requires 396.1666).

(2*S*)-2-Amino-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150g)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.040 g, 0.090 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 2 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-(4''-chlorophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150g**) (0.037 g, 100%) as a white solid. Mp 230–235 °C; ν_{max}/cm^{-1} (neat) 3457 (NH), 2928 (CH), 2361, 2168, 1975, 1682 (C=O), 1593, 1512, 1343, 1312, 1099, 853; $[\alpha]_D^{21}$ +46.9 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 4.45 (1H, dd, *J* 7.6, 5.3 Hz, 2-H), 4.78 (1H, dd, *J* 14.3, 7.6 Hz, 3-*H*H), 4.94 (1H, dd, *J* 14.3, 5.3 Hz, 3-H*H*), 7.61–7.66 (2H, m, 2''-H and 6''-H), 7.95–8.01 (2H, m, 3''-H and 5''-H), 8.29 (1H, dd, *J* 8.0, 1.3 Hz, 6'-H), 8.33 (1H, d, *J* 8.0 Hz, 5'-H), 8.55 (1H, d, *J* 1.3 Hz, 8'-H); δ_C (101 MHz, DMSO-d_6) 48.4 (CH_2), 50.9 (CH), 118.6 (C), 125.5 (CH), 125.5 (CH), 129.3 (2 × CH), 129.3 (2 × CH), 131.4 (CH), 134.3 (C), 136.4 (C), 144.3 (C), 145.6 (C), 155.3 (C), 168.4 (C); *m*/*z* (ESI) 367.0550 ([MNa–HCl]$^+$. $C_{16}H_{13}{}^{35}ClN_4NaO_3$ requires 367.0568).

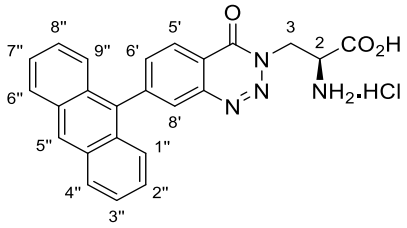
(2*S*)-2-Amino-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150h)



A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.031 g, 0.067 mmol) in 2 M aqueous hydrochloric acid (2 mL) was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* to (2*S*)-2-amino-3-[7'-(2''-napthalene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150h**) (0.021 g, 78%) as a white solid. Mp 240–245 °C; ν_{max}/cm^{-1} (neat) 3381 (NH), 2915 (CH), 1765 (C=O), 1680 (C=O), 1614, 1505, 1312, 1202, 1041, 780; $[\alpha]_D^{24}$ +75.3 (*c* 0.1, DMSO); δ_H (400 MHz, DMSO-d_6) 4.40–4.49 (1H, m, 2-H), 4.79 (1H, dd, *J* 14.3, 7.8 Hz, 3-*H*H),

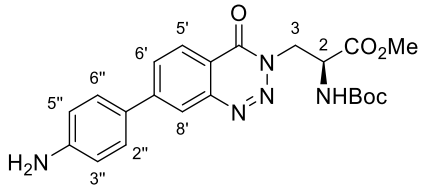
4.96 (1H, dd, *J* 14.3, 5.0 Hz, 3-*H*H), 7.55–7.66 (2H, m, 6''-H and 7''-H), 7.98–8.04 (1H, m, 8''-H), 8.06–8.16 (3H, m, 3''-H, 4''-H and 5''-H), 8.37 (1H, d, *J* 8.3 Hz, 5'-H), 8.46 (1H, dd, *J* 8.3, 1.8 Hz, 6'-H), 8.56 (1H, s, 1''-H), 8.69 (1H, d, *J* 1.8 Hz, 8'-H); δ_C (101 MHz, DMSO-d_6) 48.5 (CH_2), 50.9 (CH), 118.4 (C), 124.9 (CH), 125.4 (CH), 125.6 (CH), 126.8 (CH), 126.9 (CH), 127.1 (CH), 127.6 (CH), 128.6 (CH), 129.0 (CH), 131.6 (CH), 133.0 (C), 133.2 (C), 134.8 (C), 144.4 (C), 146.7 (C), 155.4 (C), 168.4 (C); *m*/*z* (ESI) 361.1296 ($[MH–HCl]^+$. $C_{20}H_{17}N_4O_3$ requires 361.1295).

(2*S*)-2-Amino-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (150i)



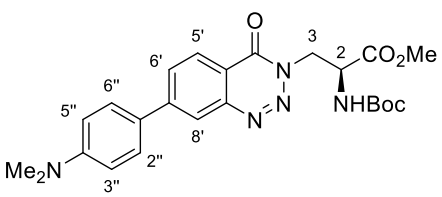
A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.030 g, 0.059 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-(10''-anthracene)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**150i**) (0.018 g, 75%) as a white solid. Mp 251–256 °C; ν_{max}/cm^{-1} (neat) 3453 (NH), 2928 (CH), 2361, 2342, 2164, 2006, 1975, 1682 (C=O), 1616, 1593, 1408, 1512, 1339, 853, 741; $[\alpha]_D^{21}$ –4.5 (*c* 0.1, MeOH); δ_H (400 MHz, DMSO-d_6) 4.23–4.35 (1H, m, 2-H), 4.76 (1H, dd, *J* 14.0, 9.3 Hz, 3-*H*H), 5.03 (1H, dd, *J* 14.0, 4.0 Hz, 3-H*H*), 7.40–7.52 (4H, m, 4 × Ar-H), 7.53–7.66 (2H, m, 2 × Ar-H), 8.00 (1H, dd, *J* 8.0, 1.5 Hz, 6'-H), 8.20–8.26 (2H, m, 2 × Ar-H), 8.30 (1H, d, *J* 1.5 Hz, 8'-H), 8.51 (1H, d, *J* 8.0 Hz, 5'-H), 8.82 (1H, s, 5''-H); δ_C (101 MHz, DMSO-d_6) 49.4 (CH_2), 51.7 (CH), 119.2 (C), 125.2 (CH), 125.2 (CH), 125.3 (CH), 125.5 (CH), 125.6 (CH), 126.6 (CH), 126.6 (CH), 127.8 (CH), 128.7 (CH), 128.7 (CH), 129.2 (C), 129.2 (C), 130.2 (CH), 130.8 (C), 130.8 (C), 133.5 (C), 135.9 (CH), 143.9 (C), 145.5 (C), 155.5 (C), 167.8 (C); *m*/*z* (ESI) 433.1270 ($[MNa–HCl]^+$. $C_{24}H_{18}N_4NaO_3$ requires 433.1271).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (151)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-nitrophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.21 mmol) in ethyl acetate (3.1 mL) was added tin(II) dichloride dihydrate (0.24 g, 1.1 mmol) and pyridine (0.086 mL, 1.1 mmol). The reaction mixture was heated under reflux for 18 h. The mixture was cooled to room temperature and sodium bicarbonate (50 mL) was added. The reaction mixture was filtered through a pad of Celite® and diluted with ethyl acetate (40 mL). The organic layer was washed with sodium bicarbonate (3 × 60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**151**) (0.081 g, 86%) as a yellow solid. Mp 130–135 °C; ν_{max}/cm^{-1} (neat) 3368 (NH), 2978 (CH), 2342, 1678 (C=O), 1597, 1157; $[\alpha]_D^{22}$ –1.2 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.80 (3H, s, OCH_3), 4.74 (1H, dd, *J* 13.2, 6.7 Hz, 3-*H*H), 4.85–4.98 (2H, m, 2-H and 3-H*H*), 5.47 (1H, d, *J* 7.6 Hz, 2-NH), 6.77–6.85 (2H, m, 3''-H and 5''-H), 7.53–7.58 (2H, m, 2''-H and 6''-H), 7.97 (1H, dd, *J* 8.3, 1.8 Hz, 6'-H), 8.25 (1H, d, *J* 1.8 Hz, 8'-H), 8.33 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 50.6 (CH_2), 53.1 (CH_3), 53.1 (CH), 80.4 (C), 115.6 (2 × CH), 117.3 (C), 124.7 (CH), 125.7 (CH), 128.4 (C), 128.7 (2 × CH), 130.7 (CH), 144.9 (C), 147.8 (C), 148.2 (C), 155.3 (C), 156.2 (C), 170.6 (C); *m*/*z* (ESI) 440.1927 (MH^+. $C_{22}H_{26}N_5O_5$ requires 440.1928).

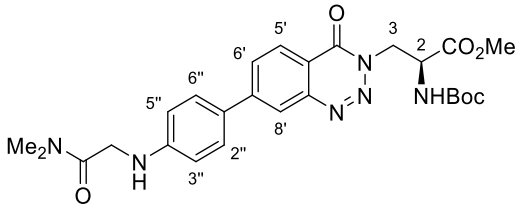
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (152)



A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.084 g, 0.19 mmol) in acetonitrile (2 mL) was degassed under argon for 0.1 h. To this was added paraformaldehyde

(0.058 g, 1.9 mmol), sodium cyanoborohydride (0.036 g, 0.56 mmol) and water (0.4 mL). Acetic acid was added dropwise until the reaction mixture reached pH 7. The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in sodium bicarbonate (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**152**) (0.059 g, 66%) as a yellow solid. Mp 169–174 °C; ν_{max}/cm^{-1} (neat) 3368 (NH), 2928 (CH), 2342, 1748 (C=O), 1678 (C=O), 1597, 1366, 1161; $[\alpha]_D^{21}$ +20.8 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.06 (6H, s, 2 × $N(CH_3)_2$), 3.80 (3H, s, OCH_3), 4.70–4.79 (1H, m, 3-*H*H), 4.85–4.97 (2H, m, 2-H and 3-H*H*), 5.48 (1H, d, *J* 7.5 Hz, 2-NH), 6.81–6.90 (2H, m, 3''-H and 5''-H), 7.63–7.69 (2H, m, 2''-H and 6''-H), 8.00 (1H, dd, *J* 8.3, 1.8 Hz, 6'-H), 8.26 (1H, d, *J* 1.8 Hz, 8'-H), 8.32 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 40.6 (2 × CH_3), 50.5 (CH_2), 53.0 (CH_3), 53.1 (CH), 80.4 (C), 112.9 (2 × CH), 117.0 (C), 124.3 (CH), 125.7 (CH), 128.4 (2 × CH), 130.5 (CH), 145.0 (2 × C), 148.2 (C), 151.1 (C), 155.3 (C), 156.2 (C), 170.6 (C); *m*/*z* (ESI) 468.2240 (MH^+. $C_{24}H_{30}N_5O_5$ requires 468.2241).

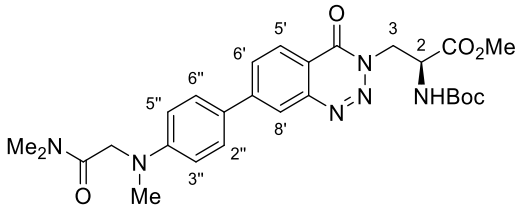
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (153)



A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.050 g, 0.11 mmol) in acetonitrile (0.15 mL) was added *N,N*-diisopropylamine (0.040 mL, 0.23 mmol). The reaction mixture was heated under reflux and a solution of 2-bromo-*N,N*-dimethylacetamide (0.012 mL, 0.11 mmol) in acetonitrile (0.1 mL) was added dropwise over 2 h. The reaction mixture was heated under reflux for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column

chromatography, eluting with 80% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**153**) (0.028 g, 47%) as a yellow solid. Mp 175–180 °C; ν_{max}/cm^{-1} (neat) 3368 (NH), 2928 (CH), 1743 (C=O), 1709 (C=O), 1655 (C=O), 1597, 1161; $[\alpha]_D^{22}$ –16.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.33 (9H, s, 3 × CH_3), 3.06 (3H, s, NCH_3), 3.07 (3H, s, NCH_3), 3.80 (3H, s, OCH_3), 3.93 (2H, br s, $CH_2CON(CH_3)_2$), 4.67–4.79 (1H, m, 3-*H*H), 4.82–4.97 (2H, m, 2-H and 3-H*H*), 5.25 (1H, br s, 4''-NH), 5.50 (1H, d, *J* 7.6 Hz, 2-NH), 6.75 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 7.60 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 7.98 (1H, dd, *J* 8.3, 1.9 Hz, 6'-H), 8.24 (1H, d, *J* 1.9 Hz, 8'-H), 8.31 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 35.9 (CH_3), 36.0 (CH_3), 44.9 (CH_2), 50.5 (CH_2), 53.0 (CH_3), 53.1 (CH), 80.3 (C), 113.4 (2 × CH), 117.0 (C), 124.4 (CH), 125.7 (CH), 127.0 (C), 128.6 (2 × CH), 130.6 (CH), 145.0 (C), 148.3 (C), 148.3 (C), 155.2 (C), 156.2 (C), 168.6 (C), 170.6 (C); *m*/*z* (ESI) 525.2465 (MH^+. $C_{26}H_{33}N_6O_6$ requires 525.2456).

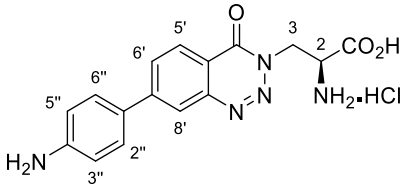
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)-*N*-methylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (154)



A solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.10 g, 0.19 mmol) in acetonitrile (2.5 mL) was degassed under argon for 0.1 h. To this was added paraformaldehyde (0.057 g, 1.9 mmol), sodium cyanoborohydride (0.0036 g, 0.57 mmol) and water (0.5 mL). Acetic acid was added dropwise until the reaction mixture reached pH 7. The reaction mixture was stirred at room temperature for 18 h. Additional paraformaldehyde (0.057 g, 1.9 mmol), sodium cyanoborohydride (0.0036 g, 0.57 mmol) and water (0.5 mL) were added and the reaction mixture was stirred for a further 18 h. The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in sodium bicarbonate (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 100% ethyl acetate gave methyl (2*S*)-2-(*tert*-

butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)-*N*-methylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (**154**) (0.070 g, 68%) as a yellow solid. Mp 160–163 °C; ν_{max}/cm^{-1} (neat) 3214 (NH), 2920 (CH), 2361, 2342, 1740 (C=O), 1678 (C=O), 1639, 1532, 1258, 1161; $[\alpha]_D^{24}$ –13.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.34 (9H, s, 3 × CH_3), 3.00 (3H, s, ArNC*H*$_3$), 3.09 (3H, s, NCH_3), 3.14 (3H, s, NCH_3), 3.80 (3H, s, OCH_3), 4.23 (2H, s, C*H*$_2$CON$(CH_3)_2$), 4.68–4.78 (1H, m, 3-*H*H), 4.84–4.97 (2H, m, 2-H and 3-H*H*), 5.48 (1H, d, *J* 7.5 Hz, 2-NH), 6.80 (2H, d, *J* 8.9 Hz, 3''-H and 5''-H), 7.62 (2H, d, *J* 8.9 Hz, 2''-H and 6''-H), 7.97 (1H, dd, *J* 8.3, 1.8 Hz, 6'-H), 8.24 (1H, d, *J* 1.8 Hz, 8'-H), 8.31 (1H, d, *J* 8.3 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 35.9 (CH_3), 36.5 (CH_3), 39.8 (CH_3), 50.5 (CH_2), 53.0 (CH_3), 53.1 (CH), 54.0 (CH_2), 80.4 (C), 112.8 (2 × CH), 117.0 (C), 124.4 (CH), 125.7 (CH), 126.4 (C), 128.5 (2 × CH), 130.6 (CH), 145.0 (C), 148.3 (C), 150.4 (C), 155.2 (C), 156.2 (C), 168.8 (C), 170.6 (C); *m*/*z* (ESI) 561.2413 (MNa^+. $C_{27}H_{34}N_6NaO_6$ requires 561.2432).

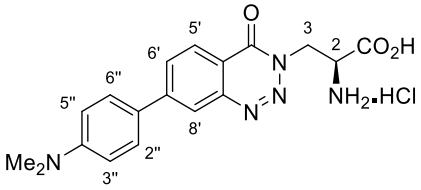
(2*S*)-2-Amino-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (155)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.060 g, 0.14 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.058 g, 0.18 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (5 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**155**) (0.037 g, 64%) as a yellow solid. A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-aminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.018 g, 0.042 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[7'-(4''-aminophenyl)-1',2',3'-

benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (0.014 g, 93%) as an orange solid. Mp 233–238 °C; ν_{max}/cm^{-1} (neat) 3410 (NH), 2924 (CH), 2542, 1744 (C=O), 1682 (C=O), 1613, 1400; $[\alpha]_D^{22}$ +19.3 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 4.63 (1H, dd, *J* 7.4, 4.5 Hz, 2-H), 4.94 (1H, dd, *J* 14.6, 7.4 Hz, 3-*H*H), 5.11 (1H, dd, *J* 14.6, 4.5 Hz, 3-H*H*), 7.59–7.64 (2H, m, 3''-H and 5''-H), 8.00–8.05 (2H, m, 2''-H and 6''-H), 8.25 (1H, dd, *J* 8.3, 1.8 Hz, 6'-H), 8.44 (1H, d, *J* 8.3 Hz, 5'-H), 8.48 (1H, d, *J* 1.8 Hz, 8'-H); δ_C (101 MHz, CD_3OD) 49.8 (CH_2), 53.3 (CH), 120.2 (C), 125.3 (2 × CH), 127.0 (CH), 127.6 (CH), 130.5 (2 × CH), 132.8 (C), 133.0 (CH), 140.7 (C), 146.0 (C), 147.9 (C), 157.6 (C), 169.2 (C); *m*/*z* (ESI) 326.1252 ($[(MH)–HCl]^+$. $C_{16}H_{16}N_5O_3$ requires 326.1248).

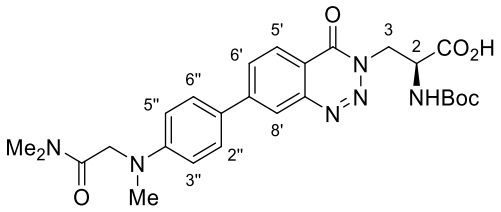
(2*S*)-2-Amino-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (156)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.059 g, 0.13 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.058 g, 0.18 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (5 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 10 mL) and ethyl acetate (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.050 g, 88%) as a yellow solid. A solution of (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (0.024 g, 0.053 mmol) in 2 M aqueous hydrochloric acid (2 mL) was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[7'-(4''-dimethylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**156**) (0.023 g, 100%) as a white solid. Mp 196–201 °C; ν_{max}/cm^{-1} (neat) 3376 (NH), 2886 (CH), 2585, 1744 (C=O), 1678 (C=O), 1613, 1466; $[\alpha]_D^{21}$ –10.7 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.36 (6H, s, 2 × $N(CH_3)_2$), 4.62 (1H, dd, *J* 7.3, 4.4 Hz, 2-H), 4.93 (1H, dd, *J* 14.7, 7.3 Hz, 3-*H*H), 5.10

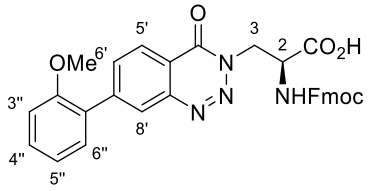
(1H, dd, *J* 14.7, 4.4 Hz, 3-H*H*), 7.89 (2H, d, *J* 8.7 Hz, 3''-H and 5''-H), 8.07 (2H, d, *J* 8.7 Hz, 2''-H and 6''-H), 8.24 (1H, dd, *J* 8.2, 1.4 Hz, 6'-H), 8.43 (1H, d, *J* 8.2 Hz, 5'-H), 8.48 (1H, d, *J* 1.4 Hz, 8'-H); δ_C (101 MHz, CD_3OD) 47.3 (2 × CH_3), 49.8 (CH_2), 53.3 (CH), 120.3 (C), 122.8 (2 × CH), 127.0 (CH), 127.7 (CH), 130.9 (2 × CH), 133.0 (CH), 141.7 (C), 144.6 (C), 146.0 (C), 147.5 (C), 157.6 (C), 169.2 (C); *m*/*z* (ESI) 354.1558 ([(MH)–HCl]$^+$. $C_{18}H_{20}N_5O_3$ requires 354.1561).

(2*S*)-2-(*tert*-Butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)-*N*-methylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (158)



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)-*N*-methylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoate (0.020 g, 0.044 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.019 g, 0.057 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (5 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[7'-(4''-(*N,N*-dimethylacetamide)-*N*-aminophenyl)methylaminophenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (**158**) (0.020 g, 100%) as a yellow solid. Mp 200–203 °C; ν_{max}/cm^{-1} (neat) 3328 (NH), 2922 (CH), 1663 (C=O), 1596, 1159; $[\alpha]_D^{24}$ –22.0 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.29 (9H, s, 3 × CH_3), 3.03 (3H, s, ArNC*H*$_3$), 3.11 (3H, s, NCH_3), 3.13 (3H, s, NCH_3), 4.24 (2H, s, C*H*$_2$CON(CH_3)$_2$), 4.49–4.59 (1H, m, 2-H), 4.65–4.75 (1H, m, 3-*H*H), 4.77–4.85 (1H, m, 3-H*H*), 5.51 (1H, d, *J* 7.7 Hz, 2-NH), 6.78 (2H, d, *J* 8.5 Hz, 3''-H and 5''-H), 7.63 (2H, d, *J* 8.5 Hz, 2''-H and 6''-H), 7.93 (1H, dd, *J* 8.4, 1.8 Hz, 6'-H), 8.17 (1H, d, *J* 1.8 Hz, 8'-H), 8.20 (1H, d, *J* 8.4 Hz, 5'-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 36.2 (CH_3), 36.6 (CH_3), 39.8 (CH_3), 50.6 (CH_2), 53.0 (CH), 54.0 (CH_2), 80.5 (C), 112.9 (2 × CH), 116.8 (C), 124.0 (CH), 125.6 (CH), 126.3 (C), 128.5 (2 × CH), 130.4 (CH), 144.8 (C), 147.9 (C), 150.3 (C), 155.7 (C), 156.2 (C), 169.5 (C), 171.7 (C); *m*/*z* (ESI) 525.2455 (MH$^+$. $C_{26}H_{33}N_6O_6$ requires 525.2456).

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid (160)



To a stirred solution of (2*S*)-2-amino-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (0.156 g, 0.414 mmol) in dioxane (1.5 mL) and water (1.5 mL) was added sodium hydrogen carbonate (0.139 g, 1.66 mmol) and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (0.137 g, 0.406 mmol). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo.* The reaction mixture was diluted in water (10 mL) and acidified to pH 2 using 1 M aqueous hydrochloric acid and extracted with diethyl ether (2 × 20 mL) and ethyl acetate (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography eluting with acetone followed by 10% methanol in acetone gave (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid hydrochloride (**160**) (0.173 g, 76%) as a white solid. Mp 181–185 °C; ν_{max}/cm^{-1} (neat) 3314 (NH), 2160, 2029, 1728 (C=O), 1670 (C=O), 1601, 1404, 1242, 1022, 741; $[\alpha]_D^{18}$ –35.9 (*c* 0.1, DMSO); δ_H (400 MHz, DMSO-d_6) 3.76 (3H, s, 2''-OCH_3), 3.85 (1H, dd, *J* 10.1, 6.7 Hz, 3-*H*H), 3.95–4.02 (1H, m, OCH_2CH), 4.07 (1H, dd, *J* 10.1, 6.7 Hz, 3-H*H*), 4.27–4.39 (2H, m, OCH_2CH), 4.91–5.04 (1H, m, 2-H), 6.76 (1H, br s, NH), 7.08 (1H, t, *J* 7.5 Hz, 5''-H), 7.18 (1H, d, *J* 8.3 Hz, 3''-H), 7.22 (1H, t, *J* 7.5 Hz, 4''-H), 7.26–7.43 (4H, m, 4 × ArH), 7.47 (2H, t, *J* 7.6 Hz, 2 × ArH) 7.60 (1H, d, *J* 7.5 Hz, 6''-H), 7.85 (2H, d, *J* 7.6 Hz, 2 × ArH), 7.94 (1H, dd, *J* 8.2, 1.7 Hz, 6'-H), 8.10 (1H, br s, 8'-H), 8.20 (1H, d, *J* 8.2 Hz, 5'-H); *m*/*z* (ESI) 563.1918 (MH^+. $C_{32}H_{27}N_4O_6$ requires 563.1925).

Cell Permeability Fluorescence Assay

HEK293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose with GlutaMAX, Gibco) supplemented with 10% (v/v) foetal bovine serum (Gibco) and 1% (v/v) penicillin/streptomycin (10,000 units/mL penicillin, 10,000 µg/mL streptomycin, Gibco). Cultured cells were maintained in a humidified incubator at 37 °C with 5% CO_2 and passaged twice weekly in Corning T-25 flasks. For cell counting, an aliquot (10 µL) of cell solution in media was added to a haemocytometer slide which was viewed using a microscope for manual inspection

and counting. For experiments, approximately 300,000 cells were seeded into CytoOne 6-well plates on 30 mm cover glass slides pre-treated with 0.1 mg/mL poly-D-lysine and left to grow for two days. This resulted in *ca*. 80% confluency before compound incubation. The media was removed, and the cells were washed with PBS prior to treatment with compound in PBS (2 h, 20 μM, 37 °C). Cells were then washed with PBS twice prior to fixing with a 4% (w/v) solution of formaldehyde in PBS (10 min, 37 °C). Cells were washed twice prior to analysis.

Fluorescence Imaging: Images were acquired on a MetaMorph/Metafluor fluorescence imaging microscope system equipped with a 40 × Superfluor objective. Image analysis and processing was performed using Fiji ImageJ.

Peptide Synthesis: General Information

All reagents were purchased from commercial sources and used without further purification unless otherwise stated. Fmoc protected amino acids were purchased from CEM Corporation and Pepceuticals. *N*,*N*-Dimethylformamide (DMF) and diethyl ether were purchased from Rathburn. Trifluoracetic acid (TFA), *N*,*N*'-diisopropylethylamine (DIPEA), *N*,*N*'-diisopropylcarbodiimide (DIC), ethyl(hydroxy)cyanoacetate (Oxyma Pure), fluorescein-5-isothiocyanate (FITC) and Fmoc-6-Ahx-OH were purchased from Fluorochem. Morpholine was purchased from Alfa Aesar. Dichloromethane was purchased from VWR. Acetonitrile (MeCN) was purchased from Fisher Scientific. ChemMatrix© Rink Amide resin was purchased from Biotage. All other reagents were purchased from Sigma Aldrich.

Synthesis of FITC labelled peptide: Peptide **159** was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using an Fmoc/*t*Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix© resin (0.45 mmol/g). Microwave-assisted SPPS was used for all coupling reactions. Following resin swelling in DMF at 70 °C for 20 min, the resin bound peptide was synthesised by loading Fmoc-Arg(Pbf)-OH to the resin and introducing the Fmoc-protected amino acids (5 equiv., 0.2 M in DMF) successively by treatment with DIC (5 equiv., 0.5 M in DMF) and Oxyma Pure (5 equiv., 0.5 M in DMF) at 90 °C for 2 min. Arginine residues were coupled at room temperature for 45 min, followed by 90 °C for 5 min, then double coupled at 90 °C for 10 min. Fmoc-6-A_{hx}-OH(A_{hx}) (2 equiv., 0.1 M in DMF) was coupled as a spacer between the *N*-terminal amino acid and the FITC. Fmoc groups were removed using morpholine (20% in DMF with 5% formic

acid, 4 mL) at 90 °C for 1 min. The resin was washed with DMF between deprotecti on and coupling (4 × 4 mL), and after coupling (2 × 4 mL). The Fmoc-deprotected resin bound peptide was suspended in DMF (2 mL) followed by addition of FITC (2 equiv.), DIPEA (8 equiv.) and DMF (4 mL for 0.1 mmol of resin). The reaction mixture was agitated at room temperature and excluding light for 16 h. The solution was filtered, and the resin was washed with DMF (2 × 5 mL) and dichloromethane (2 × 5 mL). Peptide cleavage was carried out using a cleavage cocktail (10 mL) of trifluoroacetic acid (95%), triisopropylsilane (2.5%) and water (2.5%). The reaction mixture was agitated at room temperature for 4 h. The resin was filtered, and the cleavage cocktail evaporated using a stream of nitrogen. Peptide **159** was precipitated from a solution of cold diethyl ether, centrifuged (4500 rpm for 5 min) and washed with ice-cold diethyl ether. Peptide **159** was dissolved in a mixture of H_2O and MeCN with 0.1% TFA and lyophilised on a Christ Alpha 1–2 LD plus freeze dryer.

Synthesis of benzotriazinone labelled peptide: Peptide **161** was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using an Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix© resin (0.45 mmol/g). Microwave-assisted SPPS was used for all coupling reactions. Following resin swelling in DMF at 70 °C for 20 min, the resin bound peptide was synthesised by first loading Fmoc-Arg(Pbf)-OH to the resin and by introducing the Fmoc-protected amino acids (5 equiv., 0.2 M in DMF) successively by treatment with DIC (5 equiv., 0.5 M in DMF) and Oxyma Pure (5 equiv., 0.5 M in DMF) at 90 °C for 2 min. Arg residues were coupled at room temperature for 45 min, followed by 90 °C for 5 min, then double coupled at 90 °C for 10 min. Fmoc groups were removed using morpholine (20% in DMF with 5% formic acid, 4 mL) at 90 °C for 1 min. The resin was washed with DMF between deprotection and coupling (4 × 4 mL), and after coupling (2 × 4 mL). The Fmoc-deprotected resin bound peptide was suspended in DMF (2 mL) followed by addition of unnatural amino acid **160** (2 equiv., 0.2 M in DMF), DIC (2 equiv., 0.5 M in DMF) and Oxyma Pure (2 equiv., 0.5 M in DMF). The reaction mixture was agitated at 75 °C for 10 min. The solution was filtered, and the resin was washed with DMF (2 × 5 mL) and dichloromethane (2 × 5 mL). *N*-Terminal acetylation was achieved on-resin with acetic anhydride (3 equiv.), DIPEA (4.5 equiv.) and DMF (7 mL for 0.1 mmol of resin) for 20 min with agitation. The resin was then washed with DMF (3 × 5 mL) and dichloromethane (3 × 5 mL). Peptide cleavage was carried out using a cleavage

cocktail (10 mL) of trifluoroacetic acid (95%), triisopropylsilane (2.5%) and water (2.5%). The reaction mixture was agitated at room temperature for 4 h. The resin was filtered, and the cleavage cocktail evaporated using a stream of nitrogen. Peptide **161** was precipitated from a solution of cold Et_2O, centrifuged (4500 rpm for 5 min) and washed with ice-cold diethyl ether. Peptide **161** was dissolved in a mixture of H_2O and MeCN with 0.1% TFA and lyophilised on a Christ Alpha 1-2 LD plus freeze dryer.

Synthesis of benzotriazinone labelled peptide with A_{hx} linker: Peptide **162** was synthesised on a Biotage Initiator+ Alstra peptide synthesiser using an Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix© resin (0.45 mmol/g). Microwave-assisted SPPS was used for all coupling reactions. Following resin swelling in DMF at 70 °C for 20 min, the resin bound peptide was synthesised by first loading Fmoc-Arg(Pbf)-OH to the resin and by introducing the Fmoc-protected amino acids (5 equiv., 0.2 M in DMF) successively by treatment with DIC (5 equiv., 0.5 M in DMF) and Oxyma Pure (5 equiv., 0.5 M in DMF) at 90 °C for 2 min. Arg residues were coupled at room temperature for 45 min, followed by 90 °C for 5 min, then double coupled at 90 °C for 10 min. Fmoc-6-A_{hx}-OH(A_{hx}) (2 equiv., 0.1 M in DMF) was coupled as a spacer between the *N*-terminal amino acid and the novel amino acid. Fmoc groups were removed using morpholine (20% in DMF with 5% formic acid, 4 mL) at 90 °C for 1 min. The resin was washed with DMF between deprotection and coupling (4 × 4 mL), and after coupling (2 × 4 mL). The Fmoc-deprotected resin bound peptide was suspended in DMF (2 mL) followed by addition of unnatural amino acid **160** (2 equiv., 0.2 M in DMF), DIC (2 equiv., 0.5 M in DMF) and Oxyma Pure (2 equiv., 0.5 M in DMF). The reaction mixture was agitated at 75 °C for 10 min. The solution was filtered, and the resin was washed with DMF (2 × 5 mL) and dichloromethane (2 × 5 mL). *N*-terminal acetylation was achieved on-resin with acetic anhydride (3 equiv.), DIPEA (4.5 equiv.) and DMF (7 mL for 0.1 mmol of resin) for 20 min with agitation. The resin was then washed with DMF (3 × 5 mL) and dichloromethane (3 × 5 mL). Peptide cleavage was carried out using a cleavage cocktail (10 mL) of trifluoroacetic acid (95%) triisopropylsilane (2.5%) and water (2.5%). The reaction mixture was agitated at room temperature for 4 h. The resin was filtered, and the cleavage cocktail evaporated using a stream of nitrogen. Peptide **162** was precipitated from a solution of cold Et_2O, centrifuged (4500 rpm for 5 min) and washed with ice-cold diethyl ether. Peptide **162** was dissolved in a

mixture of H_2O and MeCN with 0.1% TFA and lyophilised on a Christ Alpha 1-2 LD plus freeze dryer.

Purification: Peptide **159** was purified on a reverse-phase Biotage Isolera One purification system equipped with UV-Vis detector (monitoring at 214 nm and 280 nm), using a prepacked Biotage Sfär Bio C18–Duo 300 Å cartridge. A gradient was run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA), and collected fractions were lyophilised on a Christ Alpha 1-2 LD plus freeze dryer. Peptide **159** was analysed on a Shimadzu RP-HPLC system with Shimadzu LC-20AT pumps, a Shimadzu SIL20A autosampler and a Shimadzu SPD-20A UV-vis detector using a Phenomenex Aeris column (5 mm C18, 100 Å, 150 × 10 mm). An RP-HPLC gradient was run using a solvent system consisting of solution A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN). Two gradients were used to characterise peptide **159**, a gradient from 5–95% solution B over 50 minutes (Appendix Figure A1) and a gradient from 5–95% solution B over 20 minutes (Appendix Figure A2). Analytical RP-HPLC data is reported as column retention time (t_R) in minutes (Table A1). High-resolution mass spectrometry (HRMS) was performed on an Agilent 6546 LC/Q-TOF in positive mode (ESI+). HRMS data are reported as mass to charge ratio (*m/z*) = observed / MW (Table 15, Appendix Figure A3).

Peptides **161** and **162** were purified on a RP-HPLC using either an Agilent Technologies 1260 Infinity RP-HPLC system (monitoring at 214 nm and 280 nm) with a Phenomenex Gemini column (5 mm C18, 250 × 21.2 mm). A gradient was run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA), and collected fractions were lyophilised on a Christ Alpha 1-2 LD plus freeze dryer. Peptides **161** and **162** were analysed on a Shimadzu RP-HPLC system with Shimadzu LC-20AT pumps, a Shimadzu SIL20A autosampler and a Shimadzu SPD-20A UV-vis detector using a Phenomenex Aeris column (5 mm C18, 100 Å, 150 × 10 mm). An RP-HPLC gradient was run using a solvent system consisting of solution A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN). Two gradients were used to characterise peptides **161** and **162**, a gradient from 5–95% solution B over 50 minutes (Appendix Figures A4 and A7) and a gradient from 5–95% solution B over 20 minutes (Appendix Figures A5 and A8). Analytical RP-HPLC data is reported as column retention time (t_R) in minutes (Table A1). High-resolution mass spectrometry (HRMS) was performed on an Agilent 6546 LC/Q-TOF in positive mode (ESI+).

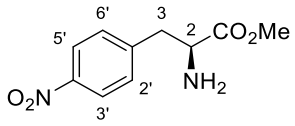
HRMS data are reported as mass to charge ratio (*m/z*) = observed / MW (Table 15, Appendix Figures A6 and A9).

Peptide Sequence	Purity by RP-HPLC (%)	Yield (%)	t_R (20 min, 50 min gradient)	Calculated *m/z*	Measured *m/z*	Error (ppm)
159 *- FITC-Ahx-YGRKKRRQRRR-NH_2*	98%	6	12.188, 21.962	$[M - 2H]^{-2}$ = 1029.0359	$[M - 2H]^{-2}$ = 1029.0348	–1.06
161 *- Ac-X-YGRKKRRQRRR-NH_2*	>99%	3	12.043, 21.646	$[M + 3H]^{+3}$ = 641.7018	$[M + 3H]^{+3}$ = 641.7013	–0.89
162 *- Ac-X- Ahx-YGRKKRRQRRR-NH_2*	>99%	20	12.549, 22.748	$[M + K + NH_4]^{+2}$ = 1046.5890	$[M + K + NH_4]^{+2}$ = 1046.5837	5.07

Table 15: Peptide sequence, % purity, % yield, *m/z* and retention time of peptides. Abbreviations: FITC = fluorescein-5-isothiocyanate, Ahx = aminohexanoic acid, NH_2 = *C*-terminal amide, Ac = acetylated and X = (2*S*)-2-amino-3-[7'-(2''-methoxyphenyl)-1',2',3'-benzotriazin-4'(3*H*)-one]propanoic acid.

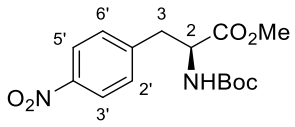
3.4 Stilbene and Biphenyl Amino Acids Experimental

Methyl (2*S*)-2-amino-3-(4'-nitrophenyl)propanoate (169)[190]



To a stirred solution of 4-nitro-L-phenylalanine (7.00 g, 33.3 mmol) in methanol (140 mL) at 0 °C was added dropwise thionyl chloride (3.40 mL, 46.6 mmol). The reaction mixture was warmed to room temperature and then heated under reflux for 3 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The reaction mixture was diluted in water (90 mL), basified to pH 8 using sodium bicarbonate and extracted with dichloromethane (3 × 90 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give methyl (2*S*)-2-amino-3-(4'-nitrophenyl)propanoate (**169**) (6.61 g, 88%) as a yellow oil. $[\alpha]_D^{20}$ +26.7 (*c* 0.1, EtOH), lit.[190] $[\alpha]_D^{24}$ +34.2 (*c* 0.1, EtOH); δ_H (400 MHz, DMSO-d_6) 2.91 (1H, dd, *J* 13.4, 7.8 Hz, 3-*H*H), 3.03 (1H dd, *J* 13.4, 5.9 Hz, 3-H*H*), 3.60 (3H, s, OCH_3), 3.67 (1H, dd, *J* 7.8, 5.9 Hz, 2-H), 7.49 (2H, d, *J* 8.7 Hz, 2'-H and 6'-H), 8.14 (2H, d, *J* 8.7 Hz, 3'-H and 5'-H); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 51.6 (CH_3), 55.2 (CH), 123.1 (2 × CH), 130.6 (2 × CH), 146.2 (C), 146.5 (C), 174.8 (C); *m/z* (ESI) 247 (MNa^+. 100%).

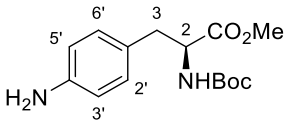
Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-nitrophenyl)propanoate (170)[191]



To a stirred solution of methyl (2*S*)-2-amino-3-(4'-nitrophenyl)propanoate (4.55 g, 20.3 mmol) in methanol (25 mL) at 0 °C was added triethylamine (8.45 mL, 60.9 mmol) and di-*tert*-butyl dicarbonate (4.88 g, 22.3 mmol). The reaction mixture was stirred at 0 °C for 0.3 h, warmed to room temperature and stirred for 3 h. The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-nitrophenyl)propanoate (**170**) (5.56 g, 84%) as a white solid. Mp 97–101 °C (lit.[191] 100–101 °C); $[\alpha]_D^{20}$ +56.2 (*c* 0.1, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 3.11 (1H, dd, *J* 13.7, 6.3 Hz, 3-*H*H), 3.27 (1H, dd, *J* 13.7, 5.7 Hz, 3-H*H*), 3.73 (3H, s, OCH_3), 4.58–4.67 (1H, m, 2-H), 5.06 (1H, d, *J* 7.4 Hz, 2-NH), 7.31 (2H, d, *J* 8.7 Hz, 2'-H and 6'-H), 8.12–8.19 (2H, m, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 38.5 (CH_2), 52.7 (CH_3), 54.2 (CH), 80.5 (C),

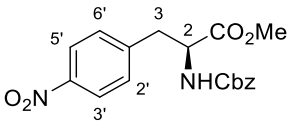
123.8 (2 × CH), 130.4 (2 × CH), 144.2 (C), 147.3 (C), 155.0 (C), 171.8 (C); *m/z* (ESI) 347 (MNa^+. 100%).

Methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-aminophenyl)propanoate (171)[192]



To a stirred solution of methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-nitrophenyl)propanoate (3.24 g, 10.0 mmol) in methanol (100 mL) was added zinc powder (6.53 g, 100 mmol) and acetic acid (5.72 mL, 100 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite®, washed with methanol (150 mL) and concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (150 mL) and was washed with water (5 × 150 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-(4'-aminophenyl)propanoate (**171**) (2.92 g, 99%) as a yellow solid. Mp 85–90 °C (lit.[192] 84–85 °C); $[\alpha]_D^{21}$ +11.9 (*c* 0.1, MeOH); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 2.90–3.03 (2H, m, 3-H_2), 3.61 (2H, br s, 4'-NH_2), 3.71 (3H, s, OCH_3), 4.46–4.54 (1H, m, 2-H), 4.93 (1H, d, *J* 8.3 Hz, 2-NH), 6.59–6.64 (2H, m, 3'-H and 5'-H), 6.87–6.93 (2H, m, 2'-H and 6'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.6 (CH_2), 52.3 (CH_3), 54.7 (CH), 79.9 (C), 115.4 (2 × CH), 125.8 (C), 130.3 (2 × CH), 145.4 (C), 155.3 (C), 172.7 (C); *m/z* (ESI) 295 (MH^+. 100%).

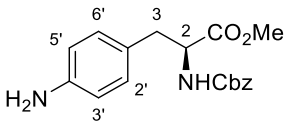
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-nitrophenyl)propanoate (173)[193]



To a stirred solution of methyl (2*S*)-2-amino-3-(4'-nitrophenyl)propanoate (4.19 g, 18.9 mmol) in water (50 mL) at 0 °C was added sodium bicarbonate (3.92 g, 46.7 mmol). A solution of benzyl chloroformate (3.16 mL, 22.4 mmol) in toluene (10 mL) was then added dropwise. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was diluted in water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and

washed with 1 M aqueous hydrochloric acid (200 mL), sodium bicarbonate (200 mL), water (200 mL) and brine (200 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-nitrophenyl)propanoate (**173**) (6.54 g, 98%) as a colourless oil. Spectroscopic data were consistent with the literature.[193] $[\alpha]_D^{21}$ +29.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.14 (1H, dd, *J* 13.8, 6.3 Hz, 3-*H*H), 3.30 (1H, dd, *J* 13.8, 5.7 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.66–4.75 (1H, m, 2-H), 5.06 (1H, d, *J* 12.1 Hz, C*H*HPh), 5.12 (1H, d, *J* 12.1 Hz, CH*H*Ph), 5.29 (1H, d, *J* 8.1 Hz, 2-NH), 7.20–7.40 (7H, m, 2'-H, 6'-H and Ph), 8.11 (2H, d, *J* 8.5 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 38.4 (CH_2), 52.8 (CH_3), 54.6 (CH), 67.3 (CH_2), 123.9 (2 × CH), 128.4 (2 × CH), 128.5 (CH), 128.7 (2 × CH), 130.3 (2 × CH), 136.1 (C), 143.8 (C), 147.3 (C), 155.6 (C), 171.4 (C); *m/z* (ESI) 381 (MNa^+. 100%).

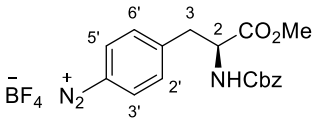
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-aminophenyl)propanoate (174)[193]



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-nitrophenyl)propanoate (9.13 g, 25.5 mmol) in methanol (160 mL) was added zinc powder (16.7 g, 255 mmol) and acetic acid (14.6 mL, 255 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite®, washed with methanol (100 mL) and concentrated *in vacuo.* The reaction mixture was diluted in ethyl acetate (300 mL) and was washed with water (5 × 250 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-aminophenyl)propanoate (**174**) (7.62 g, 94%) as a colourless oil. Spectroscopic data were consistent with the literature.[193] $[\alpha]_D^{21}$ +29.3 (*c* 0.1, $CHCl_3$) δ_H (400 MHz, $CDCl_3$) 2.97–3.02 (2H, m, 3-H_2), 3.61 (2H, br s, 4'-NH_2), 3.72 (3H, s, OCH_3), 4.55–4.63 (1H, m, 2-H), 5.08 (1H, d, *J* 12.7 Hz, OC*H*HPh), 5.11 (1H, d, *J* 12.7 Hz, OCH*H*Ph), 5.18 (1H, d, *J* 8.0 Hz, 2-NH), 6.57–6.62 (2H, m, 3'-H and 5'-H), 6.84–6.89 (2H, m, 2'-H and 6'-H), 7.28–7.40 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 37.5 (CH_2), 52.4 (CH_3), 55.1 (CH), 67.1 (CH_2), 115.5 (2 × CH), 125.5 (C), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 ×

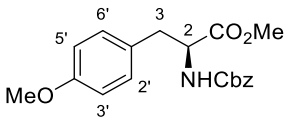
CH), 130.3 (2 × CH), 136.5 (C), 145.6 (C), 155.8 (C), 172.3 (C); *m/z* (ESI) 351 (MNa^+. 100%).

Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (176)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-aminophenyl)propanoate (0.583 g, 1.78 mmol) in 48% aqueous fluoroboroic acid (0.902 mL) and water (0.950 mL) at 0 °C was added a solution of sodium nitrite (0.174 g, 2.49 mmol) in water (0.365 mL). The reaction mixture was stirred at 0 °C for 0.3 h which resulted in formation of a red precipitate. This was filtered and washed with cold water (5 mL). Purification by recrystallisation from acetone and diethyl ether gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (**176**) (0.690 g, 91%) as a red solid. This was used immediately for subsequent reactions.

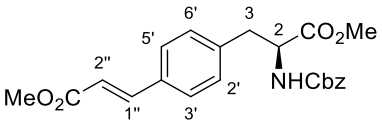
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-methoxyphenyl)propanoate (177)[194]



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.144 g, 0.337 mmol) was dissolved in methanol (1.5 mL). To this was added phenylboronic acid (0.0240 g, 0.198 mmol) and 10% palladium on carbon (0.0110 g, 0.0130 mmol, 5 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-methoxyphenyl)propanoate (**177**) (0.0990 g, 85%) as a white oil. Spectroscopic data were consistent with the literature.[194] $[\alpha]_D^{17}$ +24.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.98–3.12 (2H, m, 3-H_2), 3.72 (3H, s, OCH_3), 3.78 (3H, s, 4'-OCH_3), 4.58–4.67 (1H, m, 2-H), 5.04–5.15 (2H, m, CH_2Ph), 5.19 (1H, d, *J* 8.4 Hz, 2-NH), 6.77–6.84 (2H, m, 3'-H and 5'-H), 6.97–7.03 (2H, m, 2'-H and 6'-H), 7.27–7.40 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 37.5 (CH_2), 52.5 (CH_3), 55.1 (CH), 55.4 (CH_3), 67.1 (CH_2),

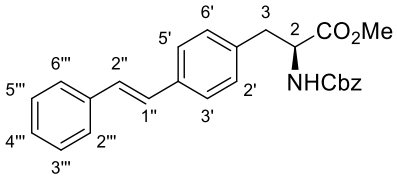
114.2 (2 × CH), 127.7 (C), 128.2 (CH), 128.3 (2 × CH), 128.7 (2 × CH), 130.4 (2 × CH), 136.4 (C), 155.8 (C), 158.9 (C), 172.2 (C); *m/z* (ESI) 366 (MNa^+. 100%).

Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-methylcinnamate)phenyl]propanoate (178a)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.31 g, 0.73 mmol) was dissolved in methanol (1.5 mL). To this was added methyl acrylate (0.091 mL, 1.5 mmol) and palladium acetate (0.0020 g, 0.0089 mmol, 1 mol%). The reaction mixture was heated to 50 °C and stirred for 0.5 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-methylcinnamate)phenyl]propanoate (**178a**) (0.21 g, 71%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3352 (NH), 2951 (CH), 1697 (C=O), 1528, 1435, 1254, 1207, 1169; $[\alpha]_D^{19}$ +60.1 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.08 (1H, dd, *J* 13.9, 6.2 Hz, 3-*H*H), 3.18 (1H, dd, *J* 13.9, 5.6 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.63–4.71 (1H, m, 2-H), 5.07 (1H, d, *J* 12.4 Hz, OC*H*HPh), 5.11 (1H, d, *J* 12.4 Hz, OCH*H*Ph), 5.27 (1H, d, *J* 8.0 Hz, 2-NH), 6.40 (1H, d, *J* 16.0 Hz, 2''-H), 7.11 (2H, d, *J* 8.1 Hz, 2'-H and 6'-H), 7.28–7.39 (5H, m, Ph), 7.42 (2H, d, *J* 8.1 Hz, 3'-H and 5'-H), 7.65 (1H, d, *J* 16.0 Hz, 1''-H); δ_C (101 MHz, $CDCl_3$) 38.2 (CH_2), 51.8 (CH_3), 52.6 (CH_3), 54.8 (CH), 67.2 (CH_2), 117.8 (CH), 128.2 (2 × CH), 128.4 (CH), 128.4 (2 × CH), 128.7 (2 × CH), 130.0 (2 × CH), 133.4 (C), 136.3 (C), 138.4 (C), 144.5 (CH), 155.7 (C), 167.5 (C), 171.9 (C); *m/z* (ESI) 398.1608 (MH^+. $C_{22}H_{24}NO_6$ requires 398.1598).

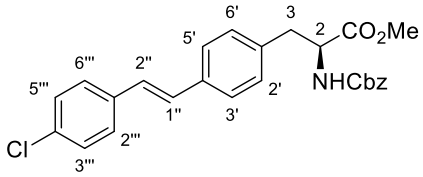
Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-(phenylethenyl)phenyl]propanoate (178b)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.18 g, 0.42 mmol) was dissolved in methanol (1.5 mL). To this

was added styrene (0.095 mL, 0.83 mmol) and palladium acetate (0.0090 g, 0.0401 mmol, 10 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% diethyl ether in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-phenylethenyl)phenyl]propanoate (**178b**) (0.12 g, 71%) as a white solid. Mp 123–128 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2955 (CH), 2920 (CH), 2025, 1717 (C=O), 1528, 1435, 1285, 1261, 1231, 1215, 1038, 756; $[\alpha]_D^{18}$ +46.4 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.09 (1H, dd, *J* 13.9, 6.0 Hz, 3-*H*H), 3.15 (1H, dd, *J* 13.9, 5.7 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.64–4.72 (1H, m, 2-H), 5.07 (1H, d, *J* 12.8 Hz, OC*H*HPh), 5.12 (1H, d, *J* 12.8 Hz, OCH*H*Ph), 5.22 (1H, d, *J* 8.3 Hz, 2-NH), 7.05–7.11 (4H, m, 2'-H, 6'-H, 1''-H and 2''-H), 7.23–7.40 (8H, m, Ph, 3'''-H, 4'''-H and 5'''-H), 7.42 (2H, d, *J* 8.1 Hz, 3'-H and 5'-H), 7.48–7.53 (2H, m, 2'''-H and 6'''-H); δ_C (101 MHz, $CDCl_3$) 38.2 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.2 (CH_2), 126.7 (2 × CH), 126.9 (2 × CH), 127.8 (CH), 128.3 (CH), 128.4 (2 × CH), 128.4 (C), 128.7 (2 × CH), 128.8 (2 × CH), 128.9 (2 × CH), 129.8 (2 × CH), 135.3 (C), 136.5 (C), 137.5 (C), 155.8 (C), 172.1 (C); *m/z* (ESI) 416.1843 (MH^+. $C_{26}H_{26}NO_4$ requires 416.1856).

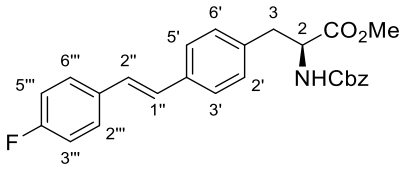
Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(4'''-chlorophenylethenyl)phenyl]propanoate (178d)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.212 g, 0.496 mmol) was dissolved in methanol (1.5 mL). To this was added 4-chlorostyrene (0.119 mL, 0.993 mmol) and palladium acetate (0.0110 g, 0.450 mmol, 10 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% diethyl ether in hexane followed by 100% diethyl ether gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(4'''-chlorophenylethenyl)phenyl]propanoate (**178d**) (0.179 g, 80%) as a white solid. Mp 110–116 °C; ν_{max}/cm^{-1} (neat) 3369 (NH), 2957 (CH), 1721 (C=O), 1707 (C=O), 1523, 1226, 1038, 821; $[\alpha]_D^{25}$ +61.9 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.08 (1H, dd, *J* 13.9, 6.1 Hz, 3-*H*H), 3.16 (1H, dd, *J* 13.9, 5.7 Hz 3-H*H*), 3.73 (3H, s, OCH_3), 4.64–4.72 (1H, m, 2-H), 5.09 (1H, d, *J* 12.0 Hz,

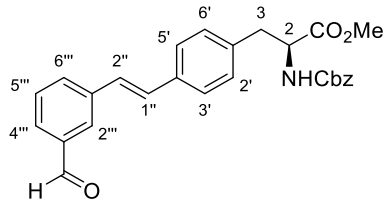
OC*H*HPh), 5.13 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 5.22 (1H, d, *J* 8.3 Hz, 2-NH), 7.00–7.04 (2H, m, 1''-H and 2''-H), 7.06–7.12 (2H, m, 2'-H and 6'-H), 7.29–7.46 (11H, m, Ph, 3'-H, 5'-H, 2'''-H, 3'''-H, 5'''-H and 6'''-H); δ_C (101 MHz, $CDCl_3$) 38.2 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.2 (CH_2), 126.9 (2 × CH), 127.5 (CH), 127.8 (2 × CH), 128.3 (CH), 128.4 (2 × CH), 128.7 (2 × CH), 129.0 (CH), 129.0 (2 × CH), 129.8 (2 × CH), 133.4 (C), 135.6 (C), 135.9 (C), 136.1 (C), 136.4 (C), 155.7 (C), 172.0 (C); *m/z* (ESI) 472.1289 (MNa^+. $C_{26}H_{24}{}^{35}ClNNaO_4$ requires 472.1286).

Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(4'''-fluorophenylethenyl)phenyl]propanoate (178e)



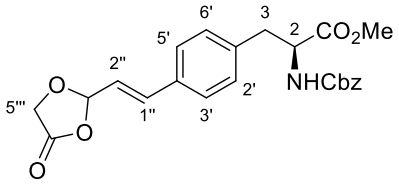
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.23 g, 0.53 mmol) was dissolved in methanol (1.5 mL). To this was added 4-fluorostyrene (0.13 mL, 1.05 mmol) and palladium acetate (0.0060 g, 0.027 mmol, 5 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% diethyl ether in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(4'''-fluorophenylethenyl)phenyl]propanoate (**178e**) (0.15 g, 66%) as a white solid. Mp 100–105 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 2955 (CH), 1705 (C=O), 1512, 1227, 1038, 83; $[\alpha]_D^{20}$ +5.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.08 (1H, dd, *J* 14.0, 6.1 Hz, 3-*H*H), 3.15 (1H, dd, *J* 14.0, 5.7 Hz, 3-H*H*), 3.73 (3H, s, OCH_3), 4.63–4.72 (1H, m, 2-H), 5.09 (1H, d, *J* 12.0 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 5.23 (1H, d, *J* 8.3 Hz, 2-NH), 6.93–7.12 (6H, m, 2'-H, 6'-H, 1''-H, 2''-H, 2'''-H and 6'''-H), 7.28–7.50 (9H, m, Ph and 3'-H, 5'-H, 3'''-H and 5'''-H); δ_C (101 MHz, $CDCl_3$) 38.2 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.2 (CH_2), 115.8 (2 × CH, $^2J_{CF}$ 21.8 Hz), 126.8 (2 × CH), 127.6 (CH), 128.1 (2 × CH, $^3J_{CF}$ 8.1 Hz), 128.1 (CH), 128.2 (2 × CH), 128.3 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 129.8 (2 × CH), 133.6 (C, $^4J_{CF}$ 3.6 Hz), 135.3 (C), 136.3 (C), 136.4 (C), 155.8 (C), 162.5 (C, $^1J_{CF}$ 247.1 Hz), 172.1 (C); *m/z* (ESI) 456.1580 (MNa^+. $C_{26}H_{24}FNNaO_4$ requires 456.1582).

Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(3'''-formylphenylethenyl)phenyl]propanoate (178f)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.204 g, 0.478 mmol) was dissolved in methanol (1.5 mL). To this was added 3-vinylbenzaldehyde (0.121 mL, 0.955 mmol) and palladium acetate (0.0110 g, 0.450 mmol, 10 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 40% diethyl ether in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(3'''-formylphenylethenyl)phenyl]propanoate (**178f**) (0.105 g, 50%) as a white solid. Mp 110–114 °C; ν_{max}/cm^{-1} (neat) 3322 (NH), 2955 (CH), 1736 (C=O), 1694 (C=O), 1528, 1296, 1250, 1022, 733; $[\alpha]_D^{20}$ +4.2 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.09 (1H, dd, *J* 13.9, 6.1 Hz, 3-*H*H), 3.17 (1H, dd, *J* 13.9, 5.8 Hz, 3-H*H*), 3.74 (3H, s, OCH_3), 4.64–4.73 (1H, m, 2-H), 5.09 (1H, d, *J* 12.0 Hz, OC*H*HPh), 5.13 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 5.24 (1H, d, *J* 8.2 Hz, 2-NH), 7.08–7.15 (3H, m, 2'-H, 6'-H and 2''-H), 7.18 (1H, d, *J* 16.0 Hz, 1''-H), 7.29–7.39 (5H, m, Ph), 7.44 (2H, d, *J* 8.1 Hz, 3'-H and 5'-H), 7.53 (1H, t, *J* 7.6 Hz, 5'''-H), 7.72–7.80 (2H, m, 4'''-H and 6'''-H), 8.02 (1H, s, 2'''-H), 10.06 (1H, s, 3'''-CHO*)*; δ_C (101 MHz, $CDCl_3$) 38.1 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.1 (CH_2), 127.0 (2 × CH), 127.1 (CH), 127.2 (CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 129.0 (CH), 129.5 (CH), 129.8 (2 × CH), 130.1 (CH), 132.4 (CH), 135.7 (C), 135.9 (C), 136.3 (C), 136.9 (C), 138.4 (C), 155.7 (C), 172.0 (C), 192.4 (CH); *m/z* (ESI) 466.1626 (MNa^+. $C_{27}H_{25}NNaO_5$ requires 466.1625).

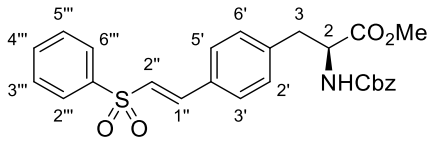
Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(2''',4'''-dioxolan-1'''-onevinyl)phenyl]propanoate (178g)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.17 g, 0.39 mmol) was dissolved in methanol (1.5 mL). To this was added 2-vinyl-1,3-dioxolan-4-one (0.075 mL, 0.78 mmol) and palladium acetate

(0.0090 g, 0.040 mmol, 10 mol%). The reaction mixture was heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 70% diethyl ether in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(2''',4'''-dioxolan-1'''-onevinyl)phenyl]propanoate (**178g**) (0.029 g, 17%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3329 (NH), 2924 (CH), 1721 (C=O), 1674 (C=O), 1516, 1215, 1126, 772; $[\alpha]_D^{23}$ +35.7 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.10 (1H, dd, *J* 13.8, 6.1 Hz, 3-*H*H), 3.21 (1H, dd, *J* 13.8, 5.7 Hz, 3-H*H*), 3.58–3.82 (4H, m, OCH_3 and 5'''-*H*H), 3.82–4.04 (1H, m, 5'''-H*H*), 4.64–4.73 (1H, m, 2-H), 5.05 (1H, d, *J* 12.0 Hz, OC*H*HPh), 5.12 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 5.25 (1H, d, *J* 8.2 Hz, 2-NH), 6.69 (1H, dd, *J* 15.9, 7.7 Hz, 2''-H), 7.16 (2H, d, *J* 8.1 Hz, 2'-H and 6'-H), 7.29–7.39 (7H, m, Ph and 1''-H), 7.47 (2H, d, *J* 8.1 Hz, 3'-H and 5'-H), 9.70 (1H, d, *J* 7.7 Hz, 3'''-H); δ_C (101 MHz, $CDCl_3$) 29.8 (CH_2), 38.4 (CH_2), 52.6 (CH_3), 54.8 (CH), 65.0 (CH), 67.2 (CH_2), 128.3 (2 × CH), 128.4 (CH), 128.7 (CH), 128.7 (2 × CH and C), 128.9 (2 × CH), 130.2 (2 × CH), 133.1 (C), 136.3 (C), 139.6 (C), 152.4 (CH), 155.7 (C), 171.8 (C), 193.8 (CH); *m/z* (ESI) 426.1566 (MH^+. $C_{23}H_{24}NO_7$ requires 426.1547).

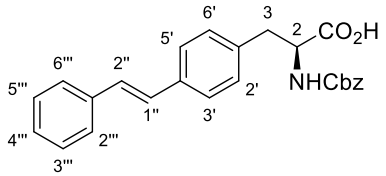
Methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(2''-phenylsulfonylvinyl)phenyl]propanoate (178h)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4'-diazophenyl)propanoate tetrafluoroborate (0.230 g, 0.539 mmol) was dissolved in methanol (1.5 mL). To this was added phenyl vinyl sulfone (0.181 g, 1.08 mmol) and palladium acetate (0.0120 g, 0.054 mmol, 10 mol%). The reaction mixture was heated to 50 °C and stirred for 5.5 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane gave methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[4'-(2''-phenylsulfonylvinyl)phenyl]propanoate (**178h**) (0.114 g, 44%) as a yellow solid. Mp 55–60 °C; ν_{max}/cm^{-1} (neat) 3314 (NH), 2585 (CH), 2180, 2025, 1717 (C=O), 1520, 1308, 1146, 752; $[\alpha]_D^{21}$ +58.4 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.06 (1H, dd, *J* 13.8, 6.2 Hz, 3-*H*H), 3.15 (1H, dd, *J* 13.8, 5.7 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 4.61–4.71 (1H, m, 2-H), 5.05 (1H, d, *J* 12.0 Hz, OC*H*HPh), 5.10 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 5.22 (1H, d, *J* 8.2 Hz, 2-NH), 6.82 (1H, d, *J* 15.4 Hz, 2''-H), 7.12 (2H, d, *J* 8.0 Hz, 2'-H and 6'-H), 7.27–7.41 (7H, m, Ph, 3'-H and 5'-H), 7.51–7.67 (4H, m,

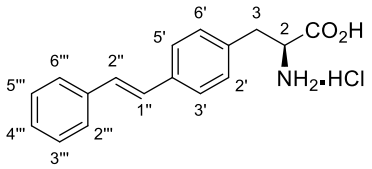
1''-H, 3'''-H, 4'''-H and 5'''-H), 7.92–7.98 (2H, m, 2'''-H and 6'''-H); δ_C (101 MHz, $CDCl_3$) 38.4 (CH_2), 52.6 (CH_3), 54.7 (CH), 67.2 (CH_2), 127.3 (CH), 127.8 (2 × CH), 128.3 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 128.9 (2 × CH), 129.5 (2 × CH), 130.2 (2 × CH), 131.4 (C), 133.5 (CH), 136.3 (C), 139.6 (C), 140.9 (C), 142.1 (CH), 155.6 (C), 171.7 (C); *m/z* (ESI) 480.1486 (MH^+. $C_{26}H_{26}NO_6S$ requires 480.1475).

(2*S*,1''*E*)-2-(Benzyloxycarbonylamino)-3-[(4'-phenylethenyl)phenyl]propanoic acid (179)



To a stirred solution of methyl (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-phenylethenyl)phenyl]propanoate (0.087 g, 0.21 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.089 g, 0.27 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (5 mL) and acidified to pH 1 using 1 M aqueous hydrochloric acid. The reaction mixture was extracted with dichloromethane (3 × 10 mL) and ethyl acetate (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-(phenylethenyl)phenyl]propanoic acid (**179**) (0.079 g, 94%) as a white solid. Mp 160–164 °C; ν_{max}/cm^{-1} (neat) 3333 (NH), 3144, 2928 (CH), 2357,1694 (C=O), 1524, 1447, 1223, 814; $[\alpha]_D^{25}$ +28.0 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 2.92 (1H, dd, *J* 13.9, 9.5 Hz, 3-*H*H), 3.19 (1H, dd, *J* 13.9, 4.8 Hz, 3-H*H*), 4.35–4.49 (1H, m, 2-H), 4.98 (1H, d, *J* 12.0 Hz, OC*H*HPh), 5.05 (1H, d, *J* 12.0 Hz, OCH*H*Ph), 7.13 (2H, s, 1''-H and 2''-H), 7.15–7.37 (10H, m, Ph, 2'-H, 6'-H, 3'''-H, 4'''-H and 5'''-H), 7.44 (2H, d, *J* 7.7 Hz, 3'-H and 5'-H), 7.53 (2H, d, *J* 7.7 Hz, 2'''-H and 6'''-H); δ_C (101 MHz, CD_3OD) 38.4 (CH_2), 56.7 (CH), 67.5 (CH_2), 127.5 (2 × CH), 127.6 (2 × CH), 128.5 (CH), 128.6 (2 × CH), 128.9 (CH), 129.4 (2 × CH), 129.4 (2 × CH), 129.7 (2 × CH), 130.7 (2 × CH), 137.4 (C), 138.1 (C), 138.2 (C), 138.9 (C), 158.4 (C), 175.1 (C); *m/z* (ESI) 402.1683 (MH^+. $C_{25}H_{24}NO_4$ requires 402.1705).

(2*S*,1''*E*)-2-Amino-3-[(4'-(phenylethenyl)phenyl]propanoic acid hydrochloride (180)

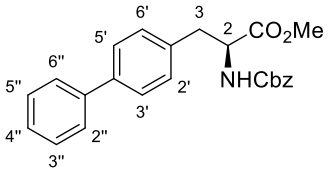


A solution of (2*S*,1''*E*)-2-(benzyloxycarbonylamino)-3-[(4'-(phenylethenyl)phenyl]propanoic acid (0.025 g, 0.062 mmol) in 4 M hydrochloric acid in dioxane (2 mL) was heated under reflux for 4 h. To this was added 6 M aqueous hydrochloric acid (2 mL) and the reaction mixture was heated under reflux for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*,1''E)-2-amino-3-[(4'-(phenylethenyl)phenyl]propanoic acid hydrochloride (**180**) (0.019 g, 100%) as a white solid. Mp 224–230 °C; ν_{max}/cm^{-1} (neat) 3364 (NH), 2913 (CH), 1736 (C=O), 1489, 1219, 826; $[\alpha]_D^{21}$ –6.0 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.14 (1H, dd, *J* 14.5, 7.6 Hz, 3-*H*H), 3.25–3.34 (1H, m, 3-H*H*), 4.20–4.28 (1H, m, 2-H), 7.15 (2H, s, 1''-H and 2''-H), 7.21 (1H, br t, *J* 7.4 Hz, 4'''-H), 7.27 (2H, d, *J* 8.0 Hz, 2'-H and 6'-H), 7.31 (2H, t, *J* 7.4 Hz, 3'''-H and 5'''-H), 7.51 (2H, d, *J* 7.4 Hz, 2'''-H and 6'''-H), 7.54 (2H, d, *J* 8.0 Hz, 3'-H and 5'-H); δ_C (101 MHz, CD_3OD) 37.1 (CH_2), 55.1 (CH), 127.6 (2 × CH), 128.2 (2 × CH), 128.7 (CH), 129.0 (CH), 129.7 (2 × CH), 130.2 (CH), 130.9 (2 × CH), 134.8 (C), 138.5 (C), 138.6 (C), 171.2 (C); *m*/*z* (ESI) 268.1325 (MH^+. $C_{17}H_{18}NO_2$ requires 268.1332).

General Procedure for the Synthesis of Biphenyl Amino Acids

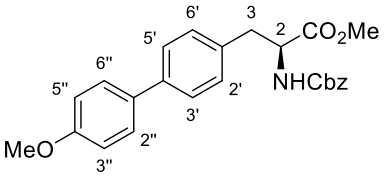
A solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (1 equiv.) and potassium phosphate (3 equiv.) in acetonitrile (3 mL mmol^{-1}) was degassed under argon for 0.2 h. To this was added perfluoro-1-butanesulfonyl fluoride (1.5 equiv.). The reaction mixture was heated to 60 °C or 80 °C and stirred for 2 h. To this was added boronic acid (1.5 equiv.), XPhos Pd G2 (1 mol%) and water (2 mL mmol^{-1}). The reaction mixture was stirred at 60 °C or 80 °C for 2 h. Additional XPhos Pd G2 (1 mol%) was added and the reaction mixture was stirred at 60 °C or 80 °C for 2 h. A final portion of XPhos Pd G2 (1 mol%) was added and the reaction mixture was stirred at 60 °C or 80 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted in ethyl acetate (30 mL) and washed with water (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography gave the biphenyl amino acids.

Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoate (175a)[195]



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), phenylboronic acid (0.083 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in acetonitrile (1.5 mL) and water (1 mL) at 60 °C. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoate (**175a**) (0.16 g, 91%) as a white solid. Mp 85–90 °C (lit.[195] 83–85 °C); $[\alpha]_D^{22}$ +35.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.14 (1H, dd, *J* 13.9, 5.9 Hz, 3-*H*H), 3.21 (1H, dd, *J* 13.9, 5.9 Hz 3-H*H*), 3.75 (3H, s, OCH_3), 4.67–4.77 (1H, m, 2-H), 5.06–5.18 (2H, m, CH_2Ph), 5.29 (1H, d, *J* 8.3 Hz, 2-NH), 7.14–7.22 (2H, m, 2'-H and 6'-H), 7.28–7.62 (12H, m, Ph, 3'-H, 5'-H, 2''-H, 3''-H, 4''-H, 5''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 38.0 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.1 (CH_2), 127.1 (2 × CH), 127.4 (CH), 127.4 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 128.9 (2 × CH), 129.8 (2 × CH), 134.9 (C), 136.4 (C), 140.1 (C), 140.8 (C), 155.8 (C), 172.1 (C); *m/z* (ESI) 412 (MNa^+. 100%).

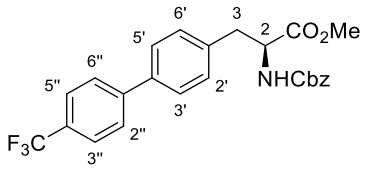
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-methoxybiphen-4'-yl)propanoate (175b)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-methoxybiphen-4'-yl)propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), 4-methoxyphenylboronic acid (0.10 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in acetonitrile (1.5 mL) and water (1 mL) at 60 °C. Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-

methoxybiphen-4'-yl)propanoate (**175b**) (0.16 g, 81%) as a white solid. Mp 102–104 °C; ν_{max}/cm^{-1} (neat) 2924 (CH), 2855, 2253, 1717 (C=O), 1501, 1246; $[\alpha]_D^{17}$ +27.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.12 (1H, dd, *J* 13.9, 6.0 Hz, 3-*H*H), 3.18 (1H, dd, *J* 13.9, 5.6 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 3.85 (4''-OCH_3), 4.65–4.73 (1H, m, 2-H), 5.06–5.16 (2H, m, CH_2Ph), 5.24 (1H, d, *J* 8.3 Hz, 2-NH), 6.94–7.00 (2H, m, 3''-H and 5''-H), 7.11–7.16 (2H, m, 3'-H and 5'-H), 7.28–7.37 (5H, m, Ph), 7.43–7.53 (4H, m, 2'-H, 6'-H, 2''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 38.0 (CH_2), 52.5 (CH_3), 54.9 (CH), 55.5 (CH_3), 67.2 (CH_2), 114.4 (2 × CH), 127.0 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.3 (2 × CH), 128.7 (2 × CH), 129.8 (2 × CH), 133.4 (C), 134.2 (C), 136.4 (C), 139.8 (C), 155.8 (C), 159.3 (C), 172.1 (C); *m/z* (ESI) 420.1805 (MH^+. $C_{25}H_{26}NO_5$ requires 420.1805).

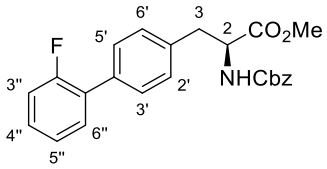
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[4''-(trifluoromethyl)biphen-4'-yl]propanoate (175d)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[4''-(trifluoromethyl)biphen-4'-yl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), 4-(trifluoromethyl)phenylboronic acid (0.13 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in acetonitrile (1.5 mL) and water (1 mL) at 80 °C. Purification by flash column chromatography, eluting with 25% ethyl acetate in gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(4''-trifluoromethyl)biphen-4'-yl]propanoate (**175d**) (0.16 g, 75%) as a white solid. Mp 125–130 °C; ν_{max}/cm^{-1} (neat) 3316 (NH), 3061, 2878 (CH), 2388, 1776 (C=O), 1696, 1537, 1392, 1335, 1267; $[\alpha]_D^{15}$ +28.2 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.14 (1H, dd, *J* 13.9, 6.0 Hz, 3-*H*H), 3.21 (1H, dd, *J* 13.9, 5.7 Hz 3-H*H*), 3.76 (3H, s, OCH_3), 4.68–4.76 (1H, m, 2-H), 5.05–5.16 (2H, m, CH_2Ph), 5.25 (1H, d, *J* 8.2 Hz, 2-NH), 7.18–7.22 (2H, m, 2'-H and 6'-H), 7.28–7.39 (5H, m, Ph), 7.48–7.53 (2H, m, 3'-H and 5'-H), 7.63–7.72 (4H, m, 2''-H, 3''-H, 5''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 38.0 (CH_2), 52.6 (CH_3), 54.9 (CH), 67.2 (CH_2), 124.9 (q, ^1J 272.1 Hz, C), 125.9 (q, ^3J 3.9 Hz, 2 × CH), 127.4 (2 × CH), 127.6 (2 × CH), 128.3 (CH), 128.4 (2 × CH), 128.7 (2 × CH), 129.5 (q, ^2J 32.6 Hz, C), 130.1 (2 × CH), 136.0 (C), 136.3 (C), 138.7

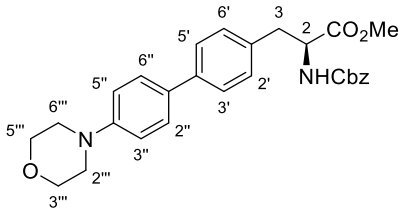
(C), 144.3 (C), 155.8 (C), 172.0 (C); *m/z* (ESI) 480.1395 (MNa^+. $C_{25}H_{22}F_3NNaO_4$ requires 480.1393).

Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoate (175f)



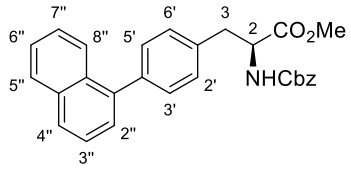
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), 2-fluorophenylboronic acid (0.096 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in acetonitrile (1.5 mL) and water (1mL) at 80 °C. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoate (**175f**) (0.17 g, 94%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3338 (NH), 2958 (CH), 2852, 1704 (C=O), 1494, 1198; $[\alpha]_D^{21}$ +70.3 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.08–3.25 (2H, m, 3-H_2), 3.75 (3H, s, OCH_3), 4.67–4.76 (1H, m, 2-H), 5.07–5.18 (2H, m, CH_2Ph), 5.28 (1H, d, *J* 8.1 Hz, 2-NH), 7.10–7.24 (4H, m, 2'-H, 6'-H, 3''-H and 6''-H), 7.27–7.39 (6H, m, Ph and 4''-H), 7.42 (1H, td, *J* 7.8, 1.8 Hz, 5''-H), 7.47 (2H, dd, *J* 8.2, 1.7 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 38.0 (CH_2), 52.5 (CH_3), 54.9 (CH), 67.2 (CH_2), 116.2 (d, ^2J 22.8 Hz, CH), 124.5 (d, ^3J 3.7 Hz, CH), 128.3 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 128.7 (d, ^2J 13.2 Hz, C), 129.1 (d, ^3J 8.2 Hz, CH), 129.4 (d, ^4J 3.7 Hz, 2 × CH), 129.5 (2 × CH), 130.8 (d, ^4J 3.5 Hz, CH), 134.8 (C), 135.3 (C), 136.4 (C), 155.8 (C), 159.9 (d, ^1J 247.8 Hz, C), 172.1 (C); *m/z* (ESI) 430.1428 (MNa^+. $C_{24}H_{22}FNNaO_4$ requires 430.1425).

Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-morpholinobiphen-4'-yl)propanoate (175g)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-morpholinobiphen-4'-yl)propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), 4-(4-morpholinyl)phenylboronic acid (0.14 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in tetrahydrofuran (1.5 mL) and water (1 mL) at 60 °C. Purification by flash column chromatography, eluting with 40% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4''-morpholinobiphen-4'-yl)propanoate (**175g**) (0.037 g, 17%) as a white solid. Mp 170–175 °C; ν_{max}/cm^{-1} (neat) 2852 (CH), 2358, 2332, 1776 (C=O), 1708 (C=O), 1485, 1322, 1200, 1122; $[\alpha]_D^{22}$ +74.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.07–3.26 (6H, m, 3-H_2, 2'''-H_2 and 6'''-H_2), 3.74 (3H, s, OCH_3), 3.85–3.92 (4H, m, 3''-H_2 and 5'''-H_2), 4.64–4.73 (1H, m, 2-H), 5.05–5.16 (2H, m, C*H*$_2$Ph), 5.23 (1H, d, *J* 8.3 Hz, 2-NH), 6.94–7.01 (2H, m, 3''-H and 5''-H), 7.10–7.15 (2H, m, 3'-H and 5'-H), 7.27–7.38 (5H, m, Ph), 7.43–7.53 (4H, m, 2'-H, 6'-H, 2''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 37.9 (CH_2), 49.3 (2 × CH_2), 52.5 (CH_3), 54.9 (CH), 67.0 (2 × CH_2), 67.1 (CH_2), 115.9 (2 × CH), 126.8 (2 × CH), 127.8 (2 × CH), 128.2 (CH), 128.3 (2 × CH), 128.7 (2 × CH), 129.8 (2 × CH), 132.2 (C), 134.0 (C), 136.4 (C), 139.8 (C), 150.7 (C), 155.8 (C), 172.1 (C); *m/z* (ESI) 475.2231 (MH^+. $C_{28}H_{31}N_2O_5$ requires 475.2227).

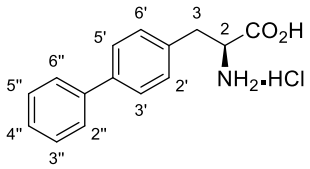
Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoate (175i)



Methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanoate (0.15 g, 0.46 mmol), perfluoro-1-butanesulfonyl fluoride (0.12 mL, 0.68 mmol), 1-naphthaleneboronic

acid (0.12 g, 0.68 mmol), XPhos Pd G2 (0.012 g, 0.015 mmol, 3 mol%) and potassium phosphate (0.29 g, 1.4 mmol) in acetonitrile (1.5 mL) and water (1 mL) at 80 °C. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoate (**175i**) (0.17 g, 84%) as a colourless oil. ν_{max}/cm^{-1} (neat) 3396 (NH), 3320, 3050, 2954, 1746 (C=O), 1696, 1506, 1249, 1057, 1014; $[\alpha]_D^{25}$ +61.4 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.19 (1H, dd, *J* 13.9, 6.0 Hz, 3-*H*H), 3.25 (1H, dd, *J* 13.9, 5.7 Hz 3-H*H*), 3.78 (3H, s, OCH_3), 4.71–4.81 (1H, m, 2-H), 5.08–5.20 (2H, m, C*H*$_2$Ph), 5.33 (1H, d, *J* 8.3 Hz, 2-NH), 7.22 (2H, d, *J* 7.8 Hz, 2'-H and 6'-H), 7.28–7.57 (11H, m, Ph, 3'-H, 5'-H, 2''-H, 3''-H, 6''-H and 7''-H), 7.83–7.96 (3H, m, 4''-H, 5''-H and 8''-H); δ_C (101 MHz, $CDCl_3$) 38.1 (CH_2), 52.6 (CH_3), 55.0 (CH), 67.2 (CH_2), 125.5 (CH), 125.9 (CH), 126.1 (CH), 126.2 (CH), 127.1 (2 × CH), 127.8 (CH), 128.3 (CH), 128.4 (2 × CH), 128.4 (CH), 128.7 (2 × CH), 129.4 (2 × CH), 130.4 (CH), 131.7 (C), 133.9 (C), 134.8 (C), 136.4 (C), 139.8 (C), 139.9 (C), 155.8 (C), 172.2 (C); *m/z* (ESI) 462.1679 (MNa^+. $C_{28}H_{25}NNaO_4$ requires 462.1676).

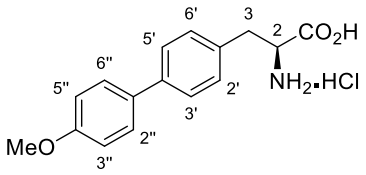
(2*S*)-2-Amino-3-(biphen-4'-yl)propanoic acid hydrochloride (186a)[196]



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoate (0.137 g, 0.352 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.149 g, 0.457 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo*, diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and then extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoic acid (0.113 g, 86%) as a yellow solid. (2*S*)-2-(Benzyloxycarbonylamino)-3-(biphen-4'-yl)propanoic acid (0.0500 g, 0.133 mmol) was dissolved in 6 M aqueous hydrochloric acid (2 mL) and heated under reflux for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-(biphen-4'-yl)propanoic acid (**186a**) (0.0380 g, 100%) as a white solid. Mp 256–258 °C (lit.[196] 256–258 °C); $[\alpha]_D^{18}$ –80.7 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.20 (1H, dd, *J* 14.5, 7.9 Hz, 3-*H*H), 3.38 (1H, dd, *J* 14.5, 5.4 Hz 3-H*H*), 4.29 (1H, dd, *J* 7.9, 5.4 Hz, 2-H), 7.38–7.49 (5H,

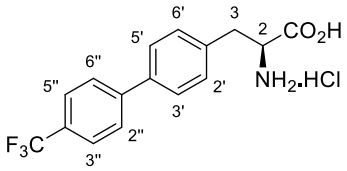
m, 2’-H, 6’-H, 3’’-H, 4’’-H and 5’’-H), 7.59–7.68 (4H, m, 3’-H, 5’-H, 2’’-H and 6’’-H); δ_C (101 MHz, CD_3OD) 36.9 (CH_2), 55.1 (CH), 127.9 (2 × CH), 128.5 (CH), 128.7 (2 × CH), 129.9 (2 × CH), 131.0 (2 × CH), 134.6 (C), 141.8 (C), 142.0 (C), 171.1 (C); *m/z* (ESI) 242 (MH^+. 100%).

(2*S*)-2-Amino-3-(4’’-methoxybiphen-4’-yl)propanoic acid hydrochloride (186b)



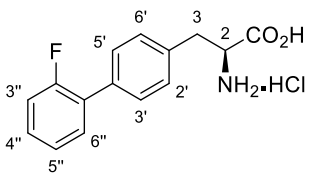
To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(4’’-methoxybiphen-4’-yl)propanoate (0.122 g, 0.290 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.123 g, 0.378 mmol). The reaction mixture was heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-(4’’-methoxybiphen-4’-yl)propanoic acid (0.0840 g, 71%) as a white solid. (2*S*)-2-(Benzyloxycarbonylamino)-3-(4’’-methoxybiphen-4’-yl)propanoic acid (0.0400 g, 0.0990 mmol) was dissolved in 6 M aqueous hydrochloric acid (2 mL) and heated under reflux for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-(4’’-methoxybiphen-4’-yl)propanoic acid (**186b**) (0.0300 g, 100%) as a white solid. Mp 249–254 °C; ν_{max}/cm^{-1} (neat) 3255 (NH), 2928 (CH), 2144, 1731 (C=O), 1495, 1248; $[\alpha]_D^{18}$ +56.5 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.19 (1H, dd, *J* 14.6, 7.9 Hz, 3-*H*H), 3.38 (1H, dd, *J* 14.6, 5.4 Hz, 3-H*H*), 3.84 (3H, s, 4’’-OCH_3), 4.28 (1H, dd, *J* 7.9, 5.4 Hz, 2-H), 6.97–7.04 (2H, m, 3’’-H and 5’’-H), 7.33–7.39 (2H, m, 3’-H and 5’-H), 7.52–7.64 (4H, m, 2’-H, 6’-H, 2’’-H and 6’’-H); δ_C (101 MHz, CD_3OD) 36.9 (CH_2), 55.1 (CH), 55.8 (CH_3), 115.3 (2 × CH), 128.2 (2 × CH), 128.9 (2 × CH), 130.9 (2 × CH), 133.8 (C), 134.2 (C), 141.7 (C), 160.9 (C), 171.2 (C); *m/z* (ESI) 272.1281 (MH^+. $C_{16}H_{18}NO_3$ requires 272.1281).

(2*S*)-2-Amino-3-[(4''-trifluoromethyl)biphen-4'-yl]propanoic acid hydrochloride (186d)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(4''-(trifluoromethyl)biphen-4'-yl]propanoate (0.100 g, 0.219 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.0930 g, 0.284 mmol). The reaction mixture was heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-[(4''-(trifluoromethyl)biphen-4'-yl]propanoic acid (0.0790 g, 81%) as a white solid. (2*S*)-2-(Benzyloxycarbonylamino)-3-[(4''-(trifluoromethyl)biphen-4'-yl]propanoic acid (0.0400 g, 0.0902 mmol) was dissolved in 6 M aqueous hydrochloric acid (2 mL) and heated under reflux for 7 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give (2*S*)-2-amino-3-[(4''-trifluoromethyl)biphen-4'-yl]propanoic acid (**186d**) (0.0330 g, 100%) as a white solid. Mp 260–263 °C; ν_{max}/cm^{-1} (neat) 2871 (CH), 1781 (C=O), 1730 (C=O), 1616, 1484, 1321, 1179, 1071; $[\alpha]_D^{16}$ +37.2 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.23 (1H, dd, *J* 14.5, 7.7 Hz, 3-*H*H), 3.39 (1H, dd, *J* 14.5, 5.4 Hz 3-H*H*), 4.30 (1H, dd, *J* 7.7, 5.4 Hz, 2-H), 7.45 (2H, d, *J* 8.2 Hz, 2'-H and 6'-H), 7.69–7.79 (4H, m, 3'-H, 5'-H, 2''-H and 6''-H), 7.83 (2H, d, *J* 7.9 Hz, 3''-H and 5''-H); δ_C (101 MHz, CD_3OD) 37.1 (CH_2), 55.2 (CH), 125.8 (q, ^1J 270.6 Hz, C), 126.8 (q, ^3J 3.9 Hz, 2 × CH), 128.5 (2 × CH), 128.9 (2 × CH), 130.5 (q, ^2J 32.8 Hz, C), 131.2 (2 × CH), 135.9 (C), 140.4 (C), 145.6 (C), 171.3 (C); *m/z* (ESI) 310.1059 (MH^+. $C_{16}H_{15}F_3NO_2$ requires 310.1049).

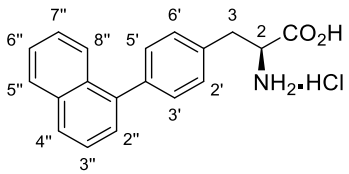
(2*S*)-2-Amino-3-(2''-fluorobiphen-4'-yl)propanoic acid hydrochloride (186f)



To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoate (0.062 g, 0.15 mmol) in methanol (3 mL), dioxane (1.75 mL) and

water (1.75 mL) was added caesium carbonate (0.064 g, 0.20 mmol). The reaction mixture was heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoic acid (0.045 g, 78%) as a colourless oil. (2*S*)-2-(Benzyloxycarbonylamino)-3-(2''-fluorobiphen-4'-yl)propanoic acid (0.045 g, 0.11 mmol) was dissolved in 6 M aqueous hydrochloric acid (2 mL) and heated under reflux for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-(2''-fluorobiphen-4'-yl)propanoic acid (**186f**) (0.025 g, 74%) as a white solid. Mp 197–202 °C; ν_{max}/cm^{-1} (neat) 2882 (CH), 1723 (C=O), 1483, 1183; $[\alpha]_D^{17}$ +11.1 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.21 (1H, dd, *J* 14.5, 7.9 Hz, 3-*H*H), 3.39 (1H, dd, *J* 14.5, 5.2 Hz 3-H*H*), 4.22–4.34 (1H, m, 2-H), 7.19 (1H, dd, *J* 8.9, 8.9, Hz, 5''-H), 7.23–7.29 (1H, m, 6''-H), 7.34–7.45 (3H, m, 2'-H, 6'-H and 4''-H), 7.45–7.52 (1H, m, 3''-H), 7.57 (2H, d, *J* 7.7 Hz, 3'-H and 5'-H); δ_C (101 MHz, CD_3OD) 37.1 (CH_2), 55.3 (CH), 117.0 (d, ^2J 22.9 Hz, CH), 125.8 (d, ^3J 3.7 Hz, CH), 129.7 (d, ^2J 13.4 Hz, C), 130.5 (d, ^3J 8.4 Hz, CH), 130.7 (2 × CH), 130.7 (d, ^4J 3.0 Hz, 2 × CH), 131.8 (d, ^4J 3.4 Hz, CH), 135.3 (C), 136.8 (C), 161.1 (d, ^1J 246.4 Hz, C), 171.4 (C); *m/z* (ESI) 260.1082 (MH^+. $C_{15}H_{14}FNO_2$ requires 206.1081).

(2*S*)-2-Amino-3-[(1''-naphthyl)phen-4'-yl]propanoic acid hydrochloride (**186h)**

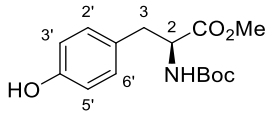


To a stirred solution of methyl (2*S*)-2-(benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoate (0.128 g, 0.291 mmol) in methanol (3 mL), dioxane (1.75 mL) and water (1.75 mL) was added caesium carbonate (0.124 g, 0.379 mmol). The reaction mixture was heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-(benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoic acid (0.113 g, 91%)

as a white solid. (2*S*)-2-(Benzyloxycarbonylamino)-3-[(1''-naphthyl)phen-4'-yl]propanoic acid (0.0500 g, 0.118 mmol) was dissolved in 6 M aqueous hydrochloric acid (2 mL) and heated under reflux for 4 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by recrystallisation from methanol and diethyl ether gave (2*S*)-2-amino-3-[(1''-naphthyl)phen-4'-yl]propanoic acid hydrochloride (**186h**) (0.0240 g, 62%) as a white solid. Mp 230–236 °C; ν_{max}/cm^{-1} (neat) 2856 (CH), 1768 (C=O), 1692, 1598, 1491, 1392, 1324, 1183, 1119; $[\alpha]_D^{16}$ –10.0 (*c* 0.1, MeOH); δ_H (400 MHz, CD_3OD) 3.25 (1H, dd, *J* 14.5, 7.7 Hz, 3-*H*H), 3.43 (1H, dd, *J* 14.5, 5.3 Hz 3-H*H*), 4.32 (1H, dd, *J* 7.7, 5.3 Hz, 2-H), 7.34–7.55 (8H, m, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 6''-H and 7''-H), 7.83–7.94 (3H, m, 4''-H, 5''-H and 8''-H); δ_C (101 MHz, CD_3OD) 37.2 (CH_2), 55.3 (CH), 126.4 (CH), 126.7 (CH), 126.9 (CH), 127.1 (CH), 127.9 (CH), 128.9 (CH), 129.4 (CH), 130.5 (2 × CH), 131.8 (2 × CH), 132.8 (C), 134.8 (C), 135.4 (C), 140.9 (C), 141.7 (C), 171.4 (C); *m/z* (ESI) 292.1342 (MH^+. $C_{19}H_{18}NO_2$ requires 292.1332).

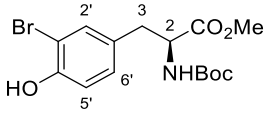
3.5 3'-Aryl tyrosine Analogues Experimental

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4'-hydroxyphenyl)propanoate (188)[197]



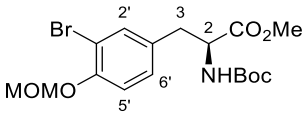
To a stirred solution of methyl (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (1.00 g, 4.33 mmol) in methanol (5 mL) at 0 °C was added triethylamine (1.80 mL, 13.0 mmol) and di-*tert*-butyl dicarbonate (1.04 g, 4.76 mmol). The reaction mixture was stirred at 0 °C for 0.2 h, then warmed to room temperature and stirred for 2 h. The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4'-hydroxyphenyl)propanoate (**188**) (1.25 g, 98%) as a white solid. Mp 99–100 °C (lit.[197] 100–102 °C); $[\alpha]_D^{20}$ +47.8 (*c* 0.1, $CHCl_3$) (lit.[197] +46.0, *c* 1.0 $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 2.97 (1H, dd, *J* 14.0, 6.1 Hz, 3-*H*H), 3.04 (1H, dd, *J* 14.0, 5.8 Hz, 3-H*H*), 3.71 (3H, s, OCH_3), 4.49–4.57 (1H, m, 2-H), 4.98 (1H, d, *J* 8.3 Hz, NH), 5.12 (1H, s, OH), 6.74 (2H, d, *J* 8.1 Hz, 3'-H and 5'-H), 6.98 (2H, d, *J* 8.1 Hz, 2'-H and 6'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.7 (CH_2), 52.4 (CH_3), 54.7 (CH), 80.3 (C), 115.6 (2 × CH), 127.9 (C), 130.6 (2 × CH), 155.1 (C), 155.4 (C), 172.7 (C); *m/z* (ESI) 318 (MNa^+ 100%).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3'-bromo-4'-hydroxyphenyl)propanoate (189)[198]



To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-hydroxyphenyl)propanoate (0.670 g, 2.27 mmol) and *p*-toluenesulfonic acid (0.0840 g, 0.227 mmol, 10 mol%) in methanol (2 mL) in a foiled round bottomed flask was added a solution of *N*-bromosuccinimide (0.444 g, 2.50 mmol) in methanol (20 mL) dropwise from a foiled dropping funnel over 0.3 h. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3'-bromo-4'-hydroxyphenyl)propanoate (**189**) (0.545 g, 64%) as a white solid. Mp 111–115 °C (lit.[198] 117–119 °C); $[\alpha]_D^{21}$ +45.7 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 2.95 (1H, dd, *J* 14.0, 5.9 Hz, 3-*H*H), 3.05 (1H, dd, *J* 14.0, 5.2 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.47–4.57 (1H, m, 2-H), 4.98 (1H, d, *J* 8.2 Hz, NH), 5.43 (1H, s, OH), 6.90–7.03 (2H, m, 5'-H and 6'-H), 7.23 (1H, d, *J* 1.5 Hz, 2'-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.4 (CH_2), 52.5 (CH_3), 54.6 (CH), 80.3 (C), 110.3 (C), 116.2 (CH), 129.9 (C), 130.2 (CH), 132.8 (CH), 151.5 (C), 155.2 (C), 172.3 (C); *m/z* (ESI) 396 (MNa^+ 100%).

Methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (191)



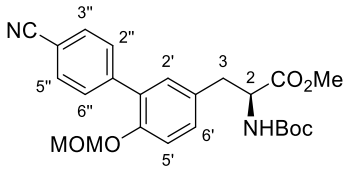
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3'-bromo-4'-hydroxyphenyl)propanoate (4.74 g, 12.7 mmol) in dichloromethane (120 mL) at 0 °C was added *N,N*-diisopropylethylamine (9.35 mL, 50.6 mmol) and bromomethyl methyl ether (3.69 mL, 44.3 mmol). The reaction mixture was stirred at 0 °C for 0.2 h, then warmed to room temperature and stirred for 2 h. The reaction mixture was diluted in dichloromethane (100 mL) and washed with 0.5 M citric acid solution (2 × 100 mL), sodium bicarbonate (2 × 80 mL) and brine (2 × 80 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate

(**191**) (5.00 g, 94%) as a colourless oil. $[\alpha]_D^{22}$ +36.8 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3364 (NH), 2978 (CH), 1746 (C=O), 1713 (C=O), 1495, 1366, 1246, 1161, 1045; δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 2.96 (1H, dd, *J* 14.0, 6.0 Hz, 3-*H*H), 3.06 (1H, dd, *J* 14.0, 5.6 Hz, 3-H*H*), 3.51 (3H, s, OCH_2OCH_3), 3.73 (3H, s, OMe), 4.49–4.57 (1H, m, 2-H), 4.99 (1H, d, *J* 8.2 Hz, NH), 5.22 (2H, s, OCH_2OCH_3), 7.00 (1H, dd, *J* 8.4, 2.0 Hz, 6-H), 7.07 (2H, d, *J* 8.4 Hz, 5-H), 7.30 (1H, d, *J* 2.0 Hz, 2'-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.3 (CH_2), 52.5 (CH_3), 54.5 (CH), 56.5 (CH_3), 80.2 (C), 95.3 (CH_2), 112.9 (C), 116.3 (CH), 129.4 (CH), 131.2 (C), 134.3 (CH), 153.0 (C), 155.2 (C), 172.2 (C); *m/z* (ESI) 440.0677 (MNa^+. $C_{17}H_{24}{}^{79}BrNNaO_6$ requires 440.0679).

General Procedure for the Synthesis of 3'-Aryl Tyrosine Ananlogues

To a solution of methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (1 equiv.), boronic acid (1.5 equiv.) and potassium phosphate (2 equiv.) in tetrahydrofuran (0.1 mL $mmol^{-1}$) and water (0.1 mL $mmol^{-1}$) was degassed under argon for 0.2 h. To this was added XPhos Pd G3 (5 mol%). The reaction mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification by flash column chromatography gave the desired products.

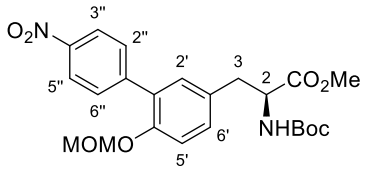
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoate (192a)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.131 g, 0.313 mmol), 4-cyanophenylboronic acid (0.0690 g, 0.470 mmol), XPhos Pd G3 (0.0130 g, 0.0154 mmol, 5 mol%) and potassium phosphate (0.133 g, 0.626 mmol) in tetrahydrofuran/water (1:1, 2 mL) at 40 °C. Purification by flash column chromatography, eluting with 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-

cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoate (**192a**) (0.106 g, 77%) as a colourless oil. $[\alpha]_D^{20}$ +27.3 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3376 (NH), 2978 (CH), 2226, 1744 (C=O), 1709 (C=O), 1493, 1157; δ_H (400 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 3.02 (1H, dd, *J* 13.9, 6.1 Hz, 3-*H*H), 3.14 (1H, dd, *J* 13.9, 5.7 Hz, 3-H*H*), 3.38 (3H, s, OCH_2OCH_3), 3.73 (3H, s, OMe), 4.54–4.64 (1H, m, 2-H), 5.01 (1H, d, *J* 8.4 Hz, NH), 5.12 (2H, s, OCH_2OCH_3), 7.05 (1H, d, *J* 2.2 Hz, 2'-H), 7.09 (1H, dd, *J* 8.5, 2.2 Hz, 6'-H), 7.15 (1H, d, *J* 8.5 Hz, 5'-H), 7.58–7.64 (2H, m, 2''-H and 6''-H), 7.66–7.71 (2H, m, 3''-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.6 (CH_2), 52.5 (CH_3), 54.5 (CH), 56.4 (CH_3), 80.2 (C), 95.1 (CH_2), 110.8 (C), 115.7 (CH), 119.2 (C), 129.8 (C), 130.1 (C), 130.4 (2 × CH), 130.8 (CH), 131.8 (CH), 131.9 (2 × CH), 143.3 (C), 153.3 (C), 155.1 (C), 172.4 (C); *m/z* (ESI) 463.1849 (MNa^+. $C_{24}H_{28}N_2NaO_6$ requires 463.1840).

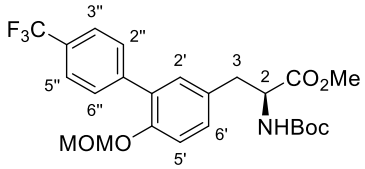
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoate (192c)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.040 g, 0.096 mmol), 4-nitrophenylboronic acid (0.024 g, 0.14 mmol), XPhos Pd G3 (0.0050 g, 0.0059 mmol, 5 mol%) and potassium phosphate (0.041 g, 0.19 mmol) in tetrahydrofuran/water (1:1, 2 mL) at 40 °C. Purification by flash column chromatography, eluting with 50% diethyl ether in hexane followed by 100% dichloromethane with 2% triethylamine gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoate (**192c**) (0.032 g, 73%) as a colourless oil. $[\alpha]_D^{23}$ +21.4 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3379 (NH), 2974 (CH), 1740 (C=O), 1713 (C=O), 1516, 1489, 1346, 1161; δ_H (400 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 3.03 (1H, dd, *J* 14.0, 6.0 Hz, 3-*H*H), 3.16 (1H, dd, *J* 14.0, 5.8 Hz, 3-H*H*), 3.39 (3H, s, OCH_2OCH_3), 3.74 (3H, s, OMe), 4.55–4.64 (1H, m, 2-H), 5.01 (1H, d, *J* 8.4 Hz, NH), 5.13 (2H, s, OCH_2OCH_3), 7.08 (1H, d, *J* 2.2 Hz, 2'-H), 7.11 (1H, dd, *J* 8.4, 2.2 Hz, 6'-H), 7.17 (1H, d, *J* 8.4 Hz, 5'-H), 7.63–7.69 (2H, m, 2''-H and 6''-H), 8.23–8.29 (2H, m, 3''-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.6 (CH_2), 52.5 (CH_3),

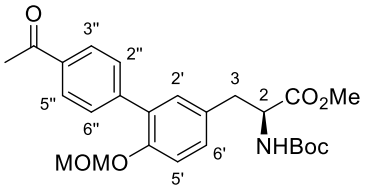
54.5 (CH), 56.4 (CH_3), 80.2 (C), 95.1 (CH_2), 115.7 (CH), 123.4 (2 × CH), 129.4 (C), 130.1 (C), 130.5 (2 × CH), 131.1 (CH), 131.8 (CH), 145.3 (C), 146.9 (C), 153.4 (C), 155.2 (C), 172.2 (C); *m/z* (ESI) 483.1738 (MNa^+. $C_{23}H_{28}N_2NaO_8$ requires 483.1738).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoate (192d)



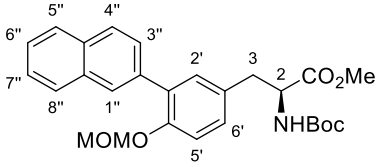
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), 4-(trifluoromethyl)phenylboronic acid (0.204 g, 1.08 mmol), XPhos Pd G3 (0.0300 g, 0.0354 mmol, 5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 40 °C. Purification by flash column chromatography, eluting with dichloromethane with 1% triethylamine followed by 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoate (**192d**) (0.222 g, 64%) as a yellow oil. $[\alpha]_D^{21}$ +70.3 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3368 (NH), 2986 (CH), 2955 (CH), 2361, 1751 (C=O), 1678 (C=O), 1497, 1327, 1223, 1157, 1111; δ_H (400 MHz, $CDCl_3$) 1.40 (9H, s, 3 × CH_3), 3.04 (1H, dd, *J* 14.0, 6.6 Hz, 3-*H*H), 3.14 (1H, dd, *J* 14.0, 5.7 Hz, 3-H*H*), 3.39 (3H, s, OCH_2OCH_3), 3.73 (3H, s, OMe), 4.54–4.64 (1H, m, 2-H), 5.02 (1H, d, *J* 8.4 Hz, NH), 5.11 (2H, s, OCH_2OCH_3), 7.04–7.12 (2H, m, 2'-H and 6'-H), 7.16 (1H, d, *J* 8.3 Hz, 5'-H), 7.57–7.69 (4H, m, 2''-H, 3''-H, 5''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.6 (CH_2), 52.4 (CH_3), 54.5 (CH), 56.3 (CH_3), 80.1 (C), 95.1 (CH_2), 115.7 (CH), 124.5 (C, q, $^1J_{CF}$ 271.9 Hz), 125.0 (2 × CH, q, $^3J_{CF}$ 3.8 Hz), 125.8 (C), 129.2 (C, q, $^2J_{CF}$ 32.2 Hz), 129.9 (2 × CH), 129.9 (C), 130.4 (CH), 131.9 (CH), 142.2 (C), 153.4 (C), 155.2 (C), 172.4 (C); *m/z* (ESI) 506.1774 (MNa^+. $C_{24}H_{28}F_3NNaO_6$ requires 506.1761).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoate (192e)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), 4-acetylphenylboronic acid (0.176 g, 1.08 mmol), XPhos Pd G3 (0.0300 g, 0.0354 mmol, 5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 40 °C. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane followed by 50% diethyl ether in hexane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoate (**192e**) (0.166 g, 51%) as a white oil. $[\alpha]_D^{23}$ +42.9 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 2917 (CH), 2362, 1742 (C=O), 1711 (C=O), 1495, 1157; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 2.64 (3H, s, 4''-$COCH_3$), 3.04 (1H, dd, *J* 14.1, 5.9 Hz, 3-*H*H), 3.14 (1H, dd, *J* 14.1, 5.7 Hz, 3-H*H*), 3.39 (3H, s, OCH_2OCH_3), 3.73 (3H, s, OMe), 4.57–4.64 (1H, m, 2-H), 5.00 (1H, d, *J* 8.3 Hz, NH), 5.11 (2H, s, OCH_2OCH_3), 7.04–7.13 (2H, m, 2'-H and 6'-H), 7.15 (1H, d, *J* 8.7 Hz, 5'-H), 7.60 (2H, d, *J* 8.2 Hz, 2''-H and 6''-H), 8.00 (2H, d, *J* 8.2 Hz, 3''-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 26.8 (CH_3), 28.4 (3 × CH_3), 37.6 (CH_2), 52.4 (CH_3), 54.6 (CH), 56.4 (CH_3), 80.1 (C), 95.1 (CH_2), 115.7 (CH), 128.2 (2 × CH), 129.8 (2 × CH), 129.9 (C), 130.4 (CH), 130.6 (C), 131.9 (CH), 135.8 (C), 143.5 (C), 153.4 (C), 155.2 (C), 172.4 (C), 198.0 (C); *m/z* (ESI) 480.1993 (MNa^+. $C_{25}H_{31}NNaO_7$ requires 480.1993).

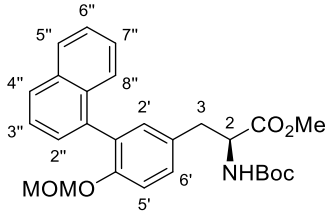
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate (192f)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general

procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), 2-naphthalene boronic acid (0.185 g, 1.08 mmol), XPhos Pd G3 (0.0150 g, 0.0177 mmol, 2.5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 40 °C for 0.5 h. To this was added additional XPhos Pd G3 (0.0150 g, 0.0177 mmol, 2.5 mol%). The reaction mixture was stirred at 40 °C for an additional 0.5 h. Purification by flash column chromatography, eluting with 20% ethyl acetate in hexane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate (**192f**) (0.263 g, 79%) as a colourless oil. $[\alpha]_D^{22}$ +27.4 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3349 (NH), 2974 (CH), 1744 (C=O), 1709 (C=O), 1497, 1157; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.07 (1H, dd, *J* 14.0, 5.9 Hz, 3-*H*H), 3.15 (1H, dd, *J* 14.0, 5.6 Hz, 3-H*H*), 3.38 (3H, s, OCH_2OCH_3), 3.74 (3H, s, OMe), 4.57–4.66 (1H, m, 2-H), 5.03 (1H, d, *J* 8.4 Hz, NH), 5.11 (2H, s, OCH_2OCH_3), 7.08 (1H, dd, *J* 8.4, 2.3 Hz, 6'-H), 7.14–7.22 (2H, m, 2'-H and 5'-H), 7.45–7.53 (2H, m, 6''H and 7''-H), 7.66 (1H, dd, *J* 8.5, 1.8 Hz, 3''-H), 7.82–7.90 (3H, m, 4''-H, 5''-H and 8''-H), 7.93 (1H, d, *J* 1.8 Hz, 1''-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.7 (CH_2), 52.4 (CH_3), 54.6 (CH), 56.3 (CH_3), 80.1 (C), 95.3 (CH_2), 116.0 (CH), 126.0 (CH), 126.2 (CH), 127.4 (CH), 127.8 (CH), 128.1 (CH), 128.2 (CH), 128.3 (CH), 129.6 (CH), 129.9 (C), 132.0 (C), 132.3 (CH), 132.6 (C), 133.5 (C), 136.2 (C), 153.6 (C), 155.3 (C), 172.5 (C); *m/z* (ESI) 488.2044 (MNa^+. $C_{27}H_{31}NNaO_6$ requires 488.2044).

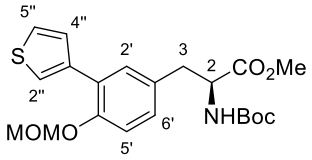
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoate (192g)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), 1-naphthalene boronic acid (0.185 g, 1.08 mmol), XPhos Pd G3 (0.0300 g, 0.0354 mmol, 5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 40 °C. Purification by flash column chromatography, eluting with 2% ethyl acetate

in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoate (**192g**) (0.258 g, 77%) as a yellow oil. $[\alpha]_D^{23}$ +54.5 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 2916 (CH), 2360, 1677 (C=O), 1265; The compounds exists as a 1:1 mixture of rotamers, δ_H (400 MHz, $CDCl_3$) 1.38 (9H, s, 3 × CH_3), 1.42 (9H, s, 3 × CH_3), 3.00–3.18 (4H, m, 2 × 3-H_2), 3.21 (6H, s, 2 × OCH_2OCH_3), 3.65 (3H, s, OMe), 3.71 (3H, s, OMe), 4.55–4.66 (2H, m, 2 × 2-H), 4.91–4.99 (4H, m, 2 × CH_2), 5.03 (1H, d, *J* 8.0 Hz, NH), 5.07 (1H, d, *J* 8.0 Hz, NH), 7.04 (2H, s, 2 × 2'-H), 7.12–7.23 (4H, m, 2 × 6'-H and 2 × 5'-H), 7.33–7.43 (4H, m, 2 × 7''-H and 2 × 8''-H), 7.43–7.62 (6H, m, 2 × 2''-H, 2 × 3''-H 2 × 4''-H), 7.83–7.92 (4H, m, 2 × 5''-H and 2 × 8''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 28.5 (3 × CH_3), 37.5 (CH_2), 37.7, (CH_2), 52.4 (CH_3), 52.4 (CH_3), 56.1 (2 × CH_3), 80.1 (2 × C), 94.8 (2 × CH_2), 94.8 (2 × CH_2), 115.3 (CH), 115.5 (CH), 125.4 (CH), 125.4 (CH), 125.8 (2 × CH), 125.9 (CH), 125.9 (CH), 126.6 (CH), 126.6 (CH), 127.3 (CH), 127.4 (CH), 127.8 (2 × CH), 128.2 (2 × CH), 129.4 (C), 129.5 (C), 129.9 (CH), 129.9 (CH), 130.8 (C), 130.8 (C), 132.3 (2 × C), 133.0 (CH), 133.2 (CH), 133.5 (C), 133.6 (C), 136.8 (C), 136.9 (C), 154.1 (2 × C), 155.2 (2 × C), 172.4 (C), 172.5 (C); *m/z* (ESI) 488.2043 (MNa^+. $C_{27}H_{31}NNaO_6$ requires 488.2044).

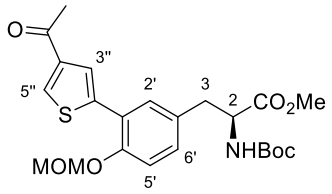
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(thiophen-3''-yl)-4'-(methoxymethoxy)phenyl]propanoate (192h)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(thiophen-3''-yl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), 3-thienylboronic acid (0.138 g, 1.08 mmol), XPhos Pd G2 (0.0280 g, 0.0354 mmol, 5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 60 °C. Purification by flash column chromatography, eluting with dichloromethane 30% ethyl acetate in hexane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(thiophen-3''-yl)-4'-(methoxymethoxy)phenyl]propanoate (**192h**) (0.271 g, 90%) as a white solid. Mp 70–74 °C; $[\alpha]_D^{23}$ +41.5 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3355 (NH), 2917 (CH), 2360, 2229, 1711 (C=O), 1496, 1157; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.03 (1H, dd, *J* 14.1, 6.0 Hz, 3-*H*H), 3.11 (1H, dd, *J* 14.1, 5.7 Hz, 3-H*H*),

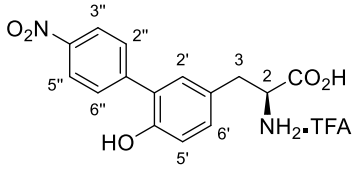
3.44 (3H, s, OCH_2OCH_3), 3.73 (3H, s, OMe), 4.53–4.64 (1H, m, 2-H), 5.00 (1H, d, *J* 8.4 Hz, NH), 5.17 (2H, s, OCH_2OCH_3), 7.00 (1H, dd, *J* 8.4 Hz, 6'-H), 7.12 (1H, d, *J* 8.4 Hz, 5'-H), 7.23 (1H, s, 2'-H), 7.31–7.37 (1H, m, 4''-H), 7.38–7.44 (1H, m, 5''-H), 7.56 (1H, d, *J* 4.0 Hz, 2''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 37.6 (CH_2), 52.4 (CH_3), 54.6 (CH), 56.3 (CH_3), 80.1 (C), 95.1 (CH_2), 115.7 (CH), 123.4 (CH), 124.7 (CH), 126.2 (C), 128.6 (CH), 129.2 (CH), 129.7 (C), 131.0 (CH), 138.3 (C), 153.4 (C), 155.2 (C), 172.5 (C); *m/z* (ESI) 444.1451 (MNa^+. $C_{21}H_{27}NNaO_6S$ requires 444.1451).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate (192i)



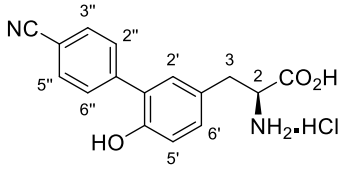
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate was synthesised as described in the general procedure using methyl (2*S*)-3-[3'-bromo-4'-(methoxymethoxy)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (0.300 g, 0.717 mmol), (4-acetylthiophen-2-yl)boronic acid (0.183 g, 1.08 mmol), XPhos Pd G3 (0.0300 g, 0.0354 mmol, 5 mol%) and potassium phosphate (0.304 g, 1.43 mmol) in tetrahydrofuran/water (1:1, 9 mL) at 40 °C. Purification by flash column chromatography, eluting with 100% dichloromethane with 1% triethylamine followed by 2.5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate (**192i**) (0.245 g, 74%) as a yellow oil. $[\alpha]_D^{21}$ +9.2 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3349 (NH), 2974 (CH), 2932 (CH), 2365, 2330, 1744 (C=O), 1713 (C=O), 1655 (C=O), 1447, 1277, 1162; δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, 3 × CH_3), 2.85 (3H, s, 4''-$COCH_3$), 3.03 (1H, dd, *J* 13.9, 6.0 Hz, 3-*H*H), 3.13 (1H, dd, *J* 13.9, 5.7 Hz, 3-H*H*), 3.50 (3H, s, OCH_2OCH_3), 3.74 (3H, s, OMe), 4.54–4.64 (1H, m, 2-H), 5.02 (1H, d, *J* 8.3 Hz, NH), 5.30 (2H, s, OCH_2OCH_3), 7.06 (1H, dd, *J* 8.5, 2.2 Hz, 6'-H), 7.17 (1H, d, *J* 8.5 Hz, 5'-H), 7.44 (1H, d, *J* 2.2 Hz, 2'-H), 7.47 (1H, d, *J* 4.0 Hz, 3''-H), 7.67 (1H, d, *J* 4.0 Hz, 5''-H); δ_C (101 MHz, $CDCl_3$) 26.9 (CH_3), 28.4 (3 × CH_3), 37.7 (CH_2), 52.5 (CH_3), 54.5 (CH), 56.6 (CH_3), 80.2 (C), 94.6 (CH_2), 115.4 (CH), 123.0 (C), 126.1 (CH), 129.6 (CH), 129.8 (C), 130.9 (CH), 132.4 (CH), 143.6 (C), 147.8 (C), 153.0 (C), 155.2 (C), 172.3 (C), 191.2 (C); *m/z* (ESI) 486.1569 (MNa^+. $C_{23}H_{29}NNaO_7S$ requires 486.1557).

(2*S*)-2-Amino-3-[3'-(4''-nitrophenyl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate acid (193a)



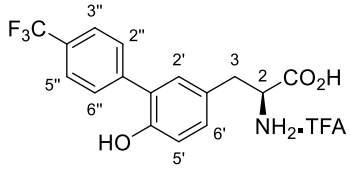
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoate (0.048 g, 0.10 mmol) in methanol (5 mL) was added dropwise a solution of lithium hydroxide (0.011 g, 0.26 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with dichloromethane (3 × 20 mL) and ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.048 g, 100%). (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(4''-nitrophenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.030 g, 0.067 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 2 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(4''-nitrophenyl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193a**) (0.030 g, 100%) as a yellow solid. Mp 99–104 °C; $[\alpha]_D^{22}$ –16.0 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 3395 (NH), 3210 (CH), 2361, 2342, 1674 (C=O), 1346, 1200, 1142; δ_H (400 MHz, CD_3OD) 3.08 (1H, dd, *J* 14.6, 7.5 Hz, 3-*H*H), 3.21 (1H, dd, *J* 14.6, 5.2 Hz, 3-H*H*), 4.13 (1H, dd, *J* 7.5, 5.2 Hz, 2-H), 6.88 (1H, d, *J* 8.3 Hz, 5'-H), 7.12 (1H, dd, *J* 8.3, 2.2 Hz, 6'-H), 7.22 (1H, d, *J* 2.2 Hz, 2'-H), 7.74–7.82 (2H, m, 2''-H and 6''-H), 8.15–8.24 (2H, m, 3''-H and 5''-H); δ_C (101 MHz, CD_3OD) 36.6 (CH_2), 55.5 (CH), 117.9 (CH), 124.0 (2 × CH), 126.9 (C), 127.8 (C), 131.3 (2 × CH), 132.1 (CH), 132.7 (CH), 146.9 (C), 147.9 (C), 155.6 (C), 171.8 (C); *m/z* (ESI) 303.0970 (MH^+. $C_{15}H_{15}N_2O_5$ requires 303.0975).

(2*S*)-2-Amino-3-[3'-(4''-cyanophenyl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (193b)



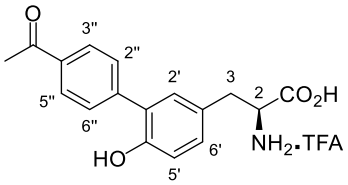
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoate (0.070 g, 0.16 mmol) in methanol (5 mL) was added dropwise a solution of lithium hydroxide (0.016 g, 0.40 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.063 g, 93%) as a white solid. (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(4''-cyanophenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.040 g, 0.094 mmol) was dissolved in 2 M aqueous hydrochloric acid (2 mL) and stirred at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* to give (2*S*)-2-amino-3-[3'-(4''-cyanophenyl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193b**) (0.031 g, 91%) as a yellow solid. Mp 190–195 °C; $[\alpha]_D^{22}$ +25.1 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 3355 (NH), 2917, 2848, 2361, 2229, 2073 (CN), 1713, 1681 (C=O), 1605, 1495, 1268, 1158; δ_H (400 MHz, CD_3OD) 3.15 (1H, dd, *J* 14.7, 7.5 Hz, 3-*H*H), 3.24 (1H, dd, *J* 14.7, 5.3 Hz, 3-H*H*), 4.25 (1H, dd, *J* 7.5, 5.3 Hz, 2-H), 6.95 (1H, d, *J* 8.3 Hz, 5'-H), 7.18 (1H, dd, *J* 8.3, 2.4 Hz, 6'-H), 7.26 (1H, d, *J* 2.4 Hz, 2'-H), 7.73–7.83 (4H, m, 2''-H, 3''-H, 5''-H and 6''-H); δ_C (101 MHz, $CDCl_3$) 36.4 (CH_2), 55.2 (CH), 111.0 (C), 117.8 (CH), 120.0 (C), 126.6 (C), 128.2 (C), 131.3 (2 × CH), 131.9 (CH), 132.6 (CH), 132.8 (2 × CH), 145.0 (C), 155.5 (C), 171.3 (C); *m/z* (ESI) 281.0931 ($(M-H)^-$. $C_{16}H_{13}N_2O_3$ requires 281.0932).

(2*S*)-2-Amino-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-hydroxyphenyl] propanoic acid trifluoroacetate (193d)



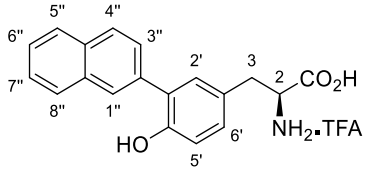
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoate (0.070 g, 0.15 mmol) in methanol (5 mL) was added dropwise a solution of lithium hydroxide (0.015 g, 0.36 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.067 g, 99%) as a colourless oil**.** (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.030 g, 0.064 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 2 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(4''-(trifluoromethyl)phenyl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193d**) (0.028 g, 100%) as a white solid. Mp 99–102 °C; $[\alpha]_D^{22}$ +10.0 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 3395 (NH), 3210 (CH), 2361, 2342, 1728 (C=O), 1667, 1323, 1188, 1111; δ_H (400 MHz, CD_3OD) 3.08 (1H, dd, *J* 14.6, 7.3 Hz, 3-*H*H), 3.21 (1H, dd, *J* 14.6, 4.7 Hz, 3-H*H*), 4.07–4.20 (1H, m, 2-H), 6.87 (1H, d, *J* 8.2 Hz, 5'-H), 7.09 (1H, dd, *J* 8.2, 2.2 Hz, 6'-H), 7.19 (1H, d, *J* 2.2 Hz, 2'-H), 7.62 (2H, d, *J* 8.1 Hz, 2''-H and 6''-H), 7.72 (2H, d, *J* 8.1 Hz, 3''-H and 5''-H); δ_C (101 MHz, CD_3OD) 36.6 (CH_2), 55.5 (CH), 117.7 (CH), 125.8 (2 × CH, q, $^3J_{CF}$ 3.7 Hz), 125.9 (C, q, $^1J_{CF}$ 270.0 Hz), 126.7 (C), 128.7 (C), 129.7 (C, q, $^2J_{CF}$ 32.2 Hz), 131.0 (2 × CH), 131.5 (CH), 132.8 (CH), 144.0 (C), 155.5 (C), 171.7 (C); *m/z* (ESI) 326.0997 (MH^+. $C_{16}H_{15}F_3NO_3$ requires 326.0999).

(2*S*)-2-Amino-3-[3'-(4''-acetylphenyl))-4'-hydroxyphenyl]propanoic acid trifluoroacetate (193e)



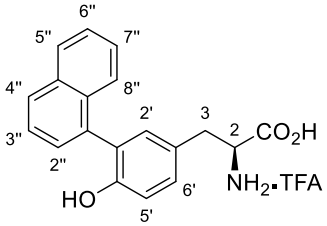
A solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoate (0.070 g, 0.15 mmol)) in methanol (4 mL) was added dropwise a solution of lithium hydroxide (0.016 g, 0.38 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.067 g, 99%) as a colourless oil. (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(4''-acetylphenyl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.030 g, 0.068 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 1 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(4''-acetylphenyl))-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193e**) (0.030 g, 100%) as a white solid. Mp 156–160 °C; $[\alpha]_D^{26}$ +9.3 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 2917 (CH), 2231, 1712 (C=O), 1680 (C=O), 1604, 1494, 1266; δ_H (400 MHz, CD_3OD) 2.59 (3H, s, 4''-$COCH_3$), 3.11 (1H, dd, *J* 14.7, 7.2 Hz, 3-*H*H), 3.24 (1H, dd, *J* 14.7, 5.0 Hz, 3-H*H*), 4.12–4.24 (1H, m, 2-H), 6.90 (1H, d, *J* 8.2 Hz, 5'-H), 7.12 (1H, br d, *J* 8.2, 2.4 Hz, 6'-H), 7.23 (1H, d, *J* 2.4 Hz, 2'-H), 7.71 (2H, d, *J* 8.1 Hz, 2''-H and 6''-H), 7.98 (2H, d, *J* 8.1 Hz, 3''-H and 5''-H); δ_C (101 MHz, $CDCl_3$) 26.7 (CH_3), 36.6 (CH_2), 55.4 (CH), 117.8 (CH), 126.6 (C), 129.0 (C), 129.2 (2 × CH), 130.6 (2 × CH), 131.4 (CH), 132.7 (CH), 136.5 (C), 145.2 (C), 155.5 (C), 171.6 (C), 200.4 (C); *m/z* (ESI) 300.1232 (MH^+. $C_{17}H_{18}NO_4$ requires 300.1230).

(2*S*)-2-Amino-3-[3'-(naphth-2''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (193f)



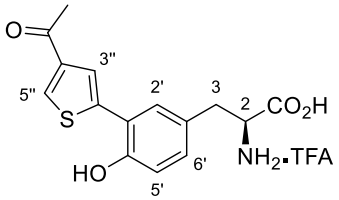
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoate (0.065 g, 0.14 mmol) in methanol (5 mL) was added dropwise a solution of lithium hydroxide (0.015 g, 0.35 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with and ethyl acetate (3 × 20 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.067 g, 100%) as a white solid. (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.030 g, 0.066 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 3 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(naphth-2''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193f**) (0.025 g, 93%) as a light green solid. Mp 98–100 °C; $[\alpha]_D^{23}$ +4.0 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 3059 (NH), 2928 (CH), 2361, 2342, 1728 (C=O), 1667, 1281, 1192, 1142; δ_H (400 MHz, CD_3OD) 3.12 (1H, dd, *J* 14.6, 7.4 Hz, 3-*H*H), 3.21–3.35 (1H, m, 3-H*H*), 4.20 (1H, dd, *J* 7.4, 5.2 Hz, 2-H), 6.91 (1H, d, *J* 8.2 Hz, 5'-H), 7.10 (1H, dd, *J* 8.2, 2.3 Hz, 6'-H), 7.30 (1H, d, *J* 2.3 Hz, 2'-H), 7.39–7.47 (2H, m, 6''-H and 7''-H), 7.71 (1H, dd, *J* 8.5, 1.7 Hz, 3''-H), 7.78–7.88 (3H, m, 4''-H, 5''-H and 8''-H), 8.00 (1H, d, *J* 1.7 Hz, 1''-H); δ_C (101 MHz, $CDCl_3$) 36.7 (CH_2), 55.4 (CH), 117.7 (CH), 126.5 (C), 126.8 (CH), 127.0 (CH), 128.2 (CH), 128.6 (CH), 128.9 (CH), 128.9 (CH), 129.1 (CH) 130.3 (C), 130.6 (CH), 133.1 (CH), 133.9 (C), 134.9 (C), 137.5 (C), 155.5 (C), 171.5 (C); *m/z* (ESI) 308.1274 (MH^+. $C_{19}H_{18}NO_3$ requires 308.1287).

(2*S*)-2-Amino-3-[3'-(naphth-1''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (193g)



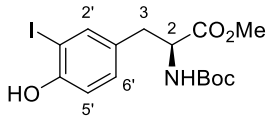
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoate (0.070 g, 0.15 mmol) in methanol (4 mL) was added dropwise a solution of lithium hydroxide (0.016 g, 0.38 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with and ethyl acetate (3 × 20 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.067 g, 99%) as a colourless oil. (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(naphth-1''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.030 g, 0.066 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(naphth-1''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193g**) (0.030 g, 100%) as a white solid. Mp 160–165 °C; $[\alpha]_D^{26}$ +3.0 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 3048 (NH), 2916, 2848, 2360, 2232, 2074, 1725 (C=O), 1667, 1607, 1498, 1187; The compounds exists as a 1:1 mixture of rotamers, δ_H (400 MHz, CD_3OD) 3.07–3.19 (2H, m, 2 × 3-*H*H), 3.20–3.27 (2H, m, 2 × 3-H*H*), 4.12–4.21 (2H, m, 2 × 2-H), 6.96 (2H, dd, *J* 8.2, 2.5 Hz, 2 × 6'-H), 7.04 (2H, d, *J* 2.5 Hz, 2 × 2'-H), 7.17–7.25 (2H, m, 2 × 5'-H), 7.32–7.56 (8H, m, 2 × 3''-H, 2 × 4''-H, 2 × 6''-H and 2 × 7''-H), 7.59–7.67 (2H, m, 2 × 2''-H), 7.80–7.92 (4H, m, , 2 × 5''-H and 2 × 8''-H); δ_C (101 MHz, CD_3OD) 36.6 (CH_2), 36.7 (CH_2), 55.5 (CH), 55.6 (CH), 117.3 (CH), 117.3 (CH), 126.2 (C), 126.4 (CH), 126.4 (CH), 126.4 (C), 126.6 (CH), 126.7 (CH), 126.7 (CH), 126.7 (CH), 127.6 (2 ×CH), 128.5 (2 × CH), 128.8 (CH), 128.8 (CH), 129.1 (CH), 129.1 (CH), 129.6 (2 × C), 130.9 (CH), 131.0 (CH), 133.4 (C), 133.5 (C), 134.0 (2 × CH), 135.1 (C), 135.1 (C), 138.0 (C), 138.1 (C), 155.8 (C), 155.8 (C), 171.6 (C), 171.7 (C); *m/z* (ESI) 352.1534 (MH^+. $C_{21}H_{22}NO_4$ requires 352.1543).

(2*S*)-2-Amino-3-[3'-(4''-acetylthiophen-2''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (193h)



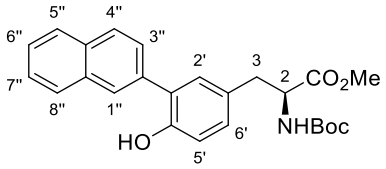
To a stirred solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen''-2-yl)-4'-(methoxymethoxy)phenyl]propanoate (0.070 g, 0.15 mmol) in methanol (5 mL) was added a solution of lithium hydroxide (0.018 g, 0.38 mmol) in water (0.3 mL). The reaction mixture was heated to 40 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction mixture was diluted in water (5 mL), acidified to pH 1 using 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen-2''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.067 g, 99%) as a yellow solid. (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3'-(4''-acetylthiophen-2''-yl)-4'-(methoxymethoxy)phenyl]propanoic acid (0.025 g, 0.056 mmol) was dissolved in dichloromethane (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h followed by room temperature for 0.5 h. Concentration *in vacuo* gave (2*S*)-2-amino-3-[3'-(4''-acetylthiophen-2''-yl)-4'-hydroxyphenyl]propanoic acid trifluoroacetate (**193h**) (0.023 g, 100%) as a yellow solid. Mp 205–210 °C; $[\alpha]_D^{23}$ –12.0 (*c* 0.1, MeOH); ν_{max}/cm^{-1} (neat) 2920 (CH), 2850 (CH), 1669 (C=O), 1623 (C=O), 1448, 1418, 1293, 1184, 1134; δ_H (400 MHz, CD_3OD) 2.54 (3H, s, 4''-$COCH_3$), 3.12 (1H, dd, *J* 14.6, 7.5 Hz, 3-*H*H), 3.26 (1H, dd, *J* 14.6, 5.3 Hz, 3-H*H*), 4.21 (1H, dd, *J* 7.5, 5.3 Hz, 2-H), 6.93 (1H, dd, *J* 8.4 Hz, 5'-H), 7.13 (1H, dd, *J* 8.4, 2.2 Hz, 6'-H), 7.62 (1H, d, *J* 2.2 Hz, 2'-H), 7.65 (1H, d, *J* 4.1 Hz, 3''-H), 7.81 (1H, d, *J* 4.1 Hz, 5''-H); δ_C (101 MHz, CD_3OD) 26.6 (CH_3), 36.6 (CH_2), 55.3 (CH), 118.0 (CH), 121.9 (C), 126.7 (C), 127.2 (CH), 130.4 (CH), 132.0 (CH), 134.7 (CH), 143.5 (C), 150.3 (C), 155.5 (C), 171.4 (C), 193.6 (C); *m/z* (ESI) 306.0791 (MH^+. $C_{15}H_{16}NO_4S$ requires 306.0795).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3’-iodo-4’-hydroxyphenyl)propanoate (194)[199]



Iron(III) chloride (0.0030 g, 0.0017 mmol) was dissolved in 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide (0.015 mL, 0.051 mmol) and stirred at room temperature for 0.5 h. This was then added to a suspension of *N*-iodosuccinimide (0.084 g, 0.37 mmol) and methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4’-hydroxyphenyl)propanoate (0.10 g, 0.34 mmol) in toluene (1 mL). The reaction mixture was heated to 70 °C and stirred for 4.5 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 5% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(3’-iodo-4’-hydroxyphenyl)propanoate (**194**) (0.047 g, 33%) as a colourless oil. $[\alpha]_D^{20}$ +37.6 (*c* 0.2, $CHCl_3$) (lit.[199] +46.0, *c* 1.0 $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 × CH_3), 2.93 (1H, dd, *J* 14.0, 6.0 Hz, 3-*H*H), 3.03 (1H, dd, *J* 14.0, 5.8 Hz, 3-H*H*), 3.72 (3H, s, OCH_3), 4.47–4.55 (1H, m, 2-H), 5.00 (1H, d, *J* 8.3 Hz, NH), 5.39 (1H, s, OH), 6.89 (1H, d, *J* 8.3 Hz, 5’-H), 6.99 (1H, dd, *J* 8.3, 2.0 Hz, 6’-H), 7.42 (1H, d, *J* 2.0 Hz, 2’-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.1 (CH_2), 52.5 (CH_3), 54.6 (CH), 80.3 (C), 85.7 (C), 115.2 (CH), 130.3 (C), 131.2 (CH), 139.0 (CH), 154.2 (C), 155.2 (C), 172.3 (C); *m/z* (ESI) 444 (MNa^+ 100%).

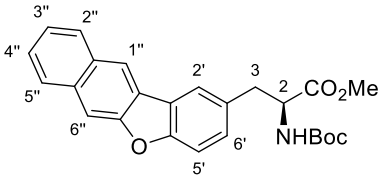
Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3’-(naphth-2’’-yl)-4’-hydroxyphenyl]propanoate (196)



Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3’-(naphth-2’’-yl)-4’-(methoxymethoxy)phenyl]propanoate (0.315 g, 0.676 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 °C. Trifluoroacetic acid (1.5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 3 h. Concentration *in vacuo* gave methyl (2*S*)-2-amino-3-[3’-(naphtha-2’’-yl)-4’-hydroxyphenyl]propanoate trifluoroacetate (0.285 g, 100%) as an off-white solid. To a solution of methyl (2*S*)-2-amino-3-[3’-(naphtha-2’’-yl)-4’-hydroxyphenyl]propanoate trifluoroacetate (0.285 g, 0.681 mmol) in methanol (3 mL)

at 0 °C was added triethylamine (0.283 mL, 2.04 mmol) and di-*tert*-butyl dicarbonate (0.164 g, 0.749 mmol). The reaction mixture was stirred at 0 °C for 0.2 h before warming to room temperature and stirring for 4 h. The reaction mixture was concentrated *in vacuo.* Purification by flash column chromatography, eluting with 2% ethyl acetate in dichloromethane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naphth-2''-yl)-4'-hydroxyphenyl]propanoate (**196**) (0.198 g, 69%) as a white solid. Mp 63–65 °C; $[\alpha]_D^{21}$ +46.4 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3341 (NH), 2972 (CH), 2362, 2331, 1717 (C=O), 1679 (C=O), 1504, 1364, 1280, 1252, 1160; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, 3 × CH_3), 3.05 (1H, dd, *J* 14.0, 5.7 Hz, 3-*H*H), 3.12 (1H, dd, *J* 14.0, 5.7 Hz, 3-H*H*), 3.73 (3H, s, OMe), 4.54–4.65 (1H, m, 2-H), 5.03 (1H, d, *J* 8.5 Hz, NH), 5.36 (1H, br s, OH), 6.95 (1H, d, *J* 8.2 Hz, 5'-H), 7.04 (1H, dd, *J* 8.2, 2.3 Hz, 6'-H), 7.09 (1H, d, *J* 2.3 Hz, 2'-H), 7.50–7.60 (3H, m, 3''-H, 6''H and 7''-H), 7.82–7.94 (3H, m, 4-''H, 5''-H and 1''-H), 7.96 (1H, d, *J* 8.5 Hz, 8''-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 37.7 (CH_2), 52.4 (CH_3), 54.7 (CH), 80.1 (C), 116.2 (CH), 126.6 (CH), 126.8 (CH), 127.2 (CH), 127.9 (2 × CH), 128.2 (CH), 128.2 (C), 128.4 (C), 129.3 (CH), 130.1 (CH), 131.5 (CH), 132.9 (C), 133.7 (C), 134.5 (C), 151.9 (C), 155.3 (C), 172.6 (C); *m/z* (ESI) 420.1817 ($[M-H]^-$. $C_{25}H_{26}NO_5$ requires 420.1816).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naptho[2,3-b]benzofuran)-4'-hydroxyphenyl]propanoate (198)



A solution of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(2''-naphthalene)-4'-hydroxyphenyl]propanoate (0.15 g, 0.35 mmol), palladium acetate (0.016 g, 0.070 mmol, 20 mol%) and 3-nitropyridine (0.0090 g, 0.070 mmol, 20 mol%) in hexafluorobenzene (0.45 mL) and *N*,*N*-dimethylimidazolidinone (0.3 mL) was degassed under argon for 0.2 h. To this was added *tert*-butyl peroxybenzoate (0.13 mL, 0.70 mmol). The reaction was heated to 90 °C and stirred for 18 h. The reaction mixture was cooled to room temperature. Purification by flash column chromatography, eluting with 25% ethyl acetate in hexane gave methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3'-(naptho[2,3-b]benzofuran)-4'-hydroxyphenyl]propanoate (**198**) (0.033 g, 22%) as a yellow solid. Mp 120–124 °C; $[\alpha]_D^{15}$ +67.2 (*c* 0.1, $CHCl_3$); ν_{max}/cm^{-1} (neat) 3357 (NH), 2921 (CH), 2848 (CH), 2358, 1700 (C=O), 1504, 1481, 1394, 1242, 1167; δ_H (400 MHz, $CDCl_3$) 1.43 (9H, s, 3 ×

CH_3), 3.23 (1H, dd, *J* 14.0, 6.2 Hz, 3-*H*H), 3.31 (1H, dd, *J* 14.0, 5.9 Hz, 3-H*H*), 3.74 (3H, s, OMe), 4.63–4.73 (1H, m, 2-H), 5.08 (1H, d, *J* 8.3 Hz, NH), 7.27 (1H, dd, *J* 8.1, 1.8 Hz, 6'-H), 7.45–7.55 (3H, m, 5'-H, 3''-H, 4''-H), 7.83 (1H, d, *J* 1.8 Hz, 2'-H), 7.90 (1H, s, 6''-H), 7.93–7.98 (1H, m, 2''-H), 8.00–8.05 (1H, m, 5''-H), 8.36 (1H, s, 1''-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 38.5 (CH_2), 52.4 (CH_3), 55.0 (CH), 80.2 (C), 107.1 (CH), 111.6 (CH), 119.3 (CH), 122.0 (CH), 124.3 (C), 124.5 (CH), 125.3 (C), 126.0 (CH), 127.9 (CH), 128.5 (CH), 129.6 (CH), 130.3 (C), 130.8 (C), 133.2 (C), 155.2 (2 × C), 157.0 (C), 172.5 (C); *m/z* (ESI) 442.1639 (MNa^+. $C_{25}H_{25}NNaO_5$ requires 442.1625).

4.0 References

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5.0 Appendices

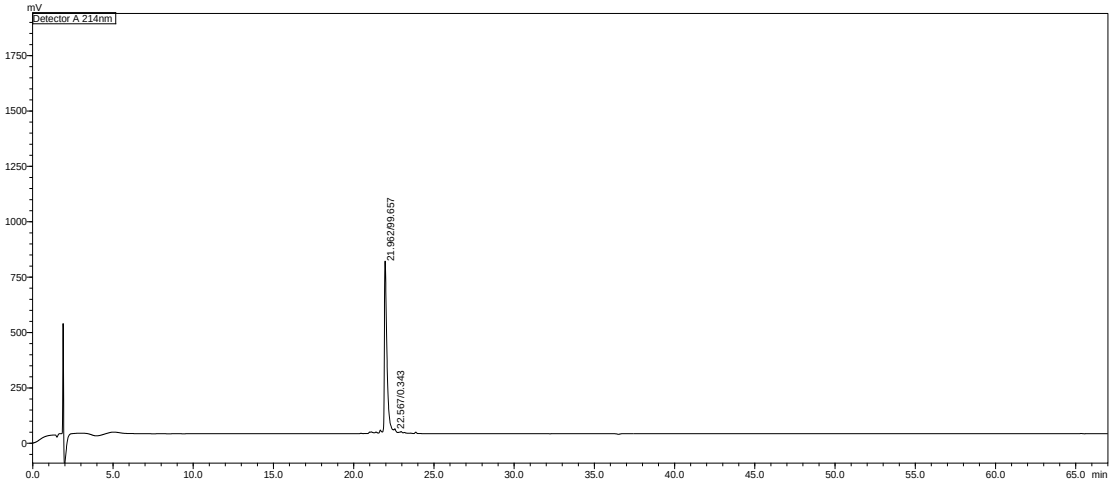

Figure A1: Peptide **159** 50 min gradient HP-LC.

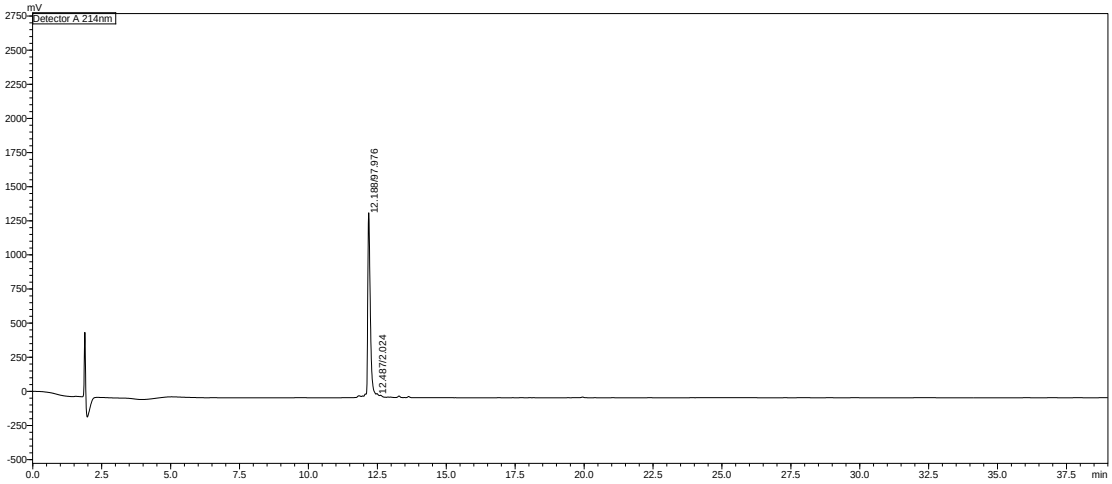

Figure A2: Peptide **159** 20 min gradient HP-LC.

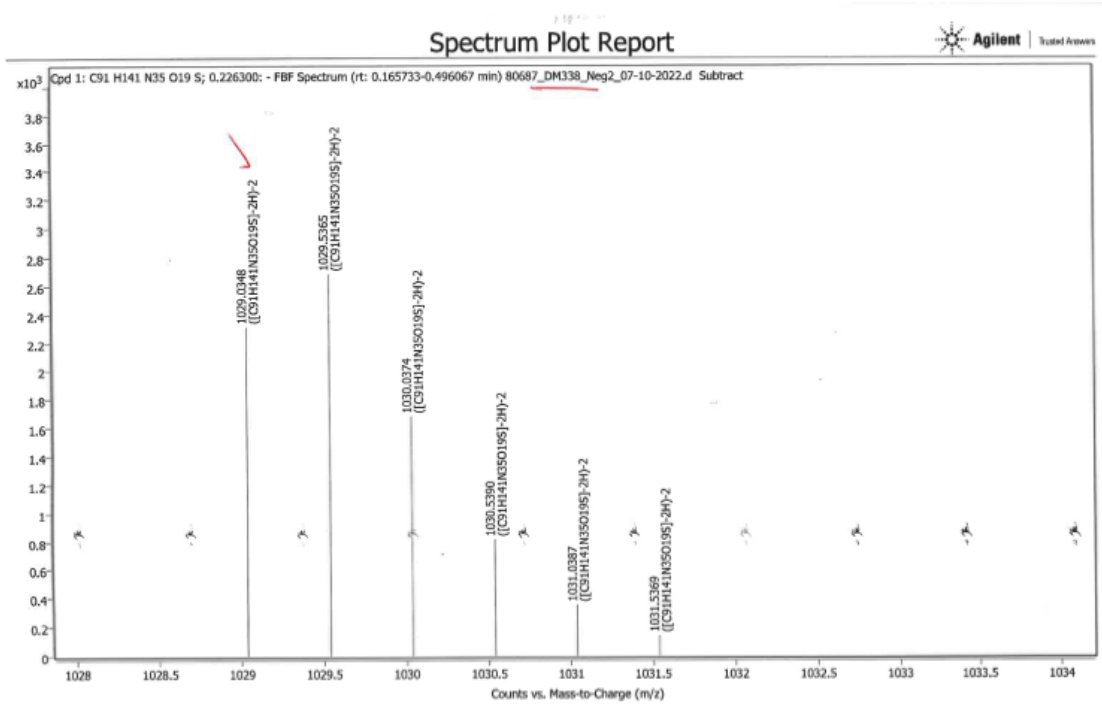

Figure A3: Peptide **159** HRMS.

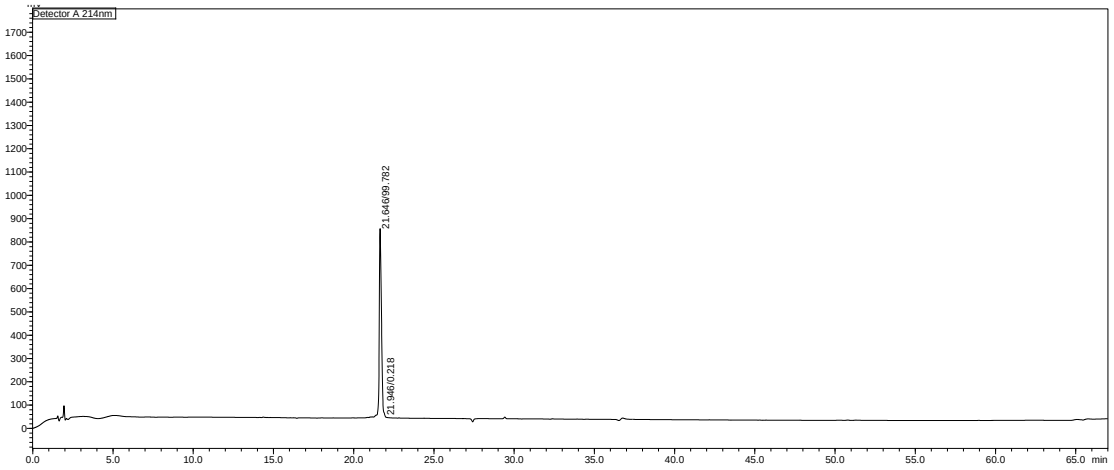

Figure A4: Peptide **161** 50 min gradient HP-LC.

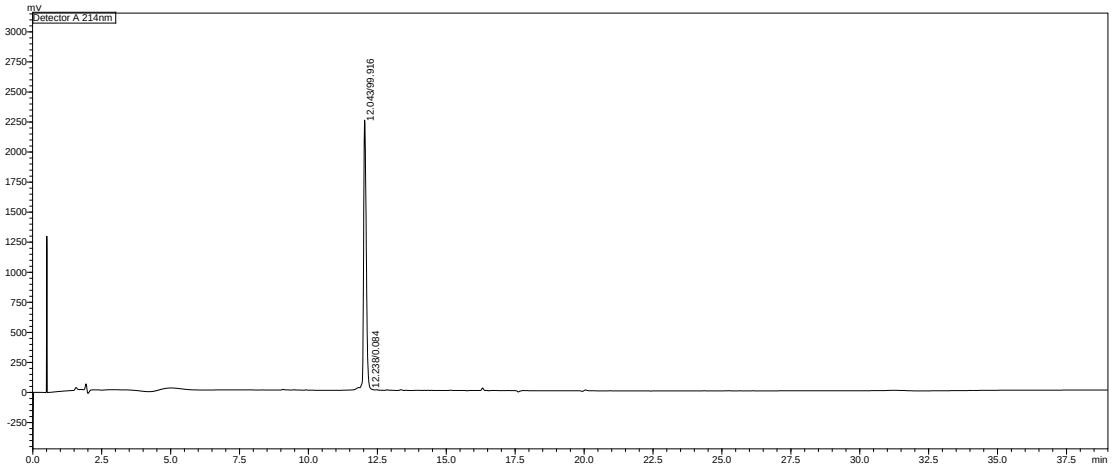

Figure A5: Peptide **161** 20 min gradient HP-LC.

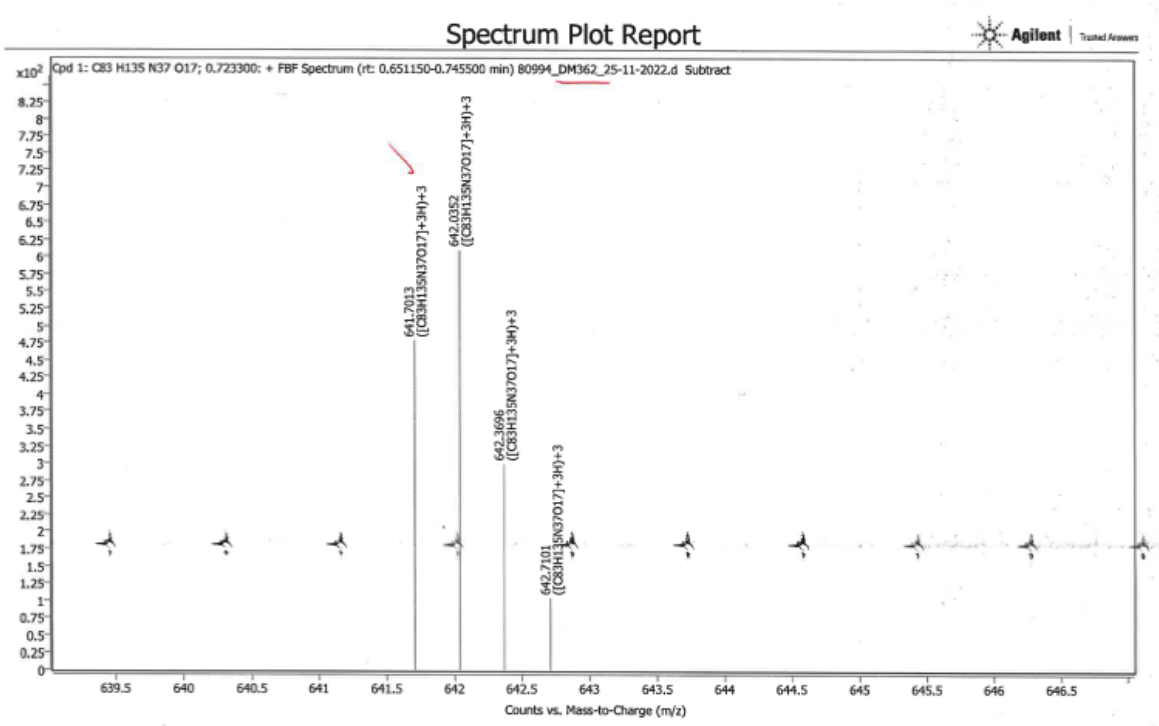

Figure A6: Peptide **161** HRMS.

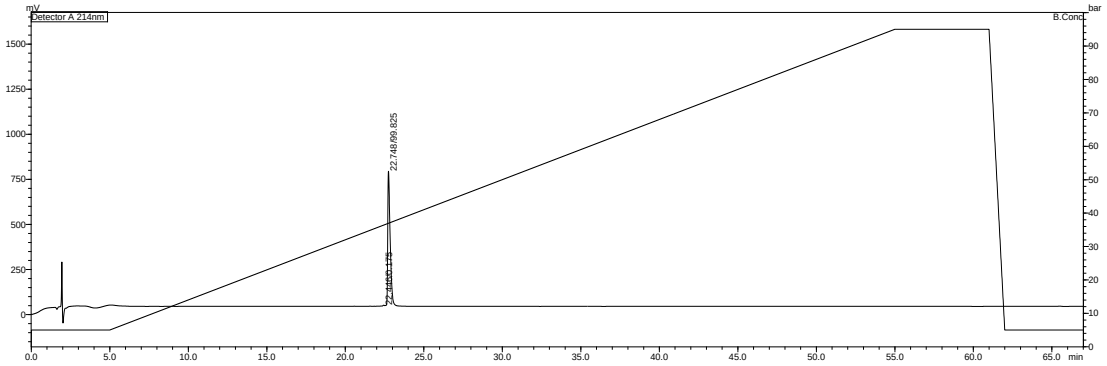

Figure A7: Peptide **162** 50 min gradient HP-LC.

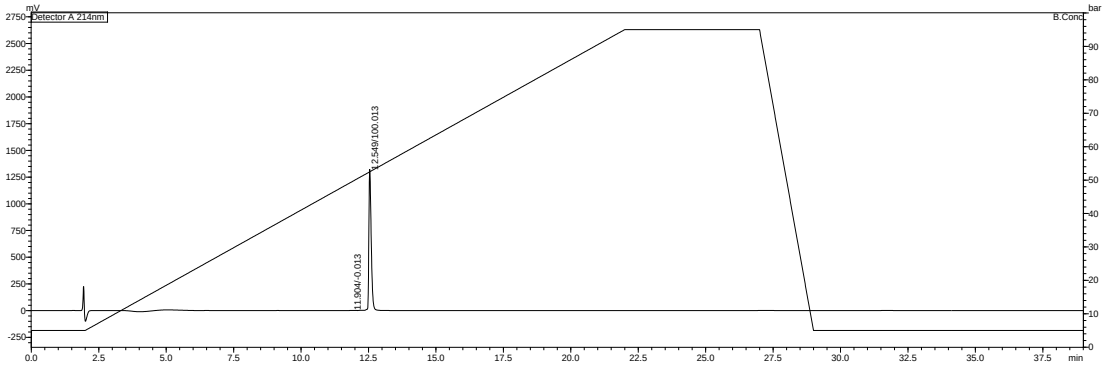

Figure A8: Peptide **162** 20 min gradient HP-LC.

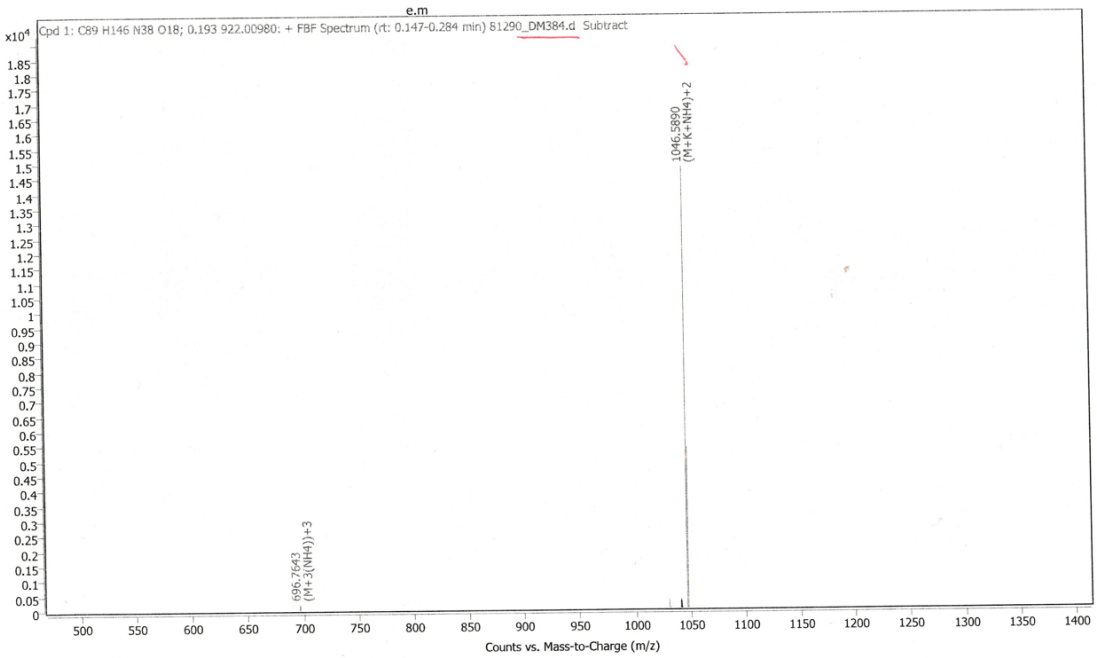

Figure A9: Peptide **162** HRMS