N-Glyoxylamides as versatile precursors for peptide mimics and heterocycles

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N-GLYOXYLAMIDES AS VERSATILE PRECURSORS FOR PEPTIDE MIMICS AND HETEROCYCLES

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

by

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August, 2014

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Thanh Le

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Last but not least, my parents. We have not always seen eye to eye but I do want to take this opportunity to thank you for leading me to where I am now.

Abstract

The work presented in this thesis aims to highlight the versatile reactions of *N*-acylisatins as precursors for the synthesis of novel peptide mimics and heterocycles.

N-arylisatins were prepared *via* Chan-Lam coupling of various electron-deficient arylboronic acids with isatin. Attempts to ring-open these *N*-arylisatins with primary alcohols were unsuccessful. However, when subjected to *tert*-butylamine in refluxing conditions, the corresponding *N*-glyoxylacid salts were obtained along with the *N*-glyoxylamides as minor products.

A novel series of first generation amphiphilic peptide mimics were prepared from the ring-opening reactions of hydrophobic *N*-acylisatins by various amines. Further modification allowed for the introduction of cationic moieties such as those mimicking positively charged amino acids as well as tertiary ammonium groups. An extension of this chemistry was used to prepare a series of second generation peptide mimics from terephthaloyl chloride. By altering the core structure of these compounds the balance of charge and hydrophobicity could be controlled.

The *N*-acylisatin ring-opening strategy was applied to the synthesis of benzene-1,3,5-tricarboxamides (BTA) allowing access to both novel BTA-*N*-glyoxylesters and amides with peripheral alkyl chains. Furthermore BTA-*N*-glyoxylamides with peripheral amino acid esters were prepared in a similar fashion demonstrating the ease with which BTAs can be functionalized using this strategy.

Various *N*-protected amino acids were coupled to isatin forming *N*-amino acyl isatins. These novel *N*-acylisatins participate in ring-opening reaction with either alcohols or amines to give the corresponding *N*-glyoxylesters or amides. Upon deprotection these precursors cyclize to form1,4-benzodiazepin-2-ones bearing C5 pendant amide or ester groups. This is a novel synthetic strategy that provides access to these biologically relevant molecules from cheap and readily accessible starting materials.

A number of strategies were investigated for the design and synthesis of novel cyclic peptide mimics incorporating the *N*-glyoxylamide moiety. Macrocyclization *via* ring closing metathesis (RCM) and macrolactonization were attempted with little success. Macrolactamization of a*N*-glyoxylmethylester containing a β-alanine moiety **6-26** afforded the first cyclic dimer containing two *N*-glyoxylamide units.

List of publications and presentations (to date) based on work described in this thesis:

T. Le, W. C. Cheah, K. Wood, D. St.C Black, M. D. Willcox and N. Kumar, Synthesis of dendrimeric N-glyoxylamide peptide mimics, *Tetrahedron Lett*,.**2011**, **52**, 3645-3647.

Le T., Cheah W.C., Wood K., Black D. StC., Kumar N. Design and synthesis of *N*-glyoxylamide peptide mimics as novel drug delivery systems and antimicrobials. RACI Natural Products One-day Symposium, UOW, Wollongong, NSW, **2011**. (Poster presentation)

Le T., Black D.S., Kumar N. Concise synthesis of 1,4-benzodiazepin-2-ones from *N*-acylamino acid isatins. The NSW Southern Highlands Conference on Heterocyclic Chemistry, Bowral, NSW, **2013**. (Poster presentation)

Le T., Black D. StC., Kumar N. *N*-Glyoxylamides as versatile synthetic precursors for medicinally relevant molecules. Medicinal Chemistry and Drug Discovery symposium, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, **2014**. (Poster presentation)

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List of important abbreviations

BTA = benzene-1,3,5-tricarboxamide

CDI = 1,1'-carbonyldiimidazole

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

DCC = *N,N'*-dicyclohexylcarbodiimide

DCM = dichloromethane

DEAD = diethyl azodicarboxylate

DIAD = diisopropyl azodicarboxylate

DIPEA = *N,N*-diisopropylethylamine

DMAP = 4-dimethylaminopyridine

DMF = *N*,*N*'-dimethylformamide

DMSO = dimethylsulfoxide

EDAC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EEDQ = *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

FA = facially amphiphilic

FDPP = pentafluorophenyl diphenylphosphinate

HBTU = *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate

HDP = host defense peptide

HC_{50} = 50% haemolytic concentration

NMR = nuclear magnetic resonance

NMM = *N*-methylmorpholine

PG = protecting group

pyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

RCM = ring-closing metathesis

THF = tetrahydrofuran

TLC = thin layer chromatography

UV-Vis = UV-Visible

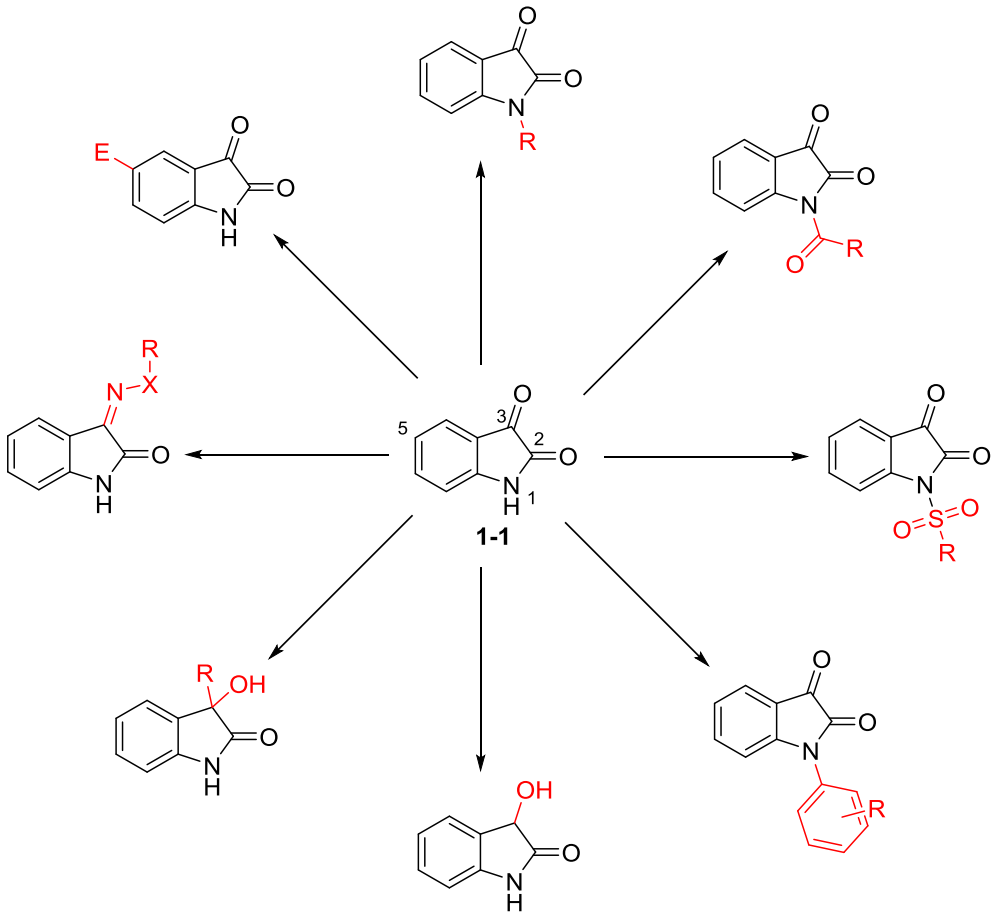
CHAPTER 1

Chapter 1: Introduction. Synthesis and applications of N-glyoxylamides derived from isatin

1.1 Ring-opening reactions of electron-deficient N1 substituted isatins

1.1.1 Isatin chemistry

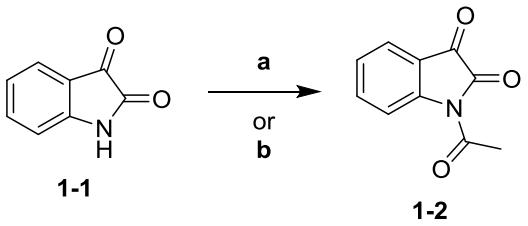
Isatin **1-1** is a synthetically versatile precursor that has been used extensively in the synthesis of biologically and pharmacologically active compounds. Reactions on the isatin skeleton occur on three key positions: N1, C3 and C5. Reactions at N1, including *N*-alkylation,[1] *N*-acylation,[3] *N*-sulphonylation[4] and *N*-arylation[5] (Scheme **1.1**) generally proceed *via* a reactive isatin anion intermediate formed from the deprotonation of isatin. Nucleophilic addition to the ketone group at C3 takes place with alkyl- or aryl-lithium or Grignard reagents to form the corresponding alcohol.[6,7] Reaction with nitrogen nucleophiles such as amines,[8] hydrazines[9] and hydroxylamines[10] also takes place at C3, giving imine products. Various methods for reducing the C3 carbonyl group into the corresponding secondary alcohol have also been reported, such as hydrogenation or hydride reduction reactions.[11,12] In addition, electrophilic aromatic substitution such as nitration and bromination reactions are possible, reacting preferentially at C5.[13,14] The work reported in this thesis will focus primarily on reactions at the C2 position of isatin **1-1** leading to the formation of *N*-acylisatins and *N*-arylisatins.



Scheme 1.1. Chemical reactivity of isatin **1.1**.

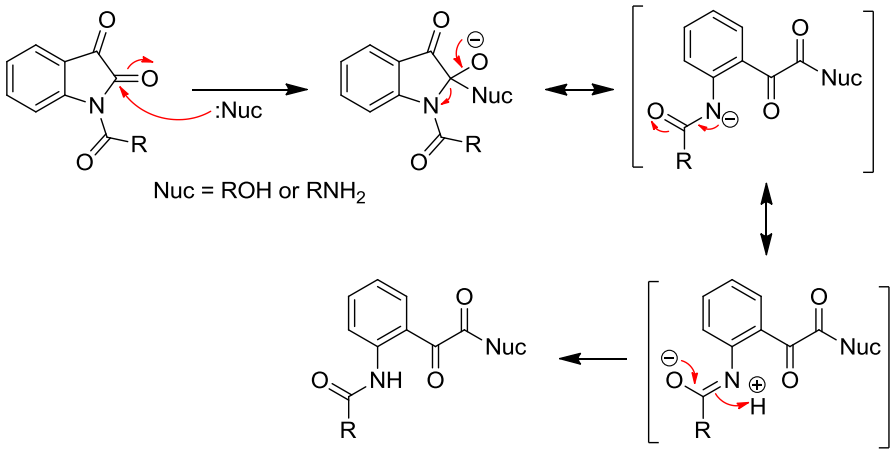
1.1.2 Synthesis and reactivity of *N*-Acylisatins

The preparation of *N*-acetylisatin **1-2** is generally achieved using one of two methods. Direct *N*-acylation with acid anhydrides[3] is possible, but a base catalyst such as triethylamine or pyridine is often added to enhance the rate of reaction (Scheme **1.2**). Alternatively, a milder and more efficient approach has been reported, involving the reaction of an isatin anion intermediate with an acid chloride to yield the desired *N*-acylisatin.[15] The reactive isatin anion intermediate, in turn formed from the deprotonation of isatin with a base such as sodium hydride, can either be isolated or reacted *in situ* with the acid chloride.



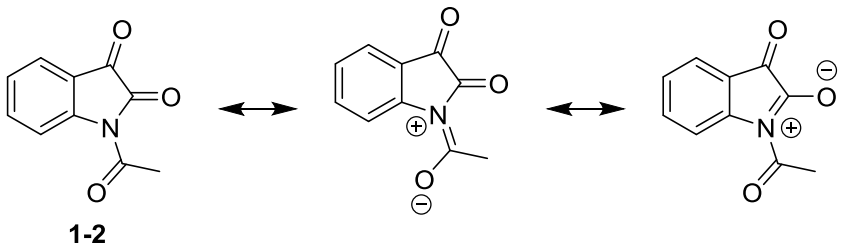
Scheme 1.2. Synthetic routes for the preparation of *N*-acetylisatin. Reagents and conditions: a) acetic anhydride, reflux b) NaH, acetyl chloride, dioxane.

The addition of the *N*-acyl substituent alters the electronic properties of the isatin scaffold and hence influences the reactivity of *N*-acylisatins. As mentioned previously, amine nucleophiles add preferentially at C3 of isatin **1-1**. In contrast, the reaction of *N*-acylisatins with amine or alcohol nucleophiles proceed preferentially at C2 to give the corresponding *N*-glyoxylamides or esters, respectively (Scheme **1.3**).



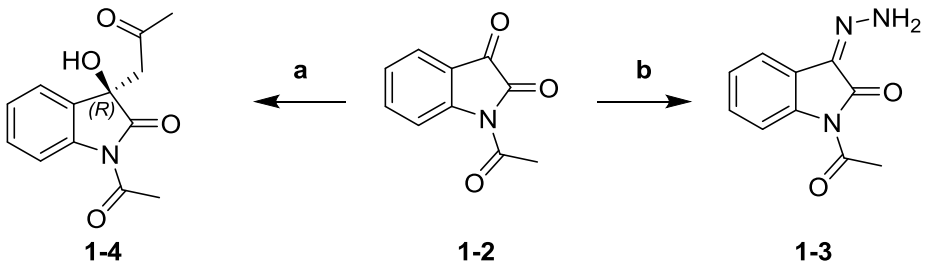
Scheme 1.3. **Ge**neral mechanism of an *N*-acylisatin ring-opening reaction.

The change in reactivity can be rationalized by considering the resonance contributors of *N*-acylisatin **1-2** (Scheme **1.4**). The electron-withdrawing effect of both the C3 carbonyl and *N*-acyl groups reduces the electron density at C2, thus causing the C2 carbonyl group to become more electrophilic compared to the carbonyl group of a typical amide. This effect is observed in the infrared spectrum of *N*-acetylisatin where the C2 carbonyl stretch appears at 1780 cm^{-1}, suggesting reactivity comparable to that of an acid halide.[16]



Scheme 1.4. Resonance contributors for *N*-acetylisatin **1-2**.

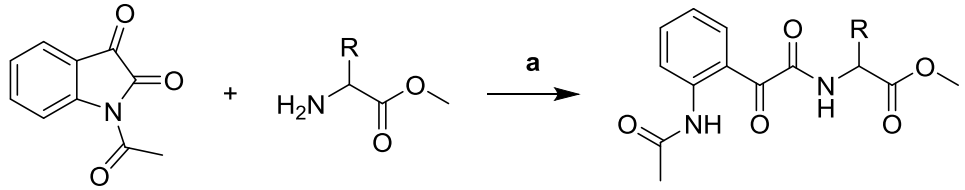
It should be noted that nucleophilic addition reactions at the C3 position of *N*-acetylisatin **1-2** can be achieved by varying the nature of the nucleophile. The reaction of **1-2** and hydrazine hydrate under microwave irradiation gave exclusive formation of the hydrazone **1-3** (Scheme **1.5**).[17] Chen et al. have reported that the *(S)*-proline catalyzed, stereoselective addition of acetone to *N*-acylisatin **1-2** afforded the alcohol **1-4**.[18] However, the work presented in this thesis will focus on reactions occurring at the C2 position of *N*-acylisatins.



Scheme 1.5. Nucleophilic addition reactions at C3 of *N*-acetylisatin **1-2**. Reagents and conditions: a) (*S*)-proline, acetone b) $NH_2NH_2.H_2O$, MeOH, microwave irradiation.

1.1.3 Synthesis of peptide mimics *via* ring-opening reactions of *N*-acylisatins

The nucleophilic ring-opening reaction of *N*-acylisatins with amino acid esters has been exploited for a variety of synthetic applications. The *N*-glyoxylamide obtained from this reaction generates two amide functional groups that make the molecule suitable for further incorporation into peptide or polymer scaffolds. Cheah et al. have reported the facile ring-opening reaction of *N*-acetylisatin **1-2** with the hydrochloride salts of various amino acid methyl esters to give the corresponding *N*-glyoxylamide peptide mimics **1-5-1-9** in excellent yields (85-98 %) (Scheme **1.6**).[19] To liberate the amino group of the hydrochloride salt, sodium bicarbonate was employed as a mild base and the reaction was conducted in a bi-phasic solvent system of CH_2Cl_2-H_2O (2:1) at a temperature of 0 °C to r.t. for 24 h. Additionally, *N*-acetylisatin **1-2** could be reacted with various dipeptides and tripeptides to generate a range of second and third generation peptidomimetics.

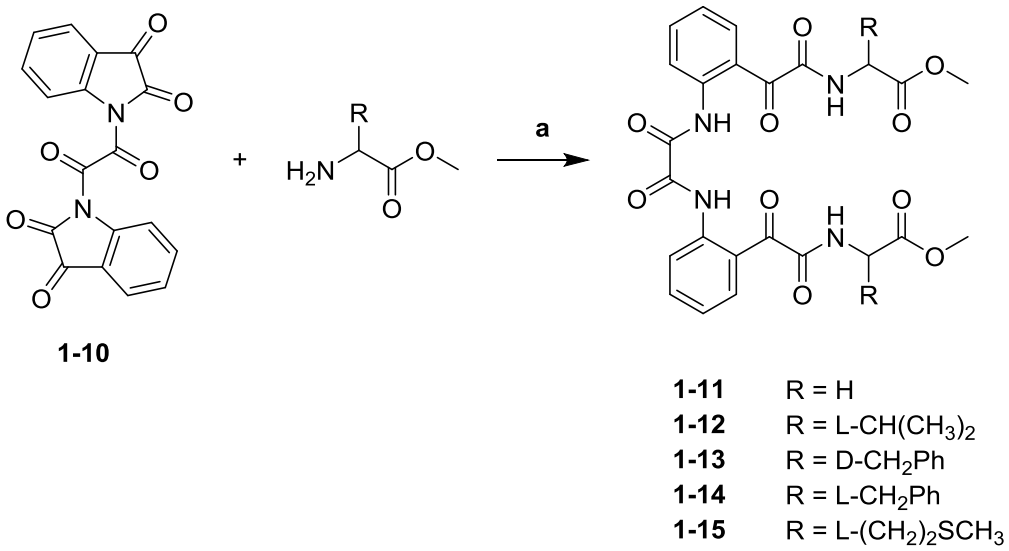


1-2

1-5 R = H
1-6 R = L-$CH(CH_3)_2$
1-7 R = D-CH_2Ph
1-8 R = L-CH_2Ph
1-9 R = L-$(CH_2)_2SCH_3$

Scheme 1.6. Synthesis of *N*-glyoxylamide based peptide mimics. Reagents and conditions: a) $NaHCO_3$, CH_2Cl_2-H_2O (v/v 2:1), 0 °C to r.t., 24 h.

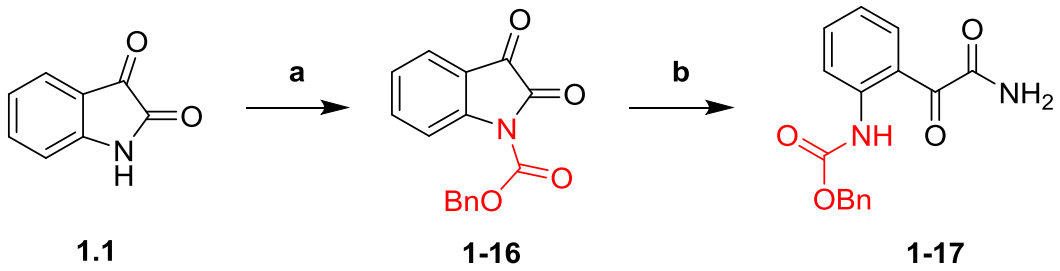
This strategy has also been extended for the preparation of bis-glyoxylamide peptidomimetics. The reactions of oxalyl bis-isatin **1-10** with a range of amino acid methyl esters gave the bis-glyoxylamide dimers **1-11-1-15** in low to medium yields (3-42 %).[20]



Scheme 1.7. Synthesis of bis-glyoxylamide peptidomimetics. Reagents and conditions: a) $NaHCO_3$, CH_2Cl_2-H_2O (4:1 v/v), 0 °C, 24 h.

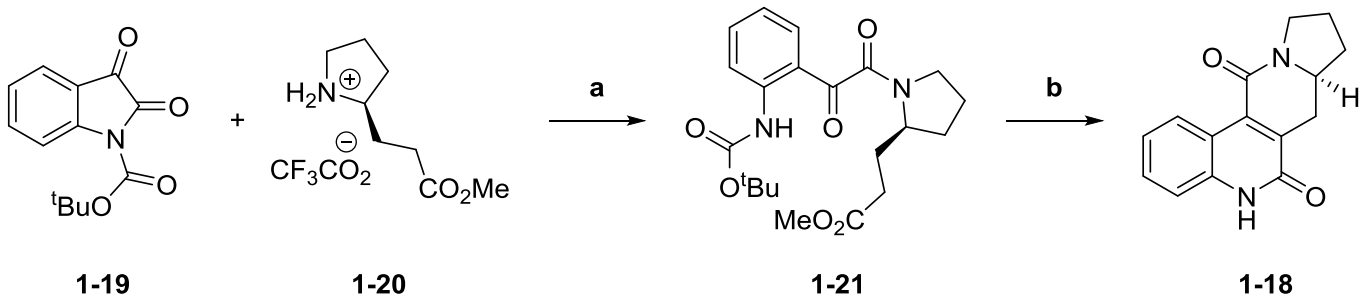
1.1.4 Ring-opening of *N*-substituted isatins containing electron-withdrawing groups at N1

As discussed above, *N*-acylisatins undergo nucleophilic ring-opening reactions with amines and alcohols to give the corresponding *N*-glyoxylamides and esters, respectively. This reaction is facilitated by the electron-withdrawing nature of the *N*-acyl group. *N*-Isatin derivatives containing a carbamate group in place of the amide group have also been shown to participate in ring-opening reactions under similar conditions (Scheme **1.8**). Black et al. have reported the synthesis of *N*-benzyloxycarbonylisatin **1-16** by reacting sodium isatin with benzylchloroformate. Reacting **1-16** with ammonia provided the ring-opened product **1-17** in excellent yield (98 %).[21]



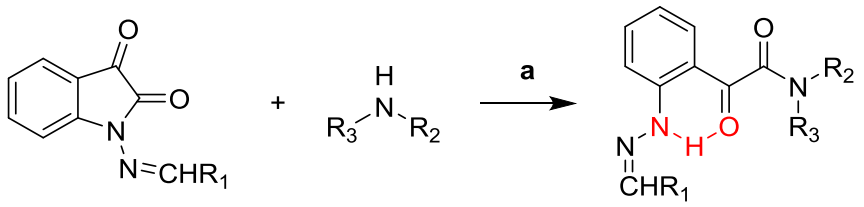
Scheme 1.8. Synthesis and ring-opening reaction of *N*-benzyloxycarbonylisatin **1-16**. Reagents and conditions: a) NaH, benzylchloroformate b) NH_3.

Employing a similar synthetic strategy, Poon and Chiu have reported the synthesis of isaindigotidione **1-18**, a natural product found in the root of *Isatis indigotica Fort.* To construct the tetracyclic carboskeleton, *N*-*tert*-butyloxycarbonylisatin **1-19** was reacted with the trifluoroacetate salt of proline derivative **1-20** in the presence of *N,N*-diisopropylethylamine to afford the ring-opened *N*-glyoxylamide **1-21**. Refluxing intermediate **1-21** with sodium methoxide in methanol gave the desired tetracyclic product **1-18** in excellent yield (96 %) (Scheme **1.9**). This reaction involved intramolecular aldol cyclization, dehydration, acylation and Boc-deprotection occurring in a one-pot operation with great efficiency.



Scheme 1.9. Construction of the isaindigotidione carboskeleton *via* the ring-opening reaction of *N*-*tert*-butyloxycarbonylisatin. Reagents and conditions: a) $^{i}Pr_2NEt$, THF b) NaOMe, MeOH.

Peterson and Heitzer have reported the reactions of 1-(arylmethyleneamino)isatins **1-22-1-24** with amines to give the corresponding *N*-glyoxylamides **1-25-1-27** (Scheme **1.10**).[22] The ring-opening reaction is promoted by the electron-withdrawing *N*-methyleneamino group in place of the *N*-acyl group as described above. The ^{1}H NMR spectrum of the ring-opened products **1-25-1-27** in d_6-DMSO exhibited a peak at approximately 11.8 ppm, corresponding to the NH proton adjacent to the methyleneamino group. The down-field shift of this signal suggests that the NH proton is hydrogen-bonded to the ketone oxygen, forming a pseudo-six-membered ring, which is characteristic of structurally similar *N*-glyoxylamides and esters formed from *N*-acylisatins (Scheme **1.10**, hydrogen bond depicted in red).

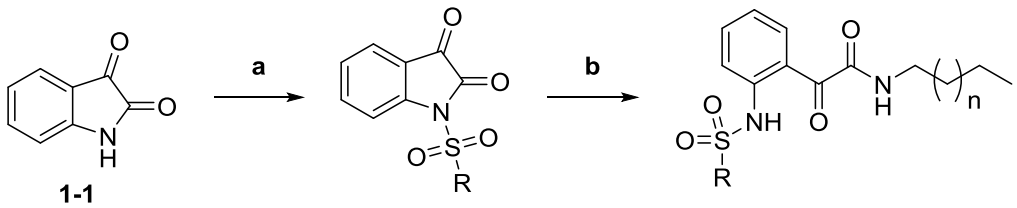


1-22 R_1 = Ph
1-23 R_1 = *p*-Cl-Ph
1-24 R_1 = *p*-NO_2-Ph

1-25 R_1 = Ph R_2 = H R_3 = 2-Furylmethyl
1-26 R_1 = *p*-Cl-Ph R_2 = H R_3 = 2-Pyridylmethyl
1-27 R_1 = *p*-NO_2-Ph R_2,R_3 = Piperidine

Scheme 1.10. Ring-opening reaction of 1-(arylmethyleneamino)isatins with amines. Reagents and conditions: a) THF or toluene, r.t.

N-sulfonylisatins **1-28** and **1-29** can be prepared from the reaction of isatin **1-1** and either methanesulfonyl chloride or benzenesulfonyl chloride in the presence of a triethylamine (Scheme **1.11**). Like the isatin derivatives described above, the *N*-sulfonyl functional group activates the C2 position towards nucleophilic attack by reducing the electron density at C2. Suryanti has demonstrated the ring-opening reactions of **1-28** and **1-29** with n-alkylamines of varying lengths (n = 3, 9 and 15), generating the corresponding *N*-methyl and *N*-phenylsulfonylglyoxylic amides **1-30-1-35** in reasonable yields (48-59 %).[23]



1-28 R = CH_3
1-29 R = Ph

1-30 R = CH_3, n = 3 59 %
1-31 R = CH_3, n = 9 53 %
1-32 R = CH_3, n = 15 48 %
1-33 R = Ph, n = 3 50 %
1-34 R = Ph, n = 9 54 %
1-35 R = Ph, n = 15 52 %

Scheme 1.11. Ring-opening reaction of *N*-sulfonylisatins with *n*-alkylamines. Reagents and conditions: a) sulfonyl chloride, dry CH_2Cl_2, NEt_3 b) CH_2Cl_2, reflux.

1.2 Antibacterial agents derived from host defense peptides

1.2.1 Antibiotic resistance

The emergence of multi-drug resistant bacteria coupled with the reduction in the number of novel antibiotics reaching clinical practice has imposed a major threat to public health.[24,25,26] The majority of conventional antibiotics used today share a common feature in that they act on specific molecular targets (e.g. protein receptors or enzymes). For example, vancomycin, usually considered as a drug of last resort for Gram-positive bacterial infections, binds to terminal D-alanyl-D-alanine motifs of *N*-acetylmuramic acid (NAM)/*N*-acetylglucosamine (NAG) peptides, thereby preventing bacterial cell wall synthesis.[27] Having very well-defined targets, these drugs act with a high degree of selectivity, minimizing unwanted side effects. However, a major limitation of antibiotics targeting a single receptor is the ease at which resistance is developed. It is well-documented that when faced with an environmental stress (i.e. the presence of an antimicrobial agent), a small proportion of a bacterial population may mutate in ways that are beneficial to their survival.[28] Mutations attributed to antibiotic resistance include alteration to target binding sites, enzymatic structural modification of drugs and reduction in permeability of a drug molecule, often through the use of active efflux pumps.[29,30 31] Therefore, efforts have been directed towards the development of compounds acting *via* novel modes of action, which may make these molecules less susceptible to existing modes of bacterial resistance.

1.2.2 Antimicrobial host defense peptides

Host defense peptides (HDPs) are a diverse array of naturally-occurring molecules that have received increasing investigation due to their bactericidal properties. HDPs are typically 10-50 amino acids in length and contain both hydrophilic cationic (i.e. lysine and arginine) and hydrophobic residues. They possess rigid secondary structures that dictate the spatial arrangement of their pendant amino acids. Typically, the cationic and hydrophobic residues are locked on opposite faces of the molecule, to create a facially amphiphilic (FA) architecture comprising α-helix (eg. LL-37) or β-sheet (eg. α- or β-defensins) regions (Figure **1.1**).

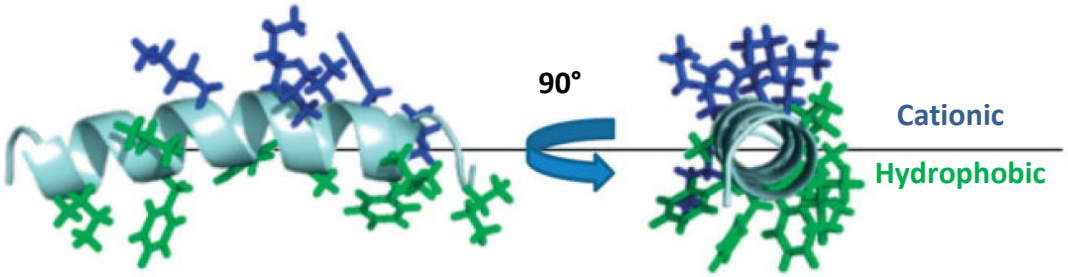


Figure 1.1. α-Helical secondary structure of HDP, magainin-2. Cationic residues are depicted in blue and hydrophobic residues are depicted in green. Both cationic and hydrophobic regions adopt a FA architecture.[32]

Unlike conventional antibiotics, HDPs act *via* non-receptor interactions. Their mechanism of action is predominately attributed to their FA structure. The hydrophilic cationic residues on one face of the HDP facilitate the initial binding of the molecule to the negatively-charged teichoic acids that comprise the bacterial cell surface. Once bound, the hydrophobic groups assist the insertion of the molecule into the membrane, altering the local distribution of membrane lipids. This can lead either to the formation of transmembrane pores causing leakage of cellular components, or the breakdown of the membrane potential, both resulting in cell death. In addition to their membrane-active properties, HDPs have been shown to act *via* other mechanisms leading to bacterial death, such as the induction of apoptosis, meditation of chemokines or activation of autoimmune responses.[33,34] Importantly, HDPs act with a high degree of selectivity towards bacterial cells over the cells of the eukaryotic host. This can be attributed to the fact that bacterial surfaces typically possess a net negative charge while host membranes are usually neutral. Thus, eukaryotic cells lack the electrostatic driving force required for surface attachment. In order for bacterial pathogens to develop resistance towards HDPs, a complete restructuring of the outer membrane chemistry is required, a more complex task that is thought to lower the propensity of HDPs to induce bacterial resistance.

Although HDPs can exhibit potent and selective antimicrobial activity, their implementation as therapeutic agents is hampered by several drawbacks. Firstly, due to the antibacterial nature of HDPs, bacterial fermentation methods cannot be used rendering solid-

phase peptide synthesis the major route of production. However, solid-phase methodologies are time and reagent-intensive and are expensive to adopt on an industrial scale. Secondly, like most peptide therapeutics, HDPs suffer from low oral bioavailability and may require the co-administration of carriers or adjuvants to achieve the desired concentrations of HDPs at the target site. Furthermore, activity assays conducted on HDPs are usually performed in well-defined media that do not accurately reflect true conditions in a biological system. In complex serum, proteins and extra-cellular structures are able to non-specifically sequester HDPs, leading to a decrease in functional activity.[35,36] These drawbacks have stimulated the development of therapeutic biomimetic agents based on antimicrobial HDPs.

1.2.3 Antibacterial peptidomimetics

To address the limitations of natural HDPs, synthetic peptidomimetics have been developed. Initially, difficulties in the design of these antibacterial agents arose due to the structural diversity of HDPs coupled with the lack of a specific molecular target. Early studies determined that the rigid secondary structure of these peptides was the key element in conferring antimicrobial function. Further studies have advanced this view by revealing that the balance of local amphiphilicity and the ability of the cationic and hydrophobic groups to self-organise on the bacterial cell surface are the major prerequisite for activity. These structural requirements are satisfied by various synthetic HDPs reported in the literature (Figure **1.2**).[37]

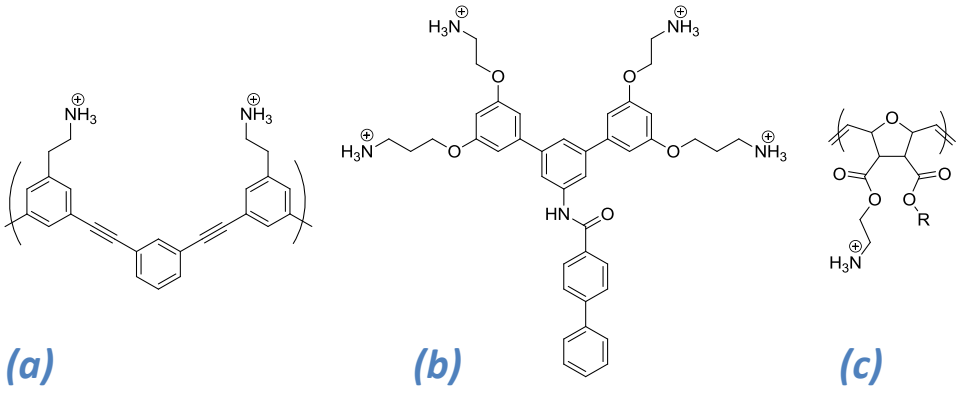
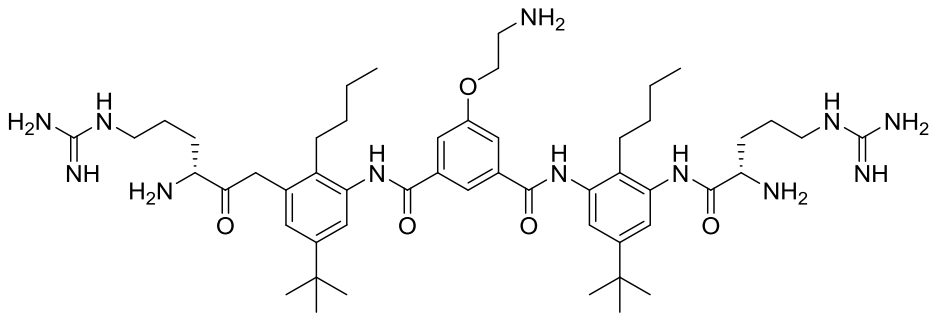


Figure 1.2. Examples of synthetic HDP mimics based on ***(a)*** polyphenylene ethynylene polymer,[38] ***(b)*** polyaryl,[37] ***(c)*** norbornene polymer.[39]

One of the major challenges faced by antimicrobial peptidomimetics is achieving selectivity for bacterial cells. Collective studies have indicated that these mimics become more potent against bacteria with increasing hydrophobicity, although a threshold is reached where their lowered solubility in aqueous media causes aggregation and precipitation. At the same time, however, these molecules tend to become more toxic to mammalian cells as hydrophobicity is increased. By altering the balance between the hydrophobic and hydrophilic groups, Yang and co-workers have been able to fine-tune the selectivity of a series of antimicrobial oligomers towards bacterial cells over erythrocytes. In particular, dimer **1-36** (Figure **1.3**) possessed remarkable selectivity, showing MIC values of 12.5 μgmL^{-1} for both E.coli and S.aureus while exhibiting an HC_{50} (50% haemolytic concentration) value of >800 μgmL^{-1} against human red blood cells.[40]



1-36

Figure 1.3. Fine-tuning of cationic and hydrophobic groups in arylamide oligomer **1-36** conferred remarkable selectivity for bacterial cells over erythrocytes.

Synthetic peptidomimetics are unable to discriminate between Gram-negative and Gram-positive bacteria due to similarities in their membrane structure and surface charge, thus these molecules exhibit broad spectrum antibacterial activity.

1.4 Benzene-1,3,5-tricarboxamides in supramolecular chemistry

1.4.1 Non-covalent interactions and their role in the cooperative self-assembly of supramolecular structures

Inter- and intra-molecular interactions play a pivotal role in the self-assembly of ordered superstructures.[41] H-bonding, electrostatic interactions, metal-ion complexation, hydrophobic interactions as well as combinations thereof can be programmed into the molecular structure of building blocks. Spontaneous organization of these functional molecules is achieved by subjecting them to specific thermodynamically controlled conditions. Hence, understanding the complexity of these non-covalent, cooperative linkages is essential for the rational design of well-defined supramolecular architectures. The successful manipulation of these weak interactions has given rise to a plethora of functional supramolecular structures with remarkable physical properties. By exploiting the hydrogen bonding networks inherent in ureido-pyrimidinones, Dankers et al. have reported the development of bioactive materials capable of binding to fibroblasts in a strong and specific manner (Figure **1.4**). These materials have potential uses in regenerative medicine, drug delivery, and tissue engineering applications.[42,43]

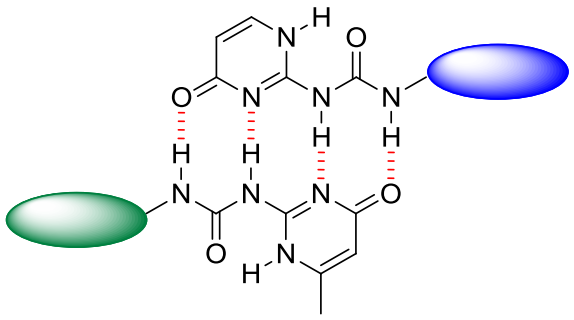


Figure 1.4. Quadruple hydrogen bonding network between ureido-pyrimidinone molecules reported by Dankers et al. for the development of biomaterials. Each ureido-pyrimidinone molecule is attached to a functional polypeptide fragment (depicted in green and blue) capable of cell surface recognition and binding.

1.4.2 BTAs as versatile scaffolds for supramolecular chemistry

The benzene-1,3,5-tricarboxamide (BTA) motif is an important supramolecular building block that has found use in applications ranging from nanotechnology to polymer chemistry

and biomedical applications.[44,45] BTA molecules are comprised of a benzene core with three amides appended *via* the carbonyl group or nitrogen atom at the 1, 3, and 5- positions. The variable groups attached to the amide linkers in BTAs can be any functional group, and when all three groups are identical, the BTA molecule is C_3-symmetric. However, the introduction of different variable groups to a single BTA molecule removes this symmetry. This body of work will focus primarily on C_3-symmetric, carbonyl-centered BTAs (Figure **1.5**).

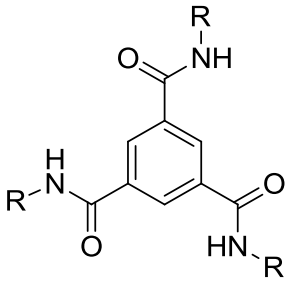


Figure 1.5. General chemical structure of a C_3-symmetric carbonyl-centered BTA molecule.

Under selected conditions, the amide groups can participate in a three-fold inter-molecular H-bonding network, where each molecule aligns and stacks parallel to the next molecule, forming a helical secondary structure (Figure **1.6**). Irrespective of the helical sense of the column, all amides point in the same direction and stack head to tail. Hence, the individual dipole moments of each amide summate to give a macroscopic dipole along the columnar structure.[46]

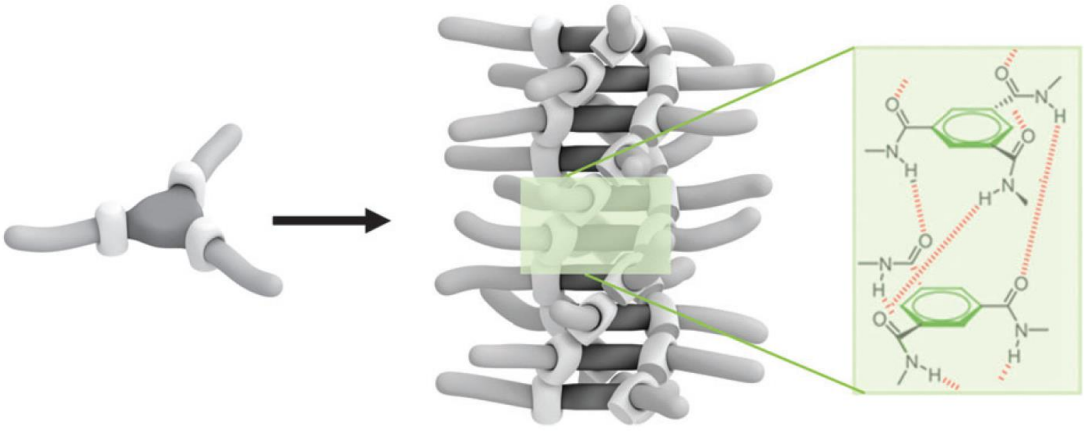


Figure 1.6. Schematic representation of BTA self-assembly into helical one-dimensional aggregates, stabilized by three-fold intermolecular H-bonding.

1.4.3 Application of BTAs

Varying the chemical nature of the substituents on the BTA core provides a means of tuning their supramolecular properties towards a specific application. This has been achieved to great effect in the literature. For example, phenylglycine and F_5-phenylglycine-based BTAs with peripheral Gd(III)-complexes **1-37** have been developed as magnetic resonance imaging contrast agents (Figure **1.7** ***(a)***).[45] Studies have established that the self-assembly of these molecules is highly dependent on the total charge of the metal chelate complexes as well as the salt concentration.[47] By manipulating these properties, nano-sized aggregates (typically 6 nm) can be formed with properties that are ideal for their purpose.

The most notable success in BTA research is the recent commercialization of **1-38** as a nucleating and clarifying agent for the bulk polymer isotactic polypropylene (*i*PP).[44,48] The addition of **1-38** in low concentrations (typically 200 ppm) increases the crystallization temperature of *i*PP, limiting its spherulite size (Figure **1.7** ***(b)***). This increases the optical transparency of the resulting polymer, which in the absence of BTA **1-38**, crystallizes as an opaque material. The addition of this nucleating agent also improves the thermal stability of the material.

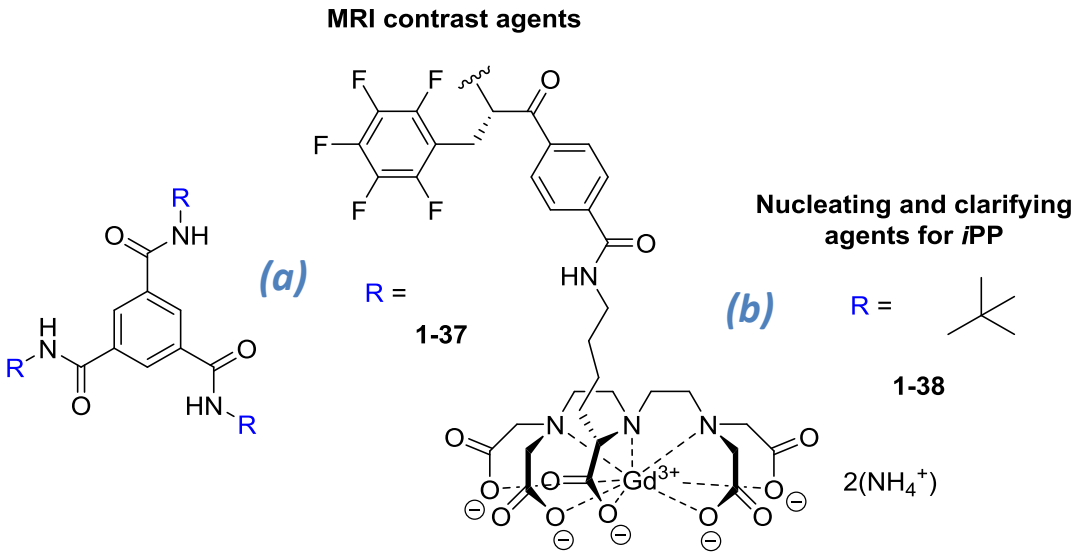
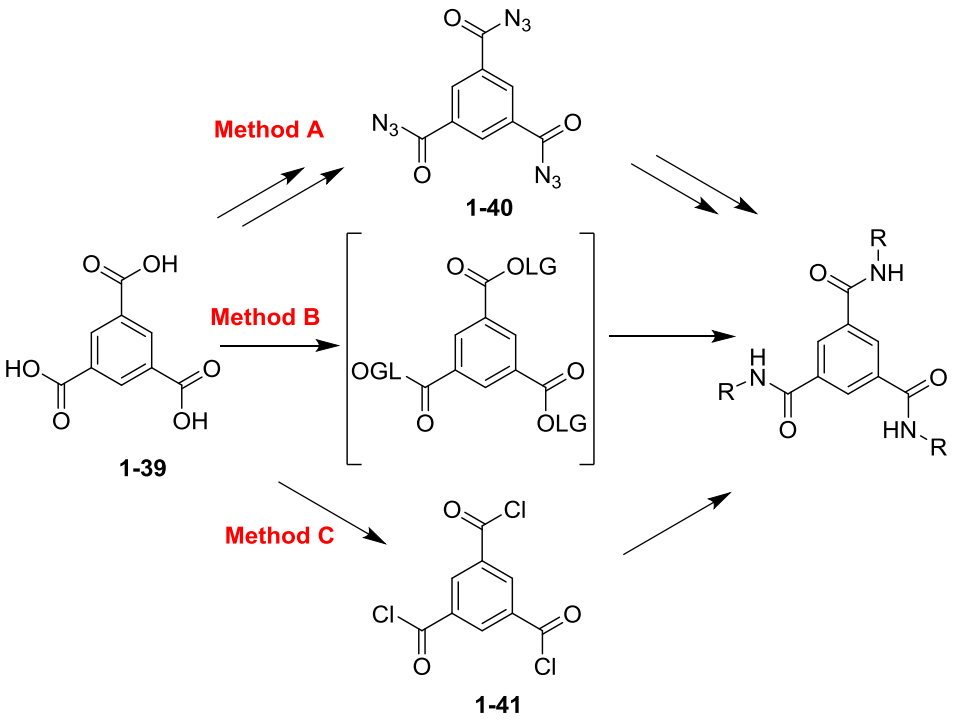


Figure 1.7. Examples of developed BTAs. The nature of the side chains appended to the BTA skeleton determines their properties and applications.

1.4.4 Synthesis of BTAs

Since their first reported synthesis in 1915 by Curtius, carbonyl-centered BTAs have been prepared *via* three strategies, all of which employ benzene-1,3,5-tricarboxylic acid (trimesic acid) **1-39** as a starting material.[49] The first involves the conversion of trimesic acid **1-39** into the reactive triacyl triazide intermediate **1-40** through a number of steps, followed by treatment with an amine to give the corresponding tri-substituted BTA (Scheme **1.12**, Method **A**).[50,51,52] However, this route suffers from a number of drawbacks. The first is that several functional group conversions are required to obtain the triacyl triazide **1-40**. Additionally, the azide intermediate **1-40** is explosive under mild pressure and heat, limiting large scale preparation.



Scheme 1.12. Synthetic strategies for substituted BTAs from trimesic acid. Method **A**: formation of a triacyl triazide intermediate **1-40**. Method **B**: use of peptide coupling reagents for the *in situ* generation of activated ester. Method **C**: Formation of reactive trimesic chloride **1-41** using thionyl chloride.

The second method provides a direct route to BTAs. The reaction of trimesic acid with a peptide coupling reagent forms an activated ester *in situ*, which is followed by the addition of

an amine to afford the BTA in a single step (Scheme **1.12**, Method **B**). A number of coupling reagents have been reported for this strategy, including DCC, EDAC and EEDQ.[53,54,55] In the third strategy, which is currently the most popular synthetic method for the synthesis of substituted BTAs, a trimesic chloride intermediate **1-41** is first prepared from the reaction of trimesic acid **1-39** with thionyl chloride. Subsequent amidation of trimesic chloride **1-41** can be achieved at room temperature with the assistance of a base. Reaction times are reduced as a result of the high reactivity of the acid chloride intermediate **1-41**.[56] As trimesic chloride **1-41** is commercially available, this method allows a single-step route to carbonyl-centered BTAs (Scheme **1.12**, Method **C**).

1.4.5 Self-assembly of the N-glyoxylamide motif and incorporation with BTAs

BTAs bearing the *N*-glyoxylamide moiety are unexplored in the literature. Incorporation of this functional unit into the well-established BTA core allows for further hydrogen bonding opportunities which can potentially assist with self-assembly owing to the presence of the additional H-bond accepting keto groups and the variable glyoxylamide torsion angle. As an example, crystal structure data of the monomeric *N*-glyoxylamide **1-42** exhibits five intra-molecular H-bond interactions N1B—H1NB---O2B (H1NB---O2B = 2.04 Å), N2B—H2NB---O2B (H2NB---O2B = 2.41 Å), C3B—H3B---O3B (H3B---O3B = 2.45 Å), C11B—H11C---O3B (H11C---O3B = 2.55 Å) and C6B—H6B---O1B (H6B---O1B = 2.30 Å) (Figure **1.8** ***(a)***, intramolecular H-bonds depicted in sky blue). In addition, intermolecular H-bonds are also present in **1-42** (Figure **1.8** ***(b)***, intermolecular H-bonds depicted in magenta). Collectively, these interactions promote the self-assembly of the basic *N*-glyoxylamide unit into an ordered crystal lattice.

Appending the *N*-glyoxylamide motif to the BTA core will add further complexity to the network of interactions present in BTAs. Hence, exploring the self-assembly of this novel class of BTAs can potentially uncover advanced, functional materials. It will also provide a more rounded understanding of how small changes in molecular structure can affect aggregation properties.

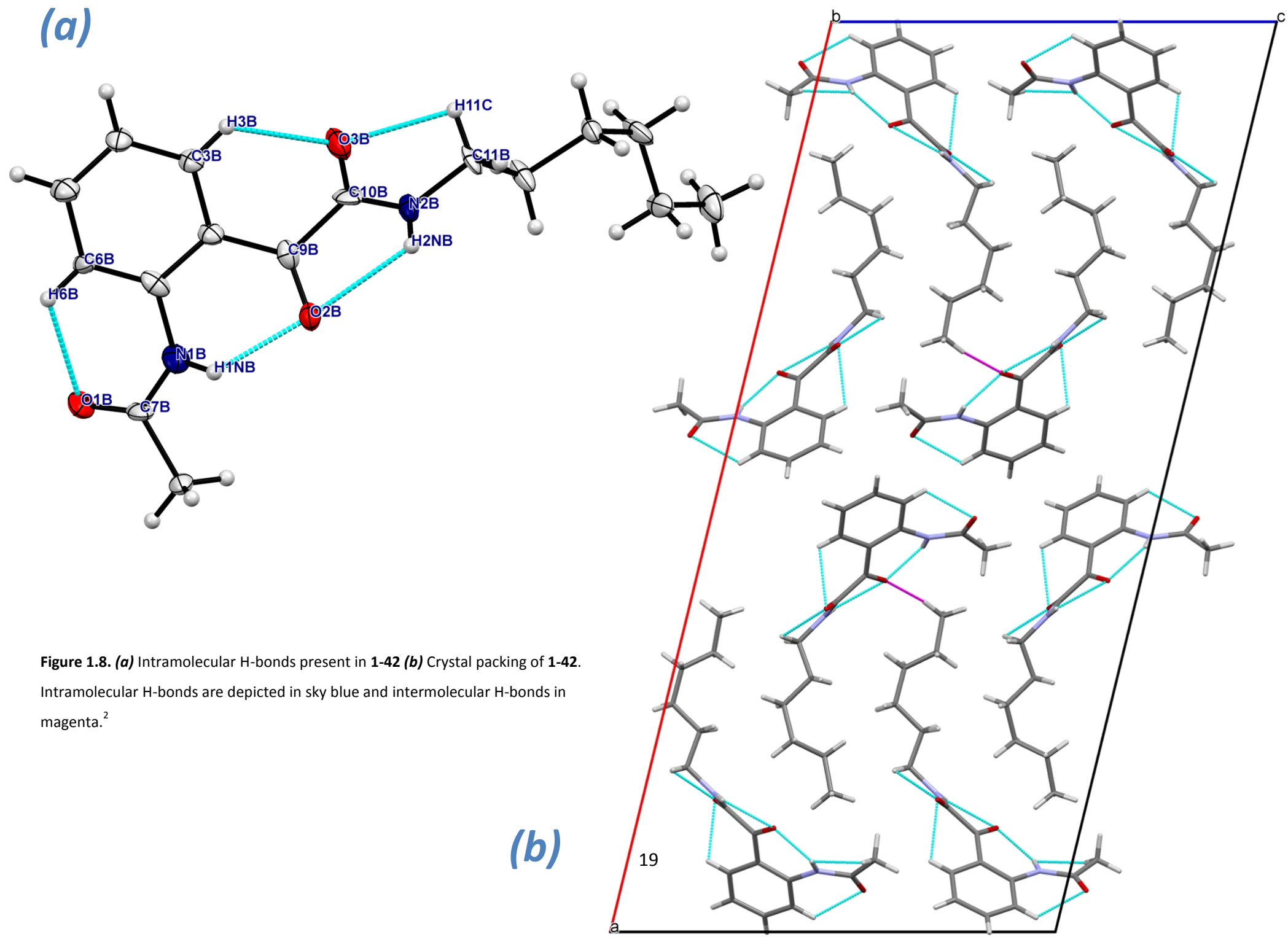


Figure 1.8. ***(a)*** Intramolecular H-bonds present in **1-42** ***(b)*** Crystal packing of **1-42**. Intramolecular H-bonds are depicted in sky blue and intermolecular H-bonds in magenta.[2]

1.5 Cyclic peptides: design and synthetic strategies

1.5.1 Cyclic peptides as therapeutic agents

Cyclic peptides represent an important class of naturally occurring macrocycles isolated from plants, microorganisms and marine sources. Screening of these unique compounds has revealed a wide spectrum of biological activity, from anticancer to antibacterial activity. For example, Kahalalide F **1-43**, a potential drug candidate about to enter phase II clinical trials, exhibits potent and selective cytotoxic activity against a panel of human prostate and breast cancer cell lines with minimal effects on non-tumor human cells (Figure **1.9**).[57,58]

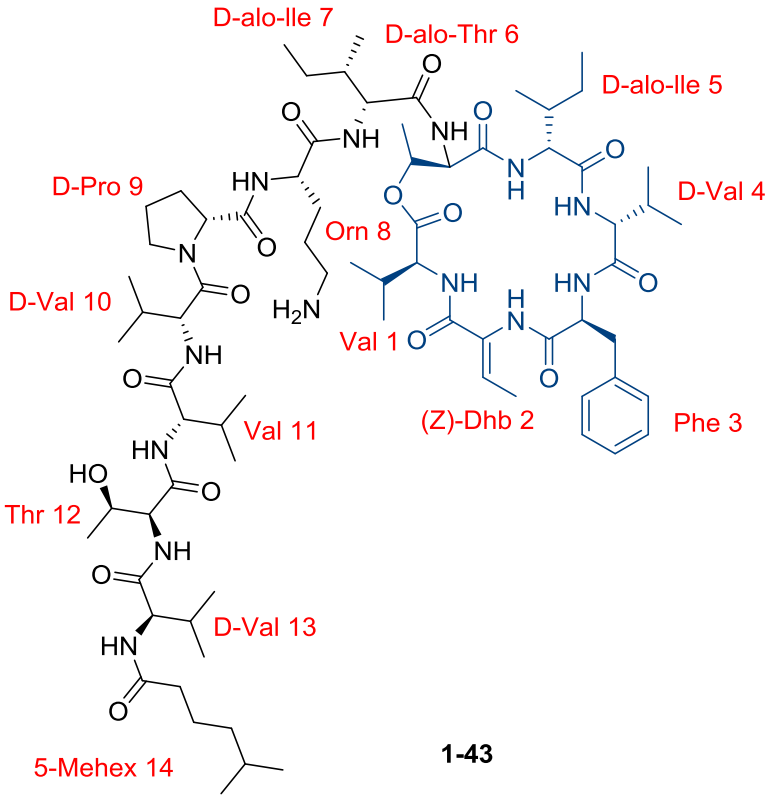


Figure 1.9. Chemical structure of natural Kahalalide F **1-43**. The cyclic peptide motif is highlighted in blue.

Many cyclic peptides have been approved for use as therapeutic agents. Examples include octreotide, calcitonin, cyclosporine A, polymixin and colistin.[59] In contrast to their linear counterparts, cyclic variants possess a higher degree of conformational rigidity and therefore

are more pre-organized. This characteristic offers a distinct advantage for the development of peptide therapeutic agents. On binding to a receptor, the loss of entropy for a cyclic peptide is lower than that of the acyclic counterpart, resulting in the cyclic variant possessing higher receptor binding affinity. In addition, conformationally constrained ligands tend to have greater receptor selectivity as they are unable to take up alternate conformations permitting competitive binding. For example, the development of cyclic peptides as inhibitors of the MCL-1 protein has delivered potent and cell-permeable drug candidates with unprecedented selectivity. Another associated advantage of a cyclic peptide structure is the decreased sensitivity to both exo- and endoprotease degradation, hence these biomolecules exhibit greater bioavailability then linear peptides.[60] This class of molecules is therefore sought after as promising lead compounds in drug development.

1.5.2 Cyclic peptides as molecular tools for investigating the functional conformation of a receptor

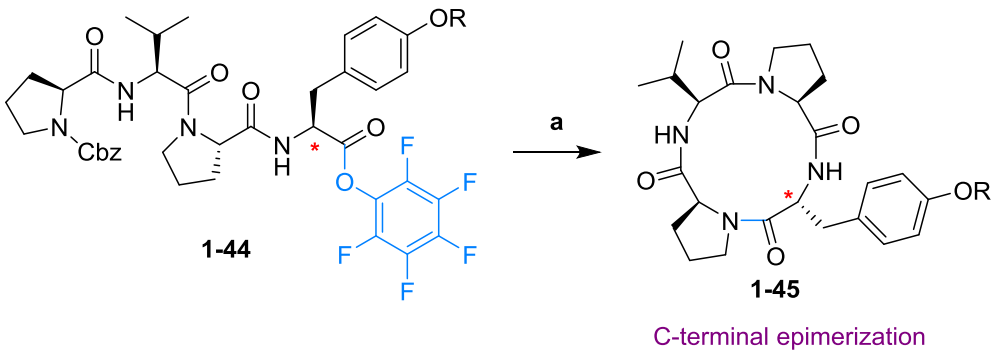
Ligand based drug discovery has been shown to be an effective strategy for generating hit compounds in the drug discovery process. Ligands with high affinity for a specific receptor target are used to create pharmacophores. These drug discovery tools incorporate the essential features required for a ligand to bind to a particular receptor and can be used to screen large chemical libraries generating hit compounds.[61] One of the major limitations associated with this approach is that flexible ligands in particularly linear peptides tend to exist in a myriad of conformations in solution. On binding to a target usually only a single conformation is used in the design of the pharmacophore, this can be difficult to determine if the ligand exists in multiple conformations. Cyclic peptide ligands offer the benefit of a rigid system, allowing the straightforward determination of the active conformation. Thus this class of macrocycles is useful for modelling effective pharmacophores.

1.5.3 General synthetic considerations for the macrocyclization of linear peptides and peptide mimics

The macrocyclization or final ring-closing reaction of a linear peptide or peptide mimic is often the most challenging step in the synthesis of both bioactive macrocyclic peptides and their mimics. The linear precursor must adopt an entropically disfavored conformation for the reactive termini to come in close spatial proximity prior to ring-closure. Depending on the functional groups present in an acyclic peptide or peptide mimic the macrocyclization can occur in four different ways: head-to tail, head-to-side chain, side chain-to-tail or side-chain-to-side-chain. The work described herein will focus on peptide mimics cyclized in a head-to-tail fashion.

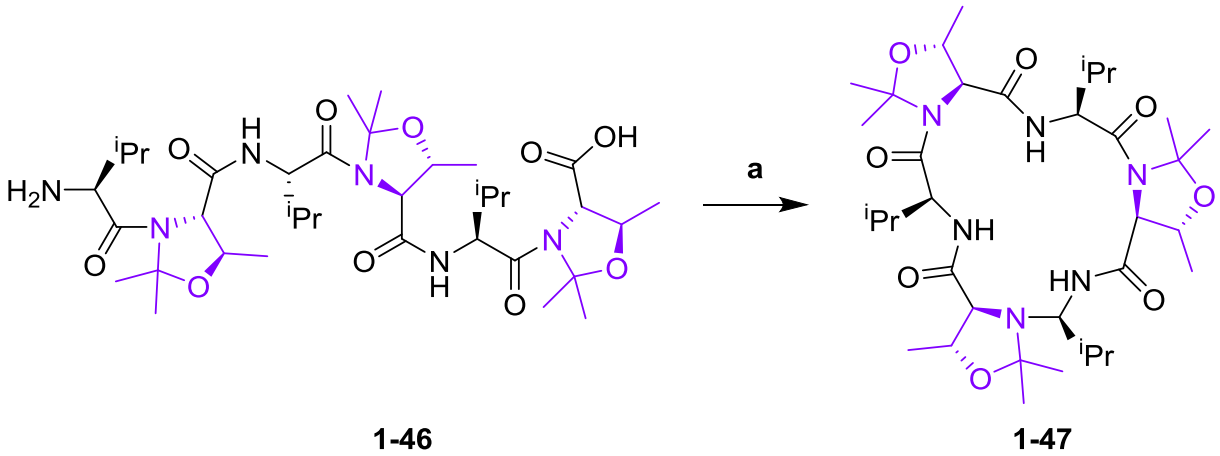
Macrocyclization is an inherently slow reaction and often the rate determining step in the synthesis of cyclic peptide and their mimics. Intramolecular cyclization reactions forming these macrocycles are best performed under high dilution (10^{-3}-10^{-4} M) to minimize undesirable oligomers and polymers. This can be achieved by two common methods. High dilution can be maintained with the use of one or multiple syringe pumps.[62] Alternately, macrocyclization can take place on solid phase support causing a pseudodilution effect as substrate molecules are less likely to encounter one another.[63,64]

To improve the rate of cyclization, internal conformational elements can be modified or incorporated into the design of the linear precursor. The ring disconnection carries significant strategic importance and can dictate the level of success of the reaction. The cyclization site should not be sterically encumbered by residues containing bulky substituents such as isoleucine or valine residues. Ring closures at *N*-terminal *N*-methyl residues usually proceed slowly hence, disconnection at these sites is not recommended. For example, the attempted macrocyclization of terminal glycine and *N*-methyl alanine to form Tentoxin by Rich, proceeded in low yield (18%).[65] Studies have shown that the rate of cyclization proceeds faster between two amino acid residues of opposing stereochemistry than that of residues with equal configuration.[66,67] Attempts to cyclize the acyclic pentafluorophenylester precursor **1-44** to all-L-isomer of *cyclo*-[Pro-Val-Pro-Typ], a known tyrosinase inhibitor by Schmidt and Langner resulted in 31% yield of the C-terminal epimerization product **1-45** (Scheme **1.13**).[68]



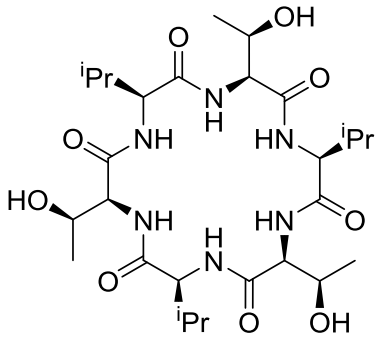
Scheme 1.13. C-terminal epimerization of tyrosinase inhibitor cyclo-[Pro-Val-Pro-Typ]. Reagents and conditions: a) H_2, Pd/C, Δ.

Mimicry of secondary protein structure, in particular reverse turns is a strategy commonly used for bringing the termini proximal in space. Introduction of a cis-amide bond in the midpoint of a peptide chain can form an analogous β-turn moiety therefore minimizing the end-to-end distances. Examples of turn inducing elements which induce cisoid conformation of the amide bond include proline, pseudo-prolines and *N*-methyl amino acids.[69,70] Jolliffe and co-workers have utilized pseudoprolines derived from the cyclocondensation reaction of threonine and acetone to facilitate the macrocyclization of linear precursor **1-46** to the protected cyclic peptide **1-47** in excellent yield (91 %)(Scheme **1.14**).



Scheme 1.14. Cyclization of pseudoproline (depicted in purple) containing linear hexapeptide **1-46**. Reagents and conditions: a) FDPP, DIPEA, CH_3CN.

Subjecting **1-47** to a solution of hydrochloric acid and dioxane or trifluoroacetic acid afforded the desired cyclic hexapeptide **1-48** devoid of turn-inducers (Figure **1.10**).[71] Interestingly, attempts to prepare target **1-48** *via* the cyclization of TBS side-chain-protected linear peptide H-Val-Thr(TBS)-Val-Thr(TBS)-Val-Thr(TBS)-OH was unsuccessful under a range of conditions.[72]



1-48

Figure 1.10 Cyclic hexapeptide **1-48**.

Hunter et al., have demonstrated the rapid cyclisation of a linear heptapeptide **1-49** with a flexible GABA residue placed strategically at the mid-point of the linear precursor. ROSEY experiments conducted on the acyclic peptide suggest the peptide exhibits sufficient conformational flexibility to bring the head and tail proximal in space (Figure **1.11**).[73] Noteworthy, both terminal residues are of opposite configuration.

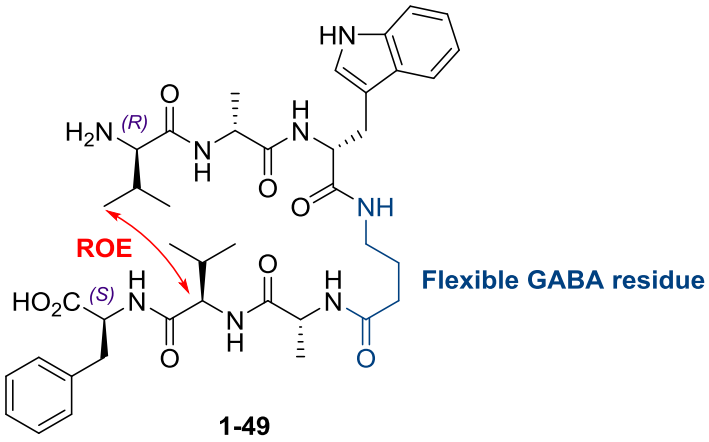


Figure 1.11 Linear heptapeptide precursor to Unguisin A. Disconnection of the cyclic peptide is made to place conformationally flexible GABA residue (depicted in blue) in the middle of the linear peptide. A long-range correlation between γ-H of valine #1 and α-H of valine #2 was observed in the ROESY spectrum indicating close proximity between the head and tail of the linear peptide (shown in red).

Intra-peptide hydrogen bonds can facilitate the angular requirement for both termini in the transition state, reducing the free energy of loop closure. These intramolecular interactions can be identified by X-ray crystallography[74] or molecular modelling. Recently Krishna et al. have incorporated non-protenogenic furan-based Z-vinylogous γ-amino acid, possessing an intra-residual hydrogen bond into various linear tetrapeptides (Figure **1.12**). Cyclization of these otherwise strained precursors proceeded in good yields (52-60%). Furthermore the intra-peptide hydrogen bond is also present in the final cyclic peptide increasing its conformational rigidity.

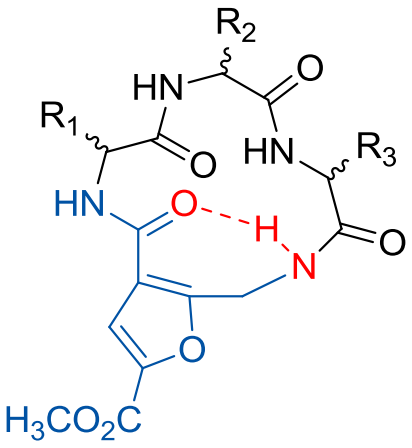


Figure 1.12 Cyclic tetrapeptides possessing non-proteinogenic furan-based Z-vinylogous γ-amino acid (depicted in blue). Intra-residual hydrogen bond depicted in red.

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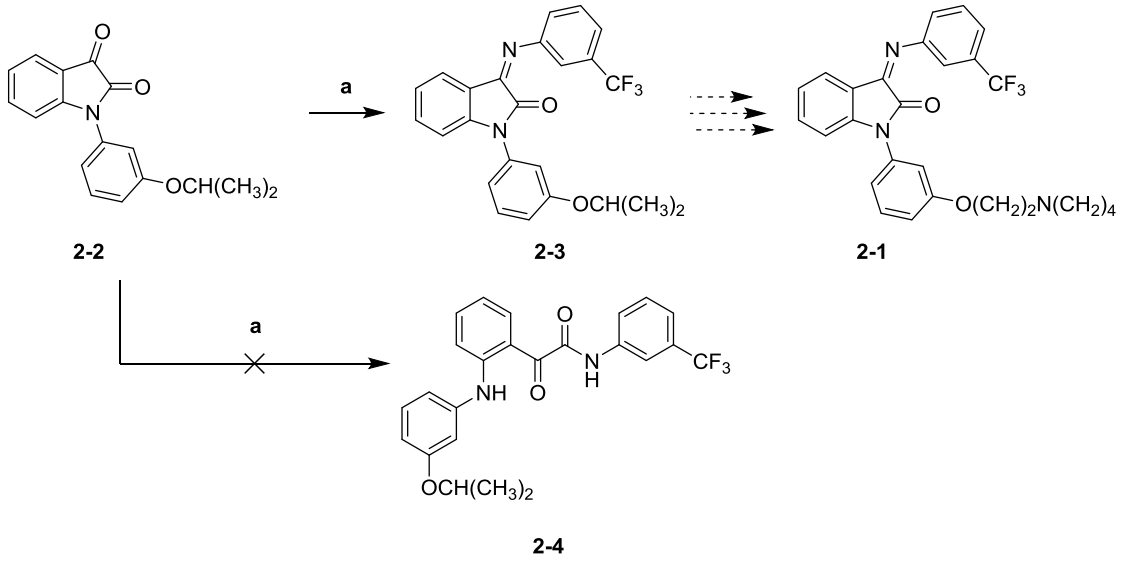
CHAPTER 2

Chapter 2: Reactivity of electron-deficient *N*-arylisatins

2.1 General introduction and chapter aims

N-Arylisatins have received considerable attention due in part to their remarkable biological properties and their use as key intermediate for the synthesis of potent drugs. For example, compound **2-1**, a high affinity ligand for the human galanin Gal_3 receptor, can be prepared from *N*-arylisatin **2-2** (Scheme **2.1**).[1] In the course of the synthesis, Konkel et al. demonstrated that the electron-rich *N*-arylisatin **2-2** reacted with 3-trifluoromethylaniline at the C3 position instead of C2 to give the *N*-(aryl)glyoxylamide **2-4**. The observed reactivity can be explained by the lack of an electron-deficient substituent on N1 of the isatin moiety, therefore preventing activation of C2 towards amines. To date, the reaction of electron-deficient *N*-arylisatins with either alcohols or amines remains unexplored. The objective of this chapter is to establish the reactivity of *N*-arylisatins containing electron-withdrawing groups at N1. By understanding the impact of an electron-withdrawing group at the N1 position of *N*-arylisatins, these molecules may find greater use as intermediates in the synthesis of more complex scaffolds.

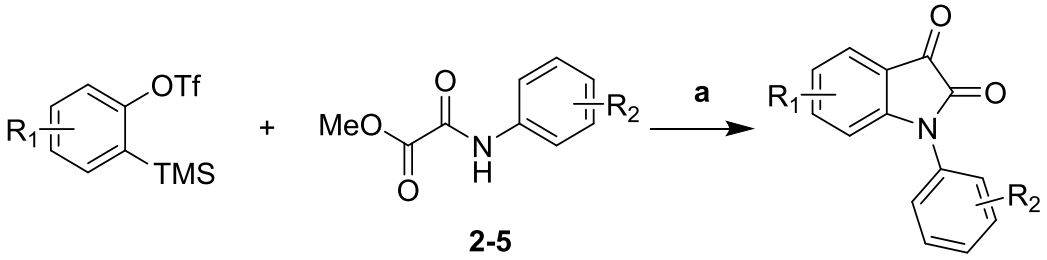


Scheme 2.1. *N*-Arylisatin **2-2** as a precursor in the synthesis of a highly potent ligand for the human galanin Gal_3 receptor **2-1**. Reaction of the electron-rich *N*-arylisatin **2-2** with 3-trifluoromethylaniline gave the C3 reaction product **2-3** instead of the C2 reaction product **2-4**.

2.2 Background, results and discussion

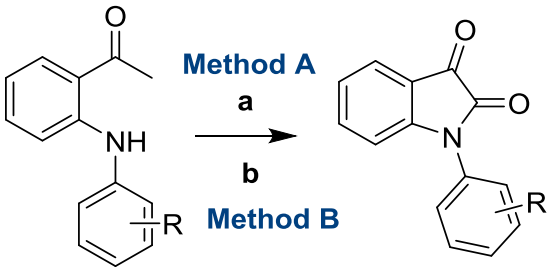
2.2.1 Synthesis of electron-deficient *N*-arylisatins

To begin the investigation, the synthesis of a range of electron-deficient *N*-arylisatins was pursued. There are only a few methods currently available for the preparation of *N*-arylisatins, and most suffer from low yields and limited diversification. For example, Larock et al. have developed a synthetic route to substituted *N*-arylisatins from the reaction of arynes with methyl 2-oxo-2(arylamino)acetates **2-5** (Scheme **2.2**). The *N*-arylisatins were generated in reasonable yields (51-90%), however only *para*- substituted electron-withdrawing groups were permitted on the pendant aryl ring.



Scheme 2.2. Synthesis of *N*-arylisatins by the reaction of arynes with methyl 2-oxo-2-(arylamino-acetates. Reagents and conditions: a) CsF, $NaHCO_3$, CH_3CN, r.t., 24 h.

Recently, two procedures for the synthesis of *N*-arylisatins from 2-(arylamino)-acetophenones have been developed. The first method involves the Cu(I)-catalyzed intramolecular oxidative C-H amination of **2-6** to afford the desired substituted heterocycles in reasonable yields (59-76 %) (Scheme **2.3**, Method **A**). Deng et al. have improved upon this procedure by employing SeO_2 as an oxidant for the intramolecular oxidative amidation of **2-6**, providing *N*-arylisatins in yields of up to 98% (Scheme **2.3**, Method **B**). Like the synthetic strategy described in Scheme **2.2**, both methods illustrated in Scheme **2.3** only permit electron-withdrawing substituents at the *para*- position of the pendant aryl ring. An additional drawback to both of these methods is the availability of the key precursors. Substituted 2-(arylamino)-acetophenones **2-6** are not commercially available and are typically prepared from the copper-catalyzed coupling of 2-amino acetophenones and aryl halides.[2]



2-6

Scheme 2.3. Synthesis of *N*-arylisatins from 2-(arylamino)-acetophenones *via* Cu(I)-catalyzed intramolecular oxidative C-H amination (Method **A**) and selenium-promoted intramolecular oxidative amidation (Method **B**). Reagents and conditions: a) CuI, 2,2'-bipyridine, o-DCB, O_2, 140 °C, 3h. b) SeO_2, dioxane, 80 °C, 24 h.

The direct *N*-arylation of isatin can be achieved by a Chan-Lam coupling.[1,3] This reaction involves the formation of a C-N bond *via* the oxidative coupling of an arylboronic acid and isatin catalyzed by a stoichiometric quantity of cupric acetate. Although the reported yields (40-74 %) for the preparation of *N*-arylisatins using this procedure are lower than those of the synthetic routes depicted in Scheme **2.2** and **2.3**, this synthetic methodology provides an efficient and straightforward method to the desired *N*-substituted heterocycles starting from commercially available precursors. Thus, a range of electron-deficient *N*-arylisatins were prepared *via* a modified Chang-Lam coupling procedure as exemplified in Table **2.1**.

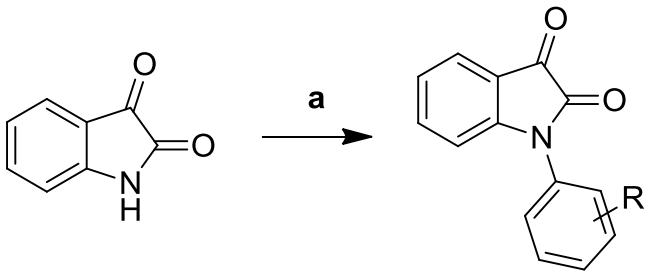


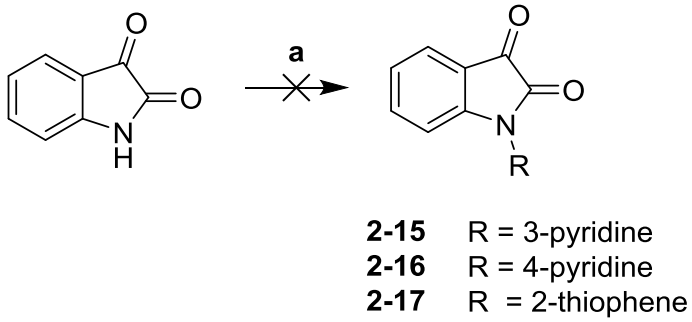
Table 2.1. Synthesis of electron-withdrawing *N*-aryl substituted isatin derivatives *via* Chan-Lam coupling. Reagents and conditions: a) Arylboronic acid, $Cu(OAc)_2$, NEt_3, pyridine, molecular sieves, CH_2Cl_2.

Compound	R	Reaction outcome (% Yield)
2-7	*p*-Br	96
2-8	*p*-Cl	67
2-9	*p*-CF_3	97
2-10	*o*-F	6
2-11	*o,p*-CF_3	15
2-12	*m*-CN	83
2-13	*p*-NO_2	75
2-14	*p*-SO_2CH_3	84

The results show that the formation of *N*-arylisatins bearing electron-withdrawing substituents at the *para* position of the pendant aryl ring (e.g. **2-7-2-9** and **2-13-2-14**) gave the highest yields (67-96%). Compounds **2-10** and **2-11** with substituents present at the *ortho* position have significantly lower yields of 6 and 15% respectively, which could presumably be due to steric effects.

The synthesis of compounds **2-7-2-14** bearing heterocyclic aryl groups was attempted in order to investigate the reactivity of *N*-arylisatins bearing electron-withdrawing heterocycles. Following the same reaction conditions exemplified in Table **2.1**, isatin was reacted with 3-pyridineboronic acid. During the course of the reaction, a gradual colour change was observed: the intense orange colour of isatin **1-1** turned to dark purple, and eventually became black. After 24 h, thin-layer chromatography analysis of the reaction mixture showed the presence of

the starting material without the formation of the desired product **2-15**. Similarly, the Chan-Lam coupling of 4-pyridineboronic acid pinacol ester or 2-thiophene boronic acid with isatin using the same conditions also failed to produce *N*-arylisatins **2-16** and **2-17** as observed by TLC analysis. Pyridines are known to coordinate to copper centers forming stable metal complexes. The unsuccessful synthesis of **2-15** and **2-17** presumably resulted from the formation of copper complexes of 3-pyridineboronic acid or 4-pyridineboronic acid pinacol ester *via* coordination of the pyridyl nitrogen atoms. These coordinate bonds were presumably more stable than those formed between the isatin anion and the Cu(II) center, hence preventing initiation of the catalytic cycle. Thus, further investigation is required to generate N1-heteroaryl-substituted isatins.

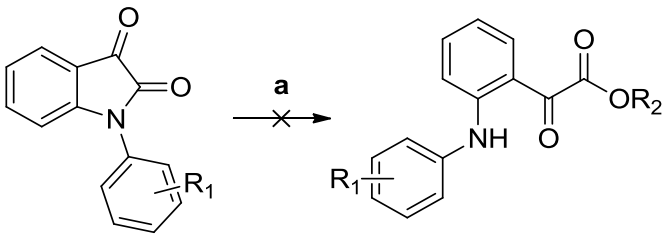


2-15 R = 3-pyridine
2-16 R = 4-pyridine
2-17 R = 2-thiophene

Scheme 2.4. Attempted synthesis of *N*-isatins bearing heterocyclic groups. Reagents and conditions: a) Heteroaryl boronic acid, $Cu(OAc)_2$, NEt_3, pyridine, molecular sieves, CH_2Cl_2.

2.2.2 Reactions of electron-deficient *N*-arylisatins with alcohols

With a variety of electron-deficient *N*-arylisatins **2-7-2-14** in hand, the reactivity of these derivatives towards primary alcohols was investigated. Thus, *N*-arylisatin **2-7** was dissolved in methanol and heated at reflux for 24 h (Scheme **2.5**). ^{1}H NMR spectroscopic analysis of the reaction mixture showed the absence of the desired product. Similarly, the reaction of the substrates **2-8-2-17** with methanol produced the same outcome, and the starting *N*-arylisatins were completely recovered in all cases. Unlike the *N*-acylisatin derivatives, these electron-deficient *N*-arylisatins did not participate in ring-opening reactions with alcohols to form the expected *N*-glyoxylmethyl esters.



Scheme 2.5. Attempted reaction of electron-deficient *N*-arylisatins with primary alcohols. Reagents and conditions: a) methanol, reflux, 24 h.

2.2.3 Reactions of electron-deficient *N*-arylisatins with amines

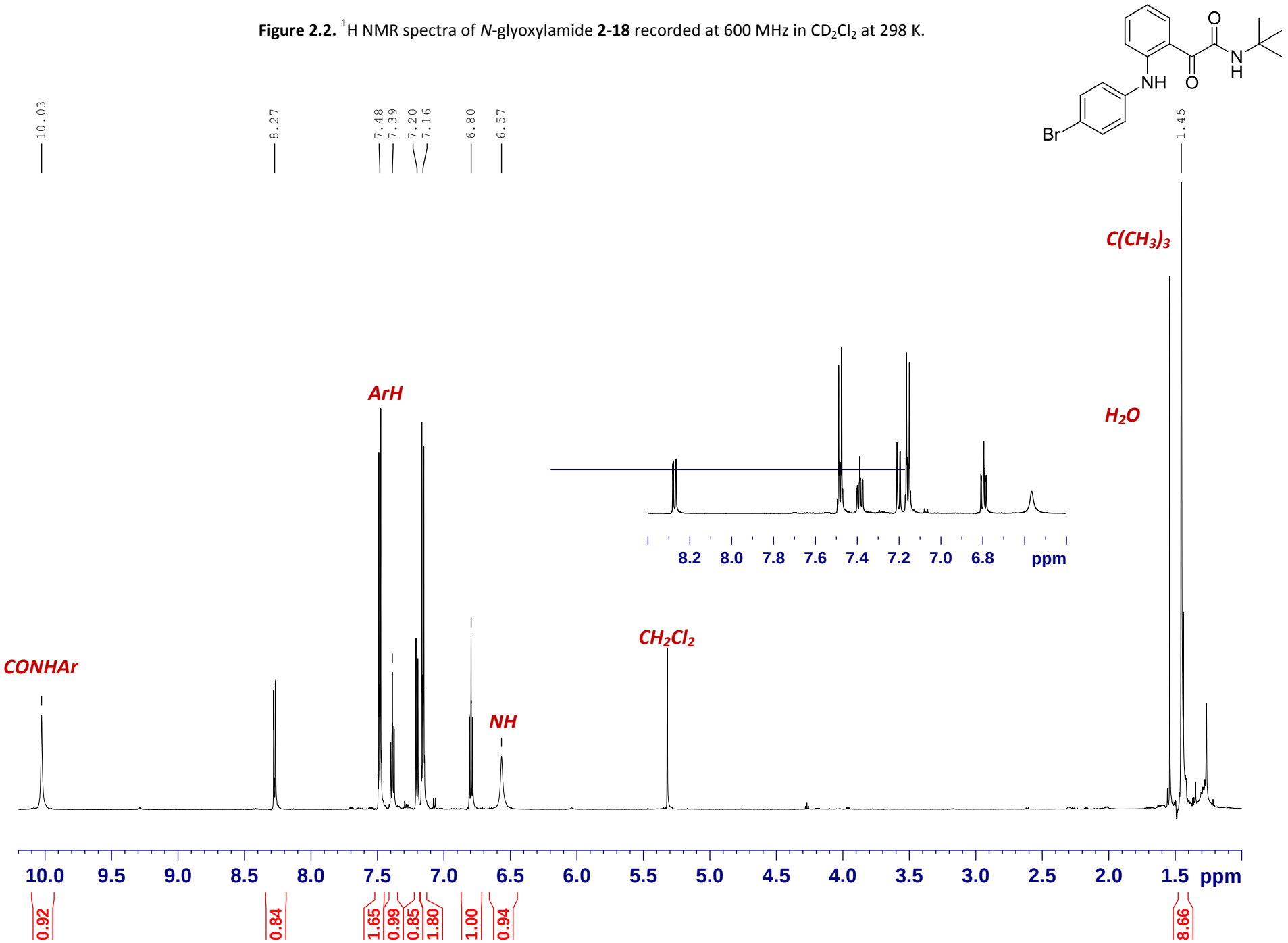
To establish the reactivity of electron-deficient *N*-arylisatins towards amines, **2-7** was stirred with *tert*-butylamine (2 equivalents) in dichloromethane at room temperature. After 8 h, the progress of the reaction was monitored by TLC analysis (1:4 ethyl acetate/n-hexane), which showed the presence of a single product as well as the starting material **2-7** at similar R_f values. To avoid difficult chromatographic separation, attempts were made to drive the reaction to completion by adding an additional equivalent of *tert*-butylamine and refluxing the reaction mixture for a further 7 h. However, ^{1}H NMR spectroscopy analysis of the reaction mixture in $CDCl_3$ still showed the incomplete conversion of the starting *N*-arylisatin **2-7** to the product. A singlet at 10.03 ppm belonging to the product molecule was identified as an NH proton by D_2O exchange. This peak was suspected to belong to a proton participating in a hydrogen bond, possibly that of NH in *N*-glyoxylamide **2-18** forming an intramolecular H-bond with the keto oxygen atom (Table **2.2**, hydrogen bond depicted in red).

With the encouraging results described above, efforts were directed towards optimizing the reaction conditions in order to achieve complete conversion of the starting material **2-7**. Thus, a neat solution of **2-7** in *tert*-butylamine was stirred at reflux. After 0.5 h, the orange colour of **2-7** changed to bright yellow. TLC analysis (1:9 ethyl acetate/n-hexane) of the reaction mixture indicated the complete consumption of the starting material along with the formation of two new compounds, both appearing as yellow spots with R_f values 0.58 and 0 (1 ethyl acetate: 4 n-hexane) respectively. The reaction mixture was concentrated and the yellow residue was redissolved in dichloromethane. After the organic layer was washed with a solution

of 2M HCl (10 mL, aq., 1 M), the colour of the solution changed from bright yellow to orange. However, TLC analysis of the organic layer indicated that the products had decomposed into isatin **1-1**, along with other minor baseline impurities. This result suggested that the reaction products were unstable in the presence of acid.

Having established the acid sensitivity of the reaction products, the reaction was repeated and the yellow residue obtained was instead subjected directly to flash chromatography on silica gel. The first compound eluted from the column (1:9 ethyl acetate/n-hexane) was isolated as a bright yellow oil. The ^{1}H NMR spectrum of this compound showed a singlet at 10.03 ppm corresponding to the aforementioned NH peak observed in the reaction mixture (Figure **2.2**). A sharp singlet at 1.45 ppm and a broad singlet at 6.57 ppm with relative integrations of nine and one protons respectively further supported the proposed *N*-glyoxylamide **2-18** structure. Analysis of the ^{13}C NMR spectrum of this compound showed peaks at 163.7 and 191.9 ppm indicating the presence of amide and ketone carbonyl atoms, respectively. High resolution mass spectrometric analysis showed a peak at 397.0536 which was consistent with the calculated mass of $(M\text{-}Na)^{+}$ for compound **2-18**.

Figure 2.2. ^{1}H NMR spectra of *N*-glyoxylamide **2-18** recorded at 600 MHz in CD_2Cl_2 at 298 K.



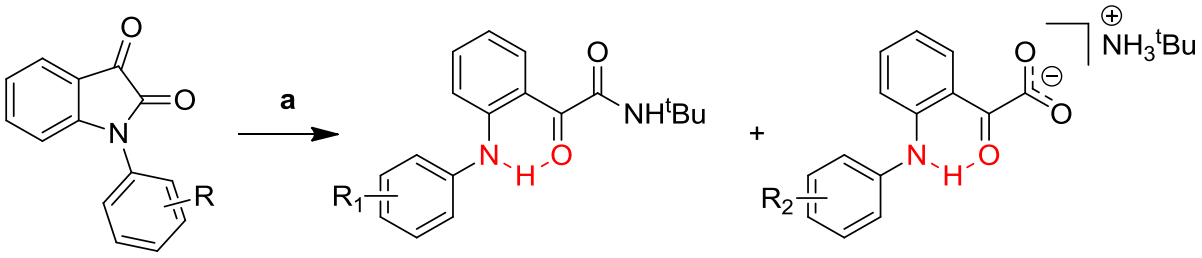


Table 2.2. Ring-opening reactions of electron-deficient *N*-arylisatins with *tert*-butylamine. The intramolecular hydrogen-bonds are depicted in red. Reagents and conditions: a) *tert*-butylamine, reflux, 0.5 h.

Compound	R_1	R_2	Reaction outcome (% Yield)
2-18	*p*-Br	-	15
2-20	*p*-Cl	-	23
2-21	*p*-CF_3	-	6
2-22	*m*-CN	-	21
2-23	*p*-NO_2	-	16
2-24	*p*-SO_2CH_3	-	11
2-19	-	*p*-Br	62
2-25	-	*p*-CF_3	54
2-26	-	*o*-F	68
2-27	-	*o,p*-CF_3	43
2-28		*m*-CN	72
2-29	-	*p*-NO_2	64

The second product isolated from the reaction of *N*-arylisatin **2-7** and *tert*-butylamine was eluted solely with ethyl acetate, possessing a higher polarity than both the starting material **2-7** and **2-18**. Structure elucidation by ^{1}H and ^{13}C NMR spectroscopy alone was unsuccessful with both spectra showing peaks corresponding to *N*-glyoxylamide **2-18**. Crystals of the unknown reaction product were grown *via* a slow evaporation of an ethyl acetate solution, giving large colourless needles. The unknown structure was unambiguously determined by single crystal X-ray analysis. Figure **2.3** ***(a)*** shows the structure of *N*-

glyoxylacetate salt **2-19**, which is consistent with both its high polarity observed in the TLC analysis and its $[M+H]^+$ peak in the mass spectrum. The asymmetrical unit of the crystal structure depicted in Figure **2.3** ***(b)*** shows two molecules doubly bridged by intramolecular H-bonds to two *tert*-butylammonium counter-ions (N1B—H1BA---O2A, N1D—H1DC---O2A, N1D—H1DA---O2C, N1B—H1BC---O2C, depicted in orange). An intramolecular H-bond (N1—H1---O1, depicted in magenta) is also present, similar to that observed in *N*-glyoxylesters and amides derived from the ring-opening reaction of *N*-acylisatins.

Having successfully demonstrated the ring-opening reaction of **2-7** with *tert*-butylamine, *N*-arylisatins **2-8-2-14** were subjected to the same conditions as exemplified in Table **2.2**. However, low yields of the novel *N*-glyoxylamide products **2-20-2-24** were obtained (6-23 % yields), with the major product (where isolated) being the *N*-glyoxylacetate salts **2-25-2-29** (43-72 % yields). The formation of the salts **2-19 and 2-25-2-29** is anticipated to be due to the presence of residual water in the *tert*-butylamine.

2.2.4 Reactivity of electron- deficient *N*-arylisatins

The results above indicate that primary alcohols such as methanol lack the reactivity to ring-open *N*-arylisatin **2-7-2-14** at the C2 position to form the corresponding *N*-glyoxylesters. On the other hand, the reaction of **2-7-2-14** with wet *tert*-butylamine formed *N*-glyoxylacetate salts **2-19, 2-25-2-29** as the major product and *N*-glyoxylamides **2-18, 2-20-2-24** as the minor product. In contrast, previous work had shown that *N*-acylisatins react preferentially with amines to form *N*-glyoxylamides as the major product in a dichloromethane: water biphasic solvent system. Further investigation is required to explain the reactivity of these electron-deficient heterocycles.

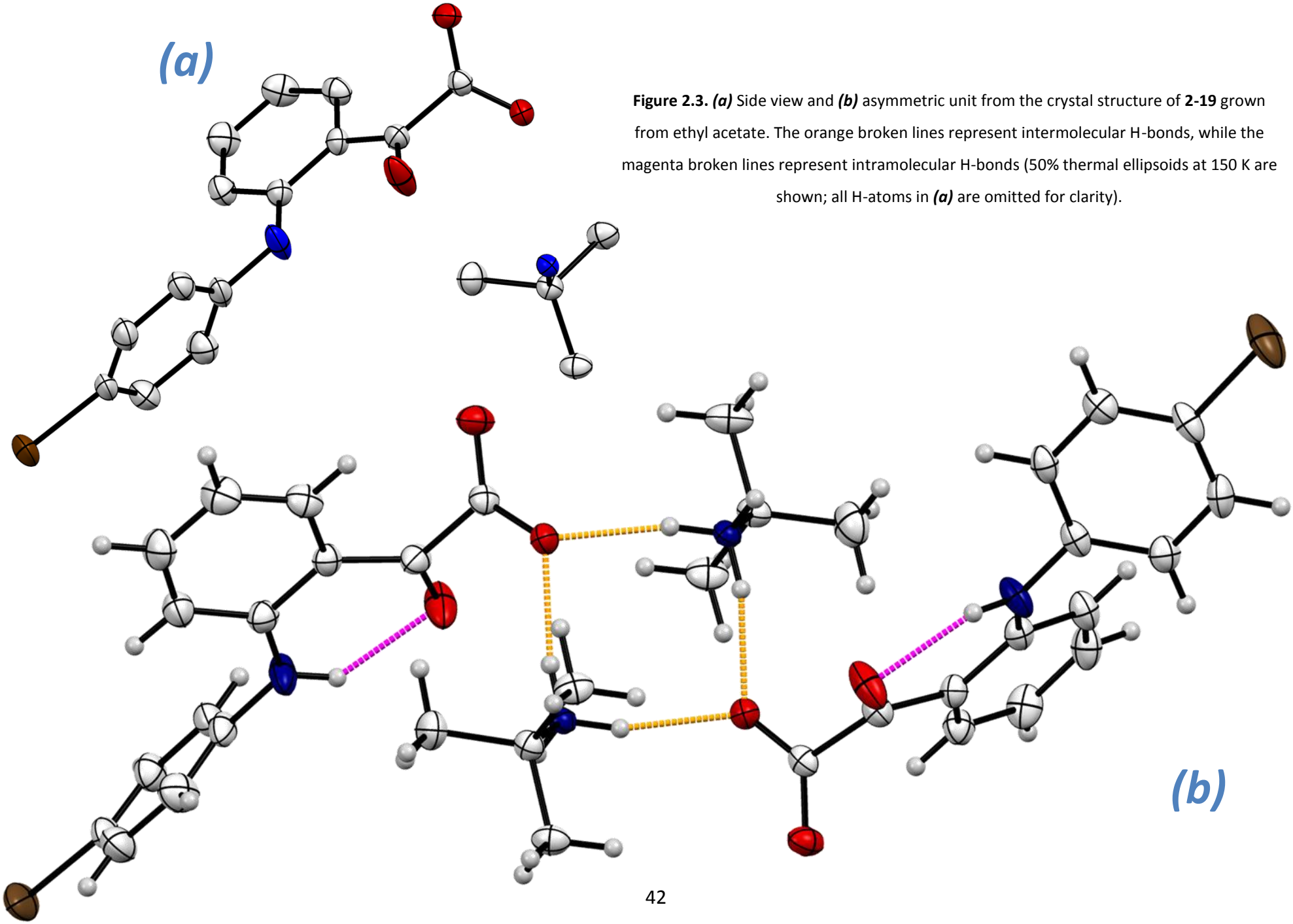


Figure 2.3. ***(a)*** Side view and ***(b)*** asymmetric unit from the crystal structure of **2-19** grown from ethyl acetate. The orange broken lines represent intermolecular H-bonds, while the magenta broken lines represent intramolecular H-bonds (50% thermal ellipsoids at 150 K are shown; all H-atoms in ***(a)*** are omitted for clarity).

2.3 Summary and future work

A series of electron-deficient *N*-arylisatins **2-7-2-14** were prepared via Chan-Lam coupling. *N*-arylisatins possessing an electron-withdrawing group at the *para* position of the pendant ring were formed in the highest yields (up to 96 %), while those with *ortho* substituents were formed in significantly lower yields (up to 15 %). Refluxing *N*-arylisatins **2-7-2-14** in methanol failed to produce the expected *N*-glyoxylesters. On the other hand, reaction of **2-7-2-14** in refluxing *tert*-butylamine gave novel *N*-glyoxylamides **2-18, 2-20-2-24** in low yields (6-23 %), with the corresponding *N*-glyoxylacetate salts **2-19, 2-25-2-29** being obtained as the major product (43-72 % yields).

Having established the reactivity of *N*-arylisatins **2-7-2-14**, future work will focus on explaining the unexpected reactivity of these derivatives towards water in preference to the more reactive primary amine groups. Synthetic strategies exploiting both the novel *N*-glyoxylamides **2-18, 2-20-2-24** and *N*-glyoxylic acids **2-19, 2-25-2-29** as precursors will be explored.

2.4 Experimental

2.4.1 General methods

Synthesis and reagents

Unless specified, reagents and solvents used in the following syntheses were purchased from Sigma-Aldrich, Alfa-Aesar, Boron Molecular or Chemimplex International and used as received. Intermediates were judged as pure by ^{1}H NMR spectroscopy and were used as described. Unless otherwise specified, preparations were performed in air. Flash and gravity chromatography was carried out on 60-200 µm silica gel.

Spectroscopy

All NMR spectra were recorded on Bruker Avance DPX-300, 400, 500 and 600 spectrometers at 298 K operating at 300, 400, 500 and 600 MHz frequency for ^{1}H NMR experiments and at 75.5, 100.6, 125.7 and 150.9 MHz for ^{13}C NMR experiments, respectively. All chemical shifts were calibrated against residual solvent signals. All coupling constants (*J*) are reported in Hertz. Signals in NMR spectra are reported as broad (b), singlets (s), doublets (d), triplets (t), quartets (q), quintets (qu), sextets (sx) or septets (sept). FT-IR spectra were recorded at a resolution of 2 cm^{-1} as KBr discs using a Nicolet Avatar 360 FT-IR spectrometer. Mass spectra were run on a Thermo Fisher Scientific Orbitrap LTQ XL ion trap mass spectrometer using a nanospray ionisation source. UV-Vis experiments were performed on a Varian Cary 50 Bio UV-Visible spectrophotometer.

Crystallography

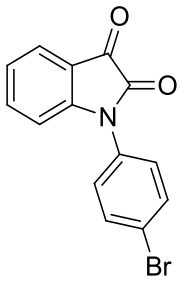
Single crystal X-ray diffraction data for **67** was obtained on the MX-1 beamline at the Australian Synchrotron. Structures were processed and refined using SHELX software.

2.4.2 Synthesis

General procedure for the synthesis of *N*-arylisatins **2-7-2-14**

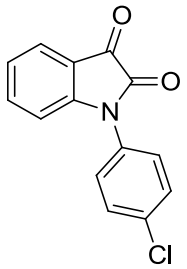
To a suspension of isatin (1 mmol) in dry dichloromethane was added anhydrous $Cu(OAc)_2$ (1 mmol), pyridine (2 mmol), triethylamine (2 mmol) and the appropriate arylboronic acid (2 mmol). A drying tube was placed over the reaction vessel and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a pad of Celite and washed with dichloromethane. The filtrate was concentrated *in vacuo* and the crude residue was subjected to flash chromatography on silica.

1-(4-Bromophenyl)indoline-2,3-dione **2-7**



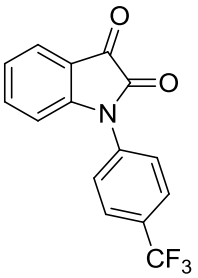
The title compound **2-7** was prepared from isatin **1-1** (0.300 g, 2.04 mmol) and 4-bromophenylboronic acid (0.820 g, 4.08 mmol) according to the general procedure outlined above. The product **2-7** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.591 g, 96 %). M.p. 182-183°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.90 (dd, *J* = 8.0, 0.8 Hz, 1H, ArH), 7.20 (ddd, *J* = 7.5, 7.5, 0.8, 1H, ArH), 7.32 (d, *J* = 8.9Hz, 2H, ArH), 7.56 (ddd, *J* = 8.0, 7.5, 1.4 Hz, 1H, ArH), 7.70-7.73 (m, 3H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 111.3 (ArCH), 117.7 (ArC), 122.7 (ArC), 124.7 (ArCH), 126.0 (ArCH), 127.7 (ArCH), 132.0 (ArC), 133.3 (ArCH), 138.6 (ArCH), 151.2 (ArC), 157.3 (CO<u>C</u>ON), 182.5 (<u>C</u>OCON); IR (ATR): ν_{max} 1731, 1608, 1460, 1361, 1311, 1178, 1069, 1009, 830, 811, 754, 711 cm^{-1}; HRMS (+ESI): Found *m/z* 323.9638, $[M+Na]^+$, $C_{14}H_8BrNO_2Na$ requires 323.9636.

1-(4-Chlorophenyl)indoline-2,3-dione **2-8**



The title compound **2-8** was prepared from isatin **1-1** (1.25 g, 8.50 mmol) and 4-chlorophenylboronic acid (2.65 g, 16.9 mmol) according to the general procedure outlined above. The product **2-8** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (1.47 g, 67 %). M.p. 204-206°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.89 (dd, *J* = 8.1, 0.8 Hz, 1H, ArH), 7.19 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 1H, ArH), 7.38 (d, *J* = 8.9 Hz, 1H, ArH), 7.48-7.61 (m, 3H, 3 x ArH), 7.70 (dd, *J* = 7.6, 1.4 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.2 (ArCH), 117.6 (ArC), 124.7 (ArCH), 125.9 (ArCH), 127.4 (ArCH), 130.3 (ArCH), 131.5 (ArC), 134.7 (ArC), 138.6 (ArCH), 151.3 (ArC), 157.3 (CO<u>C</u>ON), 182.5 (<u>C</u>OCON); IR (ATR): ν_{max} 1733, 1602, 1492, 1460, 1359, 1291, 1176, 1091, 1012, 924, 812, 753 cm^{-1}; HRMS (+ESI): Found *m/z* 280.0129, $[M+Na]^+$, $C_{14}H_8ClNO_2Na$ requires 280.0141.

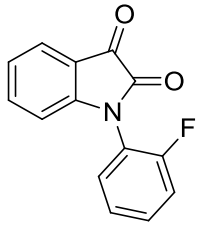
1-(4-(Trifluoromethyl)phenyl)indoline-2,3-dione **2-9**



The title compound **2-9** was prepared from isatin **1-1** (0.200 g, 1.36 mmol) and 4-(trifluoromethyl)phenylboronic acid (0.520 g, 2.72 mmol) according to the general procedure outlined above. The product **2-9** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.384 g, 97 %). M.p. 183°C; ^{1}H NMR (600 MHz, CD_2Cl_2): δ 6.97 (dd, *J* = 8.0, 0.7 Hz, 1H, ArH), 7.24 (ddd, *J* = 7.5, 7.5, 0.8 Hz,

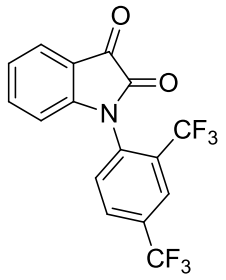
1H, ArH), 7.59-7.64 (m, 3H, 3 x ArH), 7.72 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH), 7.86 (d, *J* = 8.2 Hz, 2H, 2 x ArH); ^{13}C NMR (150 MHz, CD_2Cl_2): δ 111.6 (ArCH), 118.0 (ArC), 123.3 (ArC), 125.1 (ArCH), 126.0 (ArCH), 126.6 (ArCH), 127.4 (q, *J* = 4.0 Hz, CF_3), 130.7 (q, *J* = 33.0 Hz, ArC), 136.8 (ArC), 138.9 (ArCH), 151.1 (ArC), 157.5 (CO<u>C</u>ON), 182.6 (<u>C</u>OCON); IR (ATR): ν_{max} 1736, 1600, 1518, 1465, 1370, 1317, 1158, 1106, 1062, 926, 833, 752, 693 cm^{-1}; HRMS (+ESI): Found *m/z* 314.0395, $[M+Na]^+$, $C_{15}H_8F_3NO_2Na$ requires 314.0399.

1-(2-Fluorophenyl)indoline-2,3-dione **2-10**



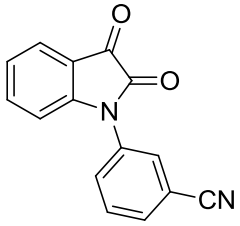
The title compound **2-10** was prepared from isatin **1-1** (1.58 g, 10.7 mmol) and 2-fluorophenylboronic acid (3.00 g, 21.4 mmol) according to the general procedure outlined above. The product **2-10** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.145 g, 6 %). M.p. 152-153°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.71 (dd, *J* = 8.0, 2.0 Hz, 1H, ArH), 7.19 (ddd, *J* = 7.5, 7.5, 0.8 Hz, 1H, ArH), 7.29-7.36 (m, 2H, 2 x ArH), 7.44 (ddd, *J* = 7.5, 7.5, 1.7 Hz, 1H, ArH), 7.47-7.52 (m, 1H, ArH), 7.56 (ddd, *J* = 7.5, 7.5, 1.4 Hz, 1H, ArH), 7.71 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 111.5 (d, *J* = 2.0 Hz, ArCH), 117.5 (d, *J* = 19.4 Hz, ArCH), 117.8 (ArC), 120.6 (d, *J* = 13.0 Hz, ArC), 124.6 (ArCH), 125.5 (d, *J* = 13.0 Hz, ArCH), 125.7 (ArCH), 129.19 (ArCH), 131.2 (d, *J* = 8.0 Hz, ArCH), 138.6 (ArCH), 151.2 (ArC), 157.2 (d, *J* = 59.2 Hz, ArC), 158.6 (CO<u>C</u>ON), 182.3 (<u>C</u>OCON); IR (ATR): ν_{max} 1736, 1601, 1505, 1463, 1366, 1303, 1234, 1185, 1106, 1031, 927, 859, 811, 754, 697 cm^{-1}; HRMS (+ESI): Found *m/z* 242.0609, $[M+H]^+$, $C_{14}H_9FNO_2Na$ requires 242.0612.

1-(2,4-Bis(trifluoromethyl)phenyl)indoline-2,3-dione **2-11**



The title compound **2-11** was prepared from isatin **1-1** (0.200 g, 1.36 mmol) and 2,4-bis(trifluoromethyl)phenylboronic acid (0.700 g, 2.72 mmol) according to the general procedure outlined above. The product **2-11** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.073 g, 15 %). M.p. 143°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.46 (d, *J* = 8.0 Hz, 1H, ArH), 7.22 (ddd, *J* = 7.5, 7.5, 0.7 Hz, 1H, ArH), 7.55 (ddd, *J* = 7.5, 7.5, 1.3 Hz, 1H, ArH), 7.62 (d, *J* = 8.0 Hz, 1H, ArH), 7.74 (dd, *J* = 7.5, 1.3 Hz, 1H, ArH), 8.07 (dd, *J* = 8.2, 1.9 Hz, 1H, ArH), 8.16 (d, *J* = 1.3 Hz, 1H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 111.4 (ArCH), 117.6 (ArC), 124.9 (ArCH), 125.8 (m, ArCH), 126.0 (ArCH), 131.3 (m, ArCH), 131.3 (q, *J* = 32.5 Hz, ArC), 132.2 (ArCH), 133.1 (q, *J* = 34.2 Hz, ArC), 134.9 (ArC), 138.9 (ArCH), 151.9 (ArC), 157.7 (CO<u>C</u>ON), 181.5 (<u>C</u>OCON); IR (ATR): ν_{max} 1734, 1609, 1508, 1464, 1371, 1271, 1171, 1127, 1078, 914, 854, 840, 762, 670 cm^{-1}; HRMS (+ESI): Found *m/z* 382.0269, $[M+Na]^+$, $C_{16}H_7F_6NO_2Na$ requires 382.0273.

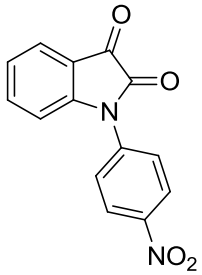
3-(2,3-Dioxoindolin-1-yl)benzonitrile **2-12**



The title compound **2-12** was prepared from isatin **1-1** (0.200 g, 1.36 mmol) and 3-cyanophenylboronic acid (0.400 g, 2.72 mmol) according to the general procedure outlined

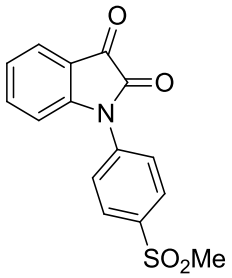
above. The product **2-12** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.280 g, 83 %). M.p. 209-210°C; ^{1}H NMR (150 MHz, d_6-DMSO): 6.95 (dd, *J* = 8.0, 0.8 Hz, 1H, ArH), 7.23 (ddd, *J* = 7.5, 7.5, 0.8 Hz, 1H, ArH), 7.64 (ddd, *J* = 8.0, 7.5, 1.4 Hz, 1H, ArH), 7.69 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH), 7.87-7.88 (m, 2H, 2 x ArH), 7.95-8.00 (m, 2H, 2 x ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.1 (ArCH), 114.4 (ArC), 117.6 (ArC), 117.7 (ArC), 125.2 (ArCH), 126.2 (ArCH), 129.3 (ArCH), 130.5 (ArCH), 131.1 (ArCH), 132.3 (ArCH), 134.1 (ArC), 138.7 (ArCH), 150.5 (ArC), 157.1 (CO<u>C</u>ON), 181.9 (<u>C</u>OCON); IR (ATR): ν_{max} 1729, 1605, 1460, 1357, 1298, 1174, 1094, 955, 891, 804, 748, 675 cm^{-1};HRMS (+ESI): Found *m/z* 271.0472, $[M+Na]^+$, $C_{15}H_8N_2O_2Na$ requires 271.0483.

1-(4-Nitrophenyl)indoline-2,3-dione **2-13**



The title compound **2-13** was prepared from isatin **1-1** (0.300 g, 2.04 mmol) and 4-nitro phenylboronic acid (0.680 g, 4.08 mmol) according to the general procedure outlined above. The product **2-13** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.410 g, 75 %). M.p. 242°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (dd, *J* = 8.1, 0.7 Hz, 1H, ArH), 7.27 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 1H, ArH), 7.63 (ddd, *J* = 8.1, 7.6, 1.4 Hz, 1H, ArH), 7.69 (d, *J* = 9.2 Hz, 2H, ArH), 7.77 (dd, *J* = 7.6, 1.4 Hz, 1H, ArH), 8.44 (d, *J* = 9.2 Hz, 2H, ArH); ^{13}C NMR (150 MHz, d_6-DMSO): δ 111.0 (ArCH), 118.1 (ArC), 124.2 (ArCH), 124.9 (ArCH), 125.1 (ArCH), 126.9 (ArCH), 137.9 (ArCH), 139.3 (ArC), 146.2 (ArC), 149.9 (ArC), 157.3 (CO<u>C</u>ON), 181.8 (<u>C</u>OCON); IR (ATR): ν_{max} 1734, 1587, 1516, 1461, 1291, 1178, 1096, 1009, 927, 824, 748, 701 cm^{-1}; HRMS (+ESI): Found *m/z* 291.0372, $[M+Na]^+$, $C_{14}H_8N_2O_4Na$ requires 291.0376.

1-(4-(Methylsulfonyl)phenyl)indoline-2,3-dione **2-14**

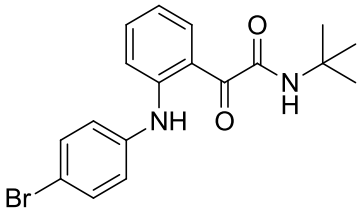


The title compound **2-14** was prepared from isatin **1-1** (0.490 g, 3.33 mmol) and 4-(methanesulfonyl)phenylboronic acid (1.00 g, 5.00 mmol) according to the general procedure outlined above. The product **2-14** was obtained by flash chromatography, eluting with 3:4 dichloromethane/n-hexane as a bright orange amorphous solid (0.843 g, 84 %). M.p. 231°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.12 (s, 3H, CH_3), 7.00 (dd, *J* = 8.1, 0.8 Hz, 1H, ArH), 7.26 (ddd, *J* = 7.5, 7.5, 0.8 Hz, 1H, ArH), 7.62 (ddd, *J* = 8.1, 7.5, 1.4 Hz, 1H, ArH), 7.70 (d, *J* = 8.9 Hz, 2H, 2 x ArH), 7.76 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH), 8.15 (d, *J* = 8.9 Hz, 2H, 2 x ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 44.7 (CH_3), 111.3 (ArCH), 117.8 (ArC), 125.3 (ArCH), 126.3 (ArCH), 126.5 (ArCH), 129.5 (ArCH), 138.0 (ArC), 138.7 (ArCH), 140.5 (ArC), 150.4 (ArC), 157.1 (CO<u>C</u>ON), 181.8 (<u>C</u>OCON); IR (ATR): ν_{max} 1735, 1610, 1463, 1373, 1288, 1198, 1144, 1085, 1033, 956, 826, 764 cm^{-1}; HRMS (+ESI): Found *m/z* 324.0298, $[M+Na]^+$, $C_{15}H_{11}NO_4SNa$ requires 324.0301.

General procedure for the synthesis of *N*-(*tert*-butyl)-2-oxo-2-(2-(phenylamino)phenyl)acetamides and 2-(2-((phenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salts **2-18, 2-20-2-24**

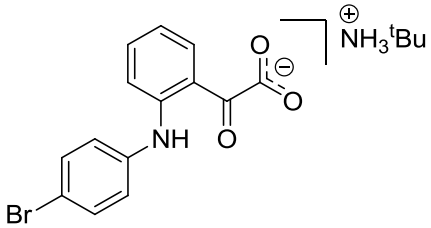
N-arylisatin (1 mmol) was refluxed in a neat solution of *tert*-butylamine for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was subjected to flash chromatography on silica gel.

N-(*tert*-Butyl)-2-(2-((4-bromophenyl)amino)phenyl)-2-oxoacetamide **2-18**



The title compound **2-18** was prepared from *N*-arylisatin **2-7** (0.100 g, 0.33 mmol) according to the general procedure outlined above. The product **2-18** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright yellow oil (0.019 g, 15 %). ^{1}H NMR (600 MHz, CH_2Cl_2): δ 1.45 (s, 9H, $C(CH_3)_3$), 6.57 (bs, 1H, CONH), 6.80 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H, ArH),7.16 (d, *J* = 8.8 Hz, 2H, 2 x ArH), 7.20 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 7.39 (ddd, *J* = 8.6, 7.0, 1.7 Hz, 1H, ArH), 7.48 (d, *J* = 8.8 Hz, 2H, 2 x ArH), 8.27 (dd, *J* = 8.2, 1.7 Hz, 1H, ArH), 10.03 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, CH_2Cl_2): δ 28.6 ($C(\underline{C}H_3)_3$), 52.2 ($\underline{C}(CH_3)_3$), 114.6 (ArCH), 116.4 (ArC), 117.1 (ArC), 117.8 (ArCH), 125.0 (ArCH), 132.8 (ArCH), 135.3 (ArCH), 136.3 (ArCH), 139.6 (ArC), 149.4 (ArC), 163.7 (CO<u>C</u>ONH), 191.9 (<u>C</u>OCONH); IR (ATR): ν_{max} 3276, 3071, 2962, 2920, 1623, 1581, 1508, 1451, 1391, 1363, 1320, 1203, 1159, 1100, 1070, 1006, 882, 838, 799, 670 cm^{-1}; HRMS (+ESI): Found *m/z* 397.0536, $[M+Na]^+$, $C_{18}H_{19}N_2O_2Na$ requires 397.0528.

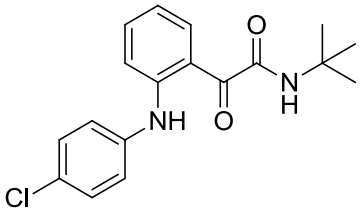
2-(2-((4-Bromophenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-19**



The title compound **2-19** was prepared from *N*-arylisatin **2-7** (0.100 g, 0.33 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate. The resulting yellow amorphous solid was recrystallized from acetonitrile to give **2-19** as large colourless needles (0.081 g, 62 %). M.p.

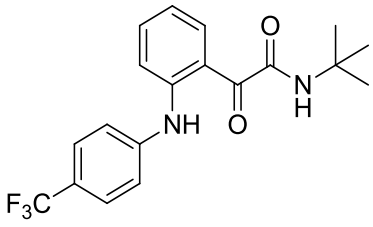
89-90°C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.25 (s, 9H, C(C<u>H</u>$_3$)$_3$), 6.81 (ddd, *J* = 8.1, 7.2, 1.1 Hz, 1H, ArH), 7.20-7.26 (m, 3H, 3 x ArH), 7.37 (ddd, *J* = 7.8, 7.2, 1.3 Hz, 1H, ArH), 7.50 (d, *J* = 8.5 Hz, 2H, 2 x ArH), 7.71 (dd, *J* = 7.8, 1.1 Hz, 1H, ArH), 7.95 (bs, 3H, NH_3), 10.35 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.1 (C(<u>C</u>H$_3$)$_3$), 50.9 (<u>C</u>(CH$_3$)$_3$), 113.7 (ArCH), 114.2 (ArC), 116.9 (ArC), 117.6 (ArCH), 122.9 (ArCH), 132.2 (ArCH), 134.1 (ArCH), 134.5 (ArCH), 139.9 (ArC), 146.2 (ArC), 169.2 (CO<u>C</u>O$_2$), 200.7 (<u>C</u>OCO$_2$). IR (ATR): ν_{max} 3255, 2977, 2906, 2825, 2732, 2623, 2529, 1626, 1511, 1452, 1393, 1319, 1206, 1160, 1127, 1067, 833, 801, 745, 674 cm^{-1}; HRMS (-ESI): Found *m/z* 317.9764, $[M]^-$, $C_{14}H_8NO_3$ requires 317.9760.

N-(*tert*-Butyl)-2-(2-((4-chlorophenyl)amino)phenyl)-2-oxoacetamide **2-20**



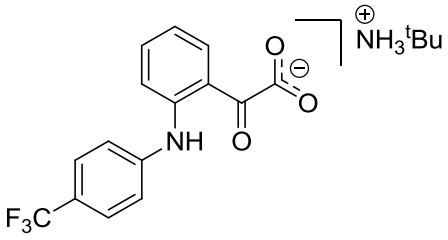
The title compound **2-20** was prepared from *N*-arylisatin **2-8** (0.200 g, 0.78 mmol) according to the general procedure outlined above. The product **2-20** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright yellow oil (0.059 g, 23 %). ^{1}H NMR (600 MHz, d_6-acetone): δ 1.48 (s, 9H, C(CH$_3$)$_3$), 6.84 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H, ArH), 7.25 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 7.32 (d, *J* = 8.8 Hz, 2H, 2 x ArH), 7.41 (d, *J* = 8.8 Hz, 2H, 2 x ArH), 7.46 (ddd, *J* = 8.6, 7.1, 1.7 Hz, 1H, ArH), 7.47-7.51 (bs, 1H, CONH), 7.98 (dd, *J* = 8.1, 1.6 Hz, 1H, ArH), 10.11 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-Acetone): δ 28.9 (C(<u>C</u>H$_3$)$_3$), 52.5 (<u>C</u>(CH$_3$)$_3$), 115.0 (ArCH), 116.9 (ArC), 118.4 (ArCH), 125.1 (ArCH), 129.5 (ArC), 130.4 (ArCH), 135.6 (ArCH), 136.7 (ArCH), 140.0 (ArC), 149.6 (ArC), 166.0 (CO<u>C</u>ONH), 194.2 (<u>C</u>OCONH); IR (ATR): ν_{max} 3279, 2922, 1623, 1585, 1509, 1451, 1320, 1226, 1160, 1088, 1010, 839, 747 cm^{-1}; HRMS (+ESI): Found *m/z* 331.1203, $[M+H]^+$, $C_{18}H_{19}N_2O_2$ requires 331.1213.

N-(*tert*-Butyl)-2-(2-((4-trifluoromethylphenyl phenyl)amino)phenyl)-2-oxoacetamide **2-21**



The title compound **2-21** was prepared from *N*-arylisatin **2-9** (0.062 g, 0.21 mmol) according to the general procedure outlined above. The product **2-21** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright yellow o (0.005 g, 6 %). M.p. 98°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H, $C(CH_3)_3$), 6.70 (s, 1H, CONH), 6.88 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H, ArH), 7.31 (d, *J* = 8.4, 2H, 2 x ArH), 7.34-7.47 (m, 2H, 2 x ArH), 7.58 (d, *J* = 8.4, 2H, 2 x ArH), 8.41 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 10.10 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.6 ($C(\underline{C}H_3)_3$), 52.1 ($\underline{C}(CH_3)_3$), 115.2 (ArCH), 117.5 (ArC), 118.8 (ArCH), 121.1 (ArCH), 125.3 (q, *J* = 32.8 Hz, ArC), 126.8 (q, *J* = 3.8 Hz, CF_3), 135.2 (ArCH), 134.0 (ArCH), 143.7 (ArC), 147.8 (ArC), 162.8 (CO<u>C</u>ONH), 191.1 (<u>C</u>OCONH); IR (ATR): ν_{max} 3270, 3096, 2968, 2920, 1526, 1322, 1247, 1206, 1159, 1106, 1065, 839, 742, 667 cm^{-1}; HRMS (+ESI): Found *m/z* 387.1288, $[M+Na]^+$, $C_{19}H_{19}F_3N_2O_2Na$ requires 387.1291.

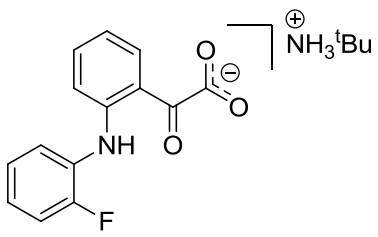
2-(2-((4-trifluoromethylphenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-25**



The title compound **2-25** was prepared from *N*-arylisatin **2-9** (0.062 g, 0.21 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate to give **2-25** as a yellow amorphous solid (0.044 g, 54%). M.p. 166-167°C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.25 (s, 9H, $C(C\underline{H}_3)_3$), 6.87-6.96 (m, 1H,

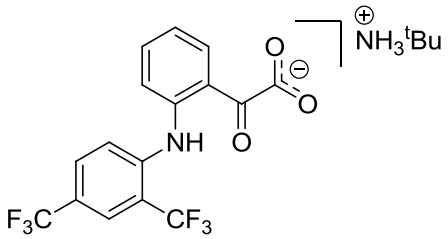
ArH), 7.41 (d, *J* = 8.5 Hz, 2H, 2 x ArH), 7.43-7.46 (m, 2H, 2 x ArH), 7.64 (d, *J* = 8.5 Hz, 2H, 2 x ArH), 7.75 (dd, *J* = 7.9, 1.4 Hz, 1H, ArH), 7.79 (bs, 3H, NH_3), 10.50 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.2 (C(<u>C</u>H_3)$_3$), 50.8 (<u>C</u>(CH_3)$_3$), 115.3 (ArCH), 118.6 (ArC), 119.0 (ArCH), 121.7 (q, *J* = 32.0 Hz, ArC), 123.7 (ArC), 125.5 (ArC), 126.6 (q, *J* = 3.7 Hz, ArCH), 134.0 (ArCH), 134.3 (ArCH), 144.7 (ArC), 169.1 (CO<u>C</u>O_2), 200.4 (<u>C</u>OCO_2), One peak not observed due to accidental equivalence; IR (ATR): ν_{max} 3290, 2980, 2906, 2829, 2637, 2537, 1589, 1526, 1450, 1375, 1322, 1249, 1216, 1159, 1109, 1065, 981, 876, 839, 793, 755 cm^{-1}; HRMS (-ESI): Found *m/z* 308.0536, $[M]^-$, $C_{15}H_9F_3NO_3$ requires 308.0540.

2-(2-((2-fluorophenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-26**



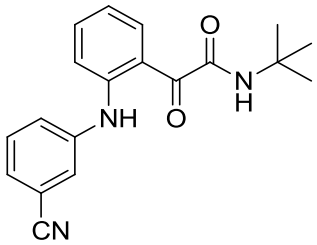
The title compound **2-26** was prepared from *N*-arylisatin **2-10** (0.070 g, 0.29 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate to give **2-26** as a yellow amorphous solid (0.066 g, 68%). M.p. 146-148°C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.26 (s, 9H, C(C<u>H</u>$_3$)$_3$), 6.81 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H, ArH), 7.08 (dd, *J* = 8.7, 1.1 Hz, 1H, ArH), 7.12-7.17 (m, 1H, ArH), 7.21 (ddd, *J* = 7.4, 7.4, 1.7 Hz, 1H, ArH), 7.32 (ddd, *J* = 11.3, 8.1, 1.4 Hz, 1H, ArH), 7.39 (ddd, *J* = 8.4, 7.2, 1.7 Hz, 1H, ArH), 7.53 (ddd, *J* = 8.4, 7.9, 1.5 Hz, 1H, ArH), 7.69 (dd, *J* = 7.4, 1.3 Hz, 1H, ArH), 8.15 (bs, 3H, NH_3), 10.33 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.2 (C(<u>C</u>H_3)$_3$), 50.8 (<u>C</u>(CH_3)$_3$), 113.4 (ArCH), 116.2 (d, *J* = 19.2 Hz, ArCH), 116.4 (ArCH), 117.46 (ArCH), 123.31 (ArCH), 124.6 (d, *J* = 7.6 Hz, ArCH), 125.0 (d, *J* = 3.6 Hz, ArCH), 128.0 (d, *J* = 11.5 Hz, ArC), 134.4 (d, *J* = 26.8 Hz, ArCH), 146.6 (ArC), 154.1 (ArC), 155.7 (ArC), 169.2 (CO<u>C</u>O_2), 200.6 (<u>C</u>OCO_2); IR (ATR): ν_{max} 3281, 2976, 2901, 2825, 2735, 2628, 2534, 1636, 1585, 1516, 1445, 1411, 1316, 1249, 1209, 1158, 1030, 988, 890, 831, 748, 706, 673 cm^{-1}; HRMS (-ESI): Found *m/z* 258.0567, $[M]^-$, $C_{14}H_9FNO_3$ requires 259.0572.

2-(2-((2,4-bis(trifluoromethyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-27**



The title compound **2-27** was prepared from the corresponding *N*-arylisatin **2-11** (0.050 g, 0.14 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate to give **2-27** as a yellow amorphous solid (0.027 g, 43%).M.p. >190°C (decomp.); ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.25 (s, 9H, C(C$\underline{H}_3)_3$), 7.02 (ddd, *J* = 8.3, 7.1, 1.1 Hz, 1H, ArH), 7.41 (dd, *J* = 8.3, 1.2 Hz, 1H, ArH), 7.47 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H, ArH), 7.79 (dd, *J* = 8.5, 1.1 Hz, 1H, ArH), 7.82-7.92 (m, 4H, NH_3 + ArH), 7.94 (dd, *J* = 8.3, 1.6 Hz, 1H, ArH), 7.96-8.00 (m, 1H, ArH), 10.87 (s, 1H, ArN$\underline{H}$Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.1 (C($\underline{C}H_3)_3$), 51.0 ($\underline{C}(CH_3)_3$), 115.9 (ArCH), 119.0 (ArC), 120.3 (ArCH), 121.4 (ArCH), 124.4 (ArC), 130.6 (ArCH), 134.1 (ArCH), 134.5 (ArCH), 143.0, (ArCH), 143.9 (ArC), 168.4 (CO$\underline{C}O_2$), 199.2 ($\underline{C}OCO_2$). Peaks for CF_3 and Ar$\underline{C}$-CF_3 were not observed due to low concentration of sample; IR (ATR): ν_{max} 2979, 1588, 1523, 1452, 1404, 1346, 1313, 1271, 1212 1162, 1110, 1050, 911, 834, 742, 670 cm^{-1}; HRMS (-ESI): Found *m/z*376.0411, $[M]^-$, $C_{16}H_8F_6NO_3$ requires 376.0414.

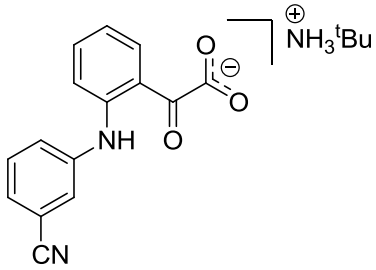
N-(*tert*-Butyl)-2-(2-((3-cyanophenyl)amino)phenyl)-2-oxoacetamide **2-22**



The title compound **2-22** was prepared from *N*-arylisatin **2-12** (0.200 g, 0.81 mmol) according to the general procedure outlined above. The product **2-22** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright yellow amorphous solid

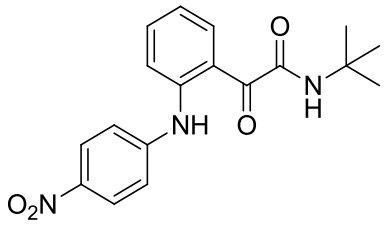
(0.054 g, 21 %). M.p. 88-90°C; ^{1}H NMR (600 MHz, CD_2Cl_2): δ 1.46 (s, 9H, C(C<u>H</u>$_3$)$_3$), 6.59 (bs, 1H, CONH), 6.68 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H, ArH), 7.27 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 7.39 (dd, *J* = 7.1, 1.6 Hz, 1H, ArH), 7.42-7.50 (m, 2H, 2 x ArH), 7.53-7.56 (m, 1H, ArH), 8.30 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 10.05 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, CD_2Cl_2): δ 28.6 (C(<u>C</u>H$_3$)$_3$), 52.3 (<u>C</u>(CH$_3$)$_3$), 113.9 (ArC), 114.9 (ArCH), 117.4 (ArC), 118.8 (ArCH), 125.3 (ArCH), 126.8 (ArCH), 127.5 (ArCH), 130.8 (ArCH), 135.4 (ArCH), 136.3 (ArCH), 141.7 (ArC), 148.2 (ArC), 163.4 (CO<u>C</u>ONH), 192.0 (<u>C</u>OCONH); IR (ATR): ν_{max} 3276, 3061, 2962, 2918, 2850, 2226, 1632, 1587, 1511, 1443, 1394, 1316, 1256, 1208, 1157, 1090, 1015, 950, 891, 852, 793, 760, 665 cm^{-1}; HRMS (+ESI): Found *m/z* 344.1360, $[M+Na]^+$, $C_{19}H_{19}N_3O_2Na$ requires 344.1369.

2-(2-((3-Cyanophenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-28**



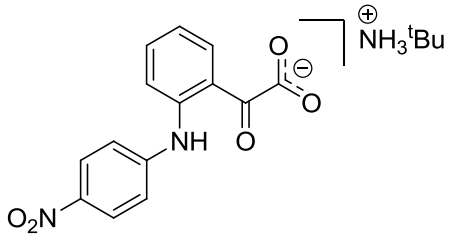
The title compound **2-28** was prepared from *N*-arylisatin **2-12** (0.200 g, 0.81 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate to give **2-28** as a yellow amorphous solid (0.197 g, 72%). M.p. 172°C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.26 (s, 9H, C(C<u>H</u>$_3$)$_3$), 6.88 (ddd, *J* = 7.9, 7.1, 1.1 Hz, 1H, ArH), 7.32 (dd, *J* = 8.5, 0.8 Hz, 1H, ArH), 7.40-7.45 (m, 2H, 2 x ArH), 7.75 (m, 1H, ArH), 7.55-7.79 (m, 1H, ArH), 7.72-7.78 (m, 1H, ArH), 7.80-8.10 (bs, 3H, NH_3), 10.40 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.2 (C(<u>C</u>H$_3$)$_3$), 50.7 (<u>C</u>(CH$_3$)$_3$), 112.3 (ArC), 114.4 (ArCH), 118.0 (CN), 118.5 (ArCH), 118.6 (ArC), 122.9 (ArCH), 124.9 (ArCH), 125.7 (ArCH), 130.6 (ArCH), 134.0 (ArCH) 134.3 (ArCH), 141.7 (ArC), 145.2 (ArC), 167.1 (CO<u>C</u>O$_2$), 200.2 (<u>C</u>OCO$_2$); IR (ATR): ν_{max} 3256, 2966, 2901, 2813, 2612, 2511, 2228, 1586, 1564, 1512, 1442, 1379, 1312, 1209, 1157, 1123, 1047, 986, 884, 843, 768, 751, 672 cm^{-1}; HRMS (-ESI): Found *m/z* 265.0614, $[M]^-$, $C_{15}H_9N_2O_3$ requires 265.0619.

N-(*tert*-Butyl)-2-(2-((4-nitrophenyl)amino)phenyl)-2-oxoacetamide **2-23**



The title compound **2-23** was prepared from *N*-arylisatin **2-13** (0.200 g, 0.75 mmol) according to the general procedure outlined above. The product **2-23** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright orange amorphous solid (0.040 g, 16 %). M.p. 134-136°C; ^{1}H NMR (600 MHz, CD_2Cl_2): δ 1.45 (s, 9H, $C(CH_3)_3$), 6.69 (bs, 1H, CONH), 7.03 (ddd, *J* = 8.2, 5.3, 2.9 Hz, 1H, ArH), 7.27 (d, *J* = 9.1 Hz, 2H, 2 x ArH), 7.51-7.55 (m, 2H, 2 x ArH), 8.17 (d, *J* = 9.1 Hz, 2H, 2 x ArH), 8.29 (dd, *J* = 8.2, 1.4 Hz, 1H, ArH), 10.05 (s, 1H, ArN<u>H</u>Ar); ^{13}C NMR (150 MHz, CD_2Cl_2): δ 28.5 ($C(\underline{C}H_3)_3$), 52.3 ($\underline{C}(CH_3)_3$), 117.6 (ArCH), 118.7 (ArCH), 120.1 (ArC), 120.9 (ArCH), 126.1 (ArCH), 135.3 (ArCH), 136.0 (ArCH), 142.3 (ArC), 145.4 (ArC), 147.7 (ArC), 162.7 (CO<u>C</u>ONH), 192.1 (<u>C</u>OCONH); IR (ATR): ν_{max} 3336, 3268, 2918, 1663, 1572, 1497, 1438, 1327, 1207, 1158, 1110, 1043, 842, 807, 742 cm^{-1}; HRMS (+ESI): Found *m/z* 364.1267, $[M+Na]^+$, $C_{18}H_{19}N_3O_4Na$ requires 364.1273.

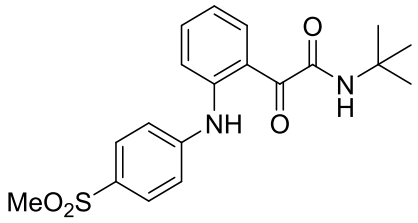
2-(2-((4-(nitrophenyl)phenyl)amino)phenyl)-2-oxoacetate *tert*-butylammonium salt **2-29**



The title compound **2-29** was prepared from *N*-arylisatin **2-13** (0.058 g, 0.22 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with ethyl acetate to give **2-29** as a yellow amorphous solid (0.050 g, 64%). M.p. 118-122°C; ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (s, 9H, $C(CH_3)_3$), 7.08 (ddd, *J* = 8.0,

6.1, 2.3 Hz, 1H, ArH), 7.33 (d, *J* = 9.2 Hz, 2H, 2 x ArH), 7.49-7.58 (m, 2H, 2 x ArH), 7.79 (dd, , *J* = 8.0, 1.1 Hz, 1H, ArH), 7.89 (bs, 3H, NH_3), 8.15 (d, *J* = 9.2 Hz, 2H, 2 x ArH), 10.63 (s, 1H, ArNHAr); ^{13}C NMR (100 MHz, d_6-DMSO): δ 27.3 ($C(\underline{C}H_3)_3$), 50.8 ($\underline{C}(CH_3)_3$), 116.6 (ArCH), 118.0 (ArCH), 121.2 (ArCH), 121.7 (ArC), 125.8 (ArCH), 133.7 (ArCH), 133.8 (ArCH), 139.8 (ArC), 142.4 (ArC), 148.4 (ArC), 169.0 ($CO\underline{C}O_2$), 199.7 ($\underline{C}OCONH$); IR (ATR): v_{max} 3262, 3023, 2896, 2813, 2727, 2614, 2511, 1572, 1522, 1487, 1451, 1401, 1301, 1250, 1214, 1182, 1109, 990, 886, 839, 749, 675 cm^{-1}; HRMS (-ESI): Found *m/z* 285.0514, $[M]^-$, $C_{14}H_9N_2O_5$ requires 285.0517.

N-(*tert*-Butyl)-2-(2-((4-(methylsulfonyl)phenyl)amino)phenyl)-2-oxoacetamide **2-24**



The title compound **2-24** was prepared from *N*-arylisatin **2-14** (0.100 g, 0.33 mmol) according to the general procedure outlined above. The product **2-24** was obtained by flash chromatography, eluting with 1:4 ethyl acetate/n-hexane as a bright yellow oil (0.014 g, 11 %). ^{1}H NMR (600 MHz, CD_2Cl_2): δ 1.45 (s, 9H, $C(C\underline{H_3})_3$), 3.03 (s, 3H, $SO_2C\underline{H_3}$), 6.68 (bs, 1H, CONH), 6.67 (ddd, *J* = 8.1, 5.9, 2.2 Hz, 1H, ArH), 7.36 (d, *J* = 8.6 Hz, 2H, 2 x ArH), 7.46-7.52 (m, 2H, 2 x ArH), 7.84 (d, *J* = 8.6 Hz, 2H, 2 x ArH), 8.28 (dd, *J* = 8.1, 1.5 Hz, 1H, ArH), 10.06 (s, 1H, ArNHAr); ^{13}C NMR (150 MHz, CD_2Cl_2): δ 28.6 ($C(\underline{C}H_3)_3$), 45.0 (SO_2CH_3), 52.3 ($\underline{C}(CH_3)_3$), 116.6 (ArCH), 119.0 (ArC), 120.0 (ArCH), 120.1 (ArCH), 129.5 (ArCH), 134.1 (ArC), 135.3 (ArCH), 136.0 (ArCH), 146.2 (ArC), 146.4 (ArC), 163.1 ($CO\underline{C}ONH$), 192.1 ($\underline{C}OCONH$); IR (ATR): v_{max} 3332, 2965, 2921, 2849, 1666, 1637, 1585, 1515, 1452, 1404, 1294, 1247, 1208, 1090, 1002, 953, 886, 842, 802, 749, 672 cm^{-1}; HRMS (+ESI): Found *m/z* 397.1188, $[M+Na]^+$, $C_{19}H_{22}N_2O_4SNa$ requires 397.1198.

2.5 References

(1) Konkel, M. J.; Packiarajan, M.; Chen, H. D.; Topiwala, U. P.; Jimenez, H.; Talisman, I. J.; Coate, H.; Walker, M. W. *Bioorg Med Chem Lett* **2006**, *16*, 3950.

(2) Wray, B. C.; Stambuli, J. P. *Org Lett* **2010**, *12*, 4576.

(3) Chan, D. M. T.; Monaco, K. L.; Wang, R. P.; Winters, M. P. *Tetrahedron Lett* **1998**, *39*, 2933.

CHAPTER 3

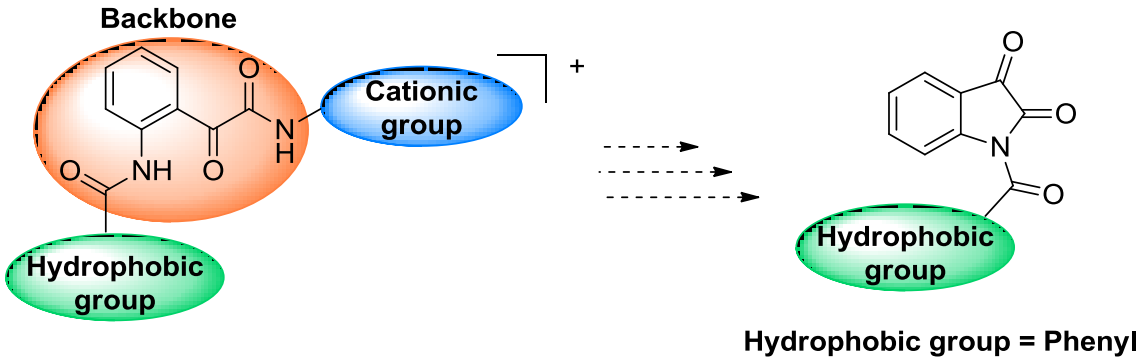
Chapter 3: *N*-Glyoxylamide based amphiphilic peptidomimetics as membrane active antimicrobials

3.1 General introduction and chapter aims

Owing to their structural resemblance to natural peptides *N*-glyoxylamides were chosen as the core scaffold for the design of novel amphiphilic peptide mimics. Preliminary biological studies have shown that this scaffold possesses low toxicity to mammalian cells, making it ideal for potential therapeutic applications.

The synthesis of *N*-glyoxylamides as described in Chapter **1** permits the facile, single-step introduction of alkyl and aryl amines, as well as amino acid esters, to the core scaffold. This strategy has been used successfully for the generation of amino acid-based peptide mimics and the extension of this methodology was envisioned to provide the desired amphiphilic peptidomimetics in an efficient manner.

In the retrosynthetic analysis it was envisaged that an *N*-acylisatin derivative with a hydrophobic substituent could be ring-opened with a primary amine incorporating a cationic moiety precursor, which could be followed by deprotection to generate the desired *N*-glyoxylamide-based amphiphilic peptidomimetic compound (Scheme **3.1**).



Scheme 3.1. Retrosynthesis of an amphiphilic peptidomimetic based on the *N*-glyoxylamide backbone (orange). The core structure contains both cationic (blue) and hydrophobic (green) moieties necessary for membrane activity.

As *N*-glyoxylamide-based biomimics have not yet been investigated in the literature, this project initially focused on varying the cationic group while keeping the hydrophobic group

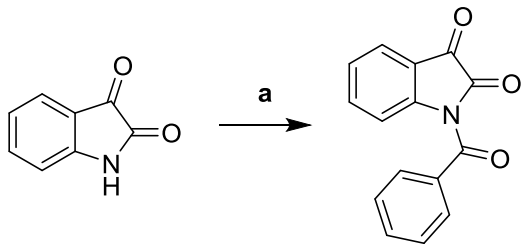
constant. To limit the number of factors affecting the properties of the peptidomimetics, a simple phenyl group was chosen for the hydrophobic substituent as a starting point for this research.[1,2]

3.2 Background, results and discussion

3.2.1 Synthesis of amphiphilic peptide mimics containing a quaternary ammonium salt

Quaternary ammonium salts are common structural motifs found in various antimicrobial agents as well as in bactericidal biomaterials.[3,4] [5] Unlike primary, secondary and tertiary ammonium salts, quaternary ammonium cations retain their positive charge irrespective of the pH in aqueous media. These groups are typically prepared by the alkylation of sterically accessible tertiary amines. Once formed, quaternary ammonium salts are no longer nucleophilic due to the lack of lone pairs on the nitrogen atom.

To begin the synthesis benzoylisatin **3-1** was prepared from isatin **1-1** and benzoyl chloride in the presence of 40% sodium hydride. The reaction proceeded without complication and the crude product was isolated in 87% yield and used in subsequent steps without further purification. With precursor **3-1** in hand, primary amines with the potential to be transformed into a cationic group were investigated for the ring-opening reaction of benzoylisatin **3-1**.

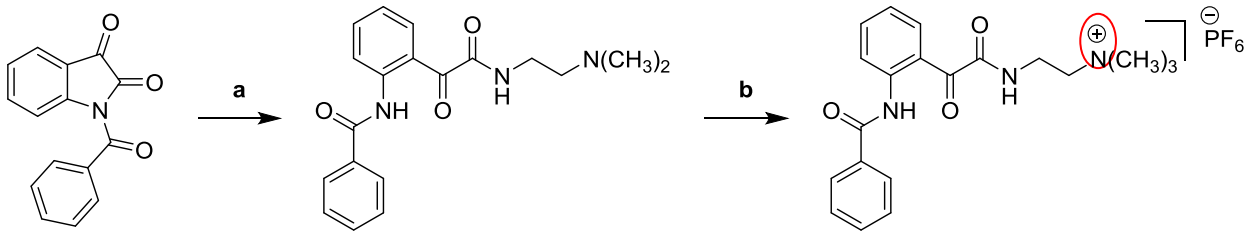


1-1 **3-1**

Scheme 3.2. Synthesis of the benzoylisatin precursor. Reagents and conditions: a) 40% NaH, benzoyl chloride, THF.

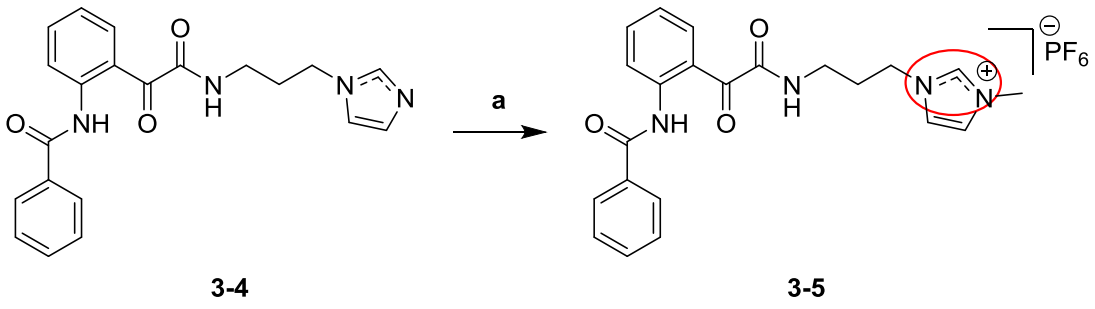
The *N,N,N,N*-ethyltrimethylammonium group is present in many bioactive molecules, such as the signaling molecule acetylcholine. To incorporate this moiety in our design, benzoyl isatin was reacted with N^1,N^1-dimethylethane-1,2-diamine to provide the intermediate **3-2**

(Scheme **3.3**). Subsequent methylation was conducted by refluxing **3-2** with a large excess of methyl iodide to obtain the iodide salt as a brown oil. A salt metathesis reaction was performed to convert the iodide salt to a more soluble PF_6^- salt, allowing the straightforward purification and isolation of **3-3** as a solid in high yield (87 %).



Scheme 3.3. Synthesis of amphiphilic derivative containing a *N,N,N,N*-ethyltrimethylammonium group. Reagents and conditions: a) N^1,N^1-dimethylethane-1,2-diamine, CH_2Cl_2 b) (i) MeI, toluene (ii) KPF_6, CH_3OH.

Similarly, the same reaction sequence using 1-(3-aminopropyl)imidazole as the primary amine gave the ring-opened product **3-4** and methylation and salt metathesis afforded **3-5** in reasonable yield (74%) (Scheme **3.4**).



Scheme 3.4. Synthesis of an amphiphilic peptide mimic **3-5** containing a quaternary ammonium cation formed from an imidazole group. Reagents and conditions: a) (i) MeI, toluene (ii) KPF_6, CH_3OH

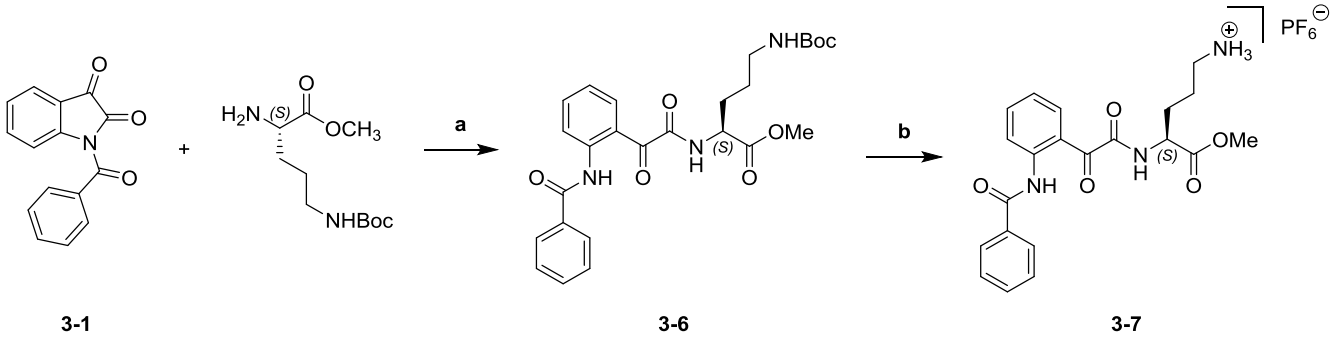
In comparing the charge distribution of molecules **3-3** and **3-5**, the positive charge on **3-3** is restricted to the nitrogen atom (Scheme **3.3**, highlighted in red), while the charge on **3-5** is delocalized over three atoms- (N1, C2 and N3) (Scheme **3.4**, positive charge highlighted in red).

Possessing different local charge distributions, the two molecules are expected to interact in a different manner towards bacterial cell membranes.

3.2.2 Synthesis of amphiphilic peptide mimics containing natural amino acids

Amino acids are often incorporated into peptide mimics and other biological molecules.[6] Integration of these natural building blocks is a convenient strategy for introducing functionality and stereochemistry into drug-like compounds. As lysine and arginine are responsible for the cationic functionality present in HDPs, these amino acids are ideal candidates for incorporation into amphiphilic *N*-glyoxylamide peptidomimetics.

To avoid selectivity issues caused by the presence of two competing nucleophilic amino groups in lysine, the pendant amino moiety was protected as a Boc group. Hence, N^d-Boc-L-ornithine methyl ester hydrochloride was selected as the ring-opening nucleophile. Following the methodology developed by Cheah,[6] benzoylisatin **3-1** was stirred with N^d-Boc-L-ornithine methyl ester hydrochloride and sodium bicarbonate in a bi-phasic solution of dichloromethane and water to afford the Boc-protected intermediate **3-6** (Scheme **3.5**). Treatment of **3-6** with TFA/CH_2Cl_2 1:4 (v/v) followed by a salt metathesis with KPF_6 afforded the desired L-ornithine peptide mimic **3-7**.

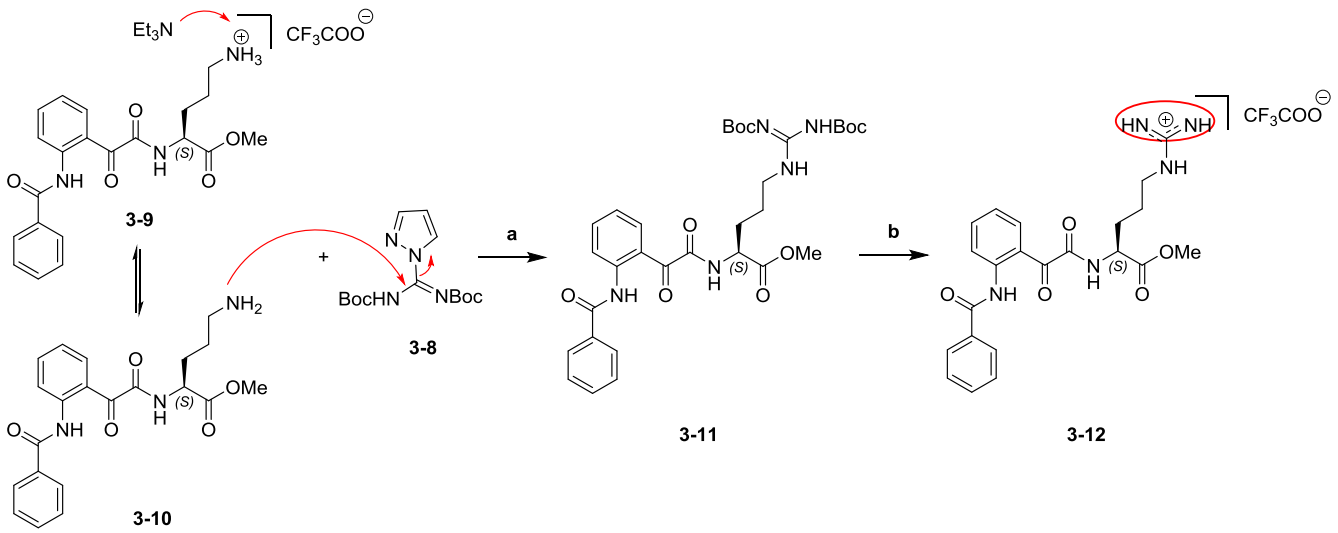


3-1 **3-6** **3-7**

Scheme 3.5. Synthesis of an amphiphilic peptide mimic containing a cationic ornithine moiety. Reagents and conditions: a) $NaHCO_3$, DCM/H_2O, b) 1. TFA/CH_2Cl_2 1:4 (v/v) 2. KPF_6, MeOH.

3.2.3 Amine guanylation reaction for the synthesis of arginine-containing amphiphilic peptide mimics

The conversion of unhindered primary and secondary amines to guanidines using *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine **3-8** has been reported by Bernatowicz.[7] The reaction of **3-8** and **3-9** proceeded in mild conditions, with the assistance of triethylamine promoting the deprotonation of the primary ammonium salt **3-9** to the primary amine **3-10** (Scheme **3.6**). Nucleophilic attack of the primary amine **3-10** onto the guanyl carbon of carboxamidine **3-8** led to the elimination of the pyrazole leaving group, forming the bis-Boc-protected guanidine intermediate **3-11**. Boc-deprotection yielded the L-arginine product **3-12** in 98% yield.



Scheme 3.6. Guanylation of **3-9**. Reagents and conditions: a) triethylamine, CH_2Cl_2 b) TFA/CH_2Cl_2 1:4 (v/v). The guanidine positive charge is distributed through three atoms (depicted in red).

Crystals of **3-12** suitable for single crystal X-ray analysis (synchrotron source) were grown by slow evaporation of a dichloromethane/n-hexane solution. It is observed that disorder is present in the crystal lattice of **3-12**, hence only one conformation is depicted for clarity in Figure **3.1**.

At physiological pH, the guanidine group exists predominately as the protonated cation, allowing the formation of an FA architecture. Like compound **3-5**, the positive charge of the guanidine cation is distributed over three atoms (Scheme **3.6**, highlighted in red).

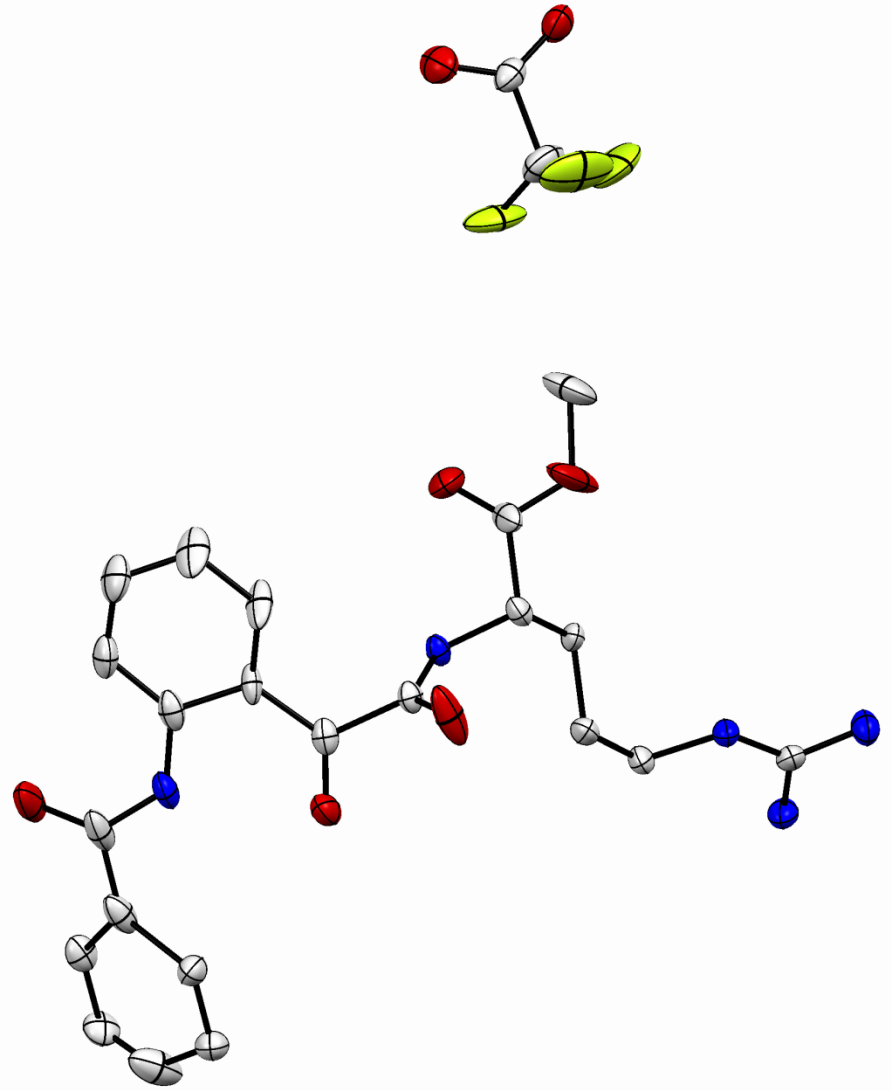
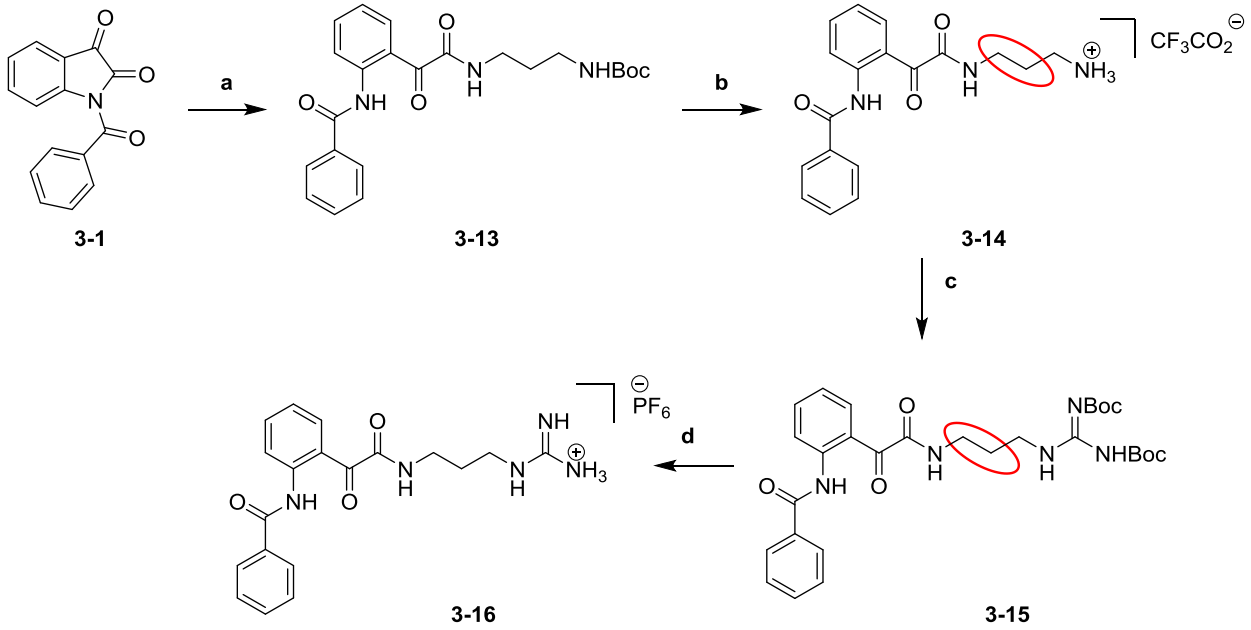


Figure 3.1. Front view of the crystal structure of the arginine-containing amphiphilic peptide mimic **3-12** (50% thermal ellipsoids at 100 K are shown; all H-atoms are omitted for clarity). Only one conformation is shown for clarity.

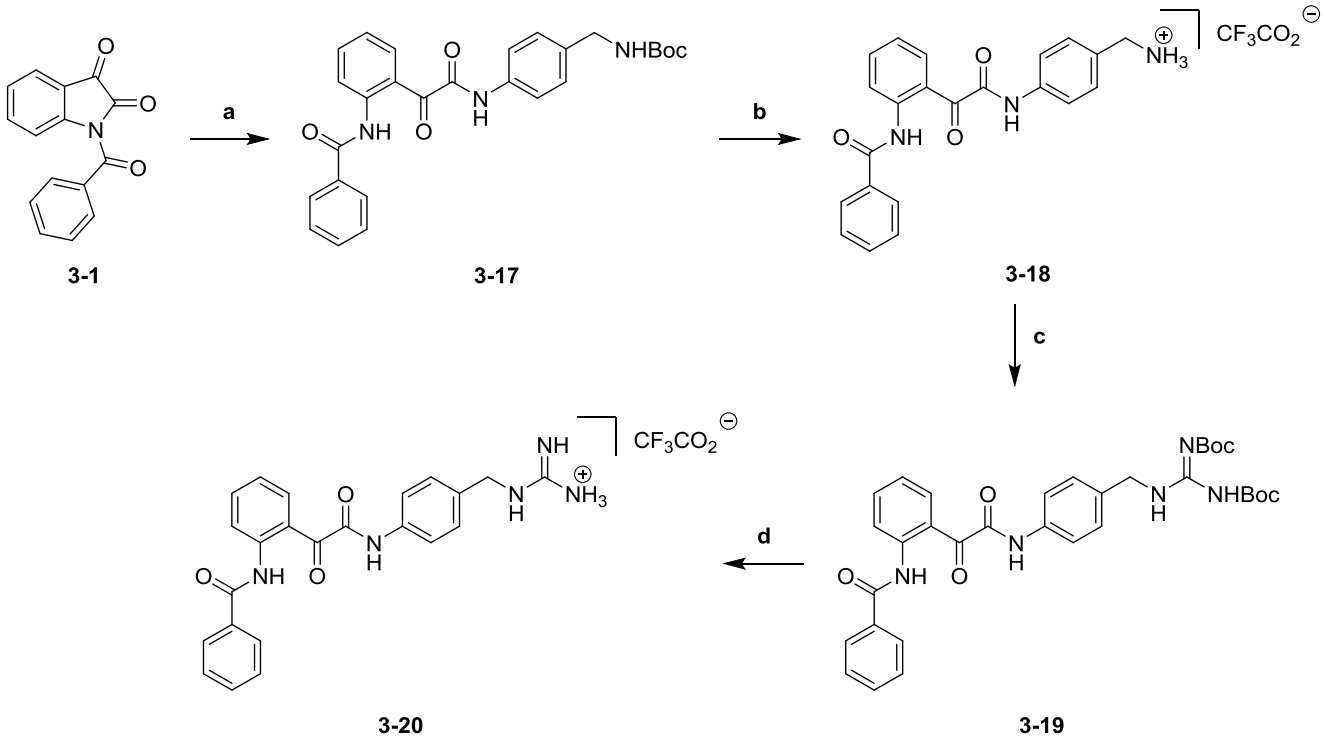
3.2.4 Simplification of ornithine and arginine amphiphilic derivatives

To simplify the structures of compounds **3-7** and **3-12**, we investigated the synthesis of derivatives lacking the non-essential α-carbon and methyl ester moieties, but retained the crucial amino acid side chains bearing the positive charge. Thus, benzoylisatin **3-1** was stirred with *N*-Boc-1,3-propanediamine to form the Boc-protected intermediate **3-13**. Deprotection of **3-13** under acidic conditions afforded the pseudo-ornithine product **3-14** (Scheme **3.7**). Subjecting **3-14** to the guanylation and deprotection sequence described previously for compound **3-12** followed by salt metathesis afforded peudo-arginine analogue **3-16** in 91% yield.



Scheme 3.7. Synthesis of pseudo-ornithine and arginine amphiphilic derivatives. Reagents and conditions: a) *N*-Boc-1,3-propanediamine, CH_2Cl_2 b) TFA/CH_2Cl_2 1:4 (v/v) c) *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine, triethylamine, CH_2Cl_2 d) 1. TFA/CH_2Cl_2 1:4 (v/v) 2.KPF_6, methanol. Alkyl linker highlighted in red.

Furthermore to explore the effects of rigidifying the alkyl chain linker (Scheme **3.7**, highlighted in red) on biological activity, the flexible alkyl chain was substituted with a *para*-phenyl group, which limits the rotation of the cationic moieties (Scheme **3.8**).

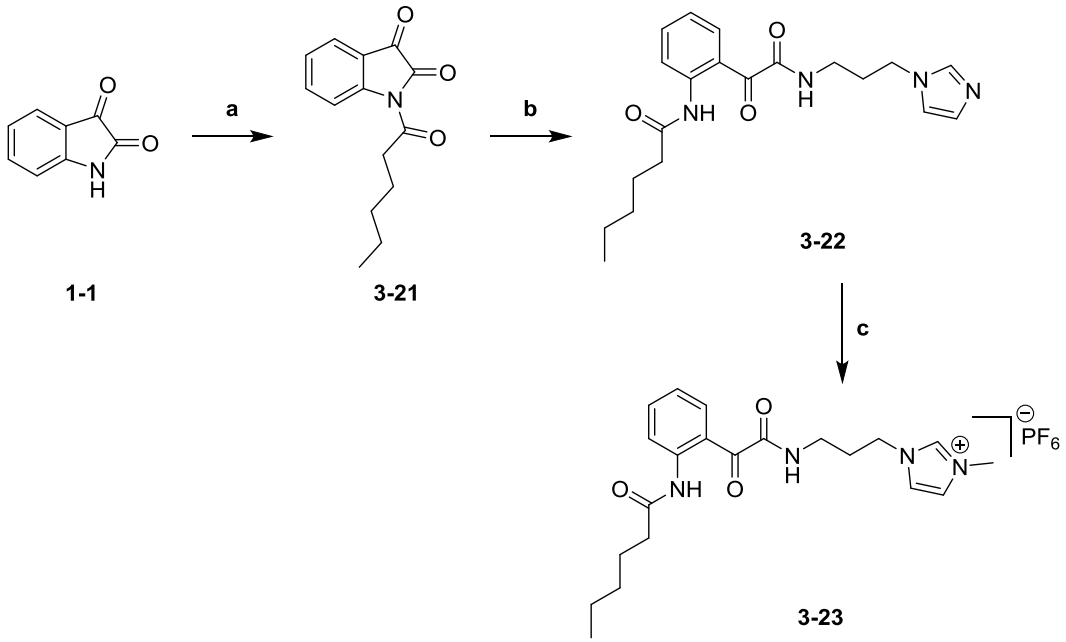


Scheme 3.8. Synthesis of *para*-phenyl amphiphilic derivatives **3-18** and **3-20**. Reagents and conditions: a) ^{t}butyl *N*-(4-aminobenzyl) carbamate, CH_2Cl_2 b) TFA/CH_2Cl_2 1:4 (v/v) c) *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine, triethylamine, CH_2Cl_2 d) TFA/CH_2Cl_2 1:4 (v/v).

3.2.5 Novel amphiphilic peptidomimetics containing hydrocarbon chains as the hydrophobic group

The previous amphiphilic peptidomimetics synthesized in this project varied in the nature of the cationic moiety, but retained the same phenyl hydrophobic group. To investigate the role of the hydrophobic group on membrane interactions, we targeted the synthesis of peptidomimetics where the phenyl group was replaced with an aliphatic chain. Aliphatic hydrophobic groups possess a greater degree of flexibility compared to aryl rings and also do not participate in aromatic π-π stacking. It was postulated that after binding to the negatively charged bacterial outer membrane, the inherent flexibility of a hydrophobic aliphatic chain could enhance the self-assembly of the peptidomimetics into the active FA conformation required for antibacterial activity.

To generate *N*-glyoxylamide amphiphilic mimics containing aliphatic hydrophobic groups, isatin **1-1** was *N*-acylated with hexanoyl chloride using the same procedure as for **3-1**. The crude intermediate **3-21** was reacted with 1-(3-aminopropyl)imidazole to form *N*-glyoxylamide **3-22**. Methylation of **3-22** followed by a salt metathesis furnished the desired amphiphilic target **3-23**. Crystals of **3-23** suitable for single crystal X-ray analysis (synchrotron source) were grown from a methanol solution. The propane linker in Figure **3.2** is bent such that the imidazolium ring lies roughly perpendicular to the plane made by the amide group. This is notable as a more linear conformation would have reduced the steric interactions between the imidazolium ring and the amide group. The interstitial distances between the atoms of the imidazolium group (N3, N4 and C20) and those of the amide carbonyl group (O1 and C1) average 3.29 Å with the shortest distance being that between O1 and C20 (3.030(2) Å). These short distances (c.f. sum of the v.d.w. radii = ca. 3.2 Å) suggests a possible stabilizing cation-π interaction from the carbonyl π molecular orbital into the positively-charged imidazolium ring, which could account for the bent conformation of the molecule.



Scheme 3.9. Synthesis of *N*-glyoxylamide amphiphlilic peptide mimic incorporating aliphatic chain as a hydrophobic group. Reagents and conditions: a) NaH, hexanoyl chloride, THF b) 1-(3-aminopropyl)imidazole, CH_2Cl_2 c) (i) MeI, toluene (ii) KPF_6, methanol .

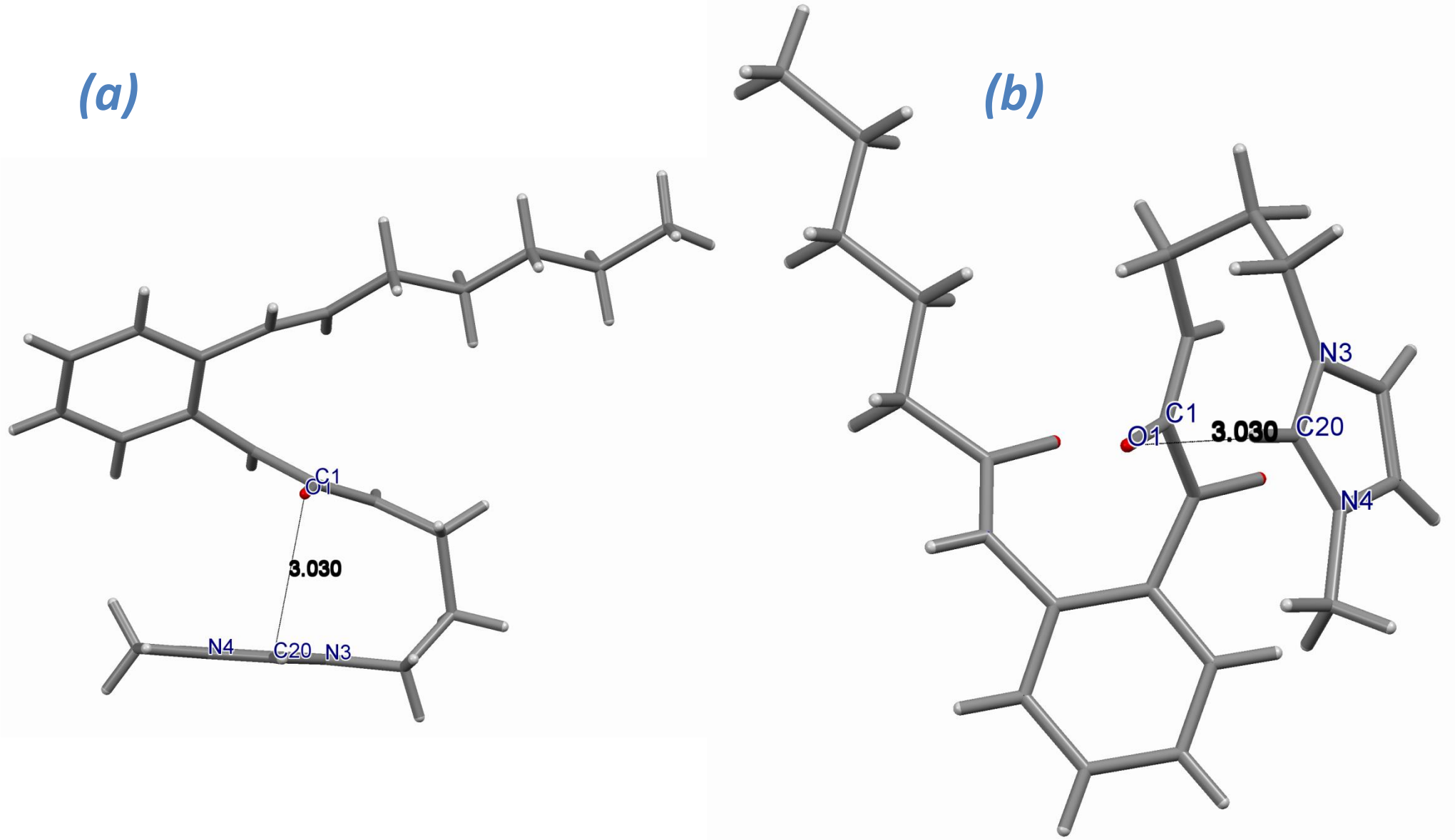


Figure 3.2. Top ***(a)*** and edge-on ***(b)*** views of the crystal structure of the cation of **3-23** (PF_6^- anion omitted for clarity). Selected distances are shown (Å): av. C20-O1 3.030(2).

3.2.6 Novel dimeric *N*-glyoxylamides as potential membrane active antibiotics

Selectivity and toxicity are major issues that must be addressed before a drug candidate can progress to clinical trials. As mentioned previously, the selectivity of these membrane-active peptidomimetics towards bacterial cells is dictated primarily by the overall balance of hydrophobic and hydrophilic groups in the molecule. With the successful synthesis of the first generation of amphiphilic *N*-glyoxylamides, attention was directed towards tuning the amphiphilicity of these compounds. It was envisaged that dimeric *N*-glyoxylamides might possess increased cationic character by virtue of having two cationic groups. Thus, molecules were designed that contained two *N*-glyoxylamide backbone moieties attached to a *para*-substituted hydrophobic core. The two cationic groups appended to the backbone confers a 2^+ charge to the overall molecule (Figure **3.3**).

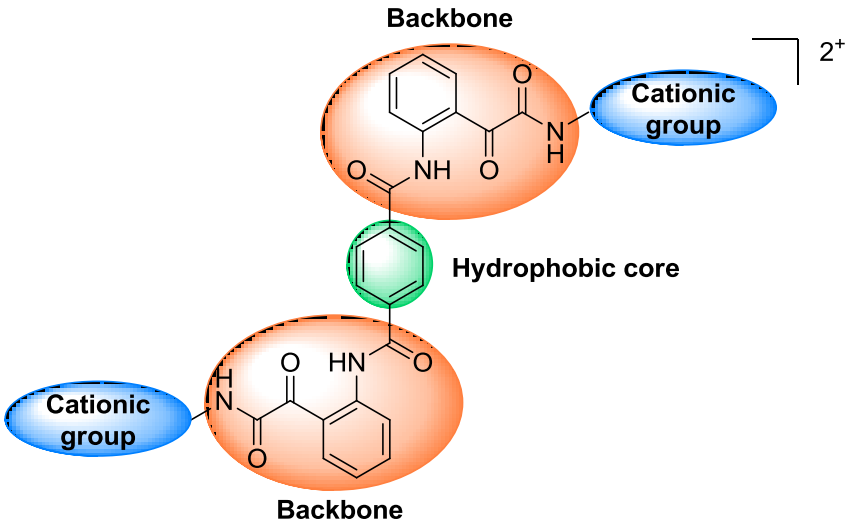
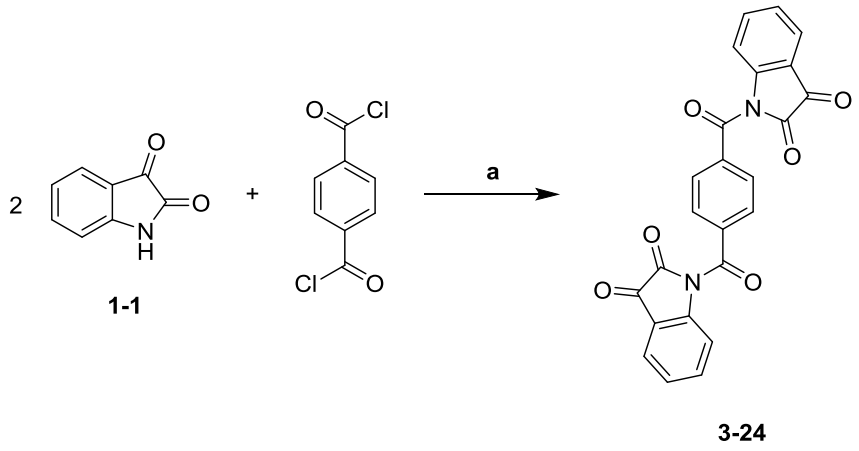


Figure 3.3. General structure of bis-*N*-glyoxylamide amphiphilic dimers.

3.2.7 Synthesis of dimeric amphiphilic peptide mimics

Dimeric compounds of the general structure depicted in Figure **3.3**, can be formed from the facile ring-opening reaction of the bis-isatin precursor **3-24**. Structurally-similar isatin dimers with varying linkers have been reported in the literature and can be synthesized *via* typical isatin *N*-acylation reactions. Thus, sodium isatide (2 equivalents) formed *in situ* from isatin and sodium hydride, was stirred with terephthaloyl chloride (1 equivalent) in THF for 0.5

h (Scheme **3.10**). Water was added and the precipitate was collected by filtration to generate the bis-isatin precursor **3-24** in 84% yield. The crude product was deemed sufficiently pure by ^{1}H NMR spectroscopy for further reactions.



Scheme 3.10. Synthesis of 1,1'-terephthaloylbis(indoline-2,3-dione) **3-24.** Reagents and conditions: a) NaH, THF.

The amine nucleophiles used to form the monomeric compounds were selected for the ring-opening reaction of isatin dimer **3-24**, allowing for a direct comparison to be made with their dimeric counterparts. The syntheses of *N*-glyoxylamide intermediates **3-25-3-28** were carried out in refluxing chloroform. The reactions were notably slower than those for the monomeric *N*-acylisatin derivatives due to the insoluble nature of the common substrate **3-24**. Formation of the cationic groups to achieve the final dimeric products **3-31-3-36** were carried out as described for their corresponding monomers. For compounds **3-31** and **3-32**, *N,N*-dimethylformamide was used to solubilize substrates **3-25** and **3-26**, before the final *N*-methylation reaction was carried out. A summary of the product yields is provided in Table **3.1**.

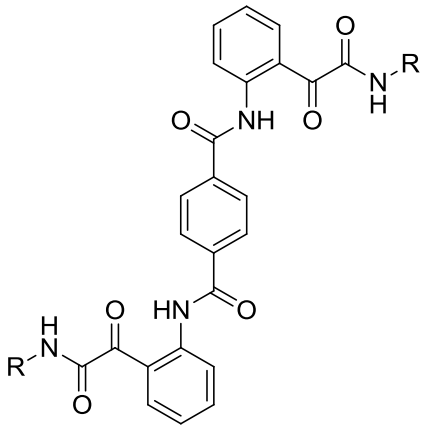
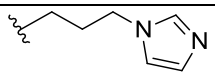
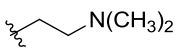
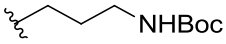
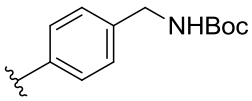
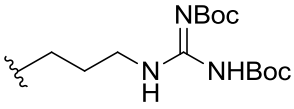
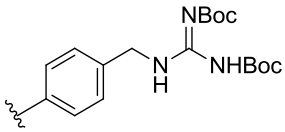
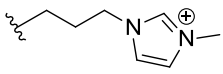
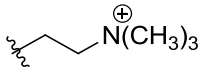
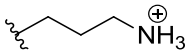
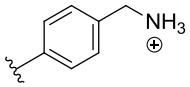
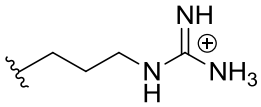
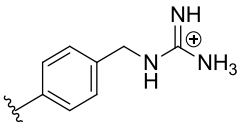
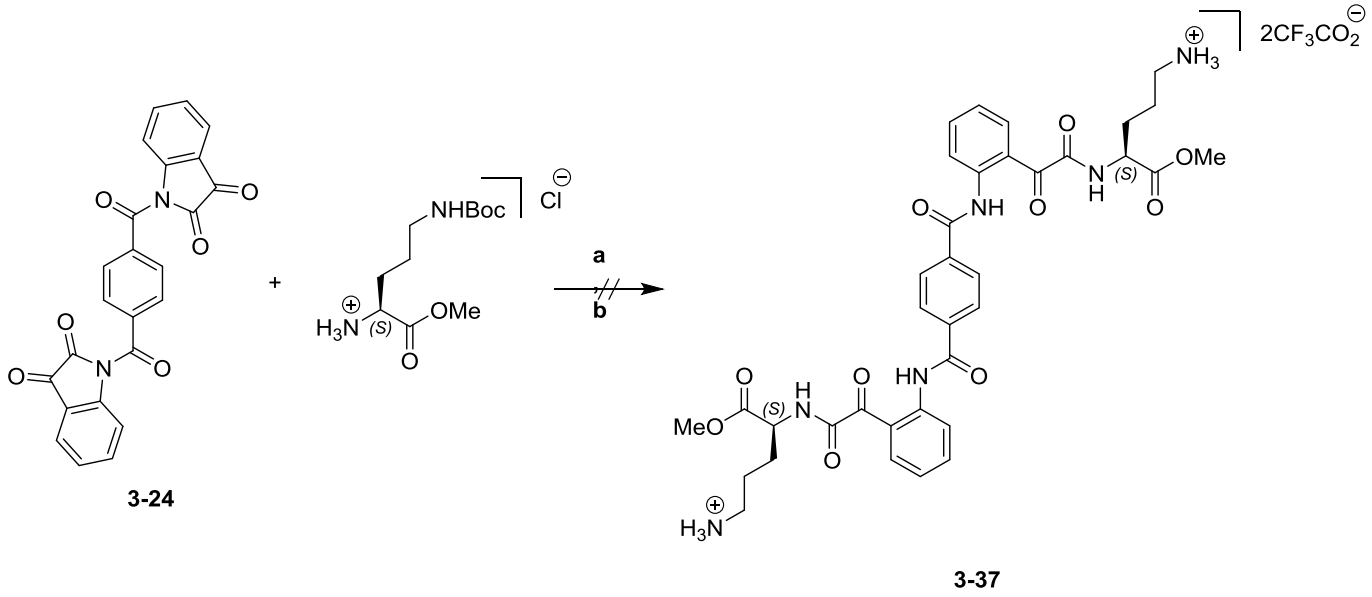


Table 3.1. Structure and product yields of dimeric intermediates **3-25**-**3-30** and di-cationic *N*-glyoxylamides **3-31**-**3-36**.

Compound	R	Counter anions ($2X^-$)	Reaction yield (%)
3-25		-	91
3-26		-	96
3-27		-	82
3-28		-	70
3-29		-	86
3-30		-	91
3-31			79

3-32		$I^{\ominus}$	37
3-33		$CF_3CO_2^{\ominus}$	97
3-34		$CF_3CO_2^{\ominus}$	90
3-35		$CF_3CO_2^{\ominus}$	88
3-36		$PF_6^{\ominus}$	74

Attempts at employing the same reaction conditions used for synthesizing the peptide mimic **3-7** failed to produce the desired di-cationic derivative **3-37**. Unreacted starting material **3-24** was recovered after stirring at room temperature for 16 h (scheme **3.11**). It was thought that the poor solubility of reactant **3-24** in dichloromethane and the lower nucleophilicity of N^{d}-Boc-L-ornithine methyl ester hydrochloride compared to the primary amines used for the synthesis of analogues **3-25-3-28** were the primary reasons for the failed reaction. Increasing the reaction temperature was not pursued as the ring opening of *N*-acylisatins by water to produce the corresponding *N*-glyoxylic acids is known to occur under weakly basic conditions at high temperatures.[8,9]



Scheme 3.11 . Attempted syntheses of a di-cationic peptide mimic **3-37** incorporating L-ornithine. Reagents and conditions: a) $NaHCO_3$, DCM/H_2O, b)TFA/CH_2Cl_2 1:4 (v/v).

Due to the unsuccessful synthesis of dimer **3-37**, the guanylation and deprotection sequence could not be carried out to produce the corresponding dimeric arginine analogue.

3.3 Summary and Future work

A novel series of first generation amphiphilic *N*-glyoxylamides has been synthesized in good to excellent yields of 66-98 %. The *N*-acylisatin chemistry employed in this section demonstrates the ease at which cationic groups can be appended to the *N*-glyoxylamide skeleton, in only 3-5 efficient steps. Derivatives with cationic groups mimicking the amino acids lysine and arginine were successfully synthesized, as well as two derivatives containing quaternary ammonium cationic groups. Using the same chemistry, a novel series of second generation, dimeric analogues were prepared from terephthaloyl chloride. These compounds incorporate the same functional groups present in the first series but possess two *N*-glyoxylamide moieties and contain two cationic groups per molecule.

With the successful synthesis of both series of amphiphilic peptide mimic candidates, future work will be directed towards evaluating their biological profiles. These compounds will be tested for their antibacterial properties among various bacterial strains. In addition, their toxicity with respect to mammalian cells will be determined.

3.4 Experimental

3.4.1 General methods

Synthesis and Reagents

A detailed account of general synthetic methodologies is provided in the experimental section for **Chapter 2**. Further details relevant to this chapter are presented below. *tert*-Butyl *N*-(4-aminobenzyl) carbamate [10] was synthesized according to literature procedures.

Spectroscopy

A detailed account of general spectroscopic methodologies is provided in the experimental section for **Chapter 2**.

Crystallography

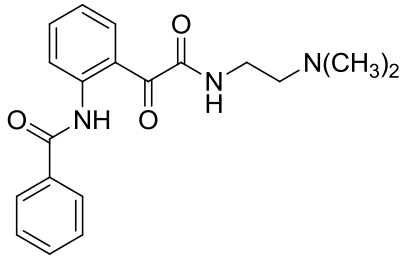
Single crystal X-ray diffraction data for **3-12** and **3-23** were obtained on the MX-1 beamline at the Australian Synchrotron. Structures were processed and refined using SHELX software.

3.4.2 Synthesis

General procedure for the ring-opening reaction of N-acylisatin with amines

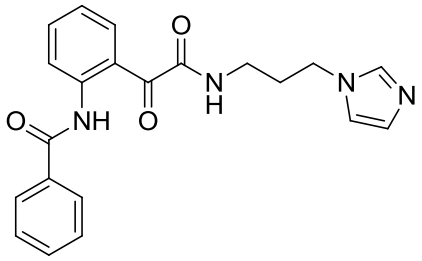
N-Acylisatin and the amine were dissolved in DCM and the reaction was stirred at room temperature for 3 h. The reaction was worked-up according to the described procedure.

N-(2-(2-((2-(Dimethylamino)ethyl)amino)-2-oxoacetyl)phenyl)benzamide **3-2**



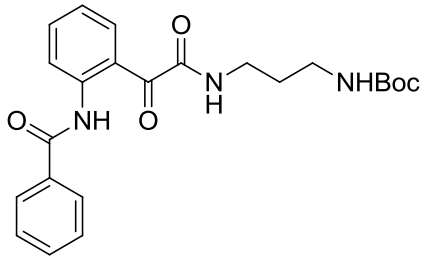
The title compound **3-2** was prepared from **3-1** (0.254 g, 1.01 mmol) and *N*′,*N*′-dimethylethane-1,2-diamine (0.12 mL, 1.06 mmol) according to the general procedure outlined above. The reaction mixture was left to cool to room temperature. HCl (30 mL, aq., 2 M) was added and the mixture was extracted with several portions of DCM. The combined organic extracts were washed with water, dried over anhydrous Na_2SO_4, filtered and the solvent was removed in vacuo. The residual oil was recrystallized from hot ethanol to afford **3-2** as light brown prisms (0.247 g, 72%); M.p. 97-99 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 6H, $N(CH_3)_2$), 2.69 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.59 (q, *J* = 5.8 Hz, 2H, $NHCH_2$), 7.18 (ddd, *J* = 8.1, 7.4, 1.2 Hz, 1H, ArH), 7.42-7.64 (m, 4H, N<u>H</u>CH_2 + 3 x ArH), 7.68 (ddd, *J* = 8.5, 7.4, 1.6 Hz, 1H, ArH), 7.98-8.10 (m, 2H, 2 x ArH), 8.38 (dd, *J* = 8.1, 1.6 Hz, 1H, ArH), 8.93 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 11.99 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.6 ($NHCH_2$), 45.0 (CH_3), 57.6 (<u>C</u>$H_2N(CH_3)_2$), 119.0 (ArC), 121.0 (ArCH), 122.8 (ArCH), 127.6 (ArCH), 129.0 (ArCH), 132.4 (ArCH), 134.7 (ArCH), 134.8 (ArC), 136.9 (ArCH), 142.8 (ArC), 163.6 (CO<u>C</u>ONH), 166.2 (<u>C</u>ONHAr), 192.7 (<u>C</u>OCONH); IR (ATR): ν_{max} 3306, 3057, 2974, 2804, 2763, 1640, 1604, 1525, 1475, 1289, 1255, 1213, 1161, 948, 931, 856, 747, 690 cm^{-1}; HRMS (+ESI): Found m/z 340.1649, $[M+H]^+$, $C_{19}H_{22}N_3O_3$ requires 340.1661.

N-(2-(2-((3-(1*H*-Imidazol-1-yl)propyl)amino)-2-oxoacetyl)phenyl)benzamide **3-4**



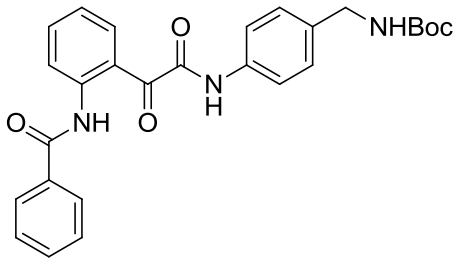
The title compound **3-4** was prepared from **3-1** (0.400 g, 1.86 mmol) and 1-(3-aminopropyl)imidazole (0.222 mL, 1.86 mmol) according to the general procedure outlined above. The solvent was removed in vacuo and the crude product was subjected to flash chromatography on silica gel, eluting with 1:1 DCM/ethyl acetate. The pale yellow amorphous solid obtained was sufficiently pure by ^{1}H NMR spectroscopy for use in subsequent steps (0.495 g, 82%). M.p. 107-110 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.86 (qu, *J* = 6.8 Hz, 2H, $CH_2C\underline{H}_2CH_2$), 3.13 (q, *J* = 6.8 Hz, 2H, $NHC\underline{H}_2$), 3.94 (t, *J* = 6.8 Hz, 2H, NCH_2), 6.89 (s, 1H, imH), 7.15 (s, 1H, imH), 7.32 (ddd, *J* = 8.5, 7.4, 1.1 Hz, 1H, ArH), 7.54-7.69 (m, 4H, 3 x ArH + imH), 7.72 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, ArH), 7.80 (dd, *J* = 8.5, 1.6 Hz, 1H, ArH), 7.95-7.99 (m, 2H, 2 x ArH), 8.20 (dd, *J* = 8.2, 1.1 Hz, 1H, ArH), 8.95 (bt, *J* = 5.6 Hz, 1H, $N\underline{H}CH_2$), 11.41 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 30.3 ($CH_2\underline{C}H_2CH_2$), 35.8 ($NHCH_2$), 43.5 (NCH_2), 119.3 (ArCH), 121.9 (ArCH), 123.6 (ArC), 123.9 (ArCH), 127.4 (ArCH), 128.4 (ArCH), 128.8 (ArCH), 132.2 (ArCH), 132.3 (ArCH), 133.9 (ArC), 134.6 (ArCH), 137.3 (ArCH), 139.2 (ArC), 163.9 ($CO\underline{C}ONH$), 165.4 ($\underline{C}ONHAr$), 192.3 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3274, 2933, 1637, 1578, 1510, 1445, 1295, 1209, 1077, 915, 818, 752, 698 cm^{-1}; HRMS (+ESI): Found m/z 399.1419, $[M+Na]^+$, $C_{21}H_{20}N_4NaO_3$ requires 399.1433.

tert-Butyl (3-(2-(2-benzamidophenyl)-2-oxoacetamido)propyl)carbamate **3-13**



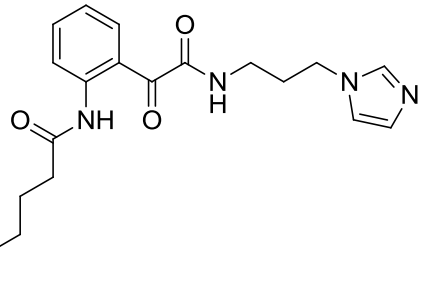
The title compound **3-13** was prepared from **3-1** (0.340 g, 1.36 mmol) and N-Boc-1,3-propanediamine (0.248 g, 1.42 mmol) according to the general procedure outlined above. The reaction mixture was left to cool to room temperature. 2M HCl was added and the mixture was extracted with several portions of DCM. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and the solvent was removed in vacuo. The crude product was purified using flash chromatography on silica gel, eluting with 1:4 ethyl acetate/n-hexane to afford **3-13** as a pale yellow amorphous powder (0.502 g, 87%). M.p. 160 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, $C(CH_3)_3$), 1.76 (qu, *J* = 6.2, 2H, $CH_2C\underline{H}_2CH_2$), 3.25 (q, *J* = 6.2, 2H, $C\underline{H}_2NHBoc$), 3.49 (q, *J* = 6.2, 2H, $CONHC\underline{H}_2$), 4.87 (bt, *J* = 6.2 Hz, 1H, $N\underline{H}Boc$), 7.18 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H, ArH), 7.43-7.62 (m, 4H, 4 x ArH), 7.68 (ddd, *J* = 8.6, 7.3, 1.7 Hz, 1H, ArH), 8.01-8.07 (m, 2H, 2 x ArH), 8.38 (bd, *J* = 6.2 Hz, 1H, $CON\underline{H}CH_2$), 8.93 (dd, *J* = 8.6, 1.2 Hz, 1H, ArH), 12.00 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5 ($C(\underline{C}H_3)_3$), 30.3 ($CH_2\underline{C}H_2CH_2$), 36.4 ($\underline{C}H_2NHBoc$), 37.3 ($CONH\underline{C}H_2$), 79.8 ($\underline{C}(CH_3)_3$), 119.0 (ArC), 121.0 (ArCH), 122.8 (ArCH), 127.6 (ArCH), 129.0 (ArCH), 132.4 (ArCH), 134.7 (ArC), 134.8 (ArCH), 137.0 (ArCH), 142.8 (ArC), 156.9 (OCONH), 163.7 ($CO\underline{C}ONH$), 166.2 ($\underline{C}ONHAr$), 192.9 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3298, 1663, 1533, 1481, 1281, 1165, 1007, 912, 763, 697 cm^{-1}; HRMS (+ESI): Found m/z 448.1832, $[M+Na]^+$, $C_{23}H_{27}N_3NaO_5$ requires 448.1848.

tert-Butyl 4-(2-(2-benzamidophenyl)-2-oxoacetamido)benzylcarbamate **3-17**



The title compound **3-17** was prepared from **3-1** (0.484 g, 1.93 mmol) and ^{t}butyl *N*-(4-aminobenzyl)carbamate (0.450 g, 2.02 mmol) according to the general procedure outlined above. The reaction mixture was left to cool to room temperature. 2M HCl was added and the mixture was extracted with several portions of DCM. The combined organic extracts were washed with water, dried over sodium sulfate, filtered and the solvent was removed in vacuo. The crude product was purified using flash chromatography on silica gel, eluting with 1:4 ethyl acetate/n-hexane to afford **3-17** as a light yellow amorphous solid (0.415 g, 67%). M.p. 178-179 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H, $C(CH_3)_3$), 4.32 (d, *J* = 5.1 Hz, 2H, $C\underline{H}_2NH$), 4.75-4.96 (m, 1H, $CH_2N\underline{H}$), 7.09-7.40 (m, 3H, 3 x ArH), 7.48-7.80 (m, 6H, 6 x ArH), 7.95-8.15 (m, 2H, 2 x ArH), 8.59-8.73 (m, 1H, ArH), 8.79 (bs, 1H, COCONH), 8.94 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 11.90 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 28.3 ($C(\underline{C}H_3)_3$), 43.0 (CH_2), 77.8 ($\underline{C}(CH_3)_3$), 120.1 (ArCH), 121.9 (ArCH), 123.2 (ArC), 124.0 (ArCH), 127.4 (ArCH), 127.4 (ArCH), 128.8 (ArCH), 132.3 (ArCH), 132.4 (ArCH), 133.9 (ArC), 134.9 (ArCH), 136.3 (ArC), 136.3 (ArC), 139.4 (ArC), 155.8 (OCONH), 162.0 (CO<u>C</u>ONH), 165.5 (<u>C</u>ONHAr), 191.4 (<u>C</u>OCONH); IR (ATR): ν_{max} 3358, 3285, 1682, 1626, 1578, 1521, 1444, 1390, 1249, 1158, 1027, 898, 754, 683 cm^{-1}; HRMS (+ESI): Found m/z 474.2020, $[M+H]^+$, $C_{27}H_{28}N_3O_5$ requires 474.2029.

N-(2-(2-((3-(1H-Imidazol-1-yl)propyl)amino)-2-oxoacetyl)phenyl)hexanamide **3-22**

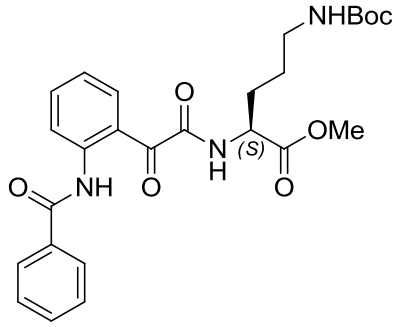


Isatin **1-1** (0.600 g, 4.08 mmol) was slowly added to a stirred suspension of sodium hydride 60% dispersion in mineral oil (0.171 g, 4.28 mmol) in THF (150 mL) at 0 °C under an inert atmosphere. At this point the suspension turned from orange to a dark purple, indicating the formation of the sodium salt of isatin. The suspension was left to stir for 5 minutes. Hexanoyl chloride (0.627 mL, 4.49 mmol) in THF (10 mL) was added dropwise and the solution was stirred for an additional hour. The solvent was reduced to 20% of the original volume in vacuo. Water (400 mL) was added and the resulting precipitate was collected by filtration. The crude product **3-21** was used in the subsequent step without further purification.

The title compound **3-22** was prepared from the crude intermediate **3-21** and 1-(3-aminopropyl)imidazole (0.535 mL, 4.49 mmol) according to the general procedure outlined above. The solvent was removed in vacuo and the crude product was subjected to flash chromatography on silica gel, eluting with 1:19 NEt_3/ethyl acetate to afford **3-22** as a beige amorphous powder (0.620 g, 82%). M.p. 104-106 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 0.86 (t, *J* = 6.9 Hz, 3H, CH_3), 1.22-1.33 (m, 4H, $C\underline{H_2}C\underline{H_2}CH_3$), 1.56 (qu, *J* = 7.5 Hz, 2H, $NCH_2C\underline{H_2}CH_2$), 1.94 (qu, *J* = 6.9 Hz, 2H, $COCH_2C\underline{H_2}CH_2$), 2.31 (t, *J* = 7.5 Hz, 2H, NCH_2), 3.16 (q, *J* = 6.7 Hz, 2H, $NHC\underline{H_2}$), 4.01 (t, *J* = 6.9 Hz, 2H, $COCH_2$), 6.90 (s, 1H, imH), 7.20 (s, 1H, imH), 7.23 (ddd, *J* = 7.8, 7.4, 1.1 Hz, 1H, ArH), 7.60 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, ArH), 7.62 (dd, *J* = 7.8, 1.6 Hz, 1H, ArH), 7.66 (s, 1H, imH), 7.83 (dd, *J* = 8.2, 1.1 Hz, 1H, ArH), 8.83 (bt, *J* = 6.7 Hz, 1H, $N\underline{H}CH_2$), 10.56 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 14.3 (CH_3), 22.3 (CH_2), 24.9 (CH_2), 30.9 (CH_2), 31.2 (CH_2), 36.3 (CH_2), 37.0 (CH_2), 44.0 (CH_2), 119.8 (ArCH), 121.7 (ArCH), 123.8 (ArCH), 124.4 (ArC), 128.8 (ArCH), 132.0 (ArCH), 134.3 (ArCH), 137.8 (ArCH), 138.7 (ArC), 163.9 ($CO\underline{C}ONH$), 172.1 ($\underline{C}ONHAr$), 191.5 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3274, 3067, 2933, 2870, 1665, 1600, 1522, 1476,

1378, 1322, 1254, 1215, 1106, 1073, 956, 774, 723 cm^{-1}; HRMS (+ESI): Found m/z 393.1893, $[M+Na]^+$, $C_{20}H_{26}N_4O_3Na$ requires 393.1903.

(*S*)-Methyl 2-(2-(2-benzamidophenyl)-2-oxoacetamido)-5-((tert-butoxycarbonyl)amino)pentanoate **3-6**



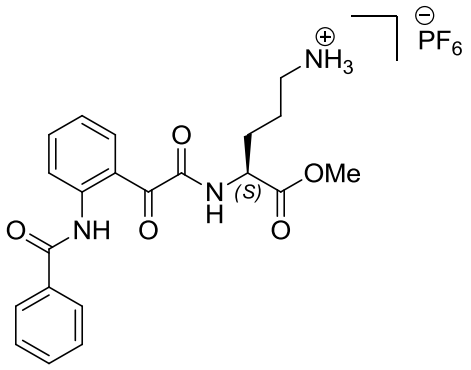
To a stirred solution of **3-1** (0.430 g, 1.71 mmol) in DCM (20 mL) was added a mixture of N^d-Boc-L-ornithine methyl ester hydrochloride (0.532 g, 1.88mmol) and Na_2SO_4 (sat., aq.) (20 mL). The reaction was stirred for 5 hours at room temperature. The organic layer was diluted with DCM and washed with HCl (30 mL, aq., 1 M) and water. The organic extract was dried over anhydrous Na_2SO_4, filtered and the solvent was removed in vacuo. The residual pale yellow oil was dissolved in a minimum volume of DCM and diluted with a large volume of n-hexane. The precipitate was collected by filtration to afford the title compound **3-6** as a pale yellow amorphous solid (0.588 g, 69%). M.p. 85-86 °C; $[\alpha]_D$ -359 (c 0.089, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, $C(CH_3)_3$), 1.75-1.92 (m, 2H, $CH_2C\underline{H_2}CH_2$), 1.94-2.11 (m, 2H, $CHC\underline{H_2}$), 3.18 (q, *J* = 6.7 Hz, 2H, $NHC\underline{H_2}$), 3.81 (s, 3H, OCH_3), 4.60 (bs, 1H, $N\underline{H}CH_2$), 4.12 (ddd, *J* = 13.1, 7.9, 5.4 Hz, 1H CH), 7.19 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H, ArH), 7.47-7.64 (m, 3H, 3 x ArH), 7.70 (ddd, *J* = 8.7, 7.3, 1.8 Hz, 1H, ArH), 7.92-8.12 (m, 2H, 2 x ArH), 8.47 (dd, *J* = 8.2, 1.8 Hz, 1H, ArH), 8.95 (dd, *J* = 8.7, 1.2 Hz, 1H, ArH), 12.00 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 25.9 ($CH_2\underline{C}H_2CH_2$), 27.8 ($CH\underline{C}H_2$), 28.3 ($C(\underline{C}H_3)_3$), 39.2 ($NHCH_2$), 51.78 (CH), 52.1 (OCH_3), 77.5 ($\underline{C}(CH_3)_3$), 120.7 (ArC), 120.9 (ArCH), 123.5 (ArCH), 127.2 (ArCH), 129.0 (ArCH), 132.4 (ArCH), 133.3 (ArCH), 133.9 (ArC), 135.8 (ArCH), 140.6 (ArC), 155.6 (OCONH), 164.8 ($CO\underline{C}ONH$), 165.2 ($\underline{C}ONHAr$), 171.7 ($\underline{C}O_2CH_3$), 193.6 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3304, 2948, 1744, 1680, 1580, 1517, 1446, 1248, 1203, 1159,

866, 752, 697 cm^{-1}; HRMS (+ESI): Found m/z 520.2042, $[M+Na]^+$, $C_{26}H_{31}N_3NaO_7$ requires 520.2060.

General procedure for Boc deproctection

N-(Boc)-protected N-glyoxylamide was stirred in TFA-CH_2Cl_2 (20% v/v) at room temperature for 0.5 h. The solvent was removed in vacuo and the resulting oil was purified according to the described procedures.

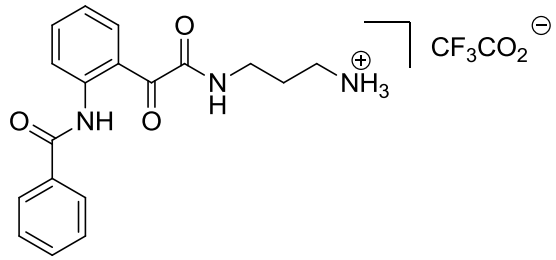
(*S*)-Methyl 5-amino-2-(2-(2-benzamidophenyl)-2-oxoacetamido)pentanoate PF_6 salt **3-7**



The title compound **3-7** was prepared from **3-6** (0.235 g, 0.154 mmol) according to the general procedure outlined above. The crude yellow oil was dissolved in a minimum volume of methanol and KPF_6 (sat., aq.) was added. The mixture was extracted with several portions of DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford **3-7** as a tan amorphous solid (0.224 g, 98%). M.p. >180 °C (decomp.); $[\alpha]_D$ 0 (c 0.17, CH_2Cl_2); ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.53-1.63 (m, 2H, $CH_AH_BC\underline{H_2}CH_2$), 1.69-1.78 (m, 1H, $C\underline{H_A}H_BCH_2CH_2$), 1.83-1.93 (m, 1H, $CH_A\underline{H_B}CH_2CH_2$), 2.73-2.83 (m, 2H, $CH_AH_BCH_2C\underline{H_2}$), 3.69 (s, 3H, OCH_3), 4.43 (ddd, *J* = 8.9, 7.6, 5.0 Hz, 1H, COCH), 7.35 (ddd, *J* = 8.0, 7.5, 0.7 Hz, 1H, ArH), 7.55-7.72 (m, 6H, NH_3 + 3 x ArH), 7.78 (ddd, *J* = 8.5, 7.5, 1.4 Hz, 1H, ArH), 7.87 (dd, *J* = 8.0, 1.4 Hz, 1H, ArH), 7.95-8.02 (m, 2H, 2 x ArH), 8.48 (dd, *J* = 8.5, 0.7 Hz, 1H,

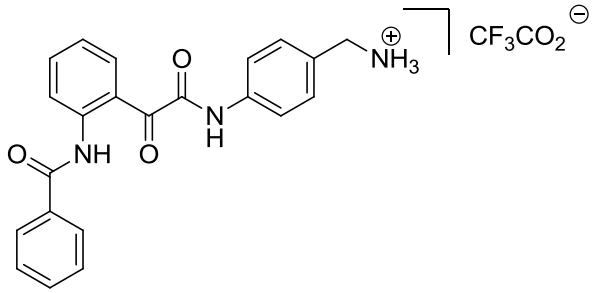
ArH), 9.35 (bd, *J* = 7.6 Hz, 1H, N<u>H</u>CH), 11.66 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, d_6-acetone): δ 24.7 ($CH_2\underline{C}H_2CH_2$), 29.1 ($CH\underline{C}H_2$), 48.1 ($C\underline{H}_2NH_3$), 52.6 (CH), 52.8 ($CO_2\underline{C}H_3$), 120.3 (ArC), 121.3 (ArCH), 123.8 (ArCH), 128.1 (ArCH), 129.8 (ArCH), 133.2 (ArCH), 135.0 (ArCH), 135.4 (ArC), 137.1 (ArCH), 142.8 (ArC), 165.5 (CO<u>C</u>ONH), 166.2 (<u>C</u>ONHAr), 172.0 ($\underline{C}O_2CH_3$), 194.6 (<u>C</u>OCONH); IR (ATR): ν_{max} 3255, 2922, 1709, 1637, 1578, 1531, 1446, 1365, 1301, 1183, 1116, 1000, 928, 838, 797, 756, 708 cm^{-1}; HRMS (+ESI): Found m/z 398.1701, $[M]^+$, $C_{21}H_{24}N_3O_5$ requires 398.1710.

3-(2-(2-Benzamidophenyl)-2-oxoacetamido)propan-1-aminium trifluoroacetate salt **3-14**



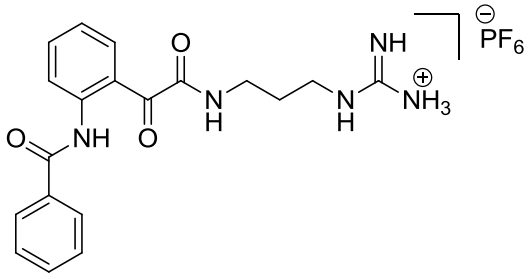
The title compound **3-14** was prepared from **3-13** (0.827 g, 1.94 mmol) according to the general procedure outlined above. The crude yellow oil was dissolved in a minimum volume of acetone and diluted with a large volume of n-hexane. The precipitate was collected by filtration to afford the title compound **3-14** as a colourless amorphous solid (0.588 g, 69%). M.p. 130-132 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.75 (qu, *J* = 6.3, 2H, $CH_2C\underline{H}_2CH_2$), 2.81 (sx, *J* = 6.3, 2H, $NH_3C\underline{H}_2$), 3.25 (q, *J* = 6.3, 2H, $NHC\underline{H}_2$), 7.32 (ddd, *J* = 7.9, 7.4, 1.1 Hz, 1H, ArH), 7.56-7.62 (m, 2H, 2 x ArH), 7.63-7.68 (m, 1H, ArH), 7.73 (ddd, *J* = 8.3, 7.4, 1.7 Hz, 1H, ArH), 7.75-7.89 (m, 4H, NH_3 + ArH), 7.94-7.98 (m, 2H, 2 x ArH), 8.22-8.25 (m, 1H, ArH), 8.95 (bt, *J* = 5.8 Hz, 1H, N<u>H</u>CH_2), 11.42 (s, 1H, COCON<u>H</u>); ^{13}C NMR (150 MHz, d_6-DMSO): δ 27.4 ($CH_2\underline{C}H_2CH_2$), 36.2 (NH_3CH_2), 37.2 ($NHCH_2$), 122.2 (ArCH), 123.7 (ArC), 124.2 (ArCH), 127.8 (ArCH), 129.3 (ArCH), 132.7 (ArCH), 132.8 (ArCH), 134.4 (ArC), 135.2 (ArCH), 139.7 (ArC), 158.7 (d, J = 33.1 Hz, $\underline{C}OCF_3$), 163.4 (CO<u>C</u>ONH), 165.8 (<u>C</u>ONHAr), 192.8 (<u>C</u>OCONH). A peak for CF_3 was not observed; IR (ATR): ν_{max} 3259, 3097, 1781, 1635, 1578, 1535, 1447, 1302, 1140, 998, 845, 797, 755, 696 cm^{-1}; HRMS (+ESI): Found m/z 326.1491, $[M]^+$, $C_{18}H_{20}N_3O_3$ requires 326.1499.

N-(2-(2-((4-(Aminomethyl)phenyl)amino)-2-oxoacetyl)phenyl)benzamide trifluoroacetate salt **3-18**



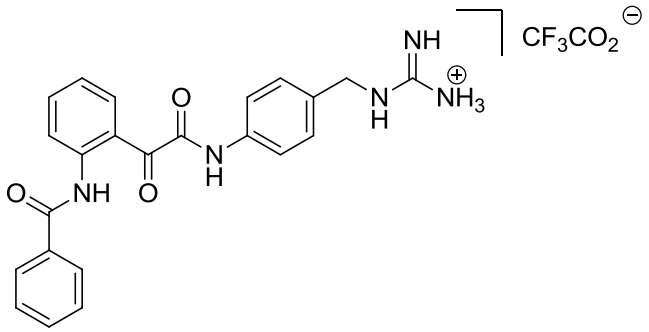
The title compound **3-18** was prepared from **3-17** (0.073 g, 0.154 mmol) according to the general procedure outlined above. The crude yellow oil was dissolved in a minimum volume of acetone and diluted with a large volume of n-hexane to afford the title compound **3-18** as a tan amorphous solid (0.065 g, 86%). M.p. 156-158 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 4.00 (q, *J* = 5.4 Hz, 2H, CH_2), 7.35 (ddd, *J* = 7.9, 7.4, 1.2 Hz, 1H, ArH), 7.41 (d, *J* = 8.6 Hz, 2H, Ar-H_{para}), 7.49-7.67 (m, 3H, 3x ArH), 7.73 (d, *J* = 8.6 Hz, 2H, ArH_{para}), 7.74-7.78 (m, 1H, ArH), 7.83 (dd, *J* = 7.9, 1.4 Hz, 1H, ArH), 7.89-8.01 (m, 2H, 2 x ArH), 8.04-8.33 (m, 4H, NH_3 + ArH), 10.90 (s, 1H, ArCONHAr), 11.33 (s, 1H, COCONH); ^{13}C NMR (75 MHz, d_6-DMSO): δ 41.9 (CH_2), 120.2 (ArCH), 122.2 (ArCH), 123.9 (ArC), 124.1 (ArCH), 127.4 (ArCH), 128.8 (ArCH), 129.5 (ArCH), 129.9 (ArC), 132.0 (ArCH), 132.3 (ArCH), 133.9 (ArC), 134.6 (ArCH), 138.0 (ArC), 138.9 (ArC), 161.9 (COCONH), 165.6 (CONHAr), 190.6 (COCONH); IR (ATR): ν_{max} 3253, 3065, 2879, 1674, 1605, 1539, 1452, 1302, 1246, 1179, 1130, 997, 897, 837, 747, 696 cm^{-1}; HRMS (+ESI): Found m/z 374.1490, $[M]^+$, $C_{22}H_{20}N_3O_3$ requires 374.1499.

1-(3-(2-(2-Benzamidophenyl)-2-oxoacetamido)propyl)guanidinium trifluoroacetate salt **3-16**



The title compound **3-16** was prepared from **3-15** (0.100 g, 0.176 mmol) according to the general procedure outlined above. The crude yellow oil was dissolved in a minimum volume of methanol and KPF_6 (sat., aq.) was added. The mixture was extracted with several portions of DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo.* The crude yellow residue was recrystallized from methanol to afford **3-16** as pale yellow prisms (0.082 g, 91%). M.p. 190-192 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.64 (qu, *J* = 6.8, 2H, $CH_2C\underline{H_2}CH_2$), 3.02-3.15 (m, 2H, $CNHNHC\underline{H_2}$), 3.16-3.26 (m, 2H, $CONHC\underline{H_2}$), 7.17 (bs, 3H, NH_3), 7.19 (ddd, *J* = 8.5, 7.4, 1.2 Hz, 1H, ArH), 7.51-7.70 (m, 4H, 3 x ArH + NH), 7.73 (ddd, *J* = 8.3, 7.3, 1.6 Hz, 1H, ArH), 7.80 (dd, *J* = 7.8, 1.6 Hz, 1H, ArH), 7.93-8.00 (m, 2H, 2 x ArH), 8.22 (dd, *J* = 8.3, 1.2 Hz, 1H, ArH), 8.88 (bt, *J* = 6.8 Hz, 1H, NH), 11.42 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, d_6-DMSO): δ 28.7 ($CH_2\underline{C}H_2CH_2$), 36.4 ($\underline{C}H_2NHCN$), 38.8($CONH\underline{C}H_2$), 122.3 (ArCH), 123.8 (ArC), 124.3 (ArCH), 127.8 (ArCH), 129.3 (ArCH), 132.7 (ArCH), 132.8 (ArCH), 134.4 (ArC), 135.2 (ArCH), 139.7 (ArC), 157.2 (C=N), 164.4 ($CO\underline{C}ONH$), 165.9 ($\underline{C}ONHAr$), 192.9 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3257, 3172, 3083, 1538, 1450, 1300, 1174, 1126, 977, 893, 820, 798, 698 cm^{-1}; HRMS (+ESI): Found *m/z* 368.1708, $[M]^+$, $C_{19}H_{22}N_5O_3$ requires 368.1717.

1-(4-(2-(2-Benzamidophenyl)-2-oxoacetamido)benzyl)guanidinium 2,2,2-trifluoroacetate **3-20**

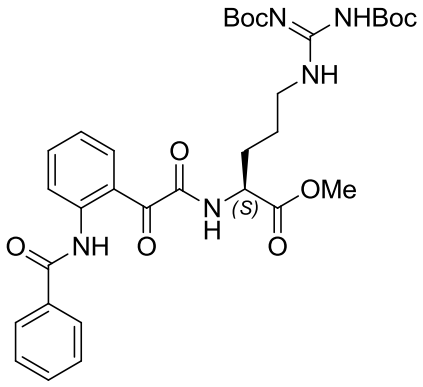


The title compound **3-20** was prepared from **3-19** (0.152 g, 0.249 mmol) according to the general procedure outlined above. The crude yellow oil was dissolved in a minimum volume of acetone and diluted with a large volume of n-hexane to afford the title compound **3-20** as a pale yellow amorphous solid (0.116 g, 89%). ^{1}H NMR (600 MHz, d_6-DMSO): δ 4.34 (d, *J* = 6.1 Hz, 2H, CH_2), 6.88-7.79 (bs, 4H, CN<u>H</u>N<u>H</u>$_3$), 7.28 (d, *J* = 8.4 Hz, 2H, 2 x ArH), 7.35 (ddd, *J* = 7.8, 7.4, 1.2 Hz, 1H, ArH), 7.51-7.56 (m, 2H, 2 x ArH), 7.60-7.63 (m, 1H, ArH), 7.69 (d, *J* = 8.4 Hz, 2H, 2 x ArH), 7.75 (ddd, *J* = 8.3, 7.4, 1.6 Hz, 1H, ArH), 7.85 (dd, *J* = 7.8, 1.6 Hz, 1H, ArH), 7.91-8.0 (m, 2H, 2 x ArH), 8.09-8.17 (m, 1H, N<u>H</u>CH_2), 8.20 (dd, *J* = 8.3, 1.2 Hz, 1H, ArH), 10.87 (s, 1H, COCONH), 11.38 (s, 1H, ArCONHAr); ^{13}C NMR (150 MHz, d_6-DMSO): δ 43.6 (CH_2), 120.3 (ArCH), 122.1 (ArCH), 123.6 (ArC), 124.0 (ArCH), 127.4 (ArCH), 127.8 (ArCH), 128.8 (ArCH), 132.2 (ArCH), 132.3 (ArCH), 133.2 (ArC), 133.9 (ArC), 134.8 (ArCH), 137.1 (ArC), 139.2 (ArC), 157.0 (C=N), 158.9 (d, *J* = 34.3 Hz, <u>C</u>OCF_3), 162.0 (CO<u>C</u>ONH), 165.6 (<u>C</u>ONHAr), 191.1 (<u>C</u>OCONH). A peak for CF_3 was not observed; IR (ATR): ν_{max} 3369, 3299, 3121, 3060, 2917, 1649, 1605, 1533, 1447, 1415, 1300, 1239, 1178, 1132, 953, 880, 839, 824, 753, 699 cm^{-1}; HRMS (+ESI): Found *m/z* 416.1709, $[M]^+$, $C_{23}H_{22}N_5O_3$ requires 416.1717.

General procedure for guanylation of mono-amines

A mixture of amine (1 equivalent), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (1.05 equivalent) and triethylamine (1 equivalent) was dissolved in dichloromethane and stirred at room temperature for 3 hours. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel, eluting with 3:7 ethyl acetate/n-hexane.

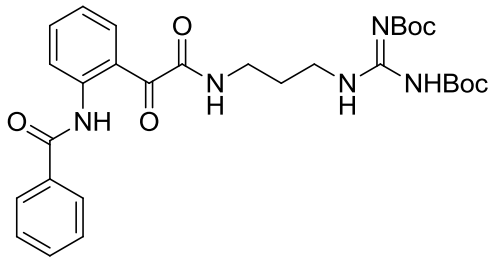
(S)-Methyl 2-(2-(2-benzamidophenyl)-2-oxoacetamido)-5-(2,3-bis(tert-butoxycarbonyl)guanidino)pentanoate **3-11**



The title compound **3-11** was prepared from **3-9** (0.210 g, 0.378 mmol), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (0.126 g, 0.396 mmol) and triethylamine (0.052 mL, 0.378 mmol) according to the general procedure outlined above. **3-11** was isolated as a light yellow amorphous powder (0.196 g, 81%). M.p. 81-82°C; $[\alpha]_D$ 4.6 (*c* 0.22, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 9H, C(CH_3)$_3$), 1.50 (s, 9H, C(CH_3)$_3$), 1.69-2.12 (m, 4H, CHCH_2CH_2), 3.49 (ddd, J = 10.0, 7.0, 3.0 Hz, 2H, NHCH_2), 3.78 (s, 3H, OCH_3), 4.70 (ddd, *J* = 8.4, 8.4, 4.9 Hz, 1H, CHCH_2), 7.28 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.56-7.71 (m, 3H, 3 x ArH), 7.77 (ddd, *J* = 8.5, 7.4, 1.7 Hz, 1H, ArH), 8.04-8.09 (m, 2H, 2 x ArH), 8.17 (dd, *J* = 8.0, 1.7 Hz, 1H, ArH), 8.36 (bt, *J* = 5.2 Hz, 1H, NHCH_2), 8.59 (bd, *J* = 7.6 Hz, 1H, NHCH), 8.88 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 11.64 (s, 1H, N$HCO_2C(CH_3)_3$), 12.03 (s, 1H, CONHAr); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.8 ($CH_2CH_2CH_2$), 28.1 (C(CH_3)$_3$), 28.3 (C(CH_3)$_3$), 28.8 ($CHCH_2$), 40.2 ($NHCH_2$), 52.5 (OCH_3), 52.8 ($CHCH_2$), 79.5 (C(CH_3)$_3$), 83.4 (C(CH_3)$_3$), 118.7 (ArC), 120.7 (ArCH), 122.9 (ArCH), 127.6 (ArCH), 129.0 (ArCH), 132.3 (ArCH), 134.5 (ArC), 134.8 (ArCH), 137.0 (ArCH), 142.8 (ArC), 153.4 (OCONH), 156.5 (OCONH), 163.4 (C=N), 163.5 (COCONH), 166.2 (CONHAr), 171.8 (CO_2CH_3), 192.2 (COCONH); IR (ATR): ν_{max}

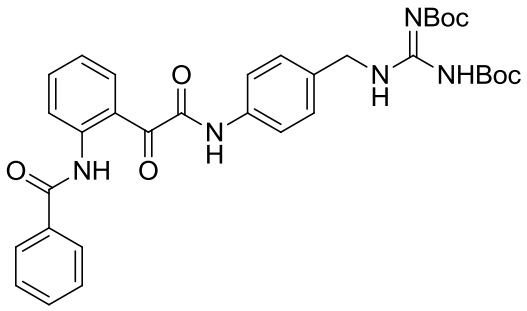
3282, 2976, 1720, 1636, 1580, 1526, 1448, 1413, 1365, 1298, 1248, 1207, 1131, 1050, 849, 801, 751, 698 cm^{-1}; HRMS (+ESI): Found *m/z* 440.1917, $[M+H]^+$, $C_{22}H_{26}N_5O_5$ requires 440.1928.

N-(2-(2-Oxo-2-(3-(*N,N'*-di-(Boc)guanadine)propylamino)acetyl)phenyl)benzamide **3-15**



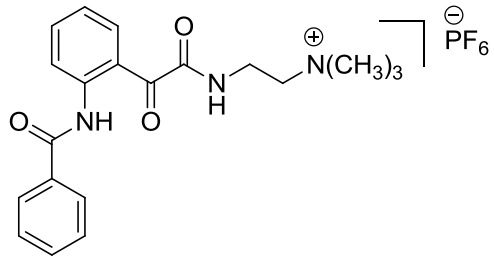
The title compound **3-15** was prepared from **3-14** (0.111 g, 0.253 mmol), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (0.082 g, 0.265 mmol) and triethylamine (0.035 mL, 0.253 mmol) according to the general procedure outlined above. **3-15** was isolated as a white amorphous powder (0.139 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 9H, C(CH_3)$_3$), 1.84 (qu, *J* = 6.3, 2H, $CH_2CH_2CH_2$), 2.05 (s, 9H, C(CH_3)$_3$), 3.50 (q, *J* = 6.3, 2H, CNNHCH_2), 3.60 (q, *J* = 6.3, 2H, CONHCH_2), 7.19 (ddd, *J* = 8.0, 7.3, 1.2 Hz, 1H, ArH), 7.48-7.63 (m, 3H, 3 x ArH), 7.68 (ddd, *J* = 8.5, 7.3, 1.6 Hz, 1H, ArH), 8.01-8.10 (m, 2H, 2 x ArH), 8.17 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.47 (t, *J* = 6.3 Hz, 1H, NH), 8.57 (bt, *J* = 6.3 Hz, 1H, NH), 8.96 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 11.43 (s, 1H, NHBoc), 12.12 (s, 1H, CONHAr). NHCHBoc not observed due to exchange; ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.1 (C(CH_3)$_3$), 28.2 (C(CH_3)$_3$), 30.1 ($CH_2CH_2CH_2$), 35.7 (C=NNHCH_2), 37.4 (CONHCH_2), 79.6 (C(CH_3)$_3$), 83.8 (C(CH_3)$_3$), 118.8 (ArC), 120.9 (ArCH), 122.8 (ArCH), 127.6 (ArCH), 129.0 (ArCH), 132.3 (ArCH), 134.6 (ArCH), 134.7 (ArC), 136.7 (ArCH), 142.8 (ArH), 153.3 (OCONH), 157.4 (C=N), 164.7 (COCONH), 166.2 (CONHAr), 194.3 (COCONH). OCONH missing due to overlap; IR (ATR): ν_{max} 3282, 2974, 1788, 1735, 1651, 1610, 1525, 1449, 1333, 1291, 1215, 1135, 1048, 856, 814, 765, 666 cm^{-1}; HRMS (+ESI): Found *m/z* 568.2767, $[M+H]^+$, $C_{29}H_{38}N_5O_7$ requires 568.2771.

N-(2-(2-Oxo-2-(3-(*N,N'*-di-(Boc)guanadine)benzylamino)acetyl)phenyl)benzamide **3-19**



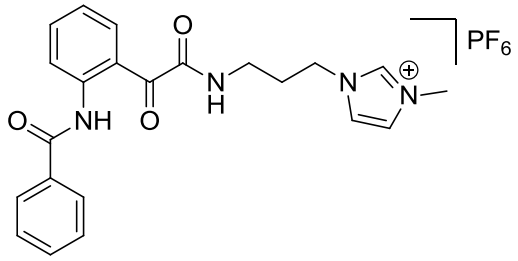
The title compound **3-19** was prepared from trifluoroacetate salt **3-18** (0.350 g, 0.718 mmol), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (0.234 g, 0.754 mmol) and triethylamine (0.100 mL, 0.718 mmol) according to the general procedure outlined above. **3-19** was isolated as a pale yellow amorphous powder (0.415 g, 96%). M.p. 148-151°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.48 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.51 (s, 9H, C(C$\underline{H}_3$)$_3$), 4.65 (d, *J* = 5.0 Hz, 2H, ArCH_2), 7.15 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H, ArH), 7.34 (d, *J* = 8.5 Hz, 2H, 2 x ArH), 7.47-7.52 (m, 2H, 2 x ArH), 7.55-7.59 (m, 1H, ArH), 7.64 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 1H, ArH), 7.71 (d, *J* = 8.5 Hz, 2H, 2 x ArH), 7.97-8.03 (m, 2H, 2 x ArH), 8.50 (dd, *J* = 8.1, 1.7 Hz, 1H, ArH), 8.68 (bs, 1H, N$\underline{H}CH_2$), 8.87 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 9.09 (s, 1H, CNN$\underline{H}$Boc), 11.54 (s, 1H, COCON$\underline{H}$), 11.93 (s, 1H, PhCON$\underline{H}$Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.2 (C($\underline{C}H_3$)$_3$), 28.4 (C($\underline{C}H_3$)$_3$), 44.8 (CH_2), 80.1 ($\underline{C}$(CH$_3$)$_3$), 83.7 ($\underline{C}$(CH$_3$)$_3$), 118.8 (ArC), 120.5 (ArCH), 120.9 (ArCH), 123.0 (ArCH), 127.5 (ArCH), 128.9 (ArCH), 129.1 (ArCH), 132.5 (ArCH), 134.4 (ArC), 134.5 (ArC), 134.9 (ArCH), 136.3 (ArC), 137.2 (ArCH), 142.8 (ArC), 153.3 (C=N), 156.1 (OCONH), 160.5 (CO$\underline{C}$ONH), 166.2 (Ar$\underline{C}$ONH), 191.7 ($\underline{C}$OCONH). One peak not observed due to accidental equivalence; IR (ATR): ν_{max} 3276, 2972, 1608, 1532, 1448, 1408, 1331, 1244, 1134, 1058, 882, 808, 749, 697 cm^{-1}; HRMS (+ESI): Found *m/z* 638.2574, [M+Na]$^{+}$, $C_{33}H_{37}N_5NaO_7$ requires 638.2591.

2-(2-(2-Benzamidophenyl)-2-oxoacetamido)-*N,N,N*-trimethylethanaminium PF_6 salt **3-3**



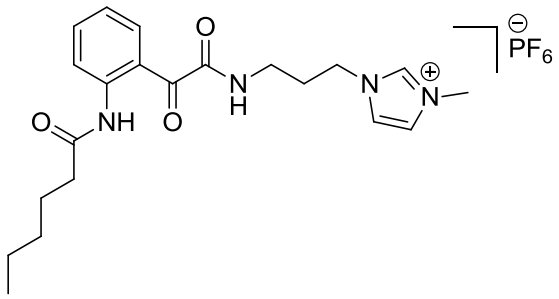
A mixture of **3-2** (0.124 g, 0.365 mmol) and methyl iodide (0.227 mL, 3.65 mmol) in toluene was heated to reflux for 3 hours. The solvent was decanted and the residue pale yellow oil was dissolved in a minimal volume of methanol and diluted with an equal amount of KPF_6 (sat., aq.) and the precipitate collected by filtration to afforded **3-3** as a colourless powder (0.160 g, 87%). M.p. 264-266 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 3.08 (s, 9H, N(C<u>H</u>$_3$)$_3$), 3.42 (t, *J* = 6.2 Hz, 2H, NC<u>H</u>$_2$), 3.61 (q, *J* = 6.2 Hz, 2H, NHC<u>H</u>$_2$), 7.33 (ddd, *J* = 7.9, 7.3, 1.2 Hz, 1H, ArH), 7.55-7.62 (m, 2H, 2 x ArH), 7.63-7.68 (m, 1H, ArH), 7.71 (ddd, *J* = 8.3, 7.3, 1.6 Hz, 1H, ArH), 7.79 (dd, *J* = 7.9, 1.6, 1H, ArH), 7.92-7.97 (m, 2H, 2 x ArH), 8.04 (dd, *J* = 8.3, 1.2 Hz, 1H, ArH), 9.13 (bt, *J* = 5.9 Hz, 1H, COCON<u>H</u>), 11.24 (bs, 1H, CON<u>H</u>Ar), ^{13}C NMR (150 MHz, d_6-DMSO): δ 33.3 (N<u>C</u>H$_2$), 52.7 (N(<u>C</u>H$_3$)$_3$), 63.3 (NHCH$_2$), 122.1 (ArCH), 123.9 (ArCH), 124.4 (ArC), 127.4 (ArCH), 128.8 (ArCH), 131.7 (ArCH), 132.3 (ArCH), 133.9 (ArC), 134.2 (ArCH), 138.4 (ArC), 163.4 (CO<u>C</u>ONH), 165.5 (Ar<u>C</u>ONH), 190.4 (<u>C</u>OCONH). IR (KBr): ν_{max} 3289, 3092, 1675, 1639, 1545, 1493, 1214, 838, 872, 697, 558 cm^{-1}; HRMS (+ESI): Found *m/z* 354.1803, $[M]^+$, $C_{20}H_{24}N_3O_3$ requires 354.1812.

1-(3-(2-(2-Benzamidophenyl)-2-oxoacetamido)propyl)-3-methyl-1H-imidazol-3-ium PF_6 salt **3-5**



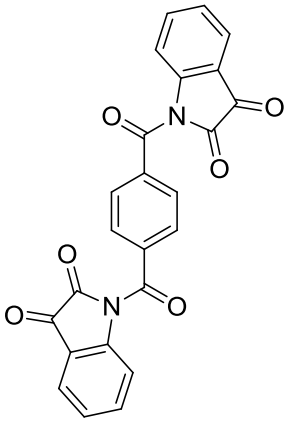
A mixture of **3-4** (0.380 g, 1.01 mmol) and methyl iodide (0.628 mL, 10.1 mmol) in toluene was heated to reflux for 3 hours. The solvent was decanted and the residue brown oil was titrated with toluene (10 mL) and dissolved completely in the minimum volume of methanol. KPF_6 (sat., aq.) was added and the mixture was extracted with several portions of DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude PF_6 salt was recrystallized from methanol to afford **3-5** as colourless microcrystals (0.401 g, 74%). M.p. 137-138°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.50 (qu, *J* = 6.7 Hz, 2H, $CH_2C\underline{H}_2CH_2$), 2.72 (q, *J* = 6.3 Hz, 2H, $NHC\underline{H}_2$), 3.39 (s, 3H, CH_3), 3.68 (t, *J* = 6.9 Hz, 2H, NCH_2), 6.88 (ddd, *J* = 8.5, 7.4, 1.2 Hz, 1H, ArH), 7.09-7.14 (m, 2H, 2 x ArH), 7.18 (ddd, *J* = 8.5, 6.6, 1.2 Hz, 1H, ArH), 7.22-7.29 (m, 3H, 3 x ArH), 7.33 (dd, *J* = 7.9, 1.4 Hz, 1H, ArH), 7.48-7.52 (m, 2H, 2 x ArH), 7.64 (dd, *J* = 8.3, 1.2 Hz, 1H, ArH), 8.47 (bt, *J* = 5.9 Hz, 1H, $NHCH_2$), 8.58-8.62 (m, 1H, ImH), 10.85 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, d_6-DMSO): δ 29.2 ($CH_2\underline{C}H_2CH_2$), 35.4 ($NH\underline{C}H_2$), 35.8 (CH_3), 46.5 (CH_2N), 122.1 (ArCH), 122.3 (ArCH), 123.6 (ArCH), 123.9 (ArCH), 124.2 (ArC), 127.4 (ArCH), 128.8 (ArCH), 131.9 (ArCH), 132.3 (ArCH), 133.9 (ArC), 134.4 (ArCH), 136.7 (ArCH), 138.7 (ArC), 163.6 (CO<u>C</u>ONH), 165.5 (CON<u>H</u>Ar), 191.5 (<u>C</u>OCONH); IR (KBr): ν_{max} 3308, 3167, 1669, 1603, 1578, 1521, 1451, 1484, 1302, 1214, 1171, 844, 767, 720, 624, 558 cm^{-1}; HRMS (+ESI): Found *m/z* 391.1755, $[M]^+$, $C_{22}H_{23}N_4O_3$ requires 391.1765.

1-(3-(2-(2-Hexanamidophenyl)-2-oxoacetamido)propyl)-3-methyl-1H-imidazol-3-ium PF_6 salt **3-23**



A mixture of **3-22** (0.475 g, 1.28 mmol) and methyl iodide (0.998 mL, 12.8 mmol) in toluene was heated to reflux for 3 hours. The solvent was decanted and the residue brown oil was triturated with toluene (10 mL) and dissolved completely in the minimum volume of methanol. KPF_6 (sat., aq.) was added and the mixture was extracted with several portions of DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude PF_6 salt was recrystallized from methanol to afford **3-23** as colourless prisms (0.449 g, 66%). M.p. 127-128 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 0.86 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H}_3$), 1.23-1.33 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_3$), 1.56 (qu, *J* = 7.4 Hz, 2H, $NCH_2C\underline{H}_2CH_2$), 2.04 (qu, *J* = 7.0 Hz, 2H, $COCH_2C\underline{H}_2CH_2$), 2.30 (t, *J* = 7.4 Hz, 2H, NCH_2), 3.22 (q, *J* = 6.4 Hz, 2H, $NHC\underline{H}_2$), 3.84 (s, 3H, NCH_3), 4.22 (t, *J* = 7.0 Hz, 2H, $COCH_2$), 7.23 (ddd, *J* = 7.8, 7.4, 1.2 Hz, 1H, ArH), 7.58-7.64 (m, 2H, 2 x ArH), 7.71 (dd, J = 1.9, 1.9 Hz, 1H, imH), 7.74-7.77 (m, 1H, ArH), 7.78 (dd, J = 1.8, 1.9 Hz, 1H, imH), 8.85 (bt, *J* = 6.7 Hz, 1H, $N\underline{H}CH_2$), 9.09 (m, 1H, imH), 10.52 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 13.8 (CH_3), 21.9 (CH_2), 24.5 (CH_2), 29.2 (CH_2), 30.7 (CH_2), 35.4 (CH_2), 35.8 (NCH_3), 36.4 (CH_2), 46.6 (CH_2), 121.4 (ArCH), 122.3 (ArCH), 123.4 (ArCH), 123.7 (ArCH), 124.4 (ArC), 131.2 (ArCH), 133.7 (ArCH), 134.8 (ArCH), 137.8 (ArC), 163.3 ($CO\underline{C}ONH$), 171.6 ($CON\underline{H}Ar$), 190.4 ($\underline{C}OCONH$); IR (KBr): ν_{max} 3400, 3287, 3085, 1696, 1672, 1607, 1534, 1483, 841, 758, 559 cm^{-1}; HRMS (+ESI): Found *m/z* 385.2226, $[M]^+$, $C_{22}H_{23}N_4O_3$ requires 385.2234.

1,1'-Terephthaloylbis(indoline-2,3-dione) **3-24**

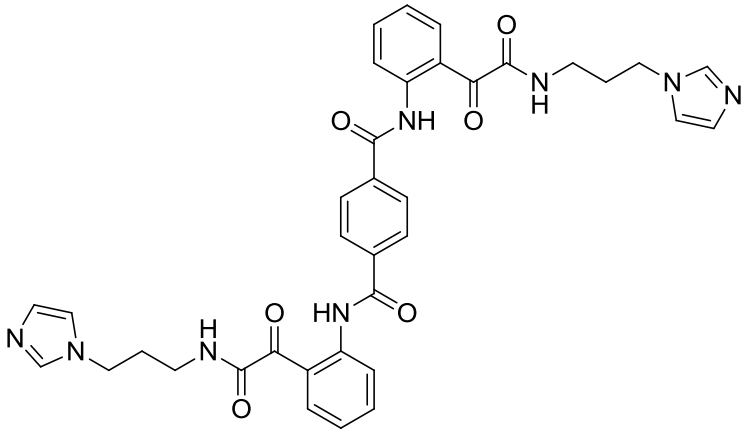


Isatin **1-1** (7.43 g, 50.5 mmol) was added to a stirred suspension of NaH (2.10 g, 51.7 mmol) in dry THF at 0 °C. The dark purple suspension of sodium isatin was stirred for 10 min under an N_2 atmosphere. A solution of terephthaloyl chloride (5.00 g, 24.6 mmol) in dry THF was added dropwise over 10 min and the resulting solution was left to stir for an additional hour. The reaction was quenched with wet THF, water was added and the solid was collected by filtration. The crude product was washed with water, dichloromethane and acetone to afford **3-24** as a bright yellow amorphous solid (8.80 g, 84%).

General procedure for compounds **3-25-3-28**

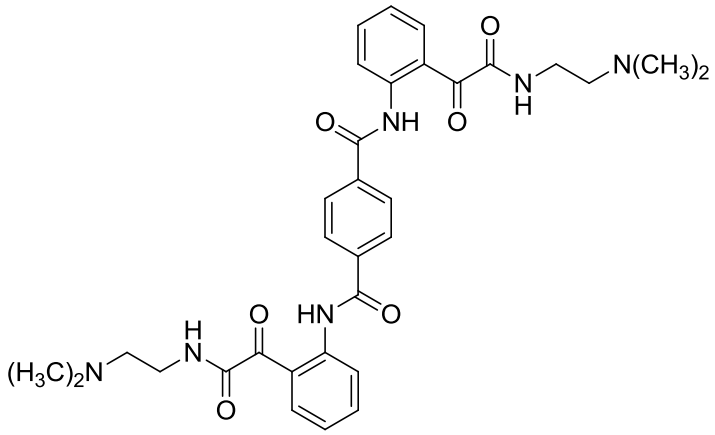
The *N*-acylisatin and the amine were dissolved in chloroform and the reaction was stirred at reflux. The reaction was worked up according to described procedure.

N[1],*N*[4]-Bis(2-(2-((3-(1H-imidazol-1-yl)propyl)amino)-2-oxoacetyl)phenyl)terephthalamide **3-25**



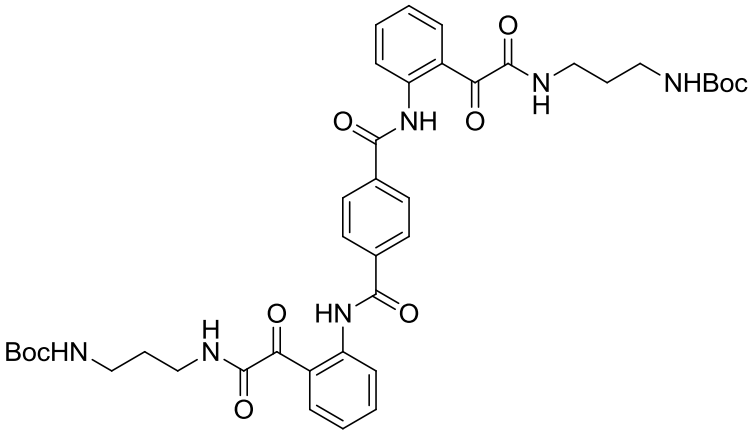
The title compound **3-25** was prepared from **3-24** (0.800 g, 1.89 mmol) and 1-(3-aminopropyl)imidazole (0.495 mL, 4.15 mmol) according to the general procedure outlined above. The reaction mixture was cooled to room temperature, 2 M HCl was added and the mixture was shaken. The resulting suspension was filtered to afford **3-25** as a light tan amorphous solid (1.16 g, 91%)**.** M.p. >235°C (decomp.); ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.85 (qu, *J* = 6.8 Hz, 4H, 2 x $CH_2C\underline{H_2}CH_2$), 3.00-3.20 (m, 4H, 2 x $NHC\underline{H_2}$), 3.93 (t, J = 6.8 Hz, 4H, 2 x NCH_2), 6.86 (s, 2H, 2 x imH), 7.13 (s, 2H, 2 x im-H), 7.36 (ddd, *J* = 8.6, 7.7, 1.1 Hz, 4H, 2 x ArH), 7.59 (s, 2H, 2 x imH), 7.74 (ddd, *J* = 8.4, 7.7, 1.6 Hz, 2H, 2 x ArH), 7.80 (dd, *J* = 8.6, 1.6 Hz, 2H, 2 x ArH), 8.03-8.18 (m, 6H, 6 x ArH), 8.94 (bt, J = 6.8 Hz, 2H, 2 x $N\underline{H}CH_2$), 11.42 (s, 2H, 2 x $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, d_6-DMSO): δ 30.3 ($CH_2\underline{C}H_2CH_2$), 35.9 ($NHCH_2$), 43.5 (CH_2N), 119.3 (ArCH), 122.3 (ArCH), 124.3 (ArCH), 124.6 (ArC), 127.8 (ArCH), 128.4 (ArCH), 132.0 (ArCH), 134.4 (ArCH), 137.0 (ArC), 137.3 (ArCH), 138.5 (ArC), 163.7 ($\underline{C}OCOAr$), 164.7 ($Ar\underline{C}ONH$), 191.8 ($CO\underline{C}OAr$); IR (KBr): ν_{max} 3302, 1666, 1529, 1504, 1482, 1448, 1325, 1288, 1215, 774, 724, 663 cm^{-1}; HRMS (+ESI): Found *m/z* 338.1375, $[M+2H]^{2+}$, $C_{36}H_{36}N_8O_6$ requires 338.1379.

N^1,N^4-Bis(2-(2-((2-(dimethylamino)ethyl)amino)-2-oxoacetyl)phenyl)terephthalamide **3-26**



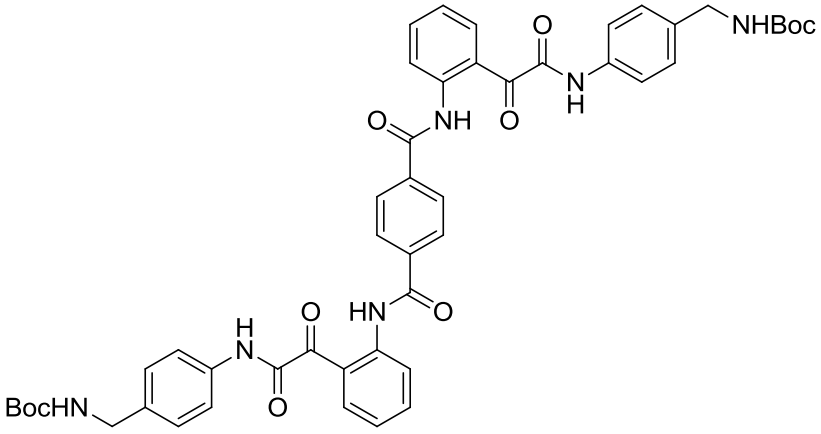
The title compound **3-26** was prepared from **3-24** (0.500 g, 1.18 mmol) and N^1,N^1-dimethylethane-1,2-diamine (0.279 mL, 2.59 mmol) according to the general procedure outlined above. The reaction mixture was cooled to room temperature, 2 M HCl was added and the mixture was shaken. The resulting suspension was filtered to afford **96** as a pale yellow amorphous solid (0.672 g, 96%)**.** M.p. >275°C (decomp.); ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.13 (s, 12H, 2 x N(C<u>H</u>$_3$)$_2$), 2.29 (t, *J* = 6.6 Hz, 4H, 2 x NC<u>H</u>$_2$), 3.26 (q, *J* = 6.6 Hz, 4H, 2 x NHC<u>H</u>$_2$), 7.35 (ddd, *J* = 7.8, 7.4, 1.2 Hz, 2H, 2 x ArH), 7.75 (ddd, *J* = 8.3, 7.4, 1.6 Hz, 2H, 2 x ArH), 7.85 (dd, *J* = 7.8, 1.6 Hz, 2H, 2 x ArH), 8.15 (s, 4H, 4 x ArH), 8.23 (dd, J = 8.3, 1.2 Hz, 2H, 2 x ArH), 11.54 (s, 2H, 2 x CON<u>H</u>Ar); ^{13}C NMR (75 MHz, d_6-DMSO): δ 36.6 (CH_2), 45.0 ($N(CH_3)_2$), 57.6 (CH_2), 121.9 (ArCH), 123.5 (ArC), 124.2 (ArCH), 127.9 (ArCH), 132.5 (ArCH), 134.8 (ArCH), 137.1 (ArC), 139.1 (ArC), 164.00 (CO<u>C</u>ONH), 164.6 (Ar<u>C</u>ONHAr), 192.9 (<u>C</u>OCONH); IR (KBr): ν_{max} 3305, 2820, 2767, 1661, 1535, 1484, 1449, 1329, 1300, 1214, 750, 674 cm^{-1}; HRMS (+ESI): Found *m/z* 601.2760, $[M+H]^+$, $C_{32}H_{37}N_6O_6$ requires 601.2775.

Di-tert-butyl (((2,2'-((terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(propane-3,1-diyl))dicarbamate **3-27**



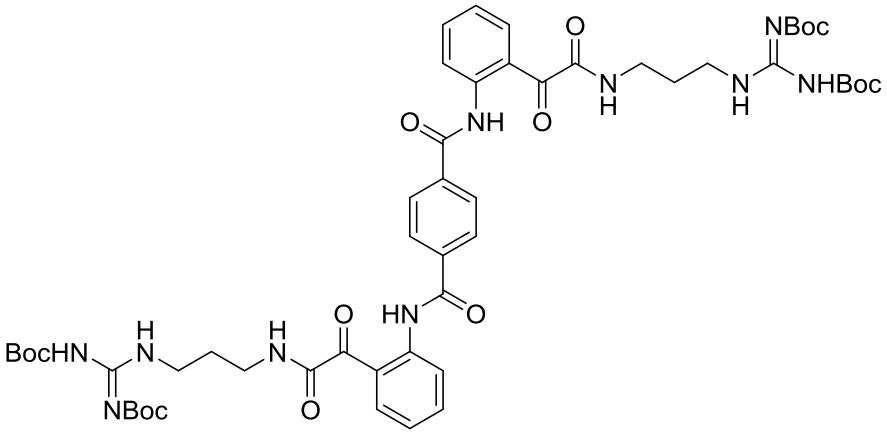
The title compound **3-27** was prepared from **3-24** (0.703 g, 1.66 mmol) and *N*-Boc-1,3-propanediamine (0.607 g, 3.48 mmol) according to the general procedure outlined above. The reaction mixture was concentrated to approximately half the volume and diluted with n-hexane. The resulting precipitate was collected by filtration and washed with cold dichloromethane to afford **3-27** as a pale yellow amorphous solid (1.05 g, 82%)**.** M.p. 183-185 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.37 (s, 18H, 2 x C(C$\underline{H}_3$)$_3$), 1.54 (qu, *J* = 6.4 Hz, 4H, 2 x C$\underline{H}_2$CH$_2$NHBoc), 2.93 (q, *J* = 6.4 Hz, 4H, 2 x CH$_2$C$\underline{H}_2$NHBoc), 3.16 (q, *J* = 6.4 Hz, 4H, 2 x CONHC$\underline{H}_2$), 6.80 (bt, *J* = 5.8 Hz, 2H, 2 x N$\underline{H}$Boc), 7.35 (ddd, *J* = 7.9, 7.4, 1.1 Hz, 2H, 2 x ArH), 7.75 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 2H, 2 x ArH), 7.82 (dd, *J* = 7.9, 1.4 Hz, 2H, 2 x ArH), 8.13 (s, 4H, 4 x ArH), 8.22 (dd, *J* = 8.2, 1.1 Hz, 2H, 2 x ArH), 8.80 (bt, *J* = 5.8 Hz, 2H, 2 x NHC$\underline{H}_2$), 11.51 (s, 2H, 2 x CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, d_6-DMSO): δ 28.2 (C($\underline{C}$H$_3$)$_3$), 29.2 (CH$_2$$\underline{C}H_2CH_2$), 36.3 ($\underline{C}H_2$NHBoc), 37.4 (NHCH$_2$), 77.5 ($\underline{C}$(CH$_3$)$_3$), 122.0 (ArCH), 123.5 (ArC), 124.1 (ArCH), 127.8 (ArCH), 132.4 (ArCH), 134.7 (ArCH), 137.1 (ArC), 139.0 (ArC), 155.6 (OCONH), 163.8 ($\underline{C}$OCOAr), 164.6 (Ar$\underline{C}$ONH), 192.6 (CO$\underline{C}$OAr); IR (KBr): ν_{max} 3349, 2976, 1686, 1585, 1534, 1449, 1366, 1300, 1254, 1218, 1166, 757, 709, 674 cm^{-1}; HRMS (+ESI): Found *m/z* 795.3318, $[M+Na]^+$, $C_{40}H_{48}N_6NaO_{10}$ requires 795.3330.

Di-*tert*-butyl ((((2,2'-((terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene))dicarbamate **3-28**



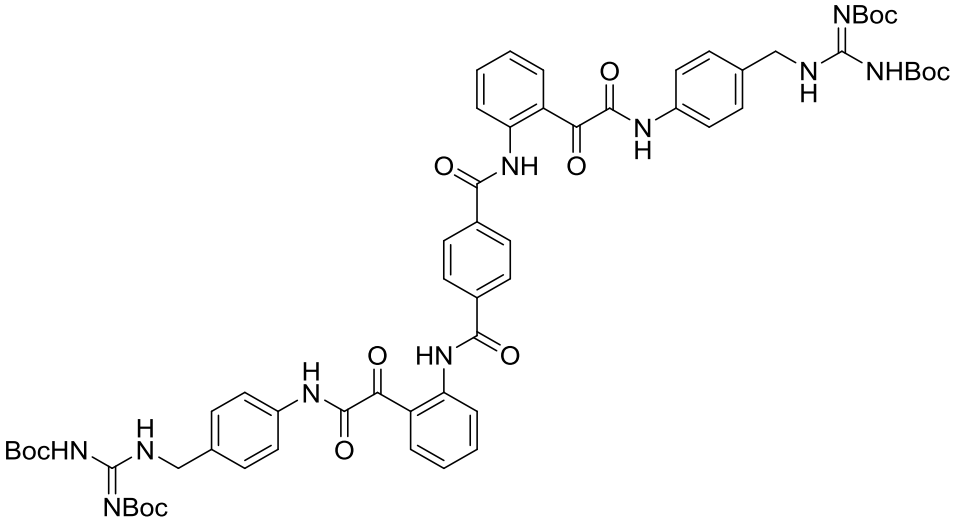
The title compound **3-28** was prepared from **3-24** (0.620 g, 1.46 mmol) and ^{t}butyl *N*-(4-aminobenzyl)-carbamate (0.607 g, 3.00 mmol) according to the general procedure outlined above. The reaction mixture was concentrated to dryness. The resulting solid was washed with two portions of cold dichloromethane to afford **3-28** as a pale yellow amorphous solid (0.888 g, 70%). M.p. 165-168 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.37 (s, 18H, 2 x C($\underline{CH_3}$)$_3$), 4.07 (d, *J* = 6.0 Hz, 4H, 2 x CH_2), 7.19 (d, *J* = 8.6 Hz, 4H, 4 x ArH), 7.36 (m, 2H, 2 x ArH), 7.37 (ddd, *J* = 8.0, 7.6, 1.2 Hz, 2H, 2 x ArH), 7.62 (d, *J* = 8.6 Hz, 4H, 4 x ArH), 7.76 (ddd, *J* = 8.4, 7.6, 1.7 Hz, 2H, 2 x ArH), 7.85 (dd, *J* = 8.0, 1.7 Hz, 2H, 2 x ArH), 8.06 (s, 4H, 4 x ArH), 8.14 (bd, *J* = 6.0 Hz, 2H, N$\underline{H}CH_2$), 10.78 (s, 2H, 2 x COCONH), 11.42 (s, 2H, 2 x ArCON$\underline{H}$Ar); ^{13}C NMR (75 MHz, d_6-DMSO): δ 28.3 (C($\underline{C}H_3$)$_3$), 43.0 (NH$\underline{C}H_2$), 77.8 ($\underline{C}$(CH$_3$)$_3$), 120.1 (ArCH), 122.3 (ArCH), 124.0 (ArC), 124.4 (ArCH), 127.4 (ArCH), 127.8 (ArCH), 132.2 (ArCH), 134.7 (ArCH), 136.3 (ArC), 136.4 (ArC), 137.0 (ArC), 138.8 (ArC), 155.8 (OCONH), 161.8 ($\underline{C}$OCOAr), 164.8 (Ar$\underline{C}$ONH), 191.0 (CO$\underline{C}$OAr); IR (KBr): ν_{max} 3302, 2977, 1685, 1530, 1449, 1300, 1248, 1164, 756, 674 673 cm^{-1}; HRMS (+ESI): Found *m/z* 891.3319, [M+Na]$^{+}$, $C_{48}H_{48}N_6NaO_{10}$ requires 891.3330.

N1,N4-bis(2-(2-((3-((Z)-2,3-di-Boc-guanidino)propyl)amino)-2-oxoacetyl)phenyl)terephthalamide **3-29**



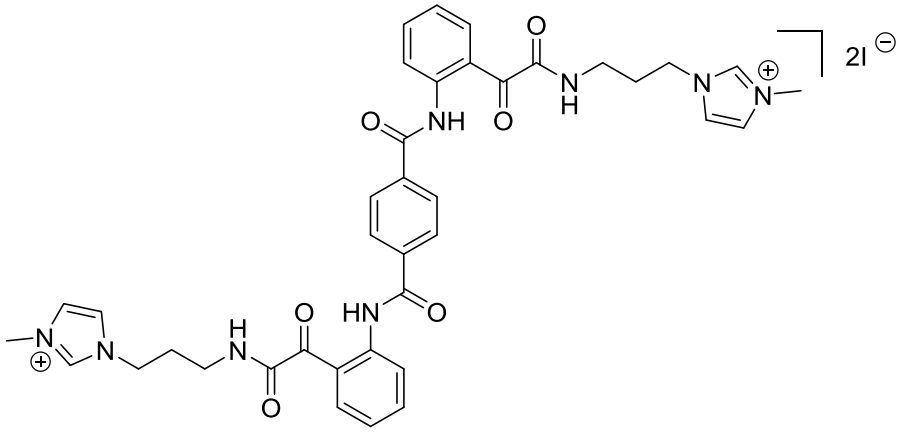
A mixture of **3-33** (0.100 g, 0.125 mmol), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (0.079 g, 0.256 mmol) and triethylamine (0.035 mL 0.250 mmol) were dissolved in dichloromethane and stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel, eluting with 3:7 ethyl acetate/n-hexane. The title compound **3-29** was isolated as a white amorphous powder (0.114 g, 86%). M.p. 235°C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 18H, 2 x C(CH_3)$_3$), 1.49 (s, 18H, 2 x C(CH_3)$_3$), 1.76-1.90 (m, 4H, 2 x $CH_2CH_2CH_2$), 3.49 (q, *J* = 6.2 Hz, 4H, 2 x CNNHCH_2), 3.58 (q, *J* = 6.2 Hz, 4H, 2 x CONHCH_2), 7.22 (ddd, *J* = 8.1, 7.3, 1.1 Hz, 2H, 2 x ArH), 7.70 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 2H, 2 x ArH), 8.18 (dd, *J* = 8.1, 1.7 Hz, 2H, 2 x ArH), 8.21 (s, 4H, 4 x ArH), 8.45-8.57 (m, 2H, 2 x COCONH), 8.96 (dd, *J* = 8.5, 1.1 Hz, 2H, 2 x ArH), 11.42 (s, 2H, 2 x CONHAr), 12.24 (s, 2H, 2 x NHBoc). NHCHBoc not observed due to exchange; ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.1 (C(CH_3)$_3$), 28.2 (C(CH_3)$_3$), 30.1 ($CH_2CH_2CH_2$), 35.7 (CNNHCH_2), 37.3 (CONHCH_2), 79.5 (C(CH_3)$_3$), 83.7 (C(CH_3)$_3$), 118.9 (ArC), 120.9 (ArCH), 123.2 (ArCH), 128.2 (ArCH), 134.7 (ArCH), 136.8 (ArCH), 137.8 (ArC), 142.5 (ArC), 153.3 (OCONH), 157.4 (OCONH), 163.0 (C=N), 164.6 (COCONH), 165.0 (ArCONH), 194.5 (COCONH); IR (KBr): ν_{max} 3322, 2979, 2938, 1721, 1643, 1533, 1449, 1416, 1330, 1137, 1052, 748 cm^{-1}; HRMS (+ESI): Found *m/z* 1057.4985, $[M+H]^+$, $C_{52}H_{69}N_{10}O_{14}$ requires 1057.4995.

N1,N4-bis(2-(2-((4-(((Z)-2,3-di-Boc-guanidino)methyl)phenyl)amino)-2-oxoacetyl)phenyl)terephthalamide **3-30**



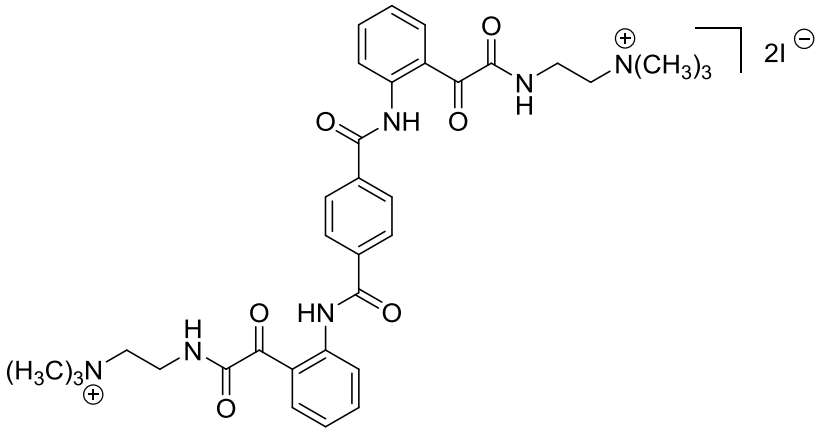
A mixture of **3-34** (0.120 g, 0.134 mmol), *N,N'*-di-Boc-1H-pyrazole-1-carboxamidine (0.085 g, 0.274 mmol) and triethylamine (0.037 mL 0.268 mmol) were dissolved in dichloromethane and stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel, eluting with 3:7 ethyl acetate/n-hexane. The title compound **3-30** was isolated as a white amorphous powder (0.140 g, 91%). M.p. >206°C (decomp.); ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.36 (s, 18H, 2 x C(C$\underline{H}_3)_3$), 1.46 (s, 18H, 2 x C(C$\underline{H}_3)_3$), 4.47 (d, *J* = 5.6 Hz, 4H, 2 x CH_2), 7.27 (d, *J* = 8.6 Hz, 4H, 4 x ArH), 7.37 (ddd, *J* = 7.7, 7.4, 1.1 Hz, 2H, 2 x ArH), 7.66 (d, *J* = 8.6 Hz, 4H, 4 x ArH), 7.76 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 2H, 2 x ArH), 7.85 (dd, *J* = 7.7, 1.6 Hz, 2H, 2 x ArH), 8.07 (s, 4H, 4 x ArH), 8.14 (dd, *J* = 8.6, 1.1 Hz, 2H, 2 x ArH), 8.62 (bt, *J* = 5.6 Hz, 2H, 2 x N$\underline{H}CH_2$), 10.82 (s, 2H, 2 x N$\underline{H}$Boc), 11.42 (s, 2H, 2 x COCONH), 11.51 (s, 2H, 2 x ArCONHAr); ^{13}C NMR (75 MHz, d_6-DMSO): δ 27.6 (C($\underline{C}H_3)_3$), 28.0 (C($\underline{C}H_3)_3$), 43.2 (CH_2), 78.3 ($\underline{C}(CH_3)_3$), 83.0 ($\underline{C}(CH_3)_3$), 120.1 (ArCH), 120.2 (ArCH), 122.3 (ArCH), 124.0 (ArC), 124.3 (ArCH), 127.8 (ArCH), 132.1 (ArCH), 134.3 (ArC), 134.6 (ArCH), 136.7 (ArC), 137.0 (ArC), 138.7 (ArC), 152.0 (OCONH), 155.3 (OCONH), 161.8 (C=N), 163.1 ($\underline{C}$OCONH), 164.7 (Ar$\underline{C}$ONH), 190.9 (CO$\underline{C}$ONH); IR (KBr): ν_{max} 3319, 2980, 1727, 1644, 1525, 1411, 1366, 1326, 1135, 1057, 753, 673 cm^{-1}; HRMS (+ESI): Found *m/z* 1153.4985, $[M+H]^+$, $C_{60}H_{69}N_{10}O_{14}$ requires 1153.4995.

1,1'-(((2,2'-((Terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(propane-3,1-diyl))bis(3-methyl-1H-imidazol-3-ium) diiodide salt **3-31**



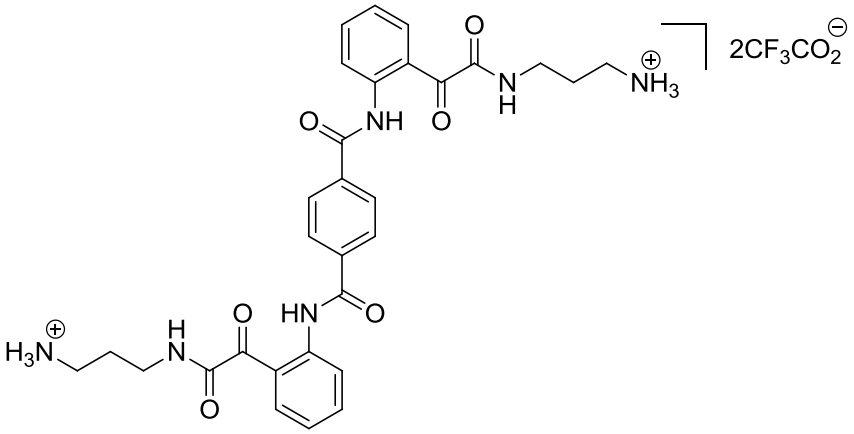
Methyl iodide (1 mL) was added to a stirred solution of **3-25** (0.109 g, 1.62 mmol) in DMF. The reaction was heated in a pressure tube to 110 °C for 16 h. Excess methyl iodide was removed *in vacuo* and dichloromethane was added to form a precipitate. The solid was washed with several portions of dichloromethane to afford **3-31** as a pale brown amorphous solid (0.122 g, 79%). M.p. >275 °C (decomp.); ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.99 (qu, *J* = 6.8 Hz, 4H, 2 x $CH_2C\underline{H}_2CH_2$), 3.19 (q, *J* = 6.8 Hz, 4H, 2 x $NHC\underline{H}_2CH_2$), 3.84 (s, 6H, 2 x NCH_3), 4.16 (t, *J* = 6.8 Hz, 4H, 2 x NCH_2), 7.37 (ddd, *J* = 7.8, 7.5, 1.1 Hz, H, 2 x ArH), 7.70 (t, *J* = 1.6 Hz, 2H, 2 x imH), 7.73-7.77 (m, 4H, 2 x (imH + ArH)), 7.80 (dd, *J* = 7.8, 1.6 Hz, 2H, 2 x ArH), 8.08-8.12 (m, 6H, 6 x ArH), 8.95 (bt, J = 5.9 Hz, 2H, 2 x $CON\underline{H}CH_2$), 9.09 (s, 2H, 2 x imH), 11.38 (s, 2H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, d_6-DMSO): δ 29.2 ($CH_2\underline{C}H_2CH_2$), 35.5 ($NHCH_2$), 35.8 (CH_3), 46.5 (CH_2N), 122.3 (ArCH), 122.3 (ArCH), 123.6 (ArCH), 124.3 (ArCH), 124.4 (ArC), 127.8 (ArCH), 132.0 (ArCH), 134.4 (ArCH), 136.7 (ArCH), 137.0 (ArC), 138.4 (ArC), 163.6 ($\underline{C}OCOAr$), 164.6 ($Ar\underline{C}ONH$), 191.5 ($CO\underline{C}OAr$); IR (ATR): ν_{max} 3216, 3080, 2945, 2869, 1662, 1583, 1535, 1446, 1303, 1211, 1160, 998, 943, 888, 851, 759, 704 cm^{-1}; HRMS (+ESI): Found *m/z* 352.1529, $[M]^{2+}$, $C_{38}H_{40}N_8O_6$ requires 352.1530.

2,2'-((2,2'-((terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(N,N,N-trimethylethan-1-aminium) diiodide salt **3-32**



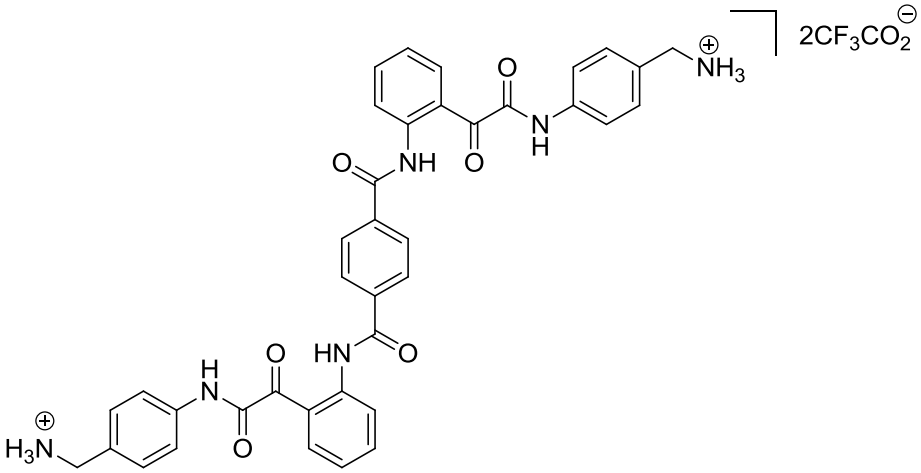
Methyl iodide (1 mL) was added to a stirred solution of **3-26** (0.063 g, 0.10 mmol) in DMF. The reaction was heated in a pressure tube to 110 °C for 16 h. Excess methyl iodide was removed *in vacuo* and dichloromethane was added to form a precipitate. The solid was washed with several portions of dichloromethane to afford **3-32** as a pale brown amorphous solid (0.034 g, 37%). M.p. 180-181 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 3.12 (s, 18H, 2 x $N(CH_3)_3$), 3.46 (t, *J* = 6.3 Hz, 4H, 2 x NCH_2), 3.62 (q, *J* = 6.3 Hz, 4H, 2 x $NHC\underline{H}_2$), 7.36 (ddd, *J* = 7.8, 7.5, 1.2 Hz, 2H, 2 x ArH), 7.74 (ddd, *J* = 8.1, 7.5, 1.6 Hz, 2H, 2 x ArH), 7.80 (dd, *J* = 7.8, 1.6 Hz, 2 x ArH), 8.04 (dd, *J* = 8.1, 1.2 Hz, 2 x ArH), 8.11 (s, 4H, 4 x ArH), 9.14 (t, *J* = 6.1 Hz, 2H, 2 x $N\underline{H}CH_2$), 11.34 (s, 2H, 2 x $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 33.3 (NCH_2), 52.7 ($N(CH_3)_3$), 63.3 ($NHCH_2$), 122.4 (ArCH), 124.3 (SrCH), 124.6 (ArC), 127.9 (ArCH), 131.7 (ArCH), 134.3 (ArCH), 137.0 (ArC), 138.0 (ArC), 163.4 ($CO\underline{C}ONH$), 164.7 ($Ar\underline{C}ONH$), 190.44 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3256, 3011, 1637, 1581, 1580, 1524, 1476, 1444, 1296, 1210, 1163, 1121, 1013, 975, 867, 754, 702, 672 cm^{-1}; HRMS (+ESI): Found *m/z* 315.1573, $[M]^{2+}$, $C_{34}H_{42}N_6O_6$ requires 315.315.1577.

3,3'-((2,2'-((Terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(propan-1-aminium) ditrifluoroacetate salt **3-33**



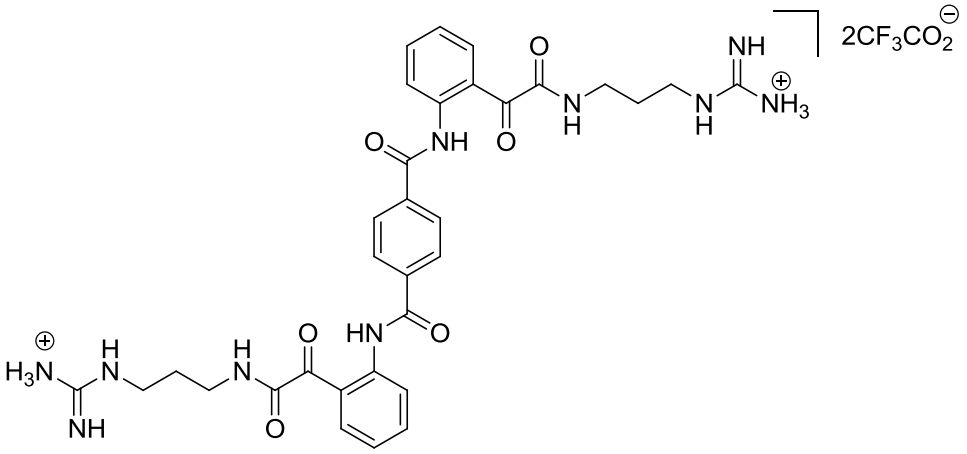
A solution of **3-27** (0.150 g, 0.194 mmol) in TFA-CH_2Cl_2 (20% v/v) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was precipitated from a solution of dichloromethane and n-hexane to afford **3-33** as a pale yellow amorphous solid (0.151 g, 97%). M.p. >225°C (decomp.); ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.76 (qu, *J* = 7.0, 4H, 2 x C$\underline{H_2}$CH$_2$CH$_2$), 2.70-2.91 (m, 4H, 2 x NHC$\underline{H_2}$), 3.16-3.30 (m, 4H, 2 x C$\underline{H_2}$NH$_3$), 7.36 (ddd, *J* = 7.9, 7.5, 1.2 Hz, 2H, 2 x ArH), 7.75 (ddd, *J* = 8.2, 7.5, 1.6 Hz, 2H, 2 x ArH), 7.79 (dd, *J* = 7.9, 1.6 Hz, 2H, 2 x ArH), 7.82-7.97 (m, 6H, 2 x NH$_3$), 8.13 (s, 4H, 4 x ArH), 8.16 (dd, *J* = 8.2, 1.2 Hz, 2H, 2 x ArH), 8.97 (bt, *J* = 5.8 Hz, 2H, 2 x N$\underline{H}$CH$_2$), 11.46 (s, 2H, 2 x CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, d_6-DMSO): δ 27.0 (CH$_2$$\underline{C}H_2CH_2$), 35.8 (NH$_3CH_2$), 36.8 (NHCH$_2$), 122.2 (ArCH), 124.0 (ArC), 124.2 (ArCH), 127.9 (ArCH), 132.1 (ArCH), 134.6 (ArCH), 137.1 (ArC), 138.7 (ArCH), 163.8 (CO$\underline{C}$ONH), 164.6 (Ar$\underline{C}$ONH), 192.0 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3278, 3067, 1631, 1580, 1529, 1445, 1296, 1182, 1124, 965, 876, 835, 753, 703 cm^{-1}; HRMS (+ESI): Found *m/z* 573.2444, $[M]^{2+}$, $C_{30}H_{33}N_6O_6$ requires 573.2451.

(((2,2'-((Terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(4,1-phenylene))dimethanaminium ditrifluoroacetate salt **3-34**



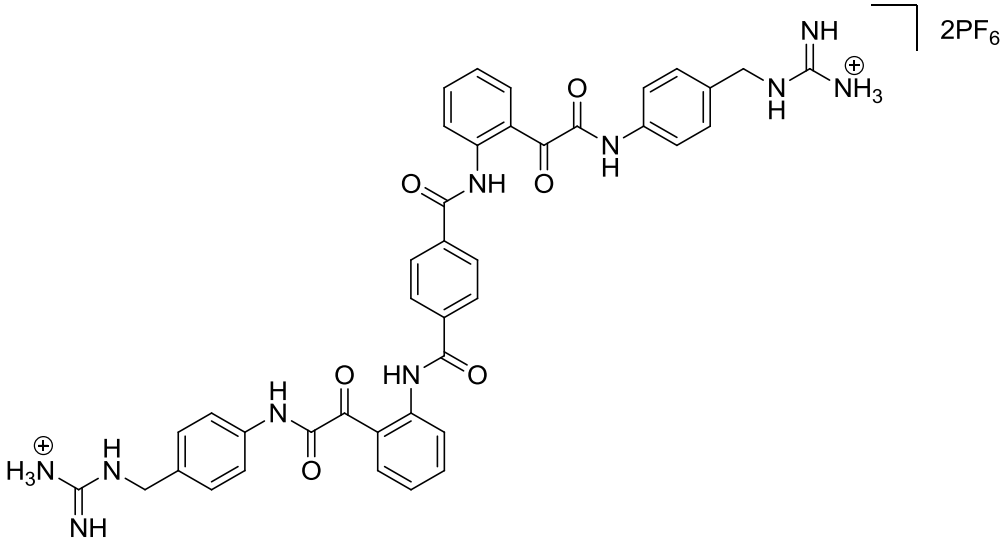
A solution of **3-28** (0.300 g, 0.345 mmol) in TFA-CH_2Cl_2 (20% v/v) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was precipitated from a solution of dichloromethane and n-hexane to afford **3-34** as a bright yellow amorphous solid (0.279 g, 90%). M.p. 184-186 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ 3.99 (s, 4H, 2 x CH_2), 7.32-7.48 (m, 6H, 6 x ArH), 7.67-7.80 (m, 6H, 6 x ArH), 7.84 (dd, *J* = 7.8, 1.0 Hz, 2H, 2 x ArH), 8.05 (s, 4H, 4 x ArH), 8.10 (d, *J* = 8.2 Hz, 2H, 2 x ArH), 8.15 (bs, 6H, 2 x NH_3), 10.92 (bs, 2H, 2 x CONHAr), 11.38 (bs, 2H, 2 x CONHAr); ^{13}C NMR (150 MHz, d_6-DMSO): δ 41.9 (CH_2), 120.1 (ArCH), 122.5 (ArCH), 124.3 (ArC), 124.4 (ArCH), 127.8 (ArCH), 129.5 (ArCH), 129.9 (ArC), 132.0 (ArCH), 134.5 (ArCH), 136.9 (ArC), 138.0 (ArC), 138.5 (ArC), 161.8 (COCONH), 164.8 (ArCONH), 190.5 (COCONH); IR (ATR): ν_{max} 3261, 3053, 1667, 1605, 1531, 1448, 1300, 1246, 1188, 1130, 892, 835, 748, 705 cm^{-1}; HRMS (+ESI): Found *m/z* 669.2437, $[M]^+$, $C_{38}H_{33}N_6O_6$ requires 669.2451.

1,1'-(((2,2'-((Terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(propane-3,1-diyl))diguanidinium ditrifluoroacetate salt **3-35**



A solution of **3-29** (0.090 g, 0.085 mmol) in TFA-CH_2Cl_2 (20% v/v) was stirred at room temperature for 1h. The solvent was removed under reduced pressure and the residue was precipitated from a solution of acetone and n-hexane to afford **3-35** as a white powder (0.066 g, 88%). M.p. 207 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.68 (qu, *J* = 6.8 Hz, 4H, 2 x $CH_2C\underline{H}_2CH_2$), 3.13 (q, *J* = 6.8 Hz, 4H, 2 x $CNNHC\underline{H}_2$), 3.23 (q, *J* = 6.8 Hz, 4H, 2 x $CONHC\underline{H}_2$), 6.82-7.32 (m, 8H, 2 x $CN\underline{H}N\underline{H}_3$), 7.35 (ddd, *J* = 7.9, 7.4, 1.2 Hz, 2H, 2 x ArH), 7.65-7.73 (m, 2H, 2 x $CNN\underline{H}CH_2$), 7.35 (ddd, *J* = 8.3, 7.4, 1.6 Hz, 2H, 2 x ArH), 7.81 (dd, *J* = 7.9, 1.6 Hz, 2H, 2 x ArH), 8.13 (s, 4H, 4 x ArH), 8.3 (dd, *J* = 8.3, 1.2 Hz, 2H, 2 x ArH), 8.91 (bt, *J* = 5.6 Hz, 2H, 2 x $CON\underline{H}CH_2$), 11.50 (bs, 2H, 2 x $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): δ 28.3 ($CH_2\underline{C}H_2CH_2$), 36.0 ($CNNH\underline{C}H_2$), 38.3 ($CONH\underline{C}H_2$), 122.1 (ArCH), 123.7 (ArC), 124.2 (ArCH), 127.9 (ArCH), 132.3 (ArCH), 134.7 (ArCH), 137.1 (ArC), 138.9 (ArC), 156.9 (C=N), 163.8 ($CO\underline{C}ONH$), 164.6 ($Ar\underline{C}ONH$), 192.3 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3259, 3191, 1637, 1608, 1536, 1449, 1303, 1170, 1118, 980, 894, 834, 752, 675 cm^{-1}; HRMS (+ESI): Found *m/z* 657.2886, $[M-H]^+$, $C_{32}H_{37}N_{10}O_6$ requires 657.2887.

1,1'-((((2,2'-((Terephthaloylbis(azanediyl))bis(2,1-phenylene))bis(2-oxoacetyl))bis(azanediyl))bis(4,1-phenylene))bis(methylene))diguanidinium diPF_6 salt **3-36**



A solution of **3-30** (0.080 g, 0.069 mmol) in TFA-CH_2Cl_2 (20% v/v) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and dissolved completely in a minimum volume of methanol. KPF_6 (sat., aq.) was added and the mixture was extracted with several portions of DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude PF_6 salt was precipitated from a solution of acetone and n-hexane to afford **3-36** as a pale yellow amorphous solid (0.050 g, 74%). M.p. 183-184 °C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 4.32 (d, *J* = 6.0 Hz, 4H, 2 x CH_2), 6.35-7.65 (m, 8H, 2 x C=NH, 2 x NH_3), 7.28 (d, *J* = 8.5 Hz, 4H, 4 x ArH), 7.38 (ddd, *J* = 7.9, 7.7, 1.0 Hz, 2H, 2 x ArH), 7.71 (d, *J* = 8.5 Hz, 4H, 4 x ArH), 7.77 (ddd, *J* = 8.3, 7.7, 1.6 Hz, 2H, 2 x ArH), 7.81 (bt, *J* = 6.1 Hz, 2H, 2 x N<u>H</u>CH_2), 7.84 (dd, *J* = 7.9, 1.6 Hz, 2H, 2 x ArH), 8.08 (s, 4H, 4 x ArH), 8.13 (dd, *J* = 8.3, 1.0 Hz, 2H, 2 x ArH), 10.86 (s, 2H, 2 x COCONH), 11.41 (s, 2H, 2 x ArCON<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 43.7 (CH_2), 120.3 (ArCH), 122.4 (ArCH), 124.1 (ArC), 124.4 (ArCH), 127.9 (ArCH), 127.9 (ArCH), 132.1 (ArCH), 133.1 (ArC), 134.7 (ArCH), 137.0 (ArC), 137.1 (ArC), 138.6 (ArC), 156.7 (C=N), 161.8 (CO<u>C</u>ONH), 164.8 (Ar<u>C</u>ONH), 190.8 (<u>C</u>OCONH); IR (ATR): ν_{max} 3475, 3362, 1655, 1604, 1526, 1446, 1265, 818 cm^{-1}; HRMS (+ESI): Found *m/z* 753.2880, $[M+H]^+$, $C_{40}H_{37}N_{10}O_6$ requires 753.2887.

3.5 References

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CHAPTER 4

Chapter 4: Synthesis of C_3-symmetric BTAs containing the *N*-glyoxylamide moiety

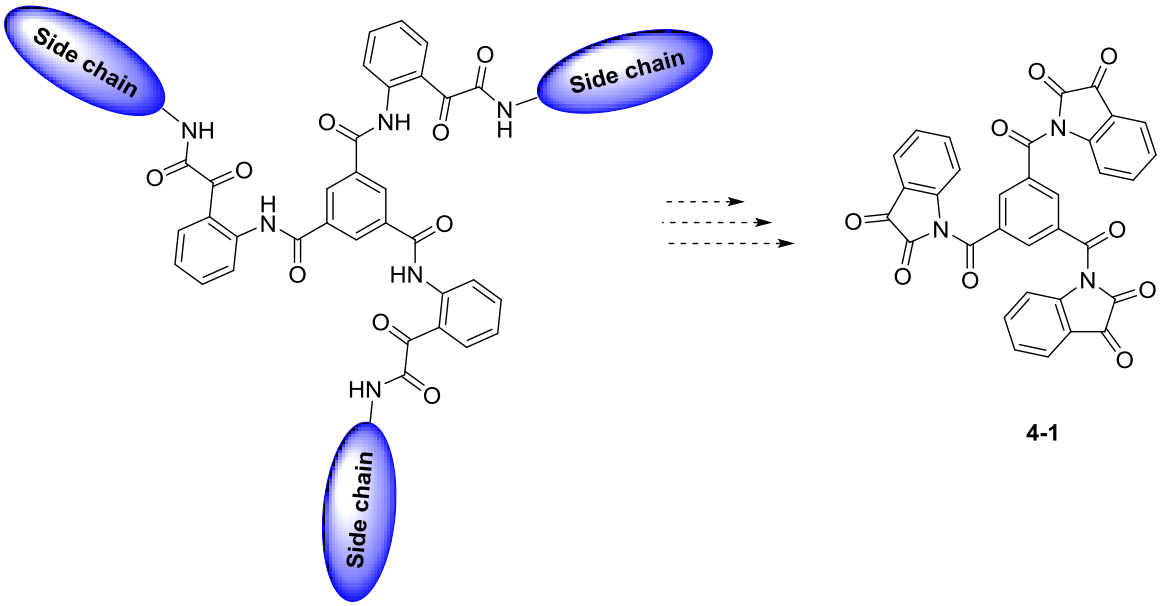
Much of this chapter has been published as a journal article: T. Le, W. C. Cheah, K. Wood, D. St.C Black, M. D. Willcox and N. Kumar, Synthesis of dendrimeric *N*-glyoxylamide peptide mimics, *Tetrahedron Lett*., 2011, **52**, 3645-3647.

4.1 Introduction

Retrosynthesis of C_3-symmetric BTAs containing the *N*-glyoxylamide moiety

We envisaged that novel C_3-symmetric BTAs containing the *N*-glyoxylamide moiety could be synthesized by elaborating the BTA core with three *N*-glyoxylamide moieties, thus creating a new BTA-*N*-glyoxylamide core. Further modification of this novel core would allow the structural tuning of these molecules to target a specific application.

In the retrosynthetic analysis the target compound could be formed from the ring-opening reaction of 1,3,5-benzenetricarbonyl-*N*-isatin **4-1** with a primary amine containing an appropriate side chain (Scheme **4.1**). The intermediate **4-1** could, in turn, be formed from the *N*-acylation of isatin **1-1** with trimesic chloride, as reported in the literature. This synthetic route would allow the desired novel structures to be prepared in two facile steps from commercially available precursors.



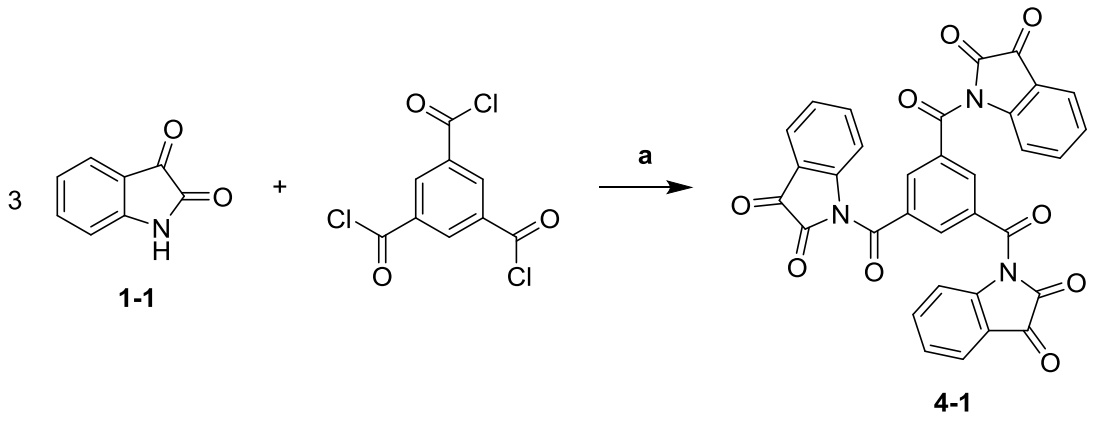
Scheme 4.1. Retrosynthesis of C_3-symmetric BTAs containing the *N*-glyoxylamide moiety from benzene tricarbonyl-*N*-isatin.

4.2 Background, results and discussion

4.2.1 Synthesis of 1,3,5-benzenetricarbonyl-*N*-isatin

The synthesis of **4-1** has been reported by *Rahmati et al*. and was achieved by the reaction of isatin with trimesic chloride and DBU in dimethyl sulfoxide.[1] In their study, the crude product obtained from the reaction was washed with several portions of methanol, and **4-1** was isolated as an orange powder in 35% yield. *N*-Acylisatins are characteristically bright yellow in colour which suggested that the sample obtained was contaminated with unreacted isatin **1-1** leading to an orange colouration. The low yield reported by Rahmati *et al*. could be due to a number of possible factors. Firstly, the reaction may not have reached completion under the described conditions. Secondly, the use of methanol for purification is far from ideal, as alcohols are known to ring-open *N*-acylisatins to form the corresponding *N*-glyoxylesters. Furthermore, Isatin **1-1** is also poorly soluble in methanol. Thus, a new procedure for **1-1** was required to improve the yield and purity of the product. Given the success of the method utilized for the synthesis of 1,4-benzene-dicarbonyl-*N*-isatin **3-24** (Chapter **3**), a similar strategy was adopted for the desired 1,3,5-benzenetricarbonyl-*N*-isatin **4-1**. Hence, sodium isatide (3

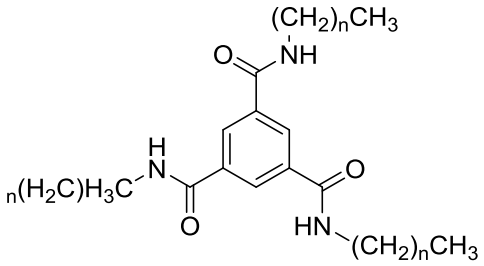
equivalents), formed *in situ* from Isatin **1-1** and sodium hydride, was stirred with trimesic chloride (1 equivalent) in THF for 4 h (Scheme **4.2**). Water was added and the precipitate was collected by filtration to generate compound **4-1** in 93% yield. The new synthesis of **4-1** represents a significant improvement over literature precedent, with respect to both yield and purity (as judged by ^{1}H NMR spectroscopy).[1] The use of NaH promotes the complete and irreversible deprotonation of isatin, which facilitates the *N*-acylation reaction.



Scheme 4.2. Modified synthesis of 1,3,5-benzenetricarbonyl-*N*-isatin **4-1**. a) 60% NaH, THF, r.t., 4h.

4.2.2 Alkyl-substituted BTAs incorporating an *N*-glyoxylamide or ester moiety

BTAs substituted with a simple linear alkyl side chain of six carbons or higher have been demonstrated to possess thermotropic liquid crystalline behavior over a wide temperature range (Figure **4.1**). These homologues self-organise into columnar hexagonally ordered mesophases.[2] As an example, the BTA derivative **4-2** with an octadecyl side chain has been demonstrated to form thin films with remnant polarization and a high surface potential as reported by Carel *et al*.[3] Such properties make these supramolecular structures ideal for use in diodes and high-density memory devices.[4]

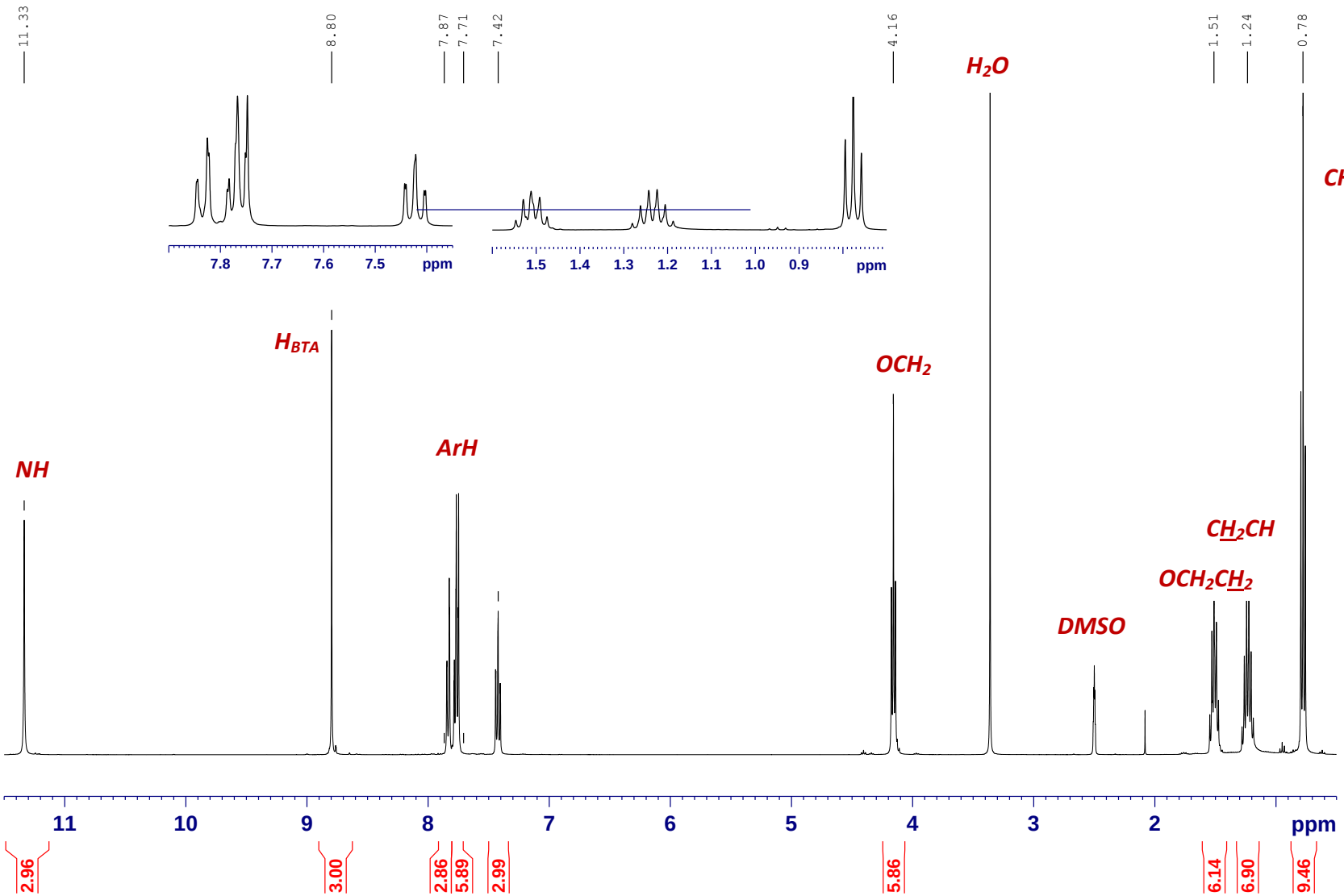


4-2 n=17

Figure 4.1. BTA substituted with an ***(a)*** hexyl, ***(b)*** ocatadecyl side chain.

With precursor **4-1** in hand, ring-opening reactions for the introduction of aliphatic chains to the novel BTA-*N*-glyoxylamide core were pursued. The first set of nucleophiles selected for these reactions were short-chain aliphatic alcohols (n = 1, 2 and 4). Thus, precursor **4-1** was dissolved in methanol, ethanol or n-butanol and heated at reflux and the reaction progress was monitored hourly by ^{1}H NMR spectroscopy analysis. In the structures of the novel ring-opened *N*-glyoxylesters **4-3**-**4-5**, the amide protons (Table **4.1**, depicted in blue) typically form intramolecular hydrogen bonds with the adjacent oxygen atoms of the keto group (Table **4.1**, depicted in red). This caused the chemical shift of the amide protons to appear downfield at approximately 11.00-13.00 ppm in d_6-DMSO. The various singly and doubly ring-opened intermediates as well as the desired products had similar solubilities in common laboratory solvents such as dichloromethane, acetone and ethyl acetate, making isolation of the trimeric *N*-glyoxylesters difficult if the reaction was not complete. Therefore, the reaction was only worked up when only one amide peak remained in the 11-13 ppm region in the ^{1}H NMR spectrum of the reaction mixture. When complete conversion was observed, typically after 2-3 h, the reaction mixtures were diluted with n-hexane and filtered to generate the novel ring-opened *N*-glyoxylesters **4-3**-**4-5** in reasonable yields (62-76%). A representative ^{1}H NMR spectrum of derivative **4-5** in d_6-DMSO is given in Figure **4.2**. Compound **4-5** is C_3-symmetric, with all symmetrical protons possessing chemical equivalence in the ^{1}H NMR spectrum. The amide proton is H-bonded to the keto oxygen atom and appeared at 11.33 ppm.

Figure 4.2. ^{1}H NMR spectra of trimeric *N*-glyoxylester BTA **4-5** recorded at 400 MHz in d_6-DMSO at 298 K.



It is known that small changes in a molecule may dramatically influence the delicate balance of non-covalent interactions between the building blocks. This may lead to the loss of reversibility of supramolecular assembly or a reduction in the stability of the self-assembled structures.[5,6] To explore the effect of minor changes in structure on the intermolecular interactions and the self-assembly properties of this novel core, a series of aliphatic *N*-glyoxylamide trimers was prepared for comparison. These compounds contain three NH groups in place of three oxygen atoms in the ester functionality. To synthesis these compounds, substrate **4-1** was stirred with 3.5 equivalents of n-alkylamine in dichloromethane at room temperature. Periodic monitoring of the reaction mixture was carried out as described above and the reactions were completed within 2-3 h. The excess alkyl amine was removed by washing with 2 M HCl, followed by water. A large quantity of n-hexane was added to the organic layer to precipitate the BTA-*N*-glyoxylamide product which was collected by filtration. In general, the yields of the tris-glyoxylamides **4-6-4-8** (48-74%) were found to decrease as the length of the alkyl chain was increased, which could be due to steric congestion lowering the rate of reaction after each successive ring-opening.

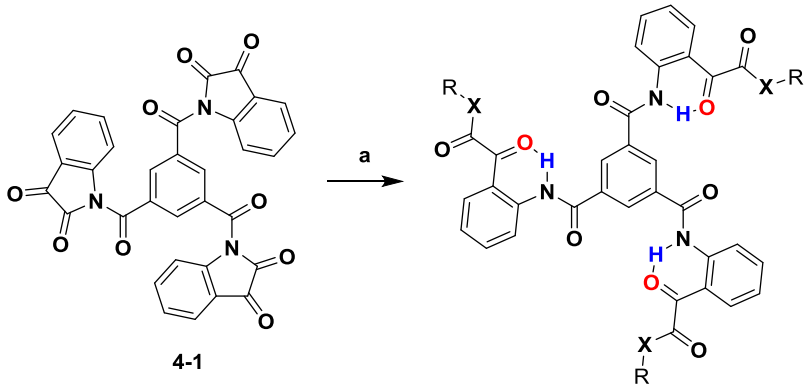


Table 4.1. Ring opening reactions of 1,3,5-benzene tricarbonyl-*N*-isatin **4-1** with *n*-alkyl alcohols and amines. Reagents and conditions: a) Alcohol, reflux or amine, rt. H-bonding of BTA amide proton (blue) and ketone oxygen (red) are highlighted. A table of yields is provided below.

Compound	X	R	Yield (%)
4-3	O	CH_3	76
4-4	O	CH_2CH_3	67
4-5	O	$(CH_2)_3CH_3$	62
4-6	NH	$(CH_2)_3CH_3$	74
4-7	NH	$(CH_2)_5CH_3$	59
4-8	NH	$(CH_2)_7CH_3$	48

4.2.3 Amino acid methyl ester functionalized *N*-glyoxylamide-BTAs

Over the past decades, BTA derivatives substituted with amino acids,[7,8] dipeptide[6] and oligopeptides[9] have been synthesized and analyzed. These molecular building blocks are widely used as structuring elements in self-assembled architectures because of their H-bonding capability, versatility, and bioactive nature. [10,11,12,7] Furthermore, the introduction of these chiral molecules into the BTA scaffold adds a new element of complexity to the building block, which is transferred to the resulting supramolecular architecture. In a study of BTAs substituted with amino acid methyl esters, Bose *et al*., demonstrated the self-assembly of these chiral building blocks into a triple helical nanofiber. Interestingly, the handedness or chirality of this nanostructure could be controlled by reversing the chiral nature of the molecular building blocks.[7] Structurally related molecules have been synthesized containing either an amino acid

or dipeptide connected directly to the BTA core with peripheral aliphatic chains tethered directly to the amino acid *via* an ester or an aryl ether linkage (Figure **4.3**).

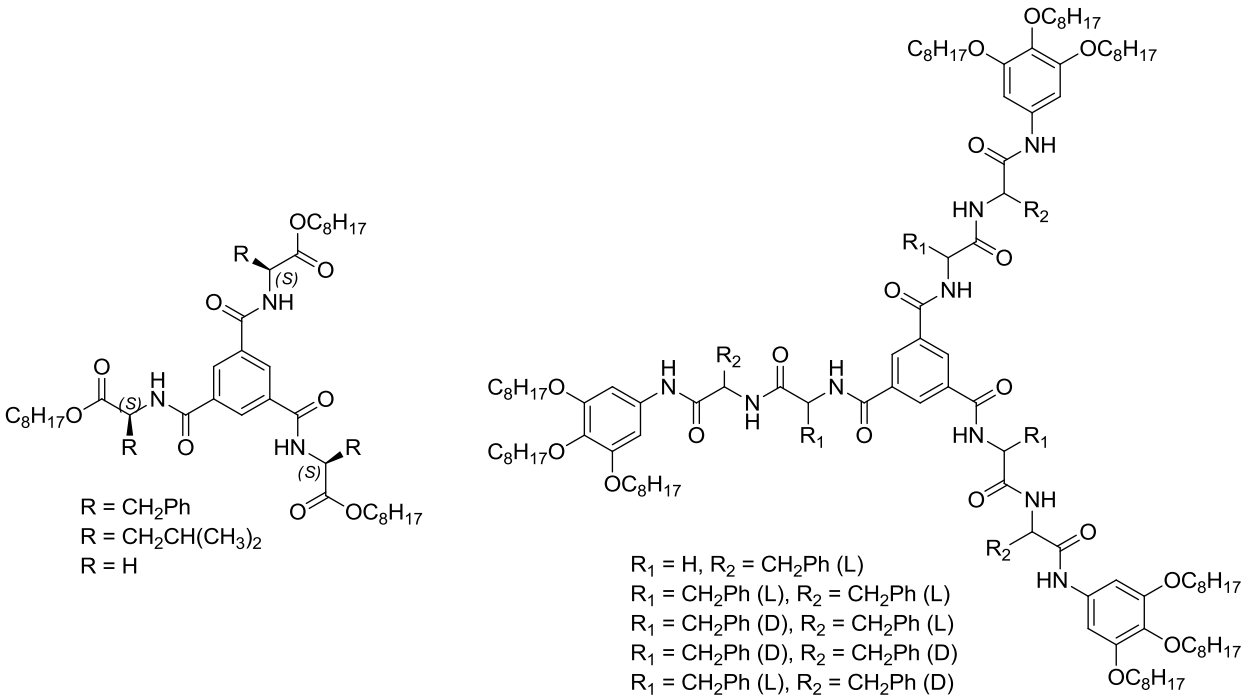


Figure 4.3. Examples of reported BTAs substituted with amino acids.

Aggregation studies conducted by Loos et al. have demonstrated the importance of the amino acid side chain with respect to self-assembly.[13] Sterically demanding groups such as the *iso*-butyl group showed poorer aggregation, while the benzyl group, though moderately bulky, was able to form π-π stacking interactions between monomers. Van den Houst et al. have shown that dipeptide BTAs containing two phenylalanines with equal chirality have greater stability compared to those with phenylalanines of opposite chirality.[6] Greater stack stability is also achieved when space confining groups such as phenylalanine are placed further away from the BTA core. These studies highlight the structural requirements influencing the stacking of these compounds as well as provide insight into the rational design of functional nanostructures.

It was hypothesized that the incorporation of the *N*-glyoxylamide moiety into structurally similar derivatives depicted in Figure **4.3** could enhance self-assembly and form superstructures with greater stability. This is because the non-proteinogenic component can

form both π- π interactions and H-bonds, capable of stabilizing columnar packing. Thus, the synthesis of these compounds was pursued.

The ring-opening reaction of 1,3,5-benzene-tricarbonyl-*N*-isatin with glycine methyl ester was conducted under previously reported conditions in a dichloromethane-water (v/v = 3:1) biphasic solvent system in the presence of sodium hydrogen carbonate. BTA **4-9** was isolated as off-white flakes in 20% yield after purification by column chromatography on silica gel. The ^{1}H NMR spectrum showed the presence of a doublet at 3.96 ppm and a singlet at 3.62 ppm, which were assigned as the methylene and methyl ester protons, respectively. High resolution mass spectrometric analysis showed a peak at 863.2166 which was consistent with the calculated mass of $(M-H)^{+}$ for compound **4-9**.

The reaction conditions were subsequently optimized with the use of a monophasic dichloromethane solution and triethylamine as the base. Using these conditions, precursor **4-1** was ring-opened with several amino acid methyl ester hydrochlorides at room temperature over 48 h to generate the *N*-glyoxylamide BTAs **4-9**-**4-12** in improved yields of 25-43% (Table **4.2**).

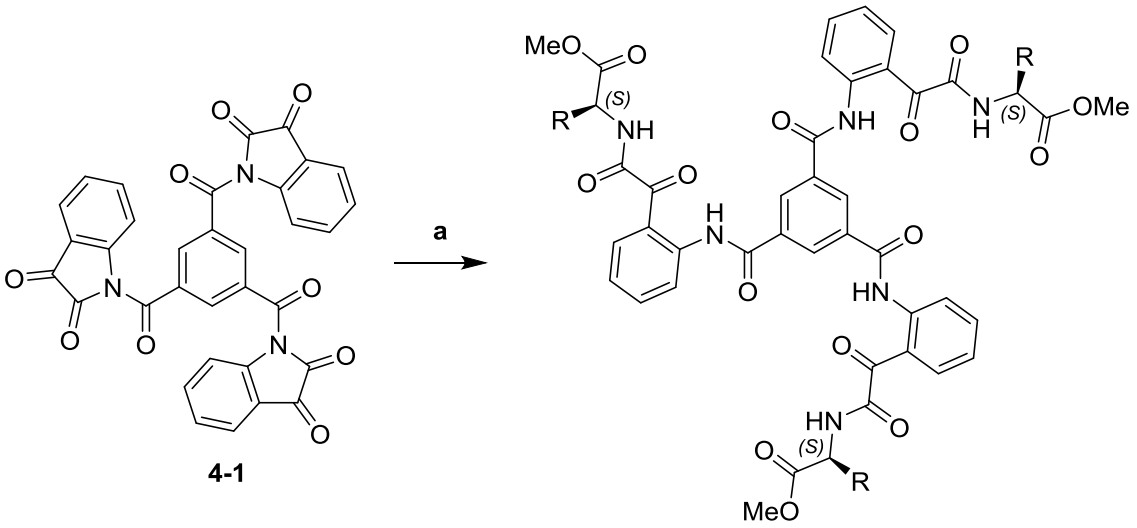


Table 4.2. Ring opening reactions of 1,3,5-benzene tricarbonyl-*N*-isatin **4-1** with amino acid methyl esters. Reagents and conditions: a) amino acid ester, triethylamine, CH_2Cl_2, rt. A table of yields is provided below.

Compound	R	Amino acid methyl ester	Yield (%)
4-9	-H	Glycine	41
4-10	-^{i}Pr	*l*-Valine	25
4-11	-$CH_2{}^{i}$Pr	*l*-Leucine	35
4-12	-$CH(CH_2)_2SCH_3$	*l*-Methionine	43

4.3 Summary and future work

The reaction yield for the preparation of 1,3,5-benzene tricarbonyl-*N*-isatin **4-1** from isatin **1-1**, sodium hydride and trimesic chloride was successfully optimized. A significant improvement in reaction yield (from 35 to 93%) and purity was achieved over the literature precedent. A series of novel BTA-*N*-glyoxylesters **4-3-4-5** with peripheral alkyl chains of varying lengths (n = 1, 2, 4) were synthesized from the ring-opening reaction of precursor **4-1** with the corresponding n-alkyl alcohols in good yields (62-76%). Using a similar strategy, novel BTA-*N*-glyoxylamides with peripheral alkyl chains of varying lengths (n = 4, 6, 8) **4-6-4-8** were synthesized in reasonable yields (48-76%) from the reaction of benzene-tricarbonyl-*N*-isatin and the appropriate n-alkylamine. Furthermore, a procedure for generating BTA-*N*-glyoxylamides with peripheral amino acid esters was developed. Using a monophasic dichloromethane solution and triethylamine as base, intermediate **4-1** was ring-opened with various amino acid methyl esters to form the novel target compounds **4-9-4-12** in moderate yields (25-43%).

With the synthetic routes to these three novel classes of BTAs established, future work will be focused on evaluating their ability to form higher order supramolecular structures. Preliminary analysis of these structures will be performed in the neat state using differential scanning calorimetry, transmission electron microscopy and X-ray diffraction, and in solution with circular dichroism, UV-visible spectroscopy and FTIR spectroscopy. The interactions made by the *N*-glyoxylester and amide moieties will be evaluated to determine if their incorporation into the BTA scaffold is justified.

4.4 Experimental

4.4.1 General methods

Synthesis and reagents

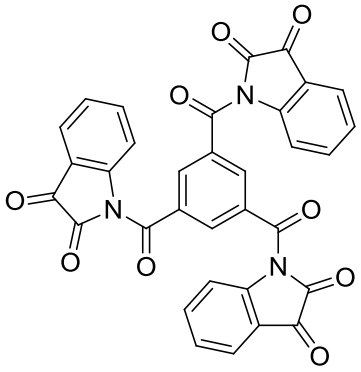
A detailed account of general synthetic methodologies is provided in the experimental section for **Chapter 2**. Further details relevant to this chapter are presented below.

Spectroscopy

A detailed account of general spectroscopic methodologies is provided in the experimental section for **Chapter 2**.

4.4.2 Synthesis

1,1',1''-Benzenetricarbonyltris(indoline-2,3-dione) **4-1**

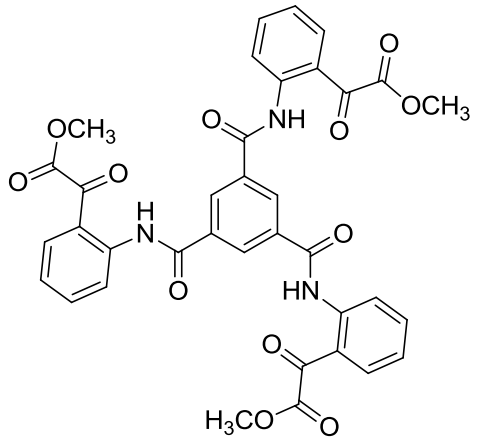


Isatin **1-1** (3.39 g, 23.0 mmol) was added to a stirred suspension of NaH (0.934 g, 23.4 mmol) in dry THF at 0 °C. The dark purple suspension of sodium isatide was stirred for 10 min under an N_2 atmosphere. A solution of trimesic chloride (2.00 g, 7.53 mmol) in dry THF was added dropwise over 10 min and the resulting solution was left to stir for an additional 3 h at room temperature. The reaction was quenched with wet THF, water was added and the solid was collected by filtration. The crude product was washed with water and cold dichloromethane to afford **4-1** as a bright yellow amorphous solid (4.72 g, 93%). M.p. 266-268°C; ^{1}H NMR (75 MHz, d_6-DMSO): 7.39 (t, *J* = 7.5 Hz, 3H, 3 x ArH), 7.84-7.77 (m, 6H, 6 x ArH), 8.03 (t, *J* = 7.5 Hz, 3H, 3 x ArH), 8.75 (s, 3H, 3 x ArH); ^{13}C NMR (75 MHz, d_6-DMSO): 116.7 (ArCH), 120.6 (ArC), 124.9 (ArCH), 126.0 (ArCH), 134.3 (ArC), 134.6 (ArCH), 138.0 (ArCH), 147.9 (ArC), 157.5 (CO<u>C</u>ON), 166.6 (Ar<u>C</u>ON), 180.3 (<u>C</u>OCON).

General procedure for the synthesis of n-alkyl substituted BTA-*N*-glyoxylesters **4-3-4-5**

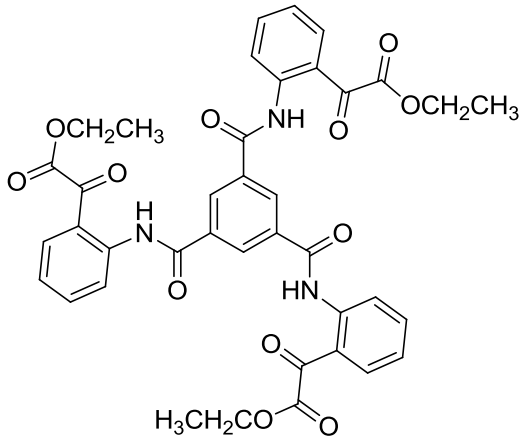
Tris-isatin **4-1** was dissolved in the appropriate alcohol and the reaction was heated at reflux. At completion, as indicated by ^{1}H NMR spectroscopy, the reaction mixture was left to cool to room temperature. An excess of n-hexane was added and the BTA-*N*-glyoxylester was collected by filtration and washed with an additional portion of n-hexane.

Trimethyl 2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetate) **4-3**



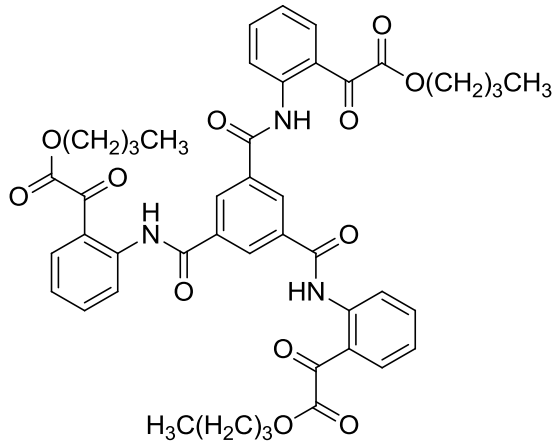
The title compound **4-3** was prepared from tris-isatin **4-1** (0.200 g, 0.335 mmol) and methanol, according to the procedure outlined above. **4-3** was isolated as a yellow amorphous powder (0.176 g, 76 %). M.p. 202-204 °C; ^{1}H NMR (75 MHz, d_6-DMSO): 3.78 (s, 9H, 3 x OCH_3), 7.33-7.83 (m, 12H, 12 x ArH), 8.75 (s, 3H, 3 x ArH), 11.30 (s, 3H, 3 x NH); ^{13}C NMR (75 MHz, d_6-DMSO): 52.8 (OCH_3), 123.9 (ArC), 125.2 (ArC), 126.9 (ArC), 130.0 (ArC), 130.5 (ArC), 134.2 (ArC), 134.5 (ArC), 137.3 (ArC), 162.6 ($CO\underline{C}O_2$), 164.4 (ArCON$\underline{H}$), 185.6 ($\underline{C}OCO_2$); IR (Nujol): ν_{max} 3280, 1735, 1705, 1660, 1605, 1595, 1540, 1345, 1305, 1235, 1215, 1170, 1125, 1110, 920, 825, 810, 750, 725, 710 cm^{-1}; UV and mass spectroscopy analysis was unable to be conducted due to the insoluble nature of the title compound **4-3** in common solvents used for these techniques.

Triethyl 2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetate) **4-4**



The title compound **4-4** was prepared from tris-isatin **4-1** (0.100 g, 0.167 mmol) and ethanol, according to the procedure outlined above. **4-4** was isolated as a yellow amorphous powder (0.082 g, 67 %)**.** M.p. 198-200 °C; ^{1}H NMR (600 MHz, $CDCl_3$): 1.43 (t, *J* = 7.1 Hz, 9H, 3 x CH_3), 4.47 (q, J = 7.1 Hz, 6H, CH_2), 7.25 (ddd, *J* = 8.0, 7.3, 1.1 Hz, 3H, 3 x ArH), 7.75 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 3H, 3 x ArH), 7.79 (dd, *J* = 8.0, 1.6 Hz, 3H, 3 x ArH), 8.97 (s, 3H, 3 x ArH), 9.07 (dd, *J* = 8.5, 1.1 Hz, 3H, 3 x ArH), 12.38 (s, 3H, 3 x CONHAr); ^{13}C NMR (150 MHz, $CDCl_3$): 14.2 (CH_3), 62.8 (CH_2), 117.9 (ArC), 121.3 (ArCH), 123.5 (ArCH), 129.9 (ArCH), 133.9 (ArCH), 136.3 (ArC), 137.4 (ArCH), 142.7 (ArC), 163.5 ($CO\underline{C}O_2$), 164.2 (ArCONH), 191.1 ($\underline{C}OCO_2$); IR (ATR): ν_{max} 3273, 1728, 1648, 1581, 1522, 1448, 1285, 1188, 1108, 1014, 948, 747, 710 cm^{-1}; HRMS (+ESI): Found *m/z* 758.1947, $[M+Na]^+$, $C_{39}H_{33}N_3O_{12}Na$ requires 758.1962.

Tributyl 2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetate) **4-5**

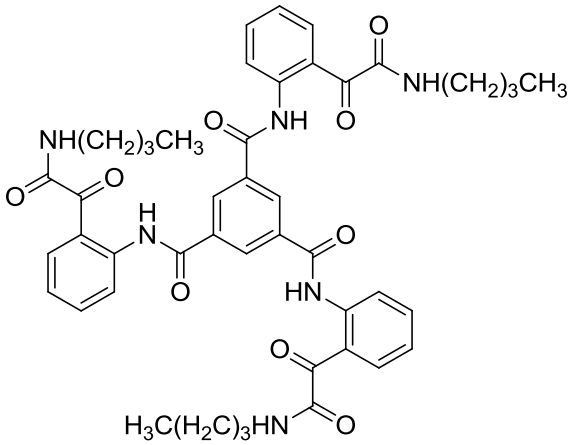


The title compound **4-5** was prepared from tris-isatin **4-1** (0.200 g, 0.335 mmol) and n-butanol, according to the procedure outlined above. **4-5** was isolated as a yellow amorphous powder (0.170 g, 62 %). M.p. 109-111 °C; ^{1}H NMR (400 MHz, d_6-DMSO): 0.78 (t, *J* = 7.4 Hz, 9H, 3 x CH_3), 1.24 (sx, *J* = 7.4 Hz, 6H, 3 x $C\underline{H}_2CH_3$), 1.51 (qu, *J* = 6.7 Hz, 6H, 3 x $C\underline{H}_2CH_2CH_3$), 4.16 (t, *J* = 6.7 Hz, 6H, $NHC\underline{H}_2$), 7.42 (ddd, *J* = 8.9, 7.3, 1.4 Hz, 3H, 3 x ArH), 7.72-7.87 (m, 9H, 9 x ArH), 8.79 (s, 3H, 3 x ArH), 11.32 (s, 3H, 3 x CON<u>H</u>Ar); ^{13}C NMR (75 MHz, d_6-DMSO): 13.4 (CH_3), 18.4 (CH_2), 29.8 (CH_2), 65.8 (CH_2), 123.8 (ArCH), 125.2 (ArCH), 126.5 (ArC), 130.1 (ArCH), 130.8 (ArCH), 134.4 (ArCH), 134.7 (ArC), 137.7 (ArC), 162.5 ($CO\underline{C}O_2$), 164.4 (ArCON<u>H</u>), 186.2 ($\underline{C}OCO_2$); IR (ATR): ν_{max} 3285, 2922, 1688, 1732, 1648, 1581, 1522, 1448, 1297, 1190, 1115, 1055, 983, 928, 834, 694 cm^{-1}; HRMS (+ESI): Found *m/z* 842.2889, $[M+Na]^+$, $C_{45}H_{45}N_3O_{12}Na$ requires 842.2895.

General procedure for the synthesis of n-alkyl substituted BTA-*N*-glyoxylamides **4-6**-**4-8**

Tris-isatin **4-1** (1 equivalent) and the appropriate primary amine (3.1 equivalents) was stirred in dichloromethane at room temperature. At completion, as indicated by ^{1}H NMR spectroscopy the reaction mixture was washed with aqueous 2M HCl and water. An excess of n-hexane was added to the organic layer, and the resulting precipitate was collected by filtration and washed with an additional portion of n-hexane to afford the desired BTA-*N*-glyoxylamide.

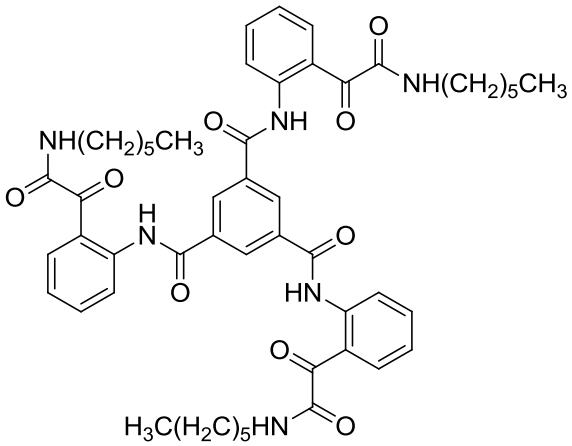
N^{1},*N*^{3},*N*^{5}-Tris(2-(2-(butylamino)-2-oxoacetyl)phenyl)benzene-1,3,5-tricarboxamide **4-6**



The title compound **4-6** was prepared from tris-isatin **4-1** (0.150 g, 0.251 mmol) and n-butylamine (0.076 mL, 0.778 mmol) according to the procedure outlined above. **4-6** was isolated as a pale yellow amorphous powder (0.151 g, 74 %). M.p. 169-171 °C; ^{1}H NMR (400 MHz, d_6-DMSO): 0.78 (t, J = 7.3 Hz, 9H, 3 x CH_3), 1.20 (sx, *J* = 7.3 Hz, 6H, 3 x C$\underline{H_2}$CH$_3$), 1.35 (qu, *J* = 7.3 Hz, 6H, 3 x C$\underline{H_2}$CH$_2$CH$_3$), 3.12 (dd, *J* = 13.1, 6.8 Hz, 6H, NHC$\underline{H_2}$CH$_2$), 7.38 (ddd, *J* = 9.0, 7.7, 1.2 Hz, 3H, 3 x ArH), 7.69-7.80 (m, 6H, 6 x ArH), 8.08 (bd, *J* = 7.9 Hz, 3H, 3 x ArH), 8.75 (bt, *J* = 5.8 Hz, 3H, 3 x CON$\underline{H}$CH$_2$), 8.79 (s, 3H, 3 x ArH), 11.54 (s, 3H, 3 x CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, d_6-DMSO): 13.6 (CH_3), 19.5 (CH_2), 30.8 (CH_2), 39.3 (CH_2), 122.6 (ArCH), 124.6 (ArCH), 124.9 (ArC), 129.7 (ArCH), 132.0 (ArCH), 134.4 (ArCH), 135.2 (ArC), 138.5 (ArC), 163.6 (CO$\underline{C}$ONH), 164.1 (ArCON$\underline{H}$), 192.2 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3274, 2927, 1635, 1580, 1522, 1445, 1296, 1207, 927, 836, 750, 702, 670 cm^{-1}; UV spectroscopy analysis was unable to be conducted due to the

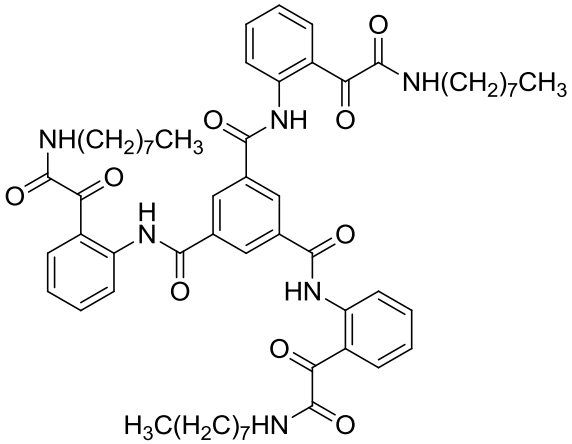
insoluble nature of the title compound **4-6** in common solvents used for this technique; HRMS (+ESI): Found *m/z* 839.3374, $[M+Na]^+$, $C_{45}H_{48}N_6O_9Na$ requires 839.3380.

N^1,*N*^{3},*N*^{5}-Tris(2-(2-(hexylamino)-2-oxoacetyl)phenyl)benzene-1,3,5-tricarboxamide **4-7**



The title compound **4-7** was prepared from tris-isatin **4-1** (0.25 g, 0.418 mmol) and n-hexylamine (0.170 mL, 1.30 mmol), according to the procedure outlined above. **4-7** was isolated as a pale yellow amorphous powder (0.222 g, 59 %). M.p. 158-161 °C; ^{1}H NMR (400 MHz, d_6-DMSO): 0.79 (t, *J* = 6.7 Hz, 9H, 3 x CH_3), 1.06-1.40 (m, 24H, 3 x $(C\underline{H}_2)_4CH_3$), 3.10 (q, *J* = 6.7 Hz, 6H, $NHC\underline{H}_2CH_2$), 7.37 (ddd, *J* = 8.8, 7.7, 1.1 Hz, 3H, 3 x ArH), 7.70-7.81 (m, 6H, 6 x ArH), 8.10 (bd, *J* = 8.1 Hz, 3H, 3 x ArH), 8.75 (bt, *J* = 6.3 Hz, 3H, 3 x $CON\underline{H}CH_2$), 8.80 (s, 3H, 3 x ArH), 11.59 (s, 3H, 3 x $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, d_6-DMSO): 13.9 (CH_3), 22.1 (CH_2), 26.3 (CH_2), 28.6 (CH_2), 28.7 (CH_2), 31.2 (CH_2), 38.6 (CH_2), 122.5 (ArCH), 124.5 (ArCH), 124.7 (ArC), 129.7 (ArCH), 132.0 (ArCH), 134.4 (ArCH), 135.1 (ArC), 138.6 (ArC), 163.6 ($CO\underline{C}ONH$), 164.0 ($ArCON\underline{H}$), 192.3 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3274, 2920, 2851, 1635, 1581, 1525, 1446, 1299, 1209, 1067, 870, 749, 718 cm^{-1}; UV and mass spectroscopy analysis was unable to be conducted due to the insoluble nature of the title compound **4-7** in common solvents used for these techniques.

N[1],*N*[3],*N*[5]-Tris(2-(2-(octylamino)-2-oxoacetyl)phenyl)benzene-1,3,5-tricarboxamide **4-8**

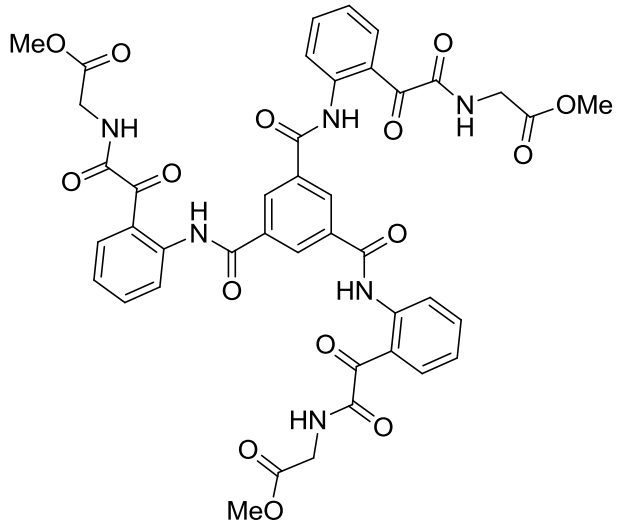


The title compound **4-8** was prepared from tris-isatin **4-1** (0.20 g, 0.335 mmol) and n-octylamine (0.171 mL, 1.04 mmol), according to the procedure outlined above. **4-8** was isolated as a pale yellow amorphous powder (0.148 g, 45 %).

General procedure for the synthesis of amino acid methyl ester functionalized *N*-glyoxylamide-BTAs **4-9-4-12**

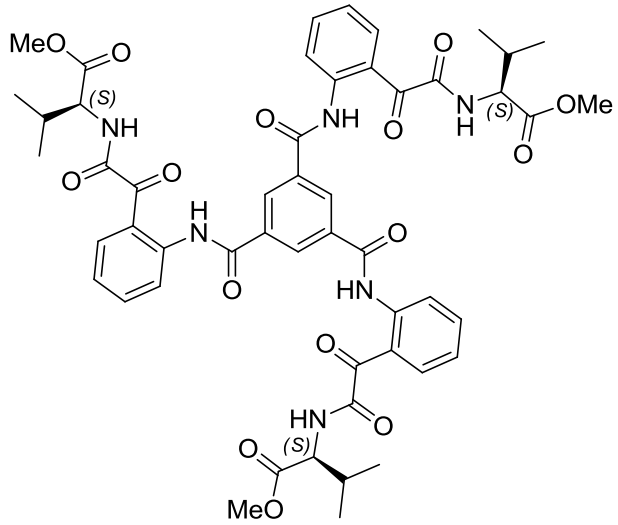
Tris-isatin **4-1** (1 equivalent), triethylamine (3.2 equivalents) and the appropriate amino acid methyl ester hydrochloride salt (3.1 equivalents) was stirred in dichloromethane at room temperature. At completion, as indicated by ^{1}H NMR spectroscopy the reaction mixture was washed with aqueous 2M HCl and water. The organic layer was concentrated *in vacuo*, and trituration of the residue afforded the desired BTA.

Trimethyl 2,2',2''-((2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetyl))tris(azanediyl))triacetate **4-9**



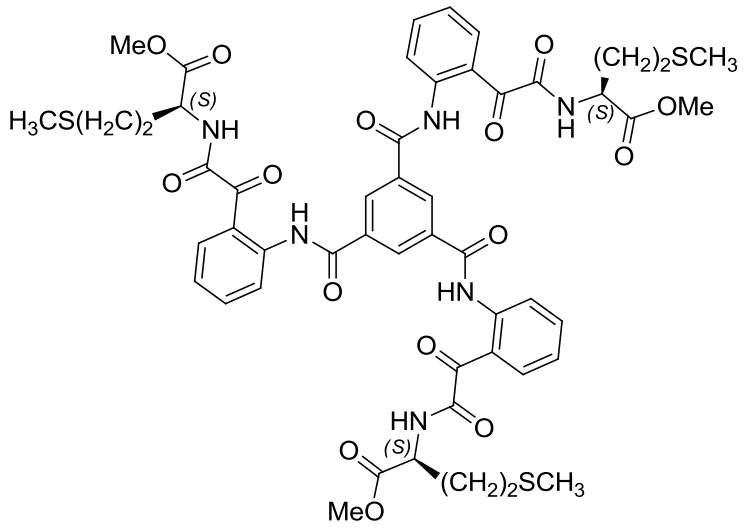
The title compound **4-9** was prepared from tris-isatin **4-1** (0.30 g, 0.502 mmol), triethylamine (0.223 mL, 1.61 mmol) and glycine methyl ester hydrochloride (0.195 g, 1.56 mmol), according to the procedure outlined above. **4-9** was isolated as fine off-white flakes (0.119 g, 41 %). M.p. 178-180°C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 3.62 (s, 9H, 3 x OCH_3), 3.96 (d, *J* = 6.0 Hz, 6H, 3 x $COCH_2NH$), 7.37 (dt, *J* = 7.5, 0.8 Hz, 3H, 3 x ArH), 7.75 (dt, *J* = 7.5, 1.5 Hz, 3H, 3 x ArH), 7.85 (dd, *J* = 7.9, 1.5 Hz, 3H, 3 x ArH), 8.16 (d, *J* = 7.9 Hz, 3H, 3 x ArH), 8.75 (s, 3H, 3 x ArH), 9.24 (t, *J* = 5.6 Hz, 3H, COCONH), 11.60 (s, 3H, 3 x CONHAr); ^{13}C NMR (75 MHz, d_6-DMSO): 40.9 ($COCH_2NH$), 52.3 (CO_2CH_3), 122.8 (ArCH), 124.3 (ArC), 124.7 (ArCH), 130.0 (ArCH), 132.6 (ArCH), 135.1 (ArCH), 135.6 (ArC), 138.9 (ArC), 164.4 ($COCONH$), 164.5 ($ArCONH$), 169.9 (CO_2CH_3), 191.9 ($COCONH$); IR (KBr): ν_{max} 3302, 2954, 1748, 1646, 1584, 1537, 1450, 1369, 1303, 1214, 1167, 956, 931, 758, 721, 674 cm^{-1}; UV spectroscopy analysis was unable to be conducted due to the insoluble nature of the title compound **4-9** in common solvents used for this technique; HRMS (+ESI): Found *m/z* 620.0700, $[M+Na]^+$, $C_{33}H_{15}N_3NaO_9$ requires 620.0706.

(*2S,2'S,2''S*)-Trimethyl 2,2',2''-((2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetyl))tris(azanediyl))tris(3-methylbutanoate) **4-10**



The title compound **4-10** was prepared from tris-isatin **4-1** (0.25 g, 0.418 mmol), triethylamine (0.186 mL, 1.34 mmol) and L-valine methyl ester hydrochloride (0.217 g, 1.30 mmol), according to the procedure outlined above. **4-10** was isolated as fine off-white flakes (0.104 g, 25 %). M.p. 195-197°C; ^{1}H NMR (300 MHz, $CHCl_3$): δ 0.96 (d, *J* = 1.5 Hz, 9H, 3 x CH(C$\underline{H}_3$)$_2$), 0.99 (d, *J* = 1.9 Hz, 9H, 3 x CH(C$\underline{H}_3$)$_2$), 2.30 (ddd, *J* = 5.3, 1.9, 1.5 Hz, 3H, 3 x C$\underline{H}$(CH$_3$)$_2$), 3.74 (s, 9H, 3 x OCH_3), 4.63 (dd, *J* = 8.8, 5.3 Hz, 3H, 3 x COC$\underline{H}$NH), 7.01 (dt, *J* = 7.9, 1.1 Hz, 3H, 3 x ArH), 7.52 (dt, *J* = 7.9, 1.5 Hz, 3H, 3 x ArH), 8.07 (d, *J* = 8.8 Hz, 3H, 3 x N$\underline{H}$CH), 8.17 (dd, *J* = 7.9, 1.1 Hz, 3H, 3 x ArH), 8.68 (d, *J* = 9.4 Hz, 3H, 3 x ArH), 8.70 (s, 3H, 3 x ArH), 12.37 (s, 3H, 3 x CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, $CHCl_3$): 17.5 (CH(C$\underline{H}_3$)$_2$), 18.9(CH(C$\underline{H}_3$)$_2$), 30.8 (C$\underline{H}$(CH$_3$)$_2$), 52.1 (OCH_3), 57.4 (CO$\underline{C}$HNH), 118.7 (ArC), 120.5 (ArCH), 123.3 (ArCH), 129.4 (ArCH), 134.5 (ArCH), 136.0 (ArC), 136.5 (ArCH), 141.2 (ArC), 163.8 (CO$\underline{C}$ONH), 171.5 ($\underline{C}$ONHAr), 192.6 ($\underline{C}O_2$Me), 206.8 ($\underline{C}$OCONH); IR (KBr): ν_{max} 3302, 2954, 1748, 1646, 1584, 1537, 1450, 1369, 1303, 1214, 1167, 956, 931, 878, 821, 756, 705, 683, 597, 552, 522, 484 cm^{-1}; UV spectroscopy analysis was unable to be conducted due to the insoluble nature of the title compound **4-10** in common solvents used for this technique; HRMS (+ESI): Found *m/z* 1013.3543, $[M+Na]^+$, $C_{51}H_{54}N_6O_{15}Na$ requires 1013.3545.

(*2S,2'S,2''S*)-Trimethyl 2,2',2''-((2,2',2''-((benzenetricarbonyltris(azanediyl))tris(benzene-2,1-diyl))tris(2-oxoacetyl))tris(azanediyl))tris(4-(methylthio)butanoate) **4-12**



The title compound **4-12** was prepared from tris-isatin **4-1** (0.25 g, 0.418 mmol), triethylamine (0.186 mL, 1.34 mmol) and L-methionine methyl ester hydrochloride (0.259 g, 1.30 mmol), according to the procedure outlined above. **4-12** was isolated as fine off-white flakes (0.196 g, 43 %). M.p. 143-145 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.82-2.14 (m, 15H, 3 x SCH_3 + 3 x SCH_2), 2.34-2.58 (m, 6H, 3 x $SCH_2C\underline{H_A}\underline{H_B}$), 3.66 (s, 9H, 3 x OCH_3), 4.52 (ddd, *J* = 12.5, 7.7, 4.9 Hz, 3H, 3 x COCH), 7.40 (ddd, *J* = 7.5, 7.4, 1.1 Hz, 3H, 3 x ArH), 7.80 (ddd, *J* = 8.4, 7.4, 1.6 Hz, 3H, 3 x ArH), 7.86 (dd, J = 7.5, 1.6 Hz, 3H, 3 x ArH), 8.81 (s, 3H, 3 x ArH), 9.33 (d, *J* = 7.6 Hz, 3H, 3 x ArH), 11.72 (s, 3H, 3 x CON<u>H</u>Ar); IR (ATR): ν_{max} 3259, 1744, 1638, 1581, 1523, 1446, 1297, 1205, 1161, 1056, 674 cm^{-1}; UV spectroscopy analysis was unable to be conducted due to the insoluble nature of the title compound **4-12** in common solvents used for this technique; HRMS (+ESI): Found *m/z* 1109.2704, $[M+Na]^+$, $C_{51}H_{54}N_6O_{15}S_3Na$ requires 1109.2707.

4.5 References

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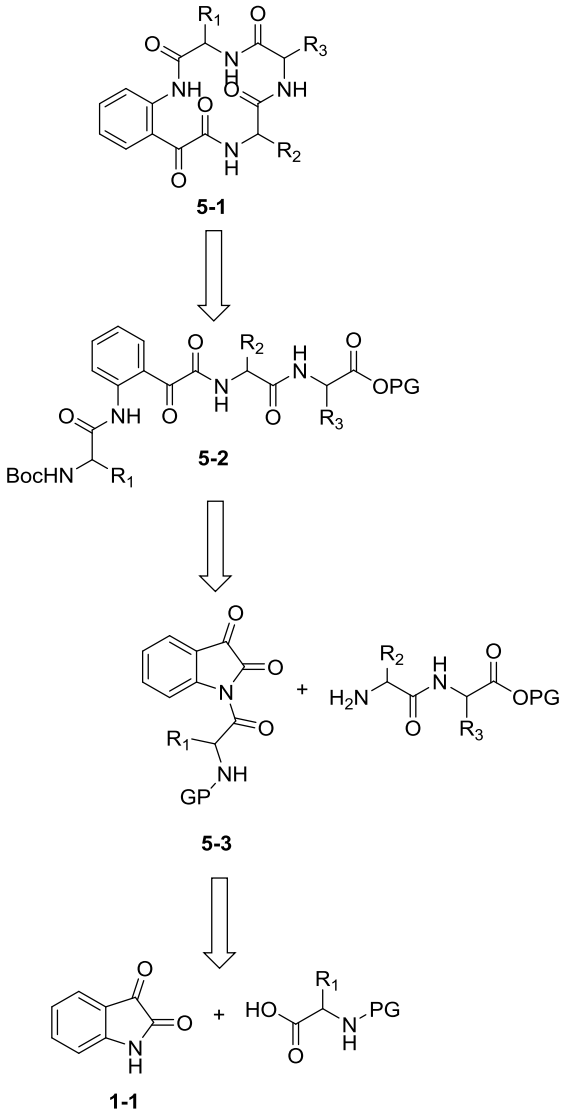
CHAPTER 5

Chapter 5: Synthesis of novel 1,4-benzodiazepin-2-ones from isatin

5.1 General introduction and chapter aims

As an extension to the work on amphiphilic peptide mimics (see chapter **3**), it was envisioned that cyclic peptide mimics incorporating the *N*-glyoxylamide moiety would be a desirable target in the search for novel antimicrobials. The pursuit of this target led to an unanticipated and novel route to 3-substituted 1,4-benzodiazepin-2-ones bearing a pendant ester or amide moiety.



Scheme 5.1. Proposed retrosynthesis of cyclic peptide mimic **5-1** incorporating an *N*-glyoxylic amide moiety.

The retrosynthetic analysis of macrocycle **5-1** (scheme **5.1**) involves the disconnection between two natural amino acids residues, to produce the protected linear precursor **5-2**. This disconnection was chosen in order to situate the *N*-glyoxylamide moiety at the center of the linear precursor, thus maximizing the turn-inducing capacity of the pseudo-amino acid as well as reducing competing cyclo-oligomerization reactions. *N*-Glyoxylamide **5-2** could arise from the facile ring-opening reaction of *N*-acylamino acid isatin **5-3** with an *O*-protected dipeptide generating two additional amide bonds. The key intermediate **5-3** could be further broken down into isatin **1-1** and *N*-protected amino acid synthons, both of which are inexpensive and readily available.

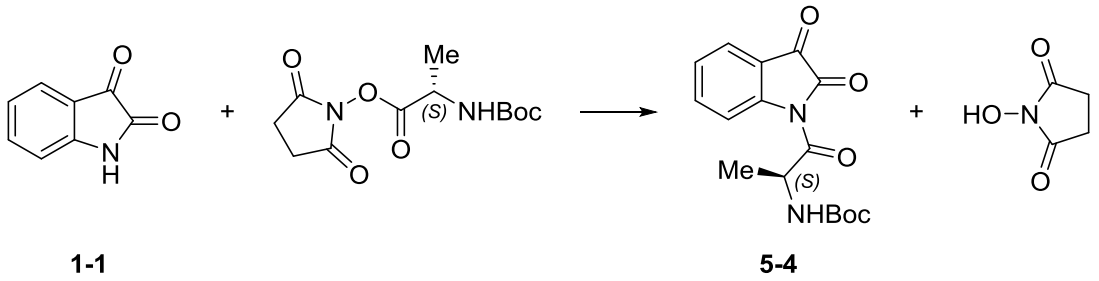
5.2 Background, results and discussion

5.2.1 Synthesis of *N*-acylamino acid isatins

As discussed in Chapter **1**, the preparation of *N*-acylisatins is generally achieved by one of two methods: *N*-acylation of isatin with an acid anhydride or carbonate ester,[1-3] or *N*-acylation of isatin with an acid chloride in the presence of a base such as sodium hydride, pyridine or DBU.[4-7] However, the preparation and reactions of amino acid chlorides have not been extensively reported in the literature. This is because the conditions required for their formation are often incompatible with many common amino acid protecting groups. In addition, their reactive nature results in a number of possible side reactions, including the loss of configuration.[8,9] Therefore, a new strategy was required for the synthesis of *N*-acylamino acid isatins.

As activated amino acid esters typically do not suffer from epimerization, methodologies for nucleophilic substitution reactiions with these compounds are well established.[10] Therefore, the reaction of isatin and an amino acid succinimide ester was first attempted. It is worth noting that the hydroxysuccinimide by-product should not be unlikely to ring-open *N*-acylisatin, as the ring-opening reaction of *N*-acylisatins with alcohols is typically slow, requiring high temperatures and prolonged reaction times.[6]

As a proof of concept, isatin **1-1** was stirred with 1.3 equivalents of *N*-(Boc)alanine hydroxysuccinimide ester in dichloromethane for 24 h (Scheme **5.2**). TLC analysis of the reaction mixture spotted against an authentic sample of *N*-acyl isatin **5-4** prepared *via* a different route (see below) revealed clean but incomplete conversion of **1-1** to **5-4**. Since the reaction was incomplete after 24 h, conditions to afford a faster reaction were sought.

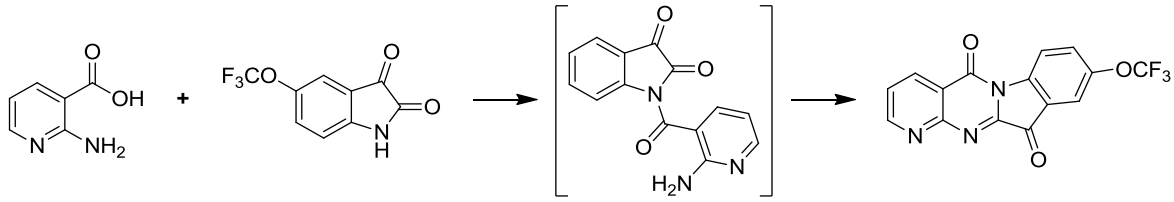


1-1 **5-4**

Scheme 5.2. Attempted synthesis of *N*-(Boc)Ala-istain *via* Boc-L-alanine *N*-succinimidyl ester. Reagents and conditions: a) DMAP, CH_2Cl_2 rt, 24h.

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) have been demonstrated to be effective coupling reagents for the synthesis of *N*-acylisatins (Scheme **5.3**).[11] This strategy permits the reaction to carried out in a single pot as the activated ester is formed *in situ*, and the urea by-product can be easily removed by an acidic work up.[12] However, the reaction of isatin **1-1** and *N*-(Boc)-L-alanine in the presence of EDAC in dichloromethane at room temperature[13] gave only recovery of the starting material. Reactions using other coupling reagents such as HBTU, pyBOP and CDI were unsuccessful or yielded only small quantities (< 5% conversion by ^{1}H NMR spectroscopy) of the desired *N*-acyl product, with the majority of isatin remaining unreacted in each case. After numerous attempts, successful *N*-acylation was achieved by reacting isatin **1-1** with *N*-(Boc)-L-alanine (1.5 equivalents) in the presence of DCC (1.5 equivalents) and a catalytic amount of DMAP (0.1 equivalents) in dichloromethane. Subsequent optimization experiments showed that using less than 1.1 equivalents of *N*-(Boc)-L-alanine or DCC resulted in incomplete reactions. During the course of the reaction, a gradual colour change was observed: the intense orange colour of isatin **1-1** was replaced by a bright yellow colour characteristic of *N*-acylisatins. TLC analysis (2:3 ethyl acetate/n-hexane) after 2 h revealed the complete consumption of isatin

1-1 with concomitant formation of a single, new product. Purification of the crude mixture using chromatography on silica gel with dichloromethane as eluent afforded the desired *N*-acyl isatin **5-4** in good yield (85%).



Scheme 5.3. Literature precedent for the *in situ* generation of *N*-acylisatin *via* HBTU coupling. Reagents and conditions: a) HBTU, NMM, DBU, DMF, rt.

Having optimized the reaction conditions for the *N*-acylation of **5-4**, the scope of the reaction was explored. Using the conditions described in Table **5.1**, Isatin **1-1** was *N*-acylated using a variety of *N*-protected amino acids. While compounds **5-4-5-11** were obtained in moderate to good yields, the reaction between isatin and *N*-(Boc)Gly.OH gave no reaction as indicated by either TLC or ^{1}H NMR spectroscopy. Furthermore, the reaction of isatin **1-1** with the dipeptide *N*-(Boc)GlyGly.OH yielded an intractable mixture of products. The reason for the lack of success for these two reactions is currently not clear and requires further investigation.

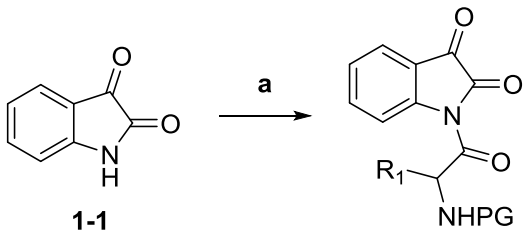
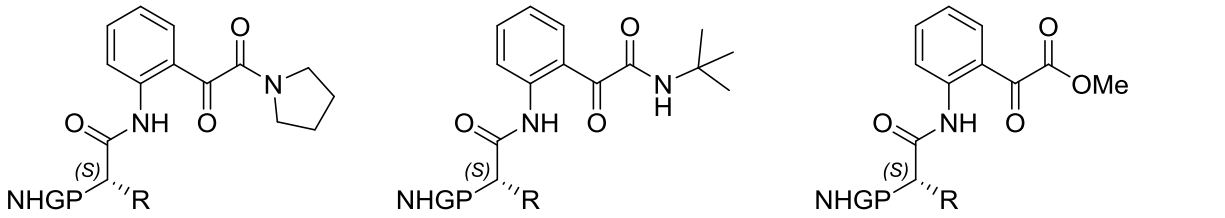


Table 5.2. Preparation of *N*-(PG)amino acid isatins *via* DCC coupling. Reagents and conditions: a) *N*-(PG)-amino acid, DCC, DMAP, CH_2Cl_2.

Compound	*N*-(PG)Amino acid	Reaction outcome (% Yield)
5-4	*N*-(Boc)Ala.OH	85
5-5	*N*-(Boc)Met.OH	49
5-6	*N*-(Boc)Phe.OH	73
5-7	*N*-(Boc)Val.OH	88
5-8	*N*-(Boc)-O-^{t}bu-L-Ser.OH	80
5-9	*N*-(Cbz)Val.OH	60
5-10	*N*-(Boc)Gly.OH	No reaction
5-11	*N*-(Boc)GlyGly.OH	Complex mixture

5.2.2 Facile ring opening reactions of *N*-amino acid isatins with nucleophiles

To establish the reactivity of these novel compounds towards various nucleophiles, the *N*-acylamino acid isatins **5-4** and **5-9** were reacted with either a primary or secondary amine. *N*-Glyoxylamides **5-12-5-14** were formed in excellent yields from the reaction of ^{t}butylamine and pyrrolidine, respectively, with the appropriate *N*-acylisatins. Additionally, refluxing *N*-acylisatins **5-6** and **5-9** in methanol for 3 h produced *N*-glyoxylesters **5-15** and **5-16** respectively.



5-12 R = Me PG = Boc
5-13 R = ^{i}Pr PG = Cbz

5-14 R = Me PG = Boc

5-15 R = CH_2Ph PG = Boc
5-16 R = ^{i}Pr PG = Cbz

Figure 5.1. Synthesis of *N*-glyoxylamides and esters *via* ring-opening reactions of *N*-(PG)amino acid isatins.

N-Acylisatins can undergo ring-opening reactions with amino acid methyl esters to yield *N*-glyoxylamide peptide mimics.[14] This reaction is typically performed in a biphasic solvent system (such as dichloromethane/water), in order to allow the solvation of an inorganic base (e.g. $NaHCO_3$), to liberate the protonated amino acid. To simplify the reaction, it was hypothesized that $NaHCO_3$ could be replaced with a non-nucleophilic organic base, so that the reaction could be performed solely in an organic solvent. It was thought this might also prevent the ring-opening of *N*-acylisatins by water to form an *N*-glyoxylic acid side product. Thus, a number of *N*-(PG)amino acid-isatins were stirred with the hydrochloride salt of various amino acid esters (1.1-2.0 equivalents) in the presence of *N,N*-diisopropylethylamine as a base at room temperature. After 2-4 h of reaction, the *N*-glyoxylamides **5-17**-**5-28**, were obtained, following a chromatographic workup, in good to excellent yield (53 to 99%) (Table **5.2**).

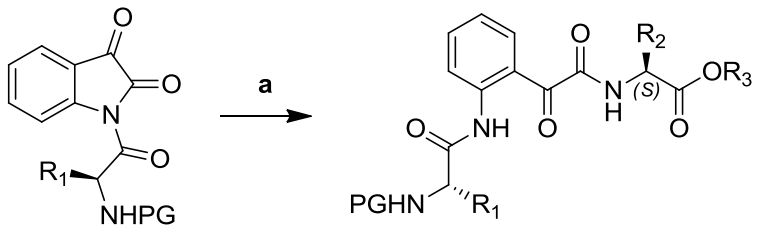


Table 5.2. Facile ring -opening reactions of *N*-(PG)amino acid isatins with various amino acid esters. Reagents and conditions: a) amino acid ester, DIPEA, CH_2Cl_2.

Compound	R_1	R_2, R_3	Reaction outcome
	***N*-(PG)amino acid)-isatin**	**Amino acid ester**	**(% Yield)**
5-17	*N*-(Boc)-L-Ala-isatin	Gly.OMe	98
5-18	*N*-(Boc)-L-Ala-isatin	Trp. OMe	81
5-19	*N*-(Boc)-L-Met-isatin	Gly.OMe	59
5-20	*N*-(Boc)-L-Met-isatin	Phe.OMe	56
5-21	*N*-(Boc)-L-Met-isatin	Trp. OMe	79
5-22	*N*-(Boc)-L-Phe-isatin	Gly.OMe	84
5-23	*N*-(Boc)-L-Phe-isatin	Met.OMe	71
5-24	*N*-(Boc)-L-Phe-isatin	Phe.OMe	73
5-25	*N*-(Boc)-L-Val-isatin	Gly.OMe	93
5-26	*N*-(Boc)-L-(O^{t}Bu)Ser-isatin	Gly.OBn	82
5-27	*N*-(Boc)-L-(OBn)Ser-isatin	Gly.OBn	53
5-28	*N*-(Cbz)-L-Val-isatin	Gly.OMe	99
5-29	*N*-(Cbz)-L-Val-isatin	Phe.OMe	89

Crystals of **5-24** suitable for single crystal X-ray analysis (synchrotron source) were grown by cooling of an ethanol solution. As depicted in Figure **5.2**, the molecule adopts a bent conformation induced by the 1,2- substitution pattern on the aromatic ring of the *N*-glyoxylamide moiety. This structural feature acts as a turn inducer, locking the conformation of the linear peptide mimic fragment. The presence of an intra-molecular hydrogen bond between H1 and O2 (1.97 Å) is consistent with the observed proton resonance at δ 11.12 in the ^{1}H NMR

spectrum. This hydrogen bond acts to further enhance the rigidity of the molecule by restricting the rotation about bonds C2-C3 and C8-N1.

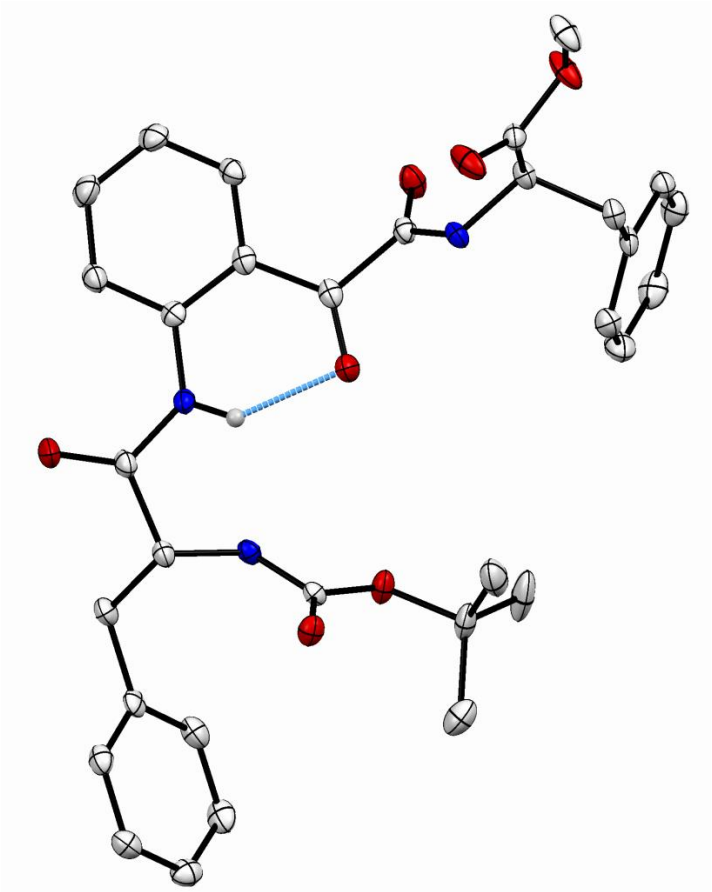
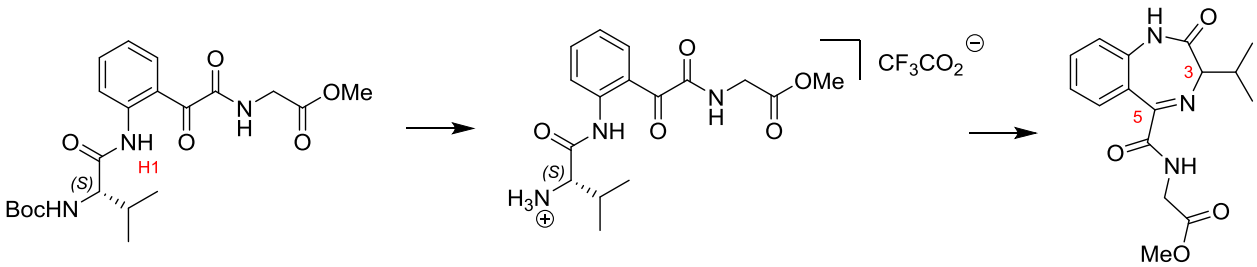


Figure 5.2. Front view from the crystal structure of *N*-glyoxylamide **5-24**, the light blue broken line represents the intramolecular H-bond N1—H1---O2 (50% thermal ellipsoids at 100 K are shown; all H-atoms are omitted for clarity).

5.2.3 Unanticipated synthesis of 1,4-benzodiazepin-2-ones

Following the successful synthesis of the *N*-protected linear peptides **5-17-5-29**, the *N*-deprotection of these intermediates were explored. Thus, derivative **5-25** was stirred in a 20% TFA in dichloromethane solution for 1 h (Scheme **5.4**). TLC analysis (2:3, ethyl acetate/n-hexane) revealed the disappearance of the starting material with the simultaneous formation of two new spots. The first spot appeared at the baseline, and was presumed to be the TFA salt of the deprotected product, as it exhibited a positive result with ninhydrin staining. Interestingly the second compound showed a negative result under ninhydrin staining, indicating the loss of the free amino group. ^{1}H NMR spectroscopic analysis of the product (Figure **5.3**) confirmed the expected loss of the ^{t}-butyloxycarbonyl group. Comparing the ^{1}H NMR spectrum of the product to that of the starting material **5-25**, H1 was observed to exhibit a significant up-field shift (δ 11.35 to 8.71), indicating the disappearance of the intramolecular hydrogen bond. Additionally, the two α-protons associated with glycine fragment appeared as two separate doublet of doublets (δ 4.07 and 4.10) with a large *J* coupling of 18.3 Hz typical of vicinal coupling. This could be due to either to the protons becoming incorporated into a ring, or to the increased proximity of the protons to the chiral carbon of the valine moiety. The ^{13}C NMR spectrum of this product showed the loss of the carbon associated with the ketone moiety (δ 190.83) and the appearance of a new quaternary carbon, confirmed by ^{13}C DEPT 135 spectroscopy.



5-25 **5-30**

Scheme 5.4. Unanticipated synthesis of 1,4-benzodiazepin-2-one **5-30** from *N*-glyoxylamide **5-25**. Reagents and conditions: a) 1. TFA/CH_2Cl_2 20% v/v 2. NEt_3/MeOH.

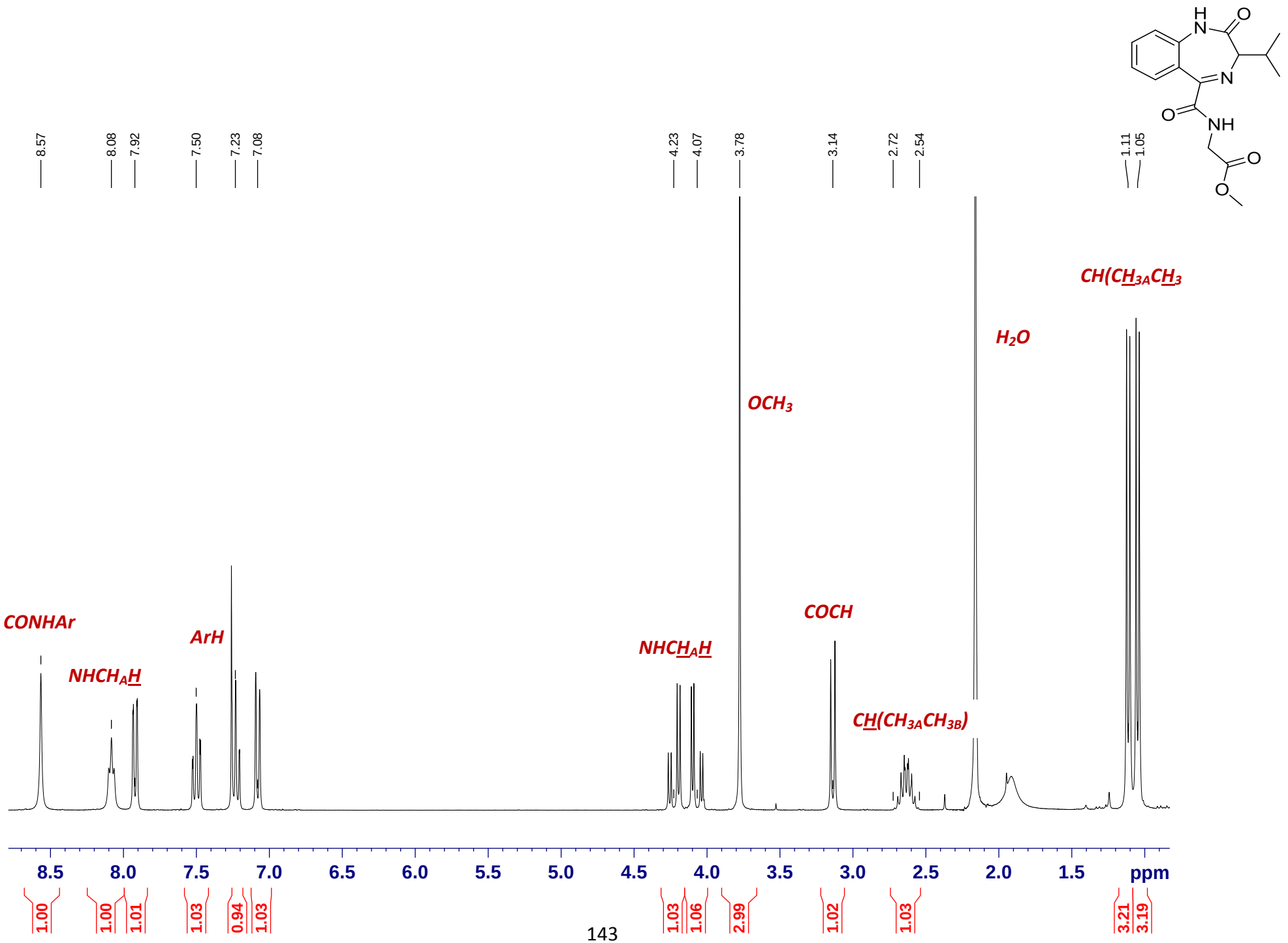


Figure 5.3. ^{1}H NMR spectra of 1,4-benzodiazepin-2-one **5-30** recorded at 300 MHz in $CDCl_3$ at 298 K.

Crystals of the unknown compound were grown *via* a slow evaporation of a toluene solution for the purpose of single crystal X-ray analysis (synchrotron source). Interestingly, as depicted in Figure **5.4,** the product **5-30** contains a 1,4-benzodiazepin-2-one ring system. Both enantiomers are present in the product, which crystallized in the centrosymmetric space group (P2), indicating the occurrence of racemization.

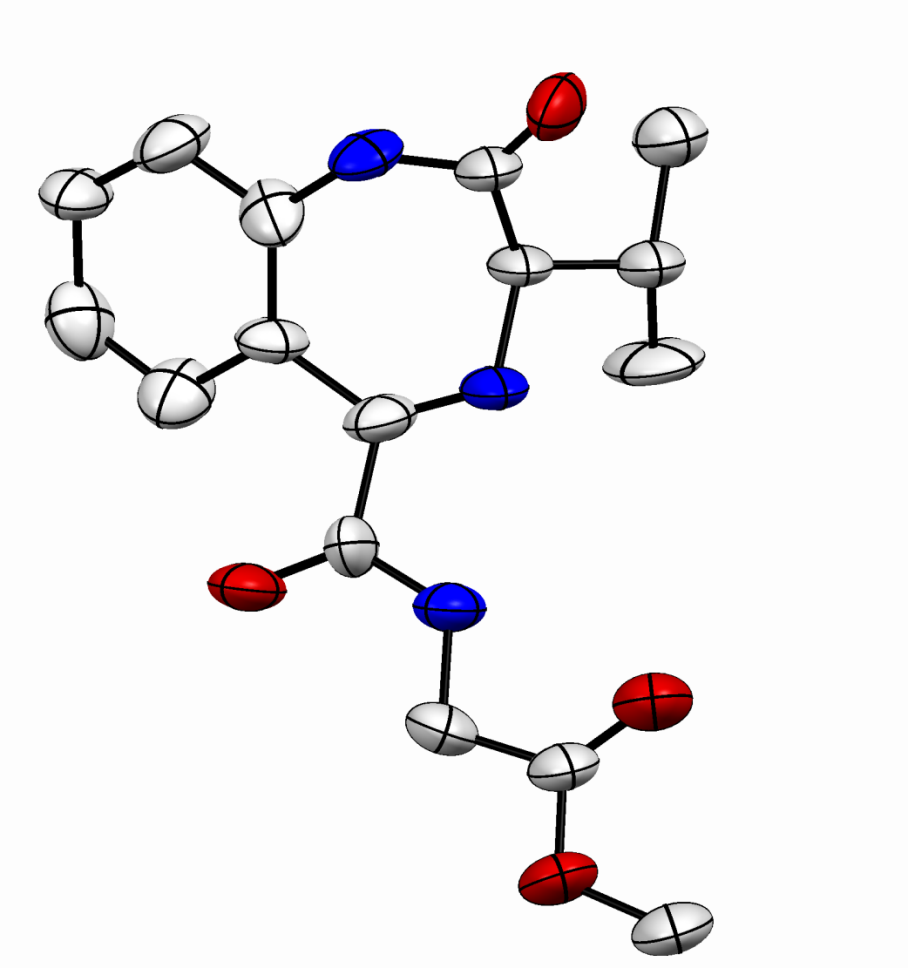
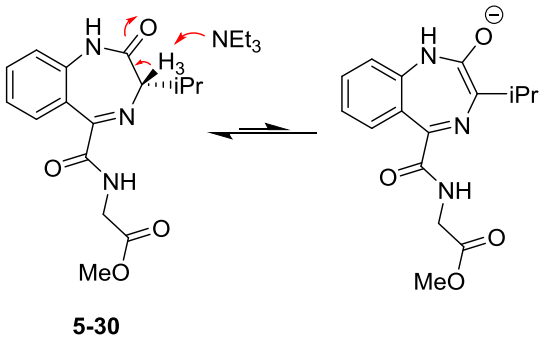


Figure 5.4. Front view from the crystal structure of 1,4-benzodiazepin-2-one **5-30** (50% thermal ellipsoids at 100 K are shown; all H-atoms are omitted for clarity).

Deprotonation of H3 was thought to be the most likely explanation for racemization due to activation of this proton by the adjacent C=N bond. However, it should be noted that deprotonation at C3 may lead to an unfavorable anti-aromatic 8π electron system (Scheme **5.5**).[15] In the literature, cyclisation of analogous substrates performed in acidic (TFA) or neutral (hydrogenation) conditions have reported no racemization at C3.[16,17]



5-30

Scheme 5.5. Deprotonation of **5-30** forming of an anti-aromatic 8π electron system.

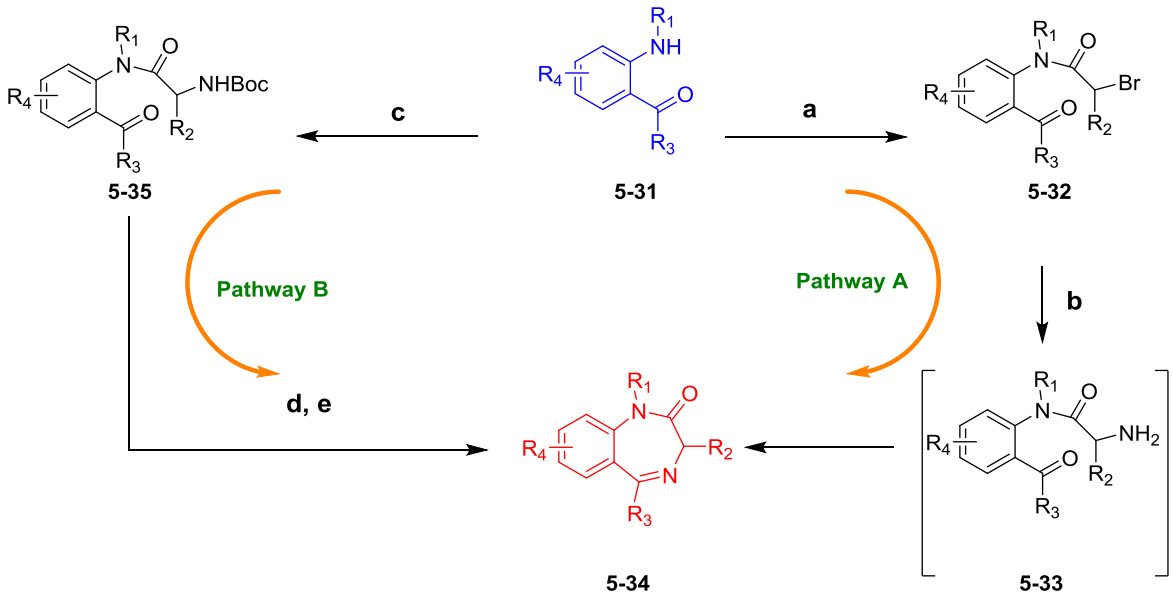
5.2.4 Benzodiazepines

Given the surprising reaction sequence uncovered above, it was of interest to further study the possibility of generating novel 1,4-benzodiazepin-2-one ring systems using this methodology. The benzodiazepine scaffold is considered as a "privileged structure" in medicinal chemistry. Drugs based on benzodiazepines are in wide clinical use as anxiolytics, hypnotics, anti-convulsants and muscle relaxants.[18] These molecules target an allosteric site on the $GABA_A$ receptor, thereby modulating the binding of endogenous GABA and potentiating CNS depression.[19] In addition, benzodiazepines have been investigated as anticancer[20,21] and anti-malarial agents.[22] Therefore the development of new synthetic routes towards novel benzodiazepines is an active area of exploration.

5.2.5 General synthetic stategies to 1,4-benzodiazepin-2-ones

Synthetic routes to 1,4-benzodiazepin-2-ones are well established in the literature. The most common methodologies for their preparation employ 2-aminobenzophenone **5-31** or 2-aminoacetophenones as a starting material. These are typically synthesized from the reaction of the corresponding 2-aminobenzonitriles with a Grignard or alkyllithium reagent.[23] This means that the C5 substituent on the final 1,4-benzodiazepin-2-one scaffold is generally restricted to an alkyl or aryl group. In contrast, the synthesis uncovered above allows for the incorporation of pendant esters or amides at C5, as well as the possibility of varying the groups at C3.

A representative synthesis of the 1,4-benzodiazpin-2-one core structure is given below (Scheme **5.6**).[24-27] Condensation of 2-aminobenzophenone **5-31** with bromoacetyl bromide forms the corresponding 2-amido-benzophenone **5-32**. Upon treatment with ammonia, the primary amine **5-33** is generated *in situ*, which can be cyclised to the final compound **5-34** in a one-pot process (Scheme **5.6**, Pathway **A**).



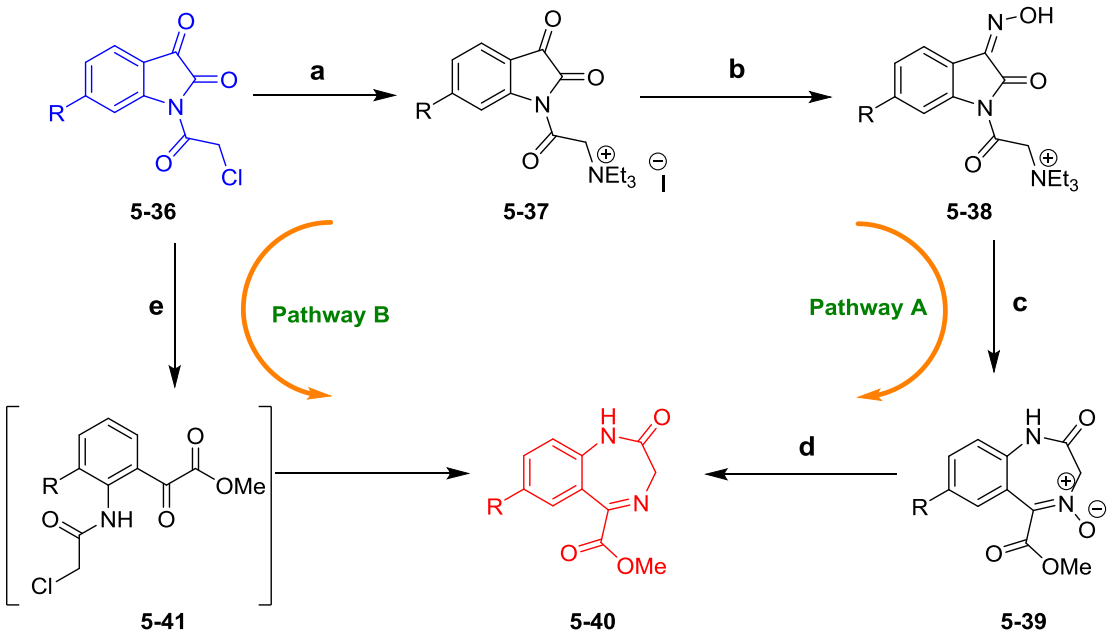
Scheme 5.6. Representative syntheses of 1,4-benzodiazepin-2-ones **5-34**. Reagents and conditions: a) bromoacetyl bromide b) NH_3 c) *N*-(Boc)amino acid, EEDQ d) TFA/CH_2Cl_2 e) $CH_3CO_2NH_4$/AcOH.

Alternately, *N*-protected amino acids can be used as precursors instead of 2-bromoacetyl bromide. For these types of compounds, carbamates (Boc[28], Fmoc,[29] Cbz[30]) are

generally the most effective class of protecting groups. Upon deprotection of the amine group, 2-amidobenzophenones **5-35** can be cyclized in a single-pot process to afford the 1,4-benzodiazepin-2-one scaffold **5-34** (Scheme **5.6**, pathway **B**). Despite sharing a similar deprotection and cyclisation sequence this synthetic route begins with aceto/benzophenone derivatives, thus limiting the substituents at C5 to either alkyl or aryl groups.

5.2.6 1,4-benzodiazepin-2-ones incorporating a pendant ester or amide moiety at C5

Literature precedents for the preparation of C5 ester or amide substituted 1,4-benzodiazepin-2-ones are sparse. Two routes that have been reported and both use *N*-chloroacetylisatin **5-36** as a precursor. In the first approach, *N*-chloroacetylisatin **5-36** was treated with triethylamine in the presence of KI to form the quaternary ammonium salt **5-37** (Scheme **5.7**, pathyway **A**). Reaction of the iodide salt **5-37** with hydroxylamine hydrochloride gave the oxime **5-38**. Installation of the methyl ester group and cyclization to the benzodiazepine *N*-oxide **5-39** was performed in a single pot by heating **5-38** in methanol. The reaction mechanism proceeds *via* nucleophilic ring-opening of **5-38** by methanol, followed by ring closure through the substitution of the triethylammonium group with the oxime nitrogen. Reduction using PCl_5 furnished the 1,4-benzodiazepin-2-one **5-40**. Unfortunately this synthesis suffers from a number of drawbacks. Introducing groups at C3 of the 1,4-benzodiazepin-2-one scaffold requires the use of 2-substituted chloroacetyl chlorides to prepare the corresponding *N*-chloroacetylisatins. However, the application of 2-substituited to chloroacetyl chlorides to this synthesis has not yet been explored. Furthermore, to install a pendant amide at C5, the ring-opening of the isatin with a primary amine is required. However, this may lead to substitution of the triethylammonium group by the primary amine, thereby preventing the key intramolecular ring-closing step.



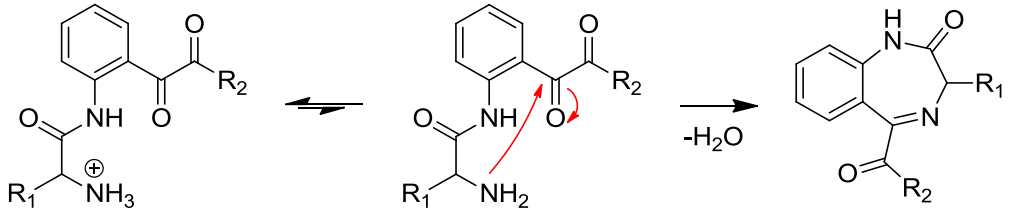
Scheme 5.7. Synthetic routes to C5 pendant methyl ester substituted 1,4-benzodiazepin-2-ones. Reagents and conditions: Pathway A a) NEt_3, KI b) $NH_2OH.HCl$ c) MeOH/DMF d) PCl_5. Pathway B e) Hexamethylenetetramine/MeOH.

The second strategy developed by Ogata and Matsumoto avoids the requirement for the benzodiazepine *N*-oxide intermediate, and therefore can be considered advantageous compared to the first approach. This synthesis entails the nucleophilic ring-opening of *N*-acylisatin **5-36** by methanol to form intermediate **5-41** followed by halide substitution with hexamethylenetetramine. Intramolecular cyclisation to the target benzodiazepine **5-40** occurs concomitantly (Scheme **5.7,** Pathway **B**).[31,32] However, this route shares similar limitations to that of the first strategy, as both employ *N*-chloroacetylisatin **5-36** as starting material.

Thus, the synthesis uncovered in this work represents a significant advancement over literature precedent, as it provides straightforward access to both C5 ester and amide-substituted 1,4-benzodiazepin-2-ones. In addition, a wide variety of C3 substituents can be installed by varying the nature of the *N*-protected amino acids used for the initial *N*-acylation of isatin **1-1**.

5.2.7 Reaction optimization of 1,4-benzodiazepin-2-ones prepared from *N*-(Boc)amino acid isatin

For this route to be a feasible pathway towards novel 1,4-benzodiazepin-2-ones, the reaction conditions for the cyclization process had to be optimized. It was thought that the acidic conditions used during the deprotection of the Boc group maintained the primary amine intermediate as the protonated ammonium salt, hence preventing efficient cyclisation (Scheme **5.8**).



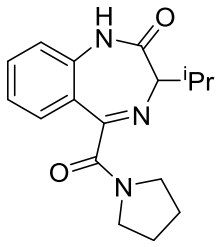
Scheme 5.8. Equilibrium hindering efficient cyclisation to 1,4-benzodiazepin-2-one.

Thus, we envisaged that the cyclisation yield and efficiency could be improved if the reaction was performed under basic conditions. Accordingly, **5-25** was again treated with TFA-CH_2Cl_2 (20% v/v) to give the Boc deprotected product. Upon consumption of the starting material (as revealed by TLC analysis), the solvent was removed *in vacuo* and the residue was redissolved in methanol. Triethylamine (10 equivalents) was then added to the reaction to convert any ammonium salts in the mixture into the corresponding free amine. The reaction was stirred at room temperature for a further 1 h, at which point the free amine was completely consumed and a new spot corresponding to the target 1,4-benzodiazapin-2-one was also observed. The crude product was subjected to chromatography on silica gel to afford the target compound **5-30** in 94% yield.

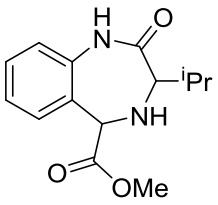
5.2.8 Preparation of 3-subtituted 1,4-benzodiazepin-2-ones bearing C5 pendant ester or amides groups

To demonstrate the scope of this novel synthetic strategy, a series of derivatives was prepared from their corresponding *N*-glyoxylesters and amides *via the* Boc deprotection and cyclisation sequence described above. The 1,4-benzodiazepin-2-one derivatives **5-30** and **5-42-5-52** were isolated in good to excellent yields (>85%)(Table **5.3**).

The formation of 1,4-benzodiazepin-2-ones could also be accomplished through the deprotection of *N*-Cbz (as opposed to *N*-Boc) protected amines. For example, deprotection of *N*-Cbz **5-13** by hydrogenation provided the corresponding 1,4-benzodiazepin-2-one **5-53**. However, subjecting the methyl ester-substituted **5-16** to identical conditions afforded the over-reduced secondary amine **5-54**. This may be due in part to a greater degree of polarization of the imine double bond induced by the ester group (c.f. an amide group). It is worth noting that C3 tends to racemise under the conditions described for the Boc deprotection and cyclisation sequence (see below). In contrast, cyclisation of structurally related *N*-Cbz substrates containing a chiral center at C3 have been shown to retain stereochemistry when subjected to neutral hydrogenation conditions. If C3 remains chiral then it may induce chirality at C5, providing a single steroisomer of the product.[17]



5-53



5-54

Figure 5.5. Hydrogenation of intermediates **5-13** and **5-16**.

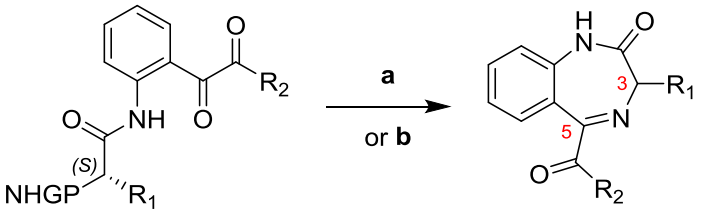


Table 5.3. Synthesis of C5-substituted pendant amides/esters *via N*-deprotection and cyclocondensation of the corresponding *N*-glyoxylic amides or esters. Reagents and conditions: a) (i) TFA, CH_2Cl_2 (ii) NEt_3, CH_3OH b) H_2, Pd/C, CH_3OH.

Compound	R_1	R_2	Reaction Yield (%)
5-30	- ^{i}Pr	- (Gly. OMe)	94
5-42	-Me	- NH^{t}Bu	87
5-43	-Me	-pyrrolidine	82
5-44	-Me	- CH_2Ferrocene	59
5-45	- Me	- (Gly. OMe)	63
5-46	-Me	- (Trp.OMe)	79
5-47	-$(CH_2)_2SCH_3$	- (Gly. OMe)	59
5-48	-$(CH_2)_2SCH_3$	- (Phe.OMe)	81
5-49	- Bn	- (Gly.OMe)	95
5-50	- Bn	- (Met.OMe)	54
5-51	- Bn	- (Phe.OMe)	88
5-52	-CH_2OH	- (Gly.OBn)	98

From closer examination of the X-ray crystal structure of compound **5-24** (Figure **5.2**), a tentative explanation can be provided for the formation of these 1,4-benzodiazepin-2-ones. If the Boc substituent is considered less important, C2 and N2 adopt a cisoid conformation, bringing them into close proximity (4.38 Å) and favoring the intramolecular cyclocondensation reaction. Several intramolecular hydrogen bonds present in the X-ray crystal lattice may contribute to stabilising this conformation. In particular, the hydrogen bond between N1—H1---O2 (H1---O2 = 1.98 Å) ∠X° is the major contributor with comparatively weaker intramolecular hydrogen bonds observed between C7—H7---O3 (H7---O3 = 2.25), N1—H1---N2 (H1---N2 = 2.27), C11—H11A---O3 (H11A---O3 = 2.5) and N2—H2--

-N1 (H2---N1 = 2.62). In contrast, the transoid conformation has been shown to be less favorable for nucleophilic attack.[15]

As an interesting aside, when **5-42** was crystallized from a solution of hot ethanol, colourless needles were obtained. However, when **5-42** was grown from a mixture of ethyl acetate and n-hexane colourless plates were obtained instead. Both of these samples were analyzed by single crystal X-ray crystallography (synchrotron source). The crystals of **5-42** grown from ethanol had a centro-symmetric unit cell containing both enantiomers and the N1—H1--O4' hydrogen bond. The crystals of **5-42** from ethyl acetate/n-hexane had a unit cell with the chiral space group $P3_2$. In the unit cell, six molecules of **5-42** were present, all with identical stereochemistry and arranged in a helical assembly.[1] This result hints that perhaps chiral resolution of this novel class of benzodiazepinones can be achieve through recrystallization alone, as opposed to less scalable and expensive methods such as chiral HPLC.

[1] Absolute sterochemistry could not be determined due to the lack of heavy atoms in **5-42**.

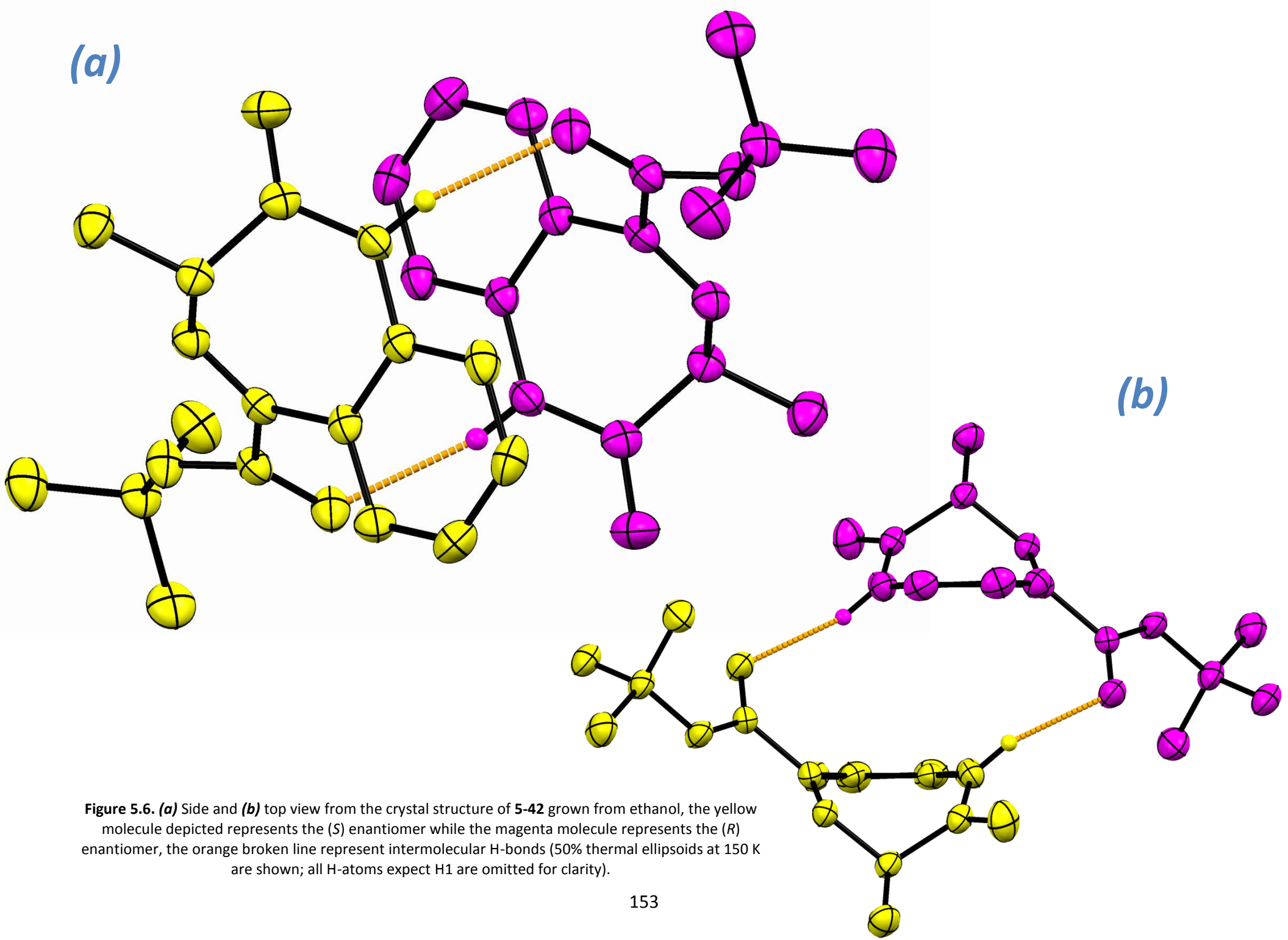


Figure 5.6. ***(a)*** Side and ***(b)*** top view from the crystal structure of **5-42** grown from ethanol, the yellow molecule depicted represents the (*S*) enantiomer while the magenta molecule represents the (*R*) enantiomer, the orange broken line represent intermolecular H-bonds (50% thermal ellipsoids at 150 K are shown; all H-atoms expect H1 are omitted for clarity).

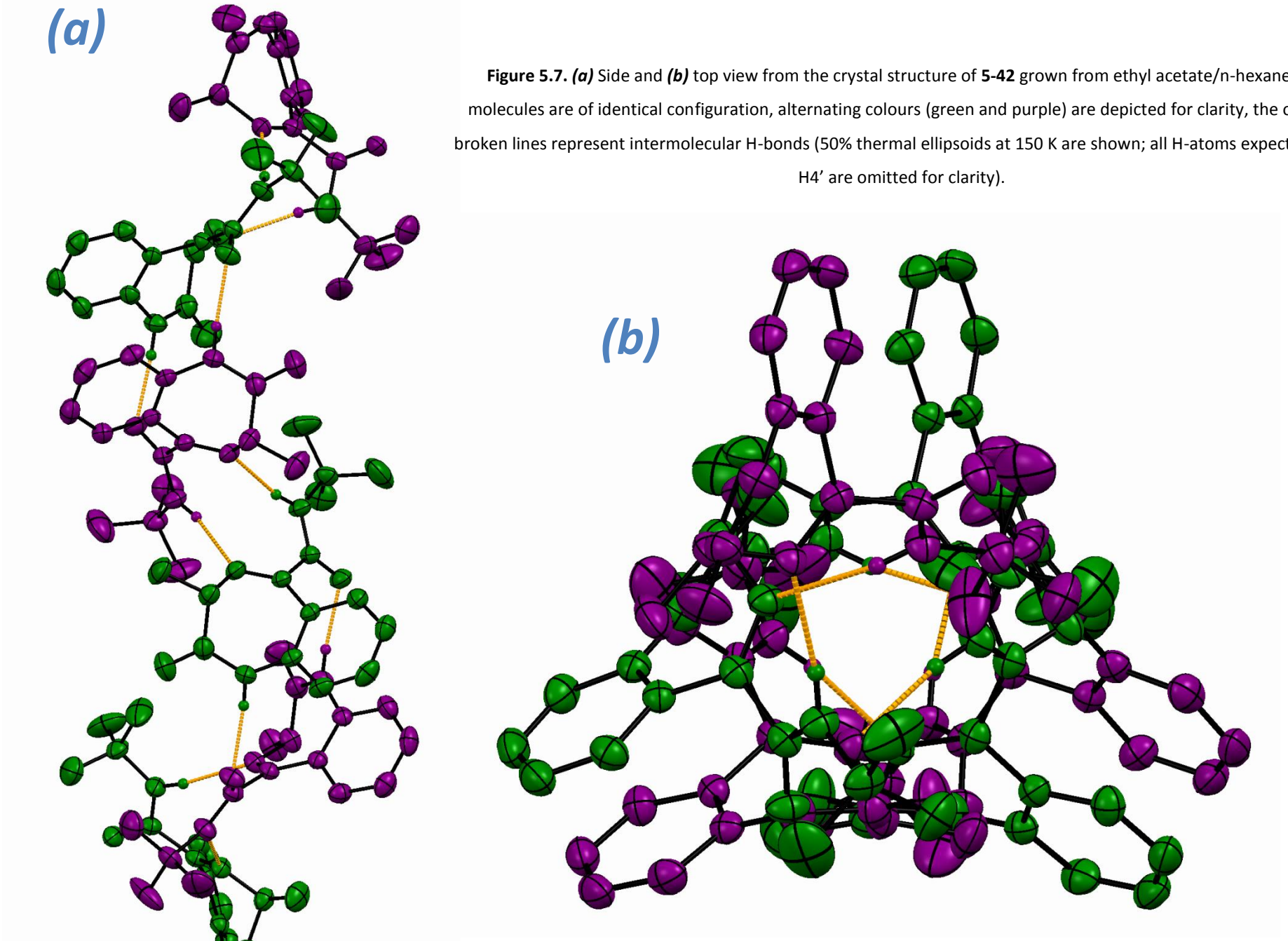


Figure 5.7. ***(a)*** Side and ***(b)*** top view from the crystal structure of **5-42** grown from ethyl acetate/n-hexane, all molecules are of identical configuration, alternating colours (green and purple) are depicted for clarity, the orange broken lines represent intermolecular H-bonds (50% thermal ellipsoids at 150 K are shown; all H-atoms expect H1 and H4' are omitted for clarity).

The synthetic path discovered permits the introduction of a wide variety of amides and esters, at C5 of the 1,4-benzodiazepin-2-one scaffold. Incorporation of these pendant C5 groups is limited only by the reactivity of the nucleophile precursor (amine or alcohol) towards the appropriate *N*-protected amino acid isatin. Diversification at the C3 position of the final scaffold can be easily achieved using amino acid precursors, which are inexpensive and readily available. Both Boc-protected and Cbz-protected amino acids are compatible with this synthesis, allowing the possibility of orthogonal deprotection at a later stage.

5.3 Summary and future work

A facile, high-yielding route to 1,4-benzodiazepin-2-ones bearing C5 pendant amide or ester groups has been uncovered. This approach starts from *N*-glyoxylamides or esters and involves an *N*-deprotection and cyclisation sequence. Twelve 1,4-benzodiazepin-2-one derivatives with variations at both C3 and C5 have been isolated and characterized using this method. This novel synthetic route provides access to compounds which would otherwise be inaccessible. As benzodiazepines are well established as GABA receptor ligands that possess diverse biological activities, future investigations will focus on determining the biological profiles of these compounds against likely protein targets.

5.4 Experimental

5.4.1 General methods

Synthesis and reagents

A detailed account of general synthetic methodologies is provided in the experimental section for **Chapter 2**. Further details relevant to this chapter are presented below.

Spectroscopy

A detailed account of general spectroscopic methodologies is provided in the experimental section for **Chapter 2**.

Crystallography

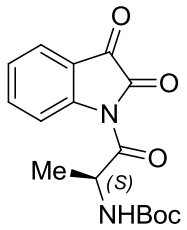
Single crystal X-Ray diffraction data for **5-24**, **5-30** and **5-42** were obtained on the MX-1 beamline at the Australian Synchrotron. Structures were processed and refined using SHELX software.

5.4.2 Synthesis

General procedure for the synthesis of *N*-protected amino acid isatins **5-4-5-9**

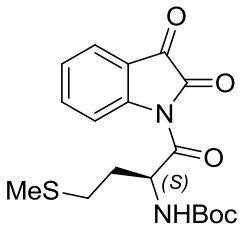
A mixture of isatin **1-1** (1 equivalent), *N*-protected amino acid (1.2 equivalents), DCC (1.2 equivalents) and DMAP (0.2 equivalents) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, *N*,*N*-dicyclohexylurea was removed by filtration and the filtrate was subjected to flash chromatography on silica gel (dichloromethane). The crude product was precipitated from a mixture of ethyl acetate and n-hexane as a bright yellow powder.

(*S*)-*tert*-Butyl (1-(2,3-dioxoindolin-1-yl)-1-oxopropan-2-yl)carbamate **5-4**



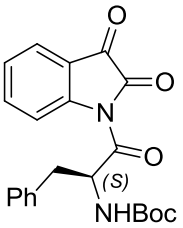
The title compound **5-4** was prepared from isatin **1-1** (4.17 g, 28.3 mmol) and *N*-(*tert*-butoxycarbonyl)-L-alanine (6.43 g, 34.0 mmol) using the general procedure described above (7.67 g, 85%). M.p. 87-89°C; $[\alpha]_D$ -73 (*c* 0.11, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C($C\underline{H}_3$)), 1.48 (d, *J* = 7.0 Hz, 3H, CH_3), 5.21 (bd, *J* = 7.0 Hz, 1H, NH), 5.43 (dq, *J* = 7.0, 7.0 Hz, 1H, CH), 7.36 (ddd, *J* = 7.5, 0.6 Hz, 1H, ArH), 7.73 (ddd, *J* = 8.5, 1.1 Hz, 1H, ArH), 7.80 (dd, *J* = 7.5, 1.1 Hz, 1H, ArH), 8.41 (dd, *J* = 8.5, 0.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$):δ 18.20 (CH_3), 28.41 (C($\underline{C}H_3)_3$), 51.19 (CH), 80.38 ($\underline{C}(CH_3)_3$), 118.56 (ArCH), 119.78 (ArC_q), 125.57 (ArCH), 126.55 (ArCH), 139.12 (ArCH), 148.55 (ArC), 155.34 (NH$\underline{C}$OO), 157.42 ($\underline{C}$ON), 174.09 (CO$\underline{C}$ON), 179.62 ($\underline{C}$OCON); IR (ATR): ν_{max}3375, 2987, 1753, 1685, 1607, 1508, 1462, 1362, 1242, 1155, 1072, 1041, 846, 758 cm^{-1}; UV–vis (CH_3CN): λ_{max} 235 nm (ε 25,800 cm^{-1} M^{-1}), 295 (8,110); HRMS (+ESI): Found *m/z* 341.1103, $[M+Na]^+$, $C_{16}H_{18}N_2O_5Na$ requires 341.1108.

(*S*)-*tert*-Butyl (1-(2,3-dioxoindolin-1-yl)-1-oxopropan-2-yl)carbamate **5-5**



The title compound **5-5** was prepared from isatin **1-1** (2.50 g, 17.0 mmol) and *N*-(*tert*-butoxycarbonyl)-L-methionine (5.51 g, 22.1 mmol) using the general procedure described above (3.15 g, 49%). M.p. 162°C; $[\alpha]_D$ -45 (*c* 0.20, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C($C\underline{H}_3$)$_3$), 1.79-1.94 (m, 1H, $SCH_2C\underline{H}_AH_B$), 2.10 (s, 3H, SCH_3), 2.16-2.29 (m, 1H, $SCH_2CH_A\underline{H}_B$), 2.67-2.73 (m, 2H, SCH_2), 5.32 (bd, *J* = 9 Hz, 1H, NH), 5.46 (ddd, *J* = 9.0, 9.0, 3.5 Hz, 1H, CH), 7.37 (ddd, *J* = 7.6, 7.6, 0.9 Hz, 1H, ArH), 7.74 (ddd, *J* = 8.4, 7.6, 1.5 Hz, 1H, ArH), 7.80 (dd, *J* = 7.6, 1.5 Hz, 1H, ArH), 8.38 (dd, *J* =8.4, 0.9 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ16.15 (SCH_3), 28.82 (C($\underline{C}H_3$)$_3$), 30.97 ($S\underline{C}H_2CH_2$), 32.79 ($SCH_2\underline{C}H_2$), 55.59 (CH), 81.03 ($\underline{C}(CH_3)_3$), 118.94 (ArCH), 120.21 (ArC), 126.06 (ArCH), 127.08 (ArCH), 139.59 (ArCH), 148.85 (ArC), 156.10 (CO$\underline{C}$ON), 173.12 (CO$\underline{C}$ON), 179.93 ($\underline{C}$OCON); IR (ATR): ν_{max}3337, 2979, 1748, 1681, 1601, 1524, 1462, 1365, 1303, 1249, 1162, 1061, 1018, 856, 758cm^{-1}; UV–vis (CH_3CN): λ_{max} 230 nm (ε 22,900 cm^{-1} M^{-1}), 295 (7,940), 670 (5450); HRMS (+ESI): Found *m/z* 401.1140, $[M+Na]^+$, $C_{18}H_{22}N_2O_5SNa$ requires 401.1142.

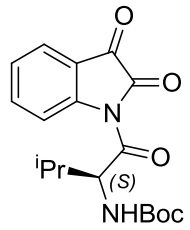
(*S*)-*tert*-Butyl (1-(2,3-dioxoindolin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate **5-6**



The title compound **5-6** was prepared from isatin **1-1** (2.00 g, 13.6 mmol) and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (4.33 g, 16.3 mmol) using the general procedure described

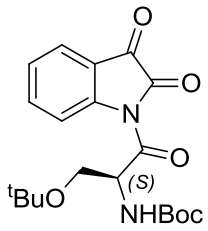
above (3.91 g, 73%). M.p. 168-170 °C; $[\alpha]_D$ -8.7 (*c* 0.12, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (s, 9H, C($C\underline{H}_3$)$_3$), 4.23 (d, *J* = 5.5 Hz, 2H, $C\underline{H}_2$Ph), 4.28-4.25 (m, 1H, $C\underline{H}CH_2$Ph), 5.10 (bd, *J* = 4.9 Hz, 1H, NH), 7.15 (ddd, *J* = 8.3, 7.4, 1.1 Hz, 1H, ArH), 7.28-7.44 (m, 5H, 5 x ArH), 7.63 (ddd, *J* = 8.7, 7.4, 1.7 Hz, 1H, ArH), 8.39 (dd, *J* = 8.3, 1.7 Hz, 1H, ArH), 8.69 (dd, *J* = 8.7, 1.1 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3 (C($\underline{C}H_3$)$_3$), 38.3 (CH_2), 56.2 (CO$\underline{C}$H), 80.3 ($\underline{C}(CH_3)_3$), 118.3 (ArCH), 119.7 (ArC), 125.5 (ArCH), 126.6 (ArCH), 127.3 (ArCH), 128.7 (ArCH), 129.5 (ArCH), 135.6 (ArC), 139.1 (ArCH), 148.4 (ArC), 155.3 (OCONH), 157.4 (CO$\underline{C}$ON), 172.7 ($\underline{C}$OCH), 179.5 ($\underline{C}$OCON); IR (ATR): ν_{max} 3339, 2974, 1778, 1748, 1722, 1685, 1605, 1521, 1463, 1363, 1304, 1245, 1163, 1077, 1049, 949, 881, 754 cm^{-1}; HRMS (+ESI): Found *m/z* 417.1413, $[M+Na]^+$, $C_{22}H_{22}N_2O_5Na$ requires 417.1426.

tert-Butyl (*S*)-(1-(2,3-dioxoindolin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate **5-7**



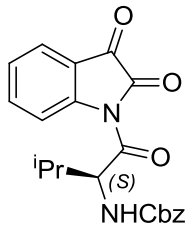
The title compound **5-7** was prepared from isatin **1-1** (2.30 g, 15.6 mmol) and *N*-(*tert*-butoxycarbonyl)-L-valine (4.08 g, 18.8 mmol) using the general procedure described above (4.76 g, 88%). M.p. 116-118 °C; $[\alpha]_D$ -54 (*c* 0.45, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, *J* = 6.9 Hz, 3H, CH($C\underline{H}_3$)$_2$), 1.11 (d, *J* = 6.8 Hz, 3H, CH($C\underline{H}_3$)$_2$), 1.42 (s, 9H, C(CH_3)$_3$), 2.09-2.22 (m, 1H, $C\underline{H}(CH_3)_2$), 5.20 (bd, *J* = 9.4 Hz, 1H, NH), 5.51 (dd, *J* = 9.4, 3.6 Hz, 1H, COCH), 7.34 (ddd, *J* = 7.6, 7.6, 0.9 Hz, 1H, ArH), 7.72 (ddd, *J* = 8.3, 7.6, 1.6 Hz, 1H, ArH), 7.77 (dd, *J* = 7.6, 1.6 Hz, 1H, ArH), 8.39 (dd, *J* = 8.3, 0.9 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.2 (CH($\underline{C}H_3$)$_2$), 20.0 (CH($\underline{C}H_3$)$_2$), 28.4 (C($\underline{C}H_3$)$_3$), 30.6 ($\underline{C}H(CH_3)_2$), 59.3 (CO$\underline{C}$H), 80.2 ($\underline{C}(CH_3)_3$), 118.5 (ArCH), 119.7 (ArC), 125.5 (ArCH), 126.5 (ArCH), 139.1 (ArCH), 148.5 (ArC), 156.0 (OCONH), 157.4 (CO$\underline{C}$ON), 173.0 ($\underline{C}$OCH), 179.6 ($\underline{C}$OCON); IR (ATR): ν_{max} 3435, 2965, 1780, 1748, 1696, 1605, 1498, 1461, 1363, 1303, 1251, 1161, 1079, 1039, 991, 929, 863, 795, 759, 704 cm^{-1}; HRMS (+ESI): Found *m/z* 369.1415, $[M+Na]^+$, $C_{18}H_{22}N_2O_5Na$ requires 369.1426.

tert-Butyl (*S*)-3-((tert-butoxycarbonyl)amino)-4-(2,3-dioxoindolin-1-yl)-4-oxobutanoate **5-8**



The title compound **5-8** was prepared from isatin **1-1** (0.800 g, 5.44 mmol) and *N*-(*tert*-butoxycarbonyl)-*O*-*tert*-butyl-L-serine (2.20 g, 6.52 mmol) using the general procedure described above (1.70 g, 80%). M.p. 102-105°C; $[\alpha]_D$ -34 (*c* 0.15, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 9H, C($\underline{C}H_3$)), 1.43 (s, 9H, C($\underline{C}H_3$)), 3.69-3.79 (m, 2H, CH$\underline{C}H_2$), 5.56-5.59 (m, 2H, NH, CH), 7.33 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 1H, ArH), 7.71 (ddd, *J* = 8.3, 7.6, 1.5 Hz, 1H, ArH), 7.77 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH), 8.31 (dd, *J* = 8.3, 0.8 Hz, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.27 (C($\underline{C}H_3)_3$), 28.37 (C($\underline{C}H_3)_3$), 55.99 (CH_2), 61.70 (CH), 73.81 ($\underline{C}(CH_3)_3$), 80.24 ($\underline{C}(CH_3)_3$), 118.31 (ArCH), 119.57 (ArC), 125.49 (ArCH), 126.37 (ArCH), 139.09 (ArCH), 148.58, (ArC), 155.75 (OCONH), 157.55 (CO$\underline{C}$ON), 171.02 ($\underline{C}$OCH), 179.74 ($\underline{C}$OCON). IR (ATR): ν_{max}3368, 2971, 1747, 1692, 1592, 1528, 1463, 1363, 1297, 1247, 1162, 1088, 945, 871, 796, 754 cm^{-1}; UV–vis (CH_3CN): λ_{max} 235 nm (ε 13,000 cm^{-1} M^{-1}), 345 (1,740); HRMS (+ESI): Found *m/z* 413.1675, $[M+Na]^+$, $C_{20}H_{26}N_2O_6Na$ requires 413.1689.

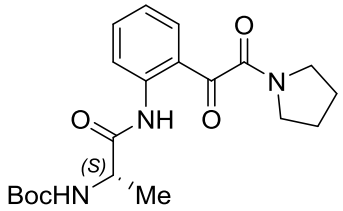
(*S*)-Benzyl (1-(2,3-dioxoindolin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate **5-9**



The title compound **5-9** was prepared from isatin **1-1** (0.500 g, 3.40 mmol), *N*-(carbobenzyloxy)-L-valine (1.03 g, 4.08 mmol) and DCC (0.841 g, 4.08 mmol) using the general procedure described above (0.779 g, 60%). M.p. 133-135°C; $[\alpha]_D$ -3.6 (*c* 0.28, CH_2Cl_2); ^{1}H NMR

(300 MHz, $CDCl_3$): δ 0.89 (d, *J* = 6.9 Hz, 3H,CH($C\underline{H}_3$)$_2$), 1.13 (d, *J* = 6.7 Hz, 3H,CH($C\underline{H}_3$)$_2$), 2.20 (dqq, *J* = 6.9, 6.7, 3.4 Hz, 1H, C$\underline{H}$(CH_3)$_2$), 5.10 (s, 2H, C$\underline{H}_2$Ph), 5.51 (bd, *J* = 9.2 Hz, 1H, NH), 5.60 (dd, *J* = 9.3, 3.3 Hz, COC$\underline{H}$NH), 7.33-7.38 (m, 6H, 6 x ArH), 7.72 (ddd, *J* = 8.4, 7.6, 1.5 Hz, 1H, ArH), 7.79 (d, *J* = 7.4, 1H, ArH), 8.40 (d, *J* = 8.4 Hz, 1H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 16.2 (CH_3), 20.0 (CH_3), 30.8 ($\underline{C}$H(CH_3)$_2$), 59.8 (CO$\underline{C}$H), 67.4 (CH_2), 118.5 (ArCH), 119.8 (ArC), 125.6 (ArCH), 126.6 (ArCH), 128.3 (ArCH), 128.4 (ArCH), 128.7 (ArCH), 136.3 (ArC), 139.1 (ArCH), 148.4 (ArC), 156.6 (OCONH), 157.4 (CO$\underline{C}$ON), 172.6 ($\underline{C}$OCH), 179.5 ($\underline{C}$OCON); IR (ATR): ν_{max} 3399, 2963, 1776, 1715, 1602, 1519, 1459, 1378, 1335, 1317, 1248, 1208, 1166, 1102, 1010, 893, 850, 801, 757, 700, 670 cm^{-1}; HRMS (+ESI): Found *m/z* 403.1256, $[M+Na]^+$, $C_{21}H_{20}N_2O_5Na$ requires 403.1270.

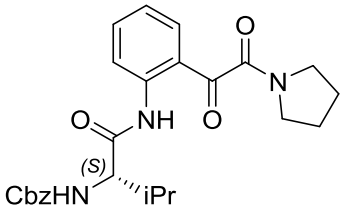
(*S*)-*tert*-Butyl (1-oxo-1-((2-(2-oxo-2-(pyrrolidin-1-yl)acetyl)phenyl)amino)propan-2-yl)carbamate **5-12**



N-(Boc)-L-Ala-isatin **5-4** (0.334 g, 1.05 mmol) and pyrrolidine (0.09 mL, 1.10 mmol) was stirred at room temperature in dichloromethane for 2h. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel, eluting with 1 : 1 ethyl acetate/n-hexane to afford **5-12** as a colourless amorphous solid (0.210 g, 82 %). M.p. 120-121°C; $[\alpha]_D$ -440 (*c* 0.014, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, C($C\underline{H}_3$)$_3$), 1.50 (d, *J* = 7.2 Hz, 3H, CHC$\underline{H}_3$), 1.91-2.02 (m, 4H, 2 x $NCH_2C\underline{H}_2$), 3.35-3.38 (m, 2H, NCH_2), 3.64-3.68 (m, 2H, NCH_2), 4.37 (dq, *J* = 7.2, 5.1 Hz, 1H, C$\underline{H}CH_3$), 5.12 (bd, *J* = 5.1 Hz, N$\underline{H}$Boc), 7.15 (ddd, *J* = 8.0, 7.3, 1.0 Hz, 1H, ArH), 7.62 (ddd, *J* = 8.7, 7.3, 1.6 Hz, 1H, ArH), 7.71 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.80 (dd, *J* = 8.7, 1.0 Hz, 1H, ArH), 11.74 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.0 (CH$\underline{C}H_3$), 24.2 ($NCH_2\underline{C}H_2$), 26.0 ($NCH_2\underline{C}H_2$), 28.5 (C($\underline{C}H_3$)$_3$), 45.4 (N$\underline{C}H_2CH_2$), 46.8 (N$\underline{C}H_2CH_2$), 51.9 (CO$\underline{C}$HNH), 80.4 ($\underline{C}$(CH_3)$_3$), 118.2 (ArC), 120.9 (ArCH), 123.2 (ArCH), 133.8 (ArCH), 136.8 (ArCH), 142.3 (ArC), 155.4 (OCONH), 164.4 (CO$\underline{C}$ONH), 172.6 ($\underline{C}$ONHAr), 195.7 ($\underline{C}$OCON); IR (ATR): ν_{max} 3277, 2971, 2931, 2873, 1714, 1627, 1576, 1508, 1444, 1365, 1280, 1245, 1158, 1054, 1011,

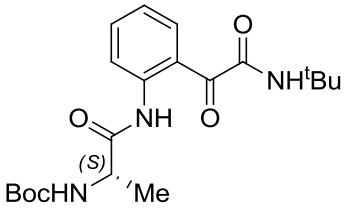
861, 805, 754, 665 cm^{-1}; HRMS (+ESI): Found *m/z* 412.1835, $[M+Na]^+$, $C_{20}H_{27}N_3O_5Na$ requires 412.1848.

(*S*)-Benzyl (3-methyl-1-oxo-1-((2-(2-oxo-2-(pyrrolidin-1-yl)acetyl)phenyl)amino)butan-2-yl)carbamate **5-13**



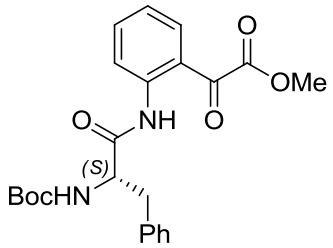
N-(Cbz)-L-Val-isatin **5-9** (0.811 g, 2.13 mmol) and pyrrolidine (0.18 mL, 2.24 mmol) was stirred at room temperature in dichloromethane for 2h. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel, eluting with 1 : 1 ethyl acetate/n-hexane to afford **5-13** as a colourless amorphous solid (0.808 g, 84 %). M.p. 50-52 °C. $[\alpha]_D$ 0 (*c* 0.025, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (d, *J* = 6.9 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.04 (d, *J* = 6.8 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.89-2.01 (m, 4H, 2 x $NCH_2C\underline{H}_2$), 2.35 (dqq, *J* = 6.9, 6.8, 4.9 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.29-3.40 (m, 2H, $NC\underline{H}_2CH_2$), 3.64-3.67 (m, 2H, $NC\underline{H}_2CH_2$), 4.31 (dd, *J* = 8.4, 4.9 Hz, 1H, COC<u>H</u>NH), 5.15 (s, 2H, $C\underline{H}_2Ph$), 5.48 (bd, *J* = 8.4 Hz, 1H, N<u>H</u>Cbz), 7.16 (ddd, *J* = 8.0, 7.4, 1.1 Hz, 1H, ArH), 7.62 (ddd , *J* = 8.8, 7.4, 1.6 Hz, 1H, ArH), 7.74 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.79 (dd, *J* = 8.8, 1.1 Hz, 1H, ArH), 11.70 (s, 1H, CON<u>H</u>Ar).^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6 ($CH(\underline{C}H_3)_2$), 19.5 ($CH(\underline{C}H_3)_2$), 24.1 ($NCH_2\underline{C}H_2$), 26.0 ($NCH_2\underline{C}H_2$), 31.2 ($\underline{C}H(CH_3)_2$), 45.4 ($N\underline{C}H_2CH_2$), 46.8 ($N\underline{C}H_2CH_2$), 61.7 (COC<u>H</u>NH), 67.3 ($C\underline{H}_2Ph$), 118.2 (ArC), 120.9 (ArCH), 123.3 (ArCH), 128.2 (ArCH), 128.3 (ArCH), 128.6 (ArCH), 133.8 (ArCH), 136.4 (ArC), 136.9 (ArCH), 142.0 (ArC), 156.6 (OCONH), 164.2 (CO<u>C</u>ON), 171.1 (<u>C</u>ONHAr), 195.8 (<u>C</u>OCON); IR (ATR): ν_{max} 3277, 2961, 2876, 1695, 1631, 1579, 1508, 1444, 1295, 1221, 1160, 1093, 1010, 923, 867, 840, 752, 696, 665 cm^{-1}; HRMS (+ESI): Found *m/z* 474.1983, $[M+Na]^+$, $C_{25}H_{29}N_3O_5$ Na requires 474.2005.

tert-Butyl (*S*)-(1-((2-(2-(tert-butylamino)-2-oxoacetyl)phenyl)amino)-1-oxopropan-2-yl)carbamate **5-14**



N-(Boc)-L-Ala-isatin **5-4** (0.899 g, 2.82 mmol) and ^{t}butylamine (0.445 mL, 4.24 mmol) was stirred at room temperature in dichloromethane for 2h. The solvent was removed *in vacuo* and the crude material was recrystallized from hot ethanol to afford **5-14** as colourless prisms (0.850 g, 77 %). M.p. 168°C; $[\alpha]_D$ -58 (*c* 0.42, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47-1.48 (m, 20H, $CO_2(C\underline{H}_3)_3$ + $NHC(C\underline{H}_3)_3$ + CH_3), 4.34-4.38 (m, 1H, CH), 5.17 (bs, 1H, NHBoc), 6.90 (bs, 1H, COCONH), 7.15 (ddd, *J* = 8.2, 7.4, 1.2 Hz, 1H, ArH), 7.60 (ddd, *J* = 8.6, 7.4, 1.6 Hz, 1H, ArH), 8.35 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 8.65 (dd, *J* = 8.6, 1.2 Hz, 1H, ArH), 11.40 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8 ($CH\underline{C}H_3$), 28.5 (2 x $C(\underline{C}H_3)_3$), 51.8 ($NH\underline{C}(CH_3)_3$), 52.2 (CH), 80.4 ($O\underline{C}(CH_3)_3$), 119.4 (ArC), 120.9 (ArCH), 122.9 (ArCH), 134.5 (ArCH), 136.3 (ArCH), 141.6 (ArC), 155.4 (OCONH), 162.2 (CO<u>C</u>ONH), 172.2 (<u>C</u>ONHAr), 192.4 (<u>C</u>OCONH); IR (ATR): ν_{max} 3345, 3298, 3243, 2980, 2928, 1687, 1640, 1580, 1507, 1445, 1365, 1210, 1153, 1043, 934, 835, 754 cm^{-1}; HRMS (+ESI): Found *m/z* 414.1991, $[M+Na]^+$, $C_{20}H_{29}N_3O_5Na$ requires 414.2005.

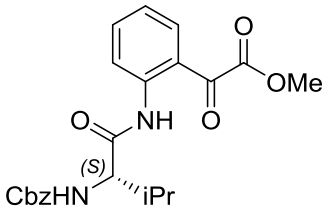
Methyl (*S*)-2-(2-(2-((*tert*-butoxycarbonyl)amino)-4-(methylthio)butanamido)phenyl)-2-oxoacetate **5-15**



N-(Boc)-L-Phe-isatin **5-6** (0.095 g, 0.241 mmol) was heated at reflux in methanol for 4 h. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel, eluting with 1 : 4 ethyl acetate/n-hexane to afford **5-15** as a pale

yellow amorphous solid (0.094 g, 92 %). M.p. 95-96 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.41 (s, 9H, $C(CH_3)_3$), 3.09-3.19 (m, 1H, $C\underline{H}_A H_B Ph$), 3.22 (dd, *J* = 14.0, 5.6 Hz, 1H, $CH_A\underline{H}_B Ph$), 3.96 (s, 3H, OCH_3), 4.49-4.61 (m, 1H, CH), 5.03-5.14 (m, 1H, N<u>H</u>Boc), 7.16 (ddd, *J* = 7.7, 7.6, 1.2 Hz, 1H, ArH), 7.18-7.31 (m, 5H, 5 x ArH), 7.59-7.72 (m, 2H, 2 x ArH), 8.81 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 11.40 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.3 ($C(\underline{C}H_3)_3$), 38.5 (CH_2), 53.0 (OCH_3), 57.3 ($\underline{C}HCH_2$), 80.6 ($\underline{C}(CH_3)_3$), 117.7 (ArC), 120.9 (ArCH), 123.1 (ArCH), 127.2 (ArCH), 128.9 (ArCH), 129.3 (ArCH), 133.6 (ArCH), 136.2 (ArC), 137.1 (ArCH), 141.9 (ArC), 155.4 (O<u>C</u>ONH), 163.9 ($\underline{C}O_2CH_3$), 171.4 (<u>C</u>ONHAr), 189.7 (<u>C</u>OCONH); IR (ATR): ν_{max} 3342, 3269, 2980, 1737, 1650, 1645, 1580, 1447, 1390, 1329, 1297, 1241, 1198, 1158, 1050, 995, 861, 800, 751, 697, 661 cm^{-1}; HRMS (+ESI): Found *m/z* 449.1682, $[M+Na]^+$, $C_{23}H_{26}N_2O_6Na$ requires 449.1689.

Methyl (*S*)-2-(2-(2-(((benzyloxy)carbonyl)amino)-3-methylbutanamido)phenyl)-2-oxoacetate **5-16**



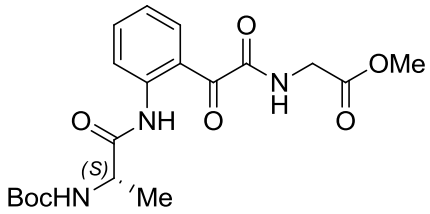
N-(Cbz)-L-Val-isatin **5-9** (0.700 g, 1.84 mmol) was heated at reflux in methanol for 4h. The solvent was removed *in vacuo* and the crude material was crystallised from a mixture of ethyl acetate and n-hexane to afford **5-16** as colourless needles (0.631 g, 83 %). M.p. 101°C; $[\alpha]_D$ 0 (*c* 0.033, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (d, *J* = 6.9 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.05 (d, *J* = 6.8 Hz, 3H, $CH(C\underline{H}_3)_2$), 2.36 (dqq, *J* = 6.9, 6.8, 4.7, 1H, $C\underline{H}(CH_3)_2$), 4.00 (s, 3H, OCH_3), 4.31 (dd, *J* = 8.2, 4.7 Hz, 1H, COC<u>H</u>NH), 5.16 (s, 2H, $C\underline{H}_2Ph$), 5.41 (bd, *J* = 8.2 Hz, 1H, N<u>H</u>Cbz), 7.18 (ddd, *J* = 8.0, 7.4, 1.1 Hz, 1H, ArH), 7.31-7.41 (m, 5H, 5 x ArH), 7.66 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H, ArH), 7.71 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.81 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 11.50 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.5 ($CH(C\underline{H}_3)_2$), 19.5 ($CH(C\underline{H}_3)_2$), 31.1 ($C\underline{H}(CH_3)_2$), 53.1 ($CO_2\underline{C}H_3$), 61.8 (COC<u>H</u>NH), 67.5 ($C\underline{H}_2Ph$), 117.6 (ArC), 120.9 (ArCH), 123.2 (ArCH), 128.3 (ArCH), 128.7 (ArCH), 133.8 (ArCH), 136.3 (ArC), 137.3 (ArCH), 142.1 (ArC), 156.6 (O<u>C</u>ONH), 163.9 ($\underline{C}O_2CH_3$), 171.2

(CONHAr), 190.3 (COCONH). One ArCH missing due to overlap; IR (ATR): ν_{max} 3278, 3055, 2952, 1740, 1661, 1577, 1522, 1446, 1349, 1285, 1243, 1196, 1122, 1039, 1003, 931, 872, 846, 747, 697, 674 cm^{-1}; HRMS (+ESI): Found *m/z* 435.1517, $[M+Na]^+$, $C_{22}H_{24}N_2O_6Na$ requires 435.1532.

General procedure for the synthesis of *compounds* **5-17-5-29**.

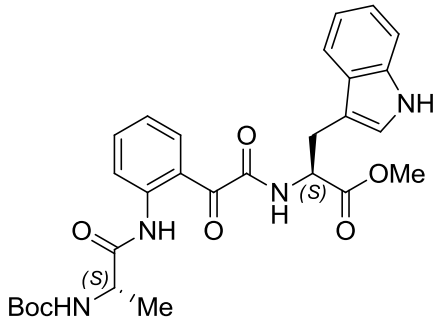
N-Protected amino acid isatin (1 equivalent) was added to a solution of the amino acid ester (1.2 equivalent) and *N,N*-diisopropylethylamine (1.2 equivalent) in dichloromethane. The reaction was stirred at room temperature for 3h and the crude reaction mixture was subjected to flash chromatography on silica gel, eluting with 2:3 ethyl acetate/n-hexane.

(*S*)-Methyl 2-(2-(2-(2-((*tert*-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetamido)acetate **5-17**



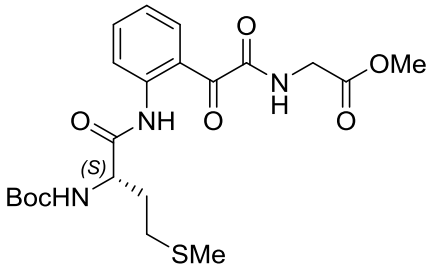
The title compound **5-17** was prepared from *N*-(Boc)-L-Ala-isatin **5-4** (1.50 g, 4.71 mmol) and glycine methyl ester hydrochloride (0.830 g, 6.60 mmol) according to the general procedure outlined above. The product **5-17** was obtained from flash chromatography as a pale yellow amorphous solid (1.89 g, 98%). M.p. 116-118°C; $[\alpha]_D$ -62 (*c* 0.24, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H, C($C\underline{H}_3)_3$), 1.49 (d, *J* = , 3H, CH_3), 4.19 (d, *J* = 5.5 Hz, 2H CH_2), 4.33-4.38 (m, 1H, $C\underline{H}CH_3$), 5.13-5.15 (m, 1H, N<u>H</u>Boc), 7.15 (ddd, *J* = 8.2, 7.4, 1.1 Hz, 1H, ArH), 7.39 (bt, *J* = 5.5 Hz, 1H, $N\underline{H}CH_2$), 7.62 (ddd, *J* = 8.6, 7.4, 1.5 Hz, 1H, ArH), 8.38 (dd, *J* = 8.2, 1.5 Hz, 1H, ArH), 8.67 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 11.36 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.7 ($CH\underline{C}H_3$), 28.4 ($C(\underline{C}H_3)_3$), 41.3 ($NH\underline{C}H_2CO$), 51.8 (OCH_3), 52.8 (CH), 80.5 ($\underline{C}(CH_3)_3$), 119.0 (ArC), 120.8 (ArCH), 123.1 (ArCH), 134.5 (ArCH), 136.8 (ArCH), 141.7 (ArC), 155.5 (OCONH), 163.1 (CO<u>C</u>ONH), 169.5 (CO_2CH_3), 172.5 (<u>C</u>ONHAr), 191.0 (<u>C</u>OCONH); IR (ATR): ν_{max} 3332, 2976, 2929, 1762, 1684, 1643, 1585, 1511, 1449, 1403, 1370, 1300, 1249, 1201, 1163, 1110, 1046, 992, 945, 858, 758, 704 cm^{-1}; HRMS (+ESI): Found *m/z* 430.1576, $[M+Na]^+$, $C_{19}H_{25}N_3O_7Na$ requires 430.1590.

(*S*)-Methyl 2-(2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetamido)-3-(1H-indol-3-yl)propanoate **5-18**



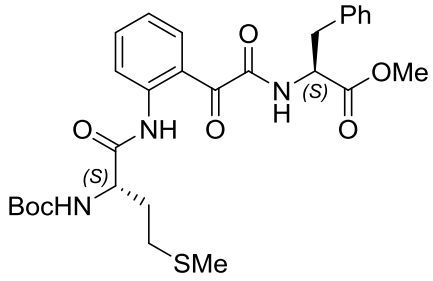
The title compound **5-18** was prepared from *N*-(Boc)-L-Ala-isatin **5-4** (0.200 g, 0.628 mmol) and L- tryptophan methyl ester hydrochloride (0.192 g, 0.754 mmol) according to the general procedure outlined above. The product **5-18** was obtained from flash chromatography as a bright yellow amorphous solid (0.273 g, 81%). M.p. 80-84°C; $[\alpha]_D$ 54 (*c* 0.055, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, C($C\underline{H}_3$)$_3$), 1.45 (d, *J* = 7.3 Hz, 3H, CH$C\underline{H}_3$), 3.40 (dd, *J* = 5.8, 0.8 Hz, 1H, CH$C\underline{H}_A H_B$), 3.41 (dd, *J* = 5.5, 0.9 Hz, 1H, CH$CH_A\underline{H}_B$), 3.73 (s, 3H, OCH_3), 4.33 (dq, *J* = 7.3, 6.9 Hz, 1H, $CHCH_3$), 5.01 (ddd, *J* = 11.4, 5.8, 5.5 Hz, 1H, C$\underline{H}CH_AH_B$), 5.18 (bd, *J* = 6.9 Hz, 1H, N$\underline{H}$Cbz), 7.05-7.10 (m, 2H, ArH + COCON$\underline{H}$), 7.09 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H, ArH), 7.17 (ddd, *J* = 8.4, 7.1, 1.3 Hz, 1H, ArH), 7.32-7.36 (m, 2H, 2 x ArH), 7.53-7.59 (m, 2H, 2 x ArH), 8.16 (dd, *J* = 8.0, 1.3 Hz, 1H, ArH), 8.36 (s, 1H, NH_{indole}), 8.62 (dd, *J* = 8.4, 1.0 Hz, 1H, ArH), 11.27 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 18.9 (CH$\underline{C}H_3$), 27.8 (CH_2), 28.4 (C($\underline{C}H_3$)$_3$), 51.8 ($\underline{C}HCH_3$), 52.8 (OCH_3), 64.6 ($\underline{C}HCH_2$), 80.4 ($\underline{C}(CH_3)_3$), 109.5 (ArC), 111.5 (ArCH), 118.6 (ArCH), 119.1 (ArC), 119.9 (ArCH), 120.9 (ArCH), 122.5 (ArCH), 123.0 (ArCH), 123.2 (ArCH), 127.4 (ArC), 134.4 (ArCH), 136.3 (ArC), 136.6 (ArCH), 141.7 (ArC), 155.4 (OCONH), 162.5 (CO$\underline{C}$ONH), 171.5 ($\underline{C}O_2CH_3$), 172.3 ($\underline{C}$ONHAr), 191.2 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3293, 2974, 1646, 1579, 1509, 1446, 1365, 1293, 1245, 1207, 1160, 1100, 1065, 1011, 911, 856, 741, 676 cm^{-1}; HRMS (+ESI): Found *m/z* 559.2148, $[M+Na]^+$, $C_{28}H_{32}N_4O_7Na$ requires 559.2169.

(*S*)-Methyl 2-(2-(2-(2-((*tert*-butoxycarbonyl)amino)-4-(methylthio)butanamido)phenyl)-2-oxoacetamido)acetate **5-19**



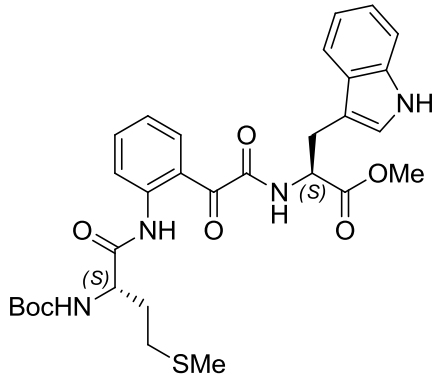
The title compound **5-19** was prepared from *N*-(Boc)-L-Met-isatin **5-5** (0.610 g, 1.61 mmol) and glycine methyl ester hydrochloride (0.202 g, 1.61 mmol) according to the general procedure outlined above. The pale yellow oil obtained from flash chromatography was crystallised from hot ethanol to afford **5-19** as a pale yellow crystalline solid (0.446 g, 59%). M.p. 104°C; $[\alpha]_D$ -49 (*c* 0.082, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, C(CH_3)$_3$), 1.97-2.05 (m, 1H, $CH_ACH_BCH_2S$), 2.11 (s, 3H, SCH_3), 2.21-2.30 (m, 1H, $CH_ACH_BCH_2S$), 2.60 (dd, *J* = 7.4, 7.4 Hz, 2H, SCH_2), 3.80 (s, 3H, OCH_3), 4.19 (d, *J* = 5.3 Hz, 2H, $COCH_2NH$), 4.44-4.45 (m, 1H, COCHNH), 4.42 (bd, *J* = 7.1 Hz, 1H, NHBoc), 7.15 (ddd, *J* = 8.2, 7.4, 1.2 Hz, 1H, ArH), 7.45 (bt, *J* = 5.3 Hz, 1H, $COCH_2NH$), 7.60 (ddd, *J* = 8.8, 7.4, 1.7 Hz, 1H, ArH), 8.34 (dd, *J* = 8.2, 1.7 Hz, 1H, ArH), 8.62 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 11.36 (s, 1H, CONHAr); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.6 (SCH_3), 28.4 (C(CH_3)$_3$), 30.4 (SCH_2), 32.0 (SCH_2CH_2), 41.3 ($NHCH_2$), 52.8 (OCH_3), 55.4 (CH), 80.6 (C(CH_3)$_3$), 119.3 (ArC), 121.0 (ArCH), 123.2 (ArCH), 134.5 (ArCH), 136.7 (ArCH), 141.5 (ArC), 155.6 (OCONH), 162.9 (COCONH), 169.5 (CO_2CH_3), 171.3 (CONHAr), 190.8 (COCONH); IR (ATR): ν_{max} 3322, 2976, 2912, 1751, 1680, 1638, 1584, 1511, 1447, 1362, 1334, 1292, 1251, 1202, 1152, 1056, 969, 861, 821, 763 cm^{-1}; HRMS (+ESI): Found *m/z* 490.1612, $[M+Na]^+$, $C_{21}H_{29}N_3O_7S$ Na requires 490.1624.

(*S*)-Methyl 2-(2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-4-(methylthio)butanamido)phenyl)-2-oxoacetamido)-3-phenylpropanoate **5-20**



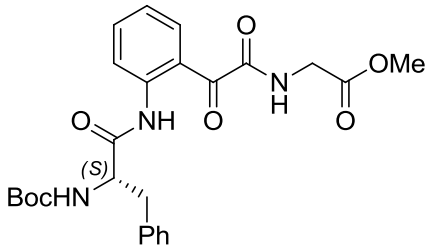
The title compound **5-20** was prepared from *N*-(Boc)Met-isatin **5-5** (0.300 g, 0.793 mmol) and phenylalanine methyl ester hydrochloride (0.171 g, 0.793 mmol) according to the general procedure outlined above. The product **5-20** was obtained from flash chromatography as a pale orange solid (0.247 g, 56%). M.p. 130°C; $[\alpha]_D$ -9.4 (*c* 0.11, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 9H, C($C\underline{H}_3)_3$), 1.94-2.06 (m, 1H, CHC$\underline{H}_A$CH_BS), 2.11 (s, 3H, SCH_3), 2.20-2.34 (m, 1H, CHCH_AC$\underline{H}_B$S), 3.15 (dd, *J* = 14.0, 6.9 Hz, 1H, C$\underline{H}_A$CH_BPh), 3.17 (dd, *J* = 14.0, 6.5 Hz, 1H, CH_AC$\underline{H}_B$Ph), 3.78 (s, 3H, OCH_3), 4.45-4.47 (m, 1H, C$\underline{H}(CH_2)_2$S), 4.99 (ddd, *J* = 8.1,6.9, 6.5, Hz, 1H, C$\underline{H}CH_2$Ph), 5.33 (bd, *J* = 5.2 Hz, 1H, N$\underline{H}$Boc), 7.12 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H, ArH), 7.16-7.35 (m, 6H, ArH + C$\underline{H}CH_2$Ph), 7.59 (ddd, *J* = 8.7, 7.4 1.5 Hz, 1H, ArH), 8.13 (dd, *J* = 8.1, 1.4 Hz, 1H, ArH), 8.64 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 11.39 (s, 1H, ArN$\underline{H}$CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.6 (SCH_3), 28.4 (C($\underline{C}H_3)_3$), 30.4 (SCH_2), 32.1 ($SCH_2\underline{C}H_2$), 38.1 ($\underline{C}H_2$Ph), 52.8 (OCH_3), 53.4 ($\underline{C}H(CH_2)_2$S), 55.4 ($\underline{C}HCH_2$Ph), 80.6 ($\underline{C}(CH_3)_3$), 119.1 (ArC), 120.9 (ArCH), 123.1 (ArCH), 127.5 (ArCH), 128.9 (ArCH), 129.4 (ArCH), 134.5 (ArCH), 135.5 (ArC), 136.7 (ArCH), 141.6 (ArC), 155.6 (OCONH), 162.3 (CO$\underline{C}$ONH), 171.1 ($\underline{C}O_2CH_3$), 171.3 ($\underline{C}$ONHAr), 191.0 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3320, 3018, 2910, 1745, 1680, 1639, 1509, 1448, 1278, 1230, 1167, 1058, 960, 929, 857, 750, 696 cm^{-1}; HRMS (+ESI): Found *m/z* 580.2083, $[M+Na]^+$, $C_{28}H_{35}N_3NaO_7S$ Na requires 580.2093.

(*S*)-Methyl 2-(2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-4-(methylthio)butanamido)phenyl)-2-oxoacetamido)-3-(1H-indol-3-yl)propanoate **5-21**



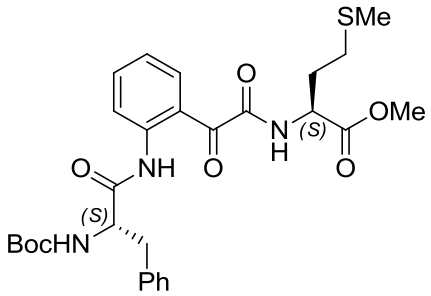
The title compound **5-21** was prepared from *N*-(Boc)Met-isatin **5-5** (0.300 g, 0.793 mmol) and L- tryptophan methyl ester hydrochloride (0.202 g, 0.793 mmol) according to the general procedure outlined above. The product **5-21** was obtained from flash chromatography as a bright yellow amorphous solid (0.385 g, 79%). M.p. 82-84°C; $[\alpha]_D$ 33 (*c* 0.09, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(CH_3)$_3$), 1.92-2.17 (m, 1H, CH_AC$H_B$$CH_2$S), 2.09 (s, 3H, SCH_3), 2.17-2.32 (m, 1H, CH_AC$H_B$$CH_2$S), 2.59 (dd, *J* = 7.4, 7.4 Hz, 2H, SCH_2), 3.40-3.42 (m, 2H, CHC$H_A$$H_B$Ar), 3.73 (s, 3H, OCH_3), 4.41-4.48 (m, 1H, CH(CH_2)$_2$S), 5.00 (ddd, *J* = 5.8, 5.8, 8.0 Hz, 1H, CHCH_2Ar), 5.34 (bd, *J* = 6.8 Hz, 1H, NHBoc), 7.07-7.12 (m, 3H, 2 x ArH + COCONH), 7.18 (ddd, *J* = 8.3, 7.1, 1.4 Hz, 1H, ArH), 7.33-7.37 (m, 2H, ArH), 7.53-7.60 (m, 2H, ArH), 7.18 (dd, *J* = 8.0, 1.4 Hz, 1H, ArH), 8.32 (bs, 1H, $NH_{(indole)}$), 8.60 (bd, *J* = 8.0 Hz, 1H, CONHCH), 11.28 (s, 1H, CONHAr); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (SCH_3), 27.7 (SCH_2), 28.4 (C(CH_3)$_3$), 30.4 (SCH_2CH_2), 32.2 (CHCH_2Ar), 52.8 (OCH_3), 53.0 (CH(CH_2)$_2$S), 55.3 (CHCH_2Ar), 80.5 (C(CH_3)$_3$), 109.5 (ArCH), 111.5 (ArCH), 118.6 (ArCH), 119.4 (ArC), 119.9 (ArCH), 121.0 (ArCH), 122.5 (ArCH), 123.2 (ArCH), 123.2 (ArC), 127.4 (ArC), 134.4 (ArCH), 136.3 (ArC), 136.5 (ArCH), 141.4 (ArC), 155.6 (OCONH), 162.4 (COCONH), 171.5 (CONHAr), 191.1 (COCONH); IR (ATR): ν_{max} 3307, 2974, 2917, 1647, 1579, 1509, 1445, 1364, 1245, 1206, 1160, 1047, 1024, 860, 741, 673 cm^{-1}; HRMS (+ESI): Found *m/z* 619.2191, $[M+Na]^+$, $C_{30}H_{36}N_4O_7S$ Na requires 619.2202.

(*S*)-Methyl 2-(2-(2-(2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)phenyl)-2-oxoacetamido)acetate **5-22**



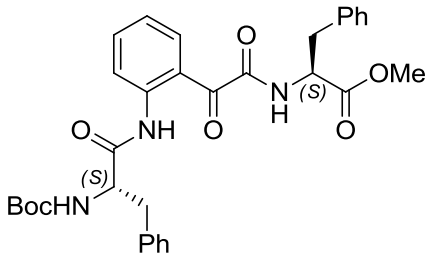
The title compound **5-22** was prepared from *N*-(Boc)Phe-isatin **5-6** (0.300 g, 0.793 mmol) and L- tryptophan methyl ester hydrochloride (0.202 g, 0.793 mmol) according to the general procedure outlined above. The product **5-22** was obtained from flash chromatography as a colourless amorphous powder (0.309 g, 84 %). M.p. 128-129°C; $[\alpha]_D$ –32 (*c* 0.062, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.44 (s, 9H, $C(C\underline{H}_3)_3$), 3.10-3.27 (m, 2H, $CHC\underline{H}_A\underline{H}_B$), 3.82 (s, 3H, OCH_3), 4.18 (d, *J* = 5.5 Hz, 2H, $COCH_2$), 4.47-4.62 (m, 1H, COCH), 5.00-5.19 (m, 1H, N<u>H</u>Boc), 7.16 (t, *J* = 7.7 Hz, 1H, ArH), 7.18-7.34 (m, 6H, 5 x ArH + $N\underline{H}CH_2$), 7.75 (t, *J* = 7.7 Hz, 1H, ArH), 8.38 (d, *J* = 7.6 Hz, 1H, ArH), 8.68 (d, *J* = 8.4 Hz, 1H, ArH), 11.11 (s, 1H, CON<u>H</u>Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.4 ($C(\underline{C}H_3)_3$), 38.8 ($\underline{C}H_2Ph$), 41.3 ($NHCH_2$), 52.8 (OCH_3), 57.3 (CO<u>C</u>H), 80.5 ($\underline{C}(CH_3)_3$), 119.2 (ArC), 121.1 (ArCH), 123.2 (ArCH), 127.2 (ArCH), 128.9 (ArCH), 129.4 (ArCH), 134.5 (ArCH), 136.3 (ArC), 136.7 (ArCH), 141.4 (ArC), 155.4 (OCONH), 162.5 (CO<u>C</u>ONH), 169.4 (<u>C</u>ONHAr), 171.0 ($\underline{C}O_2CH_3$), 190.1 (<u>C</u>OCONH). HRMS (+ESI): Found *m/z* 506.1889, $[M+Na]^+$, $C_{25}H_{28}N_2O_7Na$ requires 506.1898.

(*S*)-Methyl 2-(2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)phenyl)-2-oxoacetamido)-4-(methylthio)butanoate **5-23**



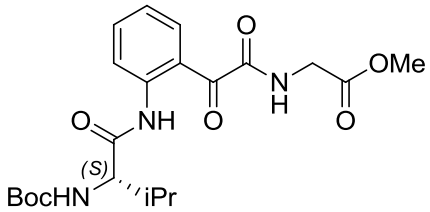
The title compound **5-23** was prepared from *N*-(Boc)-L-Phe-isatin **5-6** (0.400 g, 1.01 mmol) and L-methionine methyl ester hydrochloride (0.243 g, 1.22 mmol) according to the general procedure outlined above. The product **5-23** was precipitated as a pale yellow solid from a solution of diethyl ether and n-hexane (0.400 g, 71 %). M.p. 124-125°C; $[\alpha]_D$ –46 (*c* 0.13, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.44 (s, 9H, C(CH_3)$_3$), 2.07-2.17 (m, 4H, SCH_3, $SCH_2CH_AH_B$), 2.23-2.31 (m, 1H, $SCH_2CH_AH_B$), 2.57 (dd, *J* = 7.2, 7.1 Hz, 2H, SCH_2), 3.11-3.25 (m, 2H, CH_2Ph), 3.82 (s, 3H, OCH_3), 4.48-4.62 (m, 1H, CH$(CH_2)_2SCH_3$), 4.81 (ddd, *J* = 12.5, 7.3, 5.1 Hz, 1H, CHCH_2Ph), 5.00-5.22 (m, 1H, NHBoc), 7.16 (ddd, *J* = 8.2, 7.5, 1.3 Hz, 1H, ArH), 7.18-7.31 (m, 5H, 5 x ArH), 7.35-7.49 (m, 1H, CONHCH), 7.63 (ddd, *J* = 8.7, 7.5, 1.6 Hz, 1H, ArH), 8.37 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 8.69 (dd, *J* = 8.7, 1.3 Hz, 1H, ArH), 11.15 (s, 1H, CONHAr); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (SCH_3), 28.3 (C(CH_3)$_3$), 29.9 (CH_2S), 31.3 (CH_2CH_2S), 38.5 ($CHCH_2Ph$), 51.6 (OCH_3), 52.8 ($CHCH_2CH_2S$), 57.2 ($CHCH_2Ph$), 80.4 (C$(CH_3)_3$), 119.1 (ArC), 120.8 (ArCH), 123.0 (ArCH), 127.0 (ArCH), 128.8 (ArCH), 129.3 (ArCH), 134.3 (ArCH), 136.3 (ArC), 136.5 (ArCH), 141.3 (ArC), 155.3 (OCONH), 162.4 (COCONH), 171.0 (CONHAr), 171.4 (CO_2CH_3), 190.4 (COCONH); IR (ATR): ν_{max} 3261, 2975, 2919, 1745, 1692, 1644, 1582, 1505, 1448, 1366, 1297, 1245, 1206, 1161, 1053, 988, 851, 752, 699 cm^{-1}; HRMS (+ESI): Found *m/z* 580.2073, $[M+Na]^+$, $C_{28}H_{35}N_3O_7SNa$ requires 580.2093.

(*S*)-Methyl 2-(2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)phenyl)-2-oxoacetamido)-3-phenylpropanoate **5-24**



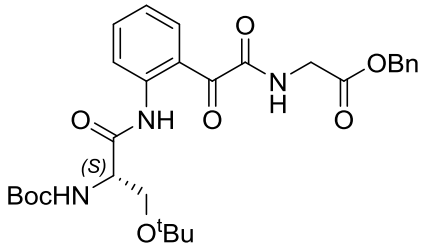
The title compound **5-24** was prepared from *N*-(Boc)Phe-isatin **5-6** (0.430 g, 1.09 mmol) and L-phenylalanine methyl ester hydrochloride (0.330 g, 1.53 mmol) according to the general procedure outlined above. The pale yellow oil obtained from flash chromatography was crystallised from hot ethanol to yield **5-24** as thin colourless needles (0.454 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, C($C\underline{H}_3$)$_3$), 3.11-3.18 (m, 3H, $C\underline{H}_2$Ph + $C\underline{H}_A\underline{H}_B$Ph), 3.27 (dd, *J* = 14.0, 5.8 Hz, 1H, $CH_A\underline{H}_B$Ph), 3.79 (s, 3H, OCH_3), 4.53-4.55 (m, 1H, CH), 4.78 (ddd, *J* = 12.2, 6.7, 5.5 Hz, 1H, CH), 5.14 (bd, *J* = 5.8 Hz, 1H, N<u>H</u>Boc), 7.09 (ddd, *J* = 8.2, 7.5, 1.2 Hz, 1H, ArH), 7.13-7.35 (m, 11H, 10 x ArH + N<u>H</u>CH), 7.59 (ddd, *J* = 8.7, 7.5, 1.5 Hz, 1H, ArH), 8.14 (dd, *J* = 8.2, 1.5 Hz, 1H, ArH), 8.66 (dd, *J* = 8.7, 1.2 Hz, 1H, ArH), 11.12 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4 (C(<u>C</u>H_3)$_3$), 38.1 (<u>C</u>H_2CHCON), 38.8 (<u>C</u>H_2CHCO$_2$), 52.8(<u>C</u>HCON), 53.4(<u>C</u>HCO$_2$), 80.5 (<u>C</u>(CH$_3$)$_3$), 119.0 (ArC), 120.9 (ArCH), 123.1 (ArCH), 127.2 (ArCH), 127.5 (ArCH), 128.9 (ArCH), 128.9 (ArCH), 129.4 (2 x ArCH), 134.4 (ArCH), 135.5 (ArC), 136.3 (ArC), 136.6 (ArCH), 141.4 (ArC), 155.4 (OCONH), 162.2 (CONHAr), 171.1 (<u>C</u>O$_2$CH$_3$), 171.1 (CO<u>C</u>ONH), 190.57 (<u>C</u>OCONH); IR (ATR): ν_{max} 3333, 2980, 1755, 1693, 1642, 1580, 1510, 1446, 1368, 1329, 1271, 1203, 1160, 1050, 988, 937, 906, 863, 806, 758, 700, 661 cm^{-1}; HRMS (+ESI): Found *m/z* 596.2359, $[M+Na]^+$, $C_{32}H_{33}N_3O_7Na$ requires 596.2373.

(*S*)-Methyl 2-(2-(2-(2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)phenyl)-2-oxoacetamido)acetate **5-25**



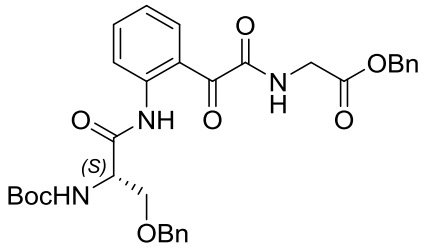
The title compound **5-25** was prepared from *N*-(Boc)-L-Val-isatin **5-7** (0.300 g, 0.866 mmol) and glycine methyl ester (0.130 g, 1.04 mmol) according to the general procedure outlined above. The product **5-25** was obtained from flash chromatography as a white amorphous solid (0.351 g, 93%). M.p. 111-112°C; $[\alpha]_D$ -46 (*c* 0.065, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (d, *J* = 6.9 Hz, 1H, CH(C$\underline{H}_3$)$_2$), 1.03 (d, *J* = 6.8 Hz, 1H, CH(C$\underline{H}_3$)$_2$), 1.46 (s, 9H, C(CH_3)$_3$), 2.25-2.36 (m, 1H, C$\underline{H}$(CH_3)$_2$), 3.81 (s, 3H, OCH_3), 4.19 (d, *J* = 5.5 Hz, 2H, CH_2), 5.17 (bd, *J* = 7.76, 1H, NHBoc), 7.15 (ddd, *J* = 8.2, 7.4, 1.1 Hz, 1H, ArH), 7.42 (bt, *J* = 5.5 Hz, 1H, N$\underline{H}CH_2$), 7.61 (ddd, *J* = 8.7, 7.4, 1.6 Hz, 1H, ArH), 8.39 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 8.70 (dd, *J* = 8.7, 1.1 Hz, 1H, ArH), 11.32 (s, 1H, CON$\underline{H}$Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.6 (CH(C$\underline{H}_3$)$_2$), 19.5 (CH(C$\underline{H}_3$)$_2$), 28.5 (C(C$\underline{H}_3$)$_3$), 31.1 (C$\underline{H}$(CH_3)$_2$), 41.3 (CO$\underline{C}H_2$NH), 52.8 (OCH_3), 61.3 (CO$\underline{C}$HNH), 80.3 (C(CH_3)$_3$), 119.0 (ArC), 120.9 (ArCH), 123.1 (ArCH), 134.6 (ArCH), 136.8 (ArCH), 141.7 (ArC), 156.0 (OCONH), 162.9 (CO$\underline{C}$ONH), 169.4 ($\underline{C}O_2$Me), 171.4 ($\underline{C}$ONHAr), 190.8 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3322, 2960, 1736, 1682, 1642, 1582, 1517, 1445, 1295, 1240, 1211, 1158, 1041, 1006, 941, 852, 833, 763 cm^{-1}; HRMS (+ESI): Found *m/z* 458.1916, $[M+Na]^+$, $C_{21}H_{29}N_3O_7Na$ requires 458.1903.

Benzyl (*S*)-(2-(2-(3-(tert-butoxy)-2-((*tert*-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetyl)glycinate **5-26**



The title compound **5-26** was prepared from *N*-(Boc)-L-(O^{t}Bu)Ser-isatin **5-8** (0.350 g, 0.896 mmol) and glycine benzyl ester *p*-toluenesulfonate (0.343 g, 1.08 mmol) according to the general procedure outlined above. The product **5-26** was obtained from flash chromatography as a pale yellow amorphous solid (0.408 g, 82%). M.p. 99-100 °C; $[\alpha]_D$ -91 (*c* 0.14, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 9H, $CO_2C(C\underline{H}_3)_3$), 1.50 (s, 9H, $CH_2OC(C\underline{H}_3)_3$), 3.55 (dd, *J* = 9.0, 4.8 Hz, 1H, $CHC\underline{H}_AH_BO$), 3.95 (dd, *J* = 9.0, 3.2 Hz, 1H, $CHCH_A\underline{H}_BO$), 4.22 (d, *J* = 5.4 Hz, 2H, $COC\underline{H}_2NH$), 4.37 (ddd, *J* = 7.6, 4.8, 3.2 Hz, 1H, $COC\underline{H}NH$), 5.24 (s, 2H, $C\underline{H}_2Ph$), 5.57 (bd, *J* = 7.6 Hz, 1H, $N\underline{H}Boc$), 7.13 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H, ArH), 7.32 (bt, *J* = 5.4 Hz, 1H, $COCON\underline{H}$), 7.33-7.41 (m, 5H, 5 x ArH), 7.61 (ddd, *J* = 8.7, 7.3, 1.6 Hz, 1H, ArH), 8.37 (dd, *J* = 8.3, 1.6 Hz, 1H, ArH), 8.72 (dd, *J* = 8.7, 1.2 Hz, 1H, ArH), 11.47 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.5 ($C(\underline{C}H_3)_3$), 28.5 ($C(\underline{C}H_3)_3$), 41.5 ($CO\underline{C}H_2NH$), 56.4 ($\underline{C}HCH_2O^tBu$), 61.8 ($CH\underline{C}H_2O^tBu$), 67.7 (CH_2Ph), 73.9 ($\underline{C}(CH_3)_3$), 80.5 ($\underline{C}(CH_3)_3$), 119.3 (ArC), 121.0 (ArCH), 122.9 (ArCH), 128.7 (ArCH), 128.8 (ArCH), 134.4 (ArCH), 135.1 (ArC), 135.8 (ArCH), 136.6 (ArCH), 141.7 (ArC), 155.7 (OCONH), 162.8 ($CO\underline{C}ONH$), 168.9 ($\underline{C}O_2Bn$), 170.7 ($\underline{C}ONHAr$), 190.3 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3397, 3349, 3250, 2975, 2931, 1755, 1688, 1645, 1575, 1498, 1448, 1363, 1285, 1248, 1192, 1084, 1029, 942, 851, 763, 696 cm^{-1}; HRMS (+ESI): Found *m/z* 578.2452, $[M+Na]^+$, $C_{29}H_{37}N_3O_8Na$ requires 578.2478.

Benzyl (*S*)-(2-(2-(3-(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetyl)glycinate **5-27**

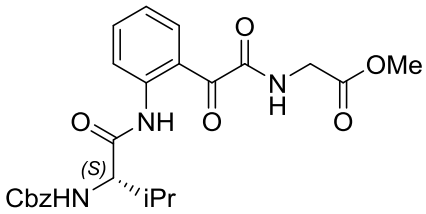


A mixture of isatin **1-1** (5.50 g, 37.4 mmol), *N-(tert*-butoxycarbonyl)-O-benzyl-L-serine (13.2 g, 44.9 mmol), DCC (9.26 g, 44.9 mmol) and DMAP (0.913 g, 7.48 mmol) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, *N*,*N*-dicyclohexylurea was removed by filtration and the filtrate was subjected to flash chromatography on silica gel (dichloromethane). The crude product was precipitated from a mixture of ethyl acetate and n-hexane as a bright yellow powder. This was used directly in the subsequent step without further purification.

The crude *N*-(Boc)-L-(OBn)Ser-isatin was added to a solution of glycine benzyl ester *p*-toluenesulfonate (15.1 g, 44.9 mmol) and *N,N*-diisopropylethylamine (7.71 mL, 44.9 mmol) in dichloromethane. The reaction was stirred at room temperature for 3h and the crude reaction mixture was subjected to flash chromatography on silica gel, eluting with 2:3 ethyl acetate/n-hexane. The product **5-27** was obtained from flash chromatography as a colourless solid (11.7 g, 53%). M.p. 70-72°C; $[\alpha]_D$ -28 (*c* 0.39, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (s, 9H, $CH(C\underline{H}_3)_2$), 3.71 (dd, *J* = 9.5, 4.5 Hz, 1H, $CHC\underline{H}_AH_BOBn$), 4.06 (dd, *J* = 9.5, 2.9 Hz, 1H, $CHCH_A\underline{H}_BOBn$), 4.20 (d, *J* = 5.5 Hz, 2H, $COC\underline{H}_2NH$), 4.47 (ddd, *J* = 7.1, 4.5, 2.9 Hz, COC<u>H</u>NH), 4.54 (m, 2H, $CH_2OC\underline{H}_2Ph$), 5.24 (s, 2H, $CO_2C\underline{H}_2Ph$), 5.59 (bd, *J* = 7.1 Hz, 1H, N<u>H</u>Boc), 7.14 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H, ArH), 7.22-7.31 (m, 6H, 6 x ArH), 7.33-7.42 (m, 5H, 4 x ArH + COCONH), 7.62 (ddd, *J* = 8.7,7.6, 1.7 Hz, 1H, ArH), 8.38 (dd, *J* = 8.2, 1.7 Hz, 1H, ArH), 8.73 (dd, *J* = 8.7, 1.2 Hz, 1H, ArH), 11.56 (s, 1H, COCONH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, ($C(\underline{C}H_3)_3$), 41.5 ($\underline{C}H_2CO_2Bn$), 56.0 (CH), 67.7 ($CO_2\underline{C}H_2Ph$), 69.8 ($CHCH_2$), 73.5 ($CH_2O\underline{C}H_2Ph$), 80.7 ($\underline{C}(CH_3)_3$), 119.2 (ArCH), 120.9 (ArC), 123.1 (ArCH), 127.9 (ArCH), 127.9 (ArCH), 128.6 (ArCH), 128.7 (ArCH), 128.9 (ArCH), 134.5 (ArCH),

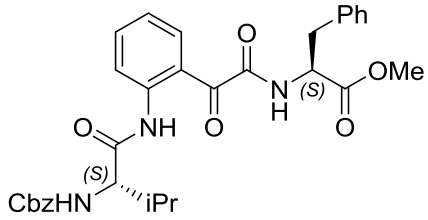
135.1 (ArC), 135.6 (ArCH) 136.7 (ArCH), 137.6 (ArC), 141.6 (ArC), 155.7 (OCONH), 162.7 (COCONH), 168.8 ($\underline{C}O_2Bn$), 170.2 ($\underline{C}ONHAr$), 190.4 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3331, 2980, 2934, 2872, 1753, 1695, 1640, 1582, 1511, 1448, 1364, 1328, 1299, 1163, 1069, 1029, 934, 863, 812, 756, 697, 663 cm^{-1}; HRMS (+ESI): Found *m/z* 612.2304, $[M+Na]^+$, $C_{32}H_{35}N_3O_8Na$ requires 612.2316.

(*S*)-Methyl 2-(2-(2-(2-(((benzyloxy)carbonyl)amino)-3-methylbutanamido)phenyl)-2-oxoacetamido)acetate **5-28**



The title compound **5-28** was prepared from *N*-(Cbz)-L-val-isatin **5-9** (0.700 g, 1.84 mmol) and glycine methyl ester hydrochloride (0.243 g, 1.93 mmol) according to the general procedure outlined above. The crude product was recrystallized from a mixture of dichloromethane and n-hexane to afford **5-28** as small colourless needles (0.855 g, 99%). M.p. 98°C; $[\alpha]_D$ -18 (*c* 0.055, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): 0.96 (d, *J* = 6.9 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.04 (d, *J* = 6.8 Hz, 3H, $CH(C\underline{H}_3)_2$), 2.34 (dqq, *J* = 6.9, 6.8, 5.0 Hz, 1H, $CH(C\underline{H}_3)_2$), 3.81 (s, 3H, OCH_3), 4.17 (d, *J* = 5.5 Hz, 2H, $COC\underline{H}_2NH$), 4.28 (dd, *J* = 8.4, 5.0 Hz, 1H, $COC\underline{H}NH$), 5.16 (rotomer, 2H, $C\underline{H}_2Ph$), 5.45 (bd, *J* = 8.4, 1H, $N\underline{H}Cbz$), 7.16 (ddd, *J* = 8.2, 7.4, 1.2 Hz, 1H, 7.30-7.38 (m, 6H, 5 x ArH + $N\underline{H}CH_2CO$), 7.62 (ddd, *J* = 8.8, 7.4, 1.7 Hz, 1H, ArH), 8.41 (dd, *J* = 8.2, 1.7 Hz, 1H, ArH), 8.69 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 11.35 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.6 ($CH(\underline{C}H_3)_2$), 19.5 ($CH(\underline{C}H_3)_2$), 31. 2 ($\underline{C}H(CH_3)_2$), 41.3 ($CO\underline{C}H_2NH$), 52.8 (OCH_3), 61.8 ($CO\underline{C}H$), 67.4 ($NH\underline{C}H_2Ph$), 119.1 (ArC), 121.0 (ArCH), 123.2 (ArCH), 128.3 (ArCH), 128.4 (ArCH), 128.7 (ArCH), 134.7 (ArCH), 136.4 (ArC), 136.9 (ArCH), 141.7 (ArC), 156.6 (OCONH), 162.7 ($CO\underline{C}ONH$), 169.4 (CO_2CH_3), 170.9 ($\underline{C}ONHAr$), 190.8 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3298, 2960, 1758, 1656, 1520, 1446, 1358, 1277, 1209, 1125, 1038, 940, 843, 753, 694 cm^{-1}; HRMS (+ESI): Found *m/z* 492.1734, $[M+Na]^+$, $C_{24}H_{27}N_3O_7Na$ requires 492.1747.

(*S*)-Methyl 2-(2-(2-((S)-2-(((benzyloxy)carbonyl)amino)-3-methylbutanamido)phenyl)-2-oxoacetamido)-3-phenylpropanoate **5-29**

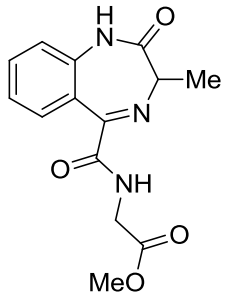


The title compound **5-29** was prepared from *N*-(Cbz)-L-val-isatin **5-9** (0.700 g, 1.84 mmol) and L-phenylalanine methyl ester hydrochloride (0.417 g, 1.93 mmol) according to the general procedure outlined above. The product **5-29** was precipitated as a pale yellow solid from a solution of diethyl ether and n-hexane (0.918 g, 89%). M.p. 142°C; $[\alpha]_D$ -61 (*c* 0.033, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.04 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 2.36 (dqq, *J* = 6.8, 6.8, 5.1, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.14 (dd, *J* = 14.0, 7.0 Hz, 1H, C$\underline{H}_A$H$_B$Ph), 3.28 (dd, *J* = 14.0, 5.5 Hz, CH$_A\underline{H}_B$Ph), 3.78 (s, 3H, OCH$_3$), 4.28 (dd, *J* = 8.5, 5.1 Hz,1H, C$\underline{H}$CH(CH$_3$)$_2$), 5.00 (ddd, *J* = 8.3, 7.5, 5.5 Hz, C$\underline{H}$CH$_A$H$_B$Ph), 5.14 (m, 2H, CO$_2$C$\underline{H}_2$Ph), 5.48 (bd, *J* = 8.5 Hz, NHCbz), 7.11 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H, ArH), 7.16-7.18 (m, 3H, 3 x ArH),7.24-7.37 (m, 8H, 7 x ArH + COCON$\underline{H}$), 7.59 (ddd, *J* = 8.8, 7.3, 1.6 Hz, 1H, ArH), 8.13 (dd, *J* = 8.2, 1.6 Hz, 1H, ArH), 8.66 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 11.32 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.6 (CH(C$\underline{H}_3$)$_2$), 19.5 (CH(C$\underline{H}_3$)$_2$), 31.3 (C(CH$_3$)$_3$), 38.2 (CHC$\underline{H}_2$Ph), 52.8 (OCH$_3$), 53.4 (C$\underline{H}$CH$_2$Ph), 61.7 (C$\underline{H}$CH(CH$_3$)$_2$), 67. 4 (CO$_2$C$\underline{H}_2$Ph), 118.8 (ArC), 120.8 (ArCH), 123.1 (ArCH), 124.8 (ArC), 127.5 (ArCH), 128.3 (ArCH), 128.7 (ArCH), 129.0 (ArCH), 129.4 (ArCH), 134.6 (ArCH), 135.5 (ArC), 136.4 (ArCH), 136.8 (ArCH), 141.7 (ArC), 156.6 (OCONH), 162.3 (CO$\underline{C}$ONH), 171.0 ($\underline{C}$O$_2$CH$_3$), 171.2 ($\underline{C}$ONHAr), 191.3 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3302, 3029, 2957, 1736, 1659, 1577, 1517, 1444, 1356, 1275, 1236, 1181, 1122, 1030, 927836, 757, 699, 660 cm^{-1}; HRMS (+ESI): Found *m/z* 582.2202, [M+Na]$^+$, $C_{31}H_{33}N_3O_7Na$ requires 582.2216.

General procedure for the synthesis of 1,4-benzodiazepin-2-ones **5-30** and **5-42-5-54**

N-(Boc) protected *N*-glyoxylamide/ester was stirred in TFA-CH_2Cl_2 (20% v/v) at room temperature for 0.5h. The solvent was removed *in vacuo.* The resulting yellow residue was redissolved in methanol, triethylamine (10 equivalents) was added and the solution was left to stir for an additional hour. The solvent was removed *in vacuo* to afford the crude material. Further purification was carried out according to described procedures.

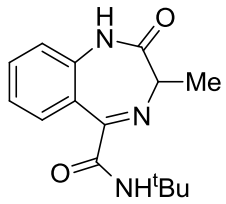
Methyl 2-(3-methyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamido)acetate **5-30**



The title compound **5-30** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-17** (0.300 g, 0.763 mmol) according to the general procedure outlined above. The crude material was dissolved in ethyl acetate and washed with two portions of water. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford **5-30** as a brown amorphous solid (0.140 g, 63 %). M.p. 176-178°C; $[\alpha]_D$ 63 (*c* 0.064, DMF); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (d, *J* = 6.5 Hz, 3H, CHC$\underline{H}_3$), 3.71 (q, *J* = 6.5 Hz, 1H, C$\underline{H}$CH$_3$), 3.78 (s, 3H, OCH$_3$), 4.07 (dd, *J* = 18.2, 5.5 Hz, 1H, NHC$\underline{H}_A$H$_B$), 4.23 (dd, *J* = 18.2, 6.2 Hz, 1H,NHCH$_A\underline{H}_B$),4.23-4.37 (m, 1H, N$\underline{H}$CH$_A$H$_B$), 7.08 (dd, *J* = 8.2, 1.2 Hz, 1H, ArH), 7.22 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.49 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.88 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 8.09 (dd, *J* = 6.2, 5.5 Hz, 1H, NHCH$_2$), 8.86 (s, 1H, CONHAr); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5 (CH$\underline{C}$H$_3$), 41.5 (NH$\underline{C}$H2CO), 52.6 (OCH$_3$), 58.9 (CH),121.1 (ArCH), 123.7 (ArC),123.7 (ArCH),131.5 (ArCH),132.4 (ArCH),138.1 (ArC),160.9 (NC$\underline{C}$ONH), 163.4 (Ar$\underline{C}$NCH),170.1 ($\underline{C}$O$_2$CH$_3$), 171.9 (NH$\underline{C}$OCH); IR (ATR): ν_{max} 3370, 3280, 3062, 2981, 2941, 2717, 1737, 1665, 1613, 1527, 1419, 1368, 1319, 1246, 1194, 1042, 962, 904, 839,

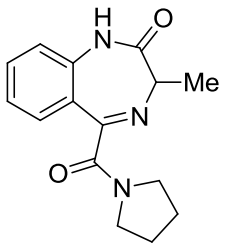
771, 724, 677 cm^{-1}; HRMS (+ESI): Found *m/z* 312.0951,$[M+H]^+$, $C_{14}H_{15}N_3O_4Na$ requires 312.0960.

(*S*)-*N*-(*tert*-butyl)-3-methyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamide **5-42**



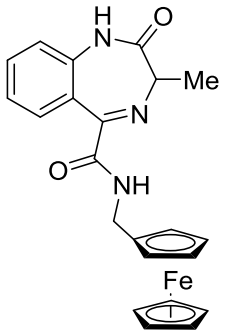
The title compound **5-42** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-14** (0.523 g, 1.34 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with 1:1 ethyl acetate/n-hexane. Crystallisation from hot ethanol gave **5-42** as colourless needles (0.318 g, 87 %). M.p. 179°C; $[\alpha]_D$ 103 (*c* 0.029, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (s, 9H, C($C\underline{H}_3)_3$), 1.33 (d, *J* = 6.5 Hz, 3H, $CHCH_3$), 3.32 (q, *J* = 6.5 Hz, 1H, CH), 6.79 (dd, *J* = 8.2, 1.2, 1H, ArH), 6.89 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.10 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, ArH), 7.55 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 9.43 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5 ($\underline{C}H_3CH$), 28.5 (C($\underline{C}H_3)_3$), 51.2 ($\underline{C}(CH_3)_3$), 58.6 ($CH_3\underline{C}H$), 120.8 (ArCH), 123.3 (ArCH), 123.9 (ArC), 131.7 (ArCH), 132.0 (ArCH), 137.9 (ArC), 161.8 (NC<u>C</u>ON), 161.9 (C=N), 171.8 (CONHAr); IR (ATR): v_{max}3204 3044, 2965, 2894, 1699, 1650, 1607, 1552, 1479, 1322, 1218, 1143, 1040, 931, 820, 752 cm^{-1}; HRMS (+ESI): Found *m/z*296.1361, $[M+Na]^+$, $C_{15}H_{19}N_3O_2Na$ requires 296.1375.

(*S*)-3-Methyl-5-(pyrrolidine-1-carbonyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **5-43**



The title compound **5-43** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-12** (0.192 g, 0.708 mmol) according to the general procedure outlined above. The product **5-43** was isolated by flash chromatography, eluting with 1:1 ethyl acetate/n-hexane as a tan amorphous powder (0.226 g, 82 %). M.p. 162-164°C; $[\alpha]_D$ –183 (*c* 0.098, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (d, *J* = 6.5 Hz, 3H, CH_3), 1.63-1.81 (m, 4H, 2 x $NCH_2C\underline{H}_2$), 2.90-2.98 (m, 1H, $N\underline{H}_AH_BCH_2$), 3.23 (m, 1H, $N\underline{H}_AH_BCH_2$), 3.37-3.55 (m, 1H, $NH_A\underline{H}_BCH_2$), 3.56 (q, *J* = 6.5 Hz, CH), 7.07 (ddd, *J* = 8.0, 7.3, 1.1 Hz, 1H, ArH), 7.12 (dd, *J* = 8.3, 1.1 Hz, 1H, ArH), 7.38 (ddd, *J* = 8.3, 7.3, 1.5 Hz, 1H, ArH), 7.69 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 9.95 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.3 (CH_3), 23.9 ($NCH_2\underline{C}H_2$), 25.7 ($NCH_2\underline{C}H_2$), 45.5 ($N\underline{C}H_2CH_2$), 47.1 ($N\underline{C}H_2CH_2$), 58.3 (CH), 121.1 (ArCH), 123.8 (ArCH +ArC), 128.8 (ArCH), 132.3 (ArCH), 137.8 (ArC), 165.2 (NH$\underline{C}$OCH), 165.7 (CN), 171.7 (NC$\underline{C}$ON). One peak not observed due to accidental equivalence; IR (ATR): ν_{max}3102, 2948, 2878, 1694, 1602, 1452, 1296, 1213, 1159, 1113, 1039, 834, 766 cm^{-1}; HRMS (+ESI): Found *m/z*272.1396, $[M+H]^+$, $C_{15}H_{18}N_3O_2$ requires 272.1399.

3-methyl-*N*-methylferrocene-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamide **5-44**

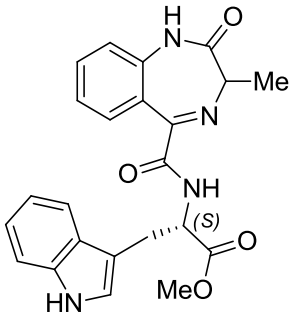


N-Protected amino acid isatin **5-4** (0.435 g, 1.37 mmol) was added to a solution of ferrocene methylamine (0.353 g, 1.64 mmol) in dichloromethane. The reaction was stirred at room temperature for 3h and the crude reaction mixture was subjected to flash chromatography on silica gel, eluting with 1:4 ethyl acetate/n-hexane as a dark yellow amorphous powder. This was used directly in the subsequent step without further purification.

The crude *N*-(Boc) protected *N*-glyoxylamide was stirred in TFA-CH_2Cl_2 (20% v/v) at room temperature for 0.5h. The solvent was removed *in vacuo.* The resulting yellow residue was redissolved in methanol, triethylamine (10 equivalents) was added and the solution was left to stir for an additional hour. The reaction mixture was washed with a saturated solution of $Na_2S_2O_4$(aq) where the organic layer changed from a dark green to a bright orange. The mixture was filtered and the two layers separated. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by gravity chromatography on silica gel, eluting with 1:1 ethyl acetate/n-hexane to give **5-44** as a bright yellow amorphous solid (0.962 g, 59 %). M.p. 194-197°C; $[\alpha]_D$ 32 (*c* 0.13, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (d, *J* = 6.5 Hz, 3H, CHC$\underline{H}_3$), 3.73 (q, *J* = 6.5 Hz, 1H, C$\underline{H}$CH$_3$), 4.15-4.24 (m, 11H, 9 x $ArH_{Ferrocene}$+ CH_2), 7.10 (dd, *J* = 8.2, 1.2 Hz, 1H, ArH), 7.24 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.49 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, ArH), 7.95 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 7.98 (bd, *J* = 10.9 Hz, 1H, N$\underline{H}$CH$_2$), 8.9 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (CH_3), 38.7 (CH_2C_p), 58.9 (CH), 68.2 (C_p), 68.2 (C_p), 68.7 (C_p), 85.3 (C_p), 121.0 (ArCH), 123.6 (ArCH), 124.0 (ArC), 131.7 (ArCH), 132.3 (ArCH), 138.2 (ArC) 161.6 (NC$\underline{C}$ON), 162.4 (Ar$\underline{C}$NCH), 171.8 (NH$\underline{C}$OCH); IR (ATR): ν_{max} 3312, 3193,

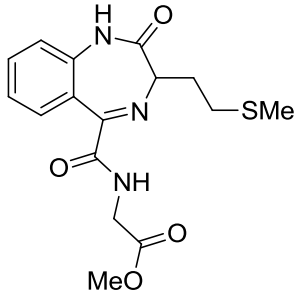
3082, 2965, 1675, 1501, 1372, 1308, 1206, 1138, 1103, 1036, 947, 808, 759, 689 cm^{-1}; HRMS (+ESI): Found *m/z* 438.0867, $[M+Na]^+$, $C_{22}H_{21}FeN_3FeO_2Na$ requires 438.0881.

Methyl ((*S*)-3-methyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carbonyl)-L-tryptophanate **5-46**



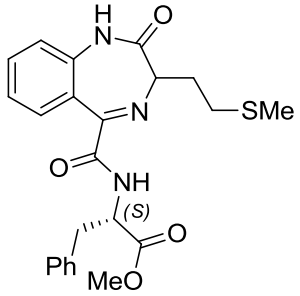
The title compound **5-46** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-18** (0.400 g, 0.745 mmol) according to the general procedure outlined above. The product **5-46** was isolated by flash chromatography, eluting with 1:1 ethyl acetate/n-hexane as a white amorphous solid (0.246 g, 79 %). M.p. 182°C; $[\alpha]_D$ 5.6 (*c* 0.36, DMF); ^1H NMR (300 MHz, d_6 DMSO): δ 1.49 (d, *J* = 6.4 Hz, 3H, CHC<u>H</u>$_3$), 3.26 (d, *J* = 6.5 Hz, 2H, CHC<u>H</u>$_2$), 3.60 (q, *J* = 6.4 Hz, 1H, C<u>H</u>CH_3), 3.66 (s, 3H, OCH_3), 4.63 (dt, *J* = 6.5, 7.5 Hz, 1H, C<u>H</u>CH_2), 6.97 (ddd, *J* = 8.0, 7.1, 1.1 Hz, 1H, ArH), 7.07 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, ArH), 7.15-7.20 (m, 2H, ArH), 7.32-7.35 (m, 1H, ArH), 7.48-7.56 (m, 2H, ArH), 8.63 (bd, *J* = 7.5 Hz, 1H, N<u>H</u>CH), 10.62 (s, 1H, CON<u>H</u>Ar), 10.92 (s, 1H, NH_{indole}). IR (ATR): ν_{max} 3279, 2922, 1730, 1670, 1612, 1505, 1437, 1365, 1310, 1276, 1205, 1101, 1008, 952, 869, 830, 793, 748 cm^{-1}; HRMS (+ESI): Found *m/z* 441.1535, $[M+Na]^+$, $C_{23}H_{22}N_4O_4Na$ requires 441.1539.

Methyl 2-(3-(2-(methylthio)ethyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamido)acetate **5-47**



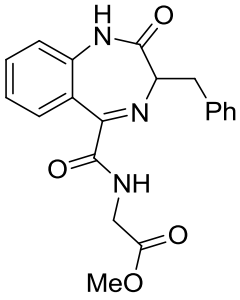
The title compound **5-47** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-19** (0.393 g, 0.330 mmol) according to the general procedure outlined above. The product **5-47** was isolated by flash chromatography, eluting with 3:2 ethyl acetate/n-hexane as a pale brown amorphous solid (0.173 g, 59 %). M.p. 97-99°C; $[\alpha]_D$ 65 (*c* 0.093, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06 (s, 3H, SCH_3), 2.43 (dd, *J* = 13.7, 6.9 Hz, 2H, SCH_2), 2.60-2.76 (m, 2H, $SCH_2C\underline{H}_A\underline{H}_B$), 3.71-3.77 (m, 4H, $C\underline{H}(CH_2)_2S$ + OCH_3), 4.06 (dd, *J* = 18.3, 5.6 Hz, 1H, $NHC\underline{H}_AH_B$), 4.20 (dd, *J* = 18.3, 6.0 Hz, 1H, $NHCH_A\underline{H}_B$), 7.08 (dd, *J* = 8.2, 1.1 Hz, 1H, ArH), 7.19 (ddd, *J* = 8.0, 7.4, 1.1 Hz, 1H, ArH), 7.46 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.87 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 8.08 (dd, *J* = 6.0, 5.6 Hz, 1H, $N\underline{H}CH_2$), 9.29 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (SCH_3), 29.8 ($S\underline{C}H_2$), 30.7 ($SCH_2\underline{C}H_2$), 41.5 ($NHCH_2$), 52.5 (OCH_3), 62.0 (CH), 121.1 (ArCH), 123.4 (ArC), 123.6 (ArCH), 131.5 (ArCH), 132.4 (ArCH), 138.1 (ArC), 161.5 ($NC\underline{C}ONH$), 163.3 (CN), 170.1 ($\underline{C}O_2CH_3$), 170.8 ($\underline{C}ONHAr$); IR (ATR): ν_{max} 3216, 3055, 2916, 1746, 1610, 1520, 1476, 1425, 1374, 1343, 1198, 1099, 1037, 976, 883, 760, 685 cm^{-1}; HRMS (+ESI): Found *m/z*372.0983, $[M+Na]^+$, $C_{16}H_{19}N_3O_4SNa$ requires 372.0994.

(2S)-Methyl 2-(3-(2-(methylthio)ethyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamido)-3-phenylpropanoate **5-48**



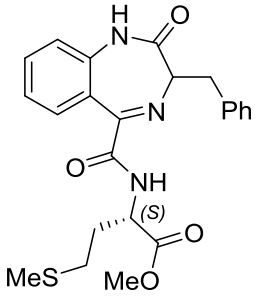
The title compound **5-48** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-20** (0.184 g, 0.330 mmol) according to the general procedure outlined above. The product **5-48** was isolated by flash chromatography, eluting with 3:2 ethyl acetate/n-hexane as a colourless amorphous solid (0.118 g, 81 %). M.p. 80-83°C; $[\alpha]_D$ 86 (*c* 0.14, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06 (s, 3H, SCH_3), 2.43 (dd, *J* = 13.6, 6.8 Hz, 2H, SCH_2), 2.56-2.72 (m, 2H, $SCH_2C\underline{H}_A\underline{H}_B$), 3.13 (dd, *J* = 13.8, 6.1 Hz, 1H, $C\underline{H}_AH_BPh$), 3.21 (dd, *J* = 13.8, 6.0 Hz, 1H, $CH_A\underline{H}_BPh$), 3.73-3.77 (m, 4H, $C\underline{H}(CH_2)_2S$ + OCH_3), 4.90 (ddd, *J* = 8.3, 6.1, 6.0 Hz, 1H, $C\underline{H}CH_2Ph$), 7.09(dd, *J* = 8.2, 1.0 Hz, 1H, ArH), 7.13 (dd, *J* = 8.0, 1.8 Hz, 2H, ArH), 7.21-7.32 (m, 4H, ArH), 7.52 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.83 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 7.94 (bd, *J* = 8.3 Hz, 1H, $N\underline{H}CHCO_2CH_3$), 8.54 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.6 (SCH_3), 30.0 (SCH_2), 30.7 ($SCH_2\underline{C}H_2$), 38.1 ($\underline{C}H_2Ph$), 52.5 (OCH_3), 53.6 ($\underline{C}HCH_2Ph$), 61.9 ($C\underline{H}(CH_2)_2S$), 121.0 (ArCH), 123.6 (ArC), 123.8 (ArCH), 127.5 (ArCH), 128.8 (2 x ArCH), 129.4 (2 x ArCH), 131.7 (ArCH), 132.6 (ArCH), 135.7 (ArC), 138.0 (ArC), 161.8 ($NC\underline{C}ONH$), 162.6 (CN), 170.6 ($\underline{C}O_2CH_3$), 171.7 ($\underline{C}ONHAr$); IR (ATR): ν_{max} 3234, 3059, 2916, 1737, 1673, 1612, 1508, 1433, 1339, 1275, 1200. 1109, 1028, 971, 865, 816, 757, 699 cm^{-1}; HRMS (+ESI): Found *m/z*462.1451, $[M+Na]^+$, $C_{23}H_{25}N_3O_4SNa$ requires 462.1463.

(*S*)-Methyl 2-(3-benzyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carboxamido)acetate **5-49**



The title compound **5-49** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-22** (0.100 g, 0.210 mmol) according to the general procedure outlined above. The product **5-49** was isolated by flash chromatography, eluting with 1:1 ethyl acetate/n-hexane as a white amorphous solid (0.067 g, 88 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.47 (dd, *J* = 13.8, 8.1 Hz, 1H, CNCHC$\underline{H}_A H_B$), 3.58 (dd, *J* = 13.8, 5.8 Hz, 1H, CNCHCH$_A\underline{H}_B$), 3.77 (dd, *J* = 8.1, 5.8 Hz, 1H, CH), 3.80 (s, 3H, OCH_3), 4.08 (dd, *J* = 18.4, 5.3 Hz, 1H, NHC$\underline{H}_A H_B$), 4.20 (dd, *J* = 18.4, 5.8 Hz, 1H, NHCH$_A\underline{H}_B$), 7.09 (dd, *J* = 8.2, 1.0 Hz, 1H, ArH), 7.20 (ddd, *J* = 8.0, 7.4, 1.0 Hz, 1H, ArH), 7.49 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.82 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 8.08 (dd, *J* = 5.8, 5.3 Hz, 1H, N$\underline{H}CH_2$), 9.30 (s, 1H, CON$\underline{H}$Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.0 (CH$\underline{C}H_2$Ph), 41.6 (NH$\underline{C}H_2$CO), 52.6 (OCH_3), 65.0 ($\underline{C}HCH_2$Ph), 121.2 (ArCH), 123.4 (ArC), 123.7 (ArCH), 126.6 (ArCH), 128.5 (ArCH), 129.8 (ArCH), 131.6 (ArCH), 132.5 (ArCH), 138.1 (ArC), 138.4 (ArC), 161.3 (NC$\underline{C}$ONH), 163.3 (Ar$\underline{C}$NCH), 170.0 ($\underline{C}O_2CH_3$), 170.8 (NH$\underline{C}$OCH). HRMS (+ESI): Found *m/z* 366.1450, $[M+H]^+$, $C_{20}H_{19}N_3O_4$ requires 366.1454.

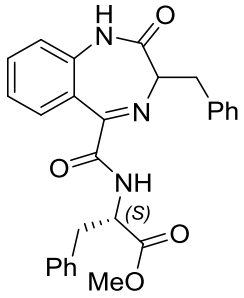
(Methyl (3-benzyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carbonyl)-L-methioninate **5-50**



The title compound **5-50** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-23** (0.573 g, 1.03 mmol) according to the general procedure outlined above. The product **5-50** was obtained by flash chromatography, eluting with 2:3 ethyl acetate/n-hexane as a bright yellow amorphous solid (0.244 g, 54 %). M.p. 68-69°C; ^{1}H NMR (600 MHz, $CDCl_3$): 2.03-2.15 (m, 4H, SCH_3 + $SCH_2C\underline{H}_A\underline{H}_B$), 2.18-2.26 (m, 1H, $SCH_2CH_A\underline{H}_B$), 2.54 (dd, *J* = 6.6, 6.4 Hz, 2H, SCH_2), 3.50 (dd, *J* = 13.9, 8.1 Hz, 1H, $C\underline{H}_A\underline{H}_BPh$), 3.56 (dd, *J* = 13.9, 5.7 Hz, 1H, $CH_A\underline{H}_BPh$), 3.77 (dd, *J* = 8.1, 5.7 Hz, 1H, $C\underline{H}CH_2Ph$), 3.82 (s, 3H, OCH_3), 4.78 (ddd, *J* = 12.6, 7.3, 5.2 Hz, 1H, $S(CH_2)_2C\underline{H}$), 7.07 (bd, *J* = 7.3 Hz, 1H, $CON\underline{H}CH$), 7.18-7.24 (m, 2H, 2 x ArH), 7.27-7.34 (m, 4H, 4 x ArH), 7.50 (ddd, *J* = 8.5, 7.5, 1.6 Hz, 1H, ArH), 7.82 (dd, *J* = 8.1, 1.6 Hz, 1H, ArH), 8.16 (d, *J* = 8.2 Hz, 1H, ArH), 8.61 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (SCH_3), 30.0 (SCH_2), 31.8 ($S\underline{C}H_2CH_2$), 37.0 ($\underline{C}H_2Ph$), 51.8 (OCH_3), 52.6 ($\underline{C}HCH_2CH_2$), 64.9 ($\underline{C}HCH_2Ph$), 120.8 (ArC), 123.4 (ArC), 123.7 (ArCH), 126.5 (ArCH), 128.5 (ArCH), 129.8 (ArCH), 131.8 (ArCH), 132.4 (ArCH), 137.8 (ArC), 138.4 (ArC), 161.1 ($NC\underline{C}ONH$), 162.5 ($Ar\underline{C}NCH$), 170.1 ($\underline{C}O_2CH_3$), 171.9 ($NH\underline{C}OCHNC$); IR (ATR): ν_{max} 3234, 3058, 2951, 2915, 1737, 1674, 1612, 1506, 1433, 1274, 1200, 1100, 1028, 986, 885, 755, 699 cm^{-1}; UV–vis (CH_3CN): λ_{max} 230 nm (ε 21,500 cm^{-1} M^{-1}); HRMS (+ESI): Found *m/z*462.1455, $[M+Na]^+$, $C_{23}H_{25}N_3O_4SNa$ requires 462.1463.

Methyl (3-benzyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carbonyl)-L-phenylalaninate

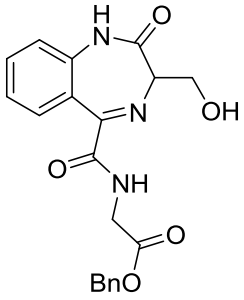
5-51



The title compound **5-51** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-24** (0.320 g, 0.558 mmol) according to the general procedure outlined above. The product **5-51** was obtained by flash chromatography, eluting with 2:3 ethyl acetate/n-hexane as a pale yellow amorphous solid (0.224 g, 88 %); M.p. 74-76°C; $[\alpha]_D$ 126 (*c* 0.095, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.13 (dd, *J* = 13.8, 6.0 Hz, 1H, NHCHC$\underline{H}_A$H$_B$), 3.22 (dd, *J* = 13.8, 5.6, 1H, NHCHCH$_A\underline{H}_B$), 3.42 (dd, J = 13.8, 8.4Hz, 1H, CNCHC$\underline{H}_A$H$_B$), 3.52 (dd, J = 13.8, 5.5 Hz, 1H,CNCHCH$_A\underline{H}_B$), 3.71 (dd, *J* = 8.4, 5.5 Hz, 1H, CNC$\underline{H}$), 3.75 (s, 3H, OCH_3), 4.92 (ddd, *J* = 11.6, 6.0, 5.6 Hz, 1H, NHC$\underline{H}$CH$_A$H$_B$), 7.04 (dd, *J* = 8.2, 1.1 Hz, 1H, ArH), 7.19 (ddd, *J* = 8.0, 7.4, 1.1 Hz, 1H, ArH), 7.17-7.36 (m, 10H, 10 x ArH), 7.49 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.74 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 7.92 (bd, *J* = 11.6 Hz, 1H, N$\underline{H}$CH), 8.54 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.0 (CH$\underline{C}$H$_2$Ph), 38.2 (CH$\underline{C}$H$_2$Ph), 52.4 (OCH_3), 53.5 ($\underline{C}$H CH$_2$), 64.8 (CN$\underline{C}$HCO), 120.9 (ArCH), 123.4 (ArC), 123.7 (ArCH), 126.4 (ArCH), 127.4 (ArCH), 128.4 (ArCH), 128.7 (ArCH), 129.4 (ArCH), 129.8 (ArCH), 131.6 (ArCH), 132.4 (ArCH), 135.6 (ArC), 137.8 (ArC), 138.3 (ArC), 161.4 (NC$\underline{C}$ONH), 162.4 (Ar$\underline{C}$NCH), 170.5 ($\underline{C}O_2CH_3$), 171.5 (NH$\underline{C}$OCHCN); IR (ATR): ν_{max} 3370, 3223, 3027, 2951, 1739, 1675, 1613, 1509, 1435, 1315, 1278, 1201, 1112, 1028, 950, 813, 745, 699 cm^{-1}; UV–vis (CH_3CN): λ_{max} 230 nm (ε 15,400 cm^{-1} M^{-1}); HRMS (+ESI): Found *m/z* 456.1911, $[M+H]^+$, $C_{27}H_{26}N_3O_4$ requires 456.1923.

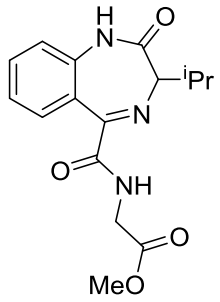
Benzyl (3-(hydroxymethyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carbonyl)glycinate

5-52



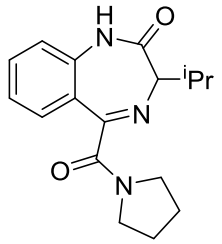
The title compound **5-52** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-26** (0.724 g, 1.30 mmol) according to the general procedure outlined above. The product **5-52** was isolated by flash chromatography, eluting with 3:2 ethyl acetate/n-hexane as a white amorphous solid (0.487 g, 98 %). M.p. 104-106°C; $[\alpha]_D$ 0 (*c* 0.072, DMF); ^{1}H NMR (400 MHz, d_6-DMSO): δ 3.49 (dd, *J* = 7.1, 5.9 Hz, 1H, $CH_A\underline{H_B}OH$) 3.96-4.10 (m, 4H, $CO\underline{CH_2}NH$ + OH + $C\underline{H_A}H_BOH$), 4.60 (dd, *J* = 7.6, 4.8 Hz, 1H, COCH), 5.11-5.23 (m, 2H, $C\underline{H_2}Ph$), 7.14-7.23 (m, 2H, 2 x ArH), 7.29-7.45 (m, 5H, 5 x ArH), 7.51-7.62 (m, 2H, 2 x ArH), 9.03 (bt, *J* = 6.1 Hz, 1H, $N\underline{H}CH_2$), 10.71 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.2 ($NHCH_2$), 61.1 (CH_2OH), 65.0 ($CHCH_2$), 66.0 ($O\underline{C}H_2Ph$), 121.1 (ArCH), 122.6 (ArCH), 123.6 (ArC), 128.0 (ArCH), 128.1 (ArCH), 128.5 (ArCH), 130.4 (ArCH), 132.1 (ArCH), 135.9 (ArC), 139.0 (ArC), 163.1 ($NC\underline{C}ONH$), 164.6 (CN), 168.8 (CO_2Bn), 169.4 ($\underline{C}ONHAr$); IR (ATR): ν_{max}3520, 3374, 3199, 3069, 1718, 1674, 1614, 1509, 1388, 1305, 1210, 1054, 935, 754 cm^{-1}; UV–vis (CH_3CN): λ_{max} 230 nm (ε 27,000 cm^{-1} M^{-1}), 315 (1,280); HRMS (+ESI): Found *m/z*404.1210, $[M+Na]^+$, $C_{20}H_{19}N_3O_5Na$ requires 404.1222

Methyl (3-isopropyl-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepine-5-carbonyl)glycinate **5-30**



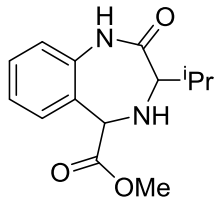
The title compound **5-30** was prepared from *N*-(Boc) protected *N*-glyoxylamide **5-25** (1.50 g, 3.44 mmol) according to the general procedure outlined above. The crude product was subjected to flash chromatography, eluting with 1:1 ethyl acetate/n-hexane. Crystallisation from hot toluene gave **5-30** as thin yellow needles (1.03 g, 94 %). M.p. 104-106°C; $[\alpha]_D$ 122 (*c* 0.041, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (d, *J* = 6.5 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.11 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 2.55-2.72 (m, 1H,C$\underline{H}$(CH$_3$)$_2$), 3.14 (d, *J* = 8.8 Hz, 1H, NHCOC$\underline{H}$N), 3.78 (s, 3H, OCH_3), 4.07 (dd, *J* = 18.3, 5.2 Hz, 1H, NHC$\underline{H}_A$H$_B$), 4.23 (dd, *J* = 18.3, 6.0 Hz, 1H, NHCH$_A$$\underline{H}_B$), 7.08 (dd, *J* = 8.2, 1.2 Hz, 1H, ArH), 7.23 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.50 (ddd, *J* = 8.2, 7.4, 1.5 Hz, 1H, ArH), 7.92 (dd, *J* = 8.0, 1.5 Hz, 1H, ArH), 8.08 (dd, *J* = 6.0, 5.2 Hz, 1H, N$\underline{H}$CH$_A$H$_B$), 8.57 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.1 (CH(C$\underline{H}_3$)$_2$), 28.6 (C$\underline{H}$(CH$_3$)$_2$), 41.6 (NHC$\underline{H}_2$CO), 52.6 (OCH_3), 69.3 (C$\underline{H}$CH(CH$_3$)$_2$), 121.1 (ArCH), 123.6 (ArC), 123.7 (ArCH), 131.6 (ArCH), 132.4 (ArCH), 138.2 (ArC), 160.9 (NC$\underline{C}$ONH), 163.3 (Ar$\underline{C}$NCH), 170.0 ($\underline{C}O_2CH_3$), 170.1 (NH$\underline{C}$OCH). IR (ATR): ν_{max} 3371, 3230, 2955, 1745, 1672, 1609, 1510, 1471, 1367, 1321, 1200, 1042, 985, 757 cm^{-1}; UV–vis (CH_3CN): λ_{max} 230 nm (ε 30,600 cm^{-1} M^{-1}), 315 (140); HRMS (+ESI): Found *m/z*318.1444, $[M+H]^+$, $C_{16}H_{20}N_3O_4$ requires 318.1454.

3-Isopropyl-5-(pyrrolidine-1-carbonyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one **5-53**



5-13 (0.750 g, 1.66 mmol) was hydrogenated for 24h at atmospheric pressure in methanol (30 mL) using 10% Pd/C (10 mol %). The reaction mixture was filtered through celite and concentrated *in vacuo*. The crude product was subjected to flash chromatography on silica gel, eluting with 4:1 ethyl acetate/n-hexane to afford **5-53** as a white amorphous solid (0.429 g, 86 %). M.p. 196-199°C; $[\alpha]_D$ –121 (*c* 0.11, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (d, *J* = 6.6 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.11 (d, *J* = 6.7 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.78-1.95 (m, 4H, 2x $NCH_2C\underline{H}_2$), 2.62 (dqq, *J* = 8.9, 6.7, 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.14 (d, *J* = 8.9 Hz, 1H, COC<u>H</u>N), 3.21-3.13 (m, 1H, $NC\underline{H}_AH_B$), 3.37-3.50 (m, 1H, $NC\underline{H}_AH_B$), 3.51-3.69 (m, 2H, $NC\underline{H}_A\underline{H}_B$), 7.09 (dd, *J* = 8.2, 1.2 Hz, 1H, ArH), 7.21 (ddd, *J* = 8.0, 7.4, 1.2 Hz, 1H, ArH), 7.50 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, ArH), 7.85 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.66 (s, 1H, CON<u>H</u>Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.2 ($CH(\underline{C}H_3)_2$), 20.4 ($CH(\underline{C}H_3)_2$), 24.3 ($NCH_2\underline{C}H_2$), 26.1 ($NCH_2\underline{C}H_2$), 28.9 ($\underline{C}H(CH_3)_2$), 45.9 ($N\underline{C}H_2$), 47.7 ($N\underline{C}H_2$), 69.1 ($\underline{C}H(CH_3)_2$), 121.0 (ArCH), 124.2 (ArCH), 124.8 (ArC), 129.4 (ArCH), 132.3 (ArCH), 137.7 (ArC), 165.4 (NC<u>C</u>ON), 165.5 (Ar<u>C</u>NCH), 169.8 (NH<u>C</u>OCH); IR (ATR): ν_{max}3069, 2952, 2879, 1694, 1609, 1465, 1376, 1323, 1273, 1211, 1168, 1019, 988, 836, 772 cm^{-1}; HRMS (+ESI): Found *m/z* 322.1522, $[M+Na]^+$, $C_{17}H_{21}N_3O_2Na$ requires 322.1531.

Methyl 3-isopropyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-5-carboxylate **5-54**



5-16 (0.559 g, 1.36 mmol) was hydrogenated for 24h at atmospheric pressure in methanol (25 mL) using 10% Pd/C (10 mol %). The reaction mixture was filtered through celite and concentrated *in vacuo* to afford **5-54** as a white amorphous solid (0.292 g, 82 %). M.p. 110-112°C; $[\alpha]_D$ 333 (*c* 0.12, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, *J* = 6.6 Hz, 3H, CH($C\underline{H}_3$)$_2$), 1.04 (d, *J* = 6.8 Hz, 3H, CH($C\underline{H}_3$)$_2$), 2.18 (dqq, *J* =7.5, 6.8, 6.6 Hz, 1H, C$\underline{H}$(CH_3)$_2$), 2.95 (d, *J* = 7.5 Hz, 1H, COC$\underline{H}$NH), 3.67 (s, 3H, OCH_3), 4.66 (s, 1H, ArC$\underline{H}$NH), 6.98 (dd, *J* = 7.9, 0.9 Hz, 1H, ArH), 7.22 (ddd, *J* = 7.9, 7.2, 1.3 Hz, 1H, ArH), 7.34- 7.40 (m, 2H, 2 x ArH), 7.76 (s, 1H, CON$\underline{H}$Ar). CHNHCH not present due to exchange; ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.6 (CH($\underline{C}H_3$)$_2$), 20.7 (CH($\underline{C}H_3$)$_2$), 28.4 ($\underline{C}$H(CH_3)$_2$), 53.0 (OCH_3), 60.6 (Ar$\underline{C}$HNH), 62.3 (CO$\underline{C}$HNH), 121.9 (ArCH), 125.9 (ArCH), 129.8 (ArCH), 129.9 (ArC), 131.6 (ArCH), 137.5 (ArC), 173.8 ($\underline{C}O_2CH_3$), 174.1 (NH$\underline{C}$OCH); IR (ATR): ν_{max} 3334, 3260, 3048, 2953, 2866, 1742, 1588, 1436, 1676, 1489, 1378, 1331, 1264, 1236, 1178, 1136, 1033, 1005, 939, 884, 798, 665 cm^{-1}; HRMS (+ESI): Found *m/z* 263.1381, $[M+H]^+$, $C_{14}H_{19}N_2O_3$ requires 263.1396.

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CHAPTER 6

Chapter 6: Design and synthesis of novel cyclic peptide mimics containing the *N*-glyoxylamide moiety

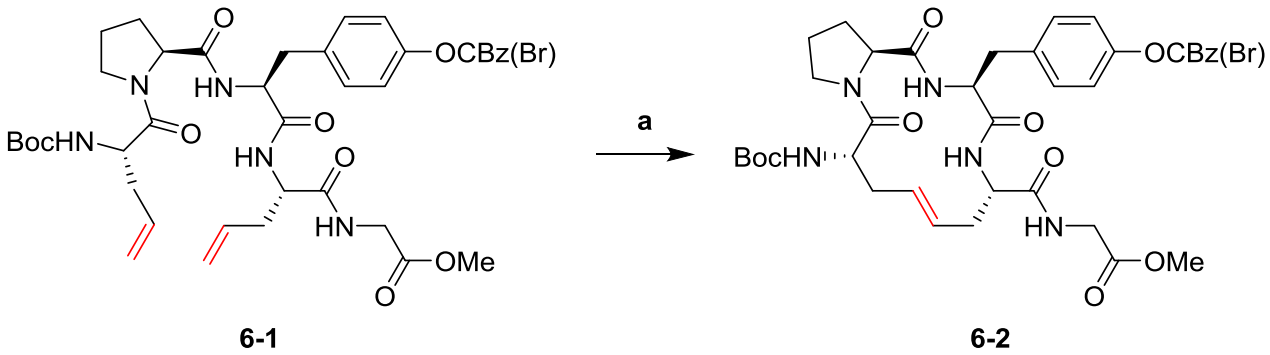
6.1 General introduction and chapter aims

As described in Chapters **1** and **5**, the ring-opening of *N*-acylisatins by a primary amine gave the corresponding *N*-glyoxylamide product. ^{1}H NMR spectroscopic and X-ray crystallographic analysis of these compounds both showed the presence of an intramolecular H-bond, locking the structure in an L-shaped conformation. This non-proteinogenic structure was hypothesized to act as a turn-inducing element that can facilitate the macrocyclization of linear peptide mimic precursors. This chapter will investigate the cyclization of such precursors using various macrocyclization reactions.

6.2 Background, results and discussion

6.2.1 Macrocyclization by ring-closing metathesis in the synthesis of cyclic peptide mimics

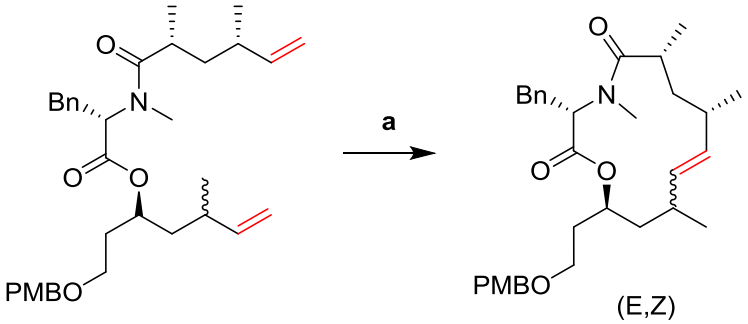
Ring-closing olefin metathesis (RCM) has quickly emerged as one of the most useful tools for the macrocyclization of complex molecules. This reaction is attractive because of its high functional group tolerance and the possibility for further transformations. Grubbs and co-workers were the first to apply the RCM strategy to conformationally rigidify amino acids and peptides. For example, exposure of pentapeptide **6-1** to Grubbs 1st generation catalyst in dichloromethane at high dilution (0.004 M) resulted in the clean side chain-to-side chain macrocyclization product **6-2** in excellent yield (80 %)(Scheme **6.1**)[1] Presumably, the turn-inducing proline residue was strategically placed at the middle of the linear precursor to encourage intramolecular cyclization.



6-1 **6-2**

Scheme 6.1. Side chain-to-side chain RCM of pentapeptide **6-1**. Reagents and conditions: a) Grubbs 1st generation catalyst, CH_2Cl_2 (0.004 M), 40 °C.

In other studies, RCM has afforded little control over the stereochemistry of the resulting alkene. For example, Chen et al. have employed this synthetic methodology as the key step in the total synthesis of spongidepsin, a depsipeptide with a remarkable anti-cancer profile. Exposure of diene **6-3** to Grubbs 2nd generation catalyst (0.1 equivalents) in refluxing toluene yielded the four possible macrocyclic 5E/Z, 7R/S diastereomers **6-4** in a combined yield of 80 %, with a > 10:1 ratio of E to Z isomers (Scheme **6.2**).



6-3 **6-4**

Scheme 6.2. RCM as the key step in the total synthesis of spongidepsin. Lack of stereochemical control is evident as the reaction formed, both E and Z isomer products (> 10:1).

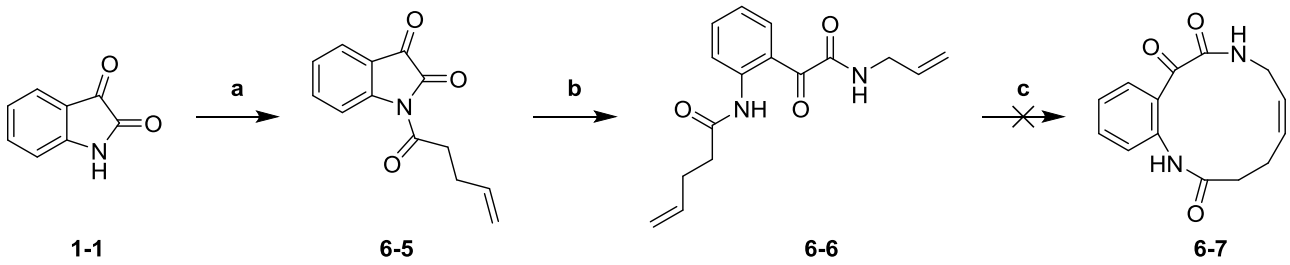
Like most macrocyclization reactions, RCM also suffers from the possibility of intermolecular side-reactions, giving rise to oligomeric and polymeric side-products. Efficient macrocyclization relies heavily on the internal conformational elements of the molecule, and hence these precursor compounds have to be carefully designed.

6.2.1.1 Preliminary studies towards the synthesis of cyclic peptide mimics *via* RCM

Although several examples of the synthesis of cyclic peptide mimics containing heterocyclic fragments such as triazoles and pyrazoles as well as aryl rings have been reported in the literature, the synthesis of macrocyclic peptidomimetics containing the *N*-glyoxylamide moiety remains an unexplored target. To investigate the feasibility of using RCM in the late-stage macrocyclization of *N*-glyoxylamide-based peptidomimetics, a model reaction was conducted. Isatin **1-1** was coupled with 4-pentenoic acid in the presence of DCC and a catalytic amount of DMAP to provide 1-(pent-4-enoyl)indoline-2,3-dione **6-5** in excellent yield (93 %) (Scheme **6.3**). Installation of the second olefin and generation of the desired *N*-glyoxylamide moiety was achieved by the nucleophilic ring-opening of **6-5** with allylamine, furnishing the diene **6-6** in 81% yield. With the model linear precursor in hand, efforts were directed to forming the twelve-membered macrocycle **6-7** *via* RCM. To initiate the cyclization, a solution of **6-6** in dichloromethane was added dropwise over 6 h to a stirred solution of Grubbs 1st generation catalyst in dichloromethane at room temperature (final concentration of **6-6** = 0.001 M). The reaction mixture was refluxed for an additional 16 h, TLC analysis showed the presence of the starting material **6-6**, along with several new spots of similar R_f values. Furthermore, prolonging the reaction time failed to achieve complete conversion of the starting material **6-6**. A ^{1}H NMR spectrum of the reaction mixture was acquired, but the desired product **6-7** could not be identified due to the large number of overlapping peaks. The decomposition of the Grubbs 1st generation catalyst was evident over the course of the reaction by a gradual colour change from pale green to black.

Advances made in the field of ruthenium carbene chemistry have facilitated the improvement of catalysts used in organic synthesis. The Grubbs 2nd generation catalyst possesses a number of improvements over its predecessor. The incorporation of the imidazolium carbene ligand into the structure has made the catalyst stable towards moisture and air, and also confers higher activity. Using the same experimental conditions, the RCM reaction of **6-6** was repeated using Grubbs 2nd generation catalyst. After 16 h, TLC analysis indicated the complete consumption of the starting *N*-glyoxylamide **6-6** along with several spots of similar R_f. However, attempts to separate the intractable mixture were not made.

Instead, to avoid a difficult isolation process and to improve the yield of the RCM reaction, focus was directed towards redesigning the internal elements of the linear precursor to favor macrocyclization.



Scheme 6.3. Model reaction for the synthesis of cyclic peptide mimics *via* RCM. Reagents and conditions: a) 4-pentenoic acid, DCC, DMAP, CH_2Cl_2, rt b) Allylamine, CH_2Cl_2, rt c) Grubbs 2nd generation catalyst, CH_2Cl_2, reflux, 16h.

6.2.1.2 Application of a polar complexing groups in macrocyclic RCM reactions

A key factor in the success of macrocycle forming RCM reactions is the presence of a polar complexing group appropriately positioned in the dienic substrate. These groups can serve as anchor points for the catalyst by assisting in olefin binding (Figure **6.1**).

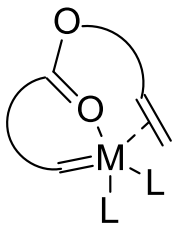


Figure 6.1. A polar complexing group can facilitate olefin binding through chelation.

Fürstner and Langemann have demonstrated the importance of a polar complexing group in the metathesis of hexadeca-2,15-diene. With the unsubstituted diene, the fourteen-membered alkene **6-8** was not formed, and only oligomeric products were produced (Figure **6.2**). When an ester functional group was introduced at the appropriate position, both the E/Z isomers of lactone **6-9** were obtained in 52% yield under identical conditions.[2]

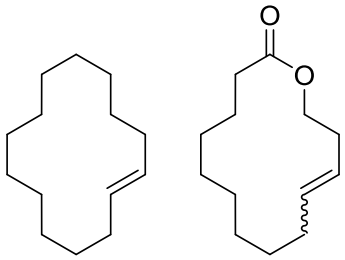
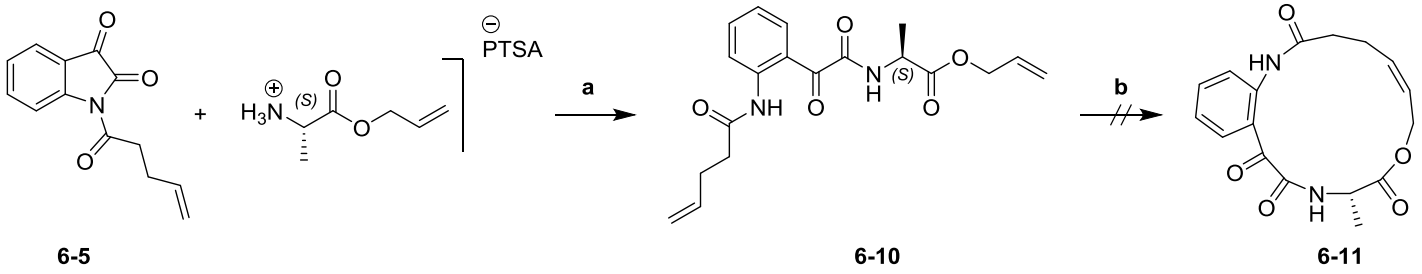


Figure 6.2. Influence of an ester group in the RCM reaction.

To adopt this approach for the generation of novel *N*-glyoxylamide-based cyclic peptides, a diene substrate was required. It was envisaged that the ring-opening reaction of **6-5** with an amino acid allyl ester would be a convenient method for introducing an ester carbonyl group, which could facilitate the subsequent RCM reaction. The first novel RCM substrate chosen for the investigation was diene **6-10**, which was prepared in 88% yield from the reaction of *N*-acylisatin **6-5** and L-alanine allyl ester *p*-toluenesulfonate *via* the general procedure for the ring opening of *N*-protected-amino acid isatins with amino acid esters, established in Chapter **5** (Scheme **6.4**).



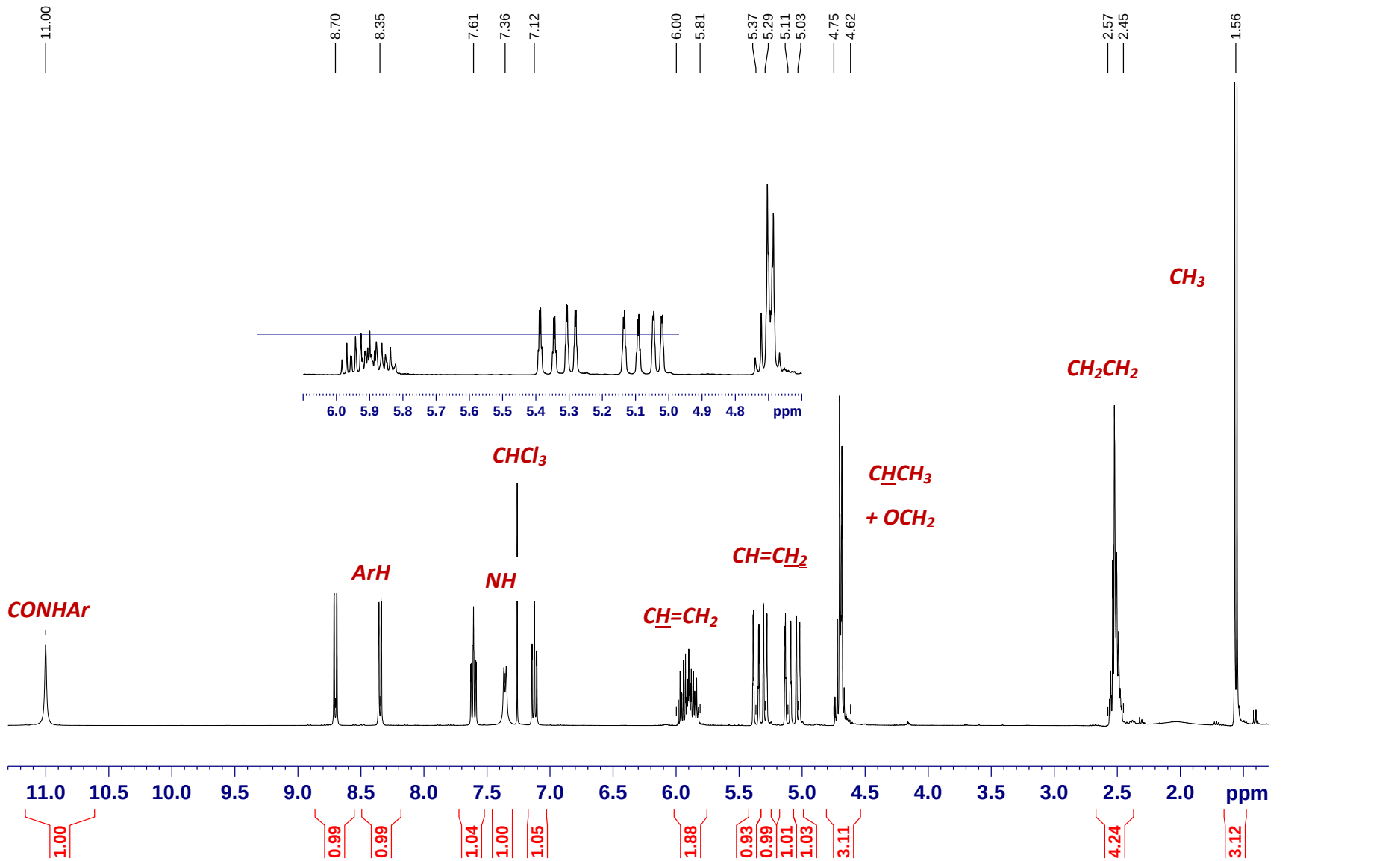
Scheme 6.4. Attempted synthesis of peptide mimic **6-11** through complexing assisted RCM. Reagents and conditions: a) $^{i}Pr_2NEt$, CH_2Cl_2, rt b) Grubbs 2nd generation catalyst, CH_2Cl_2, reflux.

^{1}H NMR spectroscopy analysis of RCM precursor **6-10** showed a doublet at 1.56 ppm with a relative integration of three protons, which were assigned as the methyl protons of the alanine moiety (Figure **6.3**). Four doublet of doublets at 5.03, 5.11, 5.29, and 5.37 ppm, all

possessing a relative integration of one proton were assigned to the four chemically non-equivalent, terminal alkene protons. The multiplet appearing in the 5.81-5.99 ppm range was assigned to the two C<u>H</u>=CH_2 protons, confirming the presence of both alkene groups.

The RCM reaction of diene **6-10** to form the fifteen-membered lactone **6-11** was attempted. Following the same high dilution (0.001 M) conditions used for precursor **6-6**, RCM substrate **6-10** was allowed to react in refluxing dichloromethane in the presence of Grubbs 2nd generation catalyst. However, a complex mixture was again obtained as observed in the ^{1}H NMR spectrum of the product mixture. Attempts to isolate the components of the reaction mixture were unsuccessful due to similarities in their solubility. The use of a non-polar solvent has been shown to promote the formation of the substrate-catalyst complex, and hence enhance the rate of RCM reactions.[3] However, the macrocyclization of **6-10** using anhydrous toluene gave a similar reaction outcome. Thus, a new target was sought

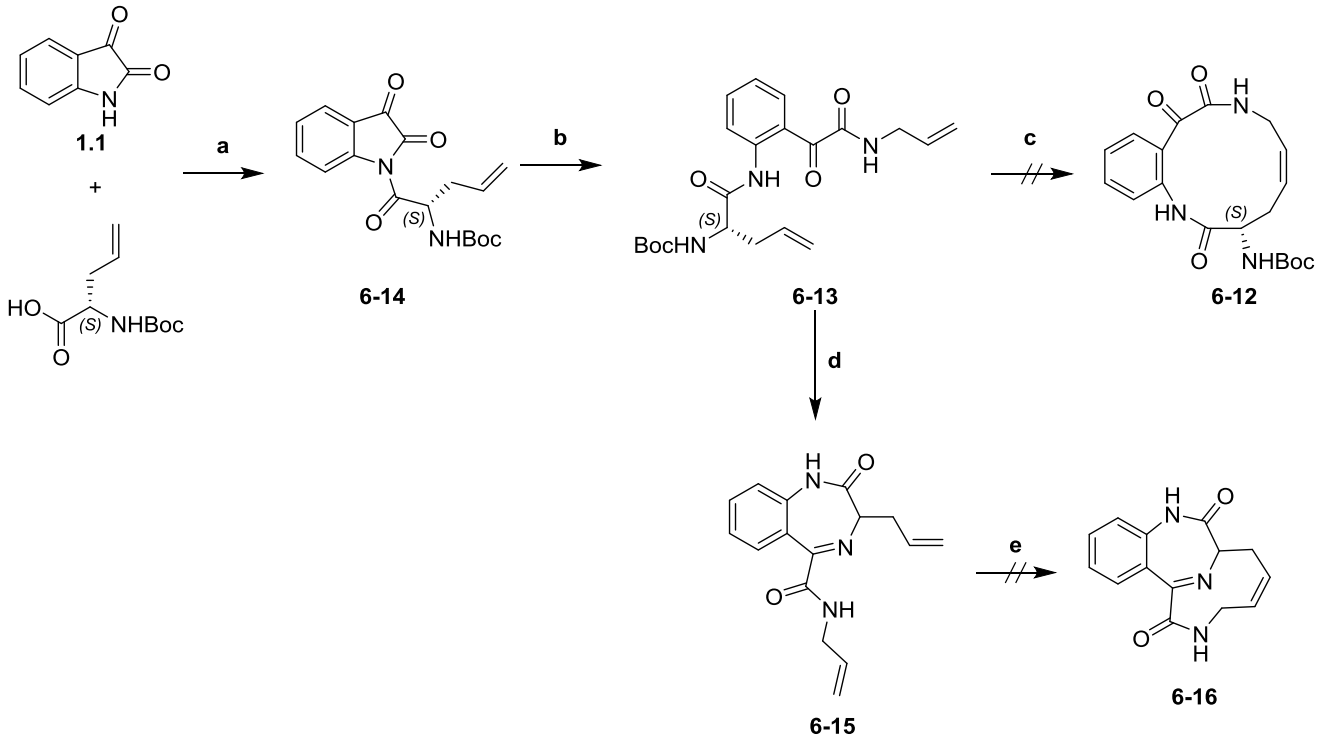
Figure 6.3. ^{1}H NMR spectra of RCM precursor **6-10** recorded at 400 MHz in $CDCl_3$ at 298 K.



6.2.1.3 Attempted synthesis of twelve-membered cyclic peptide mimics *via* RCM

As discussed in the introduction, one important factor governing the success of ring-closing reactions is the probability of the terminal functional groups coming into close proximity. The likelihood of this occurring is inversely proportional to the chain length (n > 7) of the linear precursor. Hence, larger macrocycles are often more synthetically challenging than smaller rings. Thus, the synthesis of a smaller, twelve-membered cyclic peptide mimic **6-12** incorporating the unnatural amino acid *(S)-N*-Boc-allylglycine was investigated. This could be achieved *via* the olefin metathesis of key intermediate **6-13**, which can in turn be prepared by the ring-opening reaction of *N*-(Boc)-L-allylglycine-isatin **6-14** and allylamine.

To begin the synthesis, *N*-(Boc)-L-allylglycine and isatin **1-1** were coupled *via* the general procedure established in Chapter **5** to give *N*-acylisatin **6-14** in reasonable yield (81%) (Scheme **6.5**). Subsequent ring-opening of **6-14** using allylamine furnished the RCM precursor **6-13** in 80% yield. Attempts to ring-close **6-13** using the conditions previously described led again to the generation of a complex mixture of products and isolation of the desired macrocyclic product **6-12** could not be achieved.



Scheme 6.5. Attempted synthesis of novel cyclic peptide mimic **6-12** and benzodiazein-2-one derivative **6-16.** Reagents and conditions: a) DCC, DMAP, CH_2Cl_2 b) $^{i}Pr_2NEt$, CH_2Cl_2 c) Grubbs 2nd generation catalyst, CH_2Cl_2, reflux d) TFA/CH_2Cl_2 1:4 (v/v) e) Grubbs 2nd generation catalyst, toluene, reflux.

The products obtained from the attempted RCM of **6-13** were presumably both oligomeric and cyclic structures. Rationalizing the reaction outcomes, there is a myriad of possible side-products that can be produced in addition to the desired compound **6-12**. For example, Figure **6.4** shows the six possible acyclic dimers that can be formed from the cross metathesis of an unsymmetrical diene. These dimeric side-products can either proceed to react with another equivalent of the starting diene **6-13** to give a trimer, couple together forming a linear tetramer, or cyclize to form a macrocyclic dimer. Therefore, both the large number of side-products as well as their structural similarity make the chromatographic separation of these reaction mixtures highly difficult. Hydrogenation of the reaction mixture is one strategy used to eliminate the possibility of E/Z stereoisomers, simplifying the purification process. In summary, attempts to form the targeted macrocyclic structures from diene precursors **6-6**, **6-10**

and **6-13** were unsuccessful, presumably due in part to the lack of appropriate conformation controlling elements favoring macrocyclization.

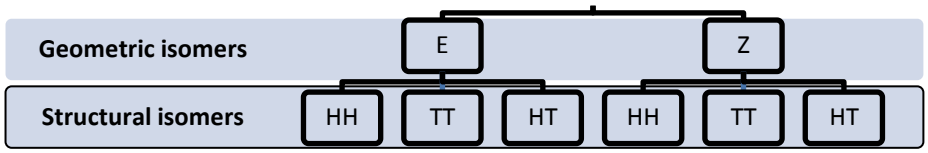


Figure 6.4. Possible acyclic dimers formed from the cross metathesis of unsymmetrical dienes.

6.2.1.4 Attempted synthesis of a novel 1,4- benzodiazepine-2-one scaffold

As an interesting aside, *N*-glyoxylamide **6-13** was cyclized to benzodiazepinone **6-15 i**n excellent yield (97%) following the established conditions described in Chapter **5**. It was envisaged that diene **6-15** could be ring-closed *via* RCM to form a nine-membered lactam ring fused with the benzodiazepine-2-one scaffold. However, subjecting diene **6-15** to Grubbs 2nd generation catalyst in refluxing toluene again provided a mixture of inseparable products (Scheme **6.5**). It is likely that the nine-membered ring lactam **6-16** has too much ring strain or a significant entropic loss which inhibits its formation. These energetic penalties are commonly experienced in the synthesis of medium-sized ring systems.[4] MM2 energy minimization was performed to investigate the hypothesis. Figures **6.5** ***(a)*** and ***(b)*** show that in the energy minimized model of **6-16**, the nine-membered lactam ring is bent in an energetically disfavored conformation. This result suggests that the desired target **6-16** possesses significant ring strain, making its formation difficult.

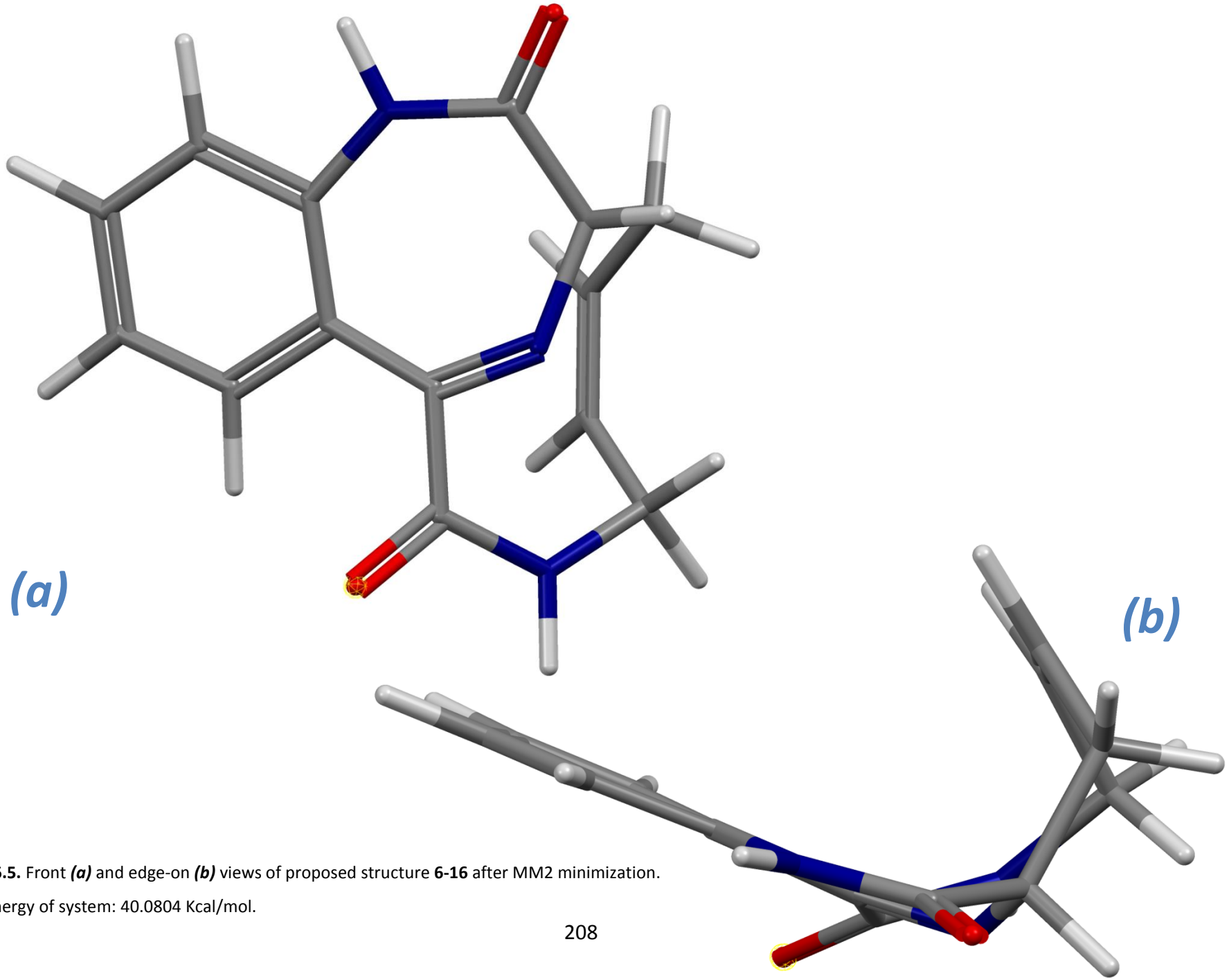
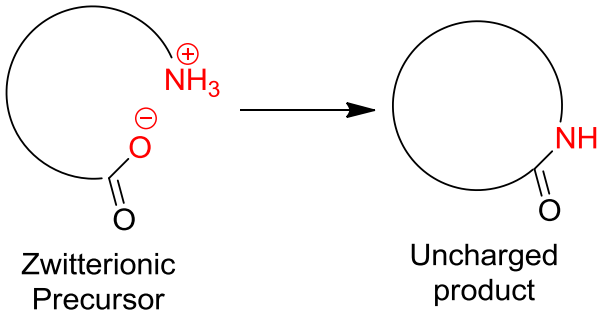


Figure 6.5. Front ***(a)*** and edge-on ***(b)*** views of proposed structure **6-16** after MM2 minimization.

Total energy of system: 40.0804 Kcal/mol.

6.2.2 Macrolactamization in the synthesis of peptide mimics containing the *N*-glyoxylamide pseudo-amino acid

Owing to structural similarities of the oligomeric side-products and the desired cyclic products obtained from the RCM reactions, difficulties in separation were encountered. To avoid this problematic purification step, an alternate macrolactamization strategy was employed for the synthesis of peptide mimics containing the *N*-glyoxylamide pseudo-amino acid. This methodology required the preparation of a zwitterionic linear precursor, which can then be cyclized to an uncharged cyclic peptide (after protection of all charged side-chains) (Figure **6.6**).



Scheme 6.6. General structure representation of the head-to-tail macrolactamization of a zwitterionic linear peptide precursor to the uncharged cyclic peptide product.

Zwitterionic compounds, due to their charged nature, are expected to possess a significantly lower R_f value on normal phase silica gel than the uncharged lactams. This difference in chemical properties should allow for the chromatographic separation of the desired macrocyclic lactams from the oligomeric side-products in a straightforward manner. However, the separation of the desired lactam from the uncharged cyclic oligomers using conventional chromatographic techniques could still pose a challenge.

6.2.2.1 Attempted synthesis of a cyclic pentapeptide mimic *via* macrolactamization

To maximize the success of the macrolactamization step, the internal elements of linear precursor **6-17** were carefully designed to reduce the distance between the head and tail. The design features incorporated in **6-17** include two non-proteinogenic *N*-glyoxylamide moieties situated between an *L*-alanine residue to maximize their turn-inducing ability. The site of cyclization was chosen to be between a glycine and an *L*-alanine residue to reduce steric hindrance. The cyclization of precursor **6-17** should yield compound **6-18** which, according to MM2 minimization, is free from unfavorable bond angles, lengths or steric interactions (Figure **6.6**). This heuristic examination suggests that compound **6-18** should have an energetically feasible structure.

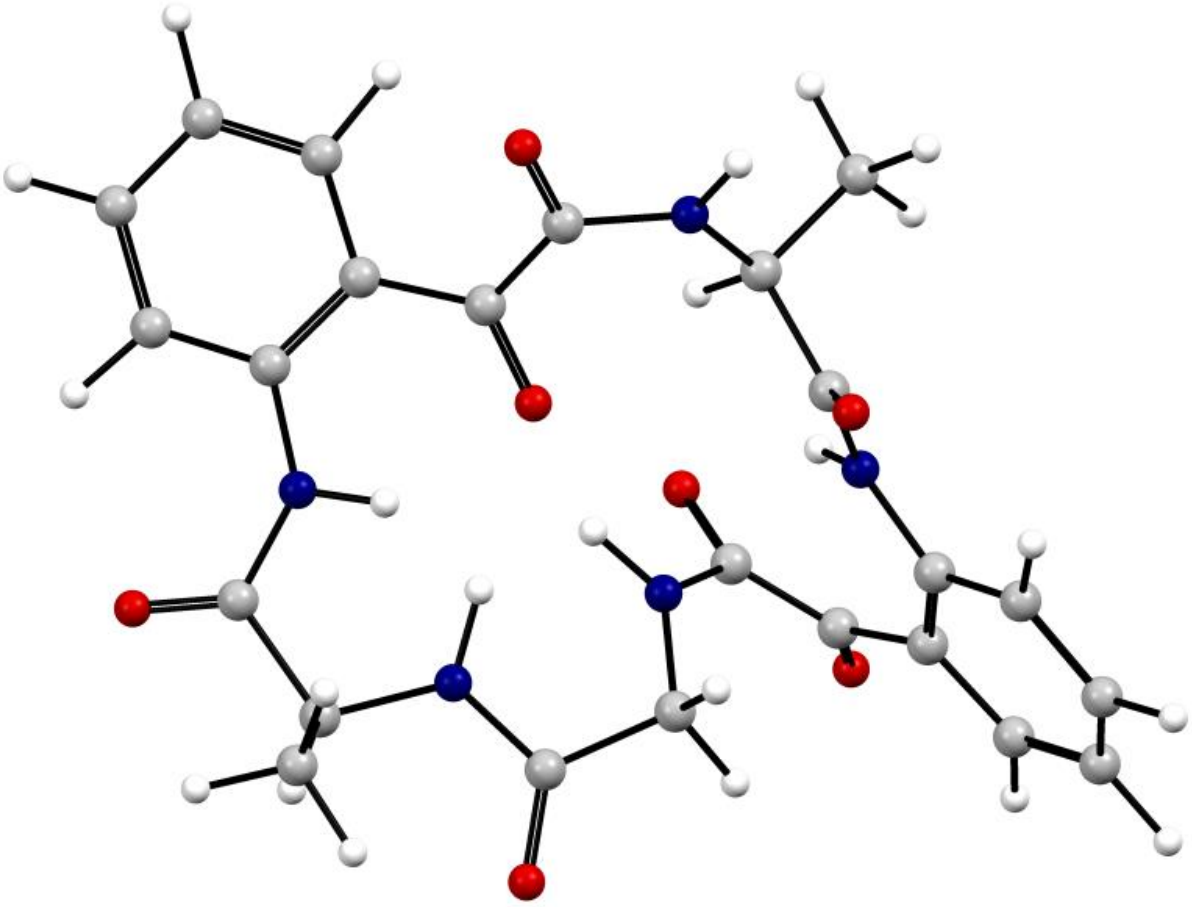
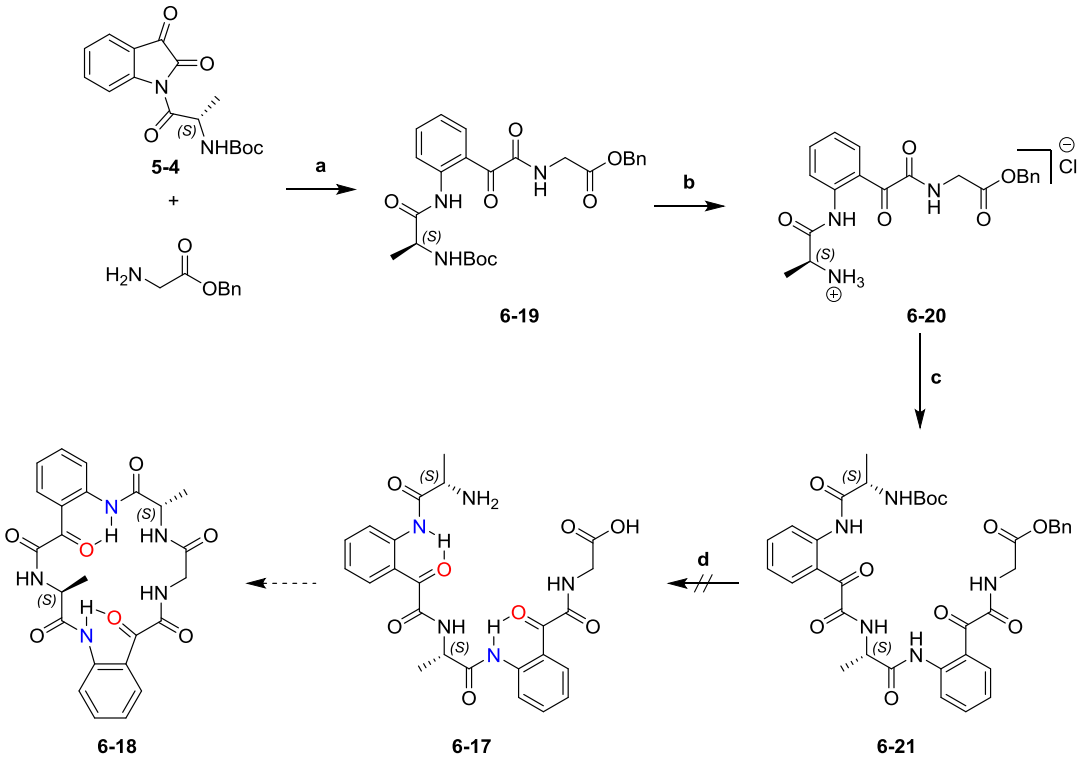


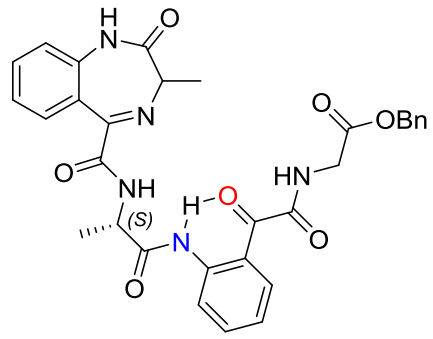
Figure 6.6. MM2 minimization of proposed cyclic peptide mimic **6-18**. Total energy of system: 43.556 Kcal/mol.

The synthetic approach to acyclic linear precursor **6-17** is depicted in Scheme **6.7**. *N*-(Boc)-alanine-isatin **5-4** and glycine benzyl ester *p*-toluene sulfonate were reacted in the presence of *N,N*-diisopropylethylamine to provide *N*-glyoxylamide intermediate **6-20**. As previously discussed in Chapter **5**, the removal of the Boc protecting group in **6-19** using TFA gives the corresponding 1,4-benzodiazepin-2-one. However, the primary amine can be trapped prior to cyclization by adding a strong acid (i.e. HCl) and a non-polar solvent, allowing the resulting HCl salt to precipitate out of the solution. Hence, subsequent Boc deprotection was achieved by stirring a solution of **6-19** in diethyl ether with gaseous hydrogen chloride to obtain **6-20** as the hydrochloride salt in 86% yield over two steps. To introduce the second turn-inducing *N*-glyoxylamide moiety, hydrochloride salt **6-20** was reacted again with *N*-(Boc)-alanine-isatin **5-4** in the presence of *N,N*-diisopropylethylamine to furnish the head and tail protected linear precursor **6-21** in 26 **%** yield. The low yield obtained for this reaction was presumably caused by the competing intramolecular cyclization reaction of the starting material **6-20** when exposed to a base.



Scheme 6.7. Attempted synthesis of a cyclic pentapeptide mimic incorporating two non-proteinogenic *N*-glyoxylamide moieties. Reagents and conditions: a) $^{i}Pr_2NEt$, CH_2Cl_2 b) $HCl_{(g)}$, Et_2O c) $^{i}Pr_2NEt$, CH_2Cl_2 d) TFA/CH_2Cl_2 1:4 (v/v).

With the benzyl and Boc-protected compound **6-21** in hand, two successive deprotection steps were required to reach the zwitterionic intermediate **6-17**. Subjecting **6-21** to TFA/CH_2Cl_2 (1:4 v/v) gave an intractable mixture of products. TLC analysis of the reaction mixture showed the presence of a major product, which exhibited a positive result under ninhydrin staining, indicating the presence of a primary amino group. A number of other side-products were also overlapped with the major product, observed on the TLC plate. Thus, it was difficult to determine the number of products and to separate them by chromatography on silica gel. One possible side-product could have been the intramolecular cyclization product **6-22** shown in Figure **6.7**.



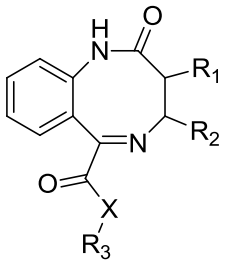
6-22

Figure 6.7. Chemical structure of possible side product **6-22**, which may result from the Boc-deprotection of **6-19** using TFA/CH_2Cl_2 1:4 (v/v) conditions.

It was thought that the successful isolation of the zwitterionic intermediate **6-17** could be obtained from **6-21** by the initial removal of the benzyl group *via* hydrogenation, followed by Boc-deprotection using gaseous hydrogen chloride, to trap **6-17** as the hydrochloride salt and prevent 1,4-benzodiazepin-2-one formation. However, the basic conditions required for the final macrolactamization step would still presumably promote the formation of **6-22** as a side product. Thus, this strategy was not pursued further.

6.2.2.2 Synthesis of an *N*-glyoxylamide-based cyclic peptide mimic

As reported in Chapter **5**, the deprotection of *N*-glyoxylamides and esters using TFA or under hydrogenation conditions formed stable seven-membered diazepinone rings. To extend the scope of this reaction, it was envisaged that replacing the *N*-protected α-amino acid used in the preparation of *N*-protected-amino acid isatins with an *N*-protected β-amino acid could lead to the formation of an 8-membered ring by following a similar synthetic route (Figure **6.8**).



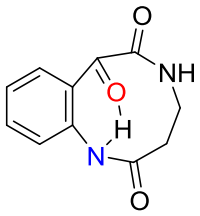
X = O, NH

Figure 6.8. Chemical structure of targeted eight-membered diazepinone ring

Thus, *N*-(Boc)-β-alanine-isatin **6-23** was prepared from the DCC coupling reaction of isatin **1-1** and *N*-Boc-β-alanine in 77% yield (Scheme **6.8**). Refluxing **6-23** in methanol for 3 h afforded the corresponding *N*-glyoxylmethylester **6-24** in excellent yield (98%). Subjecting **6-24** to the typical 1,4-benzodiazepin-2-one deprotection-cyclization sequence gave a grey precipitate suspended in a dark orange solution, after 16 h. The reaction mixture was filtered and the mother liquor was subjected to column chromatography on silica gel (2:3 ethyl acetate/n-hexane). However, isatin **1-1** was the only product isolated from the filtrate as indicated by ^{1}H NMR spectroscopy. Attempts to purify the grey precipitate by chromatography or crystallization failed due to the insoluble nature of the product in common laboratory solvents (DCM, ethyl acetate, acetonitrile or ethanol). A more polar solvent was therefore required to solubilize the unknown product. Thus, the solid was dissolved in hot *N*-methyl-2-pyrrolidone, which on cooling yielded an off-white precipitate that was collected by filtration. Due to difficulties in achieving a sufficient concentration of this unknown compound in d_6-DMSO, the aliphatic region of the ^{1}H NMR spectrum of this compound was saturated by both

DMSO and water peaks. To reveal the overlapped peaks in this region, a 1D NOSEY pre-saturation experiment was conducted with irradiation set at 3.55 ppm. The resulting spectrum revealed a singlet at 10.46 ppm that indicated the presence of an NH proton hydrogen-bonded to the oxygen atom of a carbonyl group, which is a peak that is typically observed for *N*-glyoxylamide and esters derived from *N*-acylisatins (Figure **6.10**). A quartet at 3.42 ppm, coupling with identical constants (J = 5.4 Hz) to a triplet at 2.57 ppm were assigned as $NHC\underline{H_2}CH_2$ and $NHCH_2C\underline{H_2}$ respectively. A broad triplet at 8.39 ppm $N\underline{H}CH_2CH_2$, was assigned as the $NHC\underline{H_2}CH_2$ protons despite the integration of the peak not equaling two protons. This is due to the suppression of signals in close proximity to the frequency of irradiation (3.55 ppm).

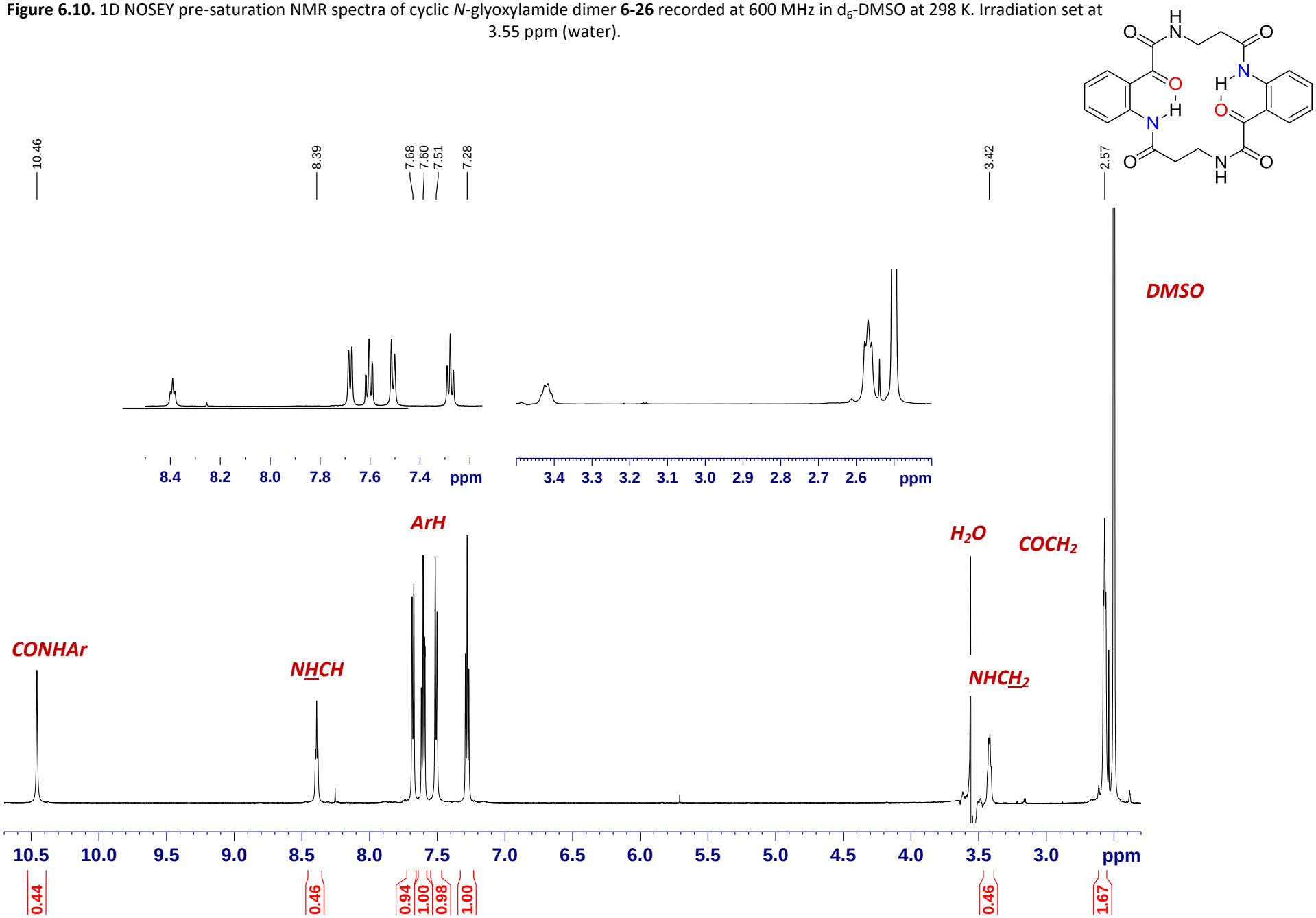
In the ^{13}C NMR spectrum of the unknown compound, a peak at 189.76 ppm corresponded to the carbon atom of the ketone that is typically observed in the spectra of *N*-glyoxylamides. This suggests that the expected intramolecular cyclization reaction forming eight-membered heterocycle **6-25** (Figure **6.8**) was unsuccessful. A peak corresponding to the methyl ester group of the starting material **6-24** was also absent in both ^{1}H and ^{13}C NMR spectra, suggesting the formation of a new amide bond through nucleophilic attack of the primary amine at the ester moiety, accompanied be the loss of methanol. The occurrence of two amide carbonyl peaks at 162.82 and 171.17 ppm initially supported the 9-membered cyclic structure **6-25** depicted in Figure **6.9**.

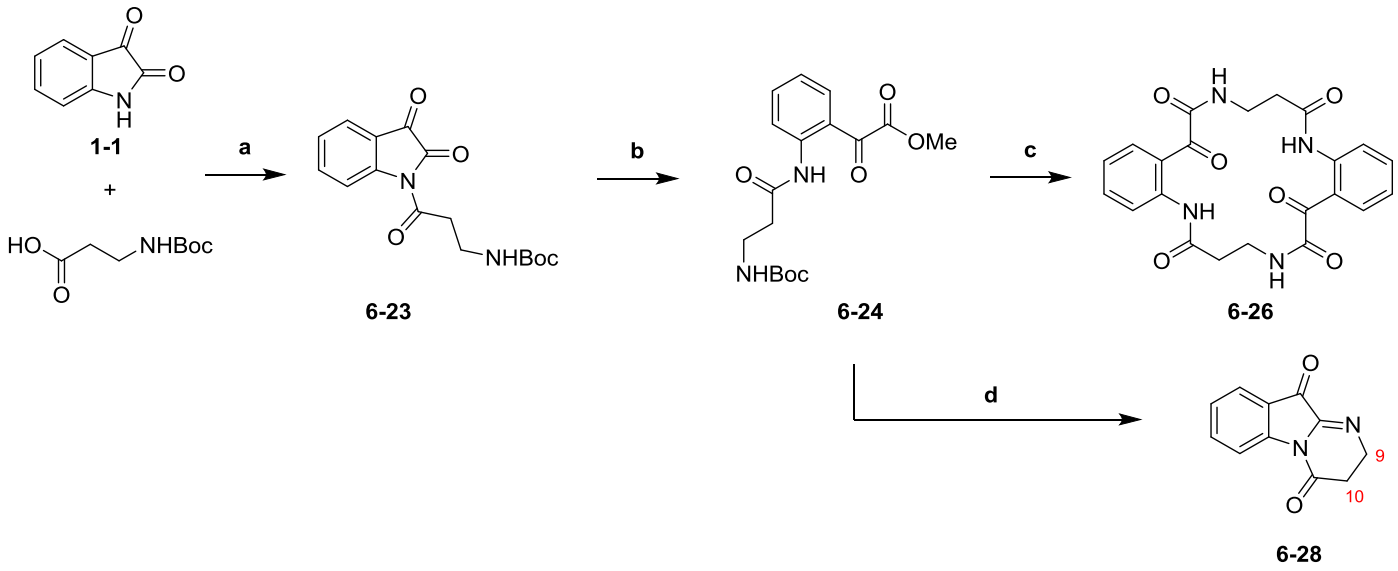


6-25

Figure 6.9. Initially proposed chemical structure of compound **6-25**.

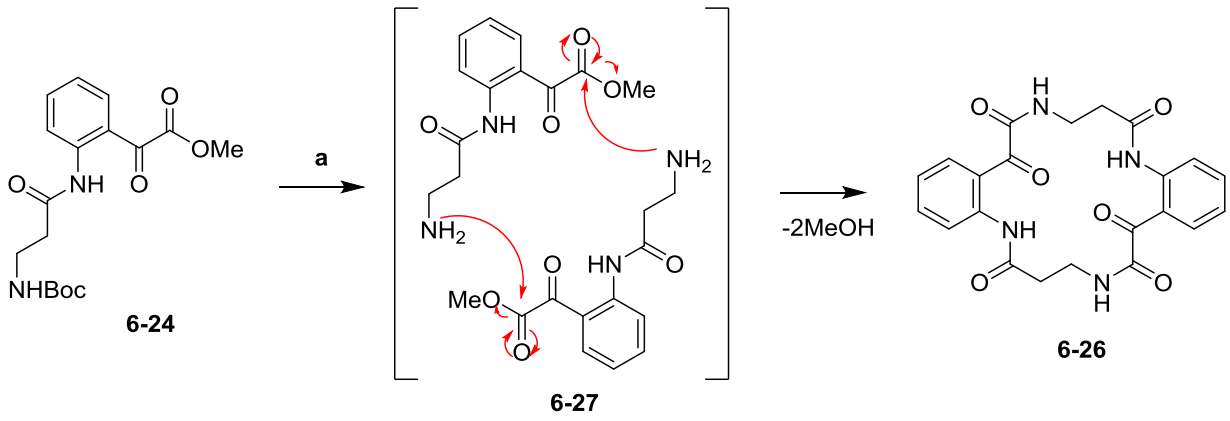
Figure 6.10. 1D NOSEY pre-saturation NMR spectra of cyclic *N*-glyoxylamide dimer **6-26** recorded at 600 MHz in d_6-DMSO at 298 K. Irradiation set at 3.55 ppm (water).





Scheme 6.8. Synthetic scheme for the synthesis of macrocyclic peptide mimic **6-26** and tricyclic hetrocycle **6-28**. Reagents and conditions: a) DCC,DMAP, CH_2Cl_2 b) MeOH, reflux c) 1. TFA/CH_2Cl_2 20% v/v 2. NEt_3/MeOH d) 1. TFA/CH_2Cl_2 20% v/v 2. NEt_3/MeOH.

However, high resolution mass spectrometric analysis showed a peak at 459.1266, which was inconsistent with the calculated mass of $(M+H)^+$: 219.0770 and $(M+Na)^+$: 241.0589 for the proposed structure **6-25**. The structure of the unknown compound was therefore revised to the eighteen-membered macrocycle **6-26** which had a calculated mass of 459.1281 for $(M+Na)^+$. This cyclic tetrapeptide mimic **6-26** is proposed to have been formed from the deprotection-cyclization sequence depicted in Scheme **6.9**. The nucleophilic attack of the ester carbonyl present in intermediate **6-27** by the primary amino group of another molecule of this intermediate generates two new amide bonds, which is accompanied by the loss of two methanol molecules. The unanticipated synthesis of macrocyclic dimer **6-26** is the first reported example of a cyclic peptide mimic containing the *N*-glyoxylamide moiety. Notably, this molecule was isolated in three simple steps from an easily accessible starting material, with only the initial *N*-(Boc)-β-alanine-isatin intermediate **6-23** requiring chromatographic techniques for isolation.



Scheme 6.9. Proposed reaction mechanism of the macrocyclization of *N*-glyoxylester **6-26**. Reagents and conditions: a) 1. TFA/CH_2Cl_2 20% v/v 2. NEt_3/MeOH.

Attempts to optimize the yield of the cyclic peptide mimic **6-26** were made by lowering the concentration of intermediate **6-24** for the cyclization step. Thus, a 7.53 M solution of the trifluoroacetate salt of intermediate **6-24** in methanol was subjected to basic cyclization conditions (Scheme **6.9**). Surprisingly, no precipitate was formed after 16 h. TLC analysis of the reaction mixture indicated the presence of a single major product. The crude product obtained after acidic aqueous work-up was subjected to slow evaporation from a solution of dichloromethane and n-hexane to give bright yellow needles. ^{1}H NMR spectroscopic analysis of this product showed the absence of a singlet at 10-13 ppm, indicating the loss of the NH proton that is hydrogen bonded to the ketone group in the starting material **6-24**. Two triplets appearing at 2.75 and 4.20 ppm both with a relative integration of two protons, were assigned as the α and β protons of the β-alanine moiety. The change in the multiplicity of one of these signals (from a quartet to a triplet) implied the loss of an adjacent proton attached to the β-alanine nitrogen of the starting material **6-24**. A down-field shift of the ketone carbonyl signal in the product relative to the starting material **6-24** was observed in the ^{13}C NMR spectrum (181.81 to 190.3 ppm).

For the purpose of structure elucidation, a sample of the product was prepared for single crystal X-ray analysis (synchrotron source). From those results, the unknown product was identified as 2,3-dihydropyrimido[1,2-a]indole-4,10-dione **6-28** (Figure **6.11**). Although

this this tricyclic compound is novel, analogous structures containing an aryl or heteroaryl ring fused at C9 and C10 have received considerable attention in the literature.[5,6,7]

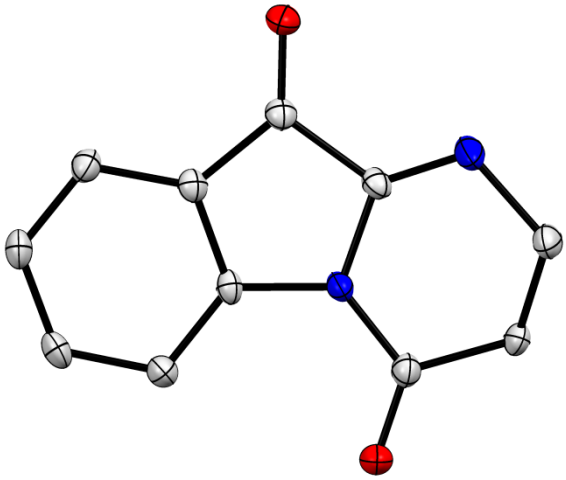
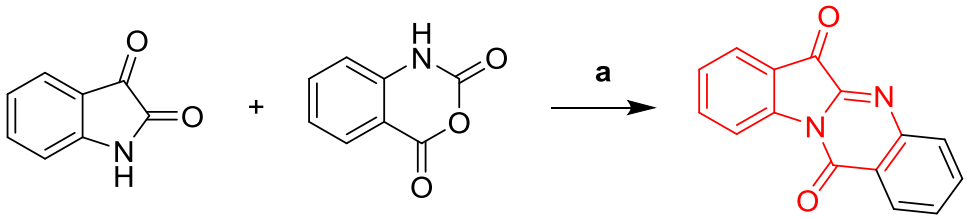


Figure 6.11. Front view from the crystal structure of *N*-glyoxylamide **6-28** (50% thermal ellipsoids at 100 K are shown; all H-atoms are omitted for clarity).

For example, tryptanthrin **6-29** is a naturally occurring alkaloid produced by several plant species that has been reported to possess various biological activities, such as antibacterial, antifungal and antileishmanial properties, and is also a potent inhibitor of COX-2 and 5-LOX. [8,9,10,11] These fused tetracyclic systems are typically prepared from the condensation of isatoic anhydride and isatin **1-1** with triethylamine in refluxing toluene (Scheme **6.10**).[5,6]

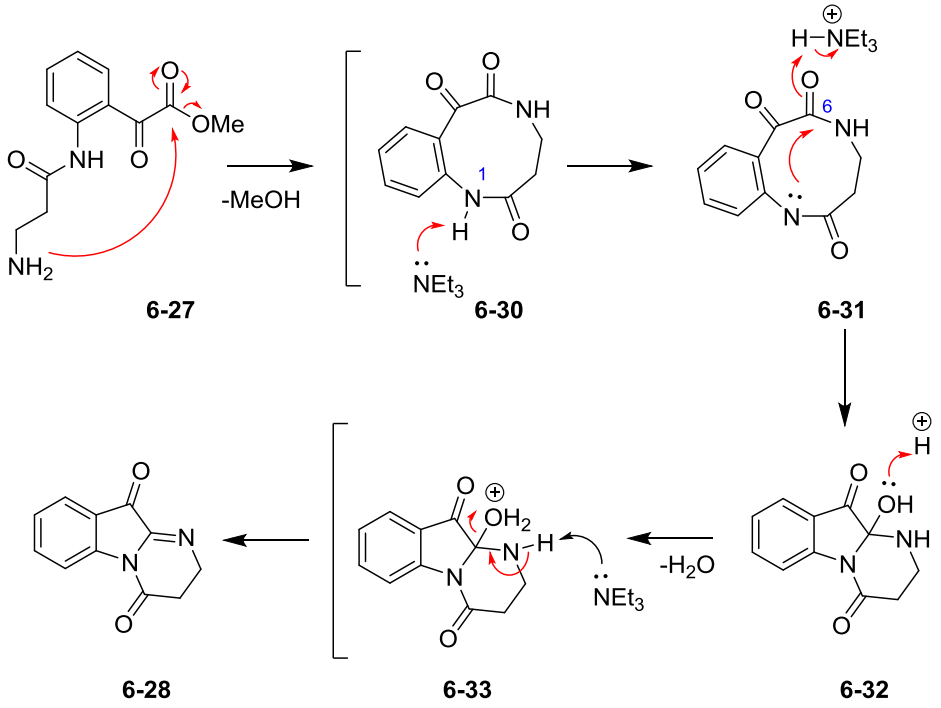


1-1 **6-29**

Scheme 6.10. Typical synthesis of tryptanthrin **6-29**. Reagents and conditions: a) NEt_3, toluene, reflux.

A tentative mechanism for the formation of **6-28** is shown in Scheme **6.11**. Intramolecular condensation of the amino group with the methyl ester group present in

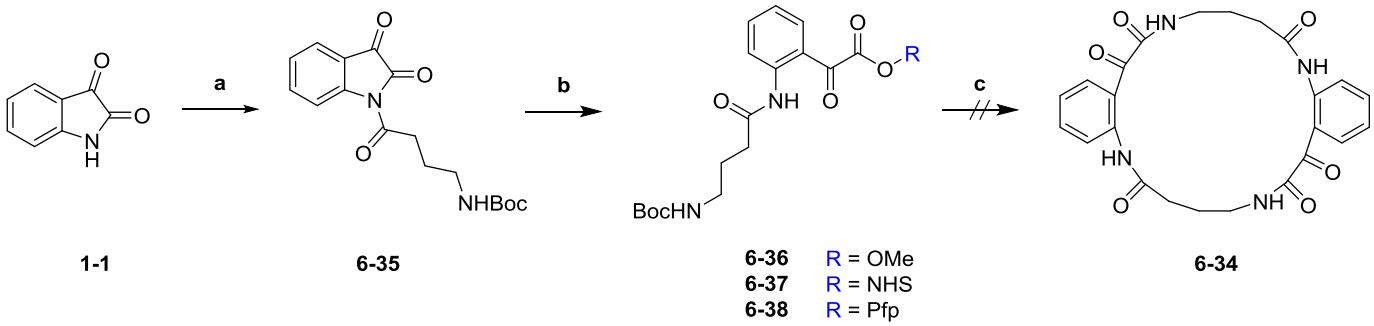
intermediate **6-27** forms a nine-membered lactam ring **6-30** with the loss of methanol. Deprotonation at N1 gives the nitrogen anion intermediate **6-31**, which undergoes intramolecular nucleophilic attack onto the amide carbonyl (C6) group to form the tricyclic alcohol intermediate **6-32**. Finally, dehydration of the intermediate **6-33** yields the fused heterocyclic system **6-28**. Notably, the choice of base used in this reaction is independent of the reaction outcome, as substituting triethylamine for DMAP and maintaining the concentration at 7.53 mM also provides **6-28** (80 % yield). Taken together, these results suggest that the choice of formation of either the eighteen-membered heterocycle **6-26** (68.4mM) or the fused tricyclic system **6-28** (7.53mM) is mainly dependent on the concentration of reactant **6-24**.



Scheme 6.11. Proposed mechanism for the formation of **6-28** from intermediate **6-27**.

With the successful synthesis of the first cyclic peptide mimic containing the *N*-glyoxylamide moiety, larger macrocycles were investigated. By substituting *N*-Boc-β-alanine with *N*-Boc-γ-aminobutyric acid, it was envisaged that a twenty-membered ring macrocycle **6-34** could be formed following the same synthetic scheme described previously. Thus, isatin **1-1** was condensed with *N*-Boc-γ-aminobutyric acid in the presence of DCC and DMAP to afford *N*-(Boc) -γ-aminobutyric **6-35** isatin in 77% yield (Scheme **6.12**). Refluxing **6-35** in

methanol provided the ring-opened methyl ester **6-36** in 72% yield. However, subjecting **6-36** to identical cyclization conditions used in the synthesis of **6-26** gave an intractable mixture of products, and the purification and identification of the product components was not pursued.



Scheme 6.12. Attempted synthesis of a twenty-membered ring macrocycle **6-34** *via* various *N*-glyoxylester intermediates. Reagents and conditions: a) *N*-Boc-γ-aminobutyric acid, DCC, DMAP b) Method 1: MeOH, reflux, Method 2: *N*-hydroxysuccinimide, $CHCl_3$, reflux, Method c: a) pentafluorophenol, $CHCl_3$, reflux.

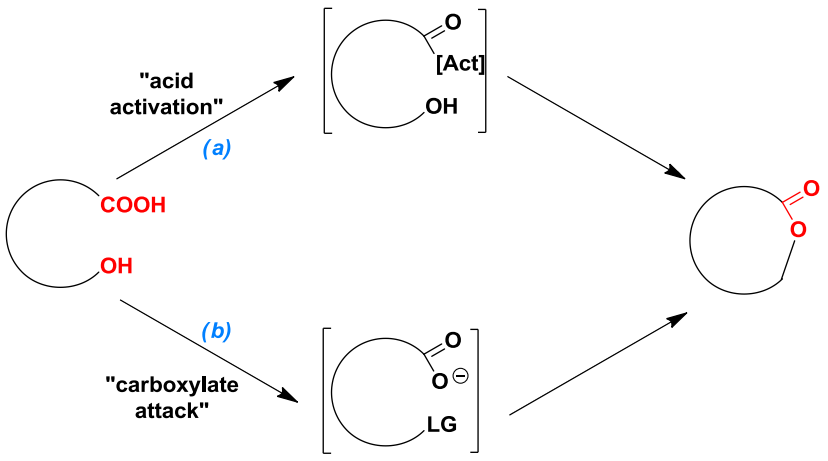
The use of methyl esters as precursors for amide formation is uncommon in contemporary peptide synthesis. Compared with many activated esters such as the pentafluorophenyl, hydroxybenzotriazole and carbodiimide esters, methyl esters are less reactive and therefore require prolonged reaction times or harsher conditions to achieve amidation and subsequent loss of methanol. To promote efficient amide bond formation, attempts were made to replace the methyl ester group with a more labile leaving group, such as succinimide or pentafluorophenol.

To investigate this approach, *N*-(Boc)-γ-aminobutyric isatin **6-35** and *N*-hydroxysuccinimide were refluxed together in chloroform (Scheme **6.12**). ^{1}H NMR analysis of the reaction mixture after 16 h showed approximately 12% conversion of **6-35** to the desired *N*-glyoxylester product **6-37**, calculated by comparison of the integrals corresponding to the Boc group present in the starting material and product. Due to the slow rate of reaction to the desired *N*-glyoxylester **6-37,** another activated ester was sought. Unfortunately, when *N*-(Boc) γ-aminobutyric isatin **6-35** and pentafluorophenol were refluxed in chloroform, TLC analysis after 16 h showed only the presence of the two reactants. The unsuccessful formation of the activated esters **6-37** and **6-38** can be

attributed to the poor nucleophilicity of both *N*-hydroxysuccinimide and pentafluorophenol, due to the presence of deactivating substituents on those nucleophiles. One general strategy, that can be employed in the future to enhance the reactivity of these alcohols is *via* the preparation of the corresponding alkoxides by deprotonation with a strong base, prior to the addition of *N*-(Boc)-γ-aminobutyric isatin **6-35**.[12,13]

6.2.3 Macrolactonization in the attempted synthesis of cyclic peptide mimics containing the *N*-glyoxylamide moiety

Macrolactonization has been used as an effective tool in the total synthesis of countless natural products. Many synthetic methodologies have been developed for this strategy, including the Yamaguchi macrocyclization where a reactive mixed anhydride is generated *in situ* using 2,4,6-trichlorobenzoyl chloride, or the Corey (Nicolaou, Brunelle) and Gerlach reactions involving the cyclization of thioesters and the use of phosphorus-based reagents to generate reactive carbon-phosphorus anhydride intermediates. These reactions can be broadly characterized into two classes based on their reaction mechanisms. The first type involves the activation of a carboxylic acid followed by the nucleophilic substitution of an alcohol (Scheme **6.13**, path ***(a)***), while in the second, a carboxylate ion performs nucleophilic attack on a leaving group (Scheme **6.13**, path ***(b)***).



Scheme 6.13. General representation of two head-to-tail macrolactamization strategies ***(a)*** acid activation ***(b)*** carboxylate attack.

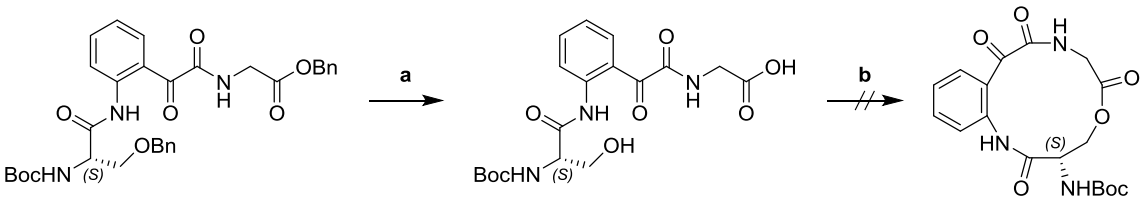
Macrolactonization for the synthesis of peptide mimics provides several advantages over the previously discussed RCM and macrolactamization strategies. If a primary alcohol is

used in the linear precursor, successful macrolactonization will produce only a single product whereas RCM can give both E and Z geometric isomers. In addition, as the precursor molecule contains two different functional groups (an alcohol and carboxylic acid as opposed to two olefins required for RCM), the number of possible oligomers is reduced. Furthermore, the primary alcohol in the macrolactonization precursor molecule is unable to participate in reactions with the ketone group forming the benzodiazepine-2-one side-product, as anticipated with the lactamization route.

6.2.3.1 Attempted side chain-to-tail macrolactonization of a linear tripeptide mimic

To install the hydroxyl group required for macrolactonization, it was envisaged that an appropriate amino acid with the desired functionality could be incorporated into the precursor molecule. Serine was chosen as it was anticipated that the second stereocentre in threonine could lead to epimerization and difficulties in determination of stereochemistry. The cyclic tripeptide **6-39** depicted in Scheme **6.14** was selected as a synthetic target. The crucial disconnection point was chosen to be between the serine side chain and the glycine carboxylic acid group to minimize the steric congestion at the site of cyclization.

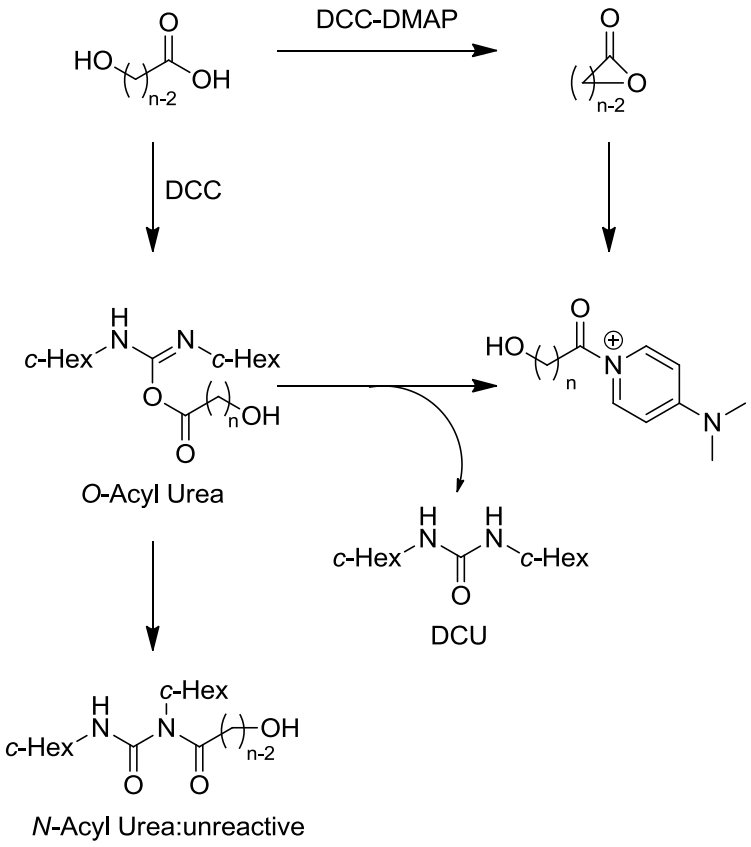
The first step in this strategy involved the deprotection of **5-27**, in order to liberate both primary alcohol and carboxylate groups. Prolonged exposure of **5-27** to typical hydrogenation conditions gave a major product **6-40** and several minor impurities as indicated by TLC analysis. Difficulties in purifying the reaction mixture by column chromatography on silica gel were encountered, due to overlap caused by the carboxylic acid group . High resolution mass spectrometric analysis showed a peak at 432.1369 which was consistent with the calculated mass of $(M\text{-}Na)^+$: 432.1383 for compound **6-40**. Finally, ^1H NMR analysis of the reaction mixture showed the loss of both benzyl protecting groups present in starting material **6-40**, suggesting the formation of the desired product **6-40**. Another minor product was also observed in the ^1H NMR spectrum, however chromatographic separation on silica gel failed to separate the two compounds. Thus, the crude product was taken to the macrolactonization step.



5-27 **6-40** **6-39**

Scheme 6.14. Attempted side chain-to-tail macrolactonization of *N*-glyoxylamide **219**. Reagents and conditions: a) H_2, 10% Pd/C, MeOH b) Method 1: DCC, DMAP, CH_2Cl_2 Method 2: DEAD, PPh_3, toluene.

Peptide coupling reagents such as DCC, PyBroP and PyBOP have been successful in the synthesis of macrolactones.[14,15,16,17] Such methodologies are classified as "acid activating" macrocyclization reactions. An initial attempt to cyclize crude **6-40** was performed under high dilution in the presence of DCC and DMAP. During the course of the reaction, a white precipitate was observed, consistent with the formation of *N'N*-dicyclohexylurea (DCU) by-product. After 36 h, the reaction was filtered and the filtrate was analyzed by TLC, which showed the presence of multiple spots with similar R_f values. Purification of the crude mixture by gravity chromatography was attempted, but the desired product **6-39** could not be detected in any of the collected fractions by ^{1}H NMR spectroscopy. Full characterization of the side products was not undertaken due to the impurity of the samples. A survey of the literature was conducted to give some insight into a possible explanation for the failed cyclization reaction. Scheme **6.15** shows the general mechanism of the DCC-DMAP macrolactonization reaction. In this mechanism, the formation of an unreactive *N*-acyl urea by-product can significantly lower the yield of the desired lactone product.[18] In a previous study, the major product in the cyclization of 15-hydroxypentadecanoic acid was the *N*-acyl urea byproduct, while the hexadecanolide was only isolated in 4% yield.[19]



Scheme 6.15. General reaction mechanism of the DCC-DMAP macrolactonization reaction. Formation of an unreactive *N*-acyl Urea byproduct is commonly observed.[18]

6.2.3.2 Attempted macrolactonization *via* the Mitsunobu reaction

Owing to the failure of the DCC-DMAP protocol reported above, an alternative approach was adopted. The Mitsunobu reaction has been used successfully in the cyclization of a number of structurally diverse 11- to 16-membered macrolactones. This methodology is based on the activation of the seco-acid alcohol using diethyl azodicarboxylate (DEAD) and triphenylphosphine. Following a procedure reported by Emmer et al., the crude precursor **6-40** was subjected to Mitsunobu conditions (Scheme **6.14**).[20] After 36 h, the reaction mixture was concentrated *in vacuo* and the crude product was subjected to column chromatography on silica gel. However, the desired macrocycle **6-39** could not be detected by ^{1}HNMR spectroscopic analysis in any of the individual fractions. One of the major drawbacks associated with the classical Mitsunobu reaction conditions (i.e. PPh_3-DEAD, toluene) is the formation of hydrazine by-products.[21] A future strategy for

overcoming this problem is to replace DEAD with the sterically hindered azodicarboxylate DIAD.

6.3 Summary and future work

The RCM reactions of precursors **6-6** and **6-10** were investigated for the synthesis of cyclic peptide mimics containing the *N*-glyoxylamide pseudo-amino acid. Purification of the resulting reaction mixtures by column chromatography on silica gel was problematic due to the sheer number of structurally similar products. Attempts to synthesize the novel tricyclic benzodiazepine-2-one **6-16** also met with the same outcome. Subsequent molecular modelling (MM2 minimization) indicated the presence of significant ring-stain in the proposed target **6-16**.

The synthesis of the linear macrolactamization intermediate **6-17** gave poor yields. Hence, the macrolactamization reaction scheme proposed for the synthesis of target macrocycle **6-18** was abandoned. It was conceived that the basic conditions required to cyclize **6-17** would also promote competing intramolecular cyclization reactions, giving rise to **6-22** as a major side product.

The synthesis of the first cyclic peptide mimic **6-26** containing the *N*-glyoxylamide moiety was achieved *via* the deprotection and cyclization of **6-24**. The concentration of the reactant was found to be the key factor in determining the outcome of the reaction. A lower concentration (7.53 mM) of **6-24** promoted the formation of **6-28**, while higher concentrations (68.4 M) favored the formation of **6-26**. To further investigate this novel scaffold, future work will focus on synthesizing derivatives possessing greater solubility in organic solvents. Structural variants of cyclic peptide mimic **6-26** can be accessed through varying the starting isatin **1-1** and β-amino acid molecules. Future direction will also be directed towards extending the structural diversity of these compounds by developing a procedure for the synthesis of unsymmetrical cyclic peptide mimics.

Attempted macrolactonization of precursors **6-40** *via* a DCC-DMAP protocol or through the Mitsunobu reaction both failed to give the desired cyclic product **6-39**, possibly due to competing side reactions. Further work is required to determine the causes of these failed reactions. The design elements of future macrocyclic targets must be carefully considered in order to maximize the success of this challenging reaction

6.4 Experimental

6.4.1 General methods

Synthesis and reagents

A detailed account of general synthetic methodologies is provided in the experimental section for **Chapter 2**. Further details relevant to this chapter are presented below.

Spectroscopy

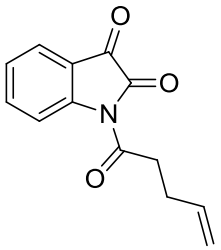
A detailed account of general spectroscopic methodologies is provided in the experimental section for **Chapter 2**.

Crystallography

Single crystal X-Ray diffraction data for **6-28** were obtained on the MX-1 beamline at the Australian Synchrotron. Structures were processed and refined using SHELX software.

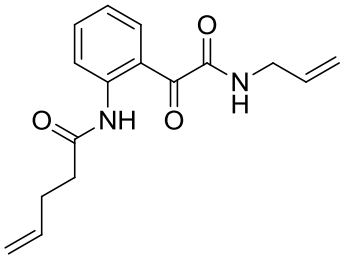
6.4.2 Synthesis

1-(Pent-4-enoyl)indoline-2,3-dione **6-5**



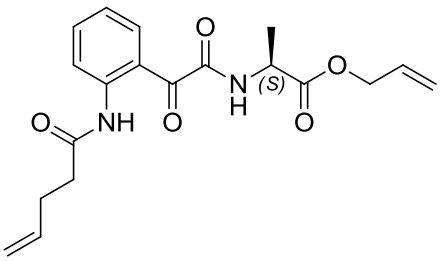
A mixture of isatin **1-1** (0.500 g, 3.40 mmol), 4-pentenoic acid (0.458 mL, 4.08 mmol), DCC (0.841 g, 4.08 mmol) and DMAP (0.083 g, 0.697 mmol) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, *N*,*N*-dicyclohexylurea was removed by filtration and the filtrate was subjected to flash chromatography on silica gel (dichloromethane). The crude product was recrystallized from a mixture of diethyl ether and n-hexane to afford **6-5** as bright yellow needles (0.625 g, 80 %). M.p. 93-96°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.44-2.51 (m, 2H, $COCH_2C\underline{H_2}$), 3.19 (t, *J* = 7.3 Hz, 2H, $CO\underline{CH_2}CH_2$), 5.00-5.05 (m, 1H, $CH=C\underline{H_A}H_B$), 5.08-5.12 (m, 1H, $CH=CH_A\underline{H_B}$), 7.31 (ddd, *J* = 7.6, 7.6, 0.9 Hz, 1H, ArH), 7.69 (ddd, *J* = 8.4, 7.6, 1.4 Hz, 1H, ArH), 7.74 (dd, *J* = 7.6, 1.4 Hz, 1H, ArH), 8.38 (dd, *J* = 8.4, 0.9 Hz, 1H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.0 ($COCH_2\underline{C}H_2$), 37.6 ($CO\underline{C}H_2CH_2$), 116.1 ($CH=\underline{C}H_2$), 118.3 (ArCH), 119.3 (ArC), 125.4 (ArCH), 126.2 (ArCH), 136.4 ($\underline{C}H=CH_2$), 139.0 (ArCH), 148.8 (ArC), 157.9 ($CO\underline{C}ON$), 172.3 ($\underline{C}OCH_2$), 180.2 ($\underline{C}OCON$); IR (ATR): ν_{max} 3132, 3081, 3010, 2913, 1778, 1747, 1701, 1639, 1602, 1455, 1386, 1330, 1299, 1253, 1206, 1155, 1126, 1083, 996, 921, 815, 758, 741 cm $^{-1}$. HRMS (+ESI): Found *m/z* 252.0644, $[M+Na]^+$, $C_{13}H_{11}NO_3Na$ requires 252.0637.

N-(2-(2-(Allylamino)-2-oxoacetyl)phenyl)pent-4-enamide **6-6**



N-Acylisatin **6-5** (1.11 g, 4.82 mmol) and allylamine (0.380 mL, 5.07 mmol) were stirred at room temperature in dichloromethane for 2 h. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel (3:7 ethyl acetate/n-hexane). Recrystallization from a mixture of ethyl acetate and n-hexane afforded **6-6** as colourless needles (1.12 g, 81%). M.p. 86°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40-2.56 (m, 4H, $COCH_2$ + $NHCH_2CH$), 4.02 (td, *J* = 5.9, 1.5 Hz, 2H CH_2CH_2CH), 4.97-5.34 (m, 4H, 2 x $CH=CH_2$), 5.77-5.97 (m, 2H, 2 x $CH=CH_2$), 7.00-7.17 (m, 2H, ArH + $CONHCH_2$), 7.56 (ddd, *J* = 8.5, 7.4, 1.7 Hz, 1H, ArH), 8.31 (dd, *J* = 8.1, 1.7 Hz, 1H, ArH), 8.63 (dd, *J* = 8.5, 1.2 Hz, 1H, ArH), 10.97 (s, 1H, CONHAr); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.3 ($COCH_2CH_2$), 37.8 ($COCH_2CH_2$), 41.9 ($NHCH_2CH$), 116.0 ($CH=CH_2$), 117.5 ($CH=CH_2$), 118.7 (ArC), 120.8 (ArCH), 122.7 (ArCH), 132.9 (ArCH), 134.5 ($CH=CH_2$), 136.6 ($CH=CH_2$), 136.7 (ArCH), 142.2 (ArC), 162.9 ($COCONH$), 171.7 ($COCH_2$), 192.1 ($COCONH$); IR (ATR): ν_{max} 3285, 3078, 2918, 1656, 1605, 1524, 1477, 1419, 1364, 1308, 1253, 1211, 1115, 993, 920, 847, 791, 756, 695 cm $^{-1}$; HRMS (+ESI): Found *m/z* 287.1396, $[M+H]^+$, $C_{16}H_{19}N_2O_3$ requires 287.1396.

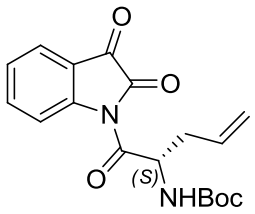
(*S*)-Allyl 2-(2-oxo-2-(2-(pent-4-enamido)phenyl)acetamido)propanoate **6-10**



N-Acylisatin **6-5** (0.703 g, 3.07 mmol) was added to a solution of the L-alanine allylester *p*-toluene sulfonate (1.11 g, 3.68 mmol) and *N,N*-diisopropylethylamine (0.640 mL,

3.68 mmol) in dichloromethane. The reaction was stirred at room temperature for 3h and the crude reaction mixture was subjected to flash chromatography on silica gel (2:3 ethyl acetate/n-hexane) to afford the title compound **6-10** as a pale yellow amorphous solid (0.963 g, 88 %). M.p. 48-50°C; $[\alpha]_D$ -6.8 (*c* 0.15, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (d, *J* = 7.2 Hz, 3H, CH_3), 2.45-2.57 (m, 4H, $COC\underline{H_2}C\underline{H_2}$), 4.62-4.75 (m, 3H, $C\underline{H}CH_3$ + OCH_2), 5.03 (dd, *J* = 10.4, 1.3 Hz, 1H, $CH=C\underline{H_A}H_B$), 5.11 (dd, *J* = 17.2, 1.5 Hz, 1H, $CH=C\underline{H_c}H_d$), 5.29 (dd, *J* = 10.4, 1.3 Hz, 1H, $CH=CH_A\underline{H_B}$), 5.37 (dd, *J* = 17.2, 1.5 Hz, 1H, $CH=CH_c\underline{H_d}$), 5.81-5.99 (m, 2H, $C\underline{H}=CH_AH_B$ + $C\underline{H}=CH_cH_d$), 7.12 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H, ArH), 7.36 (bd, *J* = 7.4 Hz, 1H, $N\underline{H}CH$), 7.61 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H, ArH), 8.35 (dd, *J* = 8.1, 1.6 Hz, 1H, ArH), 8.70 (dd, *J* = 8.6, 1.2 Hz, 1H, ArH), 11.00 (s, 1H, $CON\underline{H}Ar$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 18.2 (CH_3), 29.4 ($COCH_2\underline{C}H_2$), 37.9 ($CO\underline{C}H_2CH_2$), 48.5 (NHCH), 66.5 (OCH_2), 116.0 ($CH=\underline{C}H_2$), 118.5 (ArC), 119.3 ($CH=\underline{C}H_2$), 120.9 (ArCH), 122.7 (ArCH), 131.4 (ArCH), 134.6 (ArCH), 136.6 (ArCH), 136.9 (ArCH), 142.4 (ArC), 162.4 ($CO\underline{C}ONH$), 171.7 ($\underline{C}OCH_2$), 171.8 ($\underline{C}O_2CH_2$), 191.3 ($\underline{C}OCONH$); IR (ATR): ν_{max} 3332, 3265, 3074, 2985, 2938, 1740, 1652, 1576, 1511, 1445, 1365, 1308, 1274, 1209, 1167, 1110, 1051, 991, 928, 793, 757 cm $^{-1}$; HRMS (+ESI): Found *m/z* 381.1430, $[M+Na]^+$, $C_{19}H_{22}N_2NaO_9$ requires 381.1426.

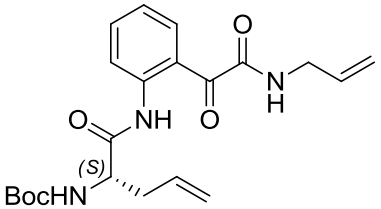
(*S*)-*tert*-Butyl (1-(2,3-dioxoindolin-1-yl)-1-oxopent-4-en-2-yl)carbamate **6-14**



A mixture of isatin **1-1** (0.146 g, 1.00 mmol), (*S*)-*N*-Boc-allylglycine (0.236 g, 1.09 mmol), DCC (0.226 g, 1.09 mmol) and DMAP (0.012 g, 0.100 mmol) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, *N*,*N*-dicyclohexylurea was removed by filtration and the filtrate was subjected to flash chromatography on silica gel (dichloromethane → 2:3 ethyl acetate/n-hexane). The crude product was precipitated from a mixture of ethyl acetate and n-hexane to afford **6-14** as a dull yellow powder (0.277 g, 81 %). M.p. 158-160°C; $[\alpha]_D$ -43 (*c* 0.046, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (s, 9H,

C($C\underline{H}_3$)$_3$), 2.41-2.49 (m, 1H, CHC$\underline{H}_A$H$_B$), 2.64-2.73 (m, 1H, CHCH$_A\underline{H}_B$), 5.14-5.23 (m, 3H,NH + CH=C$\underline{H}_2$), 5.47-5.59 (m, 1H, $\underline{C}$OCH), 5.74-5.86 (m, 1H, $\underline{C}$H=CH$_2$), 7.37 (ddd, *J* = 7.8, 7.5, 0.7 Hz, 1H, ArH), 7.73 (ddd, *J* = 8.3, 7.8, 0.9 Hz, 1H, ArH), 7.81 (dd, *J* = 7.5, 0.9 Hz, 1H, ArH), 8.40 (dd, *J* = 8.3, 0.7 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4 (C($\underline{C}$H$_3$)$_3$), 36.3 (CH$\underline{C}$H$_2$), 54.5 (CH), 80.4 ($\underline{C}$(CH$_3$)$_3$), 118.5 (ArCH), 119.8 (ArC), 119.9 (CH=$\underline{C}$H$_2$), 125.6 (ArCH), 126.6 (ArCH), 132.0 ($\underline{C}$H=CH$_2$), 139.1 (ArCH), 148.4 (ArC), 155.5 (OCONH), 157.5 (CO$\underline{C}$ON), 172.7 ($\underline{C}$OCH), 179.6 ($\underline{C}$OCON); IR (ATR): ν_{max} 3339, 3078, 2977, 1778, 1747, 1719, 1685, 1606, 1523, 1461, 1364, 1246, 1164, 1047, 994, 955, 906, 791, 754 cm^{-1}; HRMS (+ESI): Found *m/z* 367.1267, [M+Na]$^+$, $C_{18}H_{20}N_2NaO_5$ requires 367.1270.

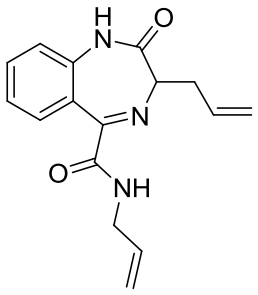
(S)-*tert*-Butyl (1-((2-(2-(allylamino)-2-oxoacetyl)phenyl)amino)-1-oxopent-4-en-2-yl)carbamate **6-13**



N-(Boc)-L-allylglycine-isatin **6-14** (0.223 g, 0.657 mmol) and allylamine (0.0533 mL, 0.712 mmol) were stirred at room temperature in dichloromethane for 2 h. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel (2:3 ethyl acetate/n-hexane) to afford **6-13** as a pale yellow amorphous solid (0.209 g, 80 %). M.p. 122-124°C; $[\alpha]_D$ -147 (*c* 0.041, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H, C($C\underline{H}_3$)$_3$), 2.53-2.69 (m, 2H, CHC$\underline{H}_A\underline{H}_B$), 4.03 (tt, *J* = 5.9, 1.6 Hz, 2H, NHC$\underline{H}_2$), 4.29-4.43 (m, 1H, N$\underline{H}$CH), 5.06-5.21 (m, 3H, CH=C$\underline{H}_A\underline{H}_B$ + COC$\underline{H}$), 5.23 (qd, *J* = 10.3, 1.4 Hz, 1H, CH=C$\underline{H}_A$H$_B$), 5.29 (qd, *J* = 17.2, 1.4 Hz, 1H, CH=CH$_A\underline{H}_B$), 5.71-5.80 (m, 1H, C$\underline{H}$=CH$_A$H$_B$), 5.85-5.94 (m, 1H, C$\underline{H}$=CH$_A$H$_B$), 6.86-6.98 (m, 1H, N$\underline{H}$CH$_2$), 7.17 (ddd, *J* = 8.3, 7.3, 1.1 Hz, 1H, ArH), 7.61 (ddd, *J* = 8.7, 7.3, 1.7 Hz, 1H, ArH), 8.35-8.48 (m, 1H, ArH), 8.68 (dd, *J* = 8.3, 1.7 Hz, 1H, ArH), 11.41 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.5 (C($\underline{C}$H$_3$)$_3$), 37.0 (CH$\underline{C}$H$_2$), 42.0 (NHCH$_2$), 55.3 ($\underline{C}$HCH$_2$), 80.5 ($\underline{C}$(CH$_3$)$_3$), 117.6 (CH=$\underline{C}$H$_2$), 119.4 (ArC), 119.7 (CH=$\underline{C}$H$_2$), 121.0 (ArCH), 123.1 (ArCH), 132.7 ($\underline{C}$H=CH$_2$), 133.0 ($\underline{C}$H=CH$_2$), 134.6 (ArCH), 136.7 (ArCH), 141.6 (ArC), 155.6 (OCONH), 162.6 (CO$\underline{C}$ONH), 171.1 ($\underline{C}$ONHAr), 191.5 ($\underline{C}$OCONH); IR (ATR): ν_{max} 3338, 3077,

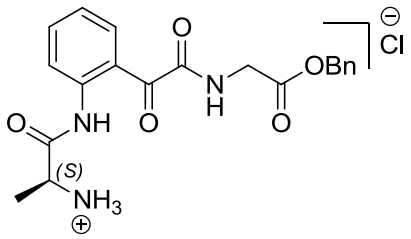
2981, 2921, 1689, 1632, 1582, 1509, 1444, 1321, 1250, 1213, 1164, 1058, 989, 906, 859, 830, 808 cm^{-1}; HRMS (+ESI): Found *m/z* 402.2015, $[M+H]^+$, $C_{33}H_{15}N_3NaO_9$ requires 402.2029.

N,3-Diallyl-2-oxo-2,3-dihydro-1H-benzo[b]azepine-5-carboxamide **6-15**



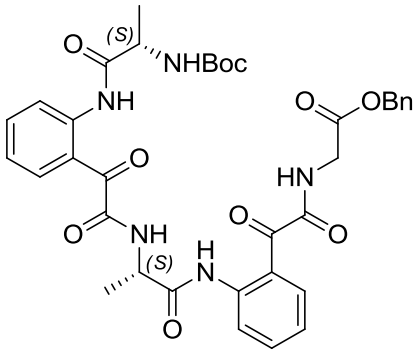
N-Glyoxylamide **6-13** (0.1793 g, 0.447 mmol) was stirred in TFA-CH_2Cl_2 (20% v/v, 10mL) at room temperature for 0.5h. The solvent was removed *in vacuo.* The resulting yellow residue was redissolved in methanol, triethylamine (excess) was added and the solution was left to stir for an additional hour. The solvent was removed *in vacuo* and the crude material was subjected to flash chromatography on silica gel (2:3 ethyl acetate/n-hexane) to afford **6-15** as a pale yellow amorphous solid (0.122 g, 97 %). M.p. 120°C; $[\alpha]_D$ 29 (*c* 0.10, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.87-2.95 (m, 1H, CHC$\underline{H}_A$H$_B$), 2.95-3.02 (m, 1H, CHCH$_A$$\underline{H}_B$), 3.62 (ddd, *J* = 8.0, 6.1, 2.1 Hz, 1H, C$\underline{H}$CH$_A$H$_B$), 3.97-4.08 (m, 2H, NHC$\underline{H}_2$), 5.08-5.13 (m, 1H, CH=C$\underline{H}_2$), 5.14-5.23 (m, 2H, 2 x CHC$\underline{H}_2$), 5.25-5.31 (m, 1H, CH=C$\underline{H}_2$), 5.86-5.97 (m, 2H, 2 x C$\underline{H}$=CH$_2$), 7.10 (dd, *J* = 8.1, 0.9 Hz, 1H, ArH), 7.26 (ddd, *J* = 8.4, 7.4, 1.1 Hz, 1H, ArH), 7.51 (ddd, *J* = 8.1, 7.4, 0.9 Hz, 1H, ArH), 7.74 (bt, *J* = 5.3 Hz, 1H, N$\underline{H}$CH$_2$), 7.95 (dd, *J* = 8.4, 0.9 Hz, 1H, ArH), 8.87 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (175 MHz, $CDCl_3$): δ 35.2 ($\underline{C}$H$_2$CH=CH$_2$), 42.2 ($\underline{C}$H$_2$CH=CH$_2$), 63.2 (CO$\underline{C}$HN), 116.8 ($\underline{C}$H=CH$_2$), 117.7 ($\underline{C}$H=CH$_2$), 121.1 (CH=$\underline{C}$H$_2$), 123.7 (CH=$\underline{C}$H$_2$), 123.8 (ArC), 131.7 (ArCH), 132.4 (ArCH), 133.8 (ArCH), 134.8 (ArCH), 138.1 (ArC), 161.8 (NC$\underline{C}$ONH), 162.8 (Ar$\underline{C}$NCH), 170.6 (NH$\underline{C}$OCH); IR (ATR): ν_{max} 3389, 3190, 3118, 3074, 2961, 2917, 1685, 1609, 1499, 1472, 1434, 1381, 1316, 1196, 1139, 1074, 1002, 912, 802, 754, 702 cm^{-1}; HRMS (+ESI): Found *m/z* 306.1213, $[M+Na]^+$, $C_{16}H_{17}N_3NaO_2$ requires 306.1218.

(*S*)-1-((2-(2-((2-(benzyloxy)-2-oxoethyl)amino)-2-oxoacetyl)phenyl)amino)-1-oxopropan-2-aminium hydrochloride salt **6-20**



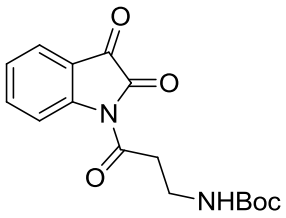
N-(Boc)-L-Ala-isatin **5-4** (3.21 g, 10.1 mmol) was added to a solution of the glycine benzylester *p*-toluene sulfonate (4.80 g, 14.1 mmol) and *N,N*-diisopropylethylamine (2.46 mL, 14.1 mmol) in dichloromethane. The reaction was stirred at room temperature for 3h and the crude reaction mixture was subjected to flash chromatography on silica gel, eluting with 2:3 ethyl acetate/n-hexane. The resulting pale yellow solid was dissolved in diethyl ether and hydrogen chloride gas (produced by adding concentrated sulfuric acid onto NaCl) was bubbled through the solution for 0.5 h. The solid was collected by filtration to afford the title compound **6-20** as a tan amorphous powder (3.64 g, 86%). M.p. 96-97°C; $[\alpha]_D$ -35 (*c* 0.28, CH_2Cl_2); ^{1}H NMR (150 MHz, d_6-DMSO): δ 1.47 (d, *J* = 7.0 Hz, 3H, CH_3), 4.08 (d, *J* = 6.0 Hz, 2H, CH_2), 4.12-4.21 (m, 1H, C<u>H</u>CH_3), 5.19 (s, 2H, OCH_2), 7.29 (ddd, *J* = 8.1, 7.8, 1.1 Hz, 1H, ArH), 7.31-7.43 (m, 5H, 5 x ArH), 7.68 (ddd, *J* = 7.8, 7.7, 1.6 Hz, 1H, ArH), 7.71 (dd, *J* = 7.7, 1.2 Hz, 1H, ArH), 7.81 (dd, *J* = 8.1, 1.6 Hz, 1H, ArH), 8.33-8.41 (m, 3H, NH_3), 9.23 (bt, *J* = 6.0 Hz, 1H, N<u>H</u>CH_2), 10.98 (s, 1H, CON<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 16.5 (CH_3), 40.8 (CO<u>C</u>H_2), 48.9 ($CHCH_3$), 66.2 (O<u>C</u>H_2Bn), 122.7 (ArCH), 124.5 (ArCH), 125.0 (ArC), 128.1 (ArCH), 128.2 (ArCH), 128.5 (ArCH), 131.8 (ArCH), 134.2 (ArCH), 135.8 (ArC), 137.0 (ArC), 163.8 (CO<u>C</u>ONH), 168.6 (CO_2<u>Bn</u>), 169.1 (<u>C</u>ONHAr), 190.4 (<u>C</u>OCONH); IR (ATR): ν_{max} 3326, 2943, 2872, 1747, 1655, 1577, 1523, 1477, 1449, 1385, 1286, 1237, 1185, 1102, 1030, 756 cm^{-1}; HRMS (+ESI): Found *m/z* 384.1553, $[M]^+$, $C_{20}H_{22}N_3O_5$ requires 384.1554.

Benzyl 2-(2-(2-((S)-2-(2-(2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetamido)propanamido)phenyl)-2-oxoacetamido)acetate **6-21**



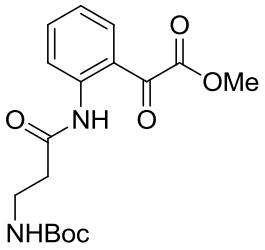
N-(Boc)-L-Ala-isatin **5-4** (0.728 g, 2.29 mmol) was added to a solution of the hydrochloride salt **6-20** (1.44 g, 3.43 mmol) and *N,N*-diisopropylethylamine (0.596 mL, 3.43 mmol) in dichloromethane. The reaction was stirred at room temperature for 3 h and the crude reaction mixture was subjected to flash chromatography on silica gel (eluting with 2:98 DCM/MeOH). Precipitation from a hot solution of methanol afforded the title compound **6-21** as an off-white amorphous solid (0.415 g, 26 %). M.p. 178-180°C; $[\alpha]_D$ -90 (*c* 0.055, CH_2Cl_2); ^{1}H NMR (600 MHz, d_6-DMSO): δ 1.30 (d, *J* = 7.1 Hz, 3H, CH_3), 1.39 (s, 9H, C(CH_3)$_3$), 1.44 (d, *J* = 7.1 Hz, 3H, CH_3), 4.00-4.08 (m, 1H, COCH), 4.10 (d, *J* = 6.0 Hz, 2H, $COCH_2NH$), 4.59 (quin, *J* = 6.9 Hz, 1H, COCH), 5.19 (s, 2H, CH_2Ph), 7.22 (tt, J = 7.6 Hz, 1.5 2H, 2 x ArH), 7.30-7.44 (m, 5H, 5 x ArH), 7.52 (d, *J* = 6.2 Hz, 1H, ArH), 7.66 (m, 2H, 2 x ArH), 7.80 (dd, *J* = 7.9, 1.6 Hz, 1H, ArH), 7.85 (dd, *J* = 8.0, 1.2 Hz, 1H, ArH), 8.21 (d, *J* = 8.4 Hz, 1H, ArH), 8.44 (bd, *J* = 8.5 Hz, 1H, NH), 9.31 (bt, *J* = 5.9 Hz, 1H, $NHCH_2$), 9.40 (bd, *J* = 6.9 Hz, 1H, NH), 11.04 (s, 1H, CONHAr), 11.30 (s, 1H, CONHAr); ^{13}C NMR (150 MHz, d_6-DMSO): δ 17.1 (CH_3), 17.2 (CH_3), 28.2 (C(CH_3)$_3$), 40.6 (CH_2), 49.7 ($CHCH_3$), 51.4 ($CHCH_3$), 66.2 (OCH_2Ph), 78.6 ($C(CH_3)_3$), 120.1 (ArCH), 121.2 (ArCH), 121.7 (ArC), 123.0 (ArCH), 123.5 (ArCH), 127.9 (ArC), 128.1 (ArCH), 128.2 (ArCH), 128.5 (ArCH), 132.8 (ArCH), 133.6 (ArCH), 135.2 (ArCH), 135.5 (ArC), 135.7 (ArCH), 139.2 (ArC), 140.1 (ArC), 155.4 (OCONH), 157.0 (COCONH), 164.7 (COCONH), 169.0 (CO_2Bn), 170.8 (CONHAr), 172.7 (CONHAr), 192.2 (COCON), 192.8 (COCON); IR (ATR): ν_{max} 3286, 2985, 2932, 1684, 1639, 1585, 1515, 1449, 1369, 1299, 1254, 1215, 1164, 1100, 1065, 1000, 903, 747, 696 cm^{-1}; HRMS (+ESI): Found *m/z* 724.2579, $[M+Na]^+$, $C_{36}H_{39}N_5O_{10}Na$ requires 724.2595.

tert-Butyl (3-(2,3-dioxoindolin-1-yl)-3-oxopropyl)carbamate **6-23**



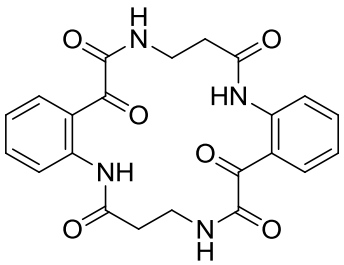
A mixture of isatin **1-1** (2.54 g, 17.3 mmol), *N*-Boc-β-alanine (4.57 g, 24.2 mmol), DCC (4.98 g, 24.2 mmol) and DMAP (0.210 g, 1.73 mmol) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, *N*,*N*-dicyclohexylurea was removed by filtration and the filtrate was subjected to flash chromatography on silica gel (dichloromethane → 2:3 ethyl acetate/n-hexane). The crude product was precipitated from a mixture of ethyl acetate and n-hexane to afford **6-23** as a bright yellow powder (4.21 g, 77 %). M.p. >108°C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, C($\underline{C}H_3)_3$), 3.32 (t, *J* = 5.9 Hz, 2H, NH$\underline{C}H_2$), 3.57 (q, *J* = 5.9 Hz, 2H, $COCH_2$), 5.02 (bt, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.35 (ddd, *J* = 7.6, 7.5, 0.9 Hz, 1H, ArH), 7.73 (ddd, *J* = 8.4, 7.6, 1.5 Hz, 1H, ArH), 7.79 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH), 8.42 (dd, *J* = 8.4, 0.9 Hz, 1H, ArH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.5 (C($\underline{C}H_3)_3$), 35.6 ($COCH_2\underline{C}H_2$), 39.4 ($CO\underline{C}H_2CH_2$), 79.8 ($\underline{C}(CH_3)$), 118.4 (ArCH), 119.5 (ArC), 125.5 (ArCH), 126.5 (ArCH), 139.1 (ArCH), 148.6 (ArC), 156.0 (OCONH), 158.0 ($CO\underline{C}ON$), 171.9 ($\underline{C}OCH_2$), 180.0 ($\underline{C}OCON$); IR (ATR): ν_{max} 3367, 3127, 2977, 2932, 2881, 1779, 1740, 1680, 1607, 1532, 1449, 1388, 1339, 1161, 1073, 990, 912, 862, 760 cm^{-1};UV–vis (CH_3CN): λ_{max} 235 nm (ε 24,400 cm^{-1} M^{-1}), 295 (4,390); HRMS (+ESI): Found *m/z* 341.1105, $[M+Na]^+$, $C_{16}H_{18}N_2NaO_5$ requires 341.1113.

Methyl 2-(2-(3-((*tert*-butoxycarbonyl)amino)propanamido)phenyl)-2-oxoacetate **6-24**



A solution of *N*-(Boc)-β-alanine-isatin **6-23** (2.00 g, 6.28 mmol) in methanol was heated at reflux in methanol for 3 h. The solvent was removed *in vacuo* and the crude material was dissolved in the minimum volume of diethyl ether. An excess of n-hexane was then added to precipitate the title compound **6-24** as a pale yellow amorphous solid (2.16 g, 98 %). M.p. 62°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 9H, C($C\underline{H}_3)_3$), 2.68 (t, *J* = 5.9 Hz, 2H, NHC$\underline{H}_2$), 3.49 (q, *J* = 5.9 Hz, 2H, $COCH_2$), 3.98 (s, 3H, OCH_3), 5.16 (bt, *J* = 5.9 Hz, 1H, N$\underline{H}CH_2$), 7.15 (ddd, *J* = 8.0, 7.4, 1.1 Hz, 1H, ArH), 7.63 (ddd, *J* = 8.6, 7.4, 1.6 Hz, 1H, ArH), 7.67 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.84 (dd, *J* = 8.6, 1.1 Hz, 1H, ArH), 11.06 (s, 1H, CON$\underline{H}$Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.5 (C($C\underline{H}_3$)), 36.4 ($COCH_2\underline{C}H_2$), 38.2 ($CO\underline{C}H_2CH_2$), 53.2 (OCH_3), 79.5 ($\underline{C}(CH_3)$), 117.3 (ArC), 120.9 (ArCH), 122.9 (ArCH), 133.8 (ArCH), 137.3 (ArCH), 142.5 (ArC), 156.0 (OCONH), 163.9 ($COCH_2$), 171.3 ($CO\underline{C}OCH_3$), 190.3 ($\underline{C}OCOCH_3$); IR (ATR): ν_{max} 3361, 2981, 2932, 1735, 1682, 1639, 1580 1510, 1445, 1387, 1324 1268, 1154, 998, 866, 808, 758, 675 cm^{-1}; HRMS (+ESI): Found *m/z* 373.1361, $[M+Na]^+$, $C_{17}H_{22}N_2NaO_6$ requires 373.1376.

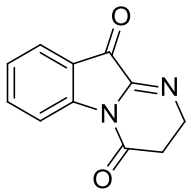
8,9,19,20-Tetrahydrodibenzo[f,o][1,5,10,14]tetraazacyclooctadecine-6,10,11,17,21,22(5H,7H,16H,18H)-hexaone **6-26**



N-Glyoxylamide **6-24** (0.120 g, 0.342 mmol) was stirred in TFA-CH_2Cl_2 (5 mL, 20% v/v) at room temperature for 0.5h. The solvent was removed *in vacuo.* The resulting yellow

residue was redissolved in methanol (5 mL), triethylamine (excess) was added and the solution was left to stir for an additional 16 h. The crude solid was collected by filtration and dissolved in hot *N*-methyl-2-pyrrolidone. Cooling of the solution gave a precipitate which was collected by filtration to give the title compound **6-26** as an off-white amorphous powder (0.036 g, 24 %). M.p. > 300°C; ^{1}H NMR (600 MHz, d_6-DMSO): δ 2.57 (t, *J* = 5.4 Hz, 4H, 2 x NHC<u>H</u>$_2$), 3.42 (q, *J* = 5.4 Hz, 4H, 2 x COC<u>H</u>$_2$), 7.28 (ddd, *J* = 7.8, 7.6, 1.2 Hz, 2H, 2 x ArH), 7.51 (dd, *J* = 8.9, 1.6 Hz, 2H, 2 x ArH), 7.60 (ddd, *J* = 8.9, 7.6, 1.6 Hz, 2H, 2 x ArH), 7.68 (dd, *J* = 7.8, 1.2 Hz, 2H, 2 x ArH), 8.39 (t, *J* = 5.4 Hz, 2H, 2 x N<u>H</u>CH$_2$), 10.46 (s, 2H, 2 x CON<u>H</u>Ar); ^{13}C NMR (150 MHz, d_6-DMSO): δ 35.2 (CH_2), 35.7 (CH_2), 122.9 (ArCH), 124.5 (ArCH), 127.2 (ArC), 130.7 (ArCH), 133.4 (ArCH), 136.8 (ArC), 162.8 (CO<u>C</u>ONH), 171.2 (<u>C</u>OCH$_2$), 189.8 (<u>C</u>OCONH); IR (ATR): ν_{max} 3297, 2948, 1688, 1647, 1581, 1523, 1451, 1356, 1319, 1292, 1255, 1210, 1166, 967, 910, 879, 834, 798, 766, 727, 676 cm^{-1}; HRMS (+ESI): Found *m/z* 459.1266, $[M+Na]^+$, $C_{22}H_{20}N_4NaO_6$ requires 459.1281.

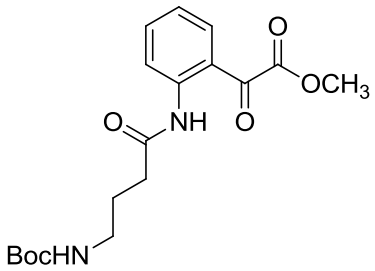
2,3-Dihydropyrimido[1,2-a]indole-4,10-dione **6-28**



N-Glyoxylamide **6-24** (0.108 g, 0.339 mmol) was stirred in TFA-CH_2Cl_2 (5mL, 20% v/v) at room temperature for 0.5h. The solvent was removed *in vacuo.* The resulting yellow residue was redissolved in DCM (45 mL), DMAP (0.0041g, 0.034 mmol) was added and the solution was left to stir for an additional 16 h. The organic layer was diluted with DCM and washed with HCl (15 mL, aq., 1 M) and water. The organic extract was dried over anhydrous Na_2SO_4, filtered and the solvent was removed *in vacuo*. The solid residue was recrystallized from hot toluene to give the title compound **6-28** as pale yellow needles (0.053 g, 78 %). M.p. >145°C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.75 (t, *J* = 7.4 Hz, 2H, $COCH_2$), 4.20 (t, *J* = 7.4 Hz, 2H, NCH_2), 7.34 (ddd, *J* = 7.7, 7.5, 0.9 Hz, 1H, ArH), 7.79 (ddd, *J* = 8.2, 7.5, 1.4 Hz, 1H, ArH), 7.85 (dd, *J* = 7.7, 1.4 Hz, 1H, ArH), 8.33 (dd, *J* = 8.2, 0.9 Hz, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.2 ($COCH_2$), 46.5 (NCH_2), 117.2 (ArCH), 121.9 (ArC), 125.0 (ArCH), 125.9

(ArCH), 138.4 (ArCH), 146.4 (ArC), 147.5 (C=N), 165.4 (CON), 181.8 (COAr); IR (ATR): ν_{max} 2969, 2894, 1700, 1673, 1603, 1526, 1375, 1300, 1219, 1160, 1002, 929, 867, 804, 755, 685 cm^{-1}; HRMS (+ESI): Found *m/z* 223.0480, $[M+Na]^+$, $C_{11}H_8N_2NaO_2$ requires 223.0483

Methyl 2-(2-(4-((tert-butoxycarbonyl)amino)butanamido)phenyl)-2-oxoacetate **6-36**



A mixture of isatin **1-1** (4.26 g, 29.0 mmol), 4-(*tert*-butoxycarbonylamino)butyric acid (8.24 g, 40.6 mmol), DCC (8.37 g, 40.6 mmol) and DMAP (0.354 g, 2.90 mmol) was stirred at 0 °C for 20 min under an inert atmosphere. The reaction was brought up to room temperature and stirred for an additional 1.5 h. At completion, the reaction mixture was filtered through a plug of silica to remove the *N*,*N*-dicyclohexylurea affording the crude **6-35** as a bright yellow solid. This was used directly in the subsequent step without further purification.

The crude *N*-Acylisatin **6-35** was dissolved in methanol and the solution was refluxed for 3 h. The solvent was removed *in vacuo* and the crude material was dissolved in the minimum volume of diethyl ether. An excess of n-hexane was then added to precipitate the title compound **6-36** as a pale yellow amorphous solid (8.86 g, 84 %). M.p. 69-70°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.42 (s, 9H, $C(CH_3)_3$), 1.94 (qu, *J* = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 2.51 (t, *J* = 7.4 Hz, 2H, $COCH_2CH_2$), 3.23 (q, J = 5.9 Hz, 2H, $NHCH_2CH_2$), 4.00 (s, 3H, OCH_3), 4.64-4.74 (m, 1H, NHBoc), 7.15 (ddd, *J* = 8.0, 7.3, 1.2 Hz, 1H, ArH), 7.65 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H, ArH), 7.68 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 8.79 (dd, *J* = 8.6, 1.2 Hz, 1H, ArH), 11.09 (s, 1H, CONHAr); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.7 ($CH_2CH_2CH_2$), 28.5 ($C(CH_3)_3$), 35.8 ($COCH_2$), 40.1 ($NHCH_2$), 53.1 (OCH_3), 79.4 ($C(CH_3)_3$), 117.3 (ArC), 121.0 (ArCH), 122.8 (ArCH), 133.7 (ArCH), 137.3 (ArCH), 142.7 (ArC), 156.1 (OCONH), 164.0 ($COCH_2$), 172.1 ($COCO_2CH_3$), 190.4 ($COCO_2CH_3$); IR (ATR): ν_{max} 3354, 3260, 2938, 2868, 1735, 1703, 1670, 1585, 1517, 1450, 1364, 1330, 1246, 1206,

1165, 1050, 1000, 858, 824, 788 cm^{-1}. HRMS (+ESI): Found *m/z* 387.1540, $[M+Na]^+$, $C_{18}H_{24}N_2O_6$ requires 387.1532.

6.5 References

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