EXPANDING BETA-TURN ANALOGS FOR MIMICKING PROTEIN-PROTEIN HOT SPOTS

A Dissertation

by

SAMUEL ONOFRE J. REYES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2006

Major Subject: Chemistry

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Approved by:

Chair of Committee,	Kevin Burgess
Committee Members,	Eric Simanek
	Coran Watanabe
	J. Martin Scholtz
Head of Department,	Emile Schweikert

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ABSTRACT

Expanding Beta-Turn Analogs for Mimicking Protein-Protein Hot Spots.

(August 2006)

Samuel Onofre J. Reyes, B.S., University of the Philippines Los Baños

Chair of Advisory Committee: Dr. Kevin Burgess

Solid-phase syntheses of two 14-membered ring peptidomimetics were done to determine whether or not a beta-turn structure can facilitate macrocyclization. NMR methods, together with CD and QMD calculations, do not fully support this assumption. However, cyclizations of more ordered structures like those of compounds **2** were more efficient than those for highly strained ring systems like **1**.

A small library of 18-membered ring peptidomimetics that accommodate an extra amino acid residue was synthesized on resin. Their syntheses were not complicated by head-to-tail dimer impurity, unlike those for previously synthesized 14-membered systems. These larger macrocycles exhibit beta-turn structures as verified by NMR, CD and QMD techniques. Moreover, two compounds in this series (**3a** and **3g**) were shown to have agonistic properties for TrkC in cell survival assays.

Dimerization of monovalent mimics was achieved first by modifying the organic template so that monovalent mimics with requisite functional groups can be synthesized. Second, the monovalent units were dimerized using sequential nucleophilic substitutions on fluorescently labeled dichlorotriazine. Our rationale to make bivalent compounds out of monovalent ones was justified when compound **4** was shown to bind TrkA with a 20 nM affinity.

Reactions of amino acids with NH_4SCN under acylating conditions produced 2-thiohydantoins in which the nitrogen of the amino acid (N^1) was acylated. This was proven by 2-D NMR which showed no cross-peak between the N*H* signal observed and the Cα-*H* of the amino acid. When the compound was deacylated, a new N*H* signal appeared and the corresponding cross-peak with the Cα-*H* was observed.

Solution-phase syntheses of non-peptidic mimics were achieved by doing a double substitution on a dihalogenated nitrobenzene scaffold. Sonogashira and S_NAr reactions were done to install the required side-chains to give the desired compounds. These non-peptidic compounds can be easily adapted to our DTAF-Inp dimerization protocol since the nitro groups can be easily reduced.

Attempts to make a spirotetracyclic peptidomimetic with three side chain mimics were done by synthesizing the spirocyclic diketopiperazine precursor. The synthesis of the DKP was achieved by making the cyclic quaternary amino acid that was coupled to another amino acid via the HOAt-EDC method. This protocol gave dipeptides in high yields. These dipeptides were deprotected and cyclized to the DKP under mildly acidic conditions in toluene.

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CHAPTER I

INTRODUCTION

1.1 The Nerve Growth Factor and the Neurotrophin Family

Neurotrophins (NTs) are dimeric polypeptide growth hormones that regulate the development, differentiation and survival of nerve cells.[1] Members of the neurotrophin family include nerve growth factor (NGF), brain-derived neurotrophin factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5) and neurotrophin-6 (NT-6).[2,3] They have approximately 50% sequence homology and dimerize non-covalently in parallel fashion where each monomer is about 13 kDa.

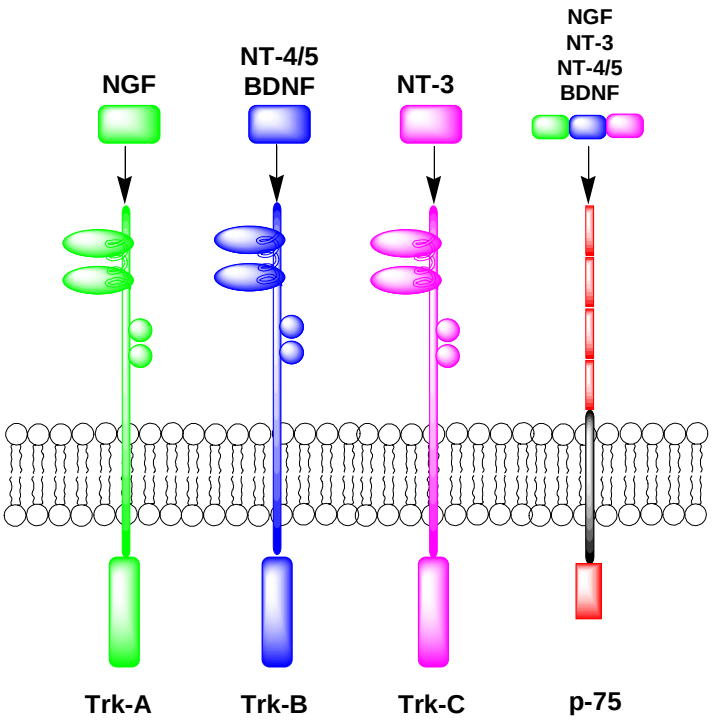


Figure 1.1. The neurotrophins and their receptors.

This dissertation follows the style of the *Journal of Organic Chemistry*.

These NTs mediate signaling by binding to two types of cell surface receptors: p75 and tyrosine kinases, Trks. p75 binds to all neurotrophins with similar affinities (Figure 1.1). It contains a cytoplasmic 'death' domain which has been shown to be involved in apoptosis.[4] Thus, in most cases, binding to p75 initiates events leading to cell death.[5] However, it can also promote neuronal survival by activating the transcription factor NF-κB in the presence of Trk activation.[6]

Tyrosine kinases, on the other hand, are more specific in binding to NTs and they do so with very high affinities (pM range). Tyrosine kinase A (TrkA) is the receptor for NGF,[7] tyrosine kinase B (Trk B) was shown to bind to BDNF[8] and NT-4/5[9], and tyrosine kinase C (TrkC) was found to bind NT-3.[10] Although these receptors bind at high affinities, TrkA and TrkB also bind NT-3 albeit at lower degrees.[11,12] Binding of NTs to Trk receptors results in dimerization of the latter. This event occurs with concomitant phosphorylation of tyrosine residues in the receptor cytoplasmic domain, triggering intracellular signaling cascades. Adapter proteins are then recruited and pathways leading to neuronal survival and neurite outgrowth are activated.[13]

NTs bind to the extracellular portion of Trk receptors which is comprised of five domains: two cysteine-rich clusters (domains 1 and 3), three leucine-rich motifs (domain 2) and two immunoglobin (Ig)-like regions (domains 4 and 5).[14] This extracellular part of Trk is not as homologous as its intracellular kinase domain. However, in domain 5, there is a stretch of 9 amino acid residues that are conserved in all Trk receptors.[15] This high degree of similarity has paramount importance as most experiments reveal the necessity and sufficiency of domain 5 in binding to neurotrophins.[2,4] In fact, the only crystal structure of a neurotrophin-Trk complex that has been solved to date contains only domain 5.[16,17] NGF and domain 5 of TrkA were shown to crystallize to form a bat-shaped complex (Figure 1.2). Two binding patches can be seen from the ligand-receptor interface: a specificity patch and a conserved patch. The specificity patch involves the *N*-terminal residues (6-9) of the NGF that form a short helix which packed itself to the ABED sheet of TrkA. This interaction is facilitated by hydrophobic residues at both regions of the pocket. It is important to point out that in crystal structures of NTs, this *N*-terminus region is disordered. Also, there is a very low degree of homology between residues at this pocket for other neurotrophins and Trks, implying that this interaction

might be unique for NGF-TrkA complex. The second patch is called a conserved patch and is formed by the central beta-sheet of NGF in contact with loops AB, C'D, and EF of TrkA. Most of the residues involved in this patch are highly conserved among the neurotrophins and Trks, hence, this patch is believed to be also present in other NT-Trk complexes.

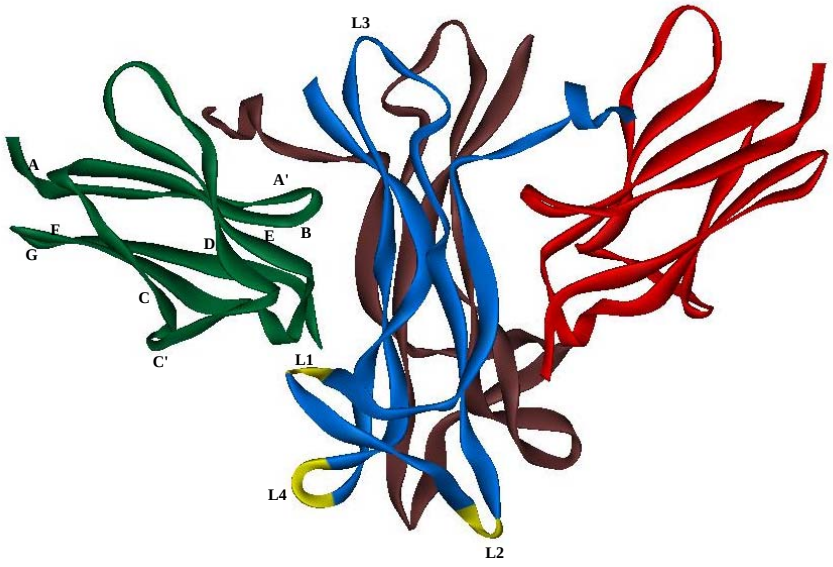


Figure 1.2. Crystal structure of dimeric nerve growth factor complexed with domain 5 of Trk A.

In contrast to NGF-TrkA complex, the crystal structure of NGF binding to p75 reveals a 2:1 NGF:p75 stoichiometry (Figure 1.3).[18] This is unexpected considering the mode of binding of NTs to Trk receptors. Another surprise is that while other tumor necrosis factor (TNF) receptors, to which p75 belongs, bind trimeric TNF ligands, p75 engage dimeric NTs. The three-dimensional structure shows that p75 positions itself in between the groove formed by two NGF monomers. There are two binding sites in the complex and most contacts involve charge complementarity between the highly negative surface of p75 and the positively charged residues of NGF. Mapping these two sites on the alignment of residues in other NTs reveal highly conserved sequences. This explains

the observed affinity of p75 to all members of NT family. Because of the way p75 binds, there is a pseudo binding site on the other side of the NGF dimer. However, this site is not accessible to another p75 because of an allosteric effect caused by the first binding. The structure also reveals an opening for Trk binding which could support experimental evidence that both TrkA and p75 are required for high affinity NGF binding sites.[6]

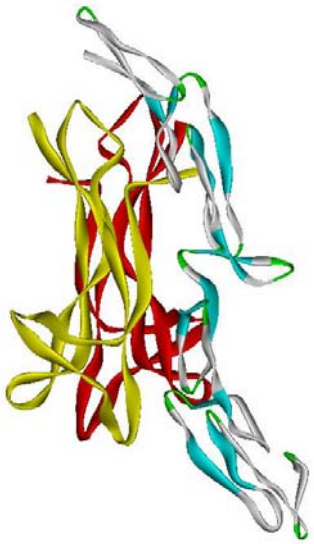


Figure 1.3. NGF-p75 crystal structure.

1.2 Neurotrophins and Neurodegenerative Diseases

Neurotrophins (NTs) vary in population through the peripheral and central nervous systems, but have a higher density in the hippocampus region of the brain. In recent years, it has been established that a decrease in trophic support plays a major role in the pathogenesis of neurodegenerative diseases.[4] Neurotrophins such as NGF and BDNF have been implicated in Alzheimer's and Huntington's disease, respectively. Although NTs have been used clinically, they have poor pharmacological properties which make them unsuitable for therapeutic use.[19] To circumvent this, more researchers have been employing small-molecule mimics, not only of NTs but of other proteins as well. In this approach, the structural and/or functional properties of a protein are being copied by a molecule much smaller in size.[20]

In the case of NTs, 'hot-spot' regions are identified as those that interact or bind to Trk receptors. These 'hot-spots' are then translated into a small molecule that could potentially possess NT activity. Ligand binding regions or sequences that interact with the receptor are determined by chimeric recombination or site-directed mutagenesis studies. For NTs and some growth factors, a lot of the 'hot-spots' involve beta-turn regions.[19] Beta-turn is one of the three common secondary structures that peptides and proteins adopt.[21] It also plays a major role in recognition events in many protein-protein interactions. Beta-turn regions in NGF are highlighted in Figure 1.2. The amino acid residues in these positions are listed in Table 1.1. By mapping similar turn regions in other neurotrophins, beta-turn residues were identified. The sequences in the loops are highly conserved among different sources of NGF. This high fidelity can also be seen for NT-3.

Table 1.1. Amino acid sequences of beta-turn residues in neurotrophins.

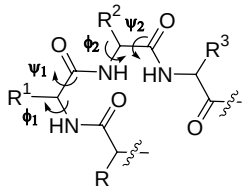
Protein	**source**	**β-turn sequence**		
		30-33	44-47	93-96
NGF	murine	DIKG	INNS	DEKQ
	human	DIKG	INNS	DGKQ
	bovine	DIKG	INNS	DNKQ
	guinea pig	DIKG	VNNN	DGKQ
		30-33	44-47	93-96
BDNF	pig	DMSG	VSKG	DSKK
		29-32	42-45	92-95
NT-3	mouse	DIRG	KTGN	ENNK
	human	DIRG	KTGN	ENNK

1.3 Beta-turn Peptidomimetics

While proteins or peptides mediate a lot of biological processes, they have undesirable pharmacological properties, like proteolytic stability, that limit their

therapeutic use. To circumvent this, researchers have turned to peptidomimetics - compounds that are not related structurally to a particular protein or peptide, but have the essential functional groups displayed in a characteristic three-dimensional pattern that will serve as the recognizing feature in binding.[22,23] Those interactions in which the spatial arrangement of side chains is critical often involve beta-turns.[24] Thus, making beta-turn mimetics is a rational approach to make small-molecule analogs of the parent protein or peptide. However, incorporating beta-turn conformations in small organic molecules to produce beta-turn peptidomimetics is a formidable challenge considering the diversity of beta-turns (Table 1.2).[21]

Table 1.2. Different types of beta-turns with corresponding dihedral angles.

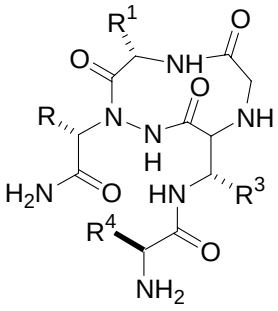


a generic β-turn

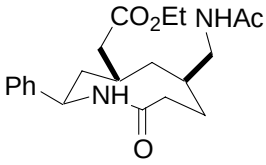
Turn type	Angles/Degrees			
	ϕ_2	ψ_2	ϕ_3	ψ_3
I	-60	-30	-90	0
I´	60	30	90	0
II	-60	120	80	0
II´	60	-120	-80	0
III	-60	-30	-60	-30
III´	60	30	60	30
IV	has 2 or more angles that differ by at least 40° from types I – III´			
V	-80	80	80	-80
V´	80	-80	-80	80
VI	has *cis*-Pro at position 3			
VII	has a kink in the chain created by ψ_2 ~180° & $\lvert\phi_3\rvert$<60° or $\lvert\psi_2\rvert$<60° and ϕ_3 ~180°			

Despite of this, a lot of groups have devoted extensive efforts to generate beta-turn mimics.[23,25] Turn mimics can be classified as internal or external.[26] In both cases,

cyclization is used as a means to enforce constraint.[27] Internal turn mimics have the pseudo C^{10} skeleton motif that incorporates side chains of interest. Examples include structures **A** and **B** which were developed by Kahn and Olson, respectively.[28,29] External turn mimics, on the other hand, are dipeptide-derived cyclic analogs that do not highlight *i*+1 and *i*+2 positions. Structures **C** and **D**, developed by Friedinger and Nagai, respectively, are prime examples.[30,31]



A



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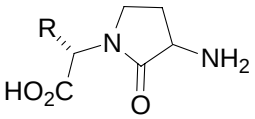
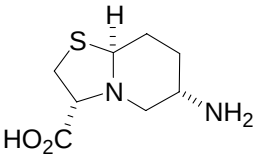


C

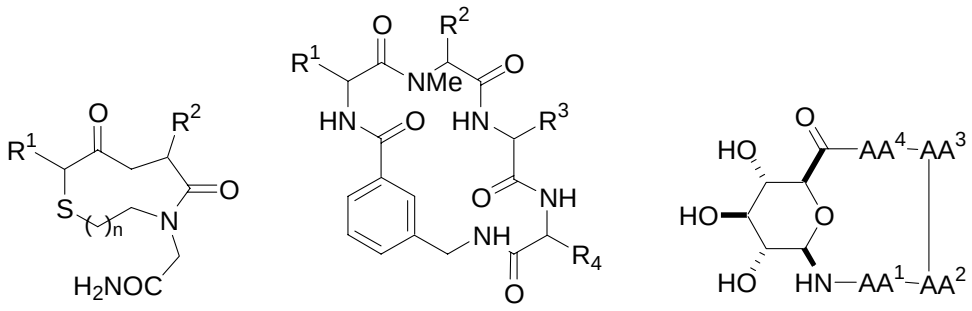


D

These early works on beta-turn peptidomimetics, although promising, have gained partial success mainly because there is simply limited information available regarding which turns are involved in the interaction. Ellman showed that this structural biology problem can be circumvented by making libraries of beta-turn mimics efficiently. By employing solid-phase chemistry and using readily available starting materials, expedient synthesis of beta-turn mimics **E** was achieved.[32]

While a library of beta-turn mimics may be useful, especially if the interaction between the ligand and the receptor is not fully understood, the hit rate for an active compound may be not very high compared to a focus library of mimics. However to have a focus library, information regarding the structural biology of the target must be available. With recent advances in crystallography and spectroscopy this information is more accessible now. Notable examples wherein a smaller, but a focus set of mimics was made based on known interactions include DeGrado's RGD mimics **F** and Kessler's sugar amino acid mimics **G**.[33,34] Compounds **F** were designed based on the known

affinity of the RGD tripeptide to glycoprotein IIb/IIIa. Mimics **G**, on the other hand, are based on the sequence of somatostatin.



Our initial approach in this context is to use amino acids in the turn regions of the neurotrophin and constrain them through cyclization using an organic template. This design is based on ring-fused C^{10} backbone motif which resembles back-to-back beta-turns of cyclic hexapeptides **H**. The extended turn of **H** can be represented by a non-peptidic moiety which will be the organic template portion of the peptidomimetic as shown in **I**.[35] The synthesis is done on solid phase for increased productivity and minimized purification procedures. Y is used as the anchoring region for resin attachment.

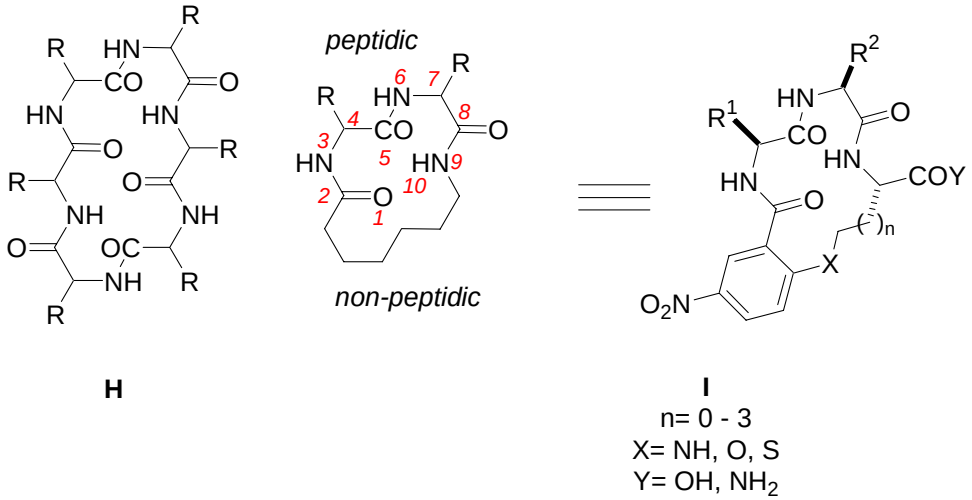


Figure 1.4. Rationale of peptidomimetic design. Half of the cyclic hexapeptides was replaced by a nonpeptidic moiety. Our first peptidomimetic design.

1.4 Conformational Studies on Beta-Turn Peptidomimetics

It is important once the synthesis of a supposed beta-turn mimetic is accomplished to determine whether or not the compound has characteristics typical of such a turn conformation. This applies strongly to cyclic peptides since their structures resemble a beta loop more than other peptidomimetics. The techniques most commonly used to elucidate peptidomimetics conformation are: NMR and CD spectroscopies, X-ray crystallography and molecular modeling. Of these, NMR, CD and molecular modeling are the ones that are routinely used in our laboratories.

1.4.1 Conformational Studies Using NMR

1.4.1.1 Coupling Constants

The dihedral angles, ϕ_1, ψ_1, ϕ_2 and ψ_2 around the backbone of the four amino acids of a beta-turn give the turn its corresponding type (i.e. Type I, II, etc.). The vicinal coupling constant ^3J is dependent on the dihedral angle[36] and their relationship is expressed in the Karplus equation:[37]

$$^3J = 6.7 \cos^2 (\phi - 60) - 1.3 (\phi - 60) + 1.5$$

In the case of the homonuclear coupling between amide N<u>H</u> and C_α<u>H</u>, the torsion angle can be easily calculated using this equation. [38] For Types I and II beta-turn, there have been generally accepted values of ^3J (4 and 9 Hz for *i + 1* and *i + 2* residues, respectively for Type I; while 4 and 5 Hz for the same residues for Type 2). However the coupling constants alone do not give an unambiguous type assignment for a particular compound especially for linear peptides because of conformational averaging under the NMR time scale.[39]

1.4.1.2 Dipolar Through Space Couplings

Nuclei which are in close proximity develop nuclear Overhauser Effect (NOE) when the saturation energy of one nucleus is transferred to a neighboring one, resulting to an enhanced NMR signal of the latter. In NOESY experiments, cross peaks between protons have proved to be useful in determining inter -hydrogen distance and orientation within a molecule.[36] One of the disadvantages of NOE is that the time it takes for the signal to build up depends on the correlation time of the distance between the two nuclei.

Correlation times in medium-sized peptides often give rise to very small NOE's. To circumvent this, the rotating frame NOE (ROESY) experiment was developed. However, in a ROESY experiment all the signals are positive that may give rise to artifacts (like TOCSY signals).[38] In general, medium to strong NOE indicates a distance of at most 5.0 Å. In case of Type I and Type II beta-turns, because of the opposite orientation of the amido group formed by *i + 2* and *i + 3* residues, cross peaks made by protons in these residues indicate what secondary structure the peptide might have. For instance, a Type I turn has a characteristic cross-peak between N*H* protons at the *i + 2* and *i + 3* positions; this is absent in Type II, which instead has a signal between $C_{\alpha}H$ at *i* + 2 and N*H* at *i* + *3*.[40]

1.4.1.3 Temperature Coefficients

Most studies on cyclic peptides indicate higher negative gradients ($\Delta\delta_{NH}/\Delta T$) on amide protons suggest these maybe solvent exposed. A less negative gradient would imply hydrogen bonding or the proton maybe oriented within the turn, shielding it from the solvent. It is widely accepted that for turn structures, values less negative than -4 ppb/°C indicate that the proton is sequestered from the solvent and is probably hydrogen-bonded.[36] While the magnitude of amide N*H* has been useful in locating reverse turns, one disadvantage is 'false positive' observations due to side chain participation.[38] Another drawback that could result to ambiguous interpretation is the change in the conformation of the molecule is not taken into account even as the temperature is increased.[41]

1.4.2 CD Spectroscopy

The difference in absorption of left and right circularly polarized lights by a chiral molecule gives the characteristic ellipticity of the compound seen on a CD spectrum. CD spectroscopy offers a fast and convenient way in analyzing alpha helical and beta sheet conformations. These secondary structures have been studied extensively and their signature CD patterns have been well established.[42,43] In the case of cyclic peptides, the p-p* and n-p* transitions of the amide chromophore give the characteristic CD spectrum for a particular type of turn.[42] Type I and type III beta-turn have a strong positive band

between 180-190 nm and a negative band centered around 205 nm. Type II on the other hand exhibits a maximum between 200 and 205 nm and a minimum at 225 nm.[44] While CD spectroscopy offers a fast and convenient way of analyzing peptide and protein secondary structure, the analysis might be complicated by other chromophores that absorb in the UV region of interest (180 – 230 nm).

1.4.3 Molecular Modeling

Computer-aided modeling is now being used almost routinely to complement the results obtained from the methods described above. Data from NMR are being applied as modeling constraints and this type of semi-empirical technique has been extensively used in molecular simulations.[45,46] One of the most common calculation techniques used as a conformational searching tool is molecular dynamics.[47] Our laboratories have been using Quenched Molecular Dynamics (QMD) to determine preferred conformations in solution but without adding any constraint from NMR data. This provides an unbiased result in assigning a secondary structure for a particular molecule that may support or contradict empirical results.

In QMD, heat is used to overcome potential energy barriers in dynamics simulations. Structures in this stage are being extracted periodically and then minimized (quenched). This step produces a collection of conformations that are grouped according to homogeneity which can be used to judge the molecule's preferred conformation.

CHAPTER II

BETA-TURN STRUCTURE AS A POSSIBLE DRIVING FORCE FOR MACROCYCLIZATION

2.1 Introduction

In medicinal chemistry, it is difficult to study relationships between conformation and biological activity if flexibility is not controlled. One way of imposing conformational constraint to linear peptides or their derivatives (like peptidomimetics) is through cyclization.[48] This process can render the cyclic compound to have several desirable biological properties over its linear counterpart. Such properties include increase in: biological activity,[49] resistance to proteolytic degradation[50,51] and specificity to a particular target. There are several ways to cyclize a linear peptide or peptidomimetic and reviews regarding this subject have been published.[52,53] A lot of these methods, although different in the approaches give an amide bond at the end of the cyclization. The examples that will be given in the next paragraphs feature synthesis of cyclic peptides/peptidomimetics that do not create amide bonds in the cyclization step.

One of the earliest techniques in making cyclic peptides is through a disulfide bond (S-S). Thiol-containing amino acids such as cysteine and penicillamine are incorporated at both ends of the linear peptide and then subjected under oxidizing conditions to form the S-S bond. An elegant example of this mode of cyclization was demonstrated by Barany and co-workers by using a solid supported oxidant.[54] In this case, a synthetic route to anchor the oxidant, Ellman's reagent (5,5´-dithiobis-2-nitrobenzoic acid, DNTB) onto a solid support was developed. After removal of the thiol protection, the linear peptide was reacted with an excess of the solid-supported reagent and was "captured" via thiol exchange reaction. A second thiol exchange created the disulfide bond within the peptide with concomitant cleavage from the resin-bound reagent (Figure 2.1). This methodology was applied to well-known disulfide-linked cyclic peptides such as oxytocin and somatostatin.[54]

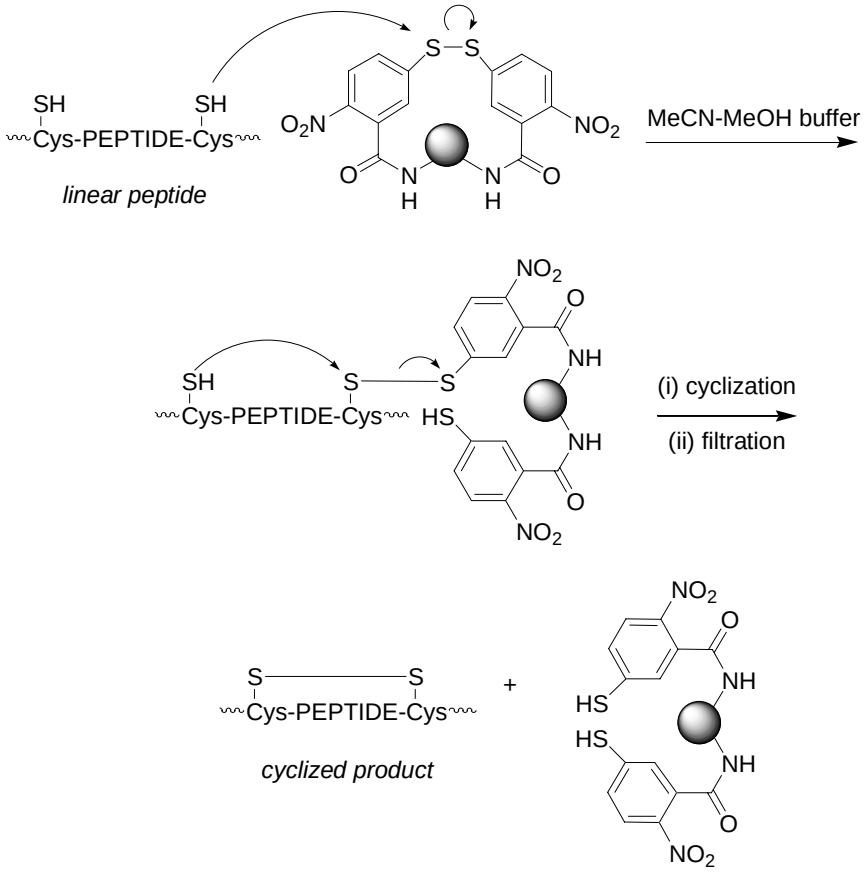


Figure 2.1. Disulfide bond formation using a solid-supported Ellman's reagent.

Another reaction that proceeds with high efficiency like disulfide bond formation is oxime bond formation. While this is not a very popular technique to make cyclic peptide analogs, Tam and co-workers have shown that linear, unprotected peptides bearing a hydroxylamine and aldehyde functionalities can be cyclized in an intramolecular fashion.[55] This methodology was applied to make a V3 loop mimic of gp120 which contains a peptide sequence being targeted as a neutralizing determinant for developing vaccines. The linear precursor was made on Wang resin; after TFA cleavage, the serine end of the molecule was oxidized and converted to an aldehyde, which underwent facile attack by the hydroxylamine group creating the oxime bond which cyclizes the molecule (Figure2.2). The cyclization was complete in two minutes as monitored by reverse-phase HPLC.

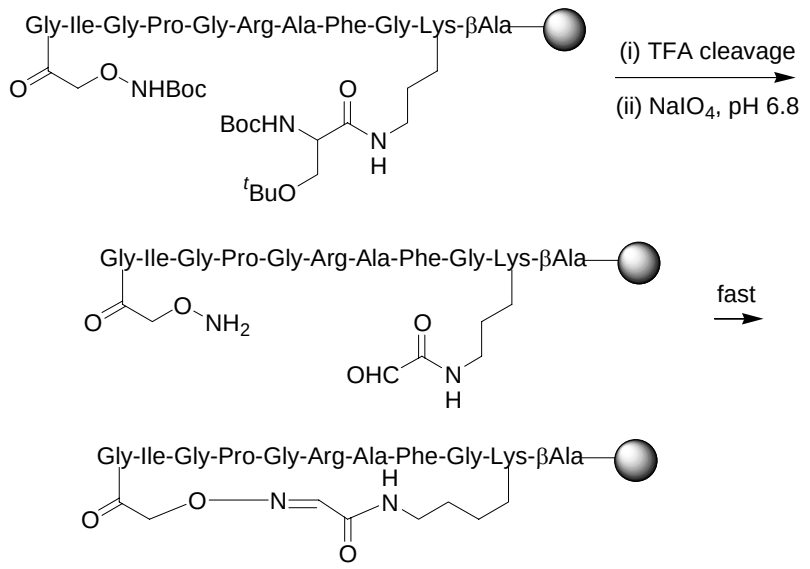


Figure 2.2. Tam's mode of cyclization via oxime bond formation.

One of the 'hottest' reactions of the last decade is ring-closing metathesis (RCM) and it is not surprising that it has found utility in the field of peptide chemistry. Several groups have employed this reaction to make cyclic peptides and they have appeared in a review elsewhere.[56] However, one example worth mentioning is the one reported by Liskamp.[57] In this work, *N*-alkylation with hydroxyalkenes was employed to yield the metathesis precursors. This method of installing the alkene group does not compromise the amino acid side chains which can be important for biological activity. Once the linear alkene precursors are made, RCM was achieved using Grubbs' first generation catalyst. One attractive feature of this work is that the site where the RCM occurs can be verified via mass spectrometry as shown in Figure 2.3.

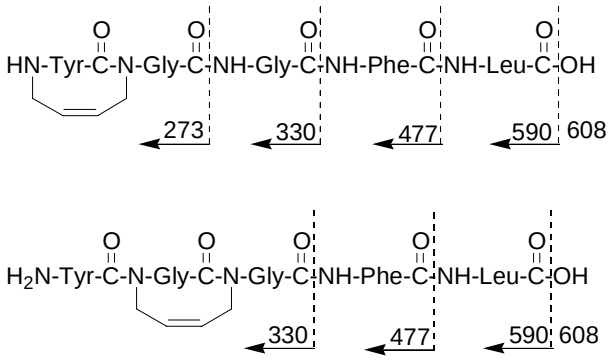


Figure 2.3. Examples of Leu enkephalin derivatives synthesized via application of ring closing metathesis.

Our group has also been involved in the synthesis of cyclic peptidomimetics and initial works in this area had involved the application of nucleophilic aromatic substitution (S_NAr) reactions to create the macrocyclic compound.

2.2 Solid Phase S_NAr Macrocyclization

Solid phase synthesis has its roots on Merrifield's invention of doing peptide synthesis on polystyrene-based support.[58] The idea of tethering an organic molecule on a solid matrix has revolutionized the field of organic synthesis and reactions other than amide bond formation were also found to be suitable on-resin.[59,60] This proved to be crucial not only in the development of combinatorial chemistry but also in the advancement of medicinal chemistry as wide range of molecules including heterocycles, peptides and carbohydrates are prepared at a faster rate.

The displacement of a leaving group in an aromatic ring by a nucleophile, otherwise known as S_NAr (substitution nucleophilic aromatic) reaction is one the most common reactions in organic chemistry. This reaction has been known since the 1900's, however, its application as a tool for macrocyclization was demonstrated only 15 years ago. In an attempt to find an alternative to synthesize the *C*-O-*D* ring of vancomycin, Buegelmanns showed that S_NAr macrocyclizations can be employed using a phenol group (Figure 2.4).[61] Interestingly, the researchers found that concentration was not a factor in the macrocyclization step. Three years after, this type of synthesis for

heterodectic compounds was applied on a solid support in a collaborative effort by Burgess and Zhu in their work on OF4949 derivatives (Figure 2.5).[62] They also found that removal of the silylether protection on the phenol functionality by TBAF was sufficient for nucleophilic attack. Thus, a milder condition for cyclization is possible which reduced epimerization that was observed under basic conditions.

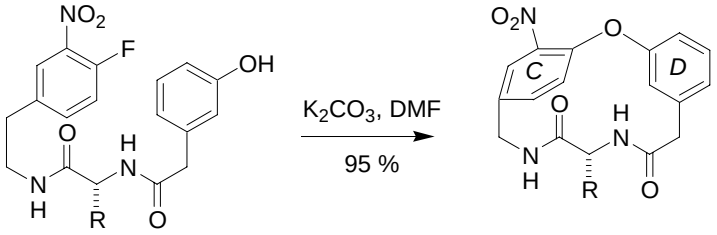


Figure 2.4. Demonstration of S_NAr macrocyclization toward the synthesis of C-O-D ring of vancomycin.

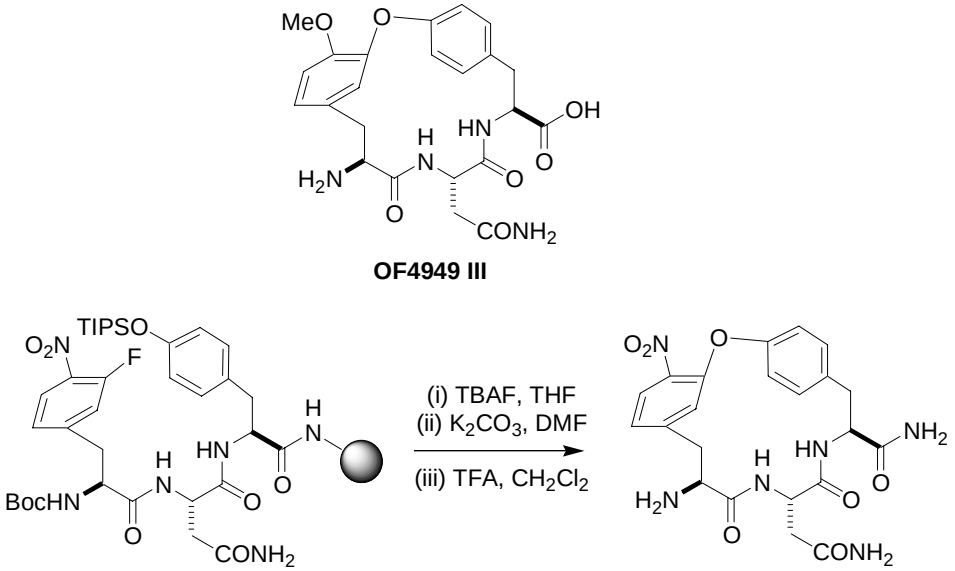
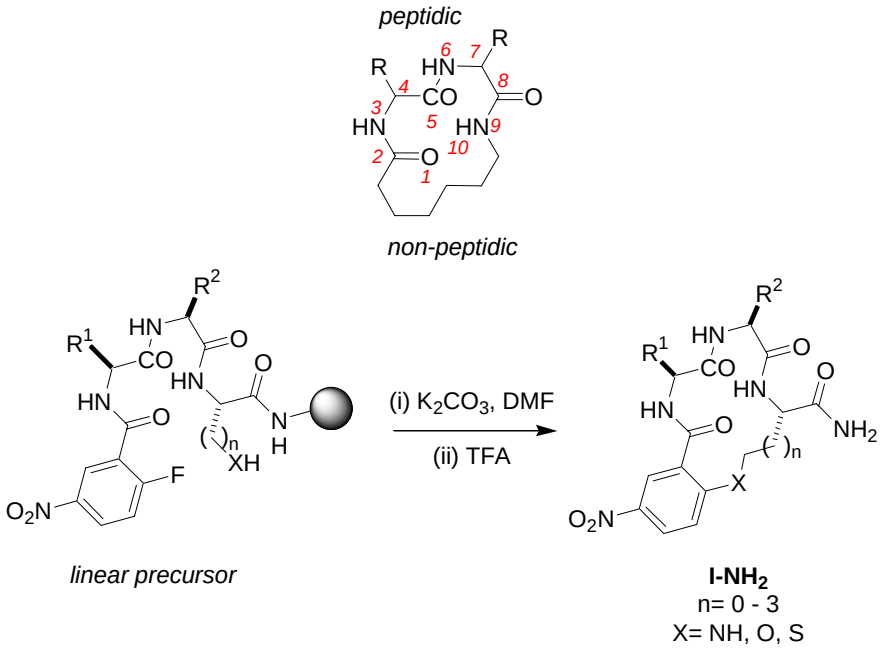


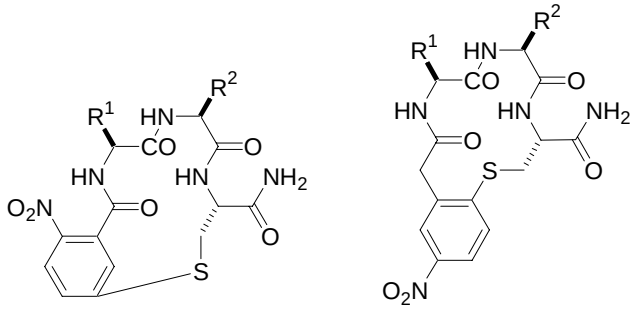
Figure 2.5. Solid-phase S_NAr macrocyclization to synthesize an OF4949 III derivative.

Our group has focused on similar type of macrocyclization to make beta-turn peptidomimetics. Early studies on beta turn peptidomimetics in our group involved a C^{10} motif in which a peptide fragment is locked by an organic moiety through the cyclization process.[35] These mimics resemble turn-extended-turn conformations of cyclic

hexapeptides.[63,64] Thirteen to 16-membered ring compounds were made with a variety of nucleophiles like alcohol (-OH), amine (-NH_2) and thiol (-SH), all of which came from amino acid side-chains.[35] The syntheses of these compounds were done on a solid support. Conventional peptide synthesis strategy based on the Fmoc protecting group was employed to make linear precursors for the macrocyclization event. In this case, intramolecular S_NAr reactions were used to give the final products **I-NH_2** shown below.[65,66] While the cyclization reactions appear to be facile, and some of the products exhibit beta-turn characteristics, it is not known whether or not an anticipated secondary structure is responsible for the efficiency of the macrocyclization process.



To investigate on this issue, we have set out to compare S_NAr cyclizations of two model compounds. Their structures are given in Figure 2.6 and their syntheses are depicted in Schemes 2.1 and 2.2. Both compounds have the same ring size (14-membered ring) but from molecular stick models, it seemed that compounds **1** are more strained and are not capable of adopting a beta-turn structure. On the other hand, compounds **2** have some flexibility but still possess rigidity and can possibly adopt a turn structure.



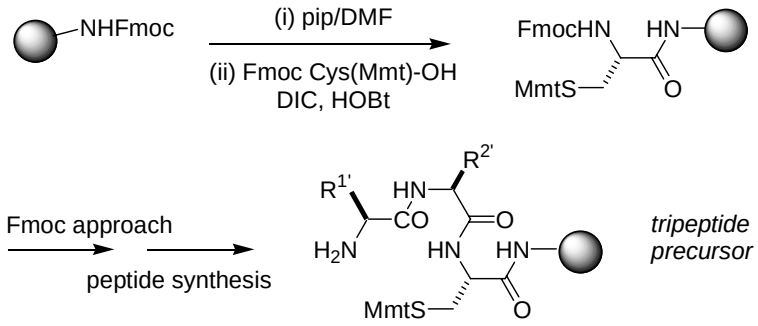
1 **2**

Figure 2.6. Structures of S_NAr products **1** and **2**.

2.3 Syntheses of Compounds 1 and 2

The syntheses of compounds **1** and **2** followed conventional solid-phase Fmoc (9-fluorenylmethoxy carbonyl) peptide synthesis approach.[67] Polystyrene resin derivatized with Rink handle was used to anchor the molecules. Monomethyltrityl (Mmt)-protected cysteine was the first amino acid to be attached followed by two more amino acids corresponding to the beta-turn sequence of the neurotrophin target (Scheme 2.1). The prime (′) symbol in the side chains (*e.g.* $R^{1'}$) denotes side chain protection where applicable. This process is the same for both compounds.

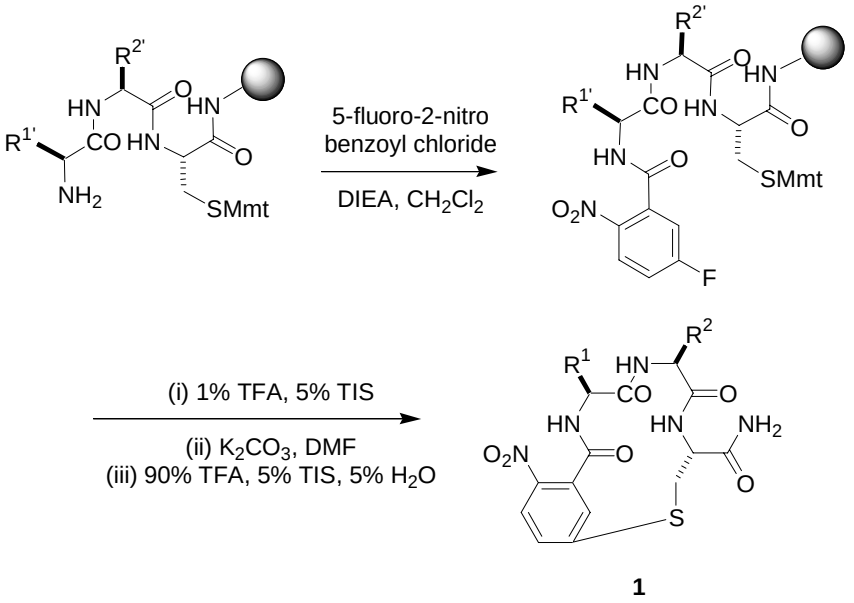
Scheme 2.1. Synthesis of tripeptide precursors of compounds 1 and 2



After the dipeptide synthesis, the Fmoc protection of the N-terminal amino acid was cleaved and the organic template, which is different for both compounds set, is

attached. For compounds **1**, 5-fluoro-2-nitrobenzoyl chloride was used and this was coupled to the free amino group giving the linear precursors. The sulfur protection was then deblocked under mild acidic conditions to liberate the free thiol that will act as nucleophile in the following macrocyclization step. This was done under basic conditions to give the cyclized compounds. Finally, the compounds were cleaved from the resin under strong acidic conditions (Scheme 2.2).

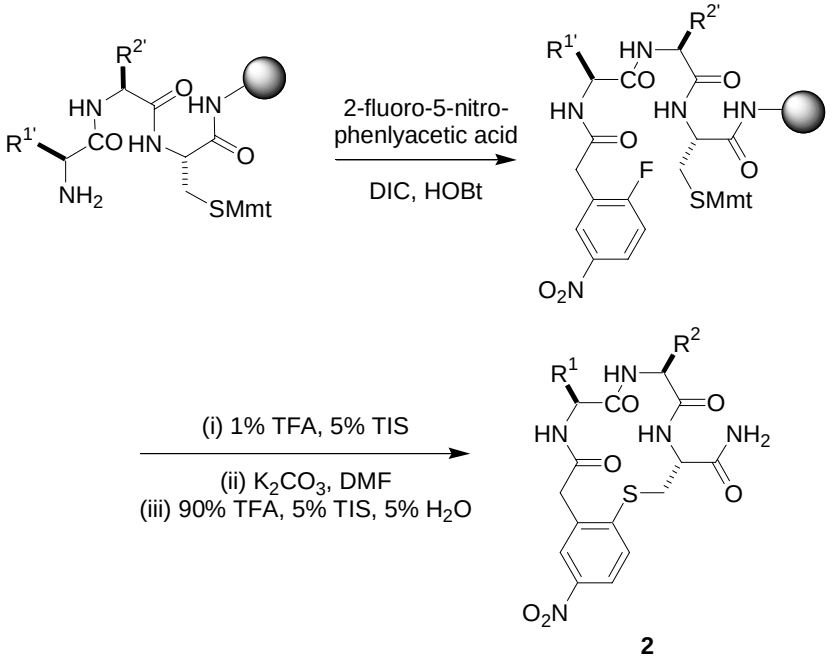
Scheme 2.2. Synthesis of compounds 1



1

Compounds **2** have 2-fluoro-5-nitrophenylacetic acid as the organic template. This one does not need to be converted to the acid chloride because it can couple smoothly to the amine using DIC/HOBt. After its attachment, the same cyclization protocol was followed for compounds **2** (Scheme 2.3).

Scheme 2.3. Synthesis of compounds 2



The yields and purities of the compounds from their syntheses are given in Tables 2.1 and 2.2. The compounds were isolated via preparative HPLC using a reverse-phase C18 column and were characterized by MALDI-MS and proton NMR. The synthesis of compounds **1a-e** is complicated by what we call 'dimer' (Figure 2.7; **1-1** in the table) formation which arises from intermolecular cyclization. This phenomenon has been observed in previous studies on peptidomimetics from our group.[68] Although it can be suppressed by using a low resin loading, it is still hard to avoid as seen in this case. As such, the purities and the yields are significantly lower than those of compounds **2**.

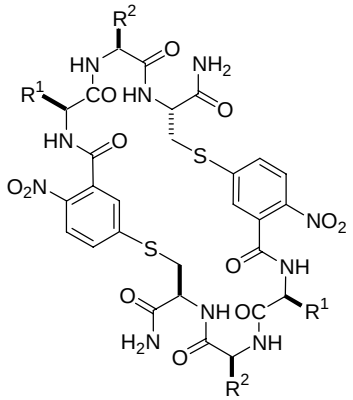


Figure 2.7. Structure of the 'dimeric' byproduct in the cyclization of compounds **1**.

The syntheses of compounds **2a-e** proved to be more efficient as the intermolecular macrocyclization byproducts are less significant. This does not mean that the competing side reaction has been completely eliminated; in fact, MS data showed that there are small amounts of the byproducts present, but HPLC data showed these are insignificant amounts that isolating them are not productive.

Table 2.1. Isolated yields and purities for **1a-e** and their corresponding dimers, **1-1**.

Compound	R^1	R^2	Purities (%)		Isolated yields (%)	
			1	**1-1**	**1**	**1-1**
1a	CH_2CONH_2	$(CH_2)_4NH_2$	72	15	28	1
1b	*(S)*-$CH(CH_3)CH_2CH_3$	$(CH_2)_4NH_2$	67	25	17	4
1c	*(S)*-$CH(CH_3)CH_2CH_3$	$(CH_2)_3NHC(=NH)NH_2$	70	29	21	5
1d	*(R)*-$CH(CH_3)(OH)$	H	62	28	26	8
1e	CH_2CO_2H	*(S)*-$CH(CH_3)CH_2CH_3$	36	60	10	4

Table 2.2. Isolated yields and purities for **2a-e**.

Compound	R^1	R^2	Purities (%)	Isolated yields (%)
2a	CH_2CONH_2	$(CH_2)_4NH_2$	89	57
2b	*(S-)*$CH(CH_3)CH_2CH_3$	$(CH_2)_4NH_2$	92	50
2c	*(S-)*$CH(CH_3)CH_2CH_3$	$(CH_2)_3NHC(=NH)NH_2$	86	37
2d	$(CH_2)_3NHC(=NH)NH_2$	H	76	40
2e	$(CH_2)_4NH_2$	*(R)*-$CH(CH_3)(OH)$	85	59

2.4 Conformational Analyses

Compounds **1a** and **2a (**Figure 2.8) were selected as representative structures for conformational analyses to assess whether or not the compounds can adopt beta-turn structures. The following experiments were done to assess beta turn propensity: CD, 1-D and 2-D NMR and quenched molecular dynamics (QMD) modeling.

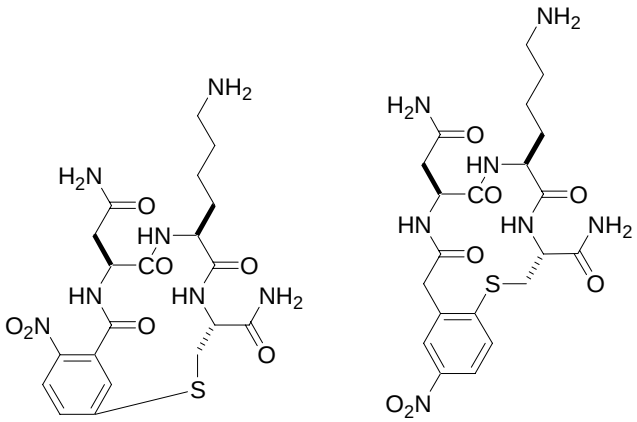


Figure 2.8. Representative compounds used for conformational studies.

Circular dichroism provides a fast and convenient method to determine whether or not a molecule possesses beta-turn structure. Compounds **1a** and **2a** were dissolved in 20% MeOH/H_2O, the ratio of which approximates the dielectric constant of DMSO used in the NMR studies. Overlaid CD spectra of the two compounds are shown in Figure 2.9. While there is a negative band around 210 nm for compound **2a**, the maxima at 190 nm is absent. Typical type I and type II turns have minima around 205 nm and 225 nm, respectively; and maxima around 190 and 205 nm, respectively.[44] Clearly, there is no definite beta-turn structure that can be deduced from these spectra.

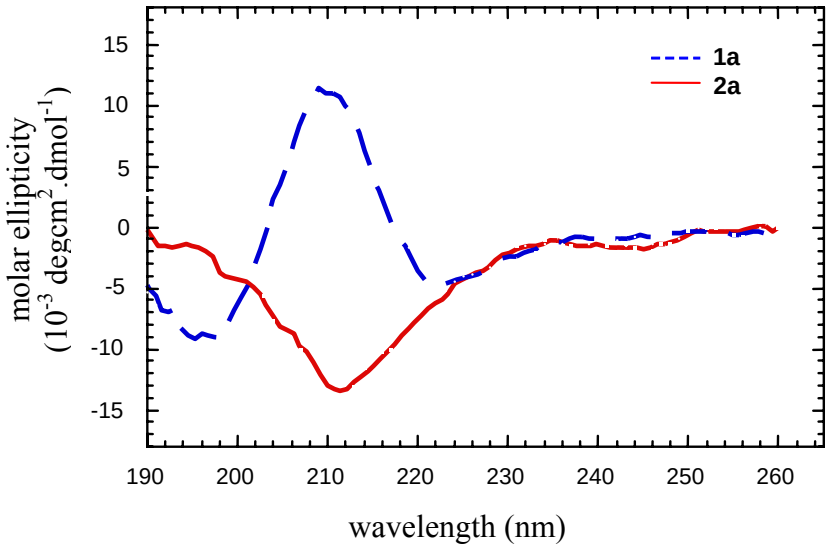


Figure 2.9. CD spectra of compounds **1a** and **2a**.

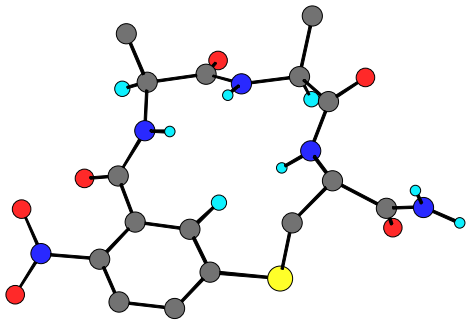
NMR experiments on the compounds were done by Dr. Mookda Pattarawarapan to evaluate relevant proton cross peaks, coupling constants and internal hydrogen bonding. Molecular simulations using QMD[69,70] were performed by Sudipta Roy. From these calculations, clusters of low energy conformers are generated without applying any constraint from NMR experiments. This provides an unbiased result so that good agreement between experimental results would validate the calculations.

Temperature coefficient values of hydrogen-bonded or solvent-shielded protons normally have values less negative than -4 ppb/K.[71,72] For compounds **1a** and **2a**, if they adopt beta turn conformation, the amino hydrogen of cysteine is expected to hydrogen-bond with the carbonyl oxygen of the template. Measured temperature coefficient values for the NH of cysteine for **1a** and **2a** (-4.49 and -7.25, respectively) reveal that this is not the case for the two compounds. This is supported by HD exchange experiments, which show that these protons are exchanged at a fast rate. There are other amine protons such as the Lys N*H* for both compounds that exhibited values that can be regarded as hydrogen-bonded or solvent-shielded (Lys N*H* for **1a** and **2a** is 3.44 and 0.79 ppb/K, respectively) but these have no implications on any turn structure. It is possible that those protons are hydrogen bonded with the carbonyl functionality of the adjacent asparagine side-chain.

QMD calculations generated four families for each compound. Table 2.3 shows the data of the lowest energy conformer family of **1a**. Coincidentally, the data for this family best match the NMR data. However, the dihedral angles measured from QMD do not correspond to any type of beta-turn. Correlation of the QMD-measured distances with those from ROE data (Figure 2.10) indicated low correspondence. These, together with temperature coefficients of amide N*H*'s and H/D exchange experiments suggest that the solution conformer is not a beta-turn.

Table 2.3. QMD results for the lowest energy conformer of **1a**.

Amino acid residue	Dihedral angle (°)	Lowest energy conformer of **1a**
Asn (R^1)	ϕ	-116.6
	ψ	-41.40
Lys (R^2)	ϕ	-168.5
	ψ	45.52
Distance (Å)C$\underline{O}_{temp.}$-N$\underline{H}_{i+3}$		5.224
Type of beta turn		none



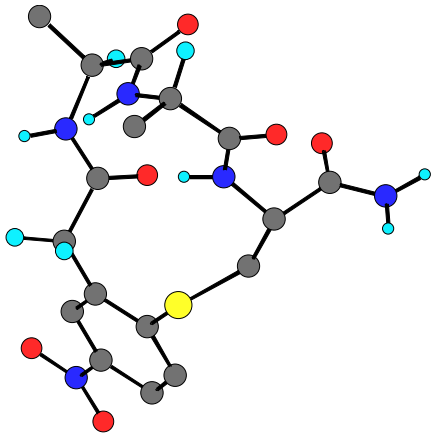
Hydrogen	ROE	QMD distance (Å)
Asn $_{NH}$ - Lys $_{NH}$	strong	1.707
Asn $_{C\alpha H}$ - Lys $_{NH}$	weak	3.518
Lys $_{NH}$ - Cys $_{NH}$	medium	2.261
Lys $_{C\alpha H}$ - Cys $_{NH}$	strong	2.656

Figure 2.10. Top: Lowest energy conformer for **1a** generated by QMD. Bottom: Correlation between NMR ROE cross-peaks and distances from QMD.

For **2a**, the lowest energy conformer of the most populated family fits poorly with NMR experimental results. While calculations support the assumption that compounds **2** can adopt a turn structure, experimental evidence suggests otherwise (Table 2.4 and Figure 2.11). It is possible that the methylene group of the template renders the macrocycle more flexible than previous designs that in solution, the preferred conformation is not a beta turn.

Table 2.4. QMD results for the lowest energy conformer of **2a.**

Amino acid residue	Dihedral angle (°)	Lowest energy conformer of **2a**
Asn	ϕ	-78.57
	ψ	-31.22
Lys	ϕ	-73.34
	ψ	-18.44
Distance (Å)$CO_{temp.}$-NH_{i+3}		2.648
Type of turn		III



Hydrogen	ROE	QMD distance (Å)
Asn $_{NH}$ - Lys $_{NH}$	medium	2.255
Asn $_{C\alpha H}$ - Lys $_{NH}$	very weak	3.486
Lys $_{NH}$ - Cys $_{NH}$	weak	2.499
Lys $_{C\alpha H}$ - Cys $_{NH}$	medium	3.443

Figure 2.11. Top: Lowest energy conformer for **2a** generated by QMD. Bottom: Correlation between NMR ROE cross-peaks and distances from QMD.

While experimental (NMR) and QMD results do not fit very well, it is possible that for compounds **2**, a more ordered structure is being adopted that facilitated the macrocyclization and minimized the dimer byproduct that is prevalent in the syntheses of compounds **1**. One final experiment was to determine the distance between the thiol (SH) nucleophile and the carbon bonded to fluorine (C-F) electrophile, the elements needed for cyclization. Open analogs of **1a** and **2a** were minimized in a normal QMD protocol and then subjected to dynamics simulation experiment. This was done for 600ps at 298 K. One conformer was downloaded every 1 ps. The distance between S and C are measured for each conformer and plotted against time (Figure 2.12). The data showed that for **2a**, there is a marked decrease in the distance after 200 ps and this distance did not go out of range of 6 – 10 Å throughout the rest of the dynamics simulation. This leveling is absent for **1a** and even though there is also a drop in distance, the variation is greater.

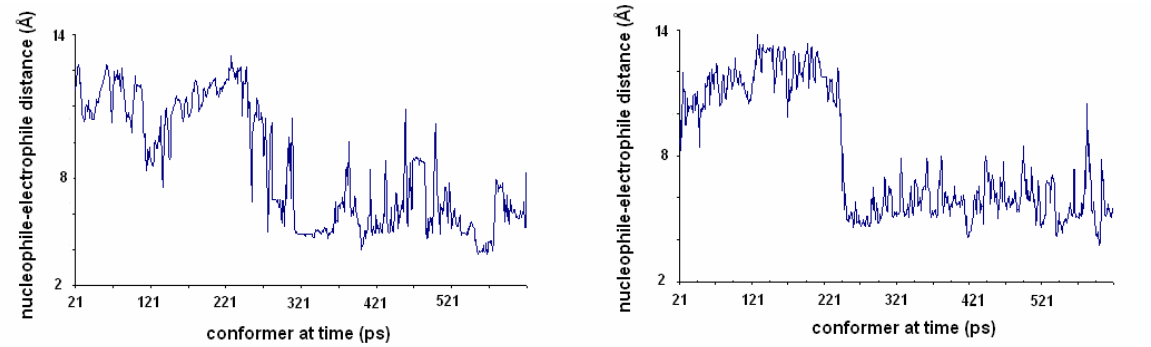


Figure 2.12. Nucleophile-electrophile distance before macrocyclization for compounds **1a** (left) and **2a** (right).

2.5 Biological Assay

Cell survival assay was done to assess the biological activity of the peptidomimetics. In this assay, cells that express the receptor are cultured in serum free media (SFM) and eventually die via apoptosis. However, the cells can be 'rescued' if functional neurotrophins are expressed within the cells. Test wells are then supplemented with suboptimal (100 pM) or optimal (2 nM) concentrations of the corresponding neurotrophin and then observed for growth or death.

Thus, these tests were designed to first check for agonism or antagonism, and then other tests such as receptor selectivity and specificity and toxicity can be conducted. Tests for agonism/antagonism use the fact that suboptimal neurotrophin concentrations afford limited protection from apoptosis (*e.g.* 25 %, throughout). Antagonism occurs when a non-cytotoxic peptidomimetic induces less than the sub-optimal 25% cell survival, and potentiation of activity occurs where survival is enhanced above that value. Additional agonism by the peptidomimetics can be tested by their ability to rescue cells from apoptosis in serum free media, in the absence of neurotrophins. Receptor specificity occurs, for instance, if a biological effect is observed for TrkC-expressing NIH cells (NIH-TrkC) but not for NIH-TrkA. Additional assays with wild type cells that do not express neurotrophin receptors are used to gauge general Trk receptor selectivity and lack of toxicity.

Table 2.5 shows the cell survival assay for compounds **1a-e** and **2a-e**. These assays were done in triplicates. The compounds did not show any significant agonistic properties even at suboptimal concentration of neurotrophin.

Table 2.5. Cell survival assay for compounds **1** and **2**.

Compound	**% survival of E25/TrkA cells**		**% survival of NIH3T3/TrkC cells**	
	SFM	NGF low	SFM	NT-3
untreated	0	0	0	0
1a	0.43 ± 0.48	-4.55 ± 2.58	0.41 ± 1.35	5.75 ± 1.68
1b	-1.14 ± 0.54	-6.0 ± 5.00	1.6 ± 0.64	-3.1 ± 1.45
1c	-1.99 ± 1.07	-13 ± 4.50	1.53 ± 1.04	-3.1 ± 1.95
1d	-2.26 ± 0.74	-11.5 ± 3.25.	-1.95 ± 1.03	-1.15 ± 2.98
1e	2.27 ± 0.93	3.5 ± 0.35	-3.8 ± 0.42	-3.25 ± 2.33
2a	-4.7 ± 0.00	-8.0 ± 0.00	-3.7 ± 1.2	-2.6 ± 3.30
2b	-1.3 ± 0.99	-5.5 ± 0.25	-0.47 ± 0.01	-2.45 ± 3.38
2c	-4.7 ± 0.14	-25.7 ± 9.85	0.18 ± 2.81	-1.3 ± 4.45
2d	-2.05 ± 1.10	-6.75 ± 3.08	-0.85 ± 3.15	4.0 ± 2.65
2e	-0.95 ± 0.25	-6.8 ± 3.30	-1.92 ± 2.04	2.85 ± 1.48

2.6 Summary

Molecular models of cyclic peptidomimetics **1** and **2** implied that the latter could adopt beta-turn structures and the former could not. This was the hypotheses at the outset of this study. However, after NMR and QMD experiments with representative structures of both compounds, it is clear that in solution, the preferred conformation for both compounds is not a beta-turn structure. It is not conclusive from NMR experiments if the asparagine side chain has affected this or the backbone has favored a different conformation other than a beta-turn. Yields from the syntheses indicated that the cyclization reactions to form compounds **2** are indeed more facile and cleaner. It is possible that this is due to some adaptation of a more ordered structure. While this is not fully supported by experimental results, theoretical calculations prove that compounds **2** have some bias toward a turn structure.

CHAPTER III

PEPTIDOMIMETICS THAT INCORPORATE THREE RESIDUES OF THE TETRAPEPTIDE BETA-TURN SEQUENCE

3.1 Introduction

The design of a peptidomimetic involves the successful incorporation of the necessary elements responsible for the biological activity of a particular protein or peptide target. In designing beta-turn mimetics, the main focus has been on the dipeptide at the tip of the turn wherein the beta-turn is centered. Inclusion of this sequence or its side-chains into a small molecule is perhaps the first goal in designing a peptide mimic. The rational for zeroing in on the beta-turn dipeptide sequence is that most interactions involved beta-turns [73,74] Thus, most small molecule mimics have featured only the beta-turn sequence or side-chains. [26 75] While this have proved to be successful, there are some biologically relevant proteins or peptides whose activities are mediated by more than two amino acid residues. Examples of such compounds include somatostatin,[76] the enkephalins[77] and the well-known RGD sequence.[78] Although more than two amino acids determine the biological activity of these compounds, the beta-turn conformation, is still present within or in the vicinity of these sequences. For example in somatostatin, a beta-turn is observed that projects the residues Phe7, Trp8 and Lys9 in the proper three-dimensional arrangement to interact with the receptor.[79] It is apparent that designing peptidomimetics which can accommodate more than a dipeptide sequence/side chain and still exhibit beta-turn conformation is very desirable. Outlined in the next few paragraphs are examples of such compounds applied to mimic biologically active peptides.

Somatostatin, or more appropriately, SRIF-14, is a cyclic tetradecapeptide which belongs to the family of somatotropin-release inhibiting factors (SIRFs).[80] They bind to G-protein-coupled receptors and in doing so, act as neuromodulators or neurotransmitters. They are also involved in inhibiting secretory pathways and cell proliferation.[81] The critical sequence that is believed to be responsible for its activity is the Phe7-Trp8-Lys9-Thr10 tetrapeptide moiety.[76] Hirschmann's group has done

extensive studies on somatostatin and they have developed several peptidic and non-peptidic mimics for the peptide.[82] A novel approach that was applied in their research is using what later would be termed to as privileged structures.[83] These are compounds that have inherent ability to adopt conformations (like alpha helices or beta-turns) of biological interest. One of their designs was the use of D-glucose as a scaffold that projects the necessary side chains of the bioactive tetrapeptide sequence of somatostatin (Figure 3.1).[84,85]

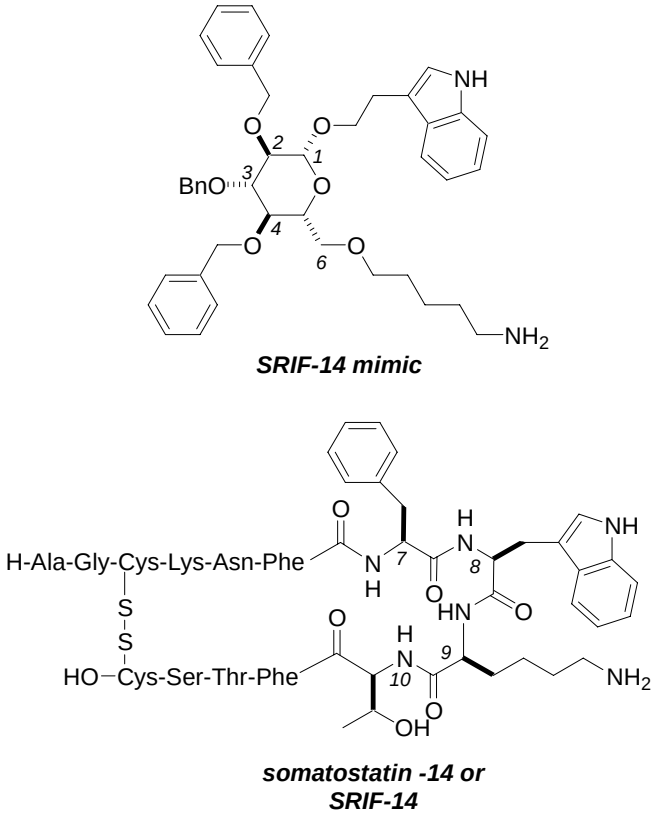


Figure 3.1. Hirschmann's somatostatin mimic based on a sugar scaffold.

Analogous to the work described above was inclusion of a sugar amino acid (SAA) in a peptide backbone. In contrast however, the pyranose structure in this case is used a dipeptide isostere. Kessler have successfully applied this approach on the same somatostatin sequence by incorporating glucosyluronic acid methylamine as the SAA moiety.[86] This work was extended by synthesizing other sugar amino acids capable of

dictating the peptide backbone to adopt a desired conformation.[34] Examples of these are shown in Figure 3.2.

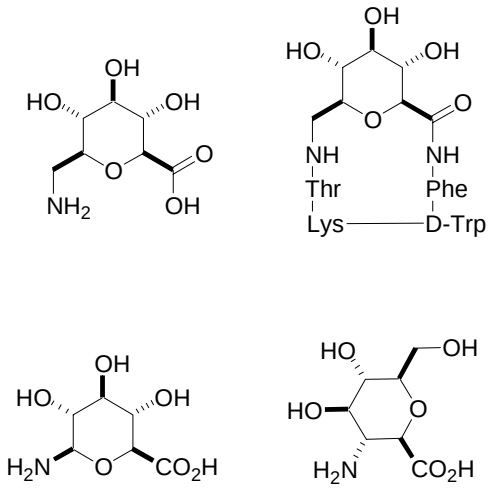


Figure 3.2. Top: Somatostatin mimic (right) that includes the sugar amino acid on the left. Bottom: Other turn-inducing dipeptide isosteres.

The highly active Arg-Gly-Asp (RDG) sequence, also known as the "universal cell recognition sequence" is found in many extracellular matrix proteins.[78,87] It is believed that the tripeptide is involved in a turn structure in the protein's bioactive conformation that is the key to binding integrins. Indeed, several X-ray structures of biologically active proteins with this sequence show that the tripeptide is found along the tip of a flexible loop region of the protein.[88] Despite of this common sequence, it was shown that the conformation around or near this sequence can dictate the specificity between different integrins.[89] Kessler have used several known dipeptide turn mimetics to make cyclic peptide analogs containing the RGD sequence.[90] These turn mimics replaced the D-Phe-Val dipeptide sequence of cyclo-(RGDfV), a highly active and $\alpha_v\beta_3$ selective cyclic peptide (Figure 3.3).[88]

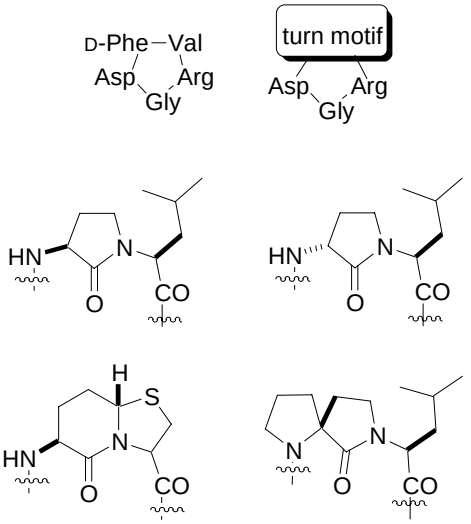


Figure 3.3. Kessler's application of turn-inducing heterocycles in making cyclic RGD mimetics.

While Kessler's work on RGD mimics have focused on dipeptide beta-turn surrogates, DeGrado's approach in designing analogs of the same sequence involved the use of an organic template to constrain the cyclic peptide.[33,91] In principle, this method is related to our approach. It is interesting to note that a change in beta-turn structure involving Arg (as the *i + 2* residue in the XX-Arg sequence) can change the specificity of the peptidomimetic to a particular integrin (Figure 3.4).[89]

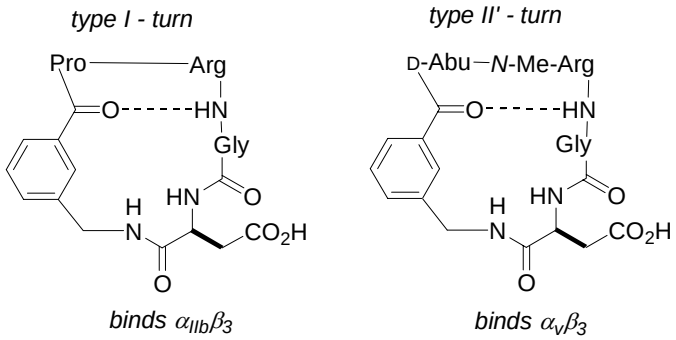


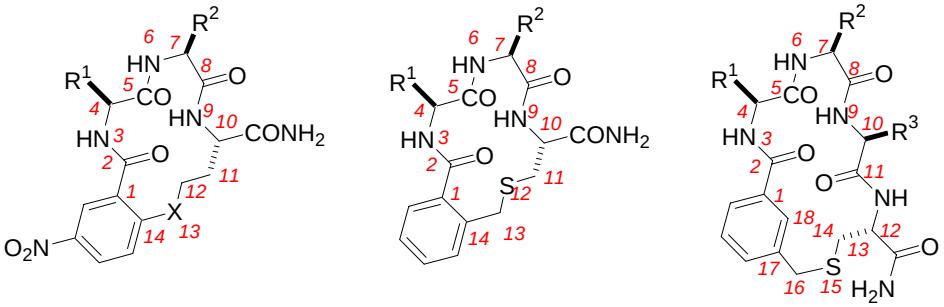
Figure 3.4. DeGrado's template-constrained cyclic RGD mimics.

It can be inferred from DeGrado's work that the organic template, *m*-aminomethyl benzoic acid (Mamb), they have used is versatile in the sense that it can, together with the amino acids in the peptide backbone, impose different types of beta-turn conformations. It is therefore, very desirable to use other templates similar in structure in our attempts to make cyclic peptidomimetics of neurotrophins.

3.2 Solid-phase S_N2 Macrocyclizations

The previous chapter has presented the rationale in our design to make beta-turn mimics of neurotrophins. Those initial mimics from our laboratories involved only dipeptides that occupy the tip of the beta-turn sequence (compounds **A** below). However, as pointed out in the discussion above, there are amino acid residues other than those in the beta-turn itself that contribute to the protein's biological activity. While this is true, the beta-turn conformation can not be discounted because the other residues can be found in the vicinity of the secondary structure. It is therefore ideal to make peptidomimetics that incorporate more than a dipeptide and still adopt a beta-turn conformation.

In our synthetic scheme, adding an extra or even two amino acids is not difficult because it is based on peptide synthesis. However, the flexibility of the molecule is being compromised with each amino acid being added. The template of choice plays a crucial role in maintaining the desired constraint within the molecule. It was mentioned before that a template similar to that of Mamb is ideal in this situation. Consequently, compounds **3** were designed. It features three amino acid residues which make the ring size larger (18-membered) than the usual 14-membered ring designs. Also, the cyclization mode is now S_N2 instead of S_NAr. Unlike for aromatic substitution, there are relatively few reports that feature an intramolecular S_N2 reaction as the macrocyclization step and those works are not related to peptide mimicry[92,93]. Our group have employed this type of ring closure strategy before with 14-membered ring S_N2 compounds.[66] From this early work, it can be noted that intermolecular cyclization still occurs at a considerable degree that significantly lowers the purities of the desired products.[94] So at the outset, it is not known if the same phenomenon will be observed with a larger ring size.



S*$_N$*Ar
X = NH, O, S
14-memebred

S*$_N$*2
14-memebred

3
18-memebred

3.3 Template Design

The template that was used for the synthesis of compounds **3** resembles the Mamb (*m*-aminomethyl benzoic acid) template that DeGrado and co-workers utilized in their work on RGD peptidomimetics.[33] These cyclic RGD mimics are also 18-membered macrocycles and were made on an oxime resin (Figure 3.5). One significant difference of our work from DeGrado's is that we did not use turn-inducing residues to force beta turn conformations on our molecules.[89]

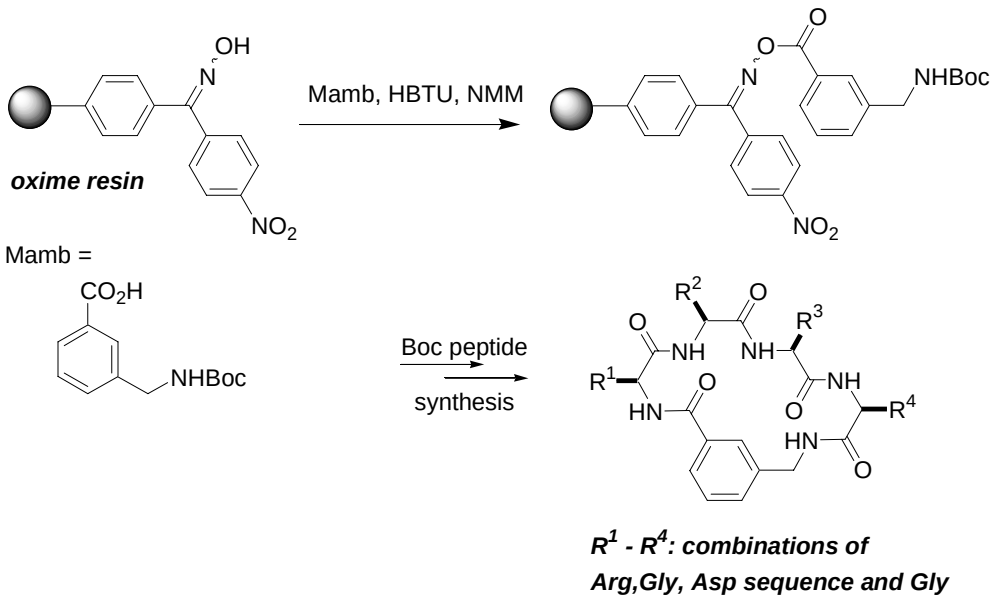
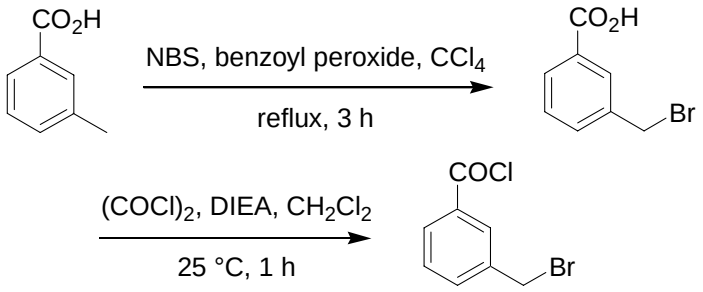


Figure 3.5. DeGrado's solid-phase synthesis of cyclic RGD mimics constrained by Mamb template.

The synthesis of the organic template used to constrain compounds **3** is shown Scheme 3.1. Radical bromination of *m*-toluic acid was done to install the benzylic bromide.[95] The carboxylic acid functionality was later converted to the acid chloride using oxalyl chloride. This makes it activated for coupling with the peptide on the solid support.

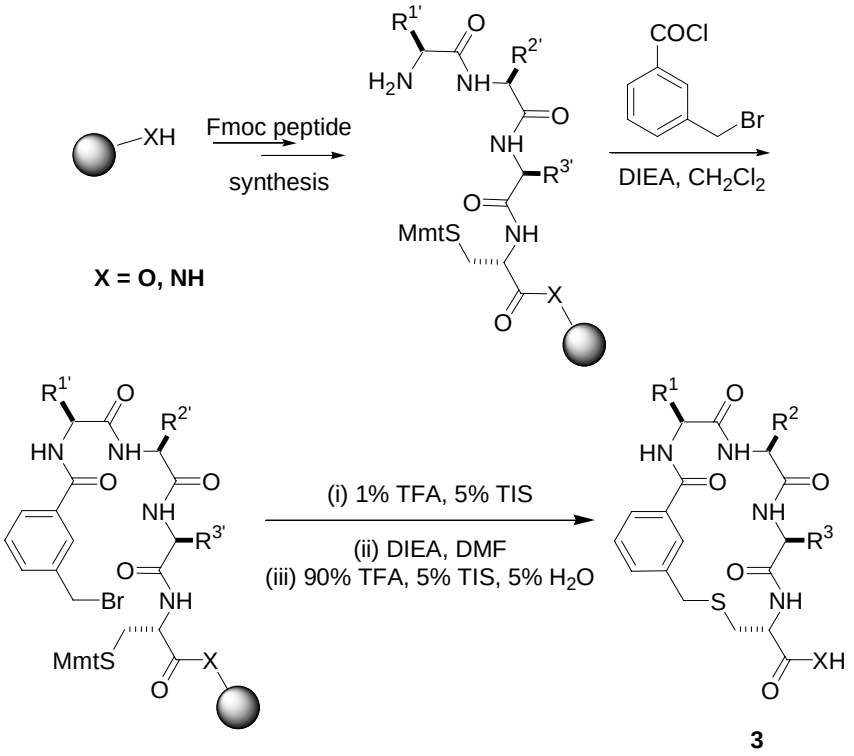
Scheme 3.1. Synthesis of the organic template



3.4 Synthesis of Compounds 3

Fmoc peptide synthesis was utilized to make the linear tetrapeptide precursors (Scheme 3.2).[96,97] FmocCys(Mmt)-OH was the first amino acid to be coupled to the resin and was followed by three amino acids that make up the three residues found in the turn regions of interest in NGF and NT-3. After the last amino acid was attached, the Fmoc group was removed and the resulting amine, which is at the *N*-terminus, was capped with the template. Removal of the thiol protection, cyclization and then cleavage from the resin afforded the final peptidomimetics which is now expanded to three amino acid residues. One main difference with regard to an S_NAr cyclization and the S_N2 mode applied here is that for the latter, ring closure can be done in milder basic conditions and also, the rate of the cyclization is faster. Another significant and somewhat surprising result is that formation of the head to tail dimer is very much suppressed. Although MS can still detect it, UV and ELS detections in HPLC suggest this side product is not significant.

Scheme 3.2. Synthesis of compounds 3



3.5 Resin Performance Comparison

Solid phase synthesis of short (less than 5 residues) linear peptides is somewhat trivial and most resins in general will give clean short linear products. It is in reactions (examples in this case would be template attachment and cyclization) that divert the peptidic nature of the compound being synthesize that resins could give varying results in terms of the purities of the product being generated. To assess the generality of the synthesis of compounds **3**, different resins were employed on three trial sequences. The resins can be divided as either having the Rink handle (amino resins) or those that have the Wang handle (hydroxyl resins).[98,99] Figure 3.6 illustrates the two types of resin used. Aside from the difference in the handle, the spacer can also be varied to give the polymer matrix swelling characteristics absent in a mainly hydrophobic polystyrene (PS) core.

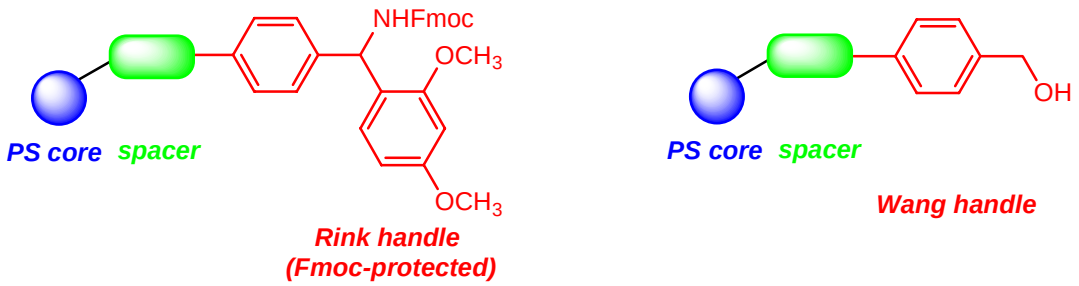


Figure 3.6. Illustrations of two major resin types used.

Table 3.1 shows the result of the resin comparison study. Six Rink-type resins and three Wang-type resins were used for three different sequences (NKK, ENN and DGK). Except for the Synphase Lanterns, all the others follow the same washing procedure. The one for the lanterns had to be modified because of its built and properties are different from the rest. It turned out that it was not a suitable solid support for this purpose as shown by the lower purities of the compounds cleaved from this type of resin. On the other hand, the rest of the amino resins gave excellent purities consistently. The hydroxyl resins are not as consistent and this is probably due to the dependence on the sequence being installed. While the loadings are different in each resin, it is not to be concerned about because we are just screening the overall applicability of the reaction; and it turned out that the reaction sequence to synthesize the compounds like **3** is tolerated well by resins with the Rink handle.

To make a small library of compounds **3**, TentaGel S Ram Fmoc resin was chosen. Table 3.2 shows the result of the syntheses. The purities and the yields are high except for about three compounds. Some of the yields are lower because of sampling of few beads after certain stages in the synthesis to monitor the progress and the efficiency of the reaction. Like in the three trial sequences used in the resin study, the formation of head to tail dimer side product is not significant in these reactions. After cleavage from the resin, the compounds were purified via reverse phase HPLC and their purities were assessed under UV and ELS (Sedex) detectors. Finally, the solutions from HPLC were concentrated in vacuo, flash frozen and freeze dried to afford solid, powdery white compounds.

Table 3.1. Resin comparison study on three trial sequences.

resin	handle	loading (mmol/g)	supplier	3-AA[1]-AA[2]-AA[3]		
				NKK	ENN	DGK
				% purity UV (Sedex)	%purity UV (Sedex)	% purity UV (Sedex)
Tenta Gel S RAM Fmoc	Rink	0.30	Advanced ChemTech	99 (96)	88 (94)	66 (75)
Tenta Gel S RAM Fmoc	Rink	0.22	Rapp Polymere	90 (93)	88 (93)	91 (97)
Argo Gel Rink NH Fmoc	Rink	0.33	Argonaut	95 (99)	88 (96)	83 (91)
Hypo Gel 400 RAM	Rink	0.53	Rapp Polymere	94 (99)	88 (95)	82 (88)
Rink Amide MBHA	Rink	0.61	NovaBiochem	95 (98)	89 (94)	85 (94)
Synphase Lantern	Rink	0.036	Mimotopes	75(91)	67 (84)	89 (92)
Tenta Gel S AC	Wang	0.24	Rapp Polymere	90 (84)	83 (97)	44 (52)
Hypo Gel 400 PHB	Wang	0.61	Rapp Polymere	82 (95)	67 (76)	56 (67)
Nova Syn TGA	Wang	0.25	NovaBiochem	90 (100)	36(29)	n/a

Table 3.2. Crude Purities and Isolated Yields for Compounds **3**.

compound	sequence	purity (%)		isolated yield (%)
		UV	ELS	
3a	IKG	92	95	27
3b	DIK	72	77	45
3c	NNS	92	97	16
3d	INN	93	92	29
3e	DGK	66	75	69
3f	GKQ	81	84	16
3g	IRG	87	91	36
3h	DIR	83	79	34
3i	KTG	91	89	68
3j	NNK	97	97	21
3k	ENN	89	94	46
3l	NKQ	64	67	36
3m	DNK	67	81	20

3.6 Conformational Analyses of Compounds 3

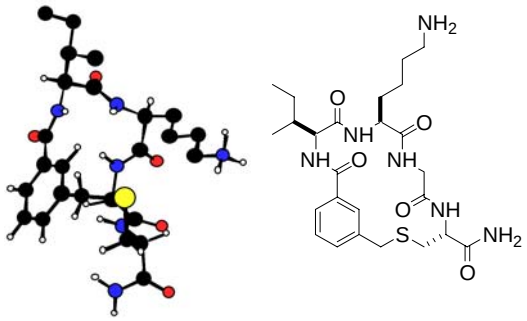
To asses if compounds **3** adopt beta-turn structures, QMD, NMR experiments (1-D, ROESY,[100] temperature coefficient), and CD were performed on two representative structures (**3a** and **3g**). These two were chosen because of their displayed bioactivity in initial cell-survival assay (see section on assay data). Table 3.3 shows the QMD analysis of compound **3a**. The lowest energy conformer belongs to the most populated family of the three families generated. The dihedral angles around the Ile and Lys residues correspond well with a type I beta-turn (-60°, -30°, -90° and 0°, normally, this last one is either omitted or the angle should be within 30 degrees). Also the distance between the oxygen of the carbonyl (C=O) of the template (*ie i* residue) and the NH hydrogen of the *i*

+ 3 residue (Gly) is within acceptable distance that could indicate H-bonding at these sites.

Table 3.3. QMD results for the lowest energy conformer of compound **3a**.

Amino acid residue	Dihedral angle (°)	Lowest energy conformer of 3a
Ile (R^1)	ϕ	-74.32
	ψ	-36.92
Lys (R^2)	ϕ	-95.28
	ψ	-22.36
Distance (Å)$CO_i - NH_{i+3}$		3.096
Type of beta-turn		Type I

One-dimensional (1-D), DQFCOSY[101], and TOCSY[102] experiments were utilized to verify the structure of the compound. To evaluate crosspeaks that suggest beta turn properties, ROESY, temperature coefficient experiments and coupling constants from the 1-D NMR were employed. Type I beta-turn in general will show correlation peak between the $i + 1$ and $i + 2$ amide hydrogens because the carbonyl of the latter is oriented in such a way that the hydrogen is *cis* to the side chains of the residues. ROESY results showed that this is indeed the case for **3a** (Figure 3.7). The observed ROE cross peaks (particularly those for Ile and Lys NH's, and Lys and Gly NH's) correspond well with accepted intensities (*eg*. medium for *i + 1* residue) for a type 1 beta-turn and these ROE's fit nicely with QMD-measured distances.



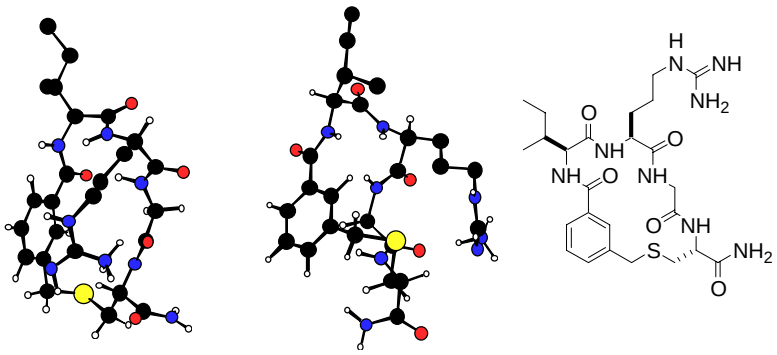
Hydrogen	ROE	QMD distance (Å)
Ile $_{NH}$ - Lys $_{NH}$	medium	2.283
Ile $_{C\alpha H}$ - Lys $_{NH}$	not obsd.	3.486
Lys $_{NH}$ - Gly $_{NH}$	weak	2.079
Lys $_{C\alpha H}$ - Gly $_{NH}$	not obsd.	3.458

Figure 3.7. Top: Lowest energy conformer of **3a** from QMD. Bottom: Correlation between ROE cross-peaks and distances from QMD.

For compound **3g**, similar results were observed in QMD but this time, two families were almost equally populated. Table 3.4 shows the results. Both families behave in almost the same way and the dihedral angles correlate very well with those for Type I turn. The ROE crosspeaks for relevant protons were measured and tabulated with distances from QMD (**Figure 3.8**). Again, the results are similar to that of **3a** indicating the **3g** adopts type 1 beta-turn structure. The main difference between the QMD structures of the two families is the orientation of the Arg side chain. For Family 1, the guanidine side chain is involved in a pi-cation interaction with the aromatic ring that made the former bend over the latter. Family 2 shows a structure in which this side chain is flipped back and away from the ring. It is possible that in solution, these two conformers are interconverting, hence, the observed coupling constants are more of an average of the calculated ones (*e.g.* for ArgNH, calculated: 8.7 and 5.7 Hz for Family 1 and 2 respectively; observed: 8.1 Hz)..

Table 3.4. QMD results for the lowest energy conformer of compound **3g**.

Amino acid residue	Dihedral angle (°)	Lowest energy conformer of 3g	
		Family 1	Family 2
Ile (R^1)	ϕ	-73.40	-73.57
	ψ	-37.13	-29.89
Arg (R^2)	ϕ	-98.3	-72.13
	ψ	-21.58	-15.93
Distance (Å)$CO_i - NH_{i+3}$		3.117	2.344
Type of beta turn		Type I	Type I



Hydrogen	ROE	QMD distance (Å)	
		Family1	Family2
Ile $_{NH}$ - Arg $_{NH}$	medium	2.310	2.234
Ile $_{C\alpha H}$ - Arg $_{NH}$	not obsd.	3.490	3.459
Arg $_{NH}$ - Gly $_{NH}$	weak	2.042	2.520
Arg $_{C\alpha H}$ - Gly $_{NH}$	not obsd.	3.453	3.418

Figure 3.8. Top: Low energy conformers of the two families of **3g** from QMD. Bottom: Correlation between ROE crosspeaks and distances from QMD.

Temperature coefficients (Tc) of the amide NH's were measured to assess internal hydrogen bonding at the carbonyl (C=O) of the template and the GlyNH. The Tc values measured were -3.3 and -2.8 for **3a** and **3g**, respectively. These values are indicative of either hydrogen bonding or solvent shielding.[36] QMD measurements of the distance between these two sites show that they are within hydrogen bonding range. The CD spectra for **3a** and **3g** are shown in Figure 3.9. They have similar patterns that can be

associated to a type 1 beta-turn profile: a negative band around 220 nm and a positive band near 190 nm.[44] The latter one is obscured because of instrument limitations. However, this spectrum is close to a class C which is associated to well-described type I beta-turn systems.[43] Thus, these temperature coefficients and CD spectra complement each other, giving a strong indication that the compounds adopt a type 1beta-turn conformation in solution.

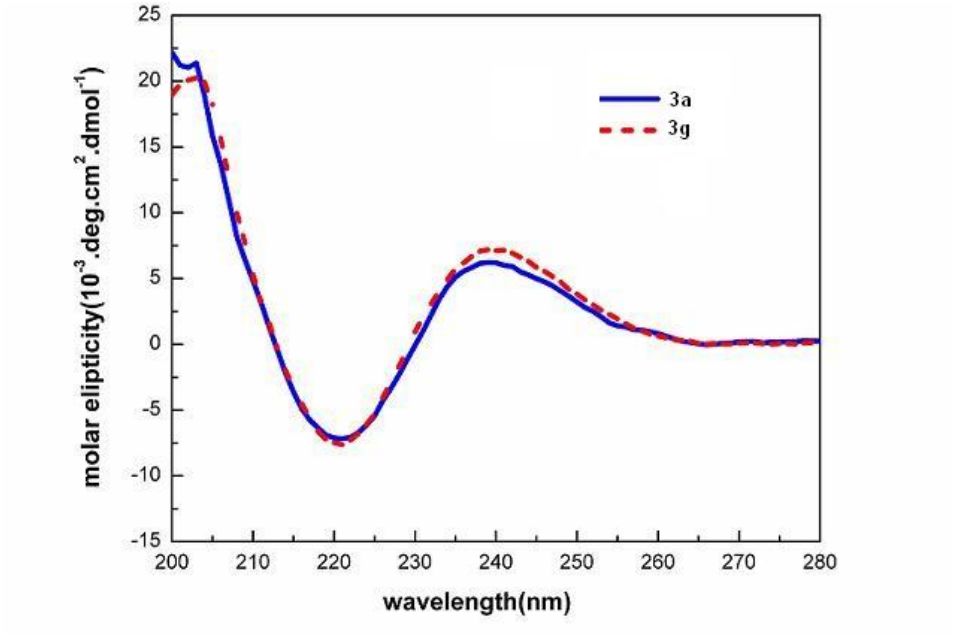


Figure 3.9. CD spectra of compounds **3a** and **3g**.

3.7 Assay Results for Compounds 3

The compounds in Table 3.2 were submitted for a cell survival assay (see previous chapter for description of the assay). However, some compounds have limited solubility and so were not assayed. The activities of those tested were tabulated in Table 3.5. Most of the compounds show no interesting activity, however, compounds **3a** and **3g** showed significant agonistic properties (% survival in bold) in TrkC expressing cells at suboptimal concentrations. It is interesting to note that **3a**, whose sequence correspond to that of NGF, did not show any activity for TrkA cells, but was active for TrkC cells.

These results showed that monovalent mimics can function as agonists to promote cell survival.

Table 3.5. Cell survival data for compounds **3**.

Compound	% survival of E25/TrkA cells		% survival of NIH3T3/TrkC cells	
	SFM	**NGF low**	**SFM**	**NT-3 low**
Untreated	0	0	0	0
3a	-3.23 ± 0.78	-14.9 ± 6.37	1.48 ± 0.62	**14.12 ± 3.99**
3b	-3.94 ± 0.48	-27.1 ± 5.23	0.21 ± 2.44	-17.8 ± 2.05
3c	-5.87 ± 2.58	-16.4 ± 3.80	3.22 ± 1.48	-9.53 ± 0.26
3e	-1.29 ± 2.18	-10.4 ± 0.00	2.17 ± 0.42	0.39 ± 0.31
3g	3.55 ± 1.12	-8.47 ± 0.88	2.65 ± 1.45	**15. 8 ± 5.76**
3j	-0.18 ± 2.14	-5.62 ± 1.15	2.83 ± 0.52	0.41 ± 2.00
3k	-6.38 ± 2.80	-20.7 ± 3.23	3.18 ± 0.76	-12.5 ± 1.71

3.8 Summary

Larger (18-membered vs. 14-membered) macrocyclic peptidomimetics **3** were made employing solid phase synthesis. Compared to previous peptidomimetics that are smaller in ring size (14-membered), the S_N2 cyclizations used to afford these larger cyclic compounds are less prone to head-to-tail dimer formation. The synthetic procedure applied to make the compounds is tolerated well by resins with the Rink handle and less by those with Wang-type resins. This assertion was verified when good to excellent purities were attained in the synthesis of a small library corresponding to NGF and NT-3 sequences was synthesized using Tenta Gel S Ram resin (Rink handle).

Conformational analyses of representative compounds show that these 18-membered macrocycles adopt type 1 beta-turn conformation. Both theoretical (QMD) and experimental (NMR and CD) experiments support this inherent bias.

Cell survival assay of the compounds reveal that **3g**, whose sequence mimics one turn of NT-3 have agonistic properties at suboptimal concentrations of NT-3.

CHAPTER IV

SYNTHESIS OF MONOVALENT PEPTIDOMIMETICS FOR DIMERIZATION

4.1 Introduction

Protein-protein interactions are wide-spread in biological systems. These interactions produce diverse effects, for example in antibody-antigen recognition, signal transduction and metabolism among others, that are critical in regulating cellular functions. A lot of these interactions are mediated by dimeric protein complexes.[103] These dimers can be regarded as homocomplexes or heterocomplexes depending on the nature of association of the individual protein components. Most homocomplexes involve permanent association while heterocomplexes are rather short-lived and transitory in nature.[104] Protein-protein interactions occur at the protein surface and these can be considered as biophysical events dictated by the conformation, chemical composition and flexibility of the proteins involved.[105] Knowing how proteins interact is paramount in understanding how these proteinaceous assemblies mediate different biological responses.

A great deal of study has been devoted on how protein complexes are formed. The findings from these contributions reveal that the total area buried by the residues in the protein-protein recognition site is about 1600 Å.[106] This is very much bigger compared to an enzyme binding pocket. Also, the surface patch at the binding interface can be described as flat.[107] It is difficult to identify lead compounds to study protein-protein interactions if there are no naturally occurring small molecules that bind these proteins. Despite of this problem, there are small molecules that can modulate protein-protein interactions. Schreiber, Crabtree et al. have shown that naturally occurring immunosuppressants (FK506, cyclosporine and rapamycin) can induce protein dimerization.[108,109] Although these compounds are not dimeric, they act as dimerizers through bivalency and were shown to affect certain pathways leading to T-lymphocyte activation.

The need for synthetic molecules to study bivalent (or multivalent) interactions has generated extensive efforts to produce dimeric compounds in scale and in high purities.[110] Bivalent compounds offer advantages that monovalent ones cannot provide. For example, they promote proximity to interacting species like in many cell surface receptors.[111] They also enhance protein-protein or protein-DNA interactions by providing an enlarged surface area which results in increased specificity [103,112]. Bivalent compounds can be used to probe ligand-receptor binding at the cell surface as in the case of neurotrophins and their Trk receptors. They can be a potential antagonist if it prevents dimerization by binding to one unit of the receptor dimer. On the other hand, it can be an agonist or a functional mimic if it induces receptor dimerization by binding at two monomeric receptor sites (Figure 4.1)

To avoid confusion, it is important early on to set a working definition for 'dimeric' and 'bivalent' that will be used in the discussion. While dimeric, refers to the chemical structure of a compound, bivalent relates more to function. For simplicity, dimeric compounds in the discussion will be regarded as 'bivalent' and whenever bivalent is used, it is regarded as a dimeric compound except for the one in Section 4.3.2.

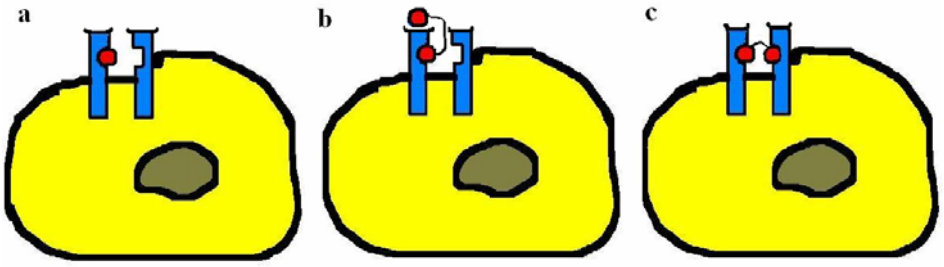


Figure 4.1. (a) A monomeric small molecule may bind to one site and act as an antagonist. (b) A bivalent analog binding at two sites on the same receptor unit can be an even stronger antagonist. (c) A bivalent analog can bridge two monomeric receptor units and act as an agonist.

While it is trivial to make just one or few dimeric compounds, the full potential of exploiting the combinatorial advantage of making dimers out of monomeric precursors will not be realized if we lack methods in making these dimers selectively. In other

words, what we want is a situation in which monomers are simply mixed in reaction vessels that will give one dimeric species exclusively (*i.e.* if one monomer is reacted with another, only one dimeric product results). This is easy for homobivalent compounds; however, the situation is not the same if heterobivalent compounds are desired. The illustration in Figure 4.2 depicts this concept.

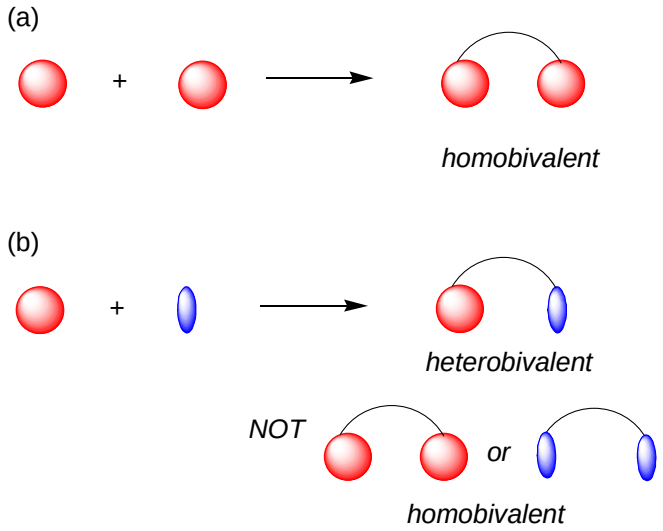


Figure 4.2. Illustration of heterobivalent selectivity.

Reactions of the kind implied above can be used to harness their potential to form bivalent compounds efficiently combinatorial chemistry. This possibility is illustrated in Figure 4.3. For n monomers, $n\,(n + 1)/2$ dimers will be produced; if such a highly efficient method exists, five different monomers, for example, can give 32 dimeric ones. This does not seem much, however, in the case of 100 monomers, 5050 dimers will be produced. Thus, the combinatorial advantage becomes very significant with larger numbers of monomers.

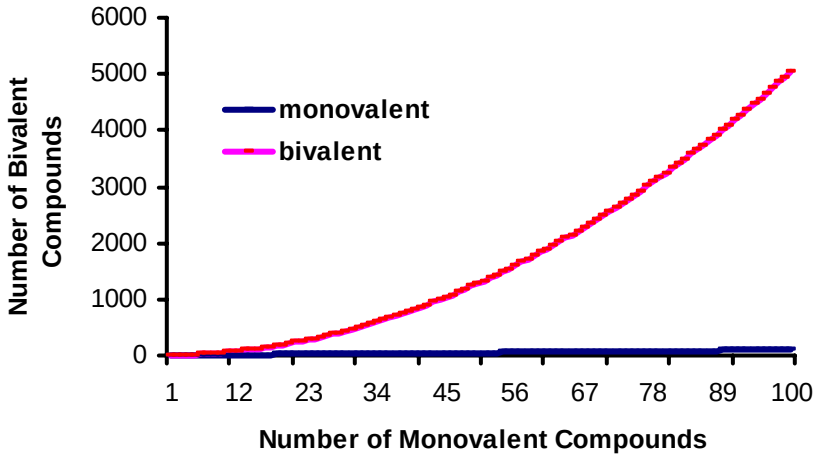


Figure 4.3. The number of bivalent compounds increases exponentially with unit increase in monovalent starting materials.

4.2 Illustrative Non-selective Approaches to Dimeric Compounds

There is a plethora of chemical reactions that can be used to make dimeric compounds. However, some of these reactions do not give exclusively one dimeric product as there are limitations (*e.g.* stereochemical issues) inherent to the reactions involved. This section gives examples of the most common techniques employed to make bivalent compound in a non-selective fashion (*i.e.* other products are formed as well).

4.2.1 Disulfide Bond Formation

One of the most common modes of dimerization is disulfide bond formation. The mild oxidative conditions used to form the S-S bond are tolerated by a lot of functional groups including those in amino acid side-chains. On the other hand, S-S bonds can be broken and reformed to make new disulfide-linked dimers. Nicolaou et al. have applied this 'combinatorial disulfide exchange' method to make dimeric analogs of the antibiotic psammaplin A.[113] The exchange however is not driven to completion as statistical mixtures of the heterodimer and the homodimeric starting materials are produced (Figure 4.4).

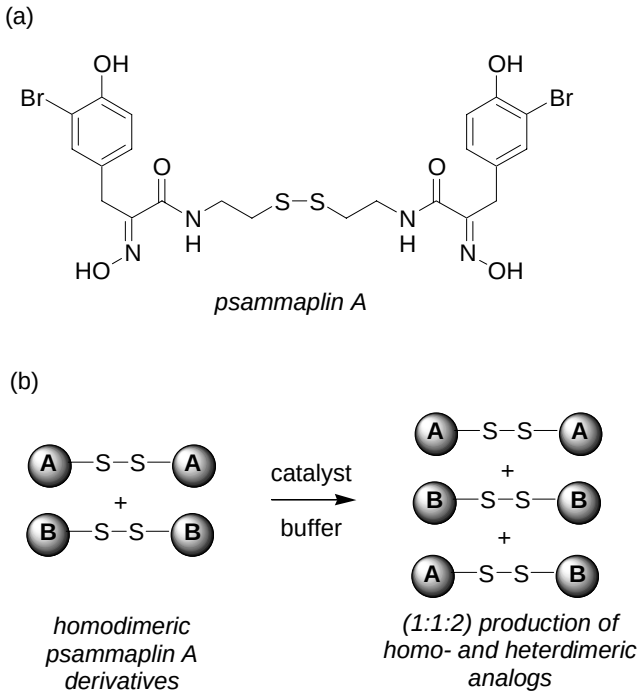


Figure 4.4. (a) Structure of psammaplin A. (b) Combinatorial disulfide exchange to produce homo-and heterodimeric psammaplin A analogs.

4.2.2 Olefin Cross-Metathesis

Boger and co-workers have employed this type of reaction to make libraries of EPO mimics (Figure 4.5).[114] A cyclic anhydride was used to attach the olefin fragment that will be used to link with another alkene via metathesis. This method can make homo- as well as heterobivalent compounds depending on the alkene monomers used, however, this method is not stereoselective as *cis* and *trans* isomers are produced.

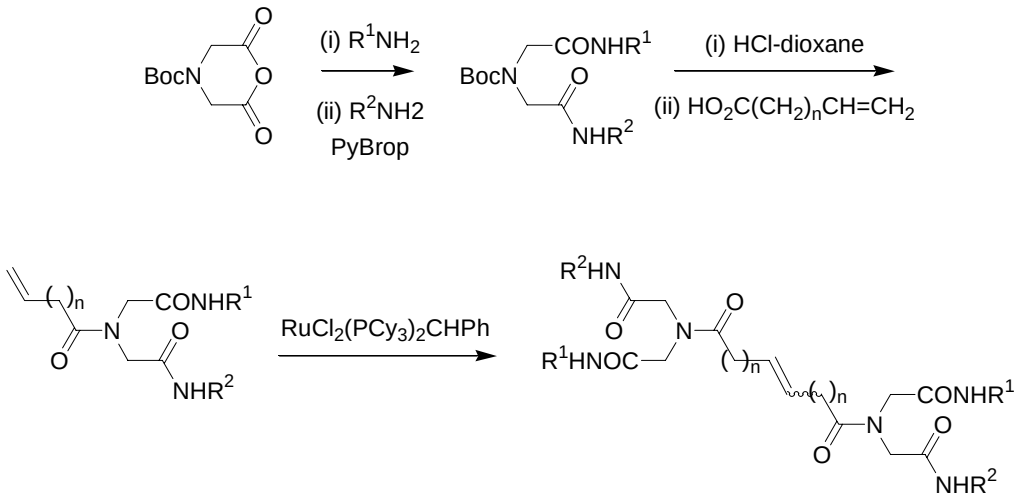


Figure 4.5. Boger's synthesis of dimeric EPO mimics via olefin methathesis.

4.2.4 Dimerization Using a Linker Molecule

The disulfide bond formation and cross-metathesis examples are dimerization methods in which there are no other starting materials needed to form the dimers other than the monomers themselves. Another way of forming dimeric species is to link two monomers via a 'bridging' molecule. One example of this approach is the work of Ellman on diaminoalkanediol-linked aldehyde monomers (Figure 4.6).[115] This approach takes advantage of the fact that oxime bond formation is fast and efficient. Also, rapid access to the dimers is made possible by using commercially available aldehyde and linker starting materials. In spite of these advantages, this process has one major draw back in that statistical mixtures of homo- and heterodimers are produced.

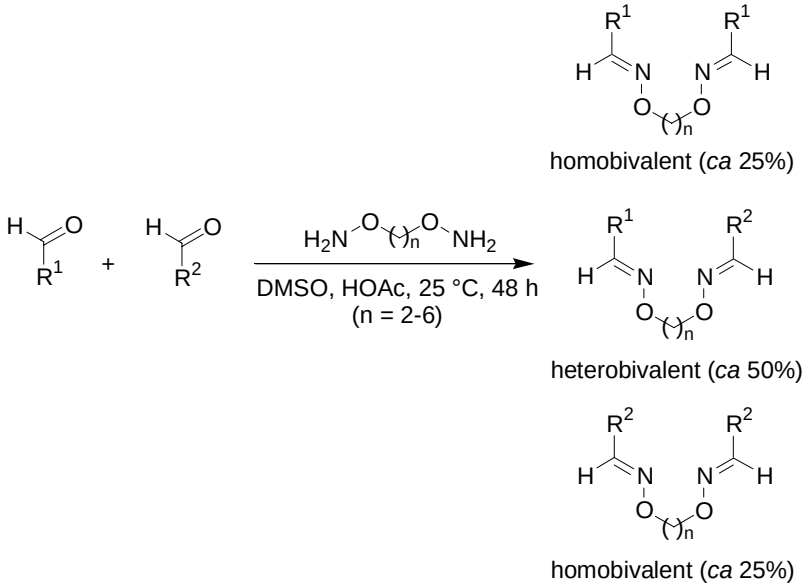


Figure 4.6. Ellman's diaminoalkanediol-linked dimers**.**

4.3 Selective Formation of Bivalent Compounds

4.3.1 Olefin Cross-Metathesis

Unlike, non-selective approaches, there are relatively few methods that have been discovered or applied to give bivalent compounds exclusively. Although it is hard to control the alkene geometry in a metathesis reaction in solution, Verdine et al. reported a solid-phase variant in which the *trans* isomer predominates very significantly (~ 96 % pure) (Figure 4.7). The starred positions were varied to produce 16 compounds that mimic endomorphine-2, an endogenous ligand for μ–opioid receptor.

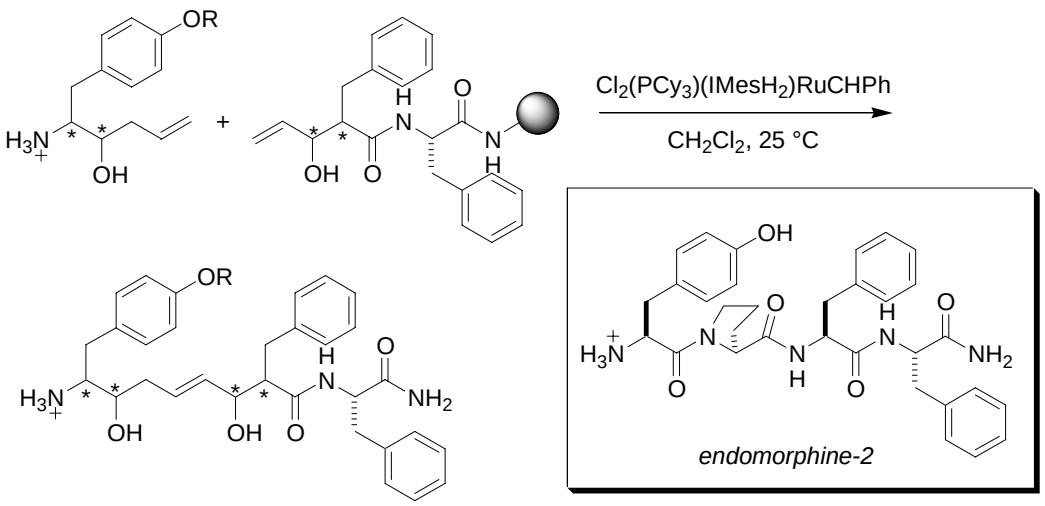


Figure 4.7. Solid-phase cross-metathesis that gives almost exclusively *trans* isomer.

4.3.2 Enzyme-catalyzed [3 + 2] Cycloadditions

One of the most used reactions recently is the [3 + 2] alkyne-azide cyclization (click chemistry).[116] While this reaction is often influenced by Cu catalysis, Sharpless, Finn, Kolb and their co-workers demonstrated that an enzyme can be used to bring the starting materials in close proximity, causing them to react without metal catalysis.[117] This approach not only produces bivalent compounds selectively, but also screens for compounds that can bind to the enzyme because only those that bind the enzyme are brought near to each other. Thus, the method serves as an *in situ* binding assay in effect. This is depicted in Figure 4.8. Five different alkyne and azide starting materials are incubated with the enzyme Acetylcholinesterase (AChE). But after incubation, only 2 of the possible 25 compounds are identified.[118] This work was extended by using different azide and alkyne pharmacophores that are previously not known to bind AchE.[119] Moreover, the applicability of this reaction with other another enzyme was demonstrated.[120]

It should be noted that while the compounds produced by this method are not dimeric, they are, however, bivalent in nature as two units interact with the enzyme.

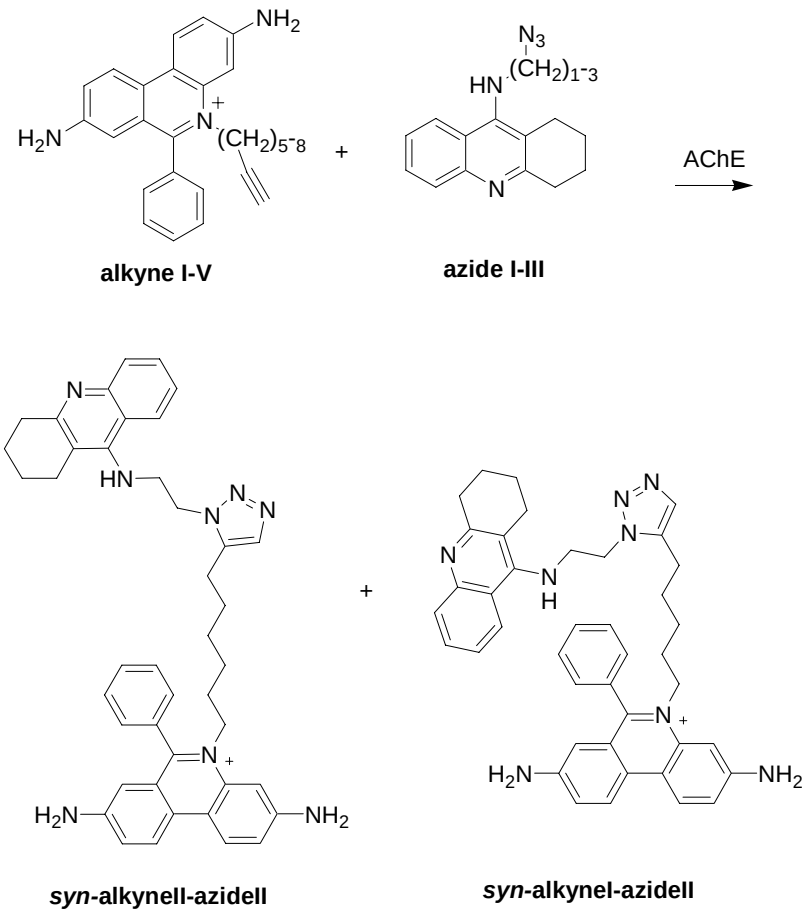


Figure 4.8. Production of bivalent ligands via click chemistry.

4.4 Sequential Nucleophilic Substitution

The techniques in Section 4.3 demonstrated that, although scarce, there are methods that can be applied to make bivalent compounds selectively. However, not all of those methods are convenient when applied to a library format. In medicinal chemistry, it is important to incorporate pharmacologically relevant functional groups in synthesis. Most of these pharmacophores can be found in amino acid side chains (*e.g.* guanidine, amine, thiol, alcohol, indole and imidazole). An ideal method of introducing diversity could be by simple substitution of leaving groups by nucleophiles which contain the desired pharmacophores. Although the idea seems obvious and simplistic, the chemistry was introduced only a few years ago. Zaragoza has coined the term sequential nucleophilic substitutions as a means of introducing diversity. This could be achieved by

using a polyelectrophile containing the leaving groups that will be replaced by nucleophilic compounds with the requisite pharmacophores [121]. The concept can be expanded by using different polyelectrophiles and most work on this area has focused on this aspect. Furthermore, the technique is greatly facilitated by employing resin-bound reagents. This will minimize laborious purification steps otherwise encountered in conventional solution-phase synthesis. Suitable compounds that have been used as polyelectrophiles are shown in Figure 4.9.[121-123]

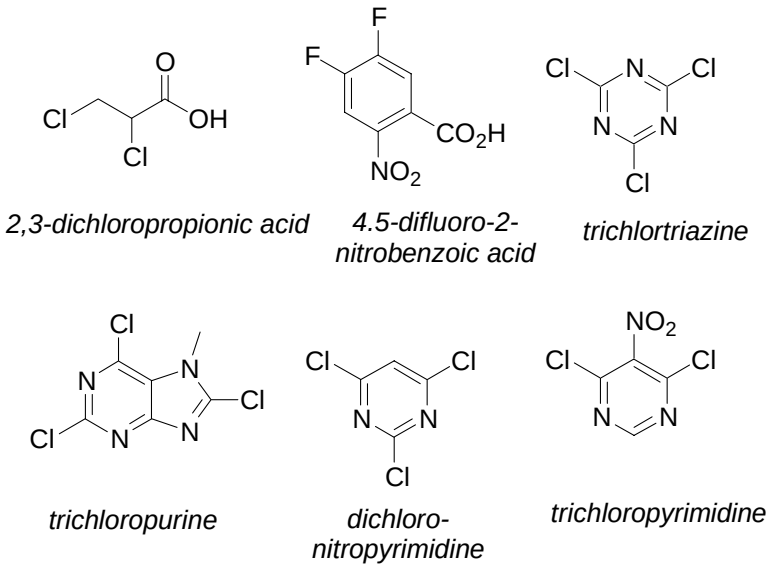


Figure 4.9. Polyelectrophiles used in sequential nucleophilic substitution reactions.

The feature common to all structures in Figure 4.9 is that they all have halogens as leaving groups. Thus, in sequential substitution reactions the mechanism is either S_N2 or S_NAr. Of all those structures, only the polychlorinated triazines, purines and pyrimidines were used to make cyclic peptidomimetics. Scharn *et al.* demonstrated that these polychloro heterocycles can be used as a template for cyclization. Cyclic peptides incorporating two to 10 amino acids were synthesized via the SPOT-synthesis[124] methodology and were cyclized using the amino group of the lysine side-chain Figure 4.10. One limitation of this technique is that if there are potential nucleophiles from amino acid side-chains (e.g. thiol, alcohol, amine or guanidine) then the cyclization reaction may give undesired products.

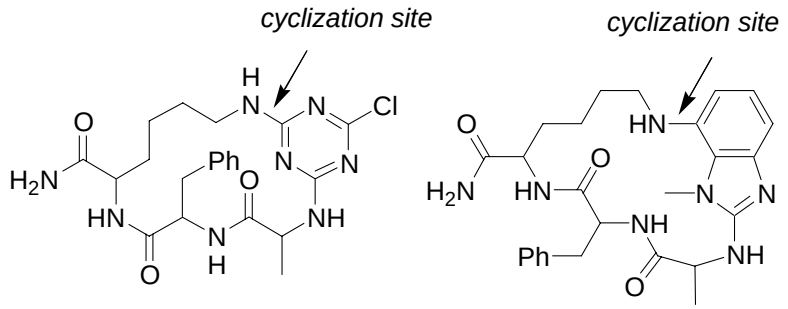
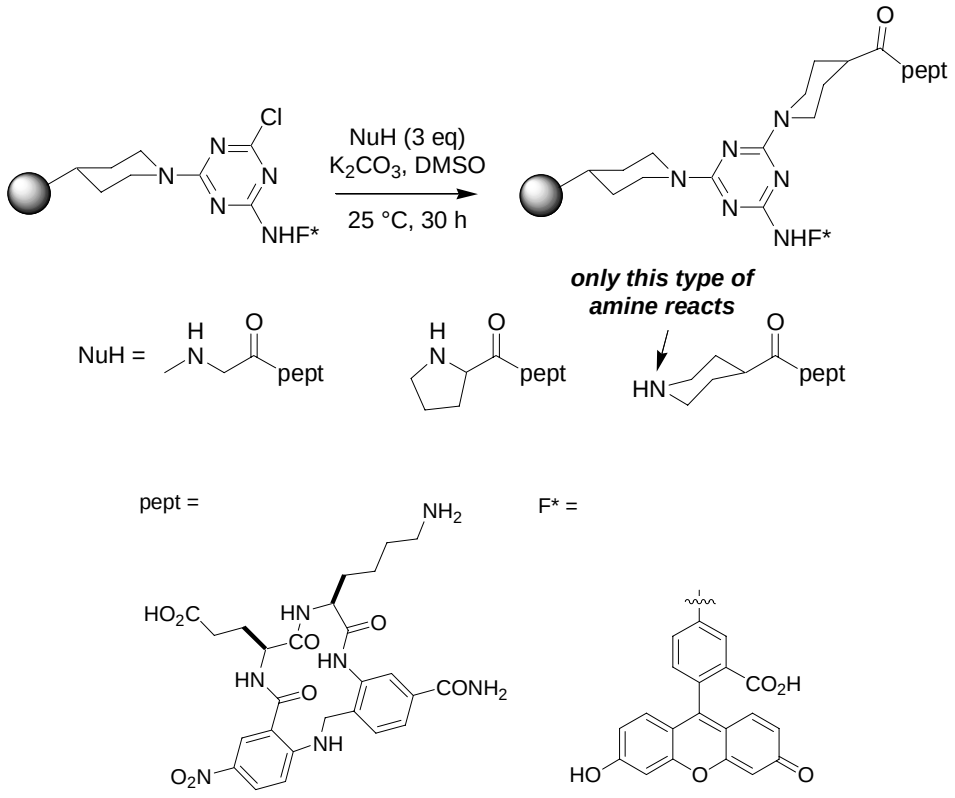


Figure 4.10. Examples of cyclic peptides using polychloro triazine and purine as templates for macrocyclizations.

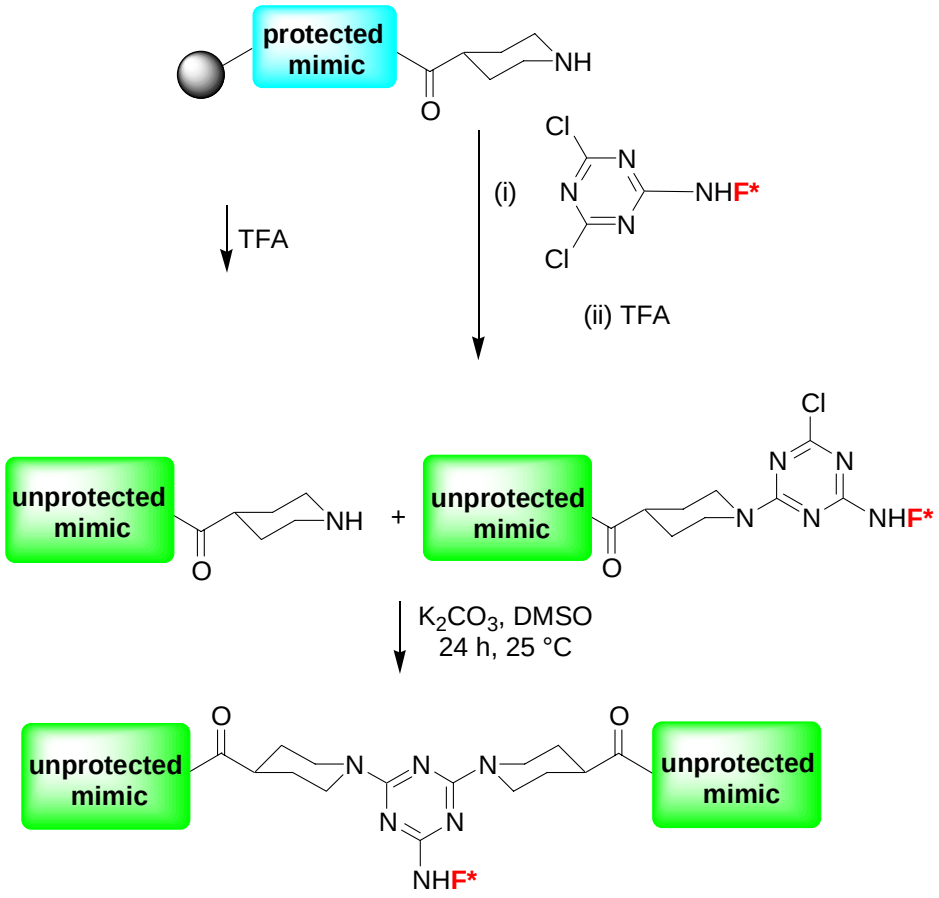
Our method to assemble dimeric compounds evolved from an interesting observation by a co-worker (Dr. Mookda Pattarawarapan) on a solid-supported triazine derivative.[125] She observed that with a monochlorinated triazine substrate, only the amino group from isonipecotic acid (Inp) was able to displace chlorine under the reaction condition used (Scheme 4.1).

Scheme 4.1. Selective displacement on monochlorinated triazine



The unusual selectivity of the piperidine amino group can be applied to make bivalent compounds in a step-wise fashion. The addition can be controlled, so one monomeric species bearing the reactive amine can be added on a fluoresceinated dichlorotriazine to give a labeled monomeric species. This in turn can be reacted with the same monomer or a different one to give fluoresceinated homo- or heterobivalent compounds (Scheme 4.2).

Scheme 4.2. Selective synthesis of homo- and heterodimers using dichlortriazinylaminofluorescein (DTAF) linker



homo- or heterodimers

4.5 Synthesis of Starting Monomers

4.5.1 Template Modification and Incorporation in Peptide Synthesis

For the monomers to be suited in the dimerization strategy depicted in Scheme 4.2, they have to have a site of attachment wherein we can link the required isonipecotic amino acid (Inp) fragment. The monomers in this case were compounds **3**a and **3g** and compound **B** (in Chapter III). These three were chosen based on their displayed activity in cell survival assays. The modification has to be on the organic template and not on the mimetic part because it is desirable to have the amino acid side-chains unencumbered,.

The design that we sought to make is to have a nitro group attached onto the benzene ring (Figure 4.11). This can later be reduced to the amine to serve as the coupling partner of Inp.

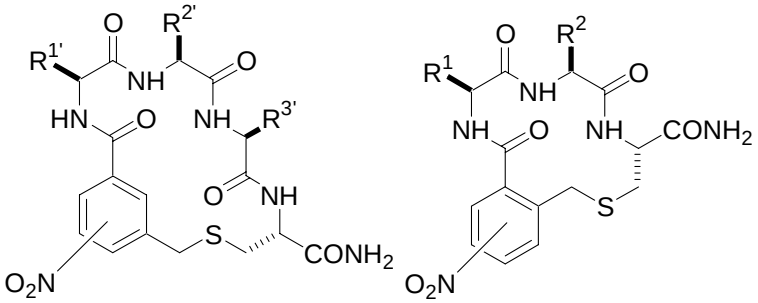
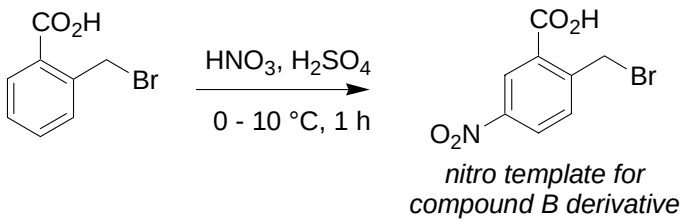


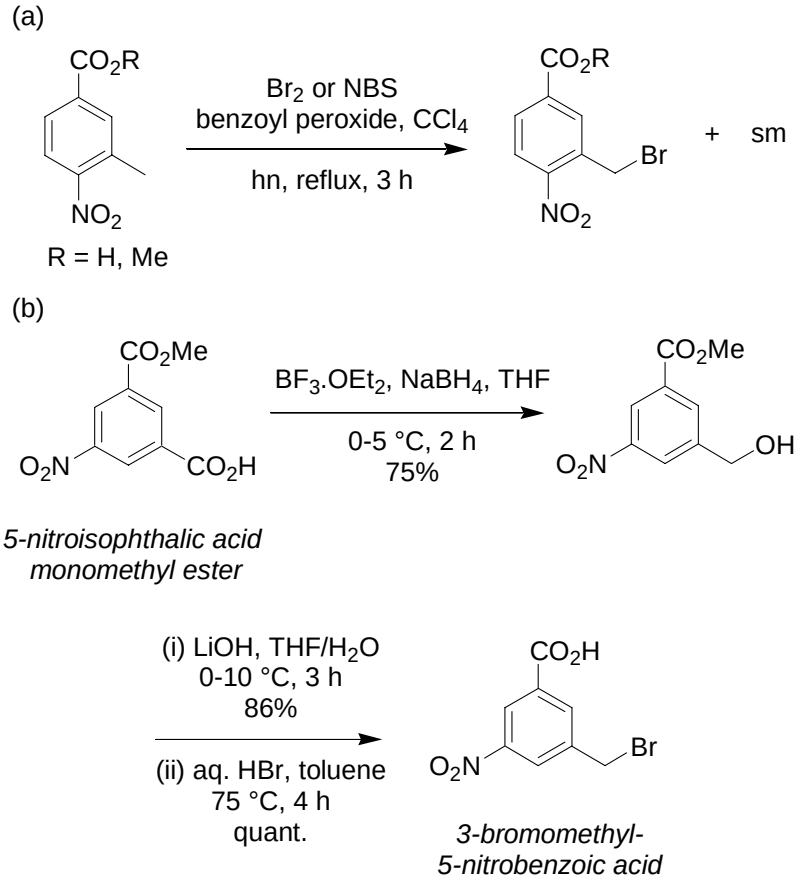
Figure 4.11. Monomer modifications for dimer assembly.

The modification to make the nitro derivative of compound **B** is straight forward (Scheme 4.3). A simple nitration procedure of the template will favor the 5-position (1 being the carboxylic acid group) because of the cooperative directing effect of the carboxyl and the bromomethyl groups. For compounds **3a** and **3g**, the nitration of the template will result in a mixture of products due to the difference in directing effects of the two groups. To circumvent this, an indirect route to modify the template was employed. Instead of doing nitration, a compound that already has the nitro group was considered. Initially, a radical bromination was attempted for 3-methyl-4-nitrobenzoic acid. However, the bromination is too slow and ineffective as TLC of the reaction showed spots for the product and starting material that are near to each other (Scheme 4.4a).

Scheme 4.3. Nitration of the organic template for compound B derivative



Scheme 4.4. Modification of the template for derivative of compounds 3a and 3g

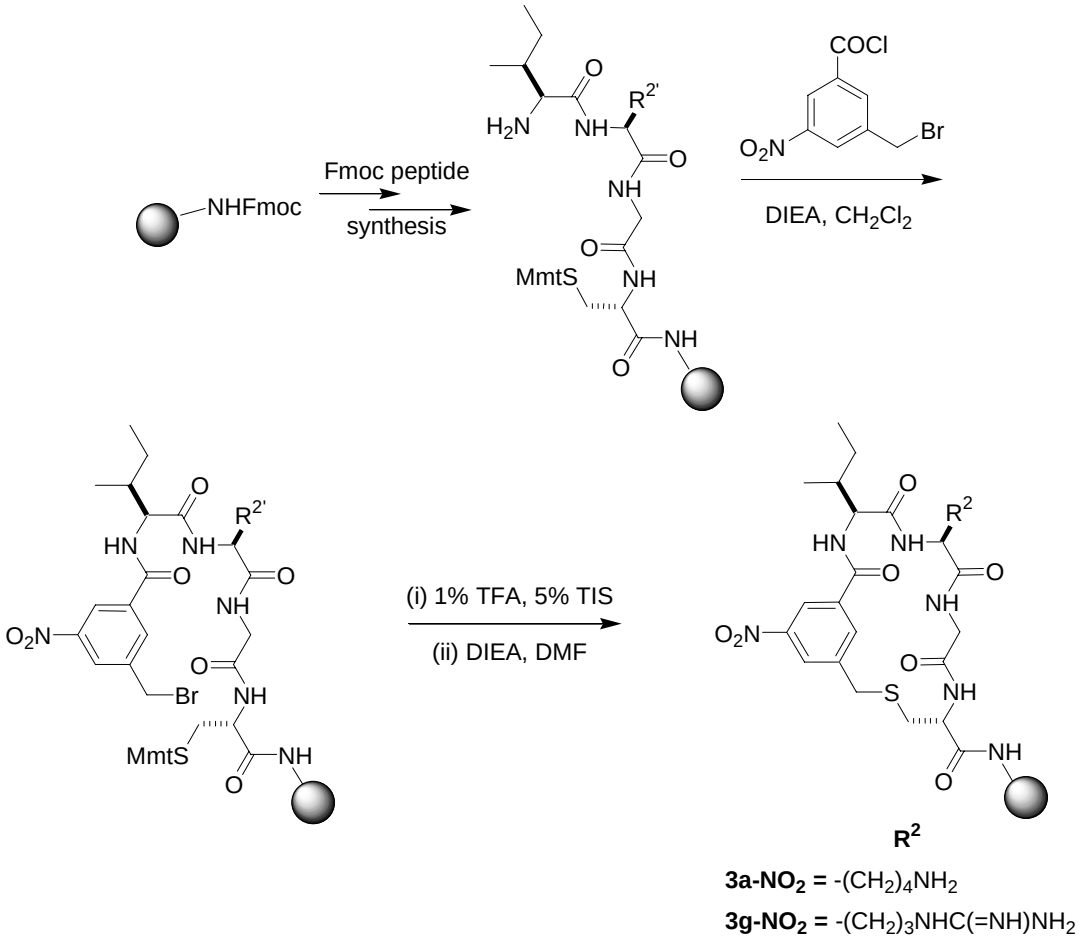


A different strategy was developed because bromination is ineffective. Starting with 5-nitroisophthalic acid monomethyl ester, the carboxylic acid part was reduced to the alcohol to give the 3-hydroxymethyl derivative. The methyl ester was then hydrolyzed under basic condition to the corresponding acid. Finally, conversion of the alcohol to the bromide was achieved using 6M HBr with toluene as a solvent. This two-phase reaction was surprisingly efficient as all of the alcohol was converted to the desired 3-bromomethyl compound (Scheme 4.4b).

With the necessary templates in hand, synthesis of the required monomeric peptidomimetics is straight forward. After conventional Fmoc peptide synthesis, the activated templates were attached to give linear precursors. Scheme 4.5 shows the

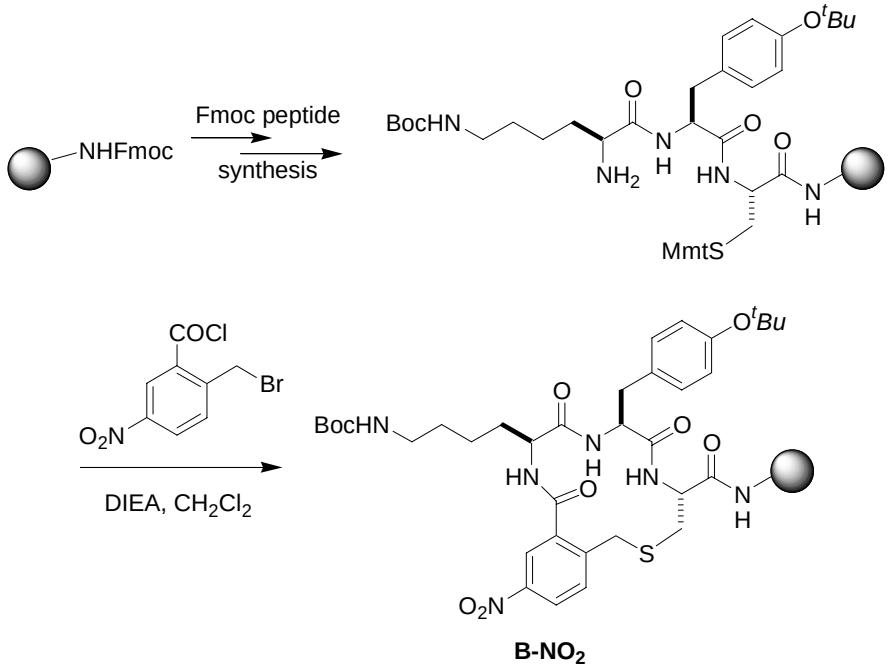
synthesis of compounds **3a-NO_2** and **3g-NO_2** (nitro analogs of compounds **3a** and **3g**, respectively).

Scheme 4.5. Synthesis of compounds 3a-NO_2 and 3g-NO_2



The synthesis of compound **B** derivative follows the same pattern and is shown in Scheme 4.6. Like our previous experience with 14-membered rings, this suffers from significant production of head-to-tail dimer.

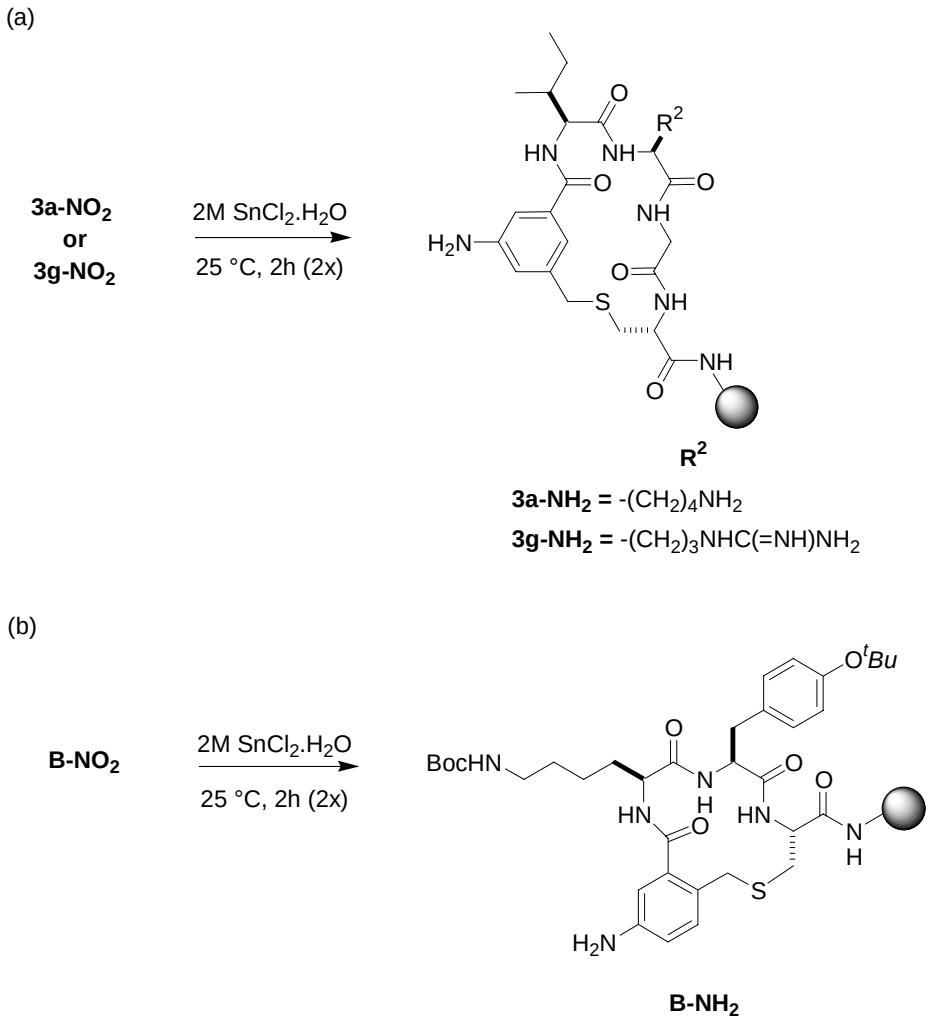
Scheme 4.6. Synthesis of compound B-NO_2



4.5.2 On-resin Reduction and Couplings with Glycine, Inp and DTAF

With the required nitro handle in place, the three cyclic peptidomimetics can now be reduced to their corresponding aniline derivatives. The use of tin(II) chloride is one of the most convenient ways to reduce a nitro group on resin. A common method is to shake the resin with the dissolved reagent in DMF for 24 hours. However, a faster and equally effective way is to do the reaction twice, each for two hours but with a higher concentration of tin.[126] This procedure was applied and the reduction proceeded smoothly to give the corresponding amino derivatives (Scheme 4.7)

Scheme 4.7. On-resin reduction of cyclic nitro peptidomimetics



Before installation of the essential piperidine moiety, FmocGly-Cl was coupled to the aniline. Glycine served as a spacer between the cyclic peptidomimetic and the triazine heterocycle which bears the fluorescent label. Other spacer molecules can be used to accommodate different loop distances in the NGF or other neurotrophin homodimer. After glycine attachment, FmocInp-Cl was then reacted. Final Fmoc deprotection gave the free secondary cyclic amine which can be cleaved from the resin, or can be reacted to DTAF to give monomeric, fluorescein-labeled peptidomimetics.

Both of these variations were done to give monomers **PEPT-Inp** and **PEPT-InpF*** (Figure 4.12**)**.

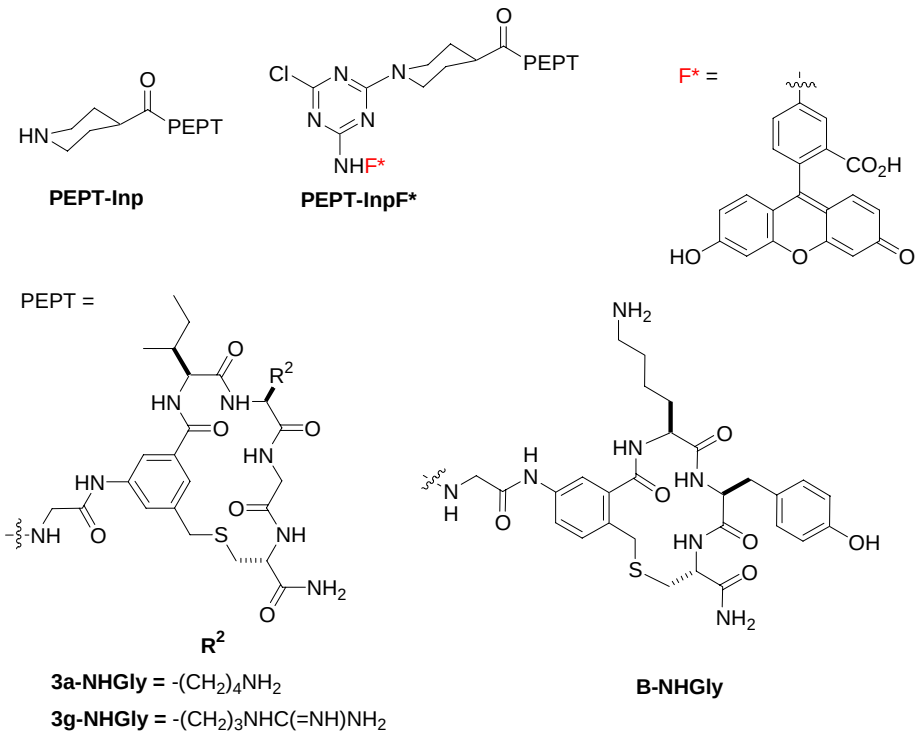


Figure 4.12. Structures of unlabeled (with only the Inp end) and labeled (with fluorescein attached) monomeric peptidomimetics.

The compounds **PEPT-Inp** and **PEPT-InpF*** were coupled to different peptidomimetics to make a 78-compound library.[125] The coupling can only be one way, that is only one monomer with the Inp couples with another that has the fluorescent label, following the one in Scheme 4.2.

4.6 Biological Assay

The compounds in the library were screened using fluorescence-activated cell sorting (FACS) assay. This technique is an application of flow cytometry. Test compounds were incubated in a binding buffer which contains receptor-expressing cells (*e.g*. NIH3T3 cells which express TrkA). After incubation, the cells travel in the instrument and intercepts with a laser of choice. The choice of fluorochrome depends on

what wavelength of excitation is being monitored. If a cell passing through the laser is fluorescent, the laser will cause excitation of the fluorescent molecule and the fluorochrome will emit at a higher wavelength typically associated to that particular compound. The emitted light passes through filters which allow only the wavelength of choice to pass. This signal is then translated and the machine is instructed whether a particular cell has to be sorted or not by charging the stream just as the cells enter the break-off point. The cells of interest leaves the break-off point charged either positive or negative and are sorted by being deflected by oppositely charged plates to collection vessels (Figure 4.13).

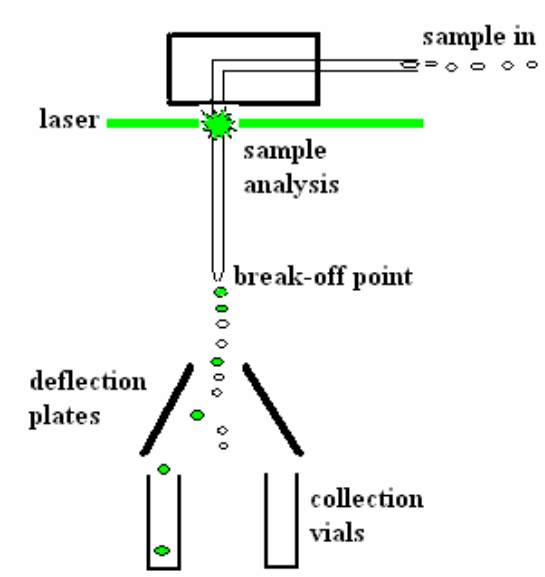
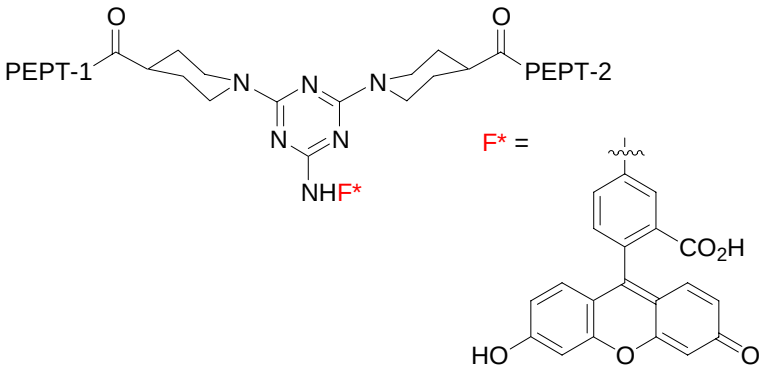


Figure 4.13. A diagram of the FACS assay.

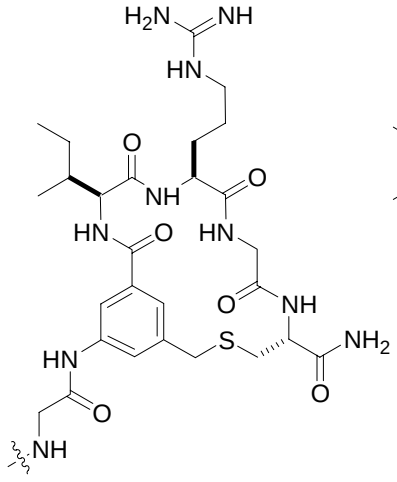
One of the compounds in the 78-member library is compound **4**. This heterobivalent compound was made by coupling **3g-NHGlyInp** and compound DTAF-labeled **5** (Figure 4.14**)**. In the initial FACS assay compound **4** showed significant staining of TrkA- and TrkC-expressing cells and was subsequently resynthesized so that further binding assays can be done. Compound **6** is a lead compound in our studies and this has already been shown to bind TrkA expressing cells with an affinity of 100 nM. A second FACS assay on heterobivalent compound **4** indicated that it binds to both TrkA

and TrkC expressing cells, with an even greater affinity for TrkC. More importantly, its binding affinity is higher than compound **6** (Figure 4.15). In a competitive assay with mAb 5C3 and I^{25}-NGF (Figure 4.16), compound **4** was measured to have an IC_{50} which is 200 times higher than NGF competing itself (2000 nM vs. 10 nM, respectively). Hence, the Kd for compound **4** was estimated at ~20 nM. These results validate our approach to make bivalent mimics from lead monovalent compounds and further, the use of fluorescent label on our mimics greatly facilitates identification of active compounds.

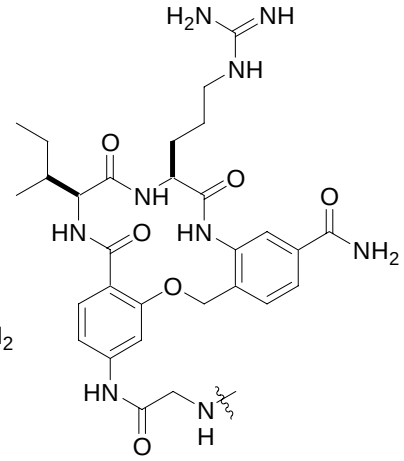


compound **4**:

PEPT-1 =

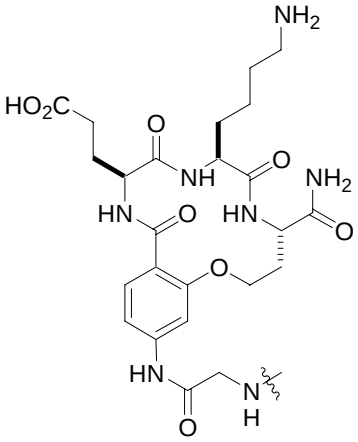


PEPT-2 =



compound **6**:

PEPT-1, PEPT-2 =



5

Figure 4.14. Structures of lead compounds **4** and **6**.

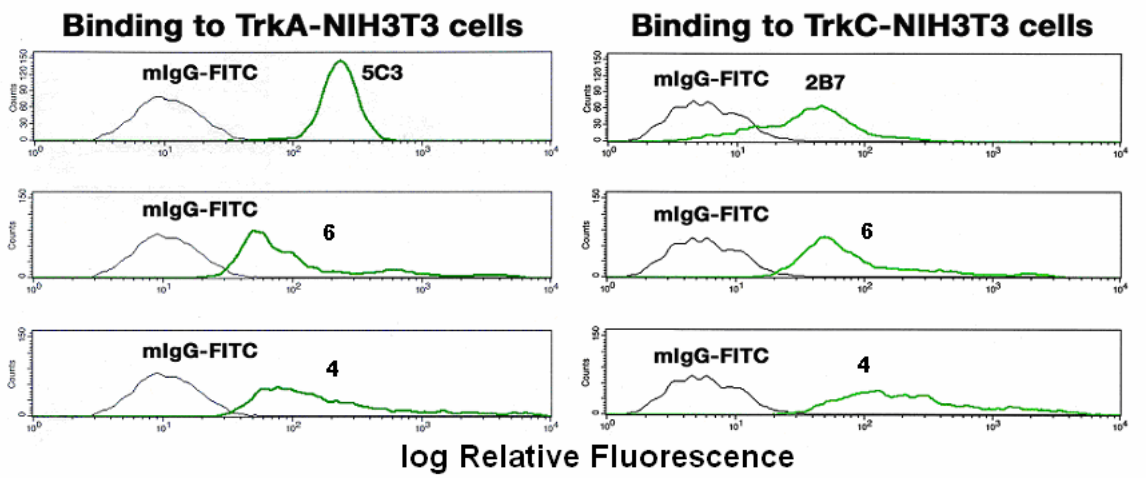


Figure 4.15. Representative data from FACS assay of compounds **4** and **6**. Left. Binding to TrkA cells with mIgG-FITC as negative and mAb 5C3 as positive controls. Right. Binding to TrkC cells with mIgG-FTIC as negative and mAb 2B7 as positive controls. A shift of the curve to the right indicates specific labeling.

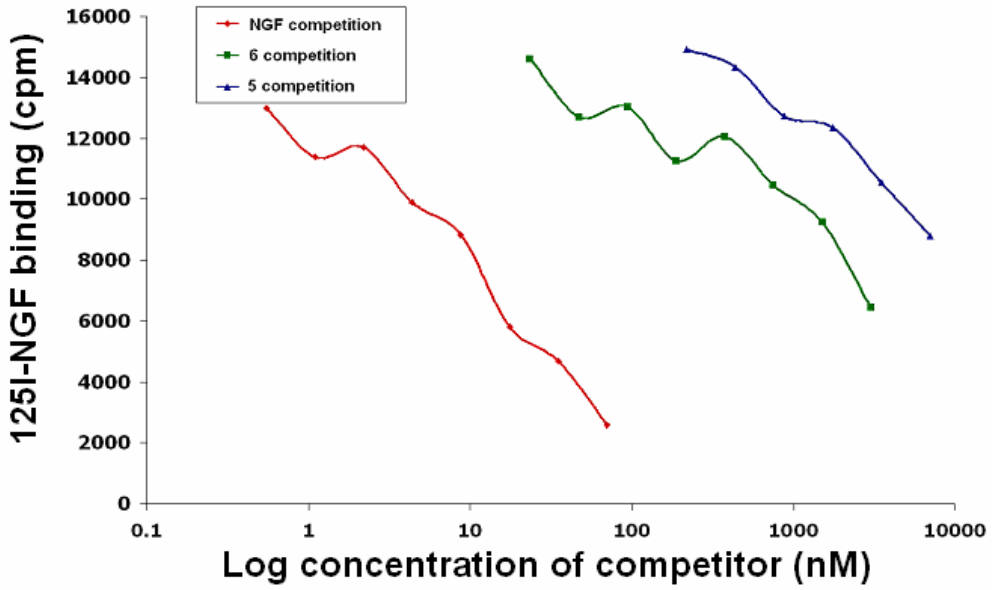


Figure 4.16. Binding affinity measurement of compounds **4** and **5** for TrkA. A shift of the curve to the left (to NGF competition) indicates higher affinity.

4.7 Summary

Protein-protein interactions are important in biological processes. Such interactions involve permanent formation of stable protein complexes, while others involve transient formations. Some of these interactions involve dimeric protein complexes. Dimerization of proteins plays a major role in regulating the function of many cell surface receptors. Synthetic small-molecules have contributed to the goal of understanding cellular pathways that are influence by dimeric protein complexes.

Although there are chemical methods available to make a specific dimeric (and bivalent) compound, there are relatively few methods that can give, exclusively, one dimeric species. Even fewer are methods that very convenient to be applied in a library format. If such a method exists, then its combinatorial advantage can be greatly appreciated as more compounds can be made easily and at a faster rate.

One of the methods that have emerged recently that was applied to meet the demand for making dimers selectively is sequential nucleophilic substitution. Our approach involves a fluoresceinated dicholoraminotriazine (DTAF) which undergoes substitution by nucleophilic groups bearing the peptidomimetics of choice. This method offers two advantages. One, the dimeric molecule has a fluorescent tag in it that greatly facilitates the assay. Two, the linker separating the cyclic peptidomimetic and the triazine scaffold can be varied so as to produce different distances in that region. These distances can correspond to different loops within the NGF or other neurotrophin homodimer.

The dimerization strategy produced lead compounds that show significant binding to cells that produces the Trk receptors. One compound, **4** was shown to have a binding affinity of 20nM. This result gives validation that our approach to targeting neurotrophin-receptor interaction can lead to compounds that have significant binding.

CHAPTER V

ATTEMPTS AT BICYCLIC GUANIDINE MIMICS AND ON THE STRUCTURE OF ACETYL 2-THIOHYDANTOIN

5.1 Introduction

The guanidine group is an important pharmacological moiety that is featured in many compounds that have diverse biological activities. Some of the therapeutic applications of molecules that possess the guanidine or guanidinium functionality are: anti-inflammatory,[127] antibacterial,[128] Na^+ and Ca^{2+} channel blockers,[129] anti-seizure agents,[130,131] and HIV-1 protease inhibitors.[132,133] Guanidine-containing drugs that have been sold in the market include cimetidine (against ulcer) and pinacidil (for hypertension).[134] While there are numerous examples of compounds with pendant guanidine, a small portion has it locked in a bicyclic form. As such, there have been few syntheses of bicyclic guanidines. Examples of these compounds are shown in Figure 5.1.[135-139] Of these, only compounds **M** were made via solid-phase (Scheme 5.1) and the rest were made in solution.

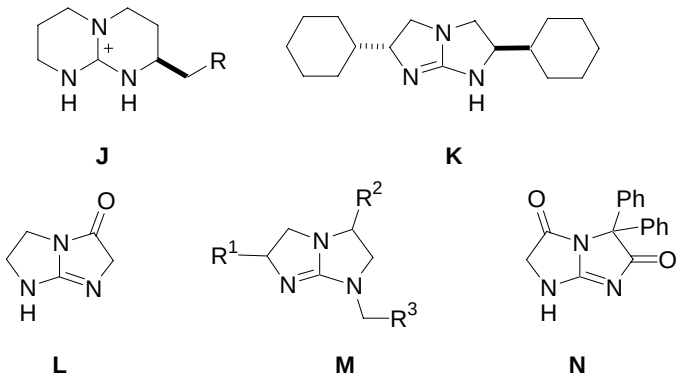
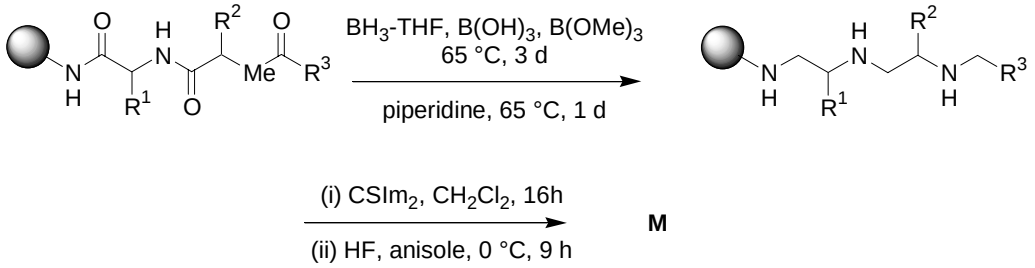


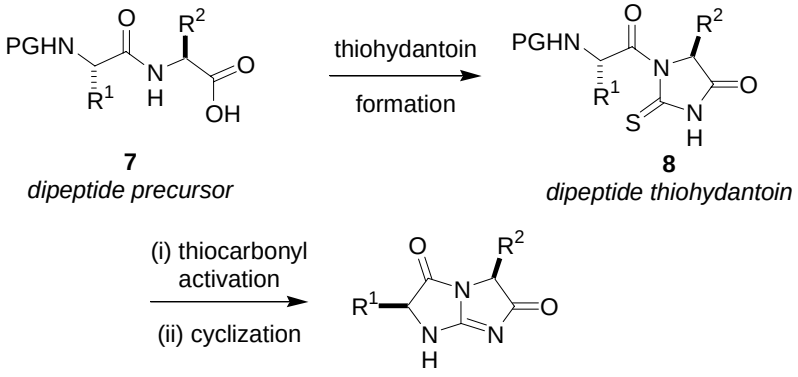
Figure 5.1 Structures of bicyclic guanidines.

Scheme 5.1. Solid-phase approach to bicyclic guanidines M



We are also interested in making bicyclic guanidines but in solution phase to facilitate the preparation of the compounds on scale. Our initial approach to achieve this is depicted in Scheme 5.2. It starts with a dipeptide with a suitable *N*-terminal protection and a free carboxylic end for thiohydantoin formation. The thiocarbonyl part is then activated, followed by deprotection of the amine to effect the cyclization. Although our mimic resembles structure **N** in Figure 5.1, we set out to make a focused library of bicyclic guanidines and not just one compound.

Scheme 5.2. Approach to bicyclic guanidine mimetics via dipeptide thiohydantoins



7
dipeptide precursor

8
dipeptide thiohydantoin

9
bicyclic guanidine mimic

The synthesis of peptide thiohydantoins is straight forward. This *C*-terminal modification of peptide chains has been used as an alternative to Edman degradation to sequence peptides from the *C*-terminus (Figure 5.2).[140] Most of the progress in this field has focused on developing new reagents that will facilitate thiohydantoin formation. Reagents include NH_4SCN,[140] trimethylsilyl isothiocyanate (TMSNCS),[141,142] ethoxycarbonyl isothiocyanate[143] and acetyl isothiocyanate.[144] The formed peptidyl thiohydantoins are then subjected to either acidic or basic condition, and sometimes at elevated temperatures, liberating the *C*-terminus amino acid thiohydantoin and the truncated peptide fragment which can be monitored by mass spectrometry.[145]

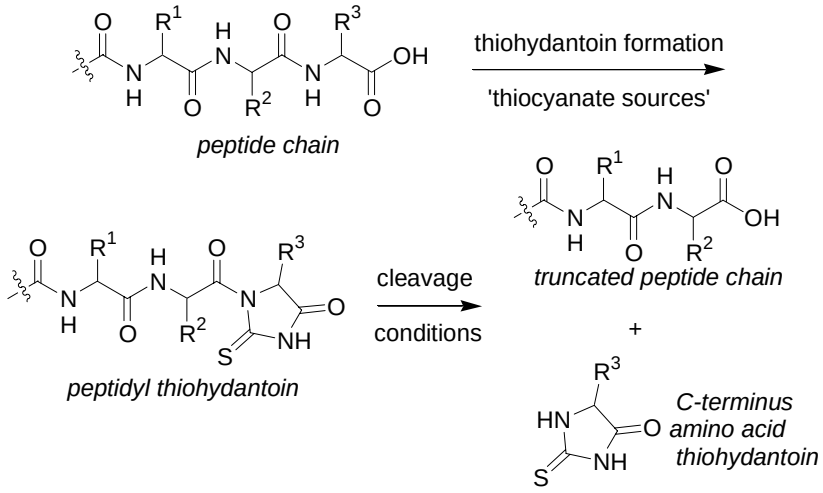


Figure 5.2. Peptide sequencing from the C-terminus.

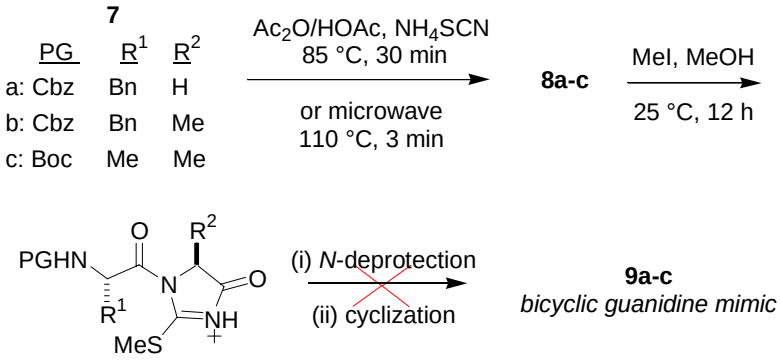
5.2 Attempts at Bicyclic Guanidines

5.2.1 The Dipeptide Thiohydantoin Approach

Carbobenzyloxycarbonyl (Cbz)-protected phenylalaninylglycine **7a** was reacted with ammonium thiocyanate in a weakly acidic medium at 85 °C to afford the dipeptide thiohydantoin **8a** (Scheme 5.3). This transformation can also be done under microwave irradiation at a higher temperature but at a shorter time. Attempts to cyclize this compound to **9a** by activating the thiocarbonyl group with MeI and then by doing hydrogenolysis to deblock the amine prior to cyclization proved to be not productive.

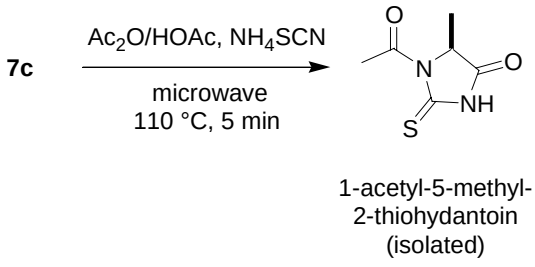
Other dipeptide thiohydantoins (**8b-c)** with different *N*-protection and side chains were also prepared but like the first one, the cyclization condition did not work as planned

Scheme 5.3. Attempts to form bicyclic guanidines via dipeptide thiohydantoins



Interestingly, increasing the reaction time by just 2 minutes under microwave irradiation for dipeptide thiohydantoin formation resulted in the cleavage of the *C*-terminal 2-thiohydantoin as shown in Scheme 5.4. This was verified by an independent synthesis of 1-acetyl-5-methyl-2-thiohydantoin from L-alanine (see Scheme 5.8). Thermal reaction for the formation of dipeptide thiohydantoin proved to be more easily controlled.

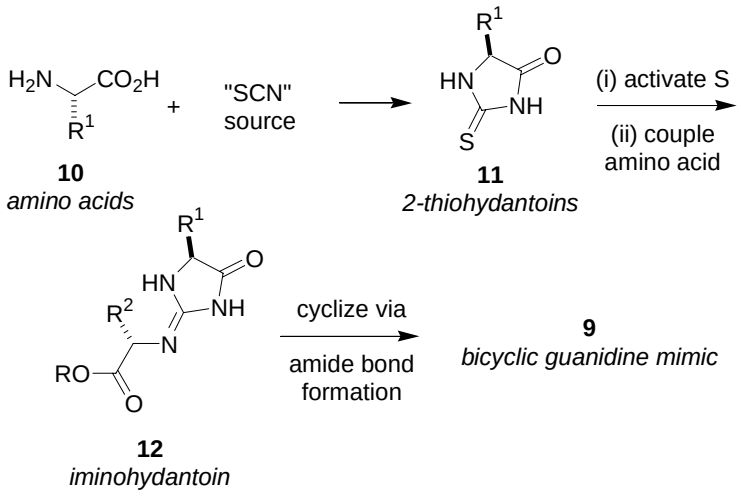
Scheme 5.4. Cleavage of C-terminal thiohydantoin amino acid by microwave irradiation



5.2.2 The 2-Thiohydantoin-Iminohydantoin Approach

It is evident at this juncture that dipeptide thiohydantoins are very labile to cleavage and so an alternative approach was considered (Scheme 5.5). Instead of making dipeptide thiohydantoins and cyclizing, amino acid thiohydantoins (*i.e.* 2-thiohydantoins, **11**) can be made and then activated to add the next amino acid. This will produce an iminohydantoin **12** which is envisioned to cyclize via amide bond formation to give the bicyclic guanidine core. This route was the one used in the synthesis of compound **N** (in Figure 5.1); and because it has the same skeletal framework as our target, it is appealing to try this approach.

Scheme 5.5. Alternative route to bicyclic guanidine via 2-thiohydantoins



The 2-thiohydantoin precursors **11** can be made from amino acids and isothiocyanate or thiocyanate sources. Figure 5.3 shows the two routes leading to 2-thiohydantoins. With an amino acid methyl ester (Route A), an alkyl isothiocyanate can be the coupling partner to give a 2-thiohydantoin which is substituted at the N^3 position. On the other hand, a free amino acid can be reacted with ammonium thiocyanate (or other thiocyanate equivalent) to give 2-thiohydantoin in which the amides are unsubstituted (Route B).

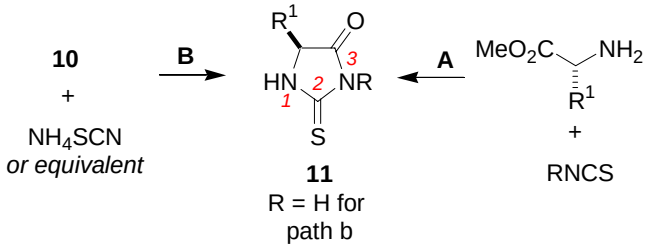
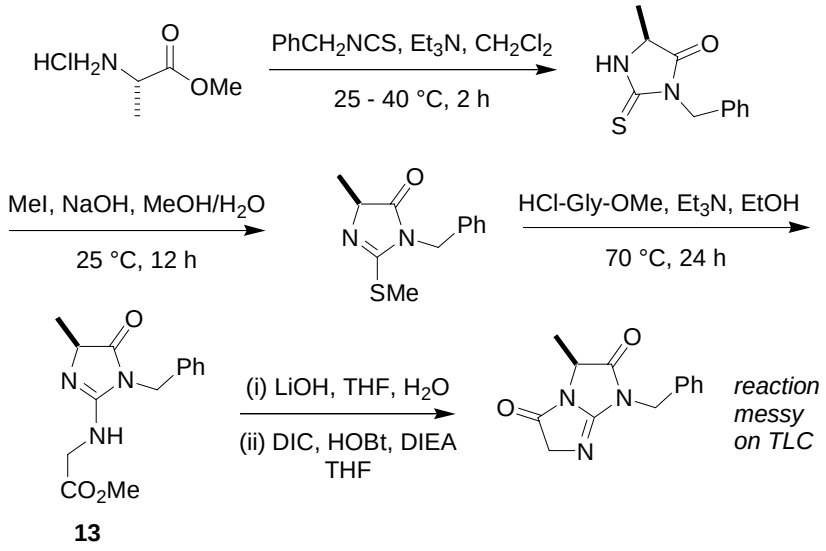


Figure 5.3. Two routes to 2-thiohydantoins.

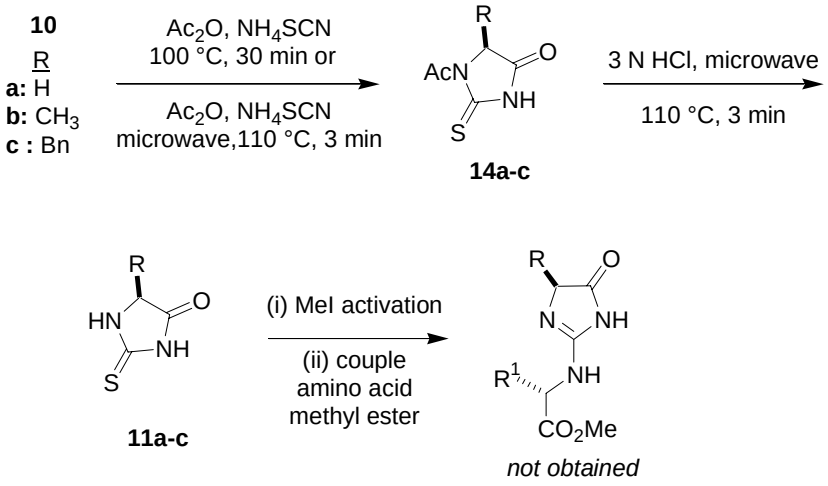
Following Route A in Figure 5.3, L-alanine methyl ester was reacted with benzylisothiocyanate to give the alkylated 2-thiohydantoin (Scheme 5.7).[146] The thiocarbonyl group was then activated with MeI and the resulting isothiourea was coupled with glycine methyl ester to give the iminohydantoin derivative **13**. The proton NMR of this compound is hard to assign but the MS shows a clear $[M + H]^+$ for the product. The methyl ester was then hydrolyzed to the acid to set the stage for cyclization. The cyclization however is not productive as was verified by a messy TLC during the monitoring of the reaction.

Scheme 5.6. Synthesis of iminohydantoin from 2-thiohydantoin



Applying Route B (Figure 5.3), amino acids **10a-c** are reacted with ammonium thiocyanate in acetic anhydride at elevated temperature to give the 2-thiohydantoins **8a-c** with an acyl group at N^1 position (Scheme 5.8)[147]. This was then deactylated under acidic condition to afford the amino acid 2-thiohydantoins **5a-c**. Activation of the 2-thiohydantoins with MeI gave the isothiourea derivatives but this supposedly activated intermediate failed to couple with an amino acid methyl ester as supported by the NMR of the crude reaction.

Scheme 5.7. Synthesis of 2-thiohydantoins from amino acids



5.3 The Acylation of 2-Thiohydantoins: 1-Acetyl or 3-Acetyl?

At this point all the plans that were employed to make the bicyclic guanidine target has been futile and the focus has shifted to an interesting discrepancy in the literature that was discovered in making the 2-thiohydantoins. The reaction where there are 'inconsistencies' found in the literature is the one in which glycine is reacted with NH_4SCN in acetic anhydride to give an acylated 2-thiohydantoin (the synthesis of **14a**, Scheme 5.7). Before going on further, it is important to give a brief background on the nomenclature system that is followed regarding 2-thiohydantoin whose structure can be regarded as a thio- derivative of 2-hydantoin. The name 2-hydantoin is a trivial name, however, 2-thiohydantoin, having the prefix thio is considered semi-trivial because one

part follows a prefix system.[148] Nevertheless, the IUPAC has recognized the advantages of using a combined trivial and semi-trivial system and has adopted their usage. Since both compounds are related, they follow the same ring atom numbering (Figure 5.4).[148] Note that positions 1 and 3 are *not the same*. With regard to numbering of the ring, there were two systems that were adopted at different time periods.[149] One is the numbering before 1907 and the other, *after* 1907. The numbering system that will be used in the following discussion refers to the post 1907 system, which is also the one that was adopted by *Chemical Abstracts*.

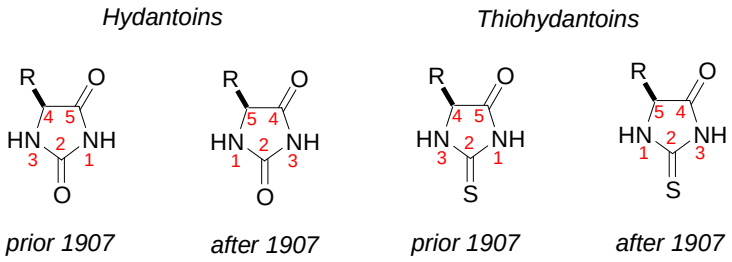
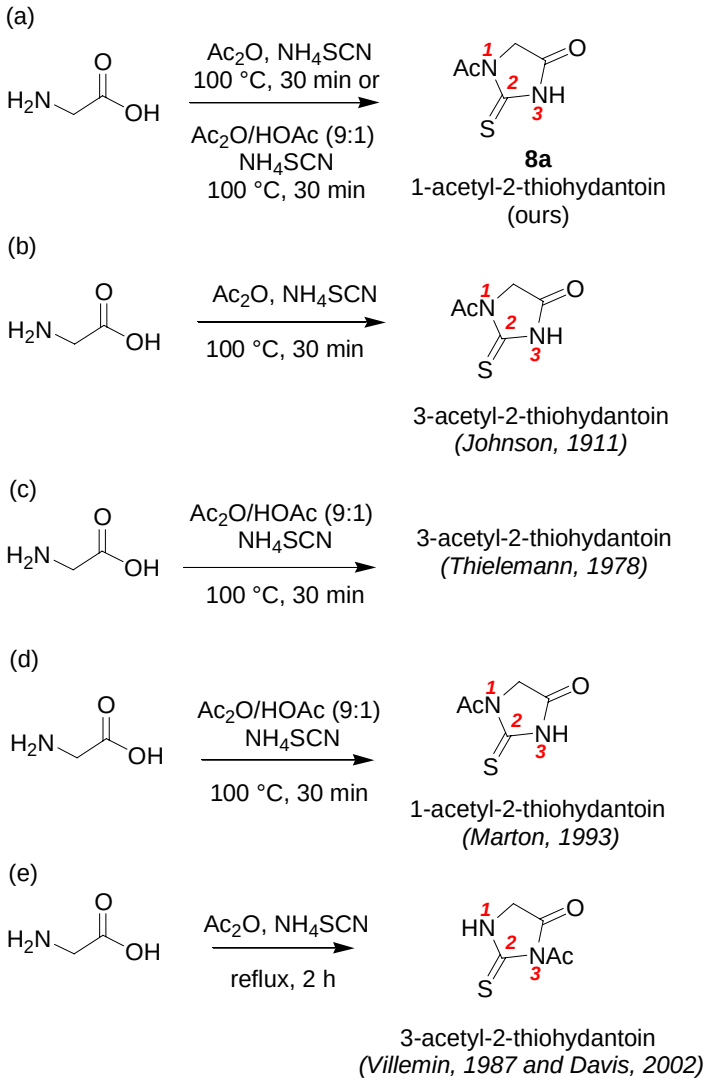


Figure 5.4. *Chemical Abstracts* numbering system on 2-hydantoin and 2-thiohydantoin.

Going back to the reaction of glycine and NH_4SCN in acetic anhydride, we depicted the structure of the product to be acylated at the N^1 position; hence, the product can be termed as 1-acetyl-2-thiohydantoin (Scheme 5.8a). The structures were redrawn instead of writing labels to give a clear depiction about the discrepancies found in the literature. That the acylation occurred at this position (N^1) was verified via NMR methods and will be discussed in a later section. The procedure that was followed in the synthesis is the one used by Johnson in 1911.[147] His reaction is shown in Scheme 5.8b. While Johnson depicted the structure of his product the same as ours, he named it 3-acetyl-2-thiohydantoin. Clearly, Johnson followed the nomenclature *before*1907. Other works on acylated 2-thiohydantoins that followed Johnson's protocol are shown in Schemes 5.8c-e. The reaction in Scheme 5.8c,[150] while showing no structure followed the name given by Johnson and has the same melting point, 175°C. This is close to our recorded value of 173-174 °C. It may be inferred that all these compounds are the same. The one in Scheme 5.8d, which was done by Marton[151] follows the correct structure and

current naming of the product, and is the same as ours. However, the one in Scheme 5.8e, done separately by Villemin[152] and Davis[153] shows the product of the same reaction to be 3-acetyl-2-thiohydantoin. Note that the name may be similar to Johnson's and is correctly depicted (by IUPAC standard), but as was hinted above, the acylation occurred at the N^1 position and *not* at N^3, hence, Villemin and Davis have written the structure of the product wrong.

Scheme 5.8. Reported syntheses of acetyl-2-thiohydantoin from glycine



To prove that the acylation occurred at the N^1 position, 2-D COSY NMR was done on the 2-thiohydantoin **8a**. A cross peak between the C^5H and N^1H would not be observed if the latter is acylated; on the other hand, a cross peak will exist if N^3 is acylated and not N^1. The COSY of the product in DMSO-d_6 is shown in Figure 5.5a. Clearly, no cross peak can be seen that will suggest acylation at N^1. However if the acetylated product is subjected to acid hydrolysis to produce 2-thiohydantoin, then a cross peak between C^5H and N^1H is observed (Figure 5.5b).

a b

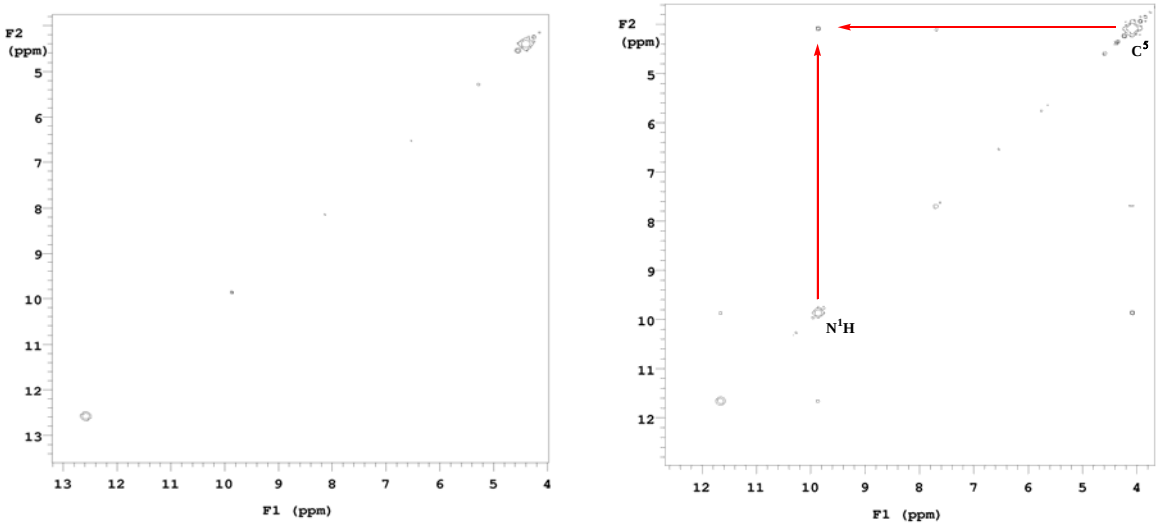
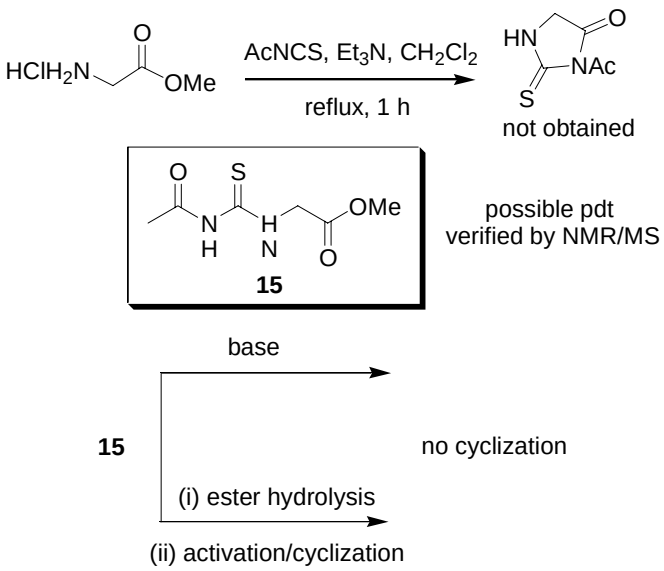


Figure 5.5. COSY spectrum of (a) acetyl-2-thiohydantoin and; (b) deacetylated product, 2-thiohydantoin.

The COSY of the isolated product shows evidence of acylation at the N^1 position. Another indication that could support this is to consider chemical shifts of the two free NH's (*i.e.* the N^1H and the N^3H of 2-thiohydantoin). The one observed for the acylated product is more downfield to where normally common cyclic thioamides resonates (*ca* 9.15 ppm)[154]and indicates that the proton is probably highly deshielded. Furthermore, when the product was deacylated, the new N*H* signal gave a chemical shift consistent with typical cyclic amides. We have tried to make 3-acetyl-2-thiohydantoin so that we could have a direct comparison with the 1-acetyl-isomer but all the syntheses we have applied failed to give the desired compound (Scheme 5.9).

Scheme 5.9. Attempts to make 3-acetyl-2-thiohydantoin



The best we could do is to use 3-benzyl-5-methyl-2-thiohydantoin (see Scheme 5.6) which has the N^1, free. This would show that (1) there is an observed cross peak between N^1H and C^5H if the former is unsubstituted and (2) the chemical shift for a free N^1H is indeed slightly upfield than that for a free N^3H (Figure 5.6).

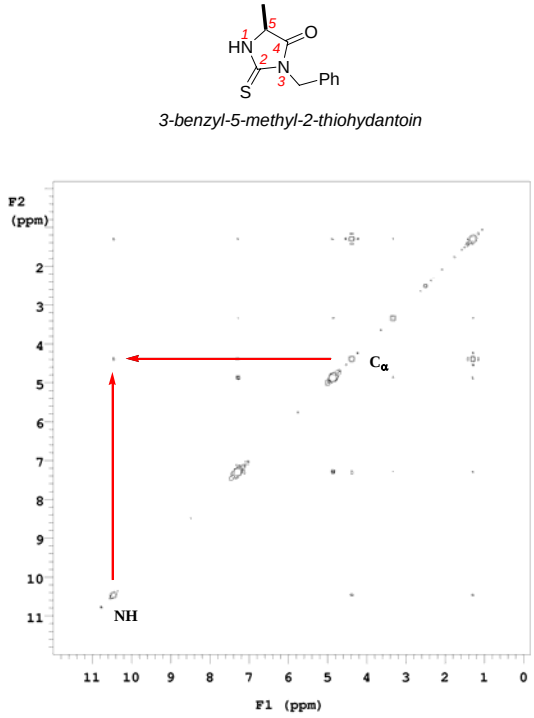


Figure 5.6. Two-dimensional COSY spectrum of 3-benzyl-5-methyl-2-thiohydantoin.

The original workers have proposed a mechanism [155] based on known observations on the behavior of glycine under the conditions employed. While they used hippuric acid to outline the transformation, an analogous mechanism can be applied to glycine (Figure 5.7). This mechanism is based on the following observations[155]:(1) glycine forms *N*-acetylglycine in Ac_2O; (2) acylated amino acids are known to form lactone anhydrides under the same conditions, and; (3) lactone anhydrides react with thiocyanic acid (HSCN), which was liberated from the reaction of Ac_2O and NH_4SCN, to form an acylisothiocyanate derivative which cyclizes to 1-acetyl-2-thiohydantoin. This last reaction is similar to reactions of lactone anhydrides with HCl to form acid chlorides.

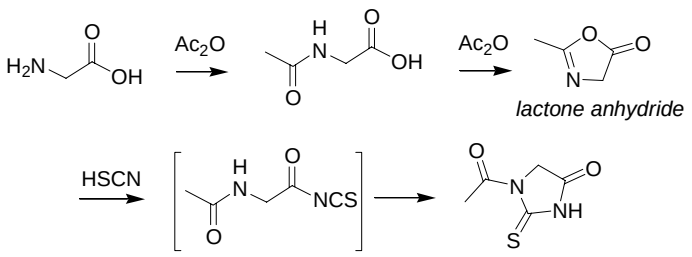


Figure 5.7. Johnson's proposed mechanism for the formation of 1-acetyl-2-thiohydantoin.

Villemin, on the other hand, proposed the mechanism[152] shown in Figure 5.8 for the formation of '3-acetyl-2-thiohydantoin':

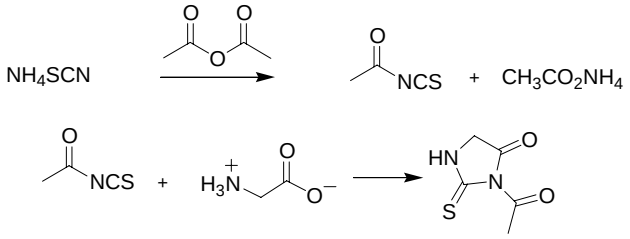
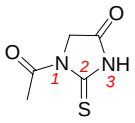


Figure 5.8. Villemin's proposed mechanism for the formation of 1-acetyl-2-thiohydantoin.

Note that the name of the compound is in quotation because Villemin's (including Davis' and Thielmann's) product should also be 1-acetyl-2-thiohydantoin. One thing that is contradictory to what Johnson has claimed is that the glycine nitrogen (N^1) was not acylated even under refluxing conditions. Table 5.1 summarizes the proton NMR chemical shifts of the 'product(s)' obtained from Villemin's , Davis', Marton's and our data. Marton's and our NMR are conspicuously the same. On the other hand, Davis' and ours, while having different structures show almost identical chemical shifts. This implied that Davis' product is the same as ours and that they just followed Villemin's drawing (Davis cited Villemin's work) without checking which site was acylated. Villemin's NMR, however, has one chemical shift that is possibly way off (the one with the 3.90 ppm). It was also not mentioned why a mixture of solvent was used in their sample. We have tried to do the same for our sample using a mixture of DMSO-d_6 and

$CDCl_3$ (*ca* 1:1)) but the N*H* peak observed was at 12.33 ppm. Although there is a disagreement in the drawing of the structures, the position of the acyl group is of no consequence since it was removed in later steps. However, one-step conversion of amino acids to heterocycles is important in medicinal chemistry and researchers have to be cautious in interpreting the literature in this area.

Table 5.1. Comparison of reported proton chemical shifts of acylated 2-thiohydantoin from glycine.

Structure	Solvent	Chemical Shift/ppm			
		N^1H	N^3H	C^5H	CH_3
 (Villemin & Davis)	$CDCl_3$/DMSO-*d6* (Villemin)	3.90		4.35	2.65
	DMSO-*d6* (Davis)	12.56		4.39	2.67
 (Marton & ours)	DMSO-*d6* (Marton)		12.60	4.42	2.68
	DMSO-*d6* (ours)		12.58	4.40	2.68

5.4 Summary

One of the easiest ways to make dipeptide thiohydantoins is using ammonium thiocyanate. While this is relatively trivial, cyclization to the target bicyclic guanidine proved to be not favorable under the conditions employed. One reason is that the formed amino acid 2-thiohydantoin at the *C*-terminus is prone to cleavage. In fact, by just microwaving the reaction, the thiohydantoin will be cleaved. This undesirable reactivity inhibits the activation of the thiocarbonyl for cyclization to bicyclic guanidines.

Acylated 2-thiohydantoins are formed from the reaction of amino acids and NH_4SCN in acetic anhydride. Two-dimensional COSY NMR experiments of the compound isolated from this reaction and the deacylated analog proved that the acyl group is in the N^1 position. Further evidence on observable vicinal coupling between C^5H and N^1H if the latter is unsubstituted is demonstrated by synthesizing 3-benzyl-5-methyl-2-thiohydantoin.

CHAPTER VI

SOLUTION PHASE SYNTHESIS OF NON-PEPTIDE MIMETICS USING DIHALOGENATED NITROBENZENE AS A SCAFFOLD AND ATTEMPTS TO MAKE SPIROTETRACYCLIC MIMICS VIA SPIROCYCLIC DIKETOPIPERAZINE

6.1 Introduction

Over the course of the last two decades, combinatorial chemistry has emerged as a separate field of organic synthesis. The genesis of doing organic reactions combinatorially can be traced to peptide synthesis.[58] Innovative methods of making peptide libraries such as those by Geysen (multi-pin),[156] Houghten (teabag),[157] Furka (split and pool)[158] and Lam (one-bead, one compound)[159] had revolutionized this area of organic synthesis that gave way to what is now known as combinatorial synthesis. Those early works can be regarded as an extension of solid-phase peptide synthesis, and thus, were done on-resin. As more reactions were applied and developed for solid-phase synthesis, it has become possible to make small molecules other than peptides in a combinatorial format. However, both solid- and solution-phase syntheses, in principle, can be applied in combinatorial chemistry. There are several advantages of doing synthesis on-resin: ease of purification, use of excess reagents and automation are some the attractive features of solid-phase synthesis. However, there are also drawbacks like cost of resin and its stability in doing a particular reaction. Indeed, most reactions are not as clean as peptide bond formation when applied to solid phase and this will require time for optimization. Because of these limitations, solution-phase approaches can not be discounted as an alternative to do combinatorial synthesis. One obvious advantage of doing solution-phase synthesis is that it does not have the restrictions resins impose during the synthesis. Those restrictions include, resin compatibility, reaction monitoring and purification at each reaction sequence. Also, solution-phase synthesis is easy to scale up to get more (*i.e.* mass) of the targeted products.

One main disadvantage in doing solution-phase combinatorial synthesis is purification after each reaction step. This is especially true, and very tedious, if the reaction is not very efficient to all substrates. Boger and co-workers have realized this problem and to circumvent this, they have employed simple liquid-liquid or liquid-solid extractions to purify intermediates at each stage of the reaction sequence.[160,161] Their strategy is based on using reactive anhydrides as templates wherein different building blocks are attached (Figure 6.1). This technique is easily adopted to make thousands of compounds. However, the reactions that can be conducted that give clean intermediates are limited to amide bond formation. Subsequent reactions that are not as efficient involved alkene cross-metathesis and Pd-catalyzed aryl halide coupling to form dimeric species.

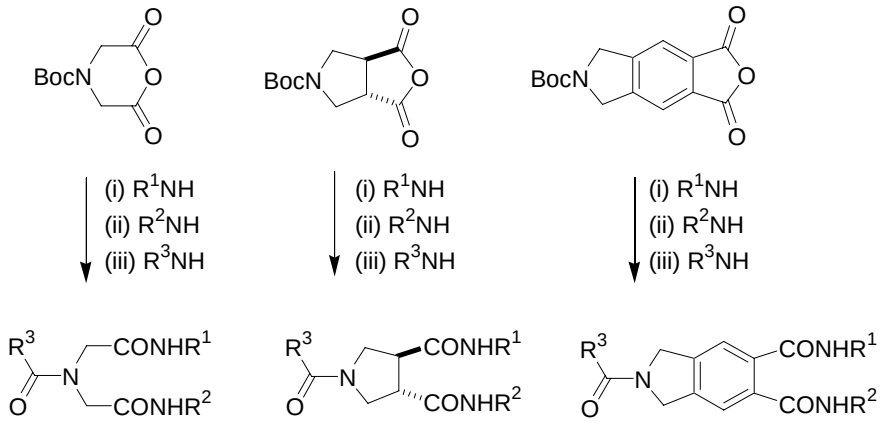


Figure 6.1. Boger's solution-phase syntheses using cyclic anhydride templates.

Another notable illustration of the potential of solution-phase combinatorial synthesis is by Pirrung *et al*.[162,163] Their work involved mixing a fixed substrate with variable number of reagents to produce a 'sublibrary' of a mixture of compounds (Figure 6.2). In their work, nine alcohols/phenols and six isocyanates are used to demonstrated the concept. For the alcohols/phenols, one of each are mixed with the six isocyanates (total number of moles of alcohols/phenols and isocyanates are the same) to give nine sublibraries. The same is done for the isocyanates to give another six sublibraries. The 15 sublibraries are then assayed for inhibition of acetylcholinesterase (Ache). The one

giving the highest activity was identified and all the members of that sublibrary were resynthesized and resubjected for assay to confirm the activity observed when it was a member of the mixture.

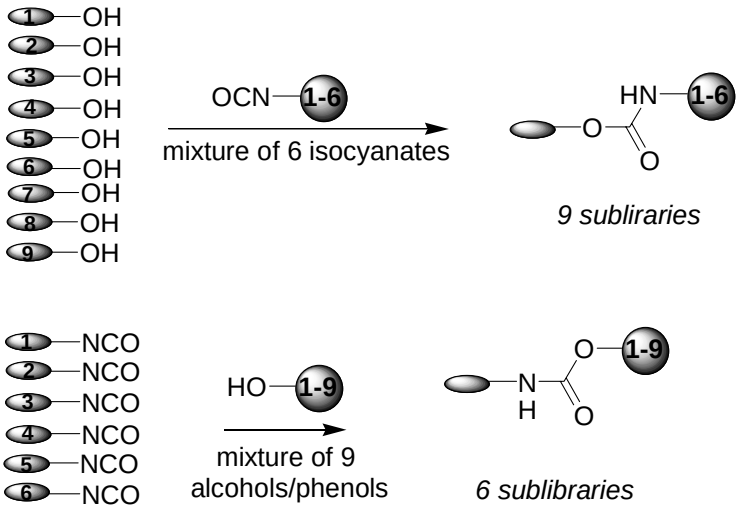


Figure 6.2. Pirrung's 'indexed' combinatorial libraries via solution-phase synthesis.

Recent developments in solution-phase combinatorial synthesis include the application of several purification strategies such as fluorous synthesis,[164]precipitation techniques,[165] resin-capture approaches,[166,167] and polymer-supported scavengers of excess reactants.[168,169] Related to using resins in solution-phase synthesis is the application of resin-bound reagents as reactants in conduction reactions.[170,171] All these factors have significantly improved productivity in doing solution-phase combinatorial synthesis and placed it in equal standing with solid-phase as a viable and robust method in doing combinatorial synthesis for drug discovery.[172]

The field of peptidomimetic chemistry have benefited from the development of combinatorial synthesis. While peptide-like compounds can be easily synthesized this way, these molecules suffer from limited proteolytic stability and bioavailability. More recently, efforts are being done to address these limitations by making compounds that are less peptidic in Nature.[173-176] In general, there are two ways to do this; one is to install an amide bond replacement and another is to use scaffolds that will serve as points

of attachment so the molecule will retain critically important side-chains. Substitution of a peptide bond by another group reduces its amide character, hence, alters the physical and chemical properties of the compound. Several modifications can be done to replace an amide bond and a review had been published regarding these methods.[177] Some well-known amine bond isosteres are shown in Figure 6.3.

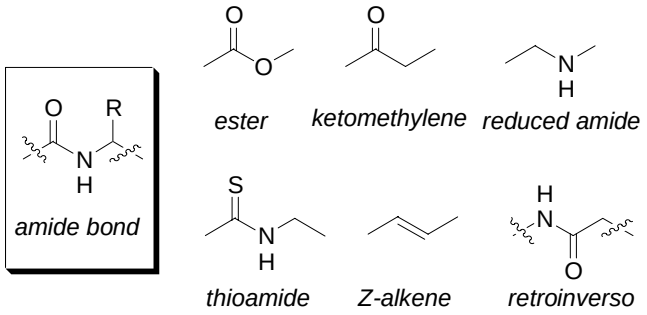


Figure 6.3. Common amide bond isosteres.

On the other hand, a scaffold utilizes cyclic compounds in which important side chains are attached and displayed to approximate the desired three-dimensional layout. Some of the common scaffolds include natural products (like steroids and sugars) and heterocycles (like diketopiperazines and benzodiazepines) (Figure 6.4). [178-180]

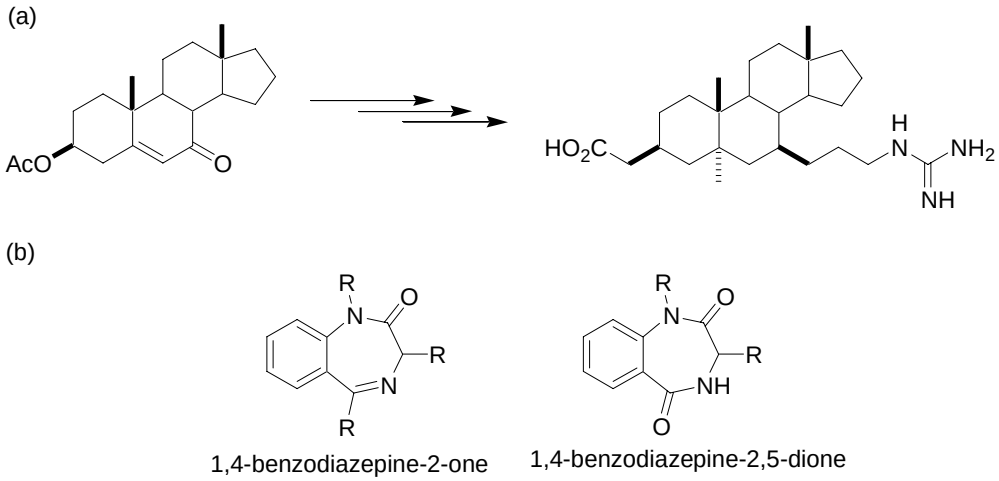


Figure 6.4. Examples of scaffolds to make non-peptide mimetics. (a) RGD mimics based on steroidal structure. (b) Mimics based on benzodiazepines.

6.2 Solution-Phase Synthesis of Non-Peptide Mimetics

Our efforts to develop compounds with less peptidic character started by using a simple substituted nitrobenzene that will serve as a scaffold to attach groups that contain side-chains found in the amino acids in the loop regions of the neurotrophins. The strategy is illustrated in Figure 6.5:

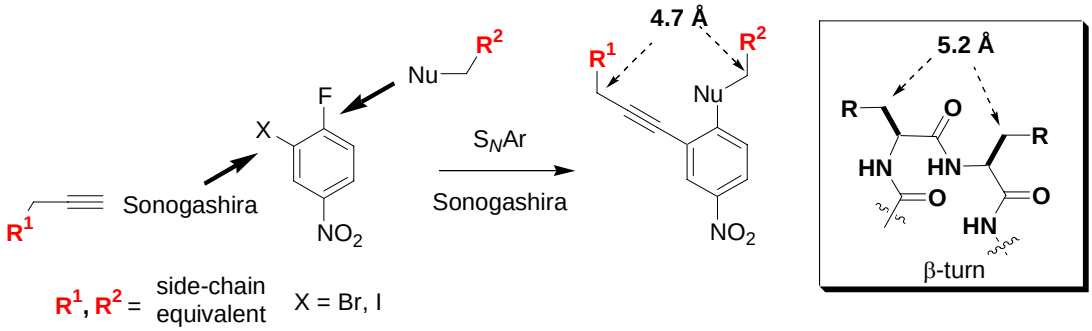


Figure 6.5. Strategy to make non-peptide mimics based on a 1,2-dihalogenated nitrobenzene scaffold.

Again, sequential substitutions[181] were utilized to append reactive groups that contain the side-chains of interest. Either the S_NAr or the Sonogashira cross-coupling[182] can be done first, provided that no complications occur for the second reaction. The resulting compounds have more conformational freedom than the cyclic peptidomimetics made previously in our group. Nevertheless, these new mimics are non-peptidic and could potentially be more stable to proteases.

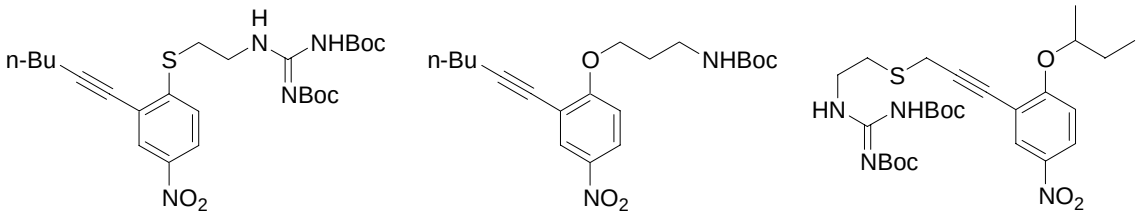
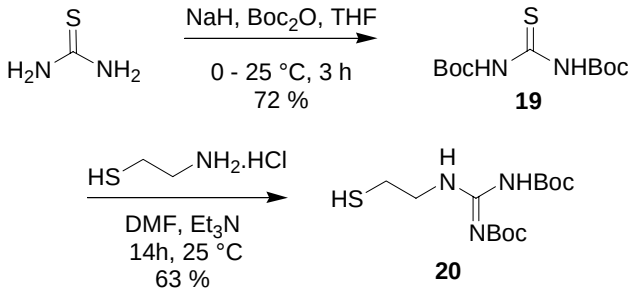


Figure 6.6. Structures of non-cyclic mimetics with important side-chains.

Part of a small library of non-peptide mimetics include compounds **16**, **17** and **18** (Figure 6.6). The side-chains that are installed in the compounds include that of norleucine, arginine and lysine (with S and O acting as methylene equivalent), and isoleucine. All the precursors for these side-chains are commercially available except for the one bearing the guandino group. Scheme 6.1 shows the synthesis of this side-chain. Double Boc protection of thiourea produced the bis-protected derivative **19** which was reacted to cysteamine to afford thiol **20** which contains the required bis Boc-protected guanidine moiety.

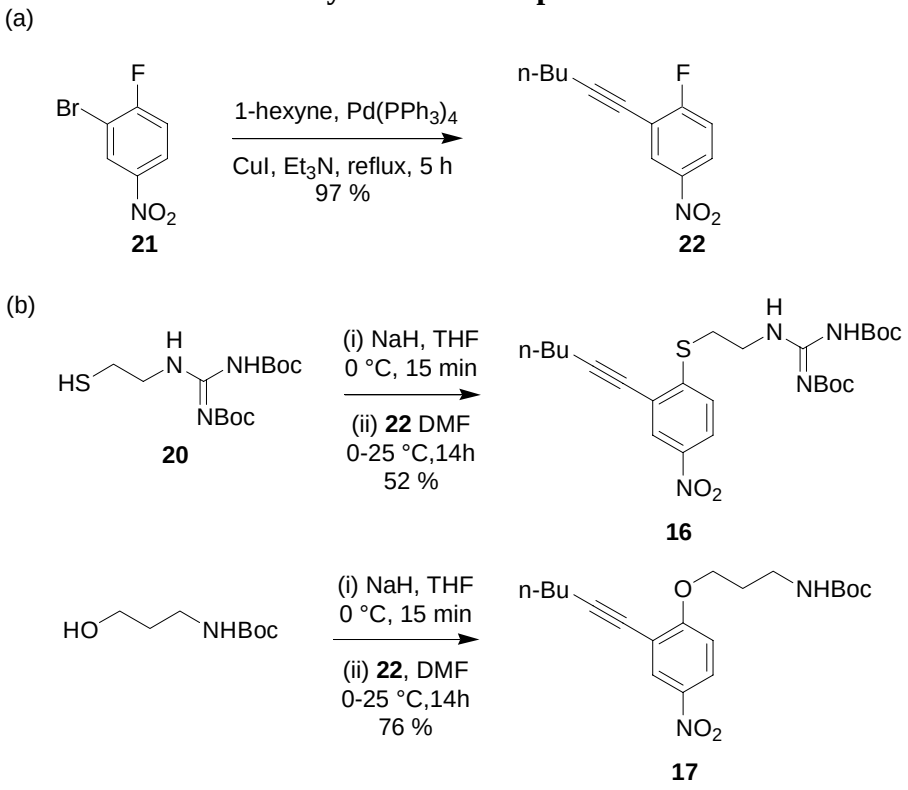
Scheme 6.1. Synthesis of guanidine side-chain mimic



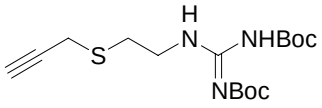
At the outset, the scaffold that we were using was 4-fluoro-3-iodonitrobenzene;[183] however, the synthesis of this compound required expensive trifluorosulfonic acid for iodination. This, plus the inherent light sensitivity of the resulting iodo compound make it unattractive for a large scale synthesis. Substituting bromine in place of iodine solved the stability and reagent cost constraints. 3-Bromo-4-fluoronitrobenzene (**21**) was synthesized by brominating the parent fluoro-nitro compound with $NaBrO_3$ and H_2SO_4 at 65 ºC for 3 h.[184] Although the yield of the bromination reaction is less than that for the iodination, it is more feasible to scale up the former due to cheaper starting materials and stability of the brominated product.

Inspection of open mimics **16** and **17** reveal that they can be made from a common intermediate **22**. Cross-coupling of **21** and n-hexyne using Pd^0 catalysis afforded compound **22** (Scheme 6.2a) in high yield. Nucleophilic aromatic substitution (S_NAr reactions of intermediate **22** with nucleophiles **20** and *N*-Boc protected 3-hydroxypropylamine yielded compounds **16** and **17**, respectively (Scheme 6.2b).

Scheme 6.2. Synthesis of compounds 16 and 17



Synthesis of compound **18** is not as straight forward. The alkyne-containing cysteamine derivative shown below poisons the Pd catalyst hence, cross-coupling with

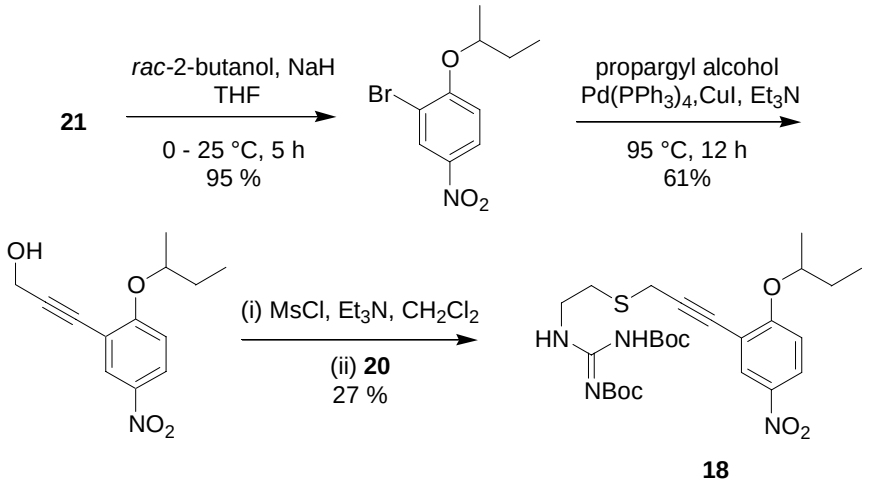


not suitable for
Sonogashira Coupling

the brominated scaffold did not work. As it turned out, installation of the necessary guanidine moiety required an indirect route. First, S_NAr reaction on scaffold **21** with (±)-2-butanol was employed to append the isoleucine side-chain. Then, the resulting intermediate was coupled with propargyl alcohol under Sonogashira conditions. This incorporated the alkyne without any complications. The alcohol was then converted to

the mesylate which was displaced by the cysteamine derivative **20** to afford compound **18** (Scheme 6.3).

Scheme 6.3. Synthesis of compound 18



The monovalent non-peptidic compounds in Figure 6.6 were combined with other compounds with different side-chain mimics installed in them to produce a small library of non-peptidic monovalent mimetics. These mimics were later dimerized by a co-worker, via the Inp-DTAF method similar to that discussed in Chapter IV (Figure 6.7). Homo- or heterobivalent non-peptidic mimics **24** can be assembled depending upon intermediate **23.**

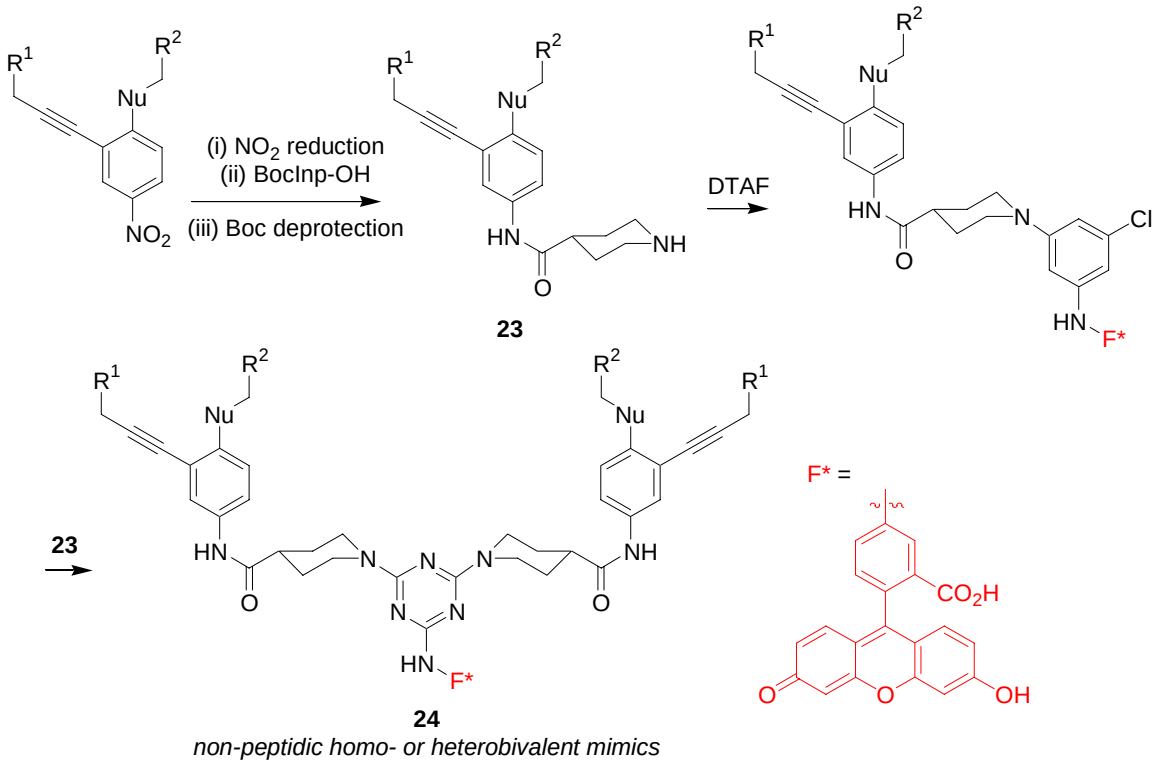


Figure 6.7. Strategy to make bivalent non-peptidic mimics using DTAF-Inp method.

6.3 Attempts to Make Peptidomimetics Based on Spirocyclic Diketopiperazine

Our efforts were shifted to prepare less peptidic mimics that can potentially contain three side chains, but unlike with the more flexible open mimics, these are cyclic ones that will have more rigidity in their structures. Consequently, we have designed and attempted to make the spirotetracyclic compound **25** shown in Figure 6.8. Retrosynthetic analysis of this compound traces it to the spirocyclic **26** which can be made from the spirocyclic diketopiperazine (DKP) **27** via activation of the carbonyl groups and two nucleophilic additions. The DKP in turn can be made from the corresponding dipeptide **28** whose one amino acid component is the α,α-disubstituted amino acid **29**.

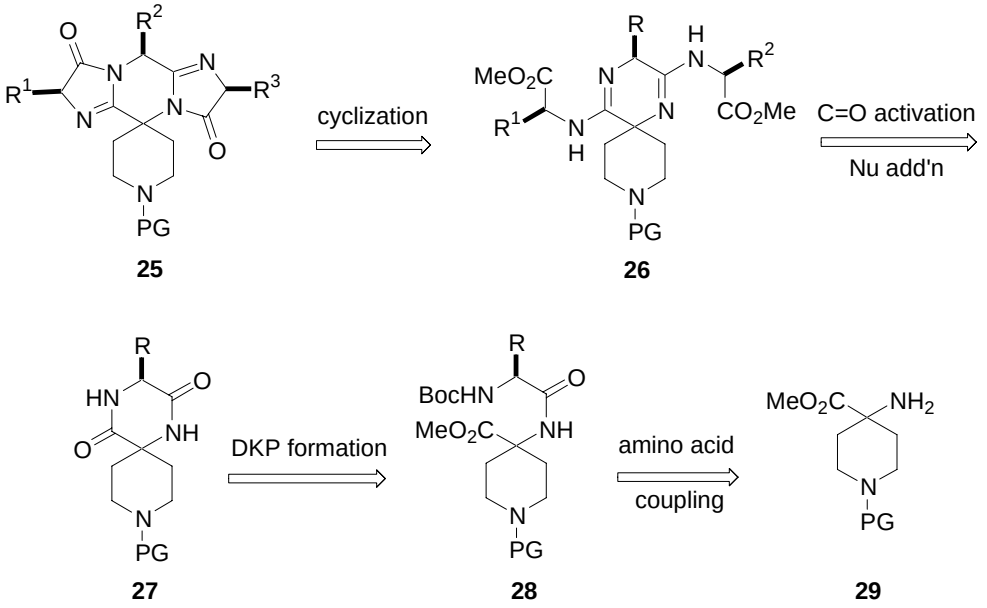


Figure 6.8. Retrosynthetic analysis toward the synthesis of peptidomimetics **25.**

Syntheses of spirocyclic diketopiperazines (DKPs) like **27**, although not as many as those for non-spirocyclic analogs have been reported and some compounds that have this fragment were found to exhibit interesting conformational and biological properties.[185,186] Published syntheses of spirocyclic DKPs involved coupling of two amino acids, one of which is the necessary α,α-disubstituted amino acid to make the linear precursors. A synthesis of a close analog of spirocyclic DKP **27** had been reported almost 15 yrs ago.[187] Compound **O** was made from the dipeptide formed by 1-amino-1-carboxymethylcyclohexane and another amino acid (Figure 6.9a).

A solid-phase approach was reported by Schafmeister *et al.* to make oligomeric DKPs based on amino acids **P** and **Q**. NMR experiments reveal that these oligomeric DKPs are capable of adopting rod-like and folded structures.(Figure 6.9b-c).[188,189]

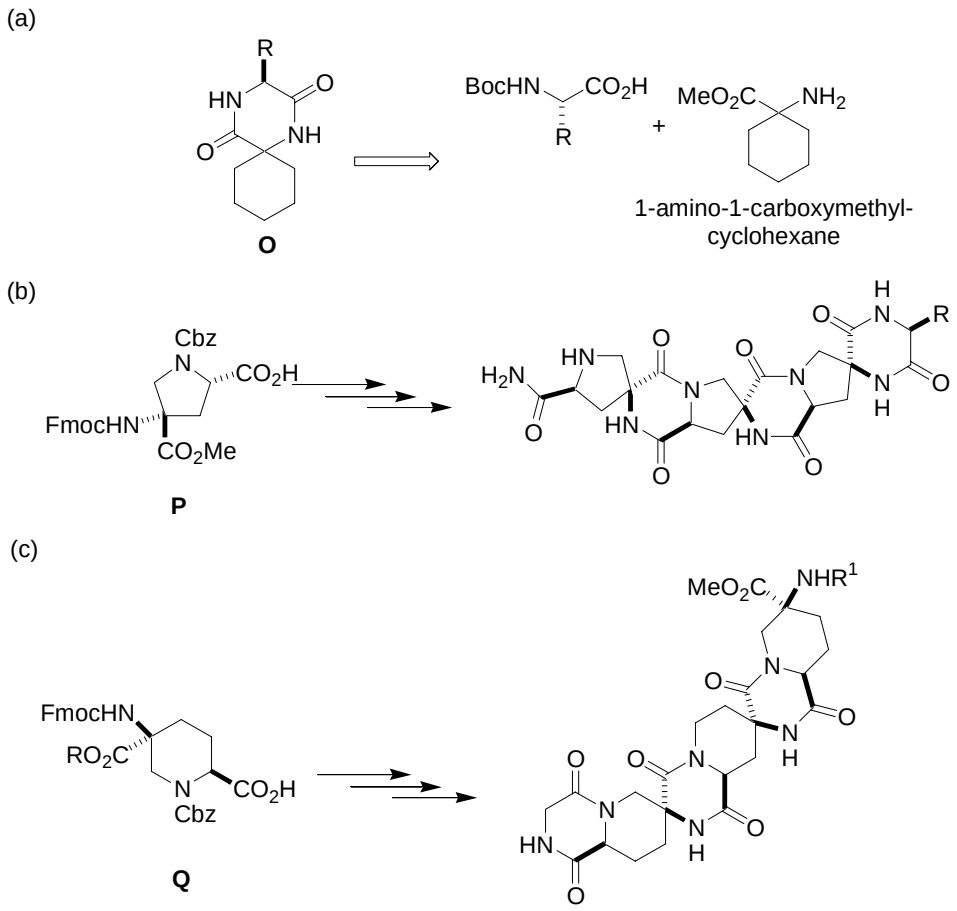


Figure 6.9. Structures of DKPs related to spirocyclic DKP **27**.

Recently, an elegant approach was reported by Herranz *et al.* to make spirocyclic 2,6-diketopiperazines (Figure 6.10).[190] A three-component Strecker reaction between cyclopentanone, a carboxyl-protected amino acid and TMSCN was conducted to give α-amino nitrile derivatives **S**. Formation of this intermediate depends on stabilizing the imine intermediate **R**. Optimum condition for imine formation required addition of $ZnCl_2$ and increasing the temperature to 65 °C. The nitrile group was converted to the amide under strong acidic conditions. This produced the amides **T** and the desired spirocyclic DKP **U** in varying yields depending on the amino acid present. This incomplete reaction can be remedied by treating the amide with NaH to make it cyclize to the DKP.

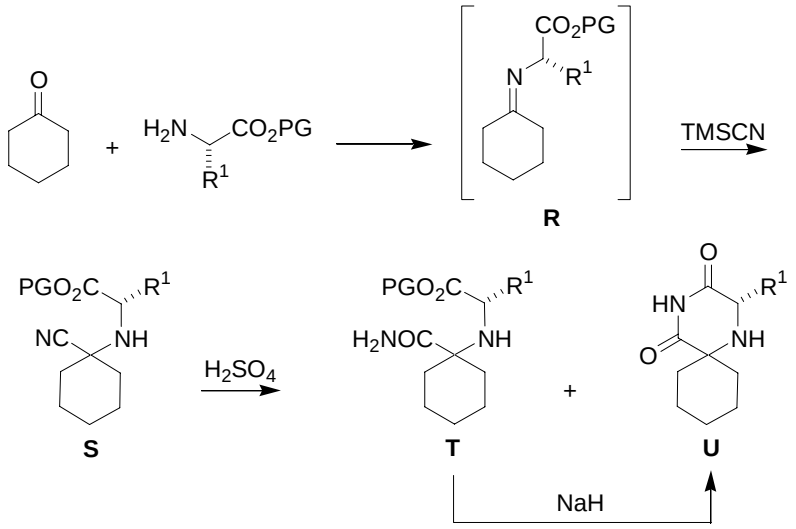


Figure 6.10. Synthesis of 2,6-DKP using a three-component Strecker reaction.

6.3.1 Attempt to Make Spirocyclic Diketopiperazines (DKPs) via Ugi Reaction

To make the synthesis of tetracyclic spiro compound **25** more amenable in parallel format, fast access to the critical spiro diketopiperazine intermediate **27** is required. As shown the Figure 6.8, this can be made from dipeptide precursor **28**.[191] Obtaining this DKP precursor via normal amino acid coupling will make the synthesis longer because the required amino acid **29** has to be independently made first. It is appealing, therefore, to try alternative routes to make the dipeptide. One of these is via a multi-component reaction of a ketone, an amine, an isonitrile, and a carboxylic acid, also known as the Ugi 4-component coupling, (4-CC).[192,193] This reaction generates a carbon skeleton of a dipeptide which, depending on the isonitrile used can be converted to several functionalities.[194,195] Hulme *et al*. have used this approach to make DKP libraries using Armstrong's 'universal' isonitrile (Figure 6.11).[196] They have found that by subjecting the dipeptide-derived Ugi product under TFA conditions and with mild heating, the very reactive *N*-acyliminium ion intermediated suffered an internal nucleophilic attack by the liberated amine to form the DKP **30** with potentially 4 sites of diversity.

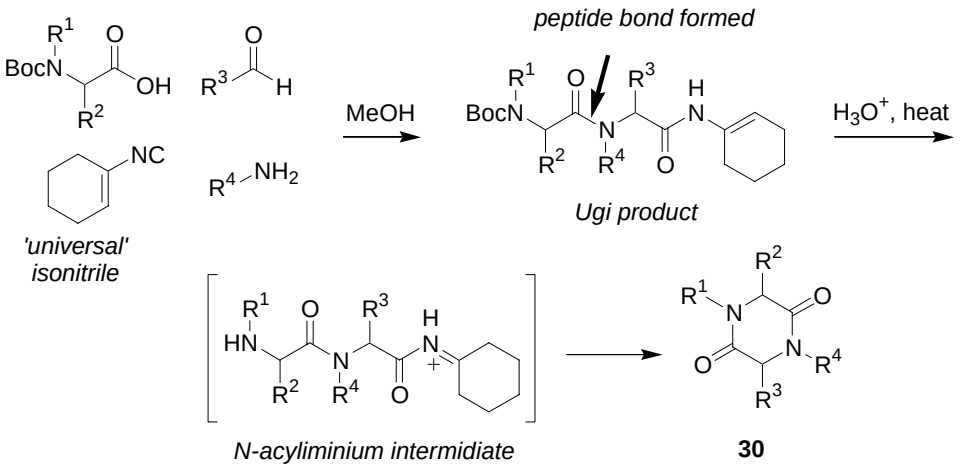
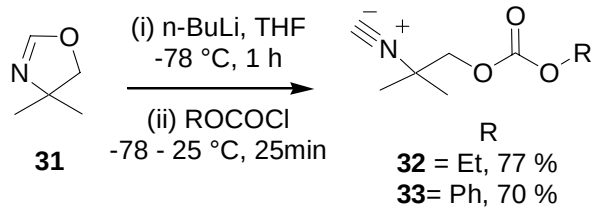


Figure 6.11. Route to 2,5-DKPs via Ugi reaction using Armstrong's 'universal' isonitrile.

Depending on the isonitrile used, the reaction can be stopped at the dipeptide stage. Other notable convertible isonitriles that were applied in this manner include **32** and **33** (Scheme 6.4). These were made from 4,4-dimethyloxazoline **31**.[197,198] Under basic conditions, the N=C*H* proton is abstracted generating the isonitrile and an alkoxide which can be quenched by a chloroformate, generating a carbonate ester at the other end of the molecule.

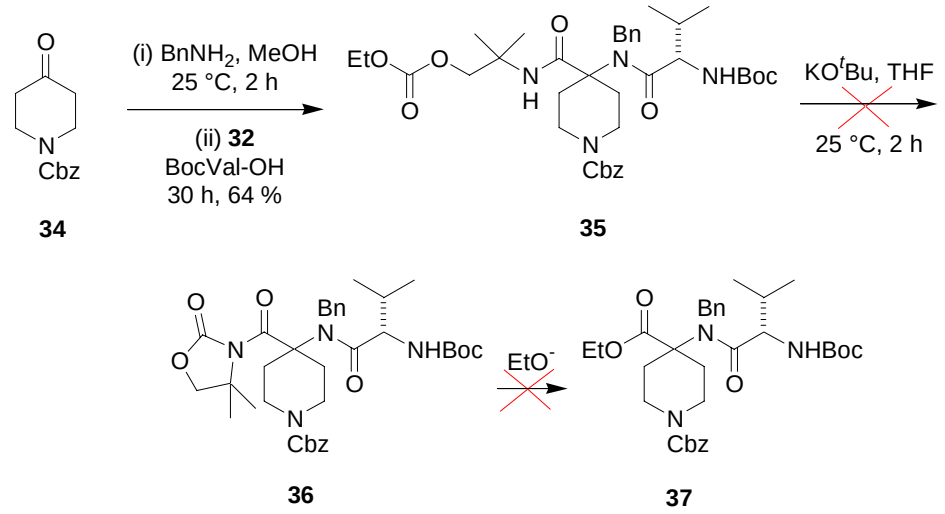
Scheme 6.4. Synthesis of convertible isonitriles 32 and 33



While applying Armstrong's convertible isonitrile for the 4-CC reaction to produce a dipeptide is very attractive, its synthesis is quite laborious and its stability is very limited even if stored under argon at -30 °C.[197] Attempts to make analog of

dipeptide **28** were therefore done using isonitrile **32** (Scheme 6.5). This isonitrile was condensed with the Schiff base generated by the reaction of benzylamine and Cbz-protected 4-piperidone **34**, then, Boc-Val-OH was added to give Ugi product **35.**

Scheme 6.5. Attempted synthesis of dipeptide analog of 28 via Ugi reaction



Its conversion to the corresponding oxazolidinone **36** using potassium *tert*-butoxide was not successful. The isolated product of the basic reaction was analyzed via NMR and it still showed an extra amide N*H* peak, plus the gem dimethyls are still observed. It is evident that deprotonation to produce the oxazolidinone did not occur; instead, hydrolysis of the carbonate was the dominant reaction observed (Figure 6.12). Other conditions (*e.g.* NaH, KO^{t}Bu/molecular sieves) were tried, but all failed. Each time, hydrolysis occurred to produce alcohol **38**.[198]

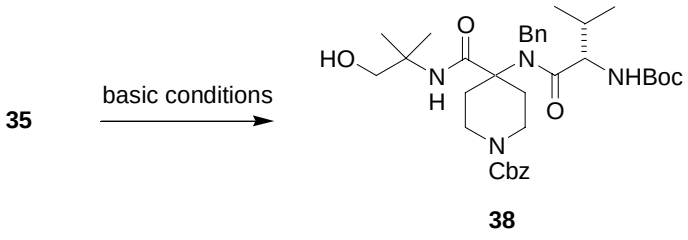
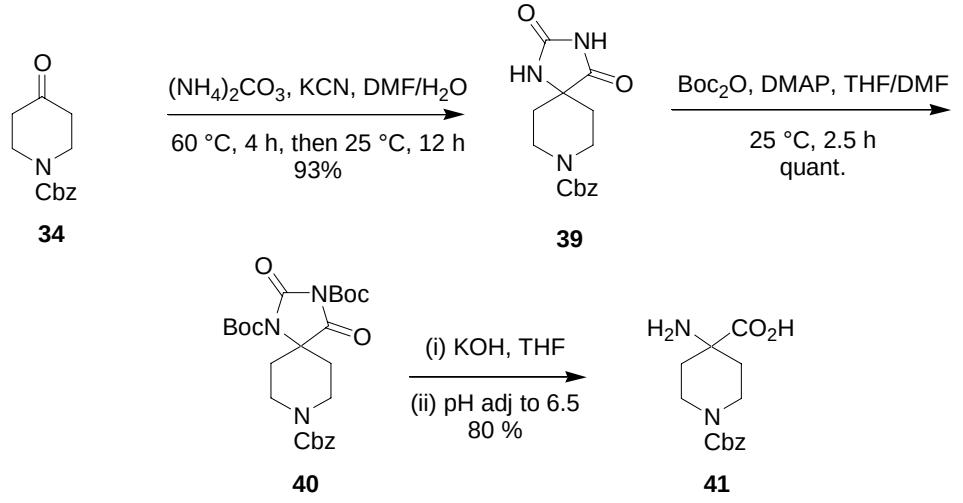


Figure 6.12. Attempts to form oxazolidinone **36** give hydrolyzed product **38**.

It is possible that deprotonation of the Ugi product **35** is very hard because of the steric hindrance contributed by the nearby piperidine ring. In this case, making the dipeptide derivative **27** has to be done via amino acid coupling. In order to apply this route, the cyclic α,α-disubstituted amino acid **41** was synthesized.

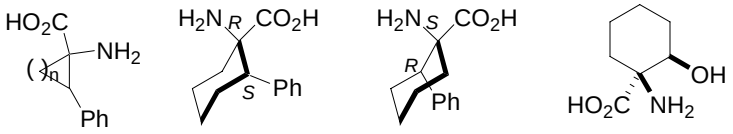
Scheme 6.6. Synthesis of Cbz-protected 4-amino-4-carboxypiperidine 41



6.3.2 Synthesis of Spiro Cyclic Diketopiperazine from Cyclic α,α-Disubstituted Amino Acid

Quaternary or α,α-disubstituted amino acids are non-natural variants that possess severe rotational freedom around the N-C-α and C-α-C=O bond. This inherent constraint imparts specific conformational preferences for inducing conformations when they are imbedded into a polypeptide chain. For example, aminoisobutyric acid (Aib) is a known inducer of helical structure.[199,200] More recently, when incorporated as part of a cyclic peptide, it was found that it can also adopt an extended conformation and can occupy the (i + 1) position of a type II′ beta-turn.[201] Alpha-methyl phenylalanine [(α-Me)Phe] has a propensity to impart both β-turn and helical conformations better than Phe.[202] The reduced conformational freedom is more pronounced with cyclic quaternary amino acids such as compound **41**. In most cases these cyclic analogs have a preference to adopt folded structures. Indeed, studies on quaternary cyclic analogs of Phe (c_nPhe) (**V**) gave evidence that these amino acids can induce beta-turn folding.[203-205]

Interestingly, the conformational restriction imposed by analogs **W** and **X** when they are used as Phe substitute in aspartame produced extreme tastes. The aspartame derivative with compound **W** was found to be sweet while that of **X** was bitter.[206] Other similar compounds is the quaternary cyclic analog of serine (c_6Ser) whose *cis*-analog **Y** was found to induce a type I beta-turn.[207].

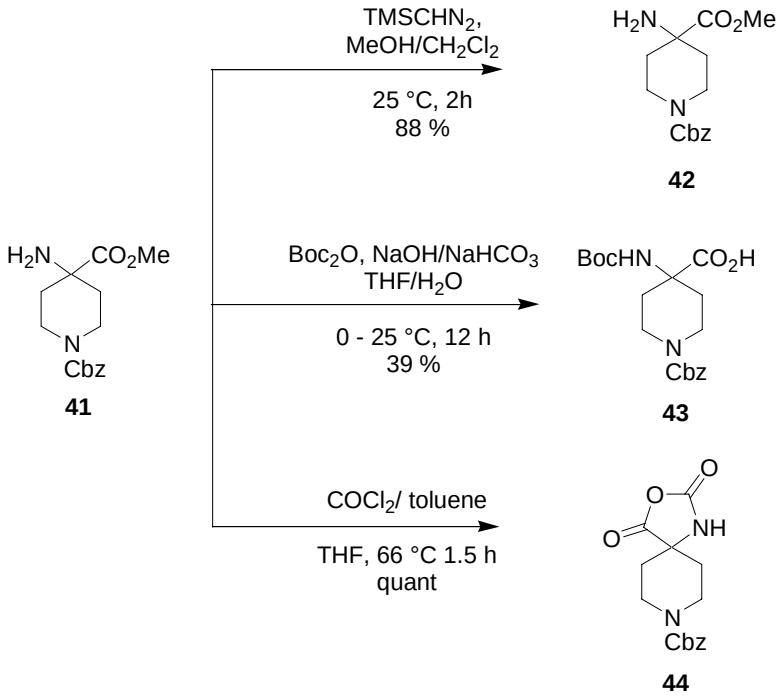


V **W** **X** **Y**

Synthesis of the required amino acid **41** utilized another multi-component reaction. This time, the Bucherer-Bergs reaction was conducted using Cbz-protected 4-piperidone **34**, ammonium carbonate and potassium cyanide (Scheme 6.6).[188,208] This reaction produced the spirocyclic hydantoin **39** which was fully Boc-protected using catalytic DMAP in THF/DMF. Full protection of the amides is required to activate the hydantoin prior to its opening under basic condition because it makes the process more facile. Ring opening will give a carboxylate salt so in order to isolate the desired compound the pH was adjusted to ~6.5 using dilute HCl. This precipitated out amino acid **41** in good yield. It is an interesting observation that if the 4-piperidone used was Boc- or Alloc-protected, there were problems along the synthesis. With Boc-protection, the spirocyclic hydantoin product after Bucherer-Bergs reaction, surprisingly, is not very soluble in THF/DMF and so global Boc protection is not efficient. In the case of Alloc-protection, the Bucherer-Bergs reaction is not clean as it gives a lot of spots on TLC.

To be able to be used in peptide synthesis, amino acid **41** has to be converted to a protected or activated form. The methyl ester derivative **42**, the Boc-protected analog **43** and the activated anhydride **44** where synthesized under the conditions indicated in Scheme 6.7. It was unusual that in making the methyl ester, normal acidic conditions like $SOCl_2$ in MeOH did not give the desired product.

Scheme 6.7. *C*- and *N*-terminal modifications on amino acid 41



To make the dipeptide derivative of **28**, protected amino acids **42** and **43** were tested in normal peptide coupling procedure using the HOBt/ EDCI method (Figure 6.13). Surprisingly, no amide bond formation occurred as indicated by TLC. In the case of the methyl ester, after considerable time, the reaction was worked-up and only the methyl ester was recovered. It seems that coupling of cyclic α,α-disubstituted amino acid is very difficult and might require different activating reagent. Indeed even the *N*-carboxyanhydride derivative **44** failed to give a dipeptide.

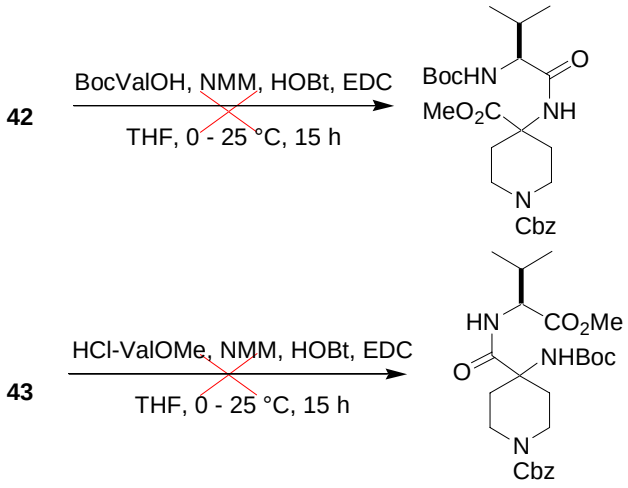
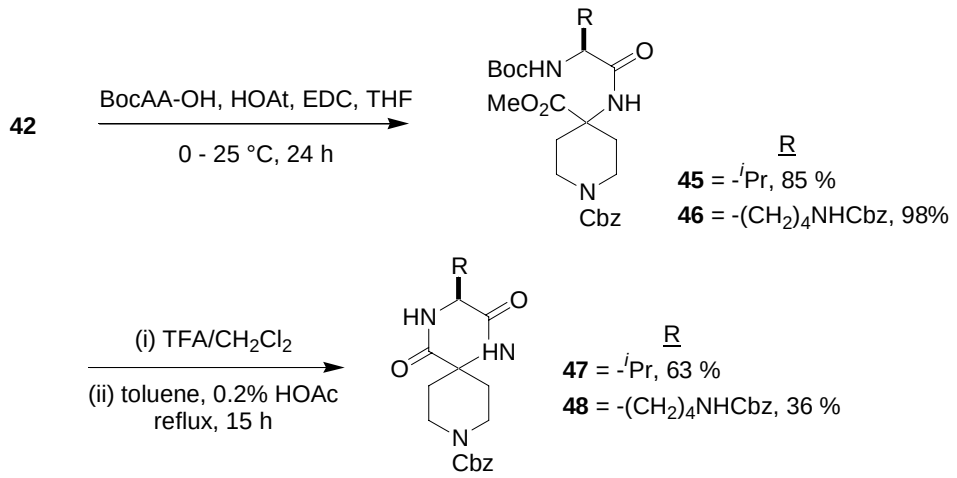


Figure 6.14. Attempts to make dipeptides of compounds **42** and **43** based on HOBt-EDC coupling.

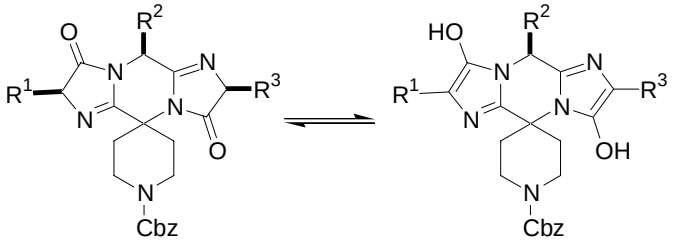
Several reports on coupling an α,α-disubstituted amino acid have indicated an inherent difficulty due to steric effects[208,209] One of the improved activating reagents used to improve coupling efficiency is HOAt. Introduced by Carpino as a substitute for HOBt, reactions mediated by this reagent were in general faster and cleaner.[210] It was noted that this aza derivative incorporates within its structure key elements of the 1:1 mixture of HOBt and a tertiary amine which drives amide bond formation faster than just using HOBt in peptide coupling reactions.[211] Gratifyingly, using HOAt to couple amino methyl ester **42** with BocVal-OH and BocLys(Boc)-OH gave the corresponding linear dipeptides **45** and **46**, respectively, in high yields (Scheme 6.8). Removal of the Boc protection followed by cyclization under weak acidic conditions in toluene gave the spirocyclic diketopiperazines **47** and **48** in modest to good yields. The yield for **48** is lower because of its solubility in toluene. On the other hand, DKP **47** is almost insoluble as it precipitated out of solution.

Scheme 6.8. Synthesis of dipeptides based on amino methyl ester 42 using HOAt/EDC method and conversion to DKP's



6.4 Future Directions

While the synthesis of the critical spirocyclic DKP took some time, the next direction should be focused on activating the 2,5-dioxo carbonyls. Normal activation of this functionality in DKP's involves $POCl_3/PCl_5$ activation. However, this reaction is not very efficient as yields of the resulting substituted compounds are low. On the other hand, other reactions can be tried as well. These include the use of Lawesson's reagent to convert to thiocarbonyl which can be activated by methylation. Another reaction that can also be tried is carbonyldiimidazole (CDI)-tetrazole/$POCl_3$ activation.[212] The fact that a general reaction for carbonyl activation could be hard to find can of course increase the time to make the ultimate target which is the spirotetracyclic mimic **25**. Another thing to consider is the stability of its structure. It is possible that it can adopt an imidazole form (**Im-25**) which will lose the chirality at the two side-chains. This in turn can affect its conformational and biological properties.



25 **(Im-25)**

6.5 Summary

Synthesis of non-peptidic mimics normally involve using peptide bond isosteres or using a scaffold wherein the critical side-chains are retained and presented in the proper three-dimensional manner to maintain the desired biological properties. One of the simplest ways to use a scaffold is to do sequential substitutions to introduce the side-chains of interest. Our first efforts toward the synthesis of non-peptide mimetics have utilized this approach. 3-Bromo-4-fluoronitrobenzene was the scaffold of choice and the side-chains were introduced via Sonogashira and S_NAr reactions. This method produced non-peptide mimics **16**, **17** and **18**. These compounds contain side-chain analogs of norleucine, isoleucine, arginine and lysine. The nitro group can be further reduced to the amine and a method for dimerization similar to the DTAF-Inp protocol can be used.

Another non-peptidic compound that we sought to make is the spirotetracyclic compound **25**. This compound can contain up to three side-chains and it already has the important amino piperidine moiety that can be used for dimerization. While this compound contains amide bonds, its rigidity could make it more resistant to proteolysis. A critical fragment in its synthesis is diketopiperazine (DKP) **27**. Attempts to make this DKP from its dipeptide precursor via Ugi reaction failed mainly because of the steric hindrance imposed by the quaternary cyclic side-chain. However, the dipeptide was made via conventional peptide synthesis using the cyclic α,α-disubstituted amino acid **42**. Using this route, two dipeptides were made and converted to their corresponding DKP derivatives.

Although the route to the DKP intermediate is slow, a proven procedure to make it has been developed. To make the eventual target **25**, the DKP has to be activated at the carbonyls and this may require time. Also, the stability of compound **25** has to be

considered. If it exists in an imidazole-like ring structure (**Im-25**), it loses its chirality at the α-carbon which may have drastic effect in its conformation and biological behavior.

CHAPTER VII

CONCLUSIONS

Solid-phase synthesis of 14-membered ring cyclic peptidomimetics using two different organic templates but the same mode of cyclization indicated that conformation can influence the ease these compounds are formed. S_NAr cyclizations that give a strained ring structure like those peptidomimetics **1** gave poor yields which are complicated by head-to-tail 'dimer' formation. On the other hand, less strained cyclic peptidomimetics like compounds **2** cyclize more efficiently, giving less amount of the 'dimer' by-product. These cyclizations were not proven unambiguously to be influenced by an anticipated beta-turn structure; although by QMD, compounds **2** were shown to be capable of adopting a type III beta-turn conformation.

Expanding the dipeptide that mimics the tip of the tetrapeptide turn sequence by adding an extra residue was done by synthesizing compounds **3**. These compounds were cyclized via an S_N2 reaction to afford 18-membered ring systems. While these are bigger in ring size than our previous mimics, their syntheses proved to be more efficient as only insignificant 'dimers' were formed. A resin comparison study showed that our protocol for solid-phase synthesis is better suited for resins that have the Rink handle. Eventually, TentaGel Rink resin was chosen to make a small library of compounds **3** that mimics NGF and NT-3 loop regions. By adding an extra residue, the chances of mimicking a turn is improved as two mimics were shown to have agonistic properties for NT-3 in cell survival assays.

Dimerization of monovalent mimics to bivalent ones can give even better mimics since the neurotrophins function as dimers. Synthesis of bivalent compounds was achieved by doing sequential substitutions on dichlortriazinylaminofluorescein (DTAF) This involved using peptidomimetics with a pendant piperidine ring from an isonipecotic acid residue that served as the nucleophile in the S_NAr reaction. Synthesis of monovalent units were done on resin and then cleaved to give either those with a reactive nucleophile (*i.e.* with the amino group of Inp) or those with an electrophile (*i.e.* with a labeled DTAF). Dimerization was done in solution by mixing solutions of one of each of the two

types of monomers. This simple technique gave homo- and heterobivalent peptidomimetics very efficiently. Bivalent mimic **4**, whose individual components are **3g** and another one that was also active on preliminary cell survival assays, were synthesized using this dimerization strategy. Our rationale that bivalent mimics made from monovalent ones can give better biological activities was justified when compound **4** was shown to bind TrkA with high affinity (20 nM). More importantly, but unsurprisingly, it also binds TrkC, since it has turn residues corresponding to NT-3, with an even higher affinity.

One-step reactions of amino acids that give products considered as pharmacophores such as thiohydantoins are important in medicinal chemistry. Acylation of amino acids with NH_4SCN in acetic anhydride was shown to give thiohydantoins that are acylated at the nitrogen of the amino acid (*i.e.* N^1 position). This was proven by 2-D NMR methods to clarify mislabeling and incorrect structure representation of the product that was reported in the literature.

Solution-phase combinatorial synthesis can be an alternative to solid-phase methods in making libraries of peptidomimetics. In our efforts to make less peptidic compounds, a simple dihalogenated nitrobenzene was used as a scaffold to attach side-chains of interest. Employing S_NAr and Sonogashira reactions, non-cyclic mimics **16**, **17** and **18** were made. These compounds contain side-chains having lipophilic and hydrophilic properties (*e.g.* isopropyl and guanidine). Because nitro group can be reduced to the amine these non-peptidic mimics can be easily dimerized by adopting the method described in Chapter III.

A compound that we have sought to make was the spirotetracyclic analog of **25**. A critically important intermediate to make this is the spirocyclic diketopiperazine (DKP) **27**. Initial approach to synthesize the DKP using Ugi reaction proved to be difficult as the 4-CC product was too hindered to be converted to the dipeptide DKP precursor. A longer route to the DKP was achieved by synthesizing the quaternary cyclic amino acid **41** via Bucherer-Bergs reaction. The methyl ester derivative of this amino acid, **42**, was found to couple smoothly only with HOAt as the activating agent. Dipeptides **45** and **46** were prepared using this coupling procedure. The dipeptides were deprotected and

cyclized in toluene under mildly acidic conditions to give the corresponding DKPs **47** and **48**.

REFERENCES

(1) Snider, W. D. *Cell* **1994**, *77*, 627-638.

(2) Wiesmann, C.; de Vos, A. M. *Cell. Mol. Life Sci.* **2001**, *58*, 748-59.

(3) Gotz, R.; Koster, R.; Winkler, C.; Raulf, F.; Lottspeich, F.; Schartl, M.; Thoenen, H. *Nature* **1994**, *372*, 266 -269.

(4) Dawbarn, D.; Allen, S. J. *Neuropathol. Appl. Neurobiol.* **2003**, *29*, 211 -230.

(5) Bibel, M.; Barde, Y. A. *Genes Dev.* **2000**, *14*, 2919-37.

(6) Hempstead, B. L.; Martin-Zanca, D.; Kaplan, D. R.; Parada, L. F.; Chao, M. V. *Nature* **1991**, *350*, 678-683.

(7) Klein, R.; Jing, S. Q.; Nanduri, V.; Orourkee, E.; Barbacid, M. *Cell* **1991**, *65*, 189-197.

(8) Klein, R.; Nanduri, V.; Jing, S. Q.; Lamabelle, F.; Tapley, P.; Bryant, S.; Cordoncardo, C.; Jones, K. R.; Reichardt, L. F.; Barbacid, M. *Cell* **1991**, *66*, 395-403.

(9) Klein, R.; Lamballe, F.; Bryant, S.; Barbacid, M. *Neuron* **1992**, *8*, 947-956.

(10) Lamballe, F.; Klein, R.; Barbacid, M. *Cell* **1991**, *66*, 967-79.

(11) Cordoncardo, C.; Tapley, P.; Jing, S. Q.; Nanduri, V.; Orourke, E.; Lamballe, F.; Kovary, K.; Klein, R.; Jones, K. R.; Reichardt, L. F.; Barbacid, M. *Cell* **1991**, *66*, 173-183.

(12) Soppet, D.; Escandon, E.; Maragos, J.; Middlemas, D. S.; Reid, S. W.; Blair, J.; Burton, L. E.; Stanton, B. R.; Kaplan, D. R.; Hunter, T.; Nikolics, K. *Cell* **1991**, *65*, 895-903.

(13) Kaplan, D. R.; Miller, F. D. *Curr. Opin. Neurobiol.* **2000**, *10*, 381-91.

(14) Schneider, R.; Schweiger, M. *Oncogene* **1991**, *6*, 1807-11.

(15) Ultsch, M. H.; Wiesmann, C.; Simmons, L. C.; Henrich, J.; Yang, M.; Reilly, D.; Bass, S. H.; de Vos, A. M. *J. Mol. Biol.* **1999**, *290*, 149-59.

(16) Wiesmann, C.; Ultsch, M. H.; Bass, S. H.; de Vos, A. M. *Nature* **1999**, *401*, 184-8.

(17) Robertson, A. G. S.; Banfield, M. J.; Allen, S. J.; Dando, J. A.; Mason, G. G. F.; Tyler, S. J.; Bennett, G. S.; Brain, S. D.; Clarke, A. R.; Naylor, R. L.; Wilcock, G. K.; Brady, R. L.; Dawbarn, D. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 131-41.

(18) He, X. L.; Garcia, K. C. *Science* **2004**, *304*, 870-875.

(19) Saragovi, H. U.; Zaccaro, M. C. *Curr. Pharm. Des.* **2002**, *8*, 2201-16.

(20) Wells, J. A. *Science* **1996**, *273*, 449-50.

(21) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467-78.

(22) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699-720.

(23) Kahn, M. *Synlett* **1993**, *11*, 821-6.

(24) Marshall, G. R. *Curr. Opin. Struct. Biol.* **1992**, *2*, 904-19.

(25) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bos, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039-49.

(26) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431-7448.

(27) Li, G. Y.; Marshall, W. J. *Organometallics* **2002**, *21*, 590-1.

(28) Gardner, B.; Nakanishi, H.; Kahn, M. *Tetrahedron* **1993**, *49*, 3433-48.

(29) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kaln, M.; Madison, V. S.; Cook, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 323-33.

(30) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656-8.

(31) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647-50.

(32) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580-1.

(33) Jackson, S.; DeGrado, W.; Dwivedi, A.; Parthasarathy, A.; Higley, A.; Krywko, J.; Rockwell, A.; Markwalder, J.; Wells, G.; Wexler, R.; Mousa, S.; Harlow, R. *J. Am. Chem. Soc.* **1994**, *116*, 3220-30.

(34) Roedern, E. G. v.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156-67.

(35) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 10768-9.

(36) Kessler, H. *Angew. Chem. Int. Ed.* **1982**, *21*, 512-23.

(37) Ludvigsen, S.; Andersen, K. V.; Poulsen, F. M. *J. Mol. Biol.* **1991**, *217*, 731.

(38) Williamson, M. P.; Waltho, J. P. *Chem. Soc. Rev.* **1992**, 227-36.

(39) Gosselin, F.; Tourwe, D.; Ceusters, M.; Meert, T.; Heylen, L.; Jurzak, M.; Lubell, W. D. *J. Pept. Res.* **2001**, *57*, 337-344.

(40) Wüthrich, K.; Billeter, M.; Braun, W. *J. Mol. Biol.* **1984**, *180*, 715-40.

(41) Andersen, N. H.; Neidigh, J. W.; Harris, S. M.; Lee, G. M.; Liu, Z.; Tong, H. *J. Am. Chem. Soc.* **1997**, *119*, 8547-61.

(42) Woody, R. W. In *Circular Dichroism Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds.; VCH: New York, 1994, p 473-96.

(43) Bush, C. A.; Sarkar, S. K.; Kopple, K. D. *Biochemistry (Mosc).* **1978**, *17*, 4951-4.

(44) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1996, p 362-364.

(45) Mierke, D. F.; Kurz, M.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 1042-9.

(46) Sefler, A. M.; Lauri, G.; Bartlett, P. A. *Int. J. Pept. Protein Res.* **1996**, *48*, 129-38.

(47) Rappé, A. K.; Casewit, C. J. *Molecular Mechanics across Chemistry*; University Science Books: Sausalito, CA 1997.

(48) Hruby, V. J. *Life Sci.* **1982**, *31*, 189-99.

(49) Shreder, K.; Zhang, L.; Dang, T.; Yaksh, T. L.; Umeno, H.; DeHaven, R.; Daubert, J.; Goodman, M. *J. Med. Chem.* **1998**, *41*, 2631-2635.

(50) Kenner, G. W.; Laird, A. H. *Chem. Commun.* **1965**, 305-6.

(51) Rozek, A.; Powers Jon-Paul, S.; Friedrich Carol, L.; Hancock Robert, E. W. *Biochemistry* **2003**, *42*, 14130-8.

(52) Li, P.; Roller, P. P. *Curr. Top. Med. Chem.* **2002**, *2*, 325-341.

(53) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc. [Perkin. 1]*. **2001**, 471-484.

(54) Annis, I.; Chen, L.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 7226-7238.

(55) Pallin, T. D.; Tam, J. P. *J. Chem. Soc., Chem. Commun.* **1995**, 2021-2.

(56) Martin, W. H. C.; Blechert, S. *Current Top. Medl Chem.* **2005**, *5*, 1521-1540.

(57) Reichwein, J. F.; Wels, B.; Kruijtzer, J. A. W.; Versluis, C.; Liskamp, R. M. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 3684-7.

(58) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

(59) Burgess, K.; Editor *Solid-Phase Organic Synthesis*; Wiley Interscience: New York, 2000.

(60) Czarnik, A. W.; Editor *Solid-Phase Organic Syntheses,* Wiley: New York, 2001, Vol.1.

(61) Beugelmans, R.; Zhu, J.; Husson, N.; Bois-Choussy, M.; Singh, G. P. *J. Chem. Soc., Chem. Commun.* **1994**, 439-40.

(62) Burgess, K.; Lim, D.; Bois-Choussy, M.; Zhu, J. *Tetrahedron Lett.* **1997**, *38*, 3345-8.

(63) Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **1991**, *113*, 9466-70.

(64) Matter, H.; Kessler, H. *J. Am. Chem. Soc.* **1995**, *117*, 3347-59.

(65) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *Chemistry - A European J.* **1999**, *5*, 3273-8.

(66) Feng, Y.; Pattarawarapan, M.; Wang, Z.; Burgess, K. *Org. Lett.* **1999**, *1*, 121-4.

(67) Chan, W. C.; White, P. D.; Editors *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press: Oxford, New York, 2000.

(68) Feng, Y.; Burgess, K. *Chemistry - A European J.* **1999**, *5*, 3261-72.

(69) Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophy. J. Biophys. Soc.* **1991**, *60*, 1540-4.

(70) O'Connor, S. D.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870-81.

(71) Kopple, K. D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264-72.

(72) Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194-202.

(73) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1-109.

(74) Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315-399.

(75) MacDonald, M.; Aube, J. *Curr. Org. Chem.* **2001**, *5*, 417-438.

(76) Veber, D. F.; Freidinger, R. M.; Perlow, D. S.; Paleveda, W. J., Jr.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C. Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Nature* **1981**, *292*, 55-8.

(77) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577-9.

(78) Pierschbacher, M. D.; Ruoslahti, E. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 5985-8.

(79) Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Proc. Natl. Acad. Sci.USA* **1978**, *75*, 2636-40.

(80) Brazeau, P.; Vale, W.; Burgus, R.; Guillemin, R. *Can. J. Biochem.* **1974**, *52*, 1067-72.

(81) Weckbecker, G.; Lewis, I.; Albert, r.; Schmid, H.; Hoyer, D.; Bruns, C. *Nat. Rev. Drug Discovery* **2003**, *2*, 999-1017.

(82) Hirschmann, R. *Peptides 1996, Proceedings of the 24th European Peptide Symposium,* Edinburgh, Sept. 8-13, 1996 **1998**, 3-17.

(83) Costantino, L.; Barlocco, D. *Curr. Med. Chem.* **2006**, *13*, 65-85.

(84) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith III, A. B.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550-68.

(85) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith III, A. B. *J. Am. Chem. Soc.* **1992**, *114*, 9217-8.

(86) Roedern, E. G. v.; Kessler, H. *Angew. Chem. Int. Ed.* **1994**, *33*, 687-9.

(87) Ruoslahti, E.; Pierschbacher, M. D. *Science* **1987**, *238*, 491-7.

(88) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461-72.

(89) Bach, A. C.; Espina, J. R.; Jackson, S. A.; Stouten, P. F. W.; Duke, J. L.; Mousa, S. A.; Degrado, W. F. *J. Am. Chem. Soc.* **1996**, *118*, 293-4.

(90) Haubner, R.; Schmitt, W.; Hölzemann, G.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7881-91.

(91) Bach, A. C.; Eyermann, C. J.; Gross, J. D.; Bower, M. J.; Harlow, R. L.; Weber, P. C.; DeGrado, W. F. *J. Am. Chem. Soc.* **1994**, *116*, 3207-19.

(92) Ramaseshan, M.; Dory, Y. L.; Deslongchamps, P. *J. Comb. Chem.* **2000**, *2*, 615-623.

(93) Soucy, P.; Dory, Y. L.; Deslongchamps, P. *Synlett* **2000**, 1123-1126.

(94) Feng, Y.; Burgess, K. *Biotech. Bioeng. Comb. Chem.* **2000**, *71*, 3-8.

(95) Litak, P. T.; Kaufmann, J. M. *J. Heterocycl. Chem.* **1994**, *31*, 457-79.

(96) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161-214.

(97) Patek, M. *Int. J. Pept. Protein Res.* **1993**, *42*, 97-117.

(98) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787-90.

(99) Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333.

(100) Bothner-By, A. A.; Stephen, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811-3.

(101) Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800-1.

(102) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820-1.

(103) Klemm, J. D.; Schreiber, S. L.; Crabtree, G. R. *Annu. Rev. Immunol.* **1998**, *16*, 569-92.

(104) Jones, S.; Thornton, J. M. *Proc. Natl. Acad. Sci.* **1996**, *93*, 13-20.

(105) Valdar, W. S. J.; Thornton, J. M. *Proteins: Struct., Funct., Genet.* **2001**, *42*, 108-124.

(106) Lo Conte, L.; Chothia, C.; Janin, J. *J. Mol. Biol.* **1999**, *285*, 2177-2198.

(107) Preissner, R.; Goede, A.; Frommel, C. *J. Mol. Biol.* **1998**, *280*, 535-550.

(108) Schreiber, S. L.; Albers, M. W.; Brown, E. J. *Acc. Chem. Res.* **1993**, *26*, 412-420.

(109) Schreiber, S. L.; Crabtree, G. R. *Immunol. Today* **1992**, *13*, 136-142.

(110) Handl, H. L.; Vagner, J.; Han, H. Y.; Mash, E.; Hruby, V. J.; Gillies, R. J. *Expert Opin. Ther. Targets* **2004**, *8*, 565-586.

(111) Heldin, C.-H. *Cell* **1995**, *80*, 213-23.

(112) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754-94.

(113) Nicolaou, K. C.; Hughs, R.; Pfefferkorn, J. A.; Barluenga, S. *Chem. Eur. J.* **2001**, *7*, 4296-4310.

(114) Boger, D. L.; Chai, W. *Tetrahedron* **1998**, *54*, 3955-70.

(115) Maly, D. J.; Choong, I. C.; Ellman, J. A. *Proc. Nat. Acad. Sci. USA* **2000**, *97*, 2419-24.

(116) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

117) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 1053-7.

(118) Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem Soc.* **2004**, *126*, 12809-12818.

(119) Krasinski, A.; Radic, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem Soc.* **2005**, *127*, 6686-6692.

(120) Mocharla, V. P.; Colasson, B.; Lee, L. V.; Roper, S.; Sharpless, K. B.; Wong, C.-H.; Kolb, H. C. *Angew. Chem. Int. Ed.* **2005**, *44*, 116-120.

(121) Zaragoza, F.; Stephensen, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 554-6.

(122) Grimstrup, M.; Zaragoza, F. *Eur. J. Org. Chem.* **2001**, 3233-3246.

(123) Scharn, D.; Germeroth, L.; Schneider-Mergener, J.; Wenschuh, H. *J. Org. Chem.* **2001**, *66*, 507-513.

(124) Frank, R. *Tetrahedron* **1992**, *48*, 9217-9232.

(125) Pattarawarapan, M.; Reyes, S.; Xia, Z.; Zaccaro, M. C.; Saragovi, H. U.; Burgess, K. *J. Med. Chem.* **2003**, *46*, 3565-7.

(126) Liu, R.; Marik, J.; Lam, K. S. *J. Am. Chem. Soc.* **2002**, *124*, 7678-80.

(127) Rachlin, S.; Bramm, E.; Ahnfelt-Roenne, I.; Arrigoni-Martelli, E. *J. Med. Chem.* **1980**, *23*, 13-20.

(128) Yang, H.; Xie, X. S. *J. Chem. Phys.* **2002**, *117*, 10965-10979.

(129) Slaughter, R. S.; Garcia, M. L.; Cragoe, E. J., Jr.; Reeves, J. P.; Kaczorowski, G. J. *Biochemistry* **1988**, *27*, 2403-9.

(130) Chapleo, C. B.; Myers, P. L.; Smith, A. C. B.; Tulloch, I. F.; Walter, D. S. *J. Med. Chem.* **1987**, *30*, 951-4.

(131) Izumi, K.; Kishita, C.; Nakagawa, K.; Huxtable, R. J.; Shimizu, T.; Koja, T.; Fukuda, T. *Eur. J. Pharmacol.* **1985**, *110*, 219-24.

(132) Jadhav, P. K.; Woerner, F. J.; Man, H.-W. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2145-2148.

(133) Breccia, P.; Boggetto, N.; Perez-Fernandez, R.; Van Gool, M.; Takahashi, M.; Rene, L.; Prados, P.; Badet, B.; Reboud-Ravaux, M.; De Mendoza, J. *J. Med. Chem.* **2003**, *46*, 5196-5207.

(134) Ganelin, C. R. *Chronicles of drug discovery*; Wiley: New York, 1982; Vol. 1.

(135) McKay, A. F.; Hatton, W. G. *J. Am. Chem Soc.* **1956**, *78*, 1618-20.

(136) Corey, E. J.; Ohtani, M. *Tetrahedron Lett.* **1989**, *30*, 5227-30.

(137) Metzger, A.; Peschke, W.; Schmidtchen, F. P. *Synthesis* **1995**, 566-70.

(138) Kiec-Kononowicz, K.; Karolak-Wojciechowska, J.; Mrozek, A.; Posel, M. *Archiv der Pharm. (Weinheim)* **1995**, *328*, 517-21.

(139) Acharya, A. N.; Ostresh, J. M.; Houghten, R. A. *Tetrahedron* **2001**, *574*, 9911-991.

(140) Waley, S. G.; Watson, J. *J. Chem. Soc. Abstracts* **1951**, *1951*, 2394-7.

(141) Bailey, J. M.; Shively, J. E. *Biochemistry.* **1990**, *296*, 3145-5.

(142) Ribeiro, P. D.; Borges, R. S. M.; Costa, P. R. R.; Alves, E. W.; Machado, O. L. T. *Protein Pept. Lett.* **1998**, *5*, 251-256.

(143) Boyd, V. L.; Bozzini, M.; Guga, P. J.; DeFranco, R. J.; Yuan, P.-M.; Loudon, G. M.; Nguyen, D. *J. Org. Chem.* **1995**, *60*, 2581-7.

(144) Mo, B.; Li, J.; Liang, S. *Anal. Biochem.* **1997**, *252*, 169-176.

(145) Suzuki, T.; Song, K.-D.; Itagaki, Y.; Tuzimura, K. *Org. Mass Spectrom.* **1976**, *11*, 557-68.

(146) LeTiran, A.; Stables, J. P.; Kohn, H. *Bioorg. Med. Chem.* **2001**, *9*, 2693-2708.

(147) Johnson, T. B.; Nicolet, B. H. *J. Am. Chem. Soc.* **1911**, *33*, 1973-8.

(148) *A Guide to IUPAC Nomenclature of Organic Chemistry, Recommendations 1993*; Blackwell Scientific Publications: Oxford, 1993.

(149) Ware, E. *Chem. Rev.* **1950**, *46*, 403-70.

(150) Thielemann, H. *Z. Chem.* **1978**, *18*, 174.

(151) Marton, J.; Enisz, J.; Hosztafi, S.; Timar, T. *J. Agric. Food Chem.* **1993**, *41*, 148-152.

(152) Villemin, D.; Ricard, M. *Synth. Commun.* **1987**, *17*, 283-9.

(153) Davis, R. A.; Aalbersberg, W.; Meo, S.; Moreira da Rocha, R.; Ireland, C. M. *Tetrahedron* **2002**, *58*, 3263-3269.

(154) Smith, D. C.; Lee, S. W.; Fuchs, P. L. *J. Org. Chem.* **1994**, *59*, 348-54.

(155) Johnson, T. B.; Nicolet, B. H. *Am. Chem. J.* **1913**, *49*, 197-204.

(156) Geysen, H. M.; Meloen, R. H.; Barteling, S. J. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3998-4002.

(157) Houghten, R. A. *Proc. Natl. Acad. Sci.USA* **1985**, *82*, 5131-5.

(158) Furka, A.; Sevestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Res.* **1991**, *37*, 487-93.

(159) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82.

(160) Goldberg, J.; Jin, Q.; Ambroise, Y.; Satoh, S.; Desharnais, J.; Capps, K.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 544-55.

(161) Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567-73.

(162) Pirrung, M. C. *Chem. Rev.* **1997**, *97*, 473-88.

(163) Pirrung, M. C.; CHen, J. *J. Am. Chem. Soc.* **1995**, *117*, 1240-5.

(164) Curran, D. P. *Angew. Chem. Int. Ed. Engl.***1998**, *37*, 1174-96.

(165) Bosanac, T.; Yang, J.; Wilcox, C. S. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1875-1879.

(166) Brown, S. D.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 6331-2.

(167) Bosanac, T.; Wilcox, C. S. *J. Am. Chem. Soc.* **2002**, *124*, 4194-4195.

(168) Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockerman, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874-81.

(169) Tripp, J. A.; Stein, J. A.; Svec, F.; Frechet, J. M. J. *Org. Lett.* **2000**, *2*, 195-198.

(170) Kaldor, S. W.; Siegel, M. G. *Curr. Opin. Chem. Biol.* **1997**, *1*, 101-106.

(171) Ley, S. V.; Baxendale, I. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 573-586.

(172) An, H. Y.; Cook, P. D. *Chem. Rev.* **2000**, *100*, 3311-3340.

(173) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Amos B. Smith, I. *J. Am. Chem. Soc.* **1992**, *114*, 9699-701.

(174) Bursavich, M. G.; West, C. W.; Rich, D. H. *Org. Lett.* **2001**, *3*, 2317-2320.

(175) Kawato, H. C.; Nakayama, K.; Inagaki, H.; Ohta, T. *Org. Lett.* **2001**, *3*, 3451-4.

(176) Gottschling, D.; Boer, J.; Schuster, A.; Holzmann, B.; Kessler, H. *Angew. Chem-Int. Ed. Engl.* **2002**, *41*, 3007-3011.

(177) Venkatesan, N.; Kim, B. H. *Curr. Med. Chem.* **2002**, *9*, 2243-2270.

(178) Ellman, J. A. *Acc. Chem. Res.* **1996**, *29*, 132-43.

(179) Abell, A. D. *Lett. Pept. Sci.* **2002**, *8*, 267-272.

(180) Maltais, R.; Tremblay, M. R.; Ciobanu, L. C.; Poirier, D. *J. Comb. Chem.* **2004**, *6*, 443-456.

(181) Stanková, M.; Lebl, M. *Mol. Diversity* **1996**, *2*, 75-80.

(182) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467-70.

(183) Olah, G. A.; Wang, Q.; Sandford, G.; Prakash, G. K. S. *J. Org. Chem.* **1993**, *58*, 3194-3195.

(184) Groweiss, A. *Org. Process Res. Dev.* **2000**, *4*, 30-33.

(185) Vinsova, J.; Kosar, K.; Kasafirek, E. *Collect. Czech. Chem. Commun.* **1993**, *58*, 2987-93.

(186) Levins, C. G.; Schafmeister, C. E. *J. Org. Chem.* **2005**, *70*, 9002-9008.

(187) Vinsova, J.; Kosar, K.; Kasafirek, E. *Collect. Czech. Chem. Commun.* **1994**, *59*, 195-202.

(188) Levins, C. G.; Schafmeister, C. E. *J. Am. Chem. Soc.* **2003**, *125*, 4702-4703.

(189) Gupta, S.; Das, B. C.; Schafmeister, C. E. *Org. Lett.* **2005**, *7*, 2861-2864.

(190) Gonzalez-Vera, J. A.; Garcia-Lopez, M. T.; Herranz, R. *J. Org. Chem.* **2005**, *70*, 3660-3666.

(191) Fischer, P. M. *J. Pept. Sci.* **2003**, *9*, 9-35.

(192) Domling, A.; Ugi, I. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3168-3210.

(193) Domling, A. *Chem. Rev.* **2006**, *106*, 17-89.

(194) Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 7842-3.

(195) Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2574-2583.

(196) Hulme, C.; Morrissette, M. M.; Volz, F. A.; Burns, C. J. *Tetrahedron Lett.* **1998**, *39*, 1113-1116.

(197) Lindhorst, T.; Bock, H.; Ugi, I. *Tetrahedron* **1999**, *55*, 7411-7420.

(198) Rikimaru, K.; Yanagisawa, A.; Kan, T.; Fukuyama, T. *Synlett* **2004**, 41-44.

(199) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747-6756.

(200) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 487-91.

(201) Jeremic, T.; Linden, A.; Heimgartner, K. M. H. *Tetrahedron* **2005**, *61*, 1871-1883.

(202) Toniolo, C.; Crisma, M.; Formaggio, F.; Polese, A.; Doi, M.; Ishida, T.; Mossel, E.; Broxterman, Q.; Kamphuis, J. *Biopolymers* **1996**, *40*, 523-7.

(203) Jimenez, A. I.; Cativiela, C.; Paris, M.; Peregrina, J. M.; Avenoza, A.; Aubry, A.; Marraud, M. *Tetrahedron Lett.* **1998**, *39*, 7841-7844.

(204) Gomez-Catalan, J.; Jimenez, A. I.; Cativiela, C.; Perez, J. J. *J. Pept. Res.* **2001**, *57*, 435-446.

(205) Lasa, M.; Lopez, P.; Cativiela, C. *Tetrahedron: Asymmetry* **2005**, *16*, 4022-4033.

(206) Alias, M.; Lasa, M.; Lopez, P.; Garcia, J. I.; Cativiela, C. *Tetrahedron* **2005**, *61*, 2913-2919.

(207) Avenoza, A.; Busto, J. H.; Cativiela, C.; Peregrina, J. M.; Rodriguez, F. *Tetrahedron Lett.* **2002**, *43*, 1429-1432.

(208) Wysong, C. L.; Yokum, T. S.; Morales, G. A.; Gundry, R. L.; McLaughlin, M. L.; Hammer, R. P. *J. Org. Chem.* **1996**, *61*, 7650-7651.

(209) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401-2.

(210) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397-8.

(211) Carpino, L. A.; Chao, H. G.; Beyermann, M.; Bienert, M. *J. Org. Chem.* **1991**, *56*, 2635-2642.

(212) Carlson, C. B.; Beal, P. A. *Org. Lett.* **2000**, *2*, 1465-1468.

APPENDIX A

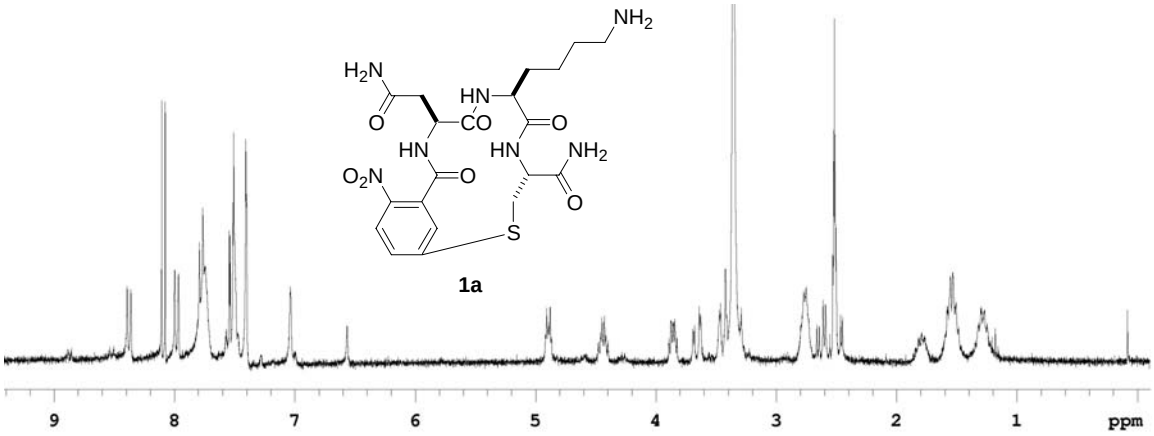
EXPERIMENTAL FOR CHAPTER II

General Methods. All α-amino acids used had the L-configuration. All chemicals were obtained from commercial suppliers and used without further purification. Diisopropylcarbodiimide (DIC), *N*-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA), oxalyl chloride, piperidine and triisopropylsilane (TIS) were purchased from Aldrich. Dimethylformamide (DMF), methanol (MeOH) and dichloromethane (DCM) were bought from EMScience. 5-Fluoro-2-nitrobenzoyl chloride was made by reacting 5-fluoro-2-nitrobenzoic acid (from Fluorochem USA) with oxalyl chloride for 30 min - 1 h. 2-Fluoro-5-nitrophenylacetic acid was obtained by nitration of 2-fluorophenylacetic acid (from Aldrich). Rink Amide MBHA resin was purchased from NovaBiochem while Tenta Gel S Ram Fmoc resin was obtained from Advanced ChemTech. All amino acids used were purchased from either NovaBiochem, Advanced ChemTech or Chem-Impex.

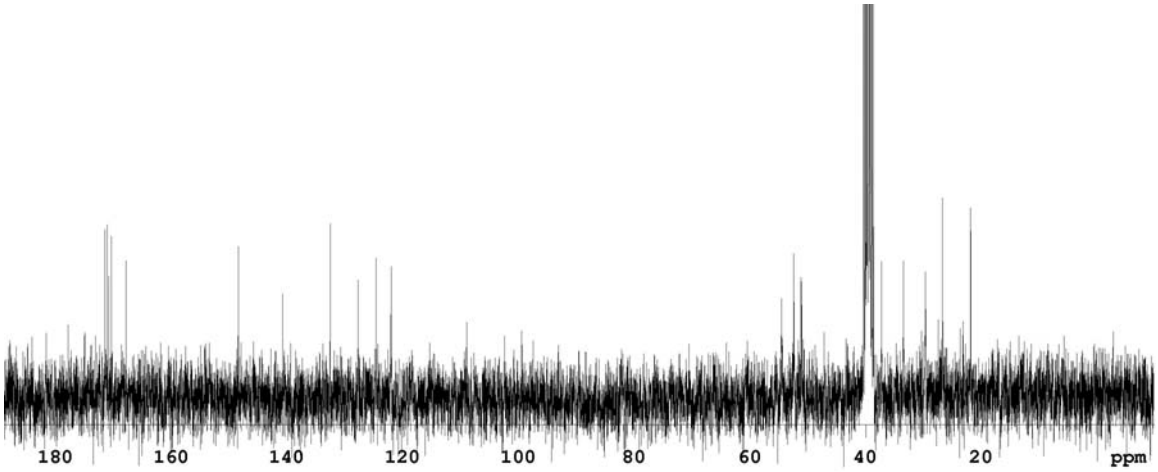
All peptidomimetic syntheses were done in a fritted polypropylene syringe (5 mL capacity) purchased from Torviq. Reverse-phase high-performance liquid chromatography (HPLC) was carried out on Vydac C-18 columns with the following dimensions: 25 x 0.46 cm for analysis and 25 x 2.2 cm for preparative work). All HPLC analyses were done using gradient conditions. Eluents used were solvents A (H_2O with 0.1% TFA) and B (CH_3CN with 0.1% TFA). Flow rates applied were 1.0 $mLmin^{-1}$ and 6.0 $mLmin^{-1}$ for analytical and preparative HPLC respectively.

General experimental procedure for the preparation of peptidomimetics: Synthesis of compound 1a. Rink Amide MBHA resin (0.1 mmol, 0.5 $mmolg^{-1}$) was swelled in DMF (*ca*10 mLg^{-1}) in a fritted syringe for 1 h. The resin was then washed with DMF (3 x at *ca* 10 mLg^{-1}, each time for 1 min and all other washings throughout, except otherwise indicated). The Fmoc protecting group on the Rink handle was removed by treating the resin with 20 % piperidine in DMF (2 x, first for 5 min, then for 25 min). The resin was washed with DMF (3x), MeOH (3x) and DCM (3x). This Fmoc removal and washings thereafter are the same throughout. Then, Fmoc-Cys(Mmt)-OH (3

eq), DIC (5 eq) and HOBt (5 eq) dissolved in DMF (1.5 - 2 mL) were added. After gentle shaking for about 4-6 h, a ninhydrin test on sample resin beads gave a negative result. The reaction mixture was then drained and the resin washed with DMF (4 x). The above deprotection, coupling and washing cycles were repeated to attach Fmoc-Lys(Boc)-OH and Fmoc-Asn(Trt)-OH, respectively. After final Fmoc deprotection and washing, the resin was washed with CH_2Cl_2 (3x) and then coupled with 5-fluoro-2-nitrobenzoyl chloride in CH_2Cl_2 and DIEA. After 2h, sample beads were tested ninhydrin negative and the resin washed with CH_2Cl_2 (5x). Removal of the Cys side-chain protecting group (Mmt, 4-methoxytrityl) was carried out using 1 % TFA and 5 % TIS in CH_2Cl_2 (6 x, each time for 10 min). The resin was then washed with CH_2Cl_2 (4 x) and was dried for 1 h prior to cyclization. Cyclization was effected by adding K_2CO_3 (5 eq) in DMF at 25°C and gently shaking the mixture for ca 24-30 h. The reaction mixture was then drained, the resin was washed with H_2O (5x), DMF (3x), MeOH (3x) and CH_2Cl_2 (3x), and then dried in *vacuo* for 6 h. The peptide was cleaved by treating the resin with a mixture of 90 % TFA, 5 % TIS and 5 % H_2O. The cleavage solution was then separated from the resin by filtration. The resin was given a final wash with CH_2Cl_2 (3x). Most of the cleavage cocktail (*ca* 90 %) was evaporated in *vacuo* after which, precipitation of the peptide was achieved via trituration with anhydrous diethyl ether. The crude peptide was then dissolved in DMF (<2 mL), purified via preparative HPLC (Rainin System, 18-20% B in 20 min) and then lyophilized to give a yellow solid (14.0 mg, 27.5 %). Analytical HPLC: homogeneous single peak, t_R = 8.3 min (8 - 70 % B in 30 min). ^{1}H NMR (500 MHz, DMSO-d_6, 25°C): δ= 8.37 (d, J= 9.0 Hz, 1H), 8.01 (d, J= 9.0 Hz, 1H), 7.97 (d, J= 9.0 Hz, 1H), 7.78 (d, J= 8.5 Hz, 1H), 7.70 (bs, 1H), 7.53 - 7.49 (unresolved, 3H), 7.51 (s, 1H), 7.49 (s, 1H), 7.40 (unresolved, 2H), 7.02 (s, 1H), 4.90 - 4.87 (m, 1H), 4.44 - 4.42 (m, 1H), 3.85 - 3.84 (m, 1 H), 3.67 - 3.42 (m, 2H), 2.77 - 2.76 (m, 2H), 2.61 - 2.47 (m, 2 H), 1.80 - 1.77 (m, 1H), 1.54 - 1.51 (m, 3H), 1.31 - 1.24 (m, 2 H). ^{13}C NMR (DMSO-d_6, 75 MHz, 25°C): δ=171.5, 171.1, 170.8, 170.4, 167.9, 148.5, 140.8, 132.6, 127.7, 124.6, 122.1, 54.5, 52.4, 51.1, 38.6 (overlap with DMSO), 37.2, 22.4, 29.7, 26.7, 21.8. MALDI MS: calcd for $C_{20}H_{27}N_7O_7S$ $(M + H)^+$ 510.5, found 510.1.

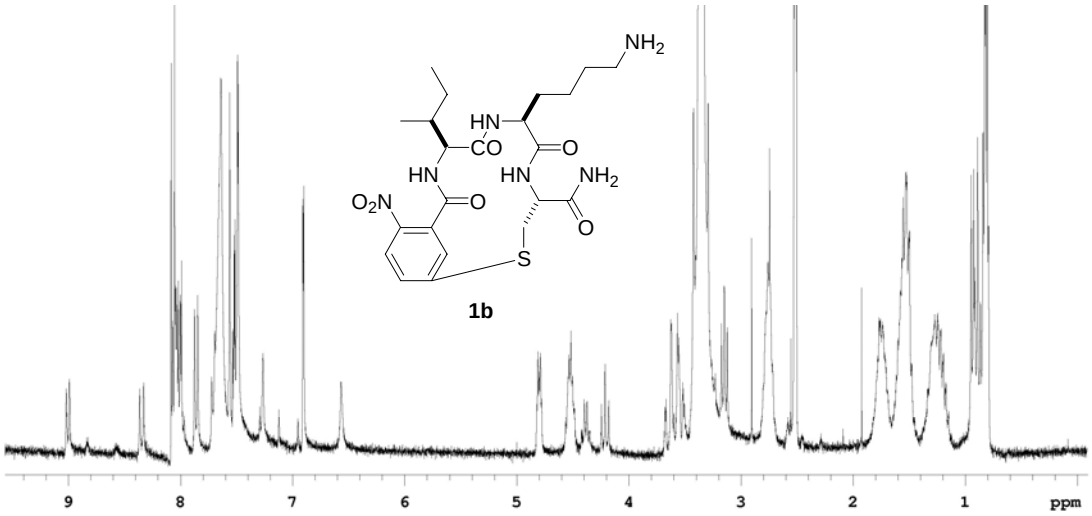


^{1}H NMR of compound **1a**



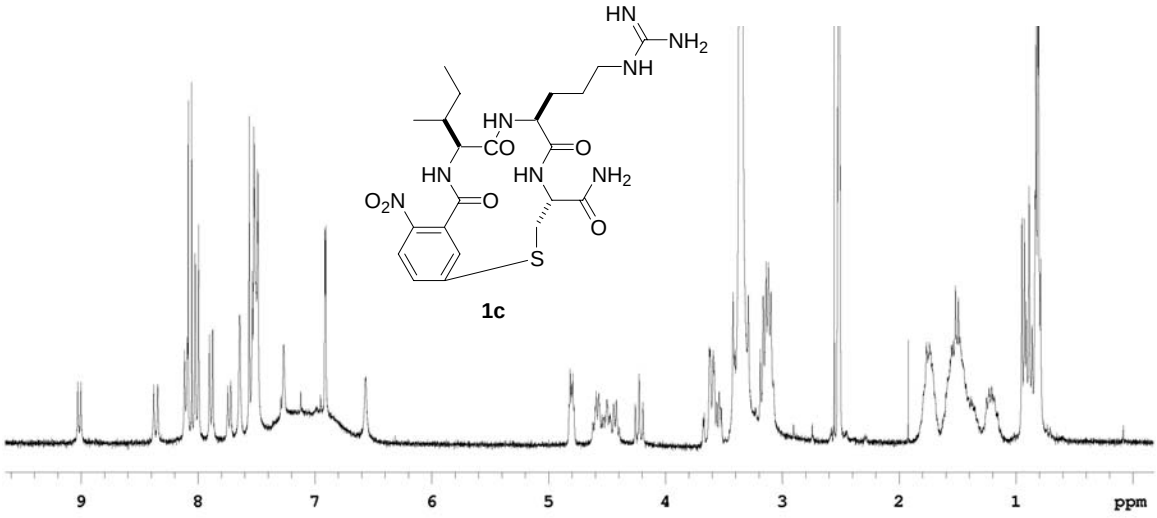
^{13}C NMR of compound **1a**

Compound 1b. Rink Amide MBHA (0.05 mmol, 0.54 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocLys(Boc)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (4.4 mg, 17.3 %). Analytical HPLC: homogeneous single peak, t_R = 14.5 min (8 - 70 % B in 30 min). ^{1}H NMR (300MHz, DMSO-d_6, 25°C): δ=8.99 (d, J=8.4 Hz, 1H), 8.33 (d, J=10.5 Hz, 1H), 8.07–7.98 (m, 3H), 7.85 (d, J=8.4 Hz, 1H), 7.70 (d, J=8.7 Hz, 1H), 7.62 (s, 3H), 6.89 (d, J=2.1 Hz, 1H), 4.80–4.77 (m, 1H), 4.52–4.50 (m, 1H), 3.65–3.51 (m, 2H) 3.16–3.11 (m, 1H), 2.74–2.72 (m, 2H), 1.74–1.72 (m, 2H), 1.54–1.48 (m, 4H), 1.26–1.16 (m, 3H), 0.931–0.775 (m, 6H). MALDI MS: calcd for $C_{22}H_{32}N_6O_6S$ $(M + H)^+$ 509.6, found 509.2.



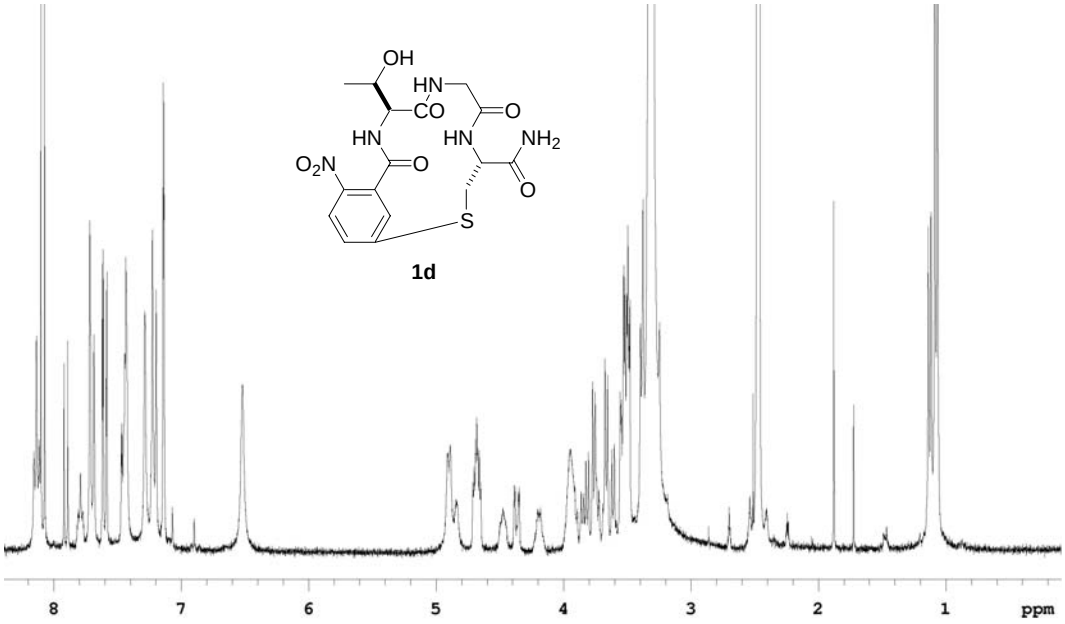
^{1}H NMR of compound **1b**

Compound 1c. Rink Amide MBHA (0.05 mmol, 0.54 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocArg(Pmc)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (5.6 mg, 20.8 %). analytical HPLC: homogeneous single peak, t_R = 15.9 min (8 - 70 % B in 30 min). ^{1}H NMR (300 MHz, DMSO-d_6, 25°C): δ= 9.00 (d, J=8.4 Hz, 1H), 8.35 (d, J=10.2 Hz, 1H)8.10–7.98 (m, 3H), 7.87 (d, J=8.7 Hz, 1H), 7.63 (s, 1H), 7.55–7.47 (m, 5H), 6.89 (d, J=2.4 Hz, 2H), 4.79–4.78 (m, 1H), 4.58–4.40 (m, 2H) 4.28–4.18 (m, 1H) 3.61–3.51 (m, 2H), 1.75–1.73 (m, 2H), 1.52–1.48 (m, 4H), 1.22–1.19 (m, 1H), 0.934–0.774 (m, 6H). MALDI MS: calcd for $C_{22}H_{32}N_8O_6S$ $(M + H)^+$ 537.6, found 537.2.



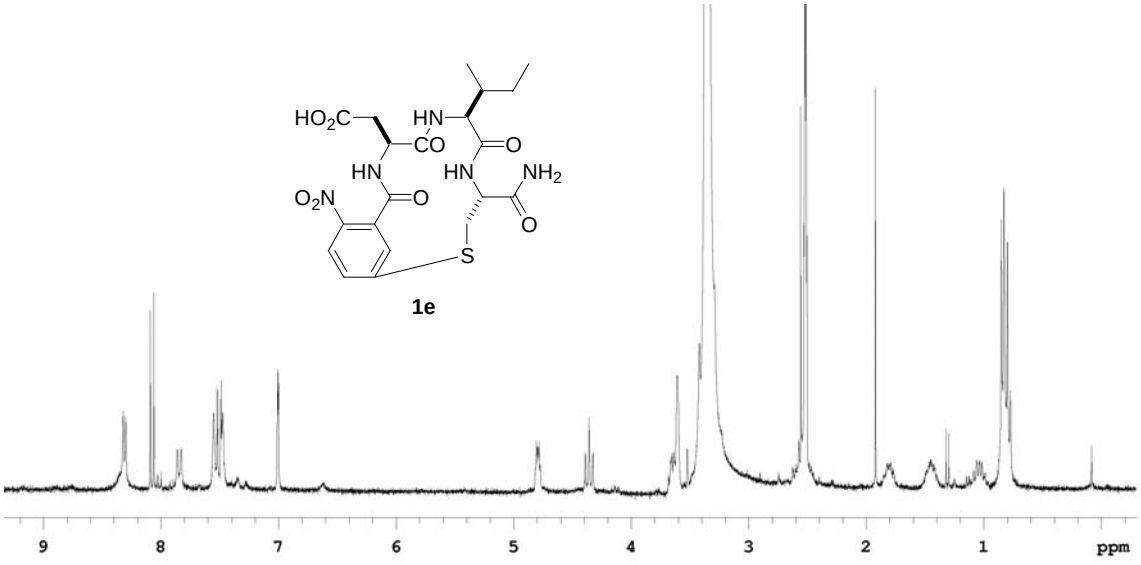
^{1}H NMR of compound **1c**

Compound 1d. Rink Amide MBHA (0.05 mmol, 0.54 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocGly-OH and FmocThr(tBu)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (5.6 mg, 26.3 %). Analytical HPLC: homogeneous single peak, t_R = 8.1 min (8 - 70 % B in 30 min). ^{1}H NMR (300 MHz, DMSO-d_6, 25°C): δ=8.15–8.09 (m, 2H), 7.72 (d, J=9.9 Hz, 1H), 7.63 (dd, J=1.8, 2.1 Hz, 1H), 7.45 (s, 1H), 7.30 (s, 1H), 7.23 (d, J=9 Hz, 1H), 7.16 (d, J=2.1 Hz, 1H), 6.53 (s, 1H), 4.91 (bs, 1H), 4.71 (bs, 1H), 4.97 (bs, 1H), 3.79, (d, J=6.3 Hz, 1H), 3.69 (d, J=5.7 Hz, 1H), 3.55–3.50 (m, 2H), 1.16–1.09 (m, 3H). MALDI MS: calcd for $C_{16}H_{19}N_5O_7S$ $(M + H)^+$ 426.4, found 426.1.



^{1}H NMR of compound **1d**

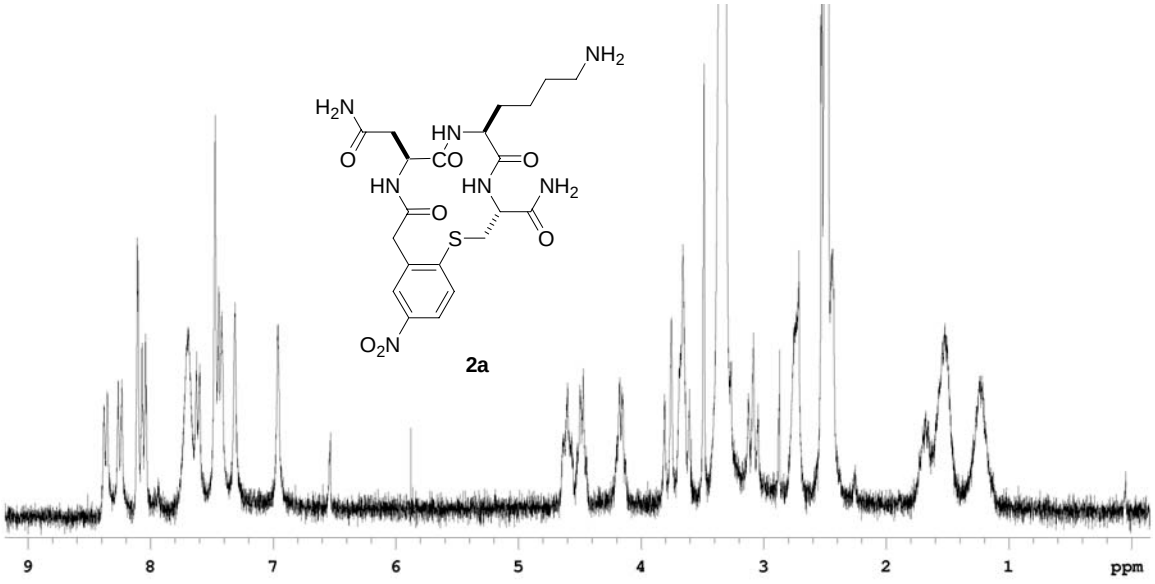
Compound 1e. Rink Amide MBHA (0.05 mmol, 0.54 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocIle-OH and FmocAsp(tBu)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (2.5 mg, 10.1 %). Analytical HPLC: homogeneous single peak, t_R = 13.0 min (8 - 70 % B in 30 min). ^{1}H NMR (300 MHz, DMSO-d_6, 25°C): δ=8.29 (d, J=6.6 Hz, 2H), 8.05 (d, J=9 Hz, 1H), 7.82 (d, J=1H), 7.53 (s, 1H), 7.48 (dd, J=2.4, 2.4 Hz, 1H), 7.45 (s, 1H) 6.98 (d, J=2.1 Hz, 1H), 4.78–4.76 (m, 1H), 4.34 (t, J=9.6 Hz, 1H), 3.64–3.59 (m, 3H), 1.77 (bs, 1H), 1.43 (bs, 2H), 1.04–0.999 (m, 2H), 0.834–0.757 (m, 6H). MALDI MS: calcd for $C_{20}H_{25}N_5O_8S$ $(M + H)^+$496.5, found 496.1.



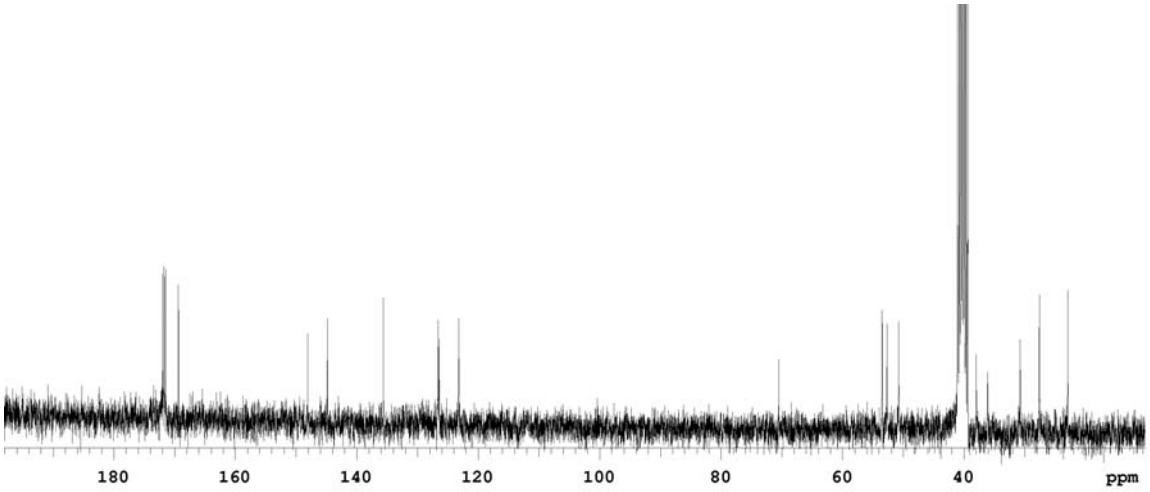
^{1}H NMR of compound **1e**

Solid-phase synthesis of Compounds 2a-e. The same procedure as in the synthesis of **2a-e** was followed except for the following variations. The resin used was Tenta Gel S RAM Fmoc (0.05 mmol, 0.26 mmolg^{-1}) and the tripeptide was coupled to 2-fluoro-5-nitrophenylacetic acid using DIC and HOBt.

Compound 2a. Tenta Gel S RAM Fmoc resin (0.05 mmol, 0.26 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocLys(Boc)-OH and FmocAsn(Trt))-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (15 mg, 57 %). Analytical HPLC: homogeneous single peak, t_R=9.5 min (8–70% B in 30 min). ^{1}H NMR (500 MHz, DMSO-d_6, 25°C): δ=8.10 (s, 1H), 8.06 (dd, J=2.5, 8.5 Hz, 1H), 7.71 (s, 3H), 7.62 (d, J=8 Hz, 1H), 7.47 (unresolved, 1H), 7.46 (s, 1H), 7.41 (s, 1H), 7.36 (d, J=8 Hz, 1H), 7.28 (bs, 1H), 7.20 (d, J=9 Hz, 1H), 6.94 (s, 1H), 4.62–4.57 (m, 1H), 4.51–4.46 (m,1H), 4.19–4.15 (m, 1H), 3.67–3.10 (m, 2H), 3.65–3.79 (m, 2H), 2.75–2.72 (m, 2H), 2.61–2.47 (m, 2H), 1.69–1.56 (m, 2H), 1.57–1.50 (m, 2H), 1.26–1.21 (m, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz, 25°C): δ=171.3, 171.0, 170.9, 170.7, 168.7, 147.4, 144.1, 134.9, 125.9, 125.7, 122.5, 69.8, 52.8, 52.0, 50.0, 40.4, 38.8, 37.3, 30.0, 26.9, 22.2. MALDI MS: calcd for $C_{21}H_{29}N_7O_7S$ $(M + H)^+$ 524.6, found 524.2.



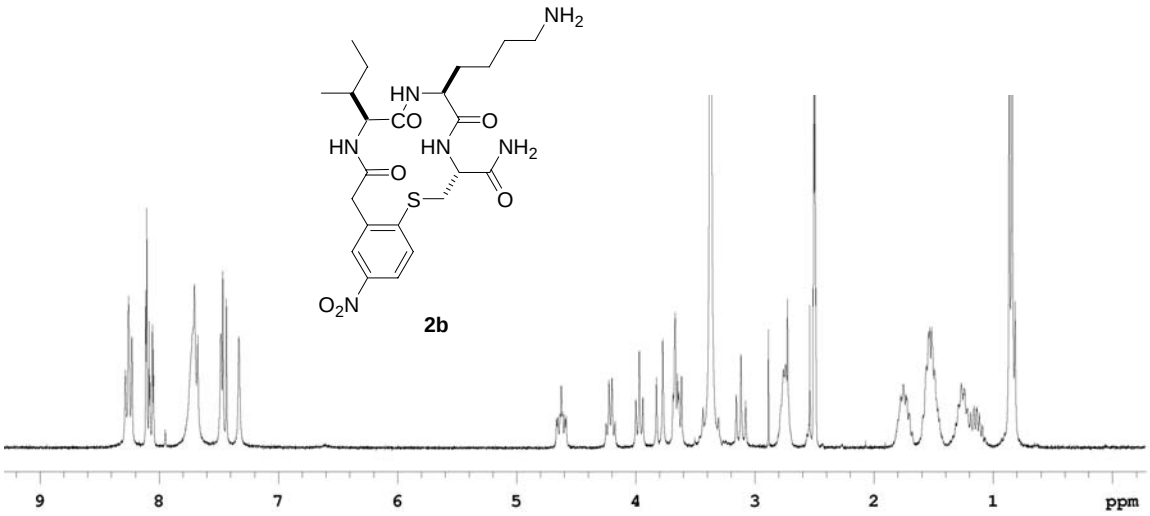
^{1}H NMR of compound **2a**



^{13}C NMR of compound **2a**

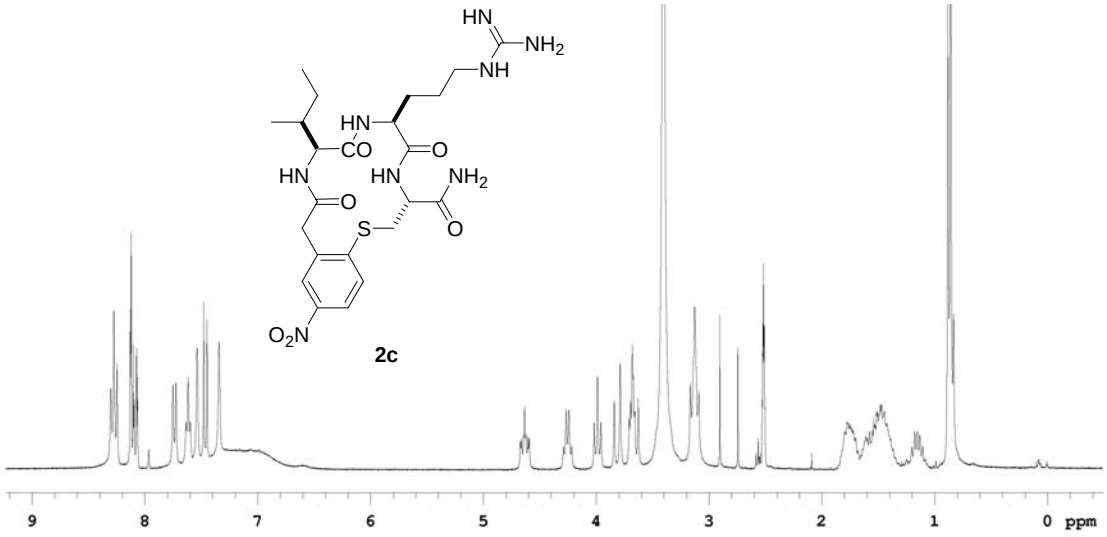
Compound 2b. Tenta Gel S RAM Fmoc resin (0.05 mmol, 0.26 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocLys(Boc)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a light yellow solid (13.1 mg, 50 %). Analytical HPLC: homogeneous single peak, t_R = 13.7 min (8 - 70 % B in 30 min). ^{1}H NMR (DMSO-d_6, 300 MHz, , 25°C): δ = 8.28 - 8.23 (m, 2H), 8.107 (d, J = 2.4 Hz, 1H), 8.07 (dd, J= 2.4 Hz, 1H), 7.70 - 7.68 (m, 4H), 7.48 - 7.44 (m, 2H), 7.33 (s, 1H), 4.66 - 4.58 (m, 1H), 4.25 - 4.18 (m, 1H), 3.97 (t, J= 9 Hz, 1H),

3.83 - 3.77 (m, 1H), 3.67 - 3.62 (m, 2 H), 3.11 (t, *J*= 11.7 Hz, 1H), 2.76 - 2.72 (m, 2 H), 1.78 - 1.71 (m, 2 H), 1.56 - 1.49 (m, 4 H), 1.29 - 1.12 (m, 3H), 0.865 - 0.815 (m, 6 H). MALDI MS: calcd for $C_{23}H_{34}N_6O_6S$ $(M + H)^+$ 523.6, found 523.2.



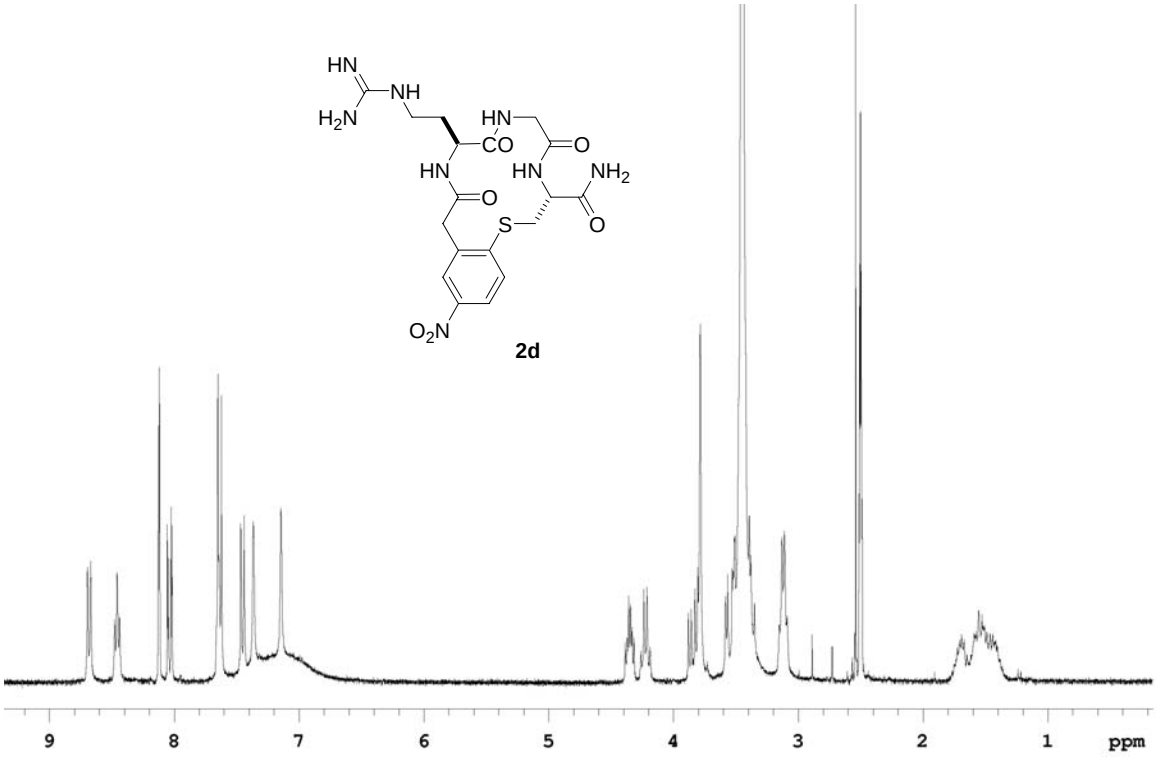
^{1}H NMR of compound **2b**

Compound 2c. Tenta Gel S RAM Fmoc resin (0.05 mmol, 0.26 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocArg(Pmc)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a light yellow solid (10.3 mg, 37 %). analytical HPLC: homogeneous single peak, t_R = 15.5 min (8 - 70 % B in 30 min). ^{1}H NMR DMSO-d_6, 300 MHz, 25°C): δ=8.29–8.23 (m, 2H), 8.11 (d, *J*=2.4 Hz, 1H), 8.07 (dd, *J*=2.4, 2.4 Hz, 1H), 7.72 (d, *J*=8.1 Hz, 1H), 7.62–7.58 (m, 1H), 7.52 (s, 1H), 7.45 (d, *J*=8.7 Hz), 7.33 (s, 1H), 4.66–4.58 (m, 1H), 4.27–4.20 (m, 1H), 4.00–3.94 (m, 1H), 3.82–3.77 (m, 1H) 3.69–3.61 (m, 2H), 3.15–3.07 (m, 3H), 1.76–1.71 (m, 2H), 1.56–1.41 (m, 4H), 1.77–1.13 (m, 1H). MALDI MS: calcd for $C_{23}H_{34}N_8O_6S$ $(M + H)^+$ 551.6, found 521.2.



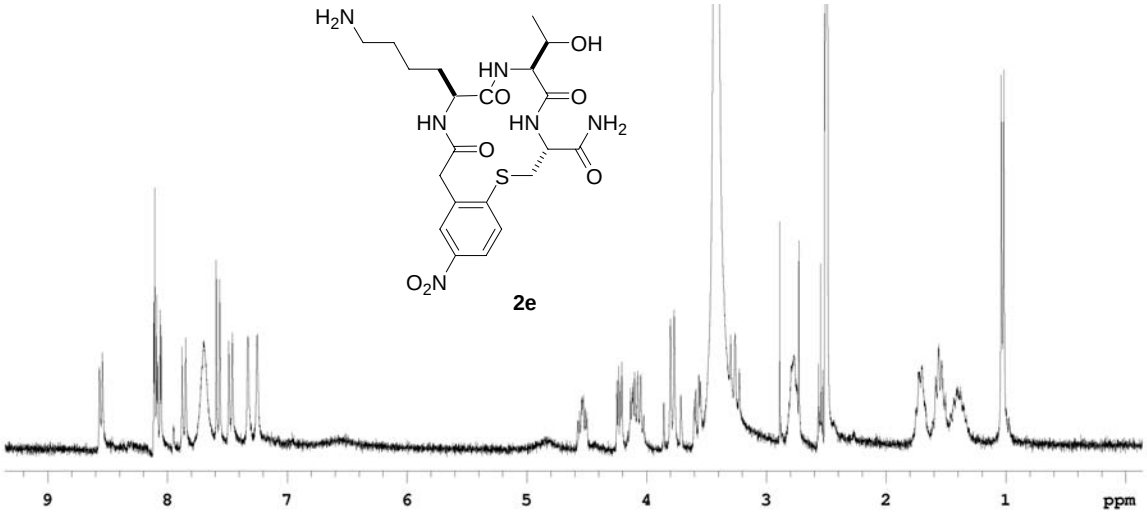
^{1}H NMR of compound **2c**

Compound 2d. Tenta Gel S RAM Fmoc resin (0.05 mmol, 0.26 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocGly-OH and FmocArg(Pmc)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a yellow solid (9.5 mg, 40 %). Analytical HPLC: homogeneous single peak, t_R = 10.3 min (8 - 70 % B in 30 min). ^{1}H NMR (DMSO-d_6, 300 MHz, , 25°C): δ= 8.68 (d, J= 7.8 Hz, 1H), 8.46 (t, J= 5.7 Hz, 1H), 8.12 (d, J= 2.7 Hz, 1H), 8.04 (dd, J= 2.4 Hz, 1 H), 7.65 - 7.62 (m, 2H), 7.37 (s, 1H), 7.15 (s, 1H), 4.36 - 4.34 (m, 1H), 4.24 - 4.21 (m, 1H), 3.88 - 3.78 (m, 3H), 3.57 (d, J= 5.1 Hz, 1H), 3.53 - 3.35 (m, 7H covered by H_2O signal), 3.13 - 3.09 (m, 2H), 1.71-1.65 (m, 1 H), 1.57 - 1.42 (m, 3H). MALDI MS: calcd for $C_{19}H_{26}N_8O_6S$ $(M + H)^+$ 495.1, found 495.0.



^{1}H NMR of compound **2d**

Compound 2e. Tenta Gel S RAM Fmoc resin (0.05 mmol, 0.26 mmolg^{-1}) was used to synthesize this compound. The second and third amino acids attached were FmocThr(tBu)-OH and FmocLys(Boc)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (15.1 mg, 59 %). Analytical HPLC: homogeneous single peak, t_R = 9.3 min (8 - 70 % B in 30 min). ^{1}H NMR (DMSO-d_6, 300 MHz, 25°C): δ= 8.56 - 8.54 (m, 1H), 8.11 (d, J= 2.4 Hz, 1H), 8.07 (dd, J= 6.3 Hz, 1H), 7.86 (d, J= 8.7 Hz, 1H) 7.7 (bs, 3H), 7.6 (d, J= 8.4 Hz, 1H), 7.5 (d, J= 9 Hz, 1 H), 7.33 (unresolved, 1H), 7.25 (unresolved, 1H), 4.54 - 4.51 (m, 1H), 4.25 - 4.20 (m, 1H), 4.13 - 4.05 (m, 2H), 3.85 - 3.71 (m, 2H), 3.60 - 3.55 (m, 1 H), 2.77 (unresolved, 3H), 2.57 - 2.53 (m, 1H), 1.73-1.70 (m, 2H), 1.59-1.50 (m, 2H), 1.41 (unresolved, 2H), 1.04 - 1.01 (m, 3H). MALDI MS: calcd for$C_{20}H_{27}N_7O_7S$ (M + H)$^+$ 1 510.5, found 510.1.



^{1}H NMR of compound **2e**

APPENDIX B

EXPERIMENTAL FOR CHAPTER III

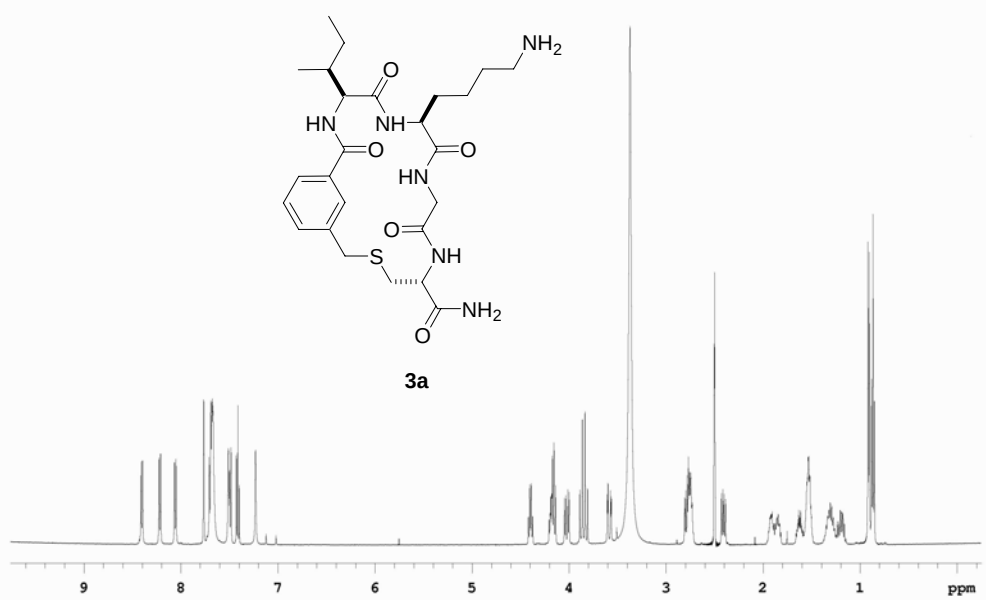
General Methods. All α-amino acids used have the L-configuration and were purchased from NovaBiochem, Advanced ChemTech or Chem-Impex. Chemicals were obtained from commercial suppliers and used without further purification. Diisopropylcabodiimide (DIC), *N*-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA), oxalyl chloride, diisopropylethylamine (DIEA), piperidine and triisopropylsilane (TIS) were purchased from Aldrich. Dimethylformamide (DMF), methanol and dichloromethane were bought from EMScience. The resins were obtained from Rapp Polymere, Advanced ChemTech, Nova Biochem, Argonaut Technologies and Chiron Mimetopes.

Peptidomimetic syntheses were performed in fritted polypropylene syringes (5 mL capacity) from Torviq. Reverse-phase high-performance liquid chromatography (HPLC) analyses were carried out using a Beckman SystemGold instrument and a Vydac C-18 column (catalog no. 218TP54, length: 250 mm; inner diameter: 4.6 mm). Preparative purifications were done on an SSI HPLC system using a Vydac C-18 column (catalog no. 2181022, length: 250 mm, inner diameter: 22 mm). All HPLC analyses were done under gradient conditions. Eluents used were solvent A (H_2O with 0. 1 % TFA) and solvent B (CH_3CN with 0.1% TFA). Flow rates applied were 1.0 and 6 mL/min for analytical and preparative HPLC respectively.

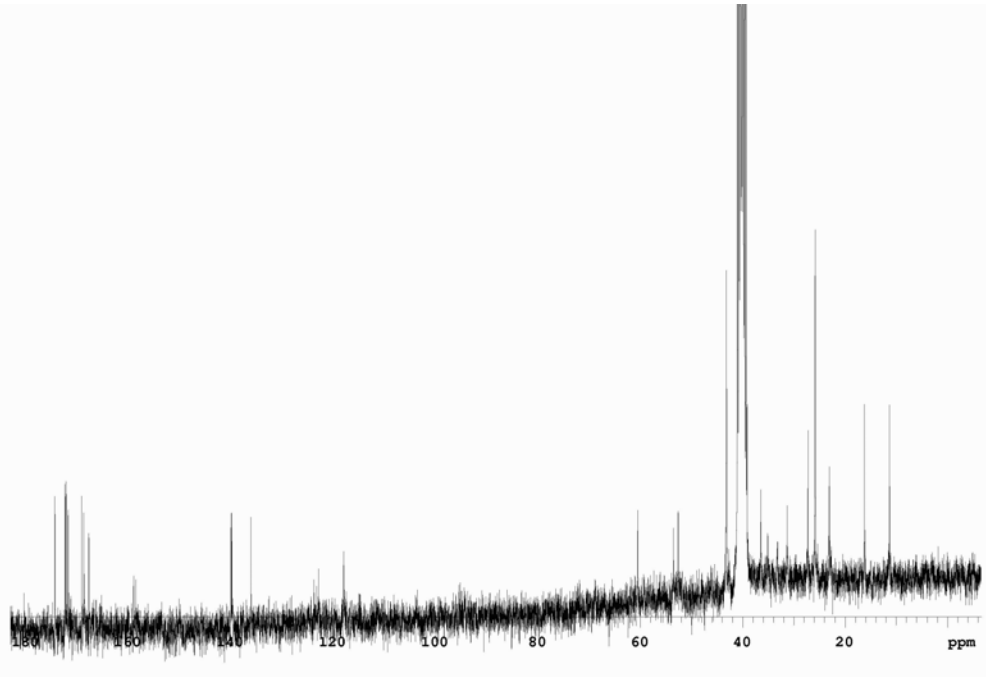
General experimental procedure for the preparation of peptidomimetics: Synthesis of compound 3a. In a 5 mL fritted syringe, TentaGel S RAM Fmoc (0.05 mmol, 0.30 mmol g^{-1}) was swelled in DMF (*ca* 10 mL g^{-1}) for 1 h. The resin was washed with DMF (3 x *ca* 10 mL g^{-1}, each time for 1 min; this amount of solvent and washing time were used for all other washings throughout). The Fmoc protecting group was removed by treating the resin with 20 % piperidine in DMF (2x, first 10 min, then for 20 min). The resin was washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x), after which a solution of FmocCys(Mmt)-OH (3 eq), DIC (5 eq) and HOBt (5 eq) in 1.5 mL DMF was

added. After shaking for 10 h, a Kaiser test[27] on sample beads indicated a negative result. The reaction mixture was drained and the resin washed with DMF (3x). The above deprotection, coupling and washing cycles were repeated to attach FmocGly-OH, FmocLys-OH and FmocIle-OH. After the final Fmoc deprotection, the resin was washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x). The resin was dried for 2 h in vacuo, then a solution of 3-bromomethylbenzoylchloride (3 eq) and DIEA (5 eq) in 2 mL CH_2Cl_2 were added. After 1 h of shaking, a Kaiser test gave a negative result. The resin was washed with CH_2Cl_2 (5x). The Mmt group on cysteine was removed by treating the resin with 1% TFA and 5 % TIS in CH_2Cl_2 (6x, each time for 10 min). The resin was then washed with CH_2Cl_2 (4x) and DMF (3x). Cyclization was carried out by adding 8 eq of DIEA in *ca* 2.0 - 2.5 mL DMF and shaking the resin for 12 h. The resin was then washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x) before it was dried for 2 h in vacuo. The product was cleaved by treating the resin with 90 % TFA, 5 % TIS and 5 % H_2O (*ca* 3.5 – 4 mL) for 2 – 4 h. The resin was then washed with 50 % TFA in CH_2Cl_2 (2x). The collected filtrate and washings were concentrated and the product was precipitated by adding Et_2O (*ca* 3 mL). The white precipitate was isolated by centrifugation of the mixture and carefully decanting the ether. The residue was washed with Et_2O two more times. The crude peptide was then dissolved in the minimum volume of H_2O with a little CH_3CN added (total volume <2.5 mL) and was purified via preparative HPLC (SSI System, 20 – 30 % B in 25 min). The dominant fraction was concentrated and lyophilized to give a white fluffy solid (7.2 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 8.41 (d, J = 7.94 Hz, 1H), 8.23 (d, J = 8.23 Hz, 1H), 8.07 (d, J = 8.30 Hz, 1H), 7.77 (s, 1H), 7.72-7.67 (m, 4H), 7.52-7.49 (m, 2H), 7.42 (t, J = 7.65 Hz, 1H), 7.24 (s, 1H), 4.41 (q, J = 7.46 Hz, 1H), 4.22-4.15 (m, 2H), 4.03 (dd, J = 7.22, 16.85 Hz, 1H), 3.90-3.82 (m, 2H), 3.59 (dd, J = 3.94, 16.85 Hz, 1H), 2.82-2.73 (m, 3H), 2.42 (dd, J = 7.44, 13.84 Hz, 1H), 1.96-1.90 (m, 1H), 1.88-1.83 (m, 1H), 1.67-1.60 (m, 1H), 1.58-1.51 (m, 3H), 1.37-1.26 (m, 2H), 1.25-1.16 (m, 1H), 0.92 (d, J = 6.80 Hz, 3H), 0.88 (t, J = 7.59 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 173.6, 171.6, 171.4, 171.1, 168.4, 168.0, 167.0, 158.3, 157.8, 139.2, 139.1, 135.4, 122.1, 117.2, 59.8, 52.8, 51.9, 42.5, 35.8, 30.6, 26.6, 25.2, 22.4, 15.6, 10.7; analytical HPLC: homogeneous single peak,

t_R=13.05 min (8-70% B in 30 min); MALDI MS: calcd for $C_{25}H_{38}N_6O_5S$ $(M + H)^+$ 535.67, found 535.20.

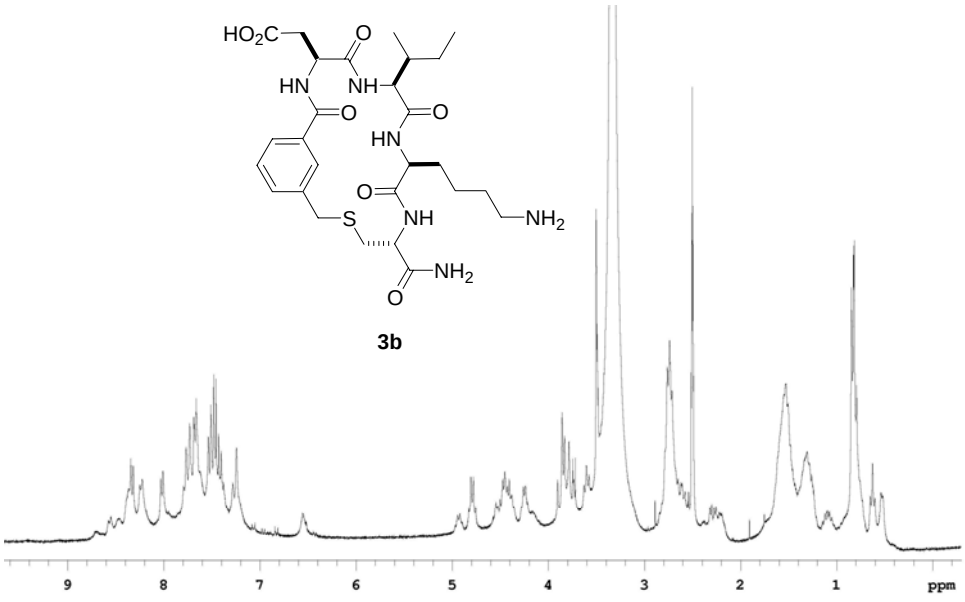


^{1}H NMR of compound **3a**



^{13}C NMR of compound **3a**

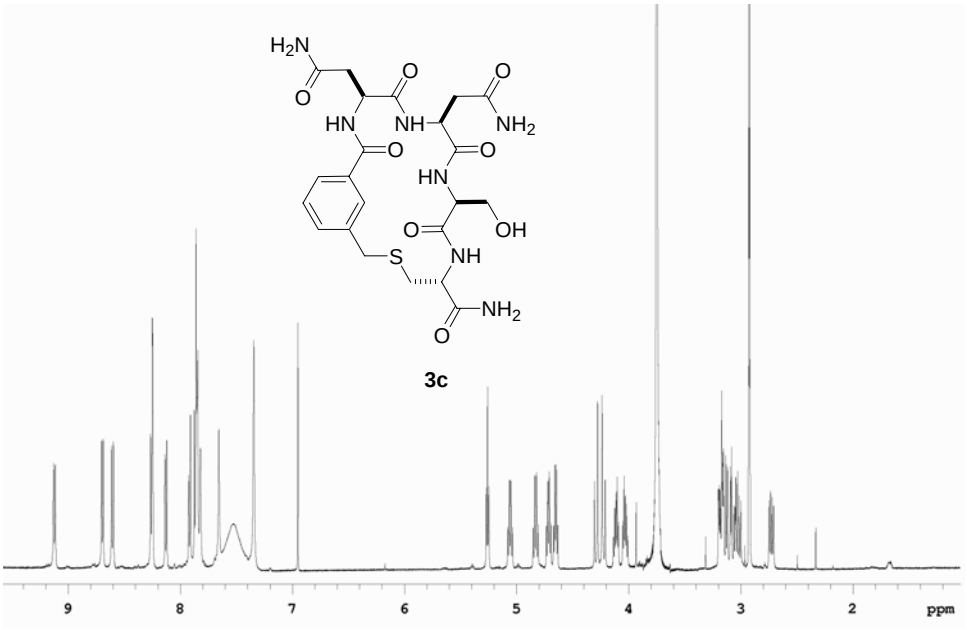
Compound 3b. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocLys(Boc)-OH, FmocIle-OH and FmocAsp(tBu)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white fluffy solid (13.2 mg, 45 %). ^{1}H NMR (300 MHz, DMSO-*d*$_6$, 25 °C): δ = 8.34 (d, *J* = 7.71 Hz, 1H), 8.25 (d, *J* = 7.71 Hz, 1H), 8.03 (d, *J* = 6.73 Hz, 1H), 7.80-7.74 (m, 2H), 7.70-7.63 (m, 2H), 7.55-7.41 (m, 3H), 7.29 (s, 1H), 7.26 (s, 1H), 4.98-4.90 (m, 1H), 4.81 (dd, *J* = 7.09, 15.05 Hz, 1H), 4.56-4.37 (m, 3H), 4.29-4.21 (m, 1H), 3.91-3.75 (m, 4H), 3.64-3.59 (m, 1H), 2.80-2.69 (m, 3H), 2.68-2.59 (m, 1H), 2.30 (dd, *J* = 6.04, 13.10 Hz, 1H), 1.63-1.46 (m, 2H), 1.41-1.23 (m, 2H), 1.17-1.03 (m, 1H), 0.85 (d, *J* = 6.88 Hz, 3H), 0.35 (d, *J* = 7.13 Hz, 3H); analytical HPLC: homogeneous single peak, t_R=10.48 min (8-70% B in 30 min); MALDI MS: calcd for $C_{27}H_{40}N_6O_5S$ (M + H)$^+$ 593.71, found 593.19.



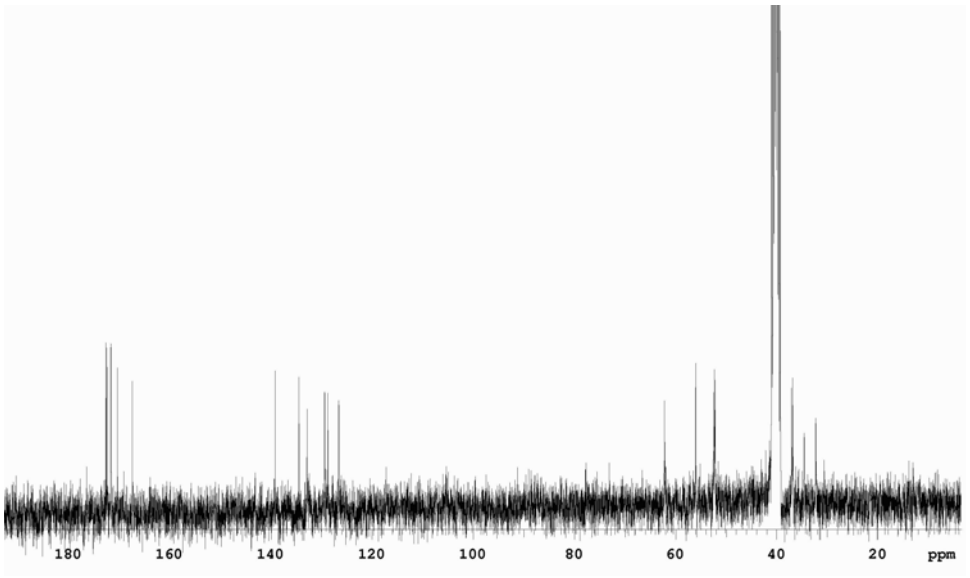
^{1}H NMR of compound **3b**

Compound 3c. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocSer(tBu)-OH, FmocAsn(Trt)-OH and FmocAsn(Trt)-OH, respectively. After

cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (4.3 mg, 16 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 8.71 (d, J = 7.83 Hz, 1H), 8.28 (d, J = 7.26 Hz, 1H), 8.19 (d, J = 8.37 Hz, 1H), 7.85-7.83 (m, 2H), 7.72-7.70 (m, 1H), 7.49-7.51 (m, 1H), 7.46-7.43 (m, 3H), 7.41 (s, 1H), 7.24 (s, 1H), 6.93 (s, 2H), 4.85 (t, J = 5.86 Hz, 1H), 4.66-4.62 (m, 1H), 4.42 (q, J = 7.25 Hz, 1H), 4.32-4.28 (m, 1H), 4.26-4.22 (m, 1H), 3.89-3.79 (m, 2H), 3.72-3.68 (m,1H), 3.64-3.59 (m, 1H), 2.79-2.74 (m, 2H), 2.72-2.69 (m, 2H), 2.65-2.58 (m, 1H) 2.31 (dd, J = 2.31, 13.80 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 172.6, 172.5, 172.3, 171.7, 171.6, 170.4, 167.4, 139.2, 134.5, 132.9, 129.4, 128.7, 126.6, 62.2, 56.0, 52.5, 52.3, 52.2, 37.1, 36.9, 34.6, 32.3; analytical HPLC: homogeneous single peak, t_R=7.46 min (8-70% B in 30 min); MALDI MS: calcd for $C_{22}H_{29}N_7O_8S$ $(M + Na)^+$ 574.57, found 574.11.

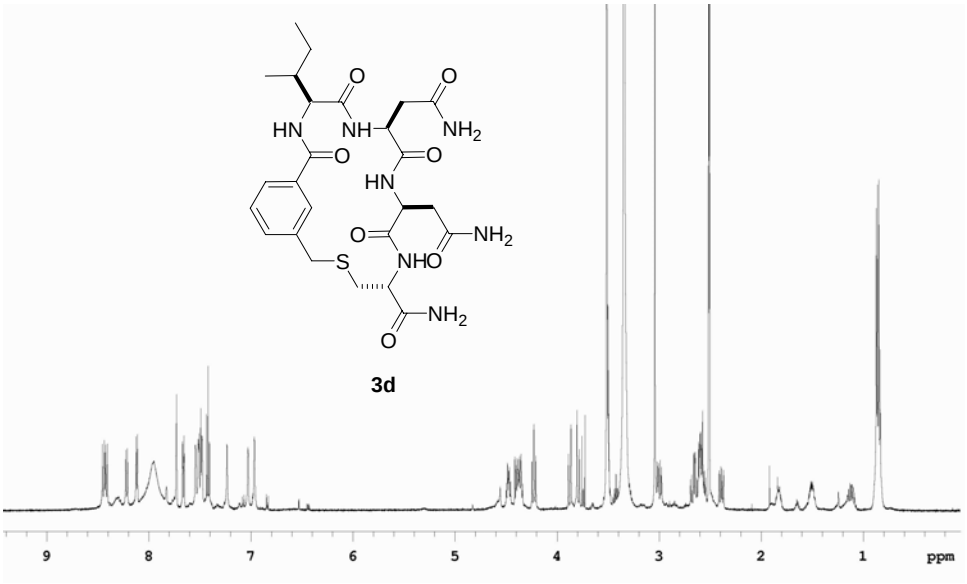


^{1}H NMR of compound **3c**



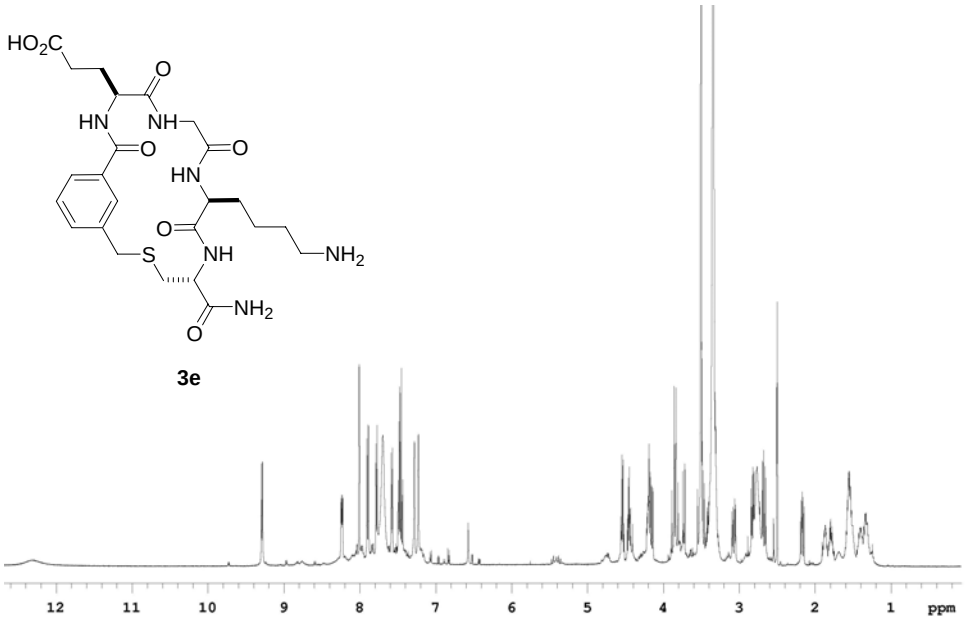
^{13}C NMR of compound **3c**

Compound 3d. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocAsn(Trt)-OH, FmocAsn(Trt)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (8.3 mg, 29 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ= 8.45 (d, J = 9.15 Hz, 1H), 8.41 (d, J = 8.42 Hz, 1H), 8.22 (d, J = 8.65 Hz, 1H), 8.12 (d, J = 6.56 Hz, 1H), 7.73 (s, 1H), 7.67-7.65 (m, 1H), 7.54 (s, 1H), 7.52-7.48 (m, 3H), 7.42 (t, J = 7.55 Hz 1H), 7.23 (s, 1H), 7.03 (s, 1H), 6.97 (s, 1H), 4.50-4.46 (m, 1H), 4.41-4.34 (m, 2H), 4.23 (t, J = 9.75 Hz, 1H), 3.89-3.78 (m, 2H), 3.44-3.41 (m, 1H), 3.0 (dd, J = 6.70, 13.86 Hz, 1H), 2.69-2.63 (m, 1H), 2.61-2.54 (m, 2H), 2.39 (dd, J = 8.51, 13.86 Hz, 1H), 1.86-180 (m, 1H), 1.54-1.47 (m, 1H), 1.16-1.07 (m, 1H), 0.87 (d, J = 5.53 Hz, 3H), 0.85 (t, J = 7.24 Hz, 3H); analytical HPLC: homogeneous single peak, t_R=12.61 min (8-70% B in 30 min); MALDI MS: calcd for $C_{25}H_{35}N_7O_7S$ $(M + Na)^+$ 600.65, found $(M + Na)^+$ 600.30.



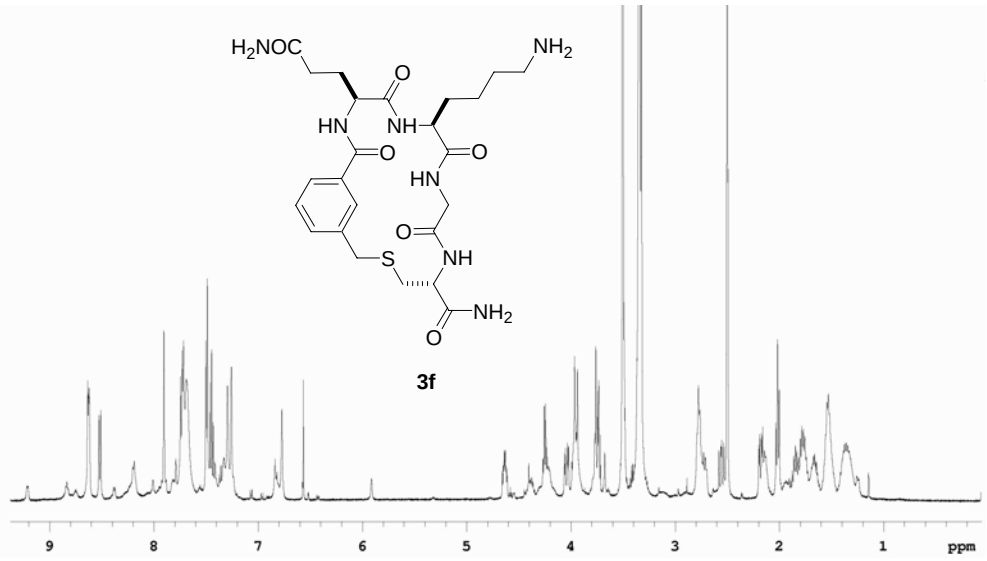
^{1}H NMR of compound **3d**

Compound 3e. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocLys(Boc)-OH, FmocGly-OH and FmocAsp(tBu)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (18.6 mg, 69 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 12.3 (bs, 1H), 9.30 (d, J = 7.64 Hz, 1H), 8.25 (dd, J = 3.86, 8.56 Hz, 1H), 8.01 (s, 1H), 7.91 (d, J = 9.10 Hz, 1H), 7.80-7.78 (m, 1H), 7.71 (bs, 2H), 7.59 (d, J = 7.79 Hz, 1H), 7.50-7.45 (m, 2H), 7.29 (s, 1H), 7.24 (s, 1H), 4.55 (q, J = 6.79 Hz, 1H), 4.49-4.44 (m, 1H), 4.23-4.15 (m, 2H), 3.90-3.82 (m, 2H), 3.75-3.73 (m, 1H), 3.54-3.50 (m, 2H), 3.08 (dd, J = 6.68, 16.21 Hz, 1H), 2.83 (dd, J = 5.28, 12.24 Hz, 1H), 2.68 (dd, J = 6.68, 16.21 Hz, 1H), 2.18 (dd, J = 8.75, 12.24 Hz, 1H), 1.93-1.84 (m, 1H), 1.83-1.77 (m, 1H), 1.60-1.56 (m, 2H), 1.46-1.38 (m, 1H), 1.37-1.34 (m, 1H); analytical HPLC: homogeneous single peak, t_R=9.26 min (8-70% B in 30 min); MALDI MS: calcd for $C_{23}H_{32}N_6O_7S$ $(M + H)^+$ 537.60, found 537.15.



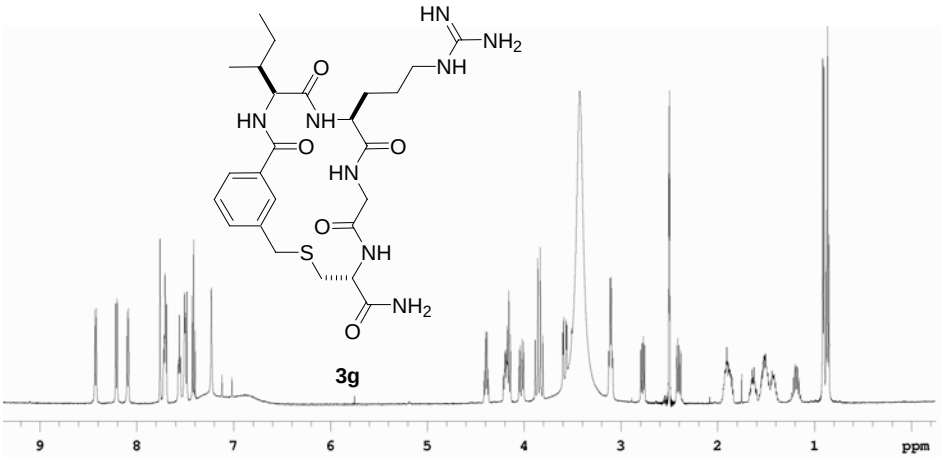
^{1}H NMR of compound **3e**

Compound 3f. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocGln(Trt)-OH, FmocLys(Boc)-OH and FmocGly-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (18.6 mg, 69 %). ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 8.64-8.63 (m, 2H), 8.52 (d, *J* = 8.68 Hz, 1H), 8.20 (d, *J* = 7.32 Hz, 1H), 7.90 (s, 1H), 7.76-7.70 (m, 4H), 7.50 (d, *J* = 7.26 Hz, 1H), 7.45 (t, *J* = 7.71 Hz, 1H), 7.29 (s, 1H), 7.26 (s, 1H), 6.77 (s, 1H), 4.67-4.62 (m, 1H), 4.28-4.24 (m, 2H), 4.04 (dd, *J* = 4.92, 14.50 Hz, 1H), 4.0-3.95 (m, 2H), 3.78-3.73 (m, 2H), 3.44-3.40 (m, 1H), 2.56 (dd, *J* = 9.89, 13.67 Hz, 1H), 2.18 (dd, *J* = 4.85, 13.67 Hz, 1H), 2.03 (t, *J* = 8.34 Hz, 2H), 1.90-1.85 (m, 1H), 1.81-1.74 (m, 2H), 1.72-1.63 (m, 1H), 1.60-1.48 (m, 2H), 1.44-1.29 (m, 2H); analytical HPLC: homogeneous single peak, t_R=8.62 min (8-70% B in 30 min); MALDI MS: calcd for $C_{24}H_{35}N_7O_6S$ $(M + Na)^+$ 572.64, found 572.15.

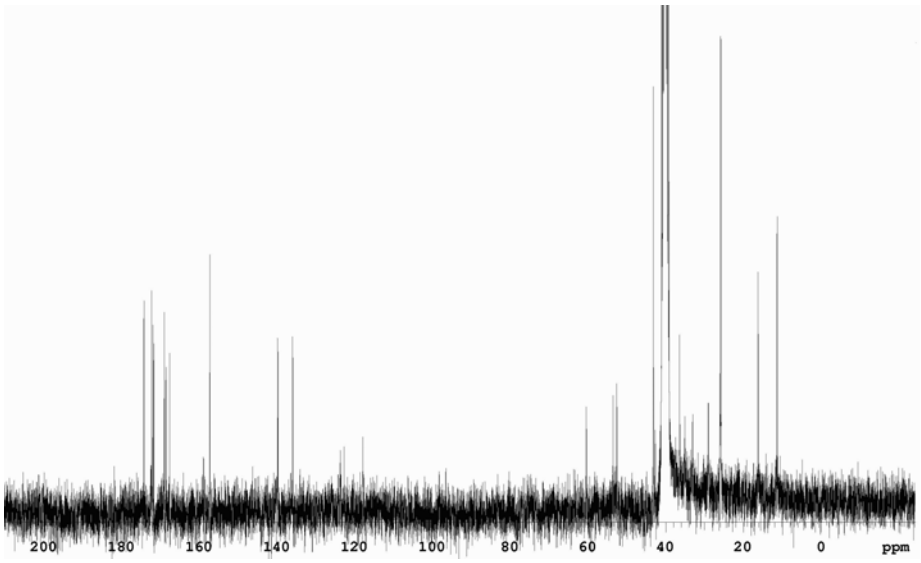


^{1}H NMR of compound **3f**

Compound 3g. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocGly-OH, FmocArg(Pmc)-OH and FmocIle-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (18.6 mg, 69 %). ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 8.44 (d, *J* = 7.90 Hz, 1H), 8.22 (d, *J* = 8.36 Hz, 1H), 8.10 (d, *J* = 8.10 Hz, 1H), 7.77 (s, 1H), 7.73-7.70 (m, 2H), 7.57 (t, *J* = 5.7 Hz, 1H), 7.52-7.51 (m, 2H), 7.49 (s, 1H), 7.24 (s, 1H), 4.40 (q, *J* = 7.15Hz, 1H), 4.22-4.15 (m, 2H), 4.04 (dd, *J* = 6.89, 16.70 Hz, 1H), 3.86 (q, *J* = 13.12 Hz, 2H), 3.59 (dd, *J* = 3.95, 16.70 Hz, 1H), 3.12 (q, 6.59 Hz, 2H), 2.79 (dd, 7.16, *J* = 14.05 Hz, 1H), 2.41 (dd, *J* = 7.16, 14.05 Hz, 1H), 1.96-1.85 (m, 2H), 1.68-1.61 (m, 1H), 1.53-1.49 (m, 2H), 1.47-1.40 (m, 1H), 1.25-1.15 (m, 1H), 0.92 (d, *J* = 6.81 Hz, 3H), 0.88 (t, *J* = 7.43 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$, 25 °C): δ = 174.1, 172.1, 171.8, 171.6, 168.9, 168.5, 167.5, 157.2, 139.7, 139.6, 135.8, 123.6, 122.6, 117.7, 60.3, 53.8, 52.4, 43.0, 36.2, 34.9, 32.9, 28.9, 25.7, 16.1, 11.1; analytical HPLC: homogeneous single peak, t_R=13.45 min (8-70% B in 30 min); MALDI MS: calcd for $C_{25}H_{38}N_8O_5S$ $(M + H)^+$ 563.69, found 563.19.



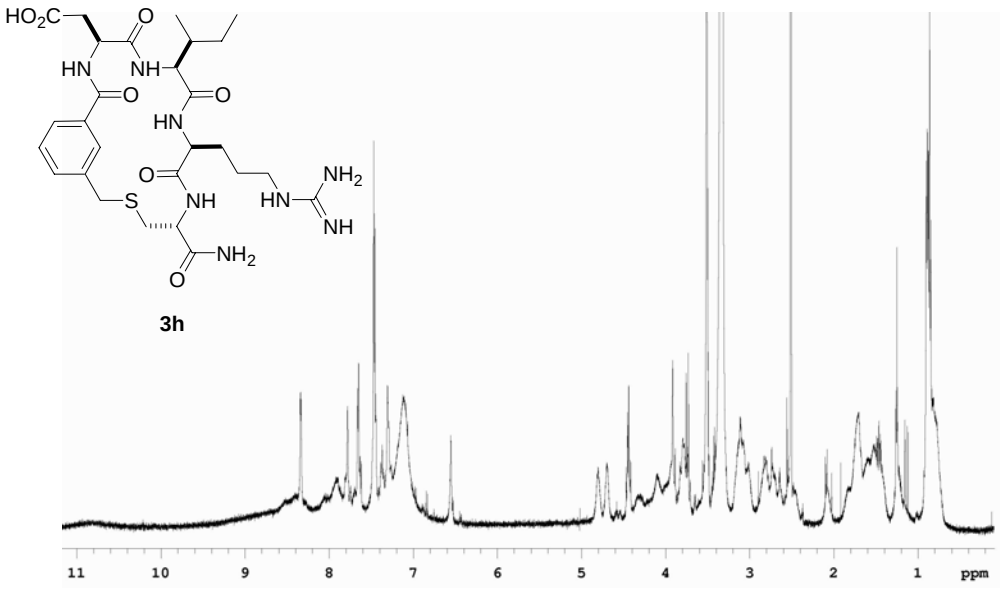
^{1}H NMR of compound **3g**



^{13}C NMR of compound **3g**

Compound 3h. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocArg(Pmc)-OH, FmocIle-OH and FmocAsp(tBu)-OH, respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (18.6 mg, 69 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 10.84 (bs, 1H), 8.34 (d, J = 6.71 Hz, 1H), 7.78 (s, 1H), 7.66 (d, J = 7.18, 1H), 7.63 (d, J = 8.50 Hz, 1H), 7.47-7.45 (m, 3H), 7.37 (t, J = 6.95 Hz, 1H), 7.32-7.25 (m,

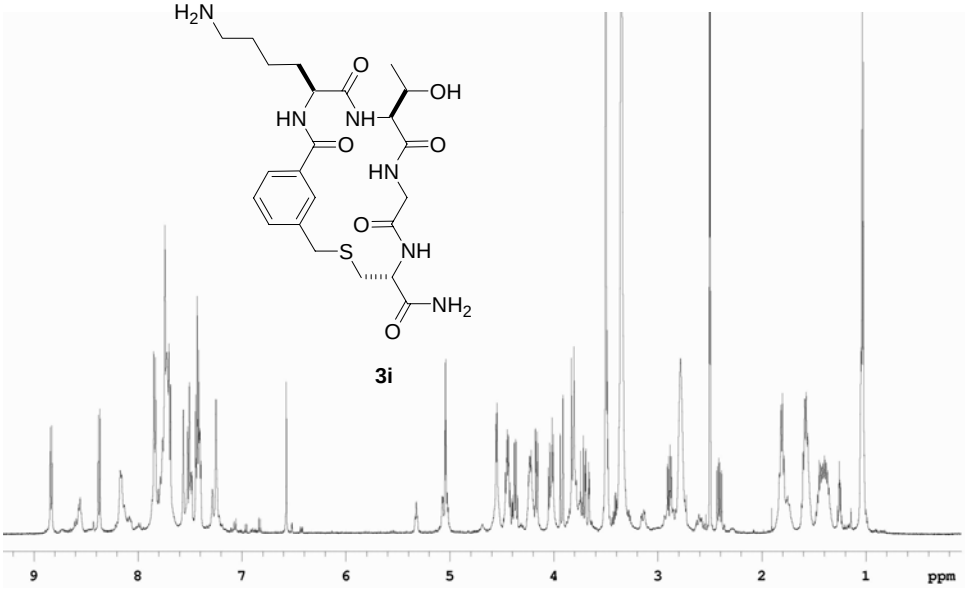
2H), 7.19-7.07 (m, 3H), 6.55 (s, 1H), 4.83-4.77 (m, 1H), 4.73-4.66 (m, 1H), 4.47-4.45 (m, 1H), 4.44 (dd, *J* = 6.54, 17.11 Hz, 1H), 3.76-3.71 (m, 3H), 3.42 (dd, *J* = 5.83, 11.10 Hz, 1H), 3.14-3.05 (m, 2H), 2.86-2.78, (m, 1H), 2.74-2.68 (m, 1H), 1.77-1.66 (m, 2H), 1.64-1.56 (m, 1H), 1.55-1.49 (m, 2H), 1.48-1.42 (m, 1H), 1.24-1.20 (m, 1H), 0.89 (d, *J* = 6.67 Hz, 3H), 0.86 (t, *J* = 7.17, 3H); analytical HPLC: homogeneous single peak, t_R=10.87 min (8-70% B in 30 min); MALDI MS: calcd for $C_{28}H_{40}N_8O_7S$ $(M + H)^+$ 621.72, found 621.25.



^{1}H NMR of compound **3h**

Compound 3i. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocGly-OH, FmocThr(tBu), FmocLys(Boc), respectively. After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (17.7 mg, 68 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 8.45 (d, *J* = 7.94 Hz, 1H), 8.38 (d, *J* = 8.51 Hz, 1H), 8.17 (d, *J* = 7.76 Hz, 1H), 7.85 (d, *J* = 8.92 Hz, 1H), 7.78-7.70 (m, 3H), 7.57 (s, 1H), 7.51 (s, 1H), 7.44 (t, 7.78 Hz, 1H), 7.43-7.40 (m, 1H), 7.26 (s, 1H), 4.50-4.41 (m, 1H), 4.40-4.36 (m, 1H), 4.27-4.20 (m, 1H), 4.18 (dd, *J* = 2.47, 8.92 Hz, 1H), 4.04 (dd, *J* = 6.29, 16.92 Hz, 1H), 3.96-3.92 (m, 1H), 3.94-3.83

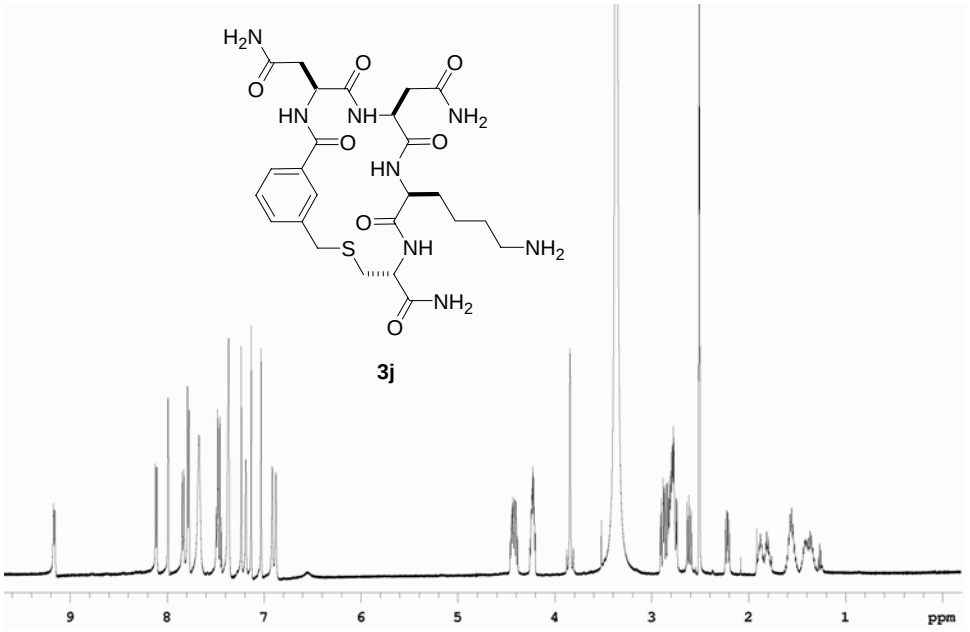
(m, 2H), 3.65 (dd, *J* = 3.71, 16.92 Hz, 1H), 2.90 (dd, *J* = 5.74, 14.03 Hz, 1H), 2.79 (unresolved, 1H), 2.42 (dd, *J* = 8.07, 14.03 Hz, 1H), 1.84-1.80 (m, 2H), 1.63-1.57 (m, 2H), 1.50-1.35 (m, 3H), 1.04 (d, *J* = 6.43 Hz, 3H); analytical HPLC: homogeneous single peak, t_R=7.51 min (8-70% B in 30 min); MALDI MS: calcd for $C_{23}H_{34}N_6O_6S$ $(M + H)^+$ 523.62, found 523.34.



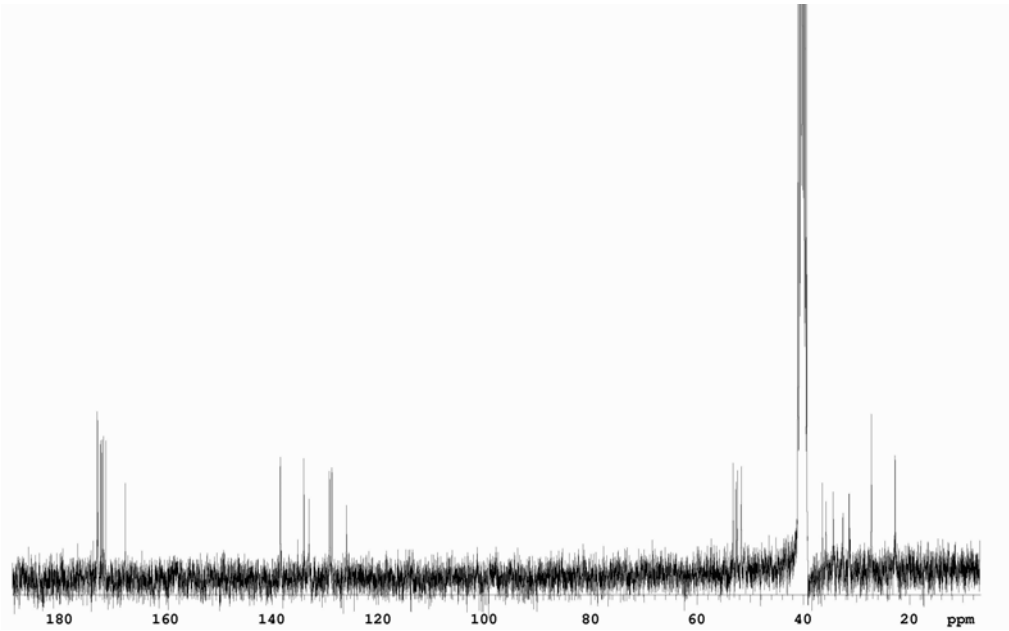
^{1}H NMR of compound **3i**

Compound 3j. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocLys(Boc)-OH, FmocAsn(Trt)-OH and FmocAsn(Trt)-OH, respectively After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (17.7 mg, 68 %). ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 9.16 (d, *J* = 7.46 Hz, 1H), 8.11 (d, *J* = 7.08 Hz, 1H), 7.99 (s, 1H), 7.84 (d, 8.86 *J* = Hz, 1H), 7.80-7.77 (m, 2H), 7.67 (unresolved, 1H), 7.49-7.44 (m, 2H), 7.36 (s, 1H), 7.23 (s,1 H), 7.19 (s, 1H), 7.13 (s, 1H), 7.03 (s, 1H), 6.91 (s, 1H), 6.88 (s, 1H), 4.46-4.38 (m, 2H), 4.26-4.20 (m, 2H), 3.86-3.83 (m, 2H), 2.91-2.85 (m, 2H), 2.84-2.74 (m, 4H), 2.61 (dd, *J* = 8.01, 15.75 Hz, 1H), 2.22 (dd, *J* = 7.78, 12.77 Hz, 1H), 1.91-1.85 (m, 1H), 1.84-1.77 (m, 1H), 1.63-1.51 (m, 2H), 1.46-1.39 (m 1H), 1.38-1.31 (m, 1H); ^{13}C NMR

(75 MHz, DMSO-d_6, 25 °C): δ = 173.0, 172.9, 172.4, 172.0 171.8, 171.3, 167.7, 138.5, 134.0, 133.1, 129.2, 128.8, 126.0, 53.2, 52.7, 52.4, 51.7, 39.5, 36.4, 35.7, 34.4, 32.5, 31.4, 27.2, 22.7 analytical HPLC: homogeneous single peak, t_R=8.19 min (8-70% B in 30 min); MALDI MS: calcd for $C_{25}H_{34}N_8O_7S$ $(M + H)^+$ 593.67, found 593.18.

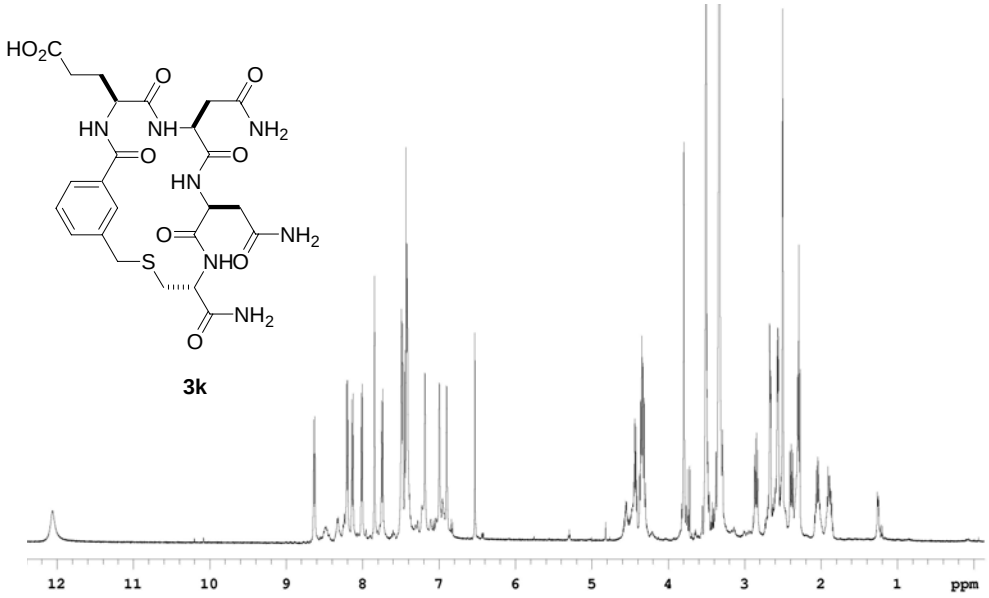


^{1}H NMR of compound **3j**

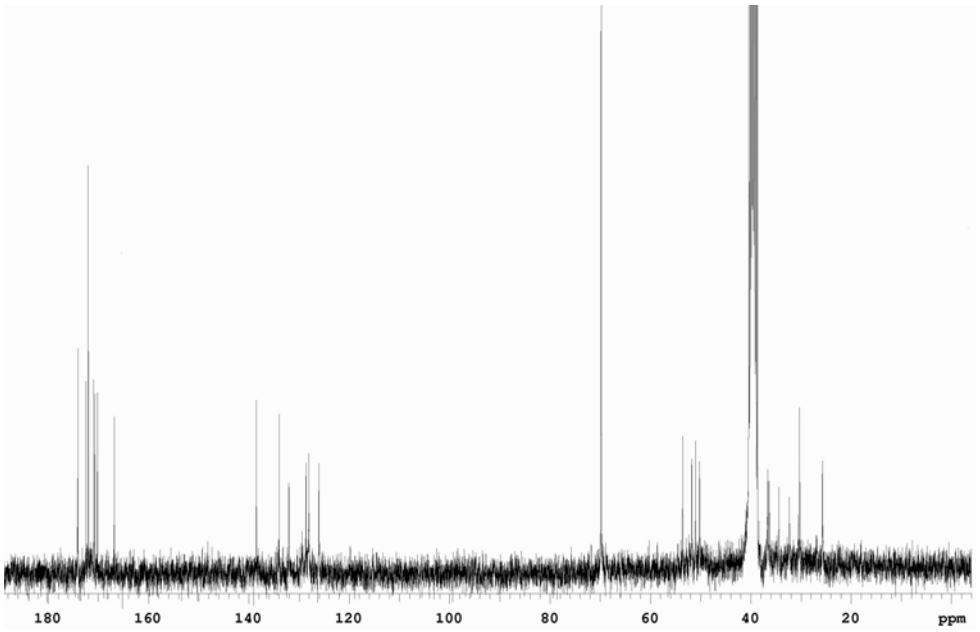


^{13}C NMR of compound **3j**

Compound 3k. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocAsn(Trt)-OH, FmocAsn(Trt)-OH and FmocGlu(tBu)-OH, respectively After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (13.7 mg, 46 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 12.07 (bs, 1H), 8.64 (d, J = 8.22 Hz, 1H), 8.21 (d, J = 7.91 Hz, 1H), 8.44 (d, J = 8.44 Hz, 1H), 8.02 (d, J = 7.60 Hz, 1H), 7.85 (s, 1H), 7.76-7.74 (m, 1H), 7.50-7.48 (m, 2H), 7.46-743 (m, 3H), 7.19 (s, 1H), 7.01 (s, 1H), 6.91 (s, 1H), 4.60-4.53 (m, 1H), 4.46-4.42 (m, 1H), 4.39-4.31 (m, 3H), 3.83-3.78 (m, 2H), 2.86 (dd, J = 6.93, 13.55 Hz, 1H), 2.69-2.66 (m, 1H), 2.59-2.56 (m, 2H), 2.40 (dd, J = 7.23, 13.55 Hz, 1H), 2.32 (t, J = 7.60, 2H), 2.09-2.02 (m, 1H), 1.94-1.85 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 174.1,172.5, 172.0, 170.9, 170.7, 170.1, 166.8, 138.5, 133.5, 132.0, 128.6, 128.0, 126.0, 69.8, 53.6, 51.7, 51.0, 50.2, 36.6, 36., 34.4, 32.4, 30.3, 25.7; analytical HPLC: homogeneous single peak, t_R=8.64 min (8-70% B in 30 min); MALDI MS: calcd for $C_{24}H_{31}N_7O_9S$ (M + Na)$^+$ 616.61, found 616.11.

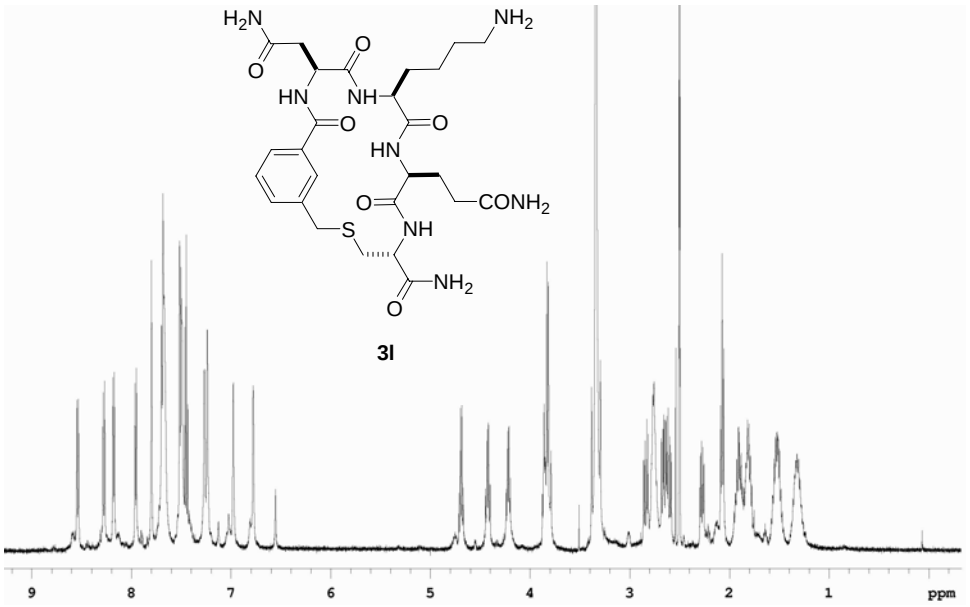


^{1}H NMR of compound **3k**



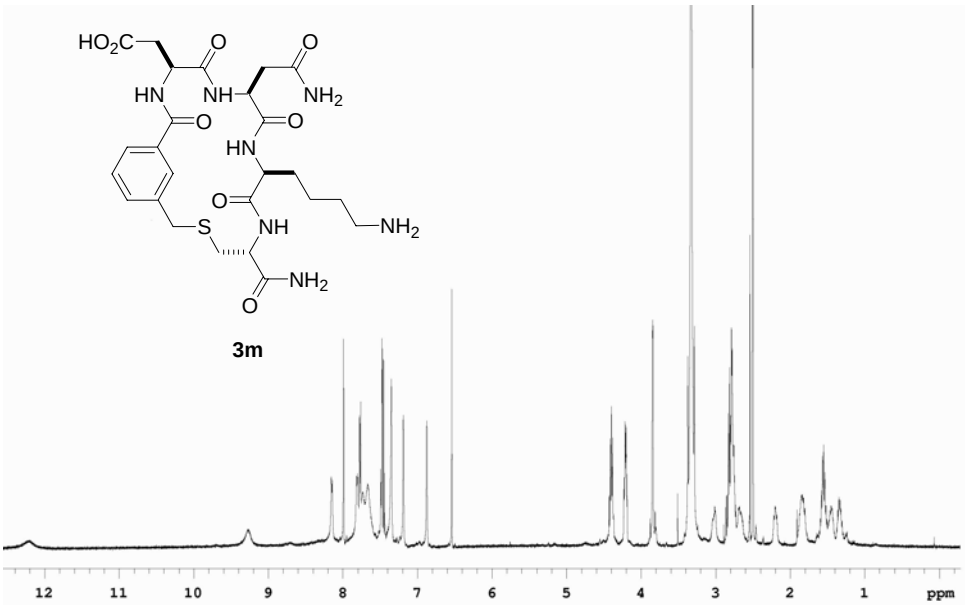
^{13}C NMR of compound **3k**

Compound 3l. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocGln(Trt)-OH, FmocLys(Boc)-OH and FmocAsn(Trt)-OH, respectively After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (10.8 mg, 36 %). ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 8.55 (d, *J* = 7.94 Hz, 1H), 8.28 (d, *J* = 8.31 Hz, 1H), 8.19 (d, *J* = 7.22 Hz, 1H), 7.97 (d, *J* = 7.20 Hz, 1H), 7.81 (s, 1H), 7.71-7.68 (m, 3H), 7.53-7.50 (m, 3H), 7.46 (t, *J* = 7.65 Hz, 1H), 7.28 (s, 1H), 7.25 (s, 1H), 6.99 (s, 1H), 6.79 (s, 1H), 4.72-4.68 (m, 1H), 4.46-4.41 (m, 1H), 4.25-4.21 (m, 1H), 3.89-3.80 (m, 3H), 2.85 (dd, *J* = 6.83, 15.57 Hz, 1H), 2.79-2.74 (m, 2H), 2.67 (dd, *J* = 8.41, 13.25 Hz, 1H), 2.61 (dd, *J* = 6.53, 15.57 Hz, 1H), 2.29 (dd, *J* = 6.13, 13.25 Hz, 1H), 2.08 (t, *J* = 8.33 Hz, 2H), 1.96-1.87 (m, 2H), 1.86-1.77 (m, 2H), 1.59-1.46 (m, 2H), 1.40-1.25 (m, 2H); analytical HPLC: homogeneous single peak, t_R=8.00 min (8-70% B in 30 min); MALDI MS: calcd for $C_{26}H_{38}N_8O_7S$ $(M + H)^+$ 607.70, found 607.23.



^{1}H NMR of compound **3l**

Compound 3m. TentaGel S RAM Fmoc resin (0.05 mmol, 0.30 mmol g^{-1}) was used to synthesize this compound. The second, third and fourth amino acids attached were FmocLys(Boc)-OH, FmocAsn(Trt)-OH and FmocAsp(tBu)-OH, respectively After cleavage from the resin, the crude peptide was purified by preparative HPLC and was lyophilized to give a white solid (6.0 mg, 20 %). ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 12.23 (bs, 1H), 8.16 (d, *J* = 7.11 Hz, 1H), 8.0 (s, 1H), 7.82 (d, *J* = 8.34 Hz, 1H), 7.79-7.77 (m, 1H), 7.75-7.68 (m, 3H), 7.5-7.45 (m, 2H), 7.36 (unresolved, 2H), 7.20 (s, 1H), 6.89 (s, 1H), 6.55 (s, 1H), 4.44-4.38 (m, 2H), 4.24-4.20 (m, 2H), 3.89-3.81 (m, 2H), 3.05-3.01 (m, 1H), 2.9-2.83 (m, 3H), 2.81-2.74 (m, 2H), 2.71-2.64 (m, 1H), 2.23-2.19 (m, 1H), 1.85-1.81 (m, 2H), 1.58-1.52 (m, 2H), 1.48-1.42 (m, 1H), 1.37-1.33 (m, 1H); analytical HPLC: homogeneous single peak, t_R=9.17 min (8-70% B in 30 min); MALDI MS: calcd for $C_{25}H_{35}N_7O_8S$ $(M + H)^+$ 594.65, found 594.22.

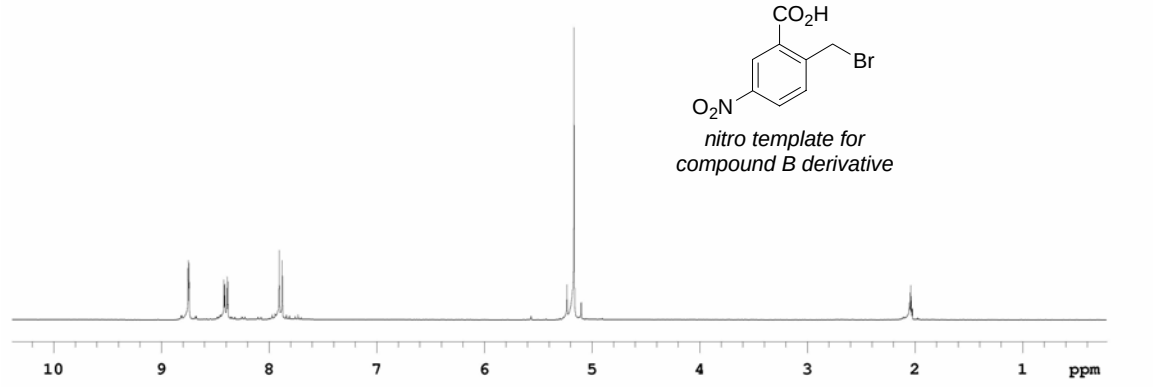


^{1}H NMR of compound **3m**

APPENDIX C

EXPERIMENTAL FOR CHAPTER IV

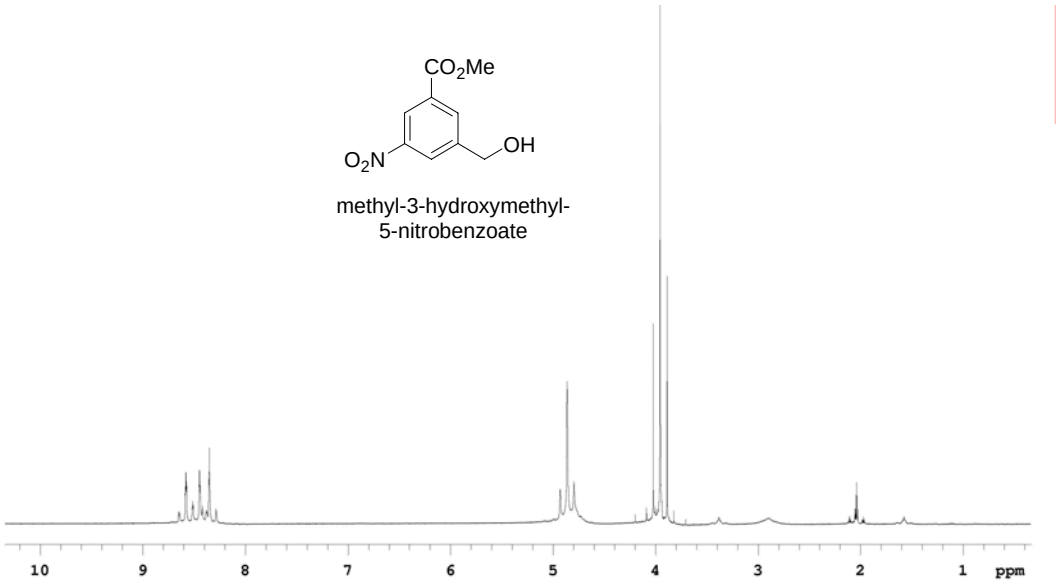
Preparation of Nitro Template for Compound B. 2-Bromomethyl benzoic acid (0.5 g, 2.34 mmol) was placed in a 25 mL round bottom flask. The flask was cooled to -10 °C and then 5 mL of conc. H_2SO_4 was added dropwise. A cold (~0 °C) mixture of H_2SO_4/HNO_3 (0.5 mL/0.3 mL) was then added dropwise via a dropping funnel to the reaction flask. The addition took ~5 min. Then the mixture was stirred for 1 hr, after which it was poured over an ice/water mixture (~25 mL). The nitrated compound which came out as white precipitate was filtered and washed with copious amount of water and then dried in vacuum. ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.75 (d, J = 2.53 Hz, 1 H), 8.40 (dd, J = 8.56, 2.49 Hz, 1 H), 7.89 (d, J = 8.57 Hz, 1 H), 5.17 (s, 2 H). MS (ESI) calcd for $C_8H_6BrNO_4$ 259.0416 $(M - H)^-$ found 259.9638.



^{1}H NMR of 2-bromoymethyl-5-nitrobenzoate

Methyl-3-hydroxymethyl-5-nitrobenzoate. In a dry 250 mL round bottom flask, 1-Nitroisophthalic acid monomethyl ester (5.0 g, 0.0222 mol) was dissolved in 20 mL dry THF. $BF_3{\cdot}OEt_2$ (5.63 mL, 0.0444 mol) was added and the flask was cooled to 0 to -5 °C. $NaBH_4$ (1.13 g, 0.02997) was added portion by portion for 10 min. The mixture was stirred for 2 hr, after which, ethanol (5 mL) was slowly added, followed by water (10 mL) and Et_2O (30 mL). The resulting layers were separated and the aqueous

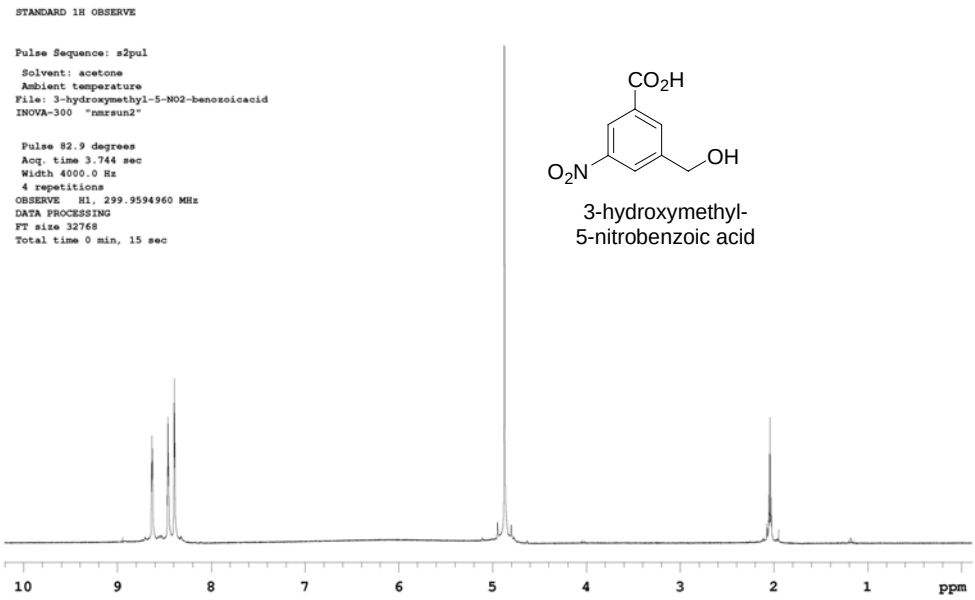
component was extracted once with Et_2O (10 mL). The organic extracts were combined and washed with 20 mL saturated $NaHCO_3$ and dried over Na_2SO_4. The colorless liquid was filtered, concentrated and dried in vacuum to give methyl-3-hydroxymethyl-5-nitrobenzoate as a white solid (3.52 g, 75 %). ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.58 (m, 1 H), 8.45 (m, 1 H), 8.35 (m, 1 H), 4.86 (s, 2 H), 3.95 (s, 3 H).



^{1}H NMR of Methyl 3-hydroxymethyl-5-nitrobenzoate

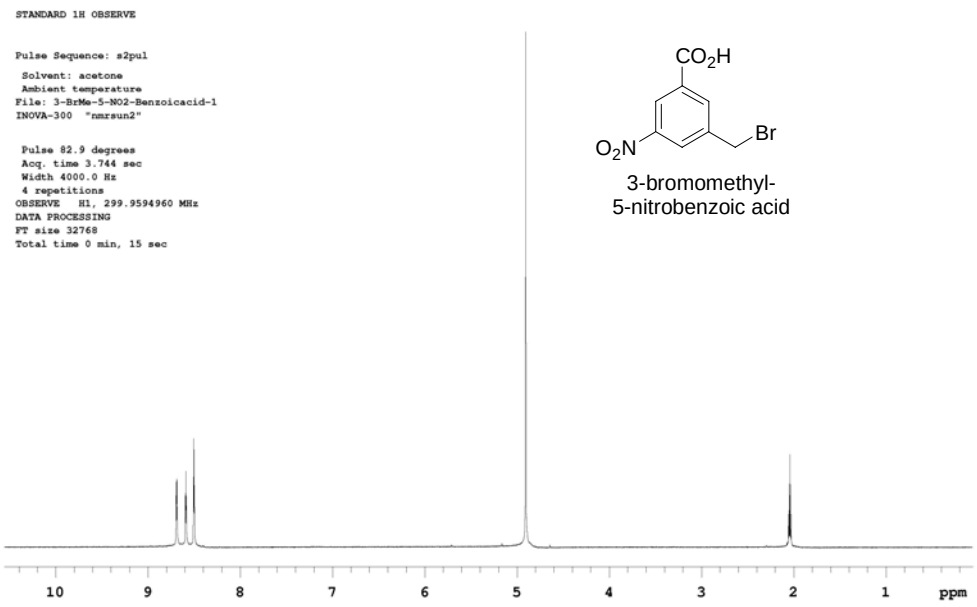
3-Hydroxymethyl-5-nitrobenzoic acid. The methyl ester alcohol (0.0167 mol, 3.52 g) product from the above procedure was placed in a 100 mL round bottom flask. Ten mL of THF was added, and then the flask was cooled to 0 °C. A cold solution of LiOH (0.840 g, 0.0200 mmol) dissolved in H_2O/THF (2 ml/8 mL) was slowly added to the reaction mixture via a dropping funnel. The addition was complete in 10 min, after which the mixture was stirred for 3 hr. After this time, TLC indicated that the reaction was competed. Then, Et_2O (20 mL) and water (20 mL) were added. The layers were separated and to the water layer, 25-30 mL of 1M HCl was added slowly until the solution is acidic. The acidic solution was then extracted with EtOAc (3x, *ca*15 mL). The EtOAc extracts were combined with the original Et_2O extract and the solution was dried over Na_2SO_4 and concentrated in vacuo to give 3-hydroxymethyl-5-nitrobenzoic

acid as a white solid (2.83 g, 86 %). ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.63 (m, 1 H), 8.46 (m, 1 H), 8.39 (m, 1 H), 4.87 (s, 2 H).



^{1}H NMR of 3-hydroxymethyl-5-nitrobenzoic acid

3-Bromomethyl-5-nitrobenzoic acid. The acid product (1.0 g, 0.005 mol) from the above procedure was placed in a three-neck 100 mL round bottom flask. Toluene (10 mL) was added (the compound is not completely soluble), followed by HBr (47 % or 6 M, 12.5 mL, 0.075 mol). The necks were sealed and the mixture was degassed for 30 min by vigorously bubbling nitrogen through a needle passing one of the necks. After this time, the flask was heated to 75 °C and stirred vigorously for 3h. The layers became clear as the temperature is increased. After 3 h, the solution was cooled to room temp and 20 mL toluene was added. The resulting layers were then separated and the organic layer was washed with water (3x, *ca*10 mL) before concentrating and drying in vacuum to give 3-bromomethyl-5-nitrobenzoic acid as white powder (1.30 g, 99 %). ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.69 (m, 1 H), 8.59 (m, 1 H), 8.50 (m, 1 H), 4.91 (s, 2 H). MS (ESI) calcd $C_8H_6BrNO_4$ 259.0416 $(M - H)^-$ found 259.9313.



^{1}H NMR of 3-bromomethyl-5-nitrobenzoate

Solid-Phase Syntheses

General Methods. All α-amino acids used have the L-configuration and were purchased from NovaBiochem, Advanced ChemTech or Chem-Impex. Chemicals were obtained from commercial suppliers and used without further purification. Diisopropylcabodiimide (DIC), *N*-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA), oxalyl chloride, diisopropylethylamine (DIEA), piperidine and triisopropylsilane (TIS) were purchased from Aldrich. Dimethylformamide (DMF), methanol and dichloromethane were bought from EMScience. The resins were obtained from Rapp Polymere, Advanced ChemTech, Nova Biochem, Argonaut Technologies and Chiron Mimetopes.

Peptidomimetic syntheses were performed in fritted polypropylene syringes (5 mL capacity) from Torviq. Reverse-phase high-performance liquid chromatography (HPLC) analyses were carried out using a Beckman SystemGold instrument and a Vydac C-18 column (catalog no. 218TP54, length: 250 mm; inner diameter: 4.6 mm). Preparative purifications were done on an SSI HPLC system using a Vydac C-18 column (catalog no. 2181022, length: 250 mm, inner diameter: 22 mm). All HPLC analyses were

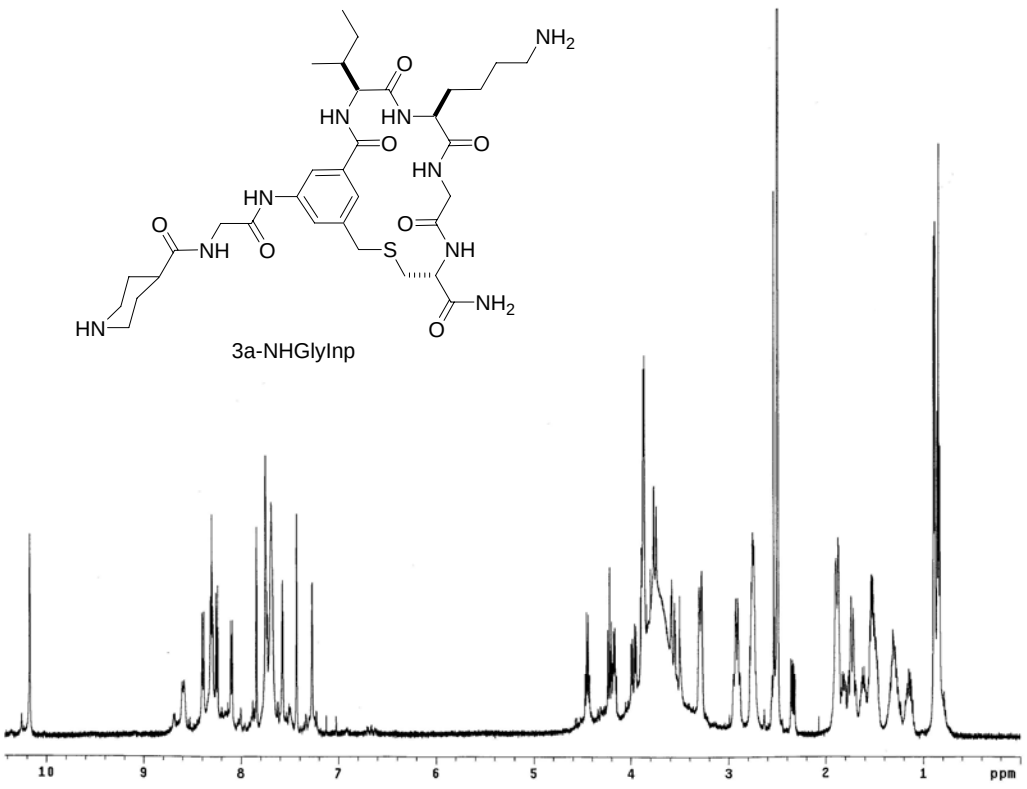
done under gradient conditions. Eluents used were solvent A (H_2O with 0. 1 % TFA) and solvent B (CH_3CN with 0.1% TFA). Flow rates applied were 1.0 and 6 mL/min for analytical and preparative HPLC respectively.

Typical solid phase syntheses of peptidomimetics. Synthesis of 3a-NO_2. In a 5 mL fritted syringe, HypoGel 400 RAM resin (0.05 mmol, 0.54 mmol g^{-1}) was swelled in DMF (*ca* 10 mL g^{-1}) for 1 h. The resin was washed with DMF (3 x *ca* 10 mL g^{-1}, each time for 1 min; this amount of solvent and washing time were used for all other washings throughout). The Fmoc protecting group was removed by treating the resin with 20 % piperidine in DMF (2x, first 10 min, then for 20 min). The resin was washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x), after which a solution of FmocCys(Mmt)-OH (3 eq), DIC (5 eq) and HOBt (5 eq) in 1.5 mL DMF was added. After shaking for 10 h, a Kaiser test on sample beads indicated a negative result. The reaction mixture was drained and the resin washed with DMF (3x). The above deprotection, coupling and washing cycles were repeated to attach FmocGly-OH, FmocLys-OH and FmocIle-OH. After the final Fmoc deprotection, the resin was washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x). The resin was dried for 2 h in vacuo, then a solution of 3-bromomethyl-5-nitrobenzoylchloride (3 eq) and DIEA (5 eq) in 2 mL CH_2Cl_2 were added. After 1 h of shaking, a Kaiser test gave a negative result. The resin was washed with CH_2Cl_2 (5x). The Mmt group on cysteine was removed by treating the resin with 1% TFA and 5 % TIS in CH_2Cl_2 (6x, each time for 10 min). The resin was then washed with CH_2Cl_2 (4x) and DMF (3x). Cyclization was carried out by adding 8 eq of DIEA in *ca* 2.0 - 2.5 mL DMF and shaking the resin for 12 h. The resin was then washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x) before it was dried for 2 h in vacuo. Sample beads were taken and compound **3a-NO_2** was cleaved by treating the resin with 90 % TFA, 5 % TIS and 5 % H_2O (*ca* 3.5 – 4 mL) for 30 min. The resin was then washed with 50 % TFA in CH_2Cl_2 (2x). The collected filtrate and washings were concentrated and the product was precipitated by adding Et_2O (*ca* 3 mL). The white precipitate was isolated by centrifugation of the mixture and carefully decanting the ether. The residue was washed with Et_2O two more times. The crude peptidomimetic was then dissolved in the minimum volume of H_2O with a little CH_3CN added (total volume 1 mL) and was assayed for purity via analytical HPLC (8 – 70 % B in 25 min).

Synthesis of 3a-NH_2. In a 5 mL fritted syringe, compound **3-NO_2** was swollen in DMF for 5 min. The solvent was then washed with DMF (2x) and then 2 mL of 2M solution of $SnCl_2 \cdot 2H_2O$ was added. The mixture was shaken for 2 h, then the solution was removed and a fresh one was added. The mixture was shaken for another 2 h, after which, the resin was drained, then washed with, H_2O (3x), DMF (3x), MeOH (3x) and CH_2Cl_2 (3x). A small sample was cleaved and subjected via analytical HPLC similar to that for **3-NO_2** to determine if the compound is pure enough for the next step.

Synthesis of 3a-NHGlyInp. In a 5 mL fritted syringe, compound **3a-NH_2** was swollen in DMF for 5 min. The solvent was then washed with DMF (2x) then FmocGly-Cl (3 eq) and DIEA (5 eq) in 2 mL CH_2Cl_2 were added. After 1 h, the resin was washed with CH_2Cl_2 (5x) and DMF (3x). The Fmoc group was deprotected by treating the resin with 20 % piperidine in DMF (2x, first 10 min, then for 20 min). The resin was washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x). A solution of FmocInp-Cl (3eq) and DIEA (5 eq) in 2mL CH_2Cl_2 were added. The resin was shaken for 1 h, at which time Kaiser test showed negative. The Fmoc group was deprotected with the same piperidine protocol and the resin was then washed with DMF (3x), MeOH (3x) and CH_2Cl_2 (3x). The resin was dried in vacuum the compound was cleaved by treating the resin with 90 % TFA, 5 % TIS and 5 % H_2O (*ca* 3.5 – 4 mL) for 30 min. The resin was then washed with 50 % TFA in CH_2Cl_2 (2x). The collected filtrate and washings were concentrated and the product was precipitated by adding Et_2O (*ca* 3 mL). The white precipitate was isolated by centrifugation of the mixture and carefully decanting the ether. The residue was washed with Et_2O two more times. The crude peptidomimetic was then dissolved in the minimum volume of H_2O with a little CH_3CN added (total volume < 2 mL) a small portion was injected into an analytical HPLC (Beckman, 8 – 70 % B in 25 min) to check the purity; the rest was purified via preparative HPLC (SSI system, 20 – 50 % B in 20 min). The fraction corresponding to **3a-NHGlyInp** was collected, concentrated and dried in vacuum to give a white fluffy solid. 1H NMR (500 MHz, DMSO-d_6): δ = 10.18 (s, 1H), 8.60 (m, 1H), 8.40 (d, J = 9.24 Hz, 1H), 8.31 (t, J = 6.01 Hz,1H), 8.26 (d, J = 8.32 Hz, 1H), 8.11 (d, J = 8.32 Hz, 1H), 7.85 (s, 1H), 7.76 (m, 1H), 7.70 (bs, 2H), 7.59 (s, 1H), 7.44 (s, 1H), 7.28 (s, 1H), 4.46 (q, J = 7.79 Hz, 1H), 4.20 (m, 2H), 3.98 (dd, J = 7.42, 10.01 Hz, 1H), 3.88 (m, 3H), 3.76 (m, 3H), 3.58 (dd, J = 3.68, 13.78 Hz, 1H), 3.51

(m, 1H), 3.30 (d, *J* = 11.94 Hz, 2H), 2.93 (m, 2H), 2.76 (m, 2H), 2.55 (m, 1H), 2.33 (m, 1H), 1.90 (m, 2H), 1.82 (m, 1H), 1.74 (m, 2H), 1.63 (m, 1H), 1.53 (m, 3H), 1.32 (m, 2H), 1.15 (m, 1H), 0.90 (d, *J* = 6.63 Hz, 3H), 0.85 (t, *J* = 7.46 Hz, 3H); LRMS (MALDI): calcd for (M+ H)+ 718, found 718; analytical HPLC: homogeneous single peak, retention time = 10.91 min (8-50 % B in 30 min).



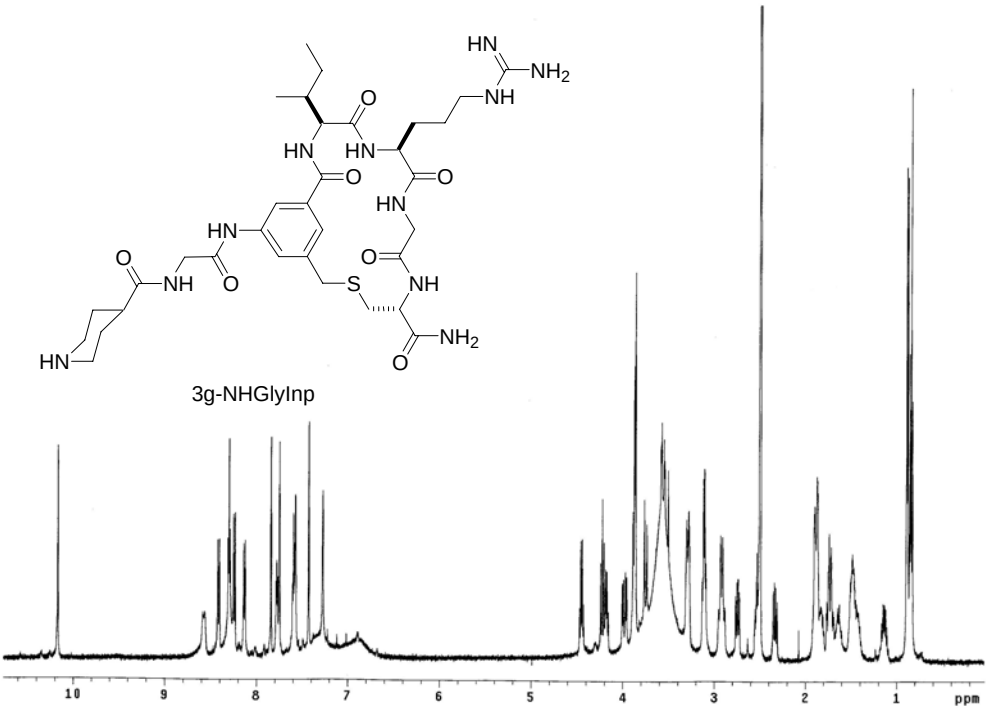
3a-NHGlyInp

^{1}H NMR of **3a-NHGlyInp**

Synthesis of 3g-NO_2. The synthesis followed the same procedure for **3a-NO_2** except that instead of FmocLys(Boc)-OH, FmocArg(Pmc)-OH was used.

Syntheses of 3g-NH_2 and 3g-NHGlyInp. The syntheses of these compounds followed that for **3a-NH_2** and **3a-NHGlyInp**, respectively. For **3a-NHGlyInp**:1H NMR (500 MHz, DMSO- d_6):): δ= 10.17 (s, 1H), 8.58 (m, 1H), 8.41 (d, *J* = 9.33 Hz, 1H), 8.30 (t, *J* = 5.50 Hz, 1H), 8.24 (d, *J* = 8.62 Hz, 1H), 8.13 (d, *J* = 7.66 Hz, 1H), 7.84 (s, 1H), 7.78 (t, *J* = 5.08 Hz, 1H), 7.75 (s, 1H), 7.59 (m, 2H), 7.43 (s, 1H), 7.27 (s, 1H), 4.55 (q, *J* =

7.55 Hz, 1H), 4.22 (t, *J* = 9.56 Hz, 1H), 4.17 (m, 1H), 3.98 (dd, *J* = 6.66, 9.76 Hz, 1H), 3.87 (m, 3H), 3.75 (d, *J* = 12.48 Hz, 2H), 3.56 (dd, *J* = 3.49, 13.31 Hz, 1H), 3.50 (m, 1H), 3.29 (d, *J* = 11.19 Hz, 2H), 3.11 (m, 2H), 2.92 (q, *J* = 11.21 Hz, 2H), 2.75 (m, 1H), 2.54 (m, 1H), 2.33 (m, 1H), 1.89 (m. 3H), 1.82 (m, 1H), 1.73 (m, 2H), 1.64 (m, 1H), 1.46 (m, 3H), 1.14 (m, 1H), 0.89 (d, *J* = 6.84 Hz, 3H), 0.85 (t, *J* = 7.41 Hz, 3H); LRMS (MALDI): calcd for $(M+ H)^+$ 746, found 746; analytical HPLC: homogeneous single peak, retention time = 11.51 min (8-50 % B in 30 min).



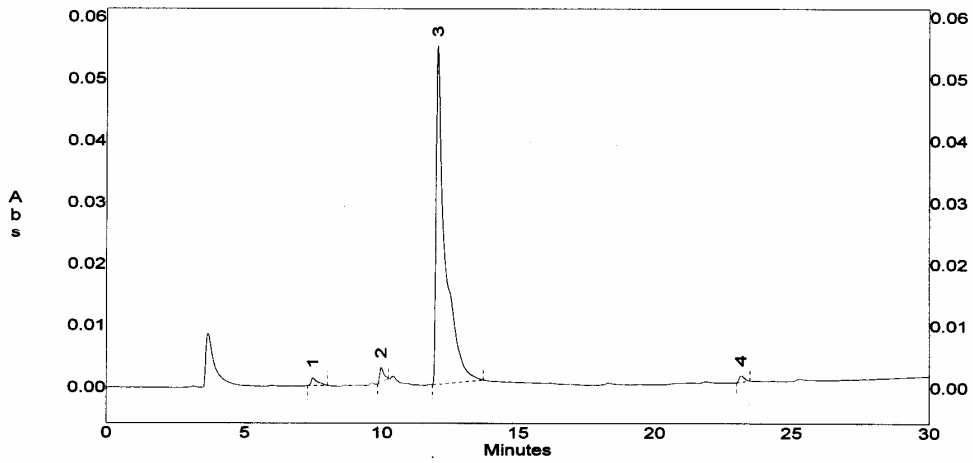
^{1}H NMR of **3g-NHGlyInp**

Synthesis of B-NO_2. The synthesis followed the same procedure for **3a-NO_2** except that after FmocCys(Mmt)-OH, FmocTyr(O^{t}Bu)-OH and FmocLys(Boc)-OH was used. Only these amino acids were coupled and the template was 2-bromomethyl-5-nitrobenzoyl chloride.

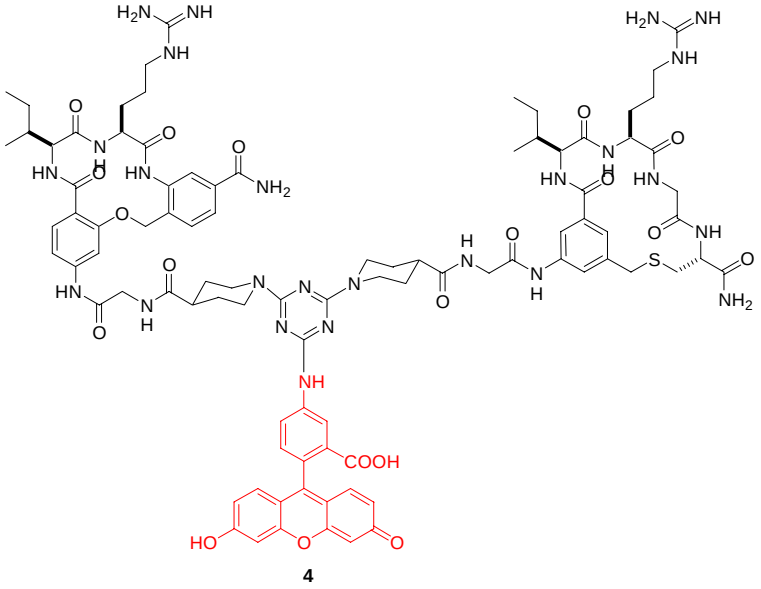
Synthesis of B-NH_2 and B-NHGlyInp. The syntheses of these compounds followed that **for 3a-NH_2** and **3a-NHGlyInp**, respectively.

Synthesis of B-NHGlyInpF*. Compound **B-NHGlyInp** was swelled in CH_2Cl_2 for 30 min. After the solvent was drained, 2 equiv. of dichlorotriazinylaminofluorescein (DTAF), DIEA (3 equiv.) in 3:1 mixture of CHCl3/ DMSO were added and the mixture was shaken for 3 h. The resin was then washed and subjected to the above cleavage condition. The resulting crude product was purified via prep HPLC (SSI system, 8 – 50 % B in 30 min) to give the dye-tagged peptidomimetic **B-NHGlyInpF***. 1H NMR (500 MHz, DMSO-d_6) δ= 10.61 (s, 1H), 10.15 (bs, 1H), 9.17 (s, 1H), 8.56 (d, J = 8.17 Hz, 1H), 8.45, (bs, 1H), 8.27 (m, 2H), 7.92 (m, 2H), 7.64 (bs, 1H), 7.48 (d, J = 9.06 Hz, 1H), 7.36 (s, 1H), 7.31 (d, J = 9.06 Hz, 1H), 7.24 (d, J = 8.49 Hz, 2H), 7.20 (s, 1H), 7.02 (d, J = 8.49 Hz, 1H), 6.98 (d, J = 8.49 Hz, 1H), 6.67 (d, J = 2.39 Hz, 2H), 6.64 (bs, 1H), 6.63 (s, 1H), 6.62 (bs, 1H), 6.60 (bs, 1H), 6.56 (m, 1H), 6.54 (m, 1H), 4.59 (m, 1H), 4.53 (d, J = 13.34 Hz, 1H), 4.41 (m, 2 H), 4. 14 (q, J = 7.40 Hz, 1H), 3.91 (m, 1H), 3.89 (bs, 1H), 3.77 (d, J = 12.26 Hz, 1H), 3.04, (m, 1H), 2.94 (m, 1H), 2.88 (m, 2H), 2.80 (m, 1H), 2.76 (m, 1H), 2.72 (m, 2H), 2.63 (m, 2H), 2.61 (m, 2H), 2.54 (m, 2H), 2.36 (m, 1H), 1.55 (m, 2H), 1.47 (m, 2H), 1.24 (m, 2H); LRMS (MALDI): calcd for $(M+ H)^+$ 1170, found 1170; analytical HPLC: homogeneous single peak, retention time = 10.20 min (8-70 % B in 30 min)

Resysnthesis of compound 4. Culture glass tubes (6 x 50 mm, VWR Scientific Products) were used as a reaction vessel. Stock solutions of all reagents in DMSO were prepared; 0.030 M of compound **3a-NHGlyInp** and 0.033 M of compound **5**. Solid K_2CO_3 (*ca* 1 mg) was first added to each tube, followed by 45 μL of a stock solution of **3a-NHGlyInp** and 45 μL of one of **5**. The reaction vessels were sealed with parafilm, gently shaken by hand and sonicated for 10 min. The mixtures were left at 25 °C without stirring for 24 h. The solutions in each vessel were transferred into 5 mL sample vials using a micropipet and then lyophilized to remove DMSO. The solid materials were then washed with H2O by adding *ca* 0.5 mL of H2O into each sample and sonicating for 5 min. The solution was left to stand for 30 min then the supernatant was carefully decanted away. The solid products were re-dissolved in 1:1 mixture of H2O/ CH3CN, then lyophilized again to give compound **4**. The crude product was analyzed by analytical HPLC **(crude 4** ~ 90% pure, retention time = 12.05 min (8-70 % B in 30 min). LRMS (MALDI) calcd for $(M+ H)^+$ 1890, found 1890.



Analytical HPLC for crude **4**.



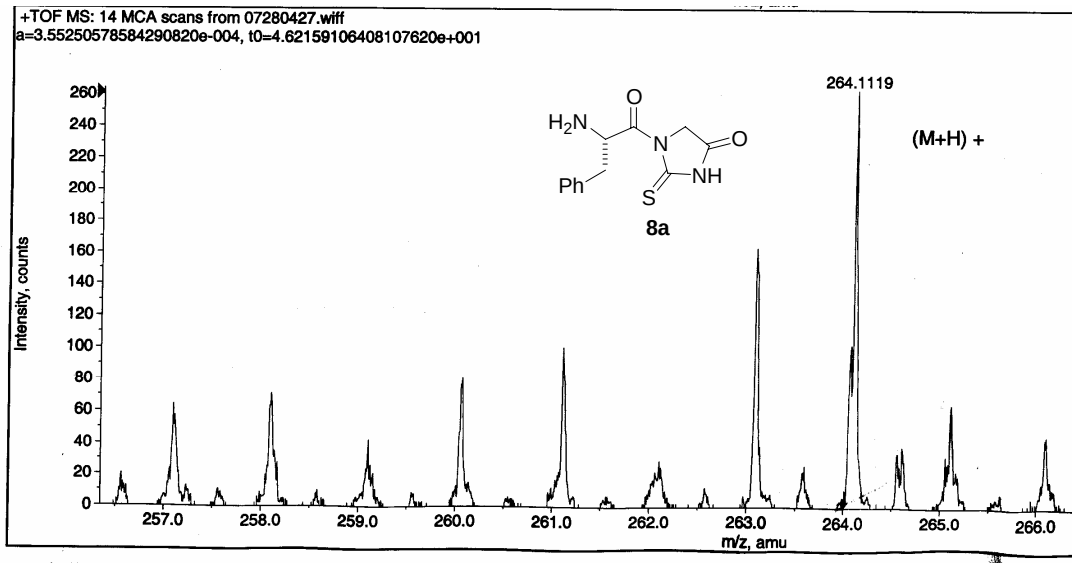
4

APPENDIX D

EXPERIMENTAL FOR CHAPTER V

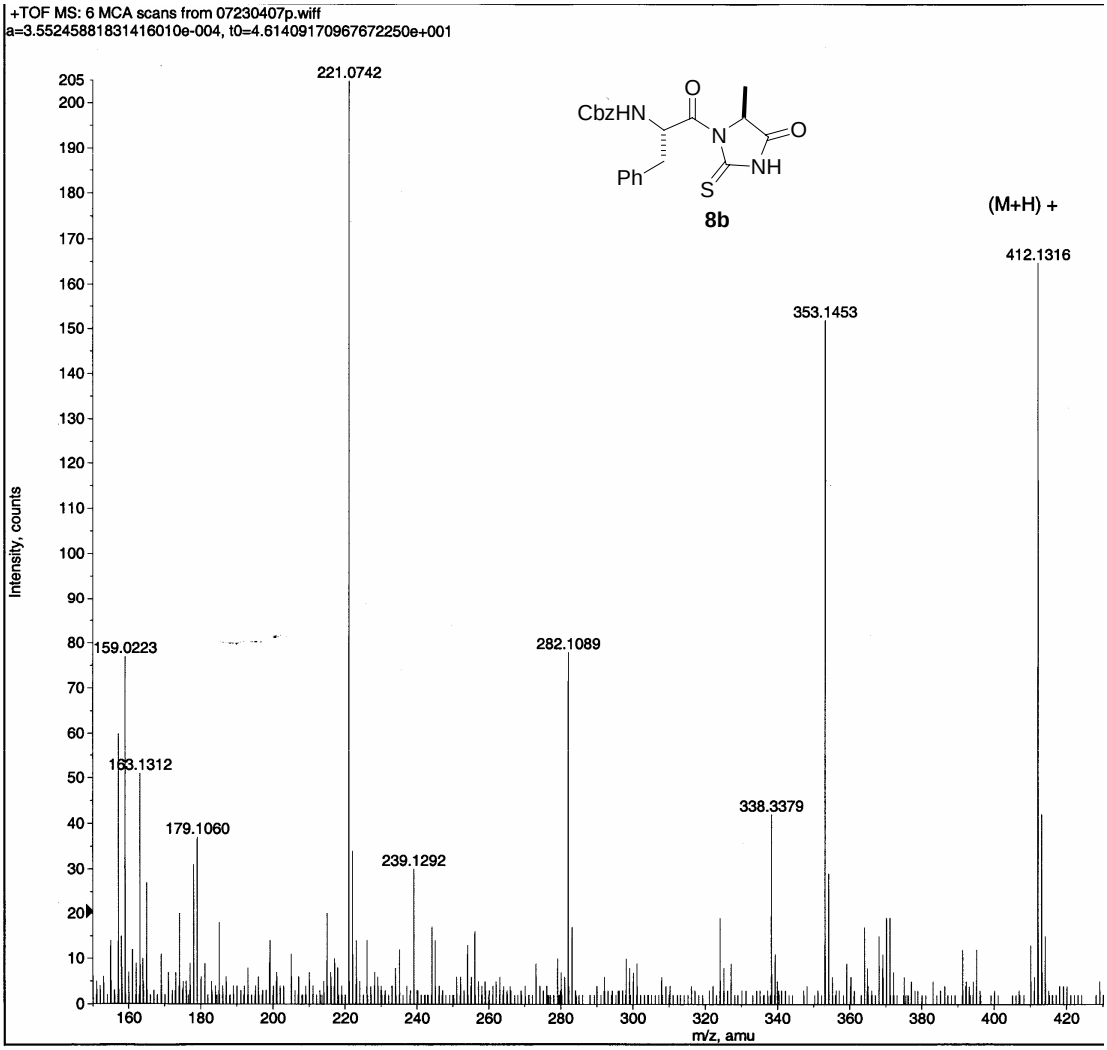
General Procedure For The Synthesis of Dipeptide Thiohydantoins.

Synthesis of 8a. CbzPheGly-OH (0.10 g, 0.28 mmol) and NH_4SCN (0.0256 g, 0.336 mmol) was placed in a 10 mL microwave tube. Ac_2O (0.30 mL) was added and the tube was capped and placed in a Discover (CEM) microwave. The tube was stirred, and irradiated to reach 110 °C. The temperature was maintained for 3 min, after which the tube was cooled to room temp. Then, 5 mL CH_2Cl_2 was added to the yellow solution followed by 5 mL water. The layers were separated and the aqueous layer was extracted 2x with 3 mL CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4 and concentrated in vacuum to give a yellow solid (crude product, 83 mg, 75 %). The proton NMR of the crude product is hard to assign. Half of the product was subjected to deprotection. The Cbz group was deprotected by dissolving the crude material in 10 mL MeOH and adding 3 mol % of 5 % Pd/C. The mixture was stirred until TLC indicates consumption of starting material. Additional 5 mL MeOH was added and the mixture was passed thru a packed celite to remove the catalyst. The solvent was evaporated to dryness and the crude solid dried in vacuum to give a light yellow material. MS (ESI): 264.1 $(M+H)^+$.



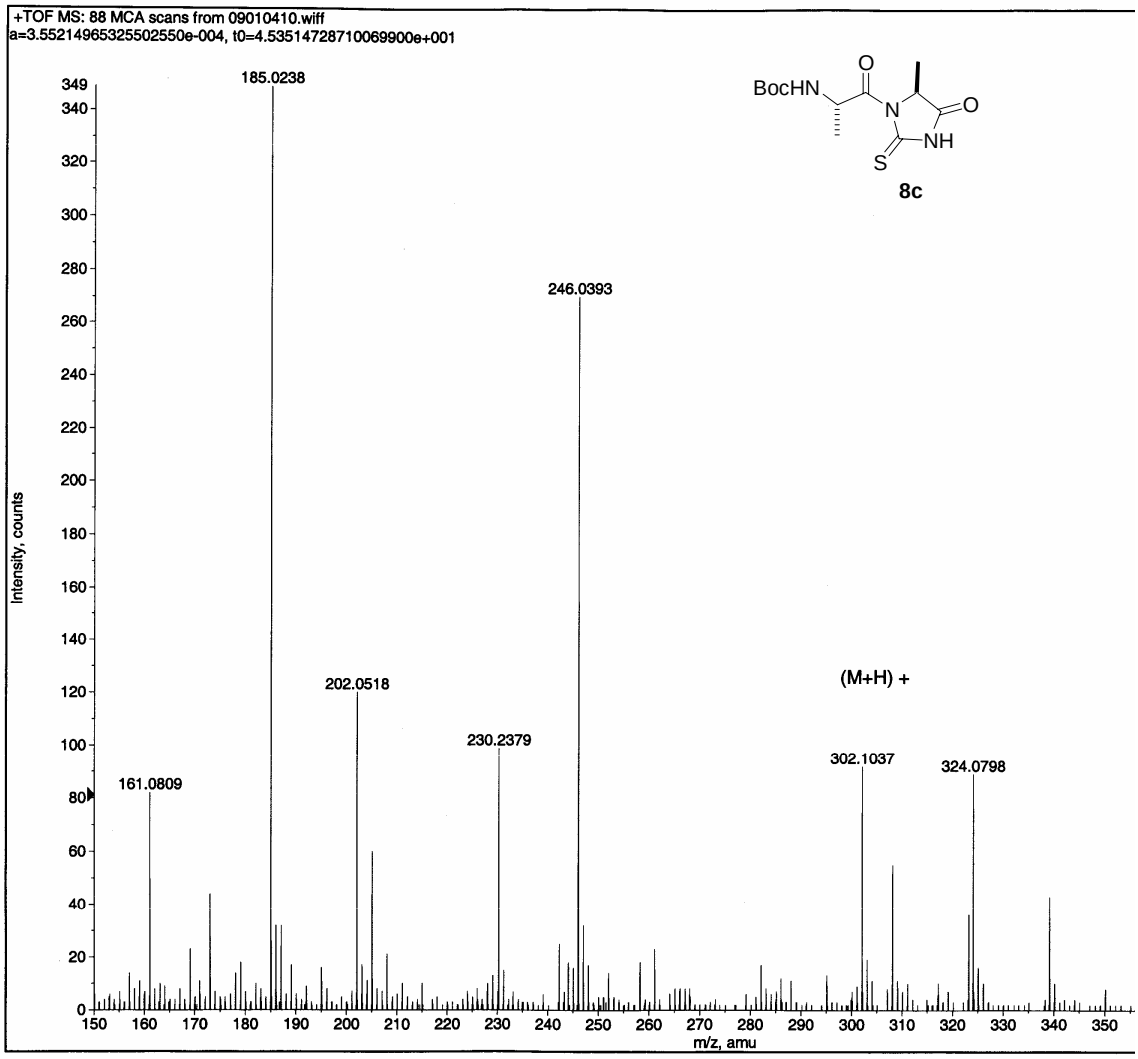
MS(ESI) of compound **8a**

Synthesis of 8b. The procedure follows that for 8a, except that the dipeptide used was CbzPheAla-OH. This time the crude product appears as yellow viscous oil (94 mg. 85 %). The proton NMR is hard to assign because of impurities but the MS shows the presence of the product. MS (ESI): 412.1 $(M+H)^+$.



MS(ESI) of compound **8b**

Synthesis of 8c. The procedure follows that for 8 a, except that the dipeptide used was BocAlaAla-OH. The crude product appears as yellow solid (98 mg, 85 %). The proton NMR is hard to assign due to impurities which can not be seen on TLC. MS (ESI): 302.1 $(M+H)^+$.

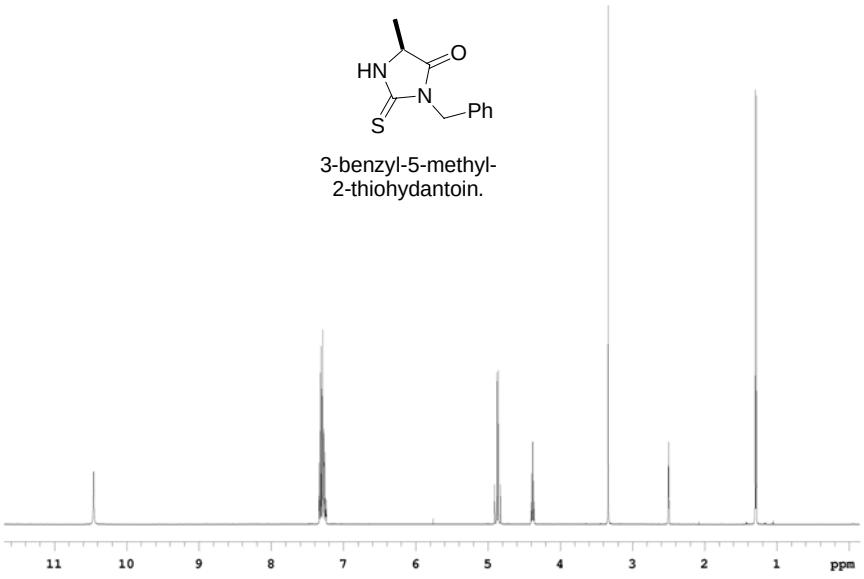


MS(ESI) of compound **8c**

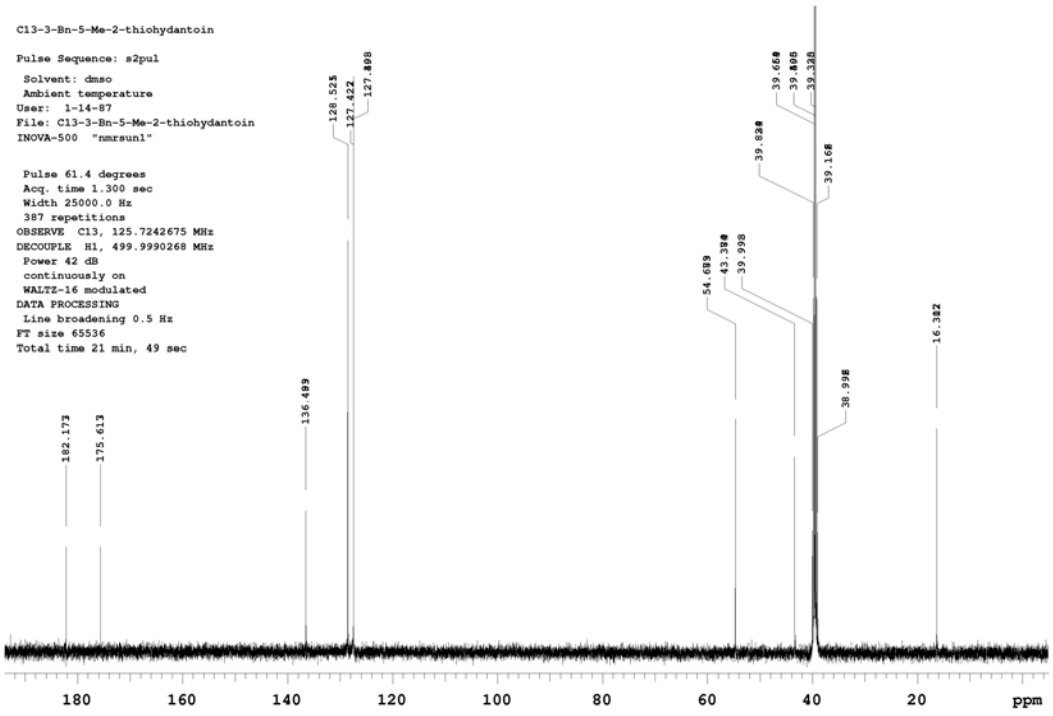
Synthesis of Iminohydantoin 13 from 3-benzyl-5-methyl-2-thiohydantoin.

Benzyl-5-Methyl-2-Thiohydantoin. To a mixture of L-alanine methyl ester hydrochloride salt (0.500 g, 3.58 mmol) and Et3N (0.5 mL, 3.58 mmol) in 10 mL CH_2Cl_2, benzylisothiocyanate (0.475 mL, 3.58 mmol) was added slowly. The mixture was stirred for 1 h at 25 °C, after which a reflux condenser was attached. The temperature was raised to 40 °C and stirring was continued for another hour. At this time, TLC revealed all of the benzylisothiocyanate has been consumed, and a new spot appeared. The solution was cooled to room temperature and the solution evaporated in

vacuo. The residue was redissolved in 15 mL CH_2Cl_2 and washed with 10 mL water and 10 mL brine and dried over Na_2SO_4. The colorless solution was concentrated and dried in vacuo to give 3-benzyl-5-methyl-2-thiohydantoin as a white tiny crystalline material (0.410 g, 52%). ^{1}H NMR (500 MHz, DMSO- d_6): δ= 10.46 (s, 1H), 7.34-7.24 (m, 5H), 4.89 (d, J = 15.0 Hz, 1H), 4.84 (d, J = 15.0 Hz, 1H), 4.38 (q, J = 7.1 Hz, 1H), 1.29 (d, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ= 182.2(C), 175.6(C), 136.5(C), 128.5(CH), 127.42(CH), 127.40(CH), 54.7(CH), 43.7(CH2), 16.3(CH3); MS (ESI): 576 $(M+H)^+$.



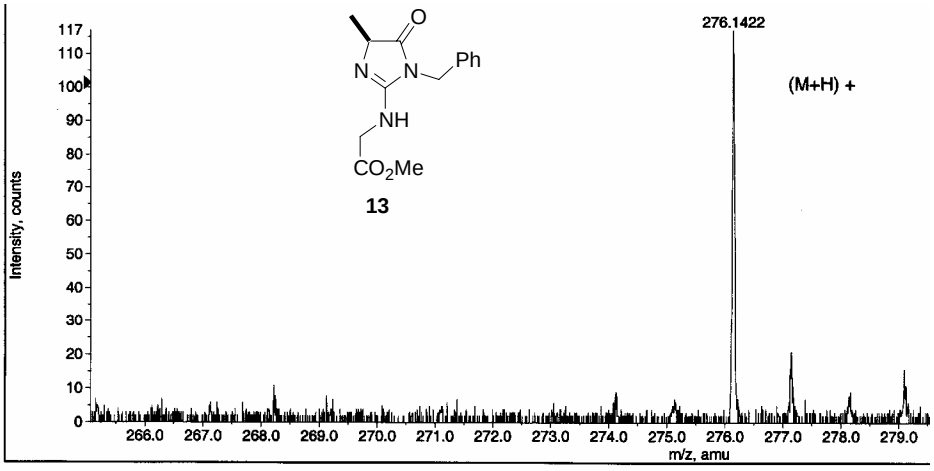
^{1}H NMR of 3-benzyl-5-methyl2-thiohydantoin



^{13}C NMR of 3-benzyl-5-methyl2-thiohydantoin

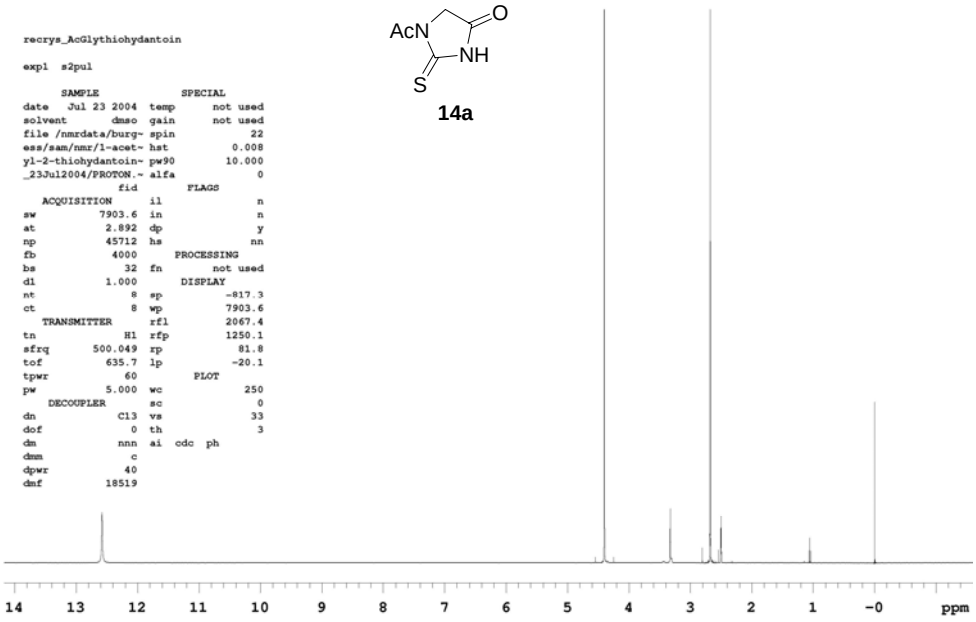
Synthesis of compound 13. In a 25 mL round bottom flask, the above product (0.10 g, 0.454 mmol) was dissolved in 8 mL MeOH and 1.5 mL CH_3CN. To this solution, NaOH (0.018 g, 0.454 mmol) dissolved in 0.5 mL water was added followed by MeI (0.113 mL, 1.82 mmol). The flask was stoppered and stirred for 18 h. After this time, 5 mL brine, 5 mL water and 30 mL $CHCl_3$ were added. The layers were separated and the aqueous layer extracted 3x with 10 mL $CHCl_3$. The organic extracts were combined, dried over Na_2SO_4 and evaporated to dryness to give the crude methyl isothiourea as a yellow solid. Methylation was verified by proton NMR. The crude product was dissolved in 10 mL ethanol, then a solution of HCl·GlyOMe (1eq) and Et_3N (2 eq) in 5 mL EtOH were added. The mixture was heated to 70 °C and stirred for 24 h. Work up was done by adding 10 mL brine and 20 mL CH_2Cl_2. The water layer was extracted 2x with 5 mL CH_2Cl_2 and the organic layers were combined and dried over Na_2SO_4. The solvent was evaporated to give a crude light yellow semi-solid product (75 mg, 60 %). A proton NMR of this crude product was taken and it still shows EtOH peaks. ^{1}H NMR (500 MHz, DMSO- d_6): δ = 7.33-7.24 (m, 6H), 4.17 (q, J = 7.16 Hz,

1H), 4.09 (t, J = 6.16 Hz, 1H), 3.17 (s, 3H), 3.30-3.23 (m, 2H), 3.22-3.18 (m, 1H), 1.23-1.19 (m, 3H). MS (ESI): 276.1 $(M+H)^+$.

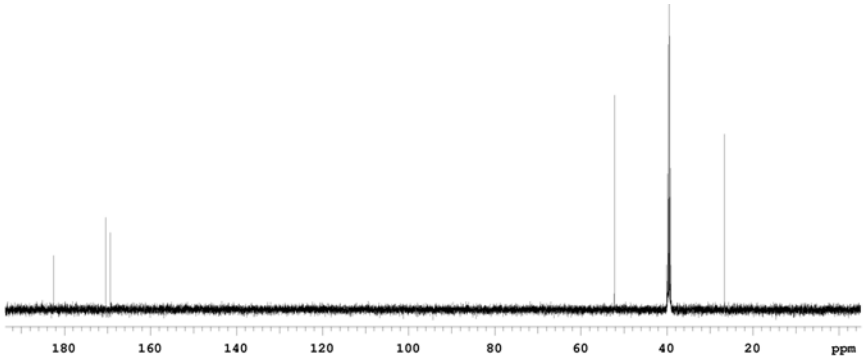


MS(ESI) of compound **13**

General Procedure for the Synthesis of Acyl-2-thiohydantoins. Synthesis of 1-Acetyl-2-thiohydantoin (14a). Glycine (1.0 g, 13.3 mmol) and NH_4SCN (1.038 g, 13.3 mmol) were ground together using a mortar and pestle. The mixed solid was transferred in a 50 mL round bottom flask and was heated in an oil bath at 100 °C for 30 min, by which time all solids were already dissolved. The light orange solution was poured into an ice/water mixture (20 mL) and stored in a freezer overnight. The resulting light orange solid was filtered and washed with cold water and dried under vacuum to afford 2a as a light orange solid which appear as tiny crystalline-like material (1.08 g, 51%): mp 173-174 °C; ^{1}H NMR (500 MHz, DMSO- d_6): δ = 12.58 (s, 1H), 4.40 (s, 2H), 2.68 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ = 182.5(C), 170.4(C), 169.3(C), 52.2(CH), 26.6(CH3); MS (ESI) 576 $(M+H)^+$.



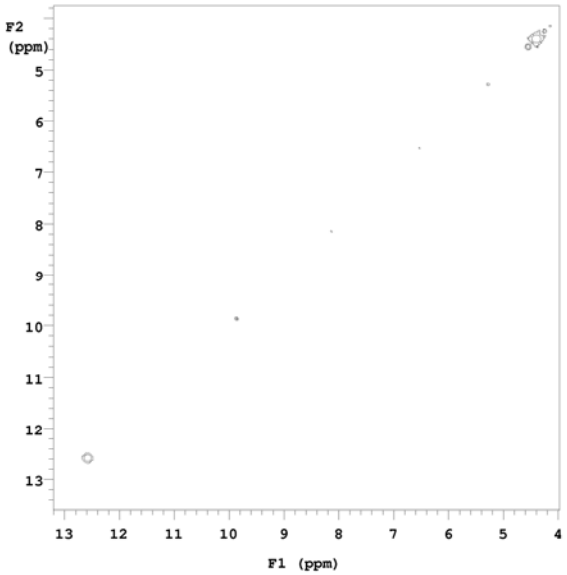
^{1}H NMR of compound **14a**



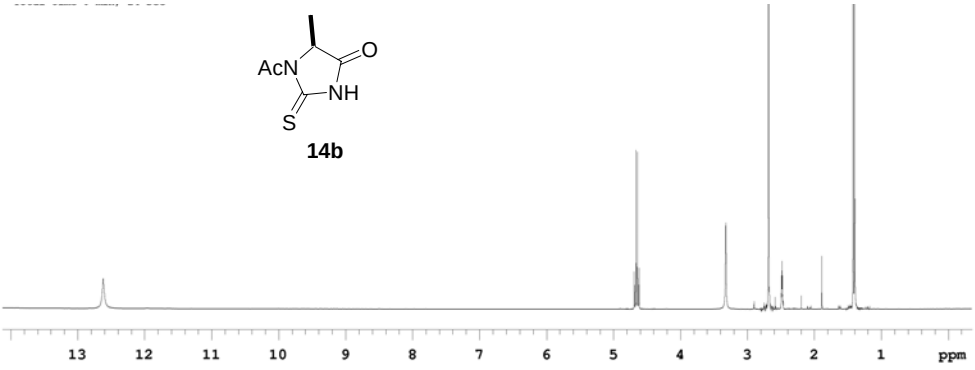
^{13}C NMR of compound **14a**

Partial COSY of compound **14a**

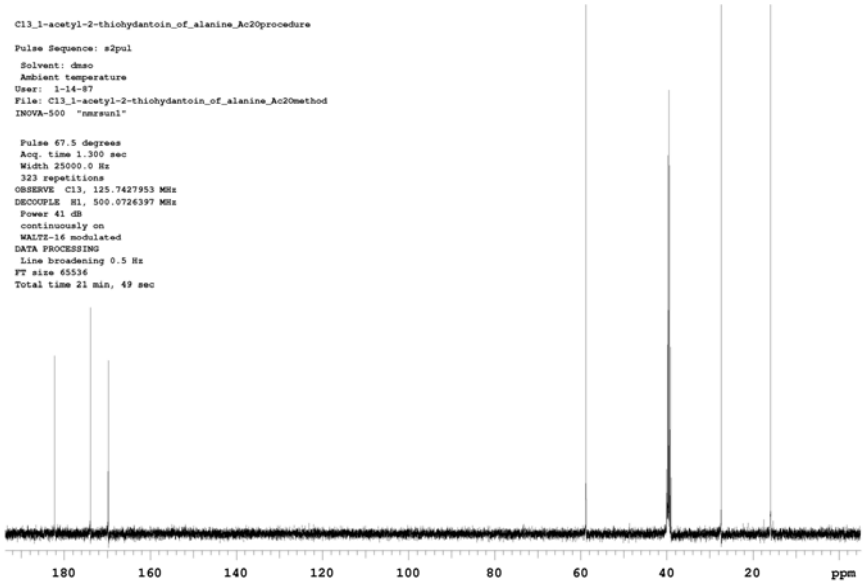
The N*H* peak around 12.6 shows that no cross peak is observed with the C^5*H* at ~4.4 ppm.



1-Acetyl-5-methyl-2-thiohydantoin (14b). L-Alanine was used to prepare this material which followed the procedure for **14a**. After filtration and washing, the off-white solid was dried under vacuum to yield **14b** (1.30 g, 68%): mp 164-166 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ= 12.63 (s, 1H), 4.67 (ddd, J = 7.1, 7.1, 7.1 Hz, 1H), 2.70 (s, 3H), 1.42 (d, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ= 182.3(C), 173.9(C), 169.7(C), 59.8(CH), 27.4(CH3), 15.9(CH3); MS (APCI): 173 $(M+H)^+$.

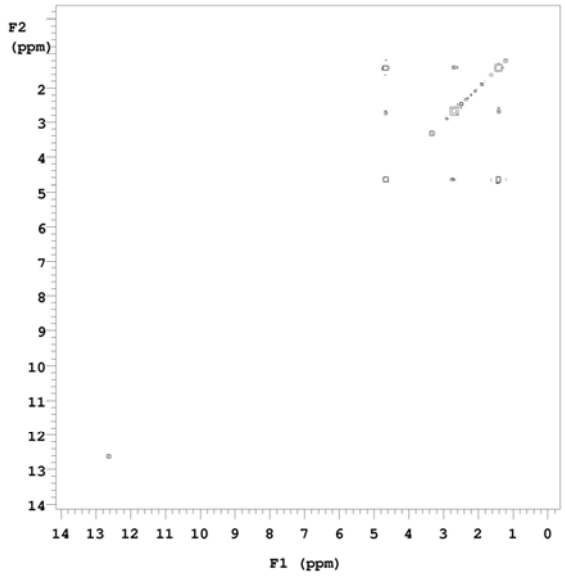


^{1}H NMR of compound **14b**

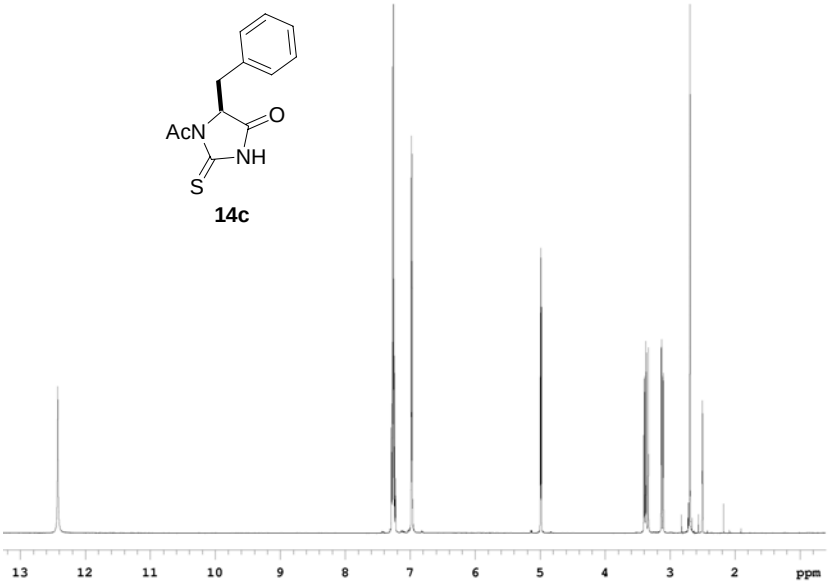


^{13}C NMR of compound **14b**

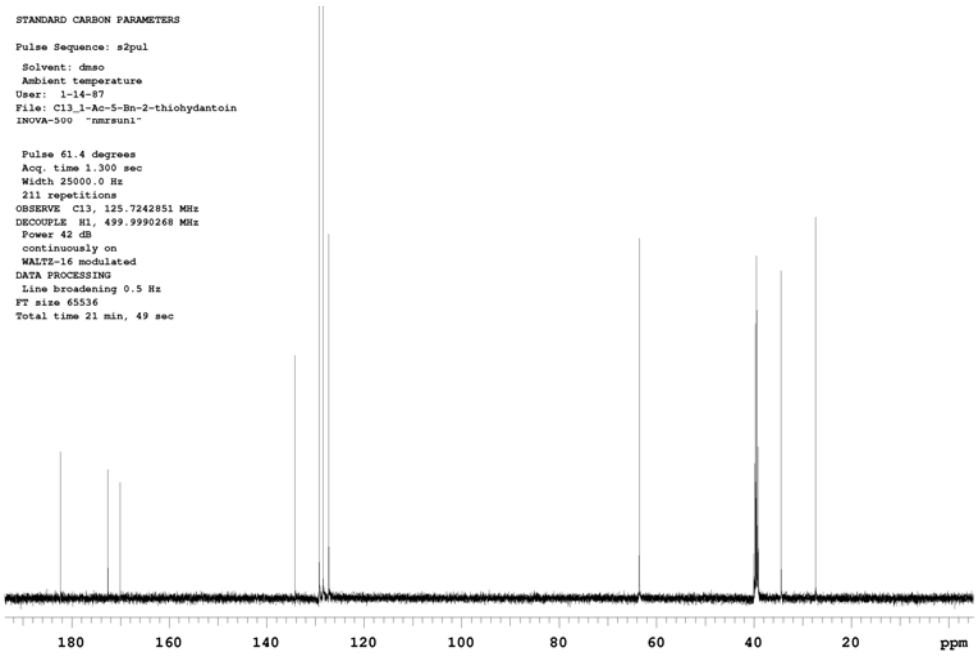
COSY of compound **14b**
The NH peak around 12.6 shows that no cross peak is observed with the C-alpha H at ~4.7 ppm



1-Acetyl-5-benzyl-2-thiohydantoin (14c). L-Phenylalanine was used to prepare this material which followed the procedure for **14a**. After filtration and washing, the white solid was dried under vacuum to afford **14c** (0.850 g, 71%): mp 168-169 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.43 (s, 1H), 7.26 (m, 3H), 6.98 (m, 2H), 4.99 (dd, J = 5.9, 2.7 Hz, 1H), 3.38 (dd, J = 13.9, 5.9 Hz, 1H), 3.12 (dd, J = 13.8, 2.7 Hz, 1H), 2.69 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 182.3(C), 172.6(C), 170.02(C), 134.2(C), 129.2(CH), 128.4(CH), 127.3(CH), 63.5(CH), 34.4(CH2), 27.4(CH3); MS (APCI) 249 $(M+H)^{+}$.

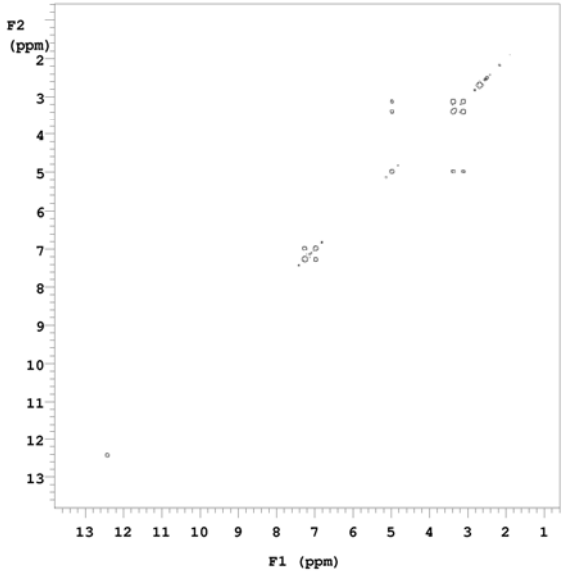


^{1}H NMR of compound **14c**

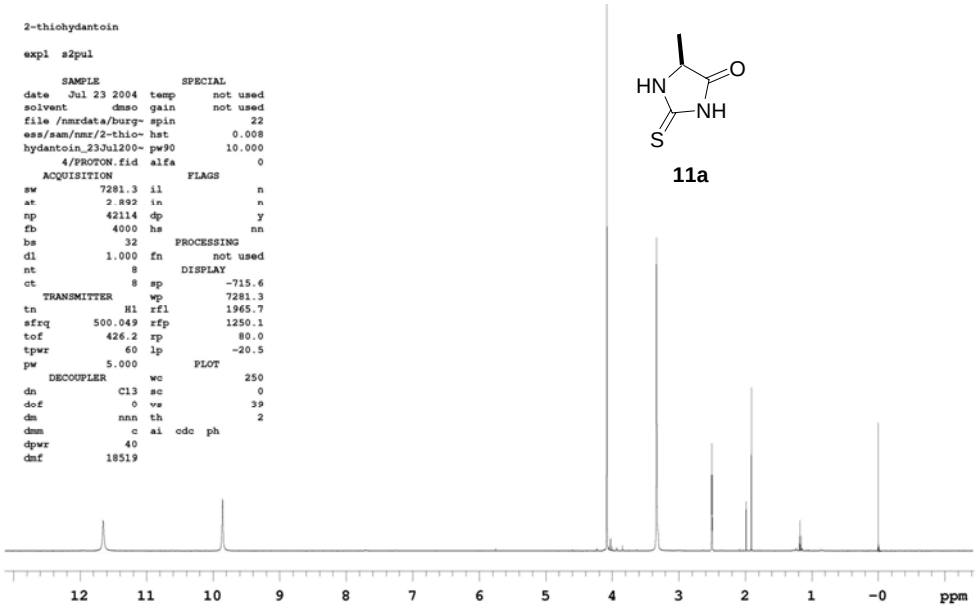


^{13}C NMR of compound **14c**

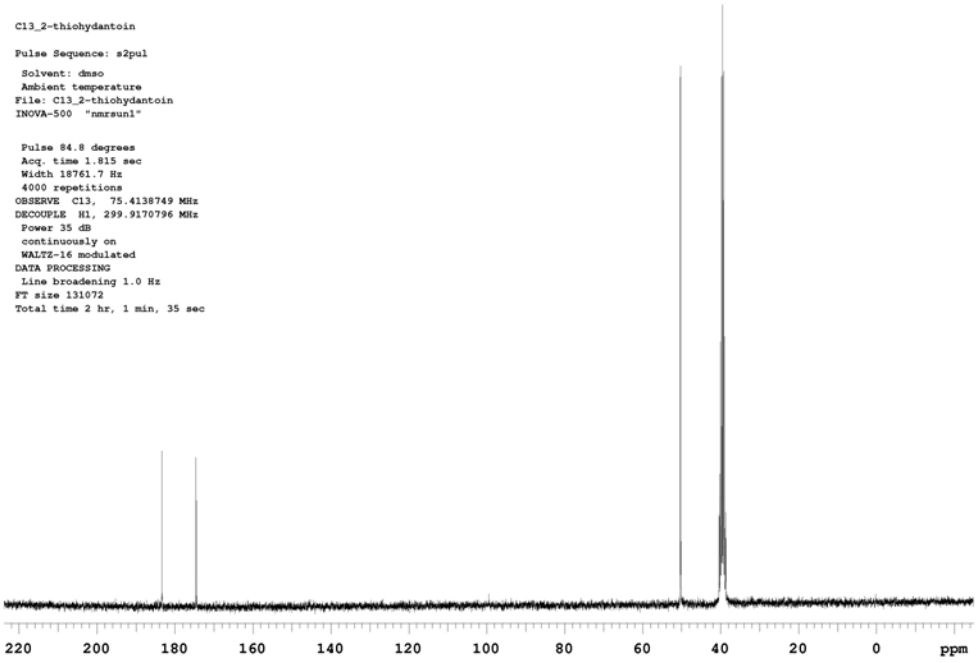
COSY of compound **14c**
The NH peak around 12.4 shows that no cross peak is observed with the C-alpha H at ~5 ppm



General Procedure for Deacylation. Synthesis of 2-Thiohydantoin (11a). A suspension of **14a** (0.148 g, 0.94 mmol) and ~5mL of 3M HCl in a microwave tube was sealed and heated under microwave irradiation using a CEM microwave (Discover) at 150 °C for 5 min. The resulting clear, yellow solution was extracted with 4 x 5ml EtOAc. The combined EtOAc extracts were dried over Na2SO4, concentrated, and dried under vacuum to give 4a as a light orange solid (0.081g, 74%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.66 (s, 1H), 9.86 (s, 1H), 4.08 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 183.4(C), 174.6(C), 50.3(CH2).

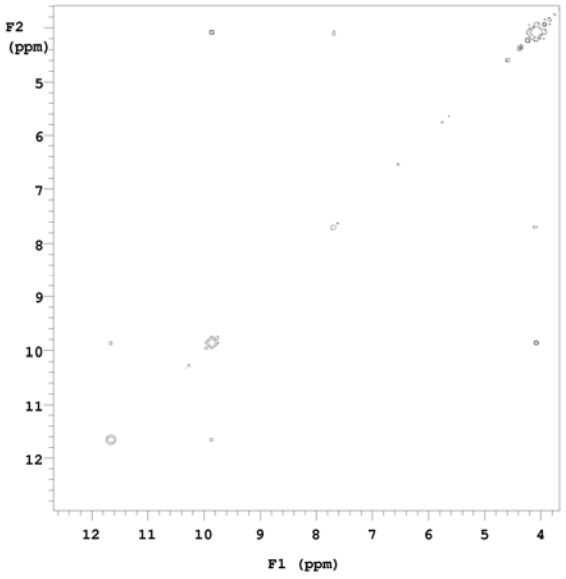


^{1}H NMR of compound **11a**

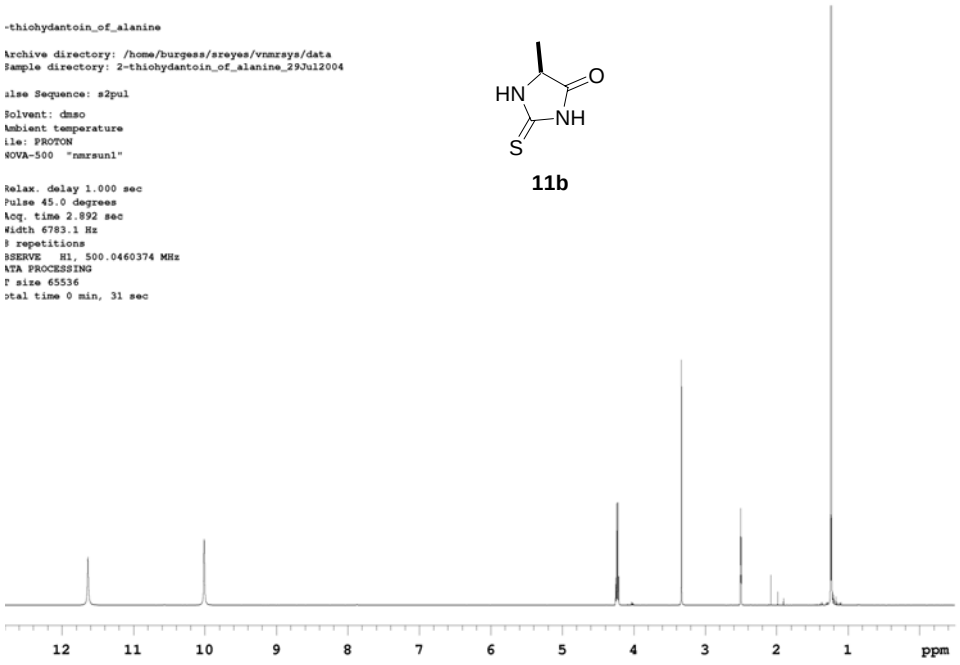


^{13}C NMR of compound **11a**

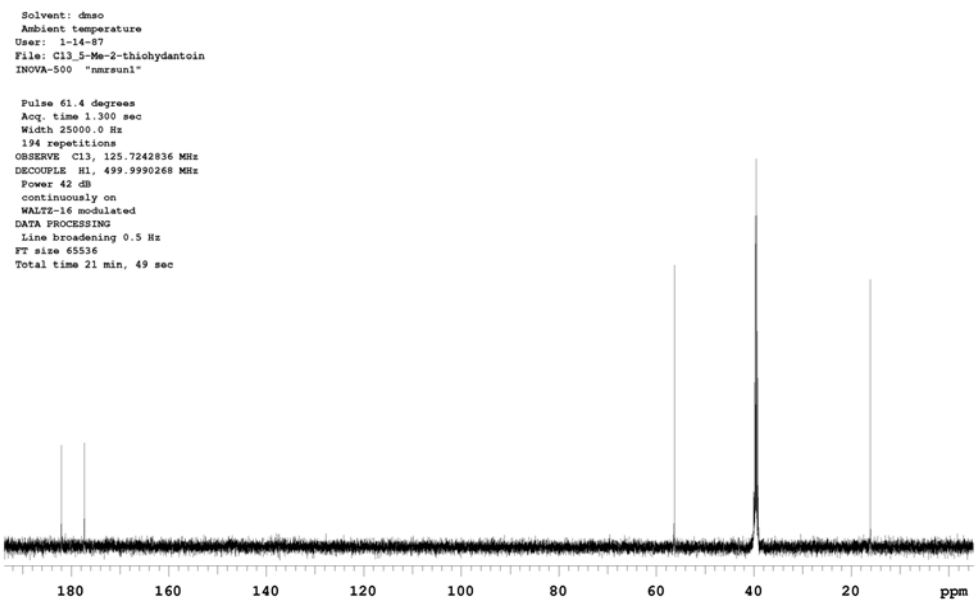
COSY of compound **11a**
After deacylation, the N1 is now protonated and resonates ~9.9 ppm. A cross peak is observed with the C-alpha H ~4 ppm.



5-Methyl-2-thiohydantoin (11b). Compound **14b** was used to prepare the title compound, which followed the above procedure. After drying of the almost colorless solution, **11b** appears as white solid (66 mg, 87 %).[1]H NMR (500 MHz, DMSO-d 6): δ= 11.64 (s, 1H), 10.01 (s, 1H), 4.23 (dddd, J = 1.1, 7.1, 7.1, 7.1 Hz, 1H), 1.24 (d, J = 7.1 Hz, 3H); [13]C NMR (125 MHz, DMSO-d_6): δ= 182.0(C), 177.3(C), 56.3(CH), 16.1(CH3).

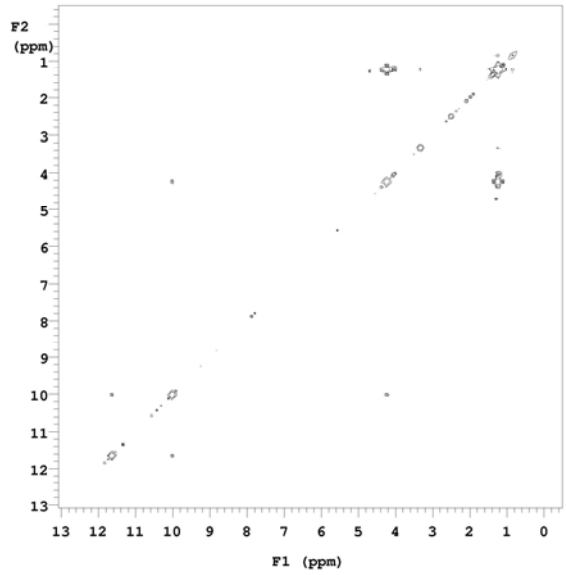


[1]H NMR of compound **11b**

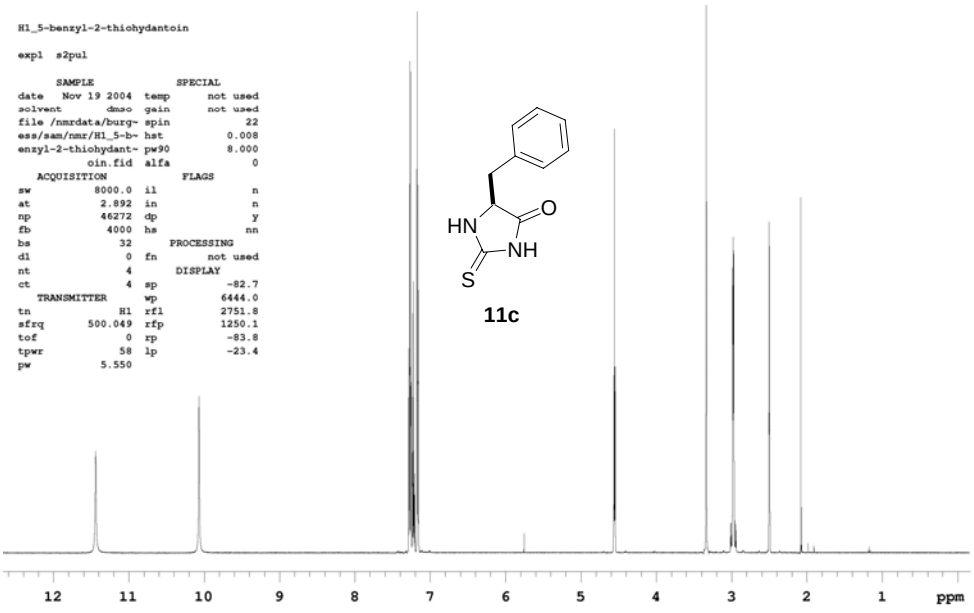


^{13}C NMR of compound **11b**

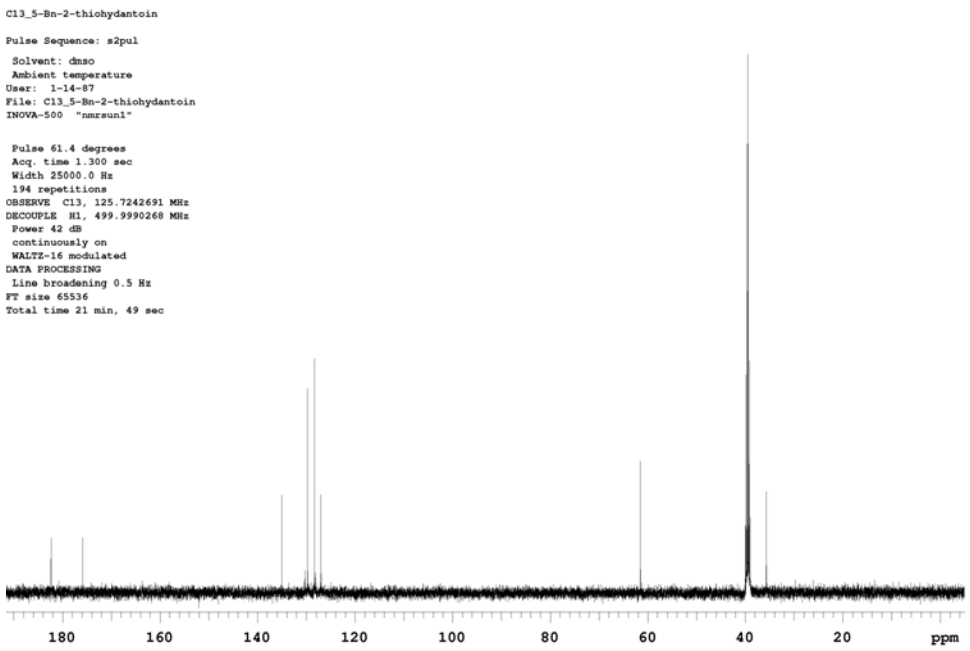
COSY of compound **11b**
After deacylation, the N^1 is now protonated and resonates ~10 ppm. A cross peak is observed with the C-alpha H ~4.2 ppm



5-Benzyl-2-thiohydantoin (11c). Compound **14c** was used to prepare the title compound which followed the above procedure. After drying of the colorless liquid, **11c** appears as white solid (0.35 g, 84%). 1H NMR (500 MHz, DMSO-d 6): δ = 11.44 (s, 1H), 10.07 (s, 1H), 7.27 (m, 2H), 7.22 (m, 1H), 7.17 (m, 2H), 4.56 (t, J = 5.1 Hz, 1H), 2.98 (dd, J = 5.0, 2.8 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 182.3(C), 175.8(C), 135.0(C), 129.7(CH), 128.3(CH), 127.0(CH), 61.5(CH), 35.7(CH2).

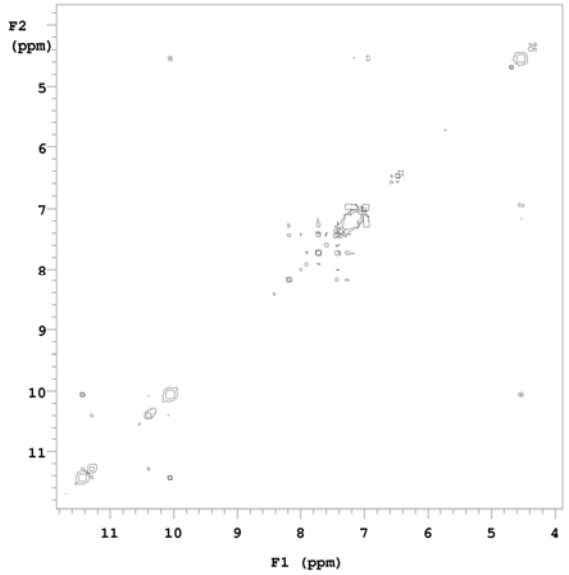


^{1}H NMR of compound **11c**



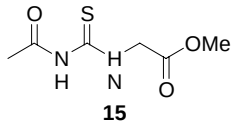
^{13}C NMR of compound **11c**

COSY of compound **11c**
After deacylation, the N^1 is now protonated and resonates ~10 ppm. A cross peak is observed with the C-alpha H ~4.5 ppm



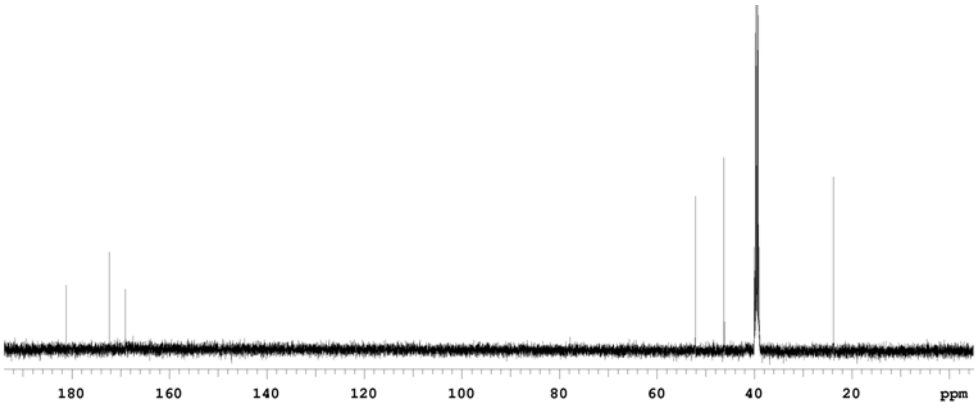
Synthesis of 5-Acetyl-4-thiohydantoic Acid Ethyl Ester (15). To a mixture of glycine methyl ester hydrochloride salt (0.50 g, 3.98 mmol) and Et_3N (0.350 mL, 3.98

mmol) in 15 mL dry CH_2Cl_2, acylisothiocyanate (0.559 mL, 3.98 mmol) was added slowly. The mixture was stirred for 1 h at 25 °C, after which a reflux condenser was attached. The temperature was raised to 40 °C and stirring was continued for another hour. After one hour, a TLC of the reaction showed a faint major spot (R_f = 0.41, 40% EtOAc/hexanes). The red brown mixture was cooled to room temperature and then evaporated in vacuo. The residue was redissolved in 15 mL CH2Cl2 and washed with 10 mL water and 10 mL brine and dried over Na2SO4. The solution was concentrated and dried in vacuo to give **15** as an off white solid (0.475 g, 75%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.36 (s, 1H), 10.85 (t, J = 5. 7 Hz, 1H), 4.37 (d, J = 5.7 Hz, 2H), 3.66 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 181.2(C), 172.3(C), 169.0(C), 52.1(CH), 46.2(CH3), 23.8(CH3); HRMS (ESI) calcd for $C_6H_{10}N_2O_3S$ 191.0412 $(M+H)^+$, found 191.0497.

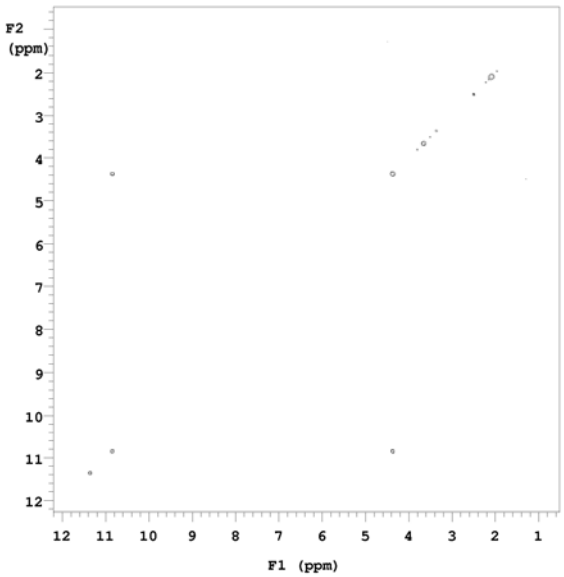


15

^{1}H NMR of compound **15**



^{13}C NMR of compound **15**

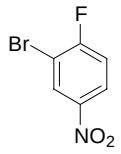


COSY of compound **15**

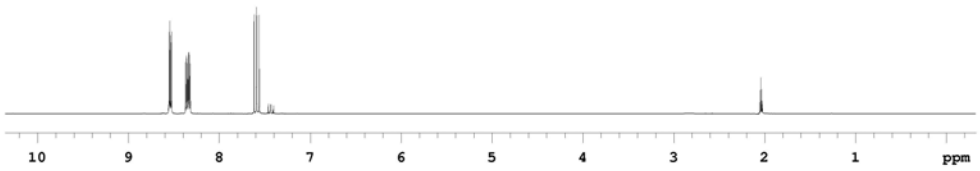
APPENDIX E

EXPERIMENTAL FOR CHAPTER VI

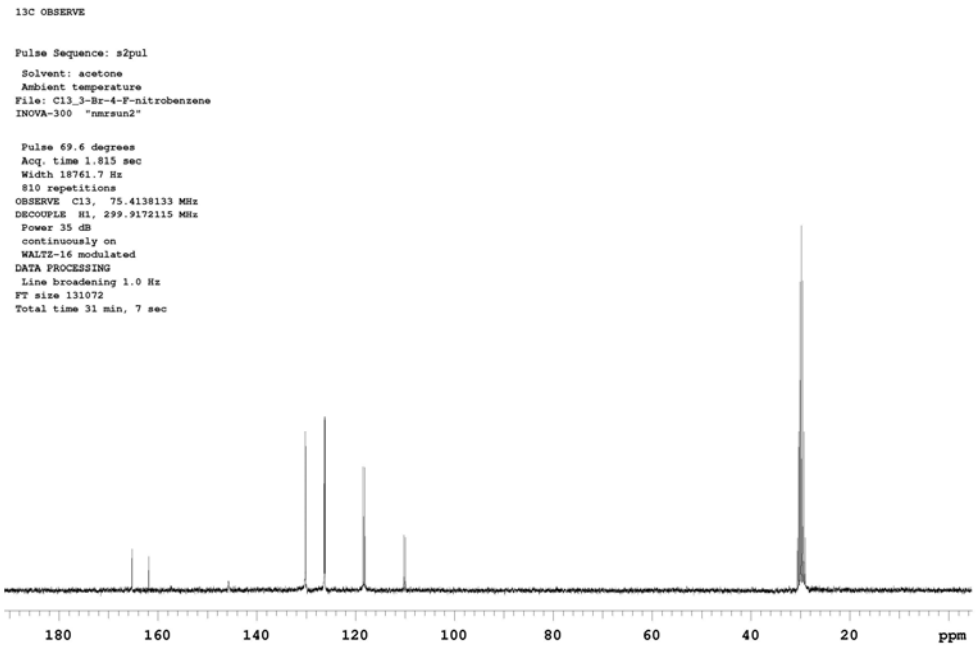
Synthesis of 3-bromo-4-fluoronitrobenezene 21. A two-neck 500 mL round bottom flask was charged with 4-fluoronitrobenzene (10 mL, 94.3 mmol), Na_2SO_4 (0. 15 g), concd H_2SO_4 (100 mL) and water (96.2 mL). The reaction flask was heated to 65 °C via oil bath and then $NaBrO_3$ (17.1 g, 113 mmol) was added in portions to maintain the temperature at 65 °C. The addition took 20 min. The mixture was stirred for another 3 h at the same temp. After this time, the flask was cooled to room temp. Two layers were produced from the reaction. The layers were separated and the aqueous layer extracted with 50 mL EtOAc (3x). The organic extracts were combined, washed with brine, dried over $Na_2SO_\$$ and concd in vacuum. The resulting liquid was placed in a vacuum pump and after one day, crystals came out. The crystals were filtered and recrystallized from hexane to give the product as light brown crystals (9.7 g, 46 %). ^{1}H NMR (300 MHz, acetone- d_6): δ = 8.52 (dd, J = 5.9, 2.8 Hz, 1 H), 8.36-8.31 (m, 1H), 7.58 (t, J = 8.6 Hz, 1H); ^{13}C NMR (75 MHz, acetone- d_6): δ = 165.2, 161.9, 130.1, 126.2, 118.3, 110.1.



21

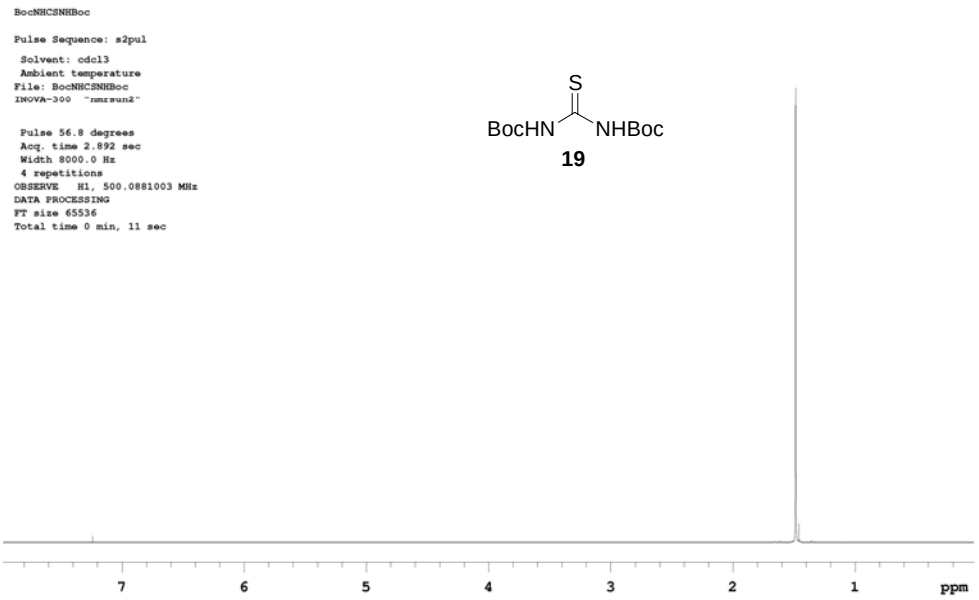


^{1}H NMR of compound **21**



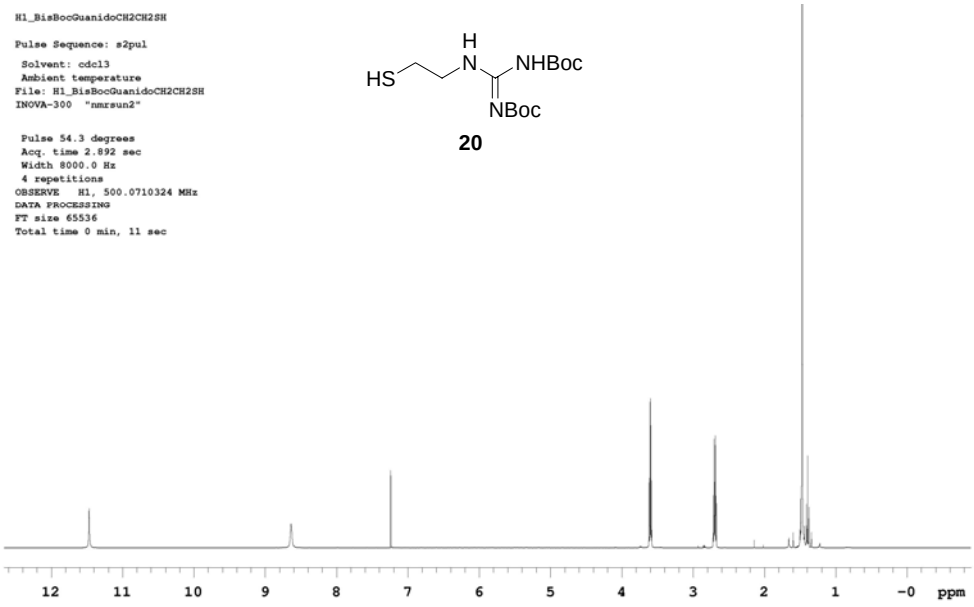
^{13}C NMR of compound **21**

Synthesis of BisBocThiourea 19. To an oven-dried two-neck 250 mL round bottom flask, thiourea, (0.571 g, 7.5 mmol) was added. The flask was stoppered and was purged with nitrogen for 5 min. After this time, 125 mL dry THF was added to dissolve thiourea. The solution was cooled to 0 °C via immersion in an ice-bath. Then, NaH (60 % in oil; 1.35 g, 33.8 mmol) was added and the mixture stirred for 5min. The flask was then removed from the ice-bath and stirring was continued for 10 min after which it was returned to the ice-bath, then Boc_2O (3.6 g, 16.5 mmol) was added. The slurry mixture was stirred for 1h at 0 °C and for 3 h at room temp. After this time, TLC indicates consumption of thiourea. The reaction was quenched by slowly adding 10 mL saturated $NaHCO_3$. The reaction mixture was then poured into 250 mL water. The layers were separated and the aqueous layer extracted 3x with 75 mL EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuum to give white solids. The crude product was recrystallized in hexane and a small amount of CH_2Cl_2 to give **19** as a white solid (1.5 g, 72 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.49 (s, 18 H).

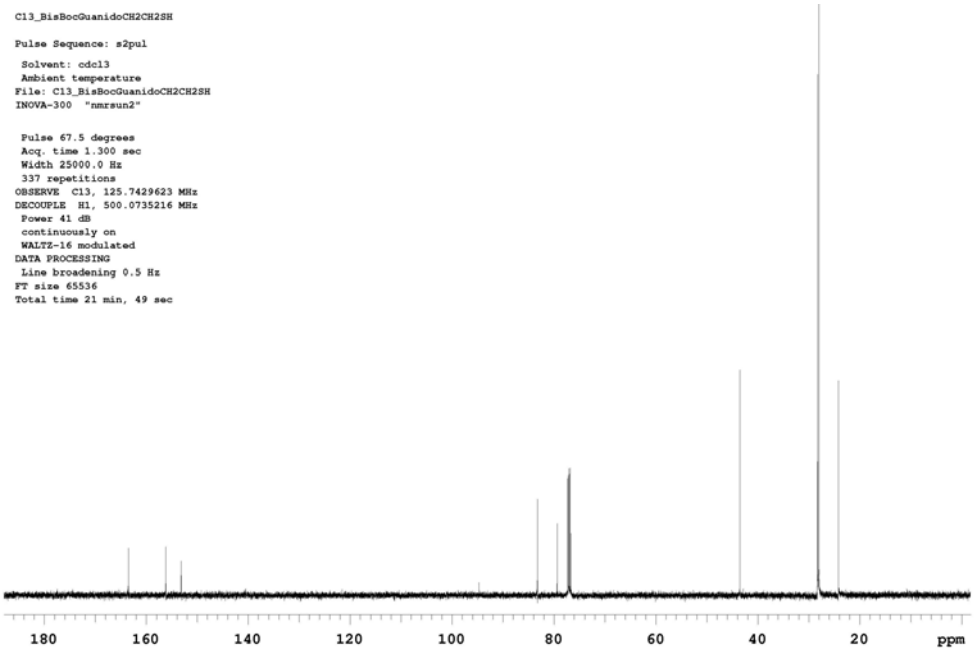


^{1}H NMR of compound **19**

Synthesis of guanidine side-chain mimic 20. A dry 100 mL round bottom flask was charged with cysteamine hydrochloride (0.51 g, 5.0 mmol) and 10 mL anhydrous DMF. To this solution was slowly added Et_3N (1.4 mL, 10.0 mmol).and a cloudy suspension formed. Another 5 mL anhydrous DMF was added followed by compound **19** (1.38 g, 5.0 mmol). The cloudy mixture was stirred for 15 h at 25 °C. After this time, water (15 mL) and EtOAc (30 mL) were added. The layers were separated and the aqueous solution extracted three more times with 15 mL EtOAc. The combined organics were dried over Na_2SO_4, concentrated and dried in vacuum to give compound **20** as a white solid (1.0 g, 63 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 11.47 (s, 1H), 8.64 (s, 1H), 3.62-3.58 (m, 2H), 2.72-2.67 (m, 2H), 1.47 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.5, 156.2, 153.1, 83.2, 79.4, 43.5, 28.2, 28.0, 24.2.



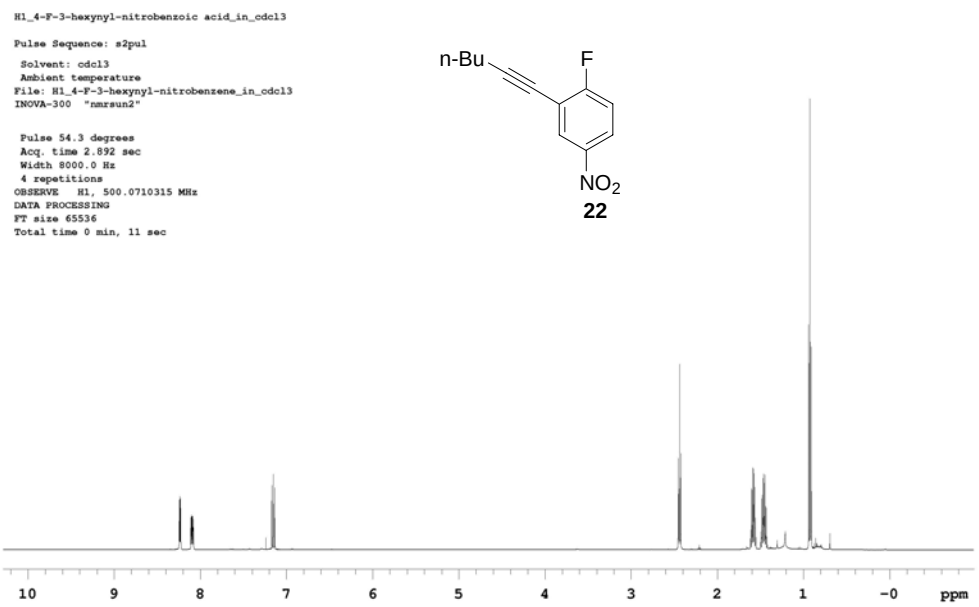
^{1}H NMR of compound **20**



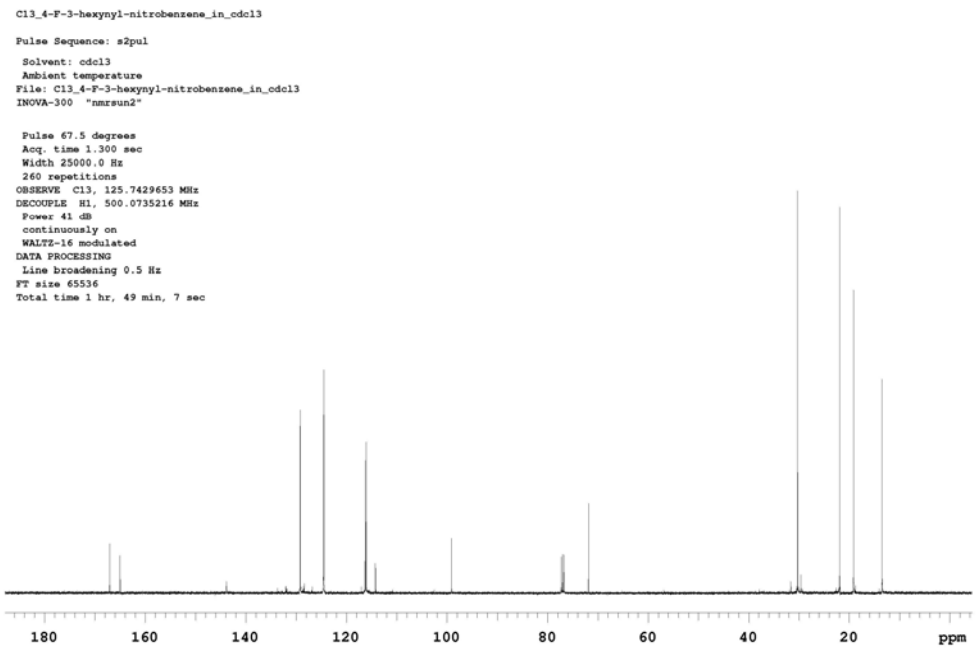
^{13}C NMR of compound **20**

Synthesis of Sonogashira intermediate 22. A dry two-neck 100 mL round bottom flask was charged with compound **21** (2.21 g, 10 mmol) and 40 mL Et_3N. The solution was deaerated by bubbling nitrogen through it with a needle for10 min. After

this time, 1-hexyne (1.16 mL, 10 mmol) was added followed by $Pd(PPh_3)_4$ (0.35 g, 3 mol %) and CuI (0.057 g, 3 mol %). A reflux condenser was attached and the mixture was heated to reflux (*ca* 100 °C) with good stirring for 10 h. After this time, the mixture was filtered on a filter paper and to the filtrate was added 15 mL Et_2O. The solution was evaporated in vacuum leaving a liquid residue. To this residue was added 20 mL Et_2O and the solution extracted three times with 20 mL saturated NH_4Cl solution. The Et_2O solution was then dried over Na_2SO_4 and purified via column chromatography using 3 % Et_2O/hexane then 5 % EtOAc/hex as eluents. Compound **22** was isolated as yellow oil (2.14 g, 97 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.24 (dd, J = 6.05, 3.02 Hz, 1H), 8.11-8.08 (m, 1H), 7.17-7.13 (m, 1H), 2.44 (t, J = 7.2 Hz, 2H), 1.66-1.55 (m, 2H), 1.49-1.42 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.0, 165.0, 129.7, 124.5, 116.1, 114.2, 99.1, 71.8, 30.3, 21.9, 19.2, 13.5.



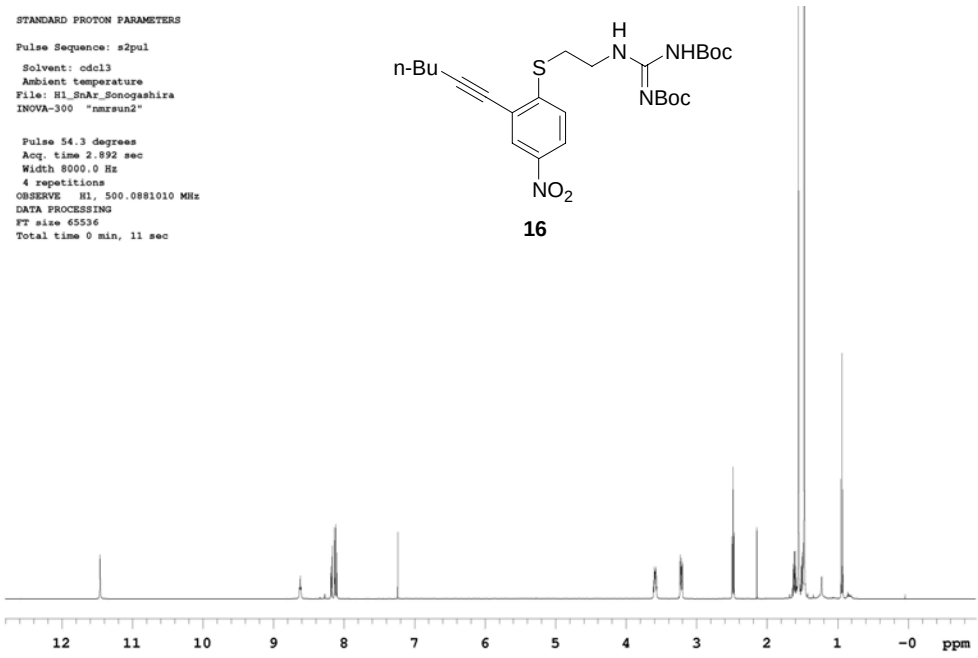
^{1}H NMR of compound **22**



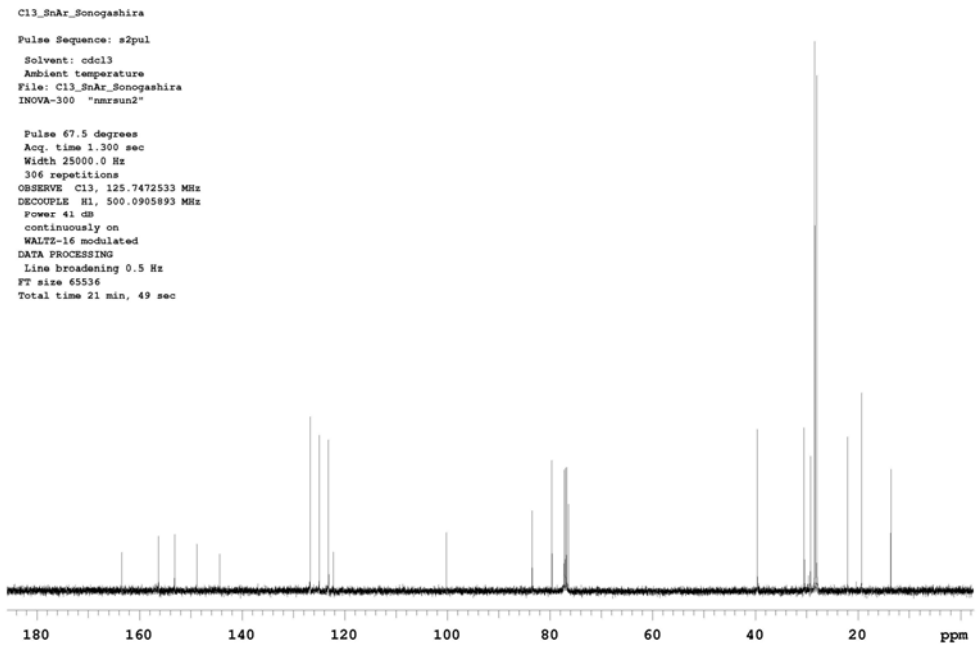
^{13}C NMR of compound **22**

Synthesis of compound 16. A dry 25 mL round bottom flask was charged with 5 mL dry THF and NaH (0.04 g, 1.0 mmol). The flask was cooled down to 0°C then a solution of compound **20** in 5 mL dry THF was added and the mixture stirred from 0 – 25 °C for 12 h (*i.e.* the ice-bath was allowed to melt and warm to 25 °C). After this time, 10 mL water was added slowly to stop the reaction, and the mixture was transferred to a separatory funnel and diluted with 10 mL EtOAc. Additional 5 mL EtOAc was used to wash the flask. The layers were separated and the aqueous layer extracted 3 times with 10 mL EtOAc. The combined organics were washed with 10 mL water (2x) and 10 mL brine (1x) dried over Na_2SO_4, concentrated in vacuum and purified via column chromatography using first 10 % Et_2O/hexanes then 10 % EtOAc/hex as eluents. The fraction corresponding to the product was collected, concentrated and dried in vacuum to give compound **16** as yellow solid (0.27 g, 52 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 11.46 (s, 1H), 8.62 (t, J = 5.9 Hz, 1H), 8.19-8.17 (m, 1H), 8.14-8.10 (m, 2H), 3.61-3.57 (m, 2H), 3.23-3.20 (m, 2H), 2.48 (t, J = 7.0 Hz, 2H), 1.64-1.58 (m, 2H), 1.55 (s, 9H), 1.53-1.49 (m, 2H), 1.47 (s, 9H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}H NMR (125 MHz, $CDCl_3$): δ

= 163.4, 156.3, 153.1, 148.8, 144.4, 126.7, 124.5, 123.1, 122.2, 100.2, 83.5, 79.6, 76.4, 39.6, 30.5, 29.2, 28.4, 28.0, 22.0, 19.3, 13.6.

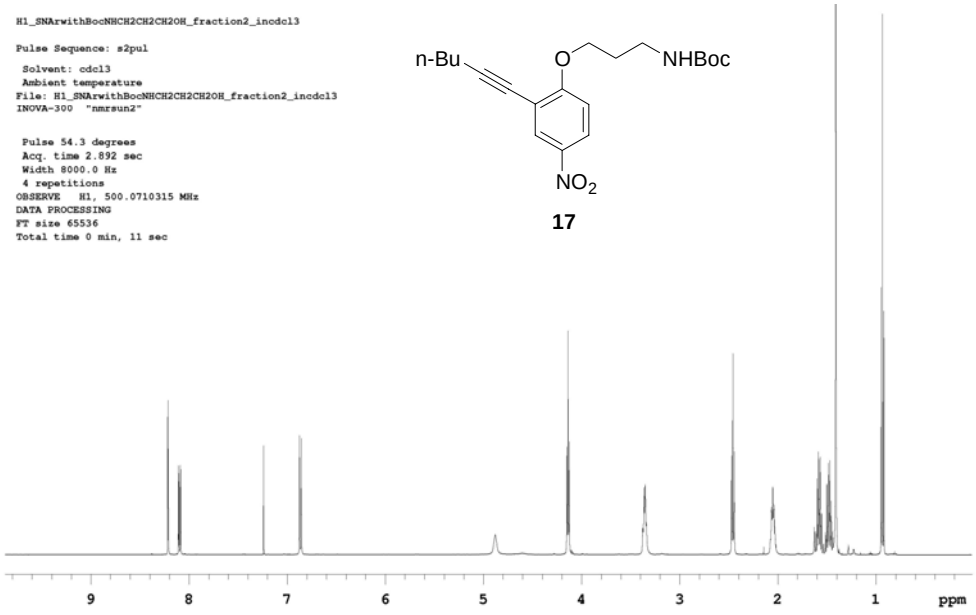


^{1}H NMR of compound **16**

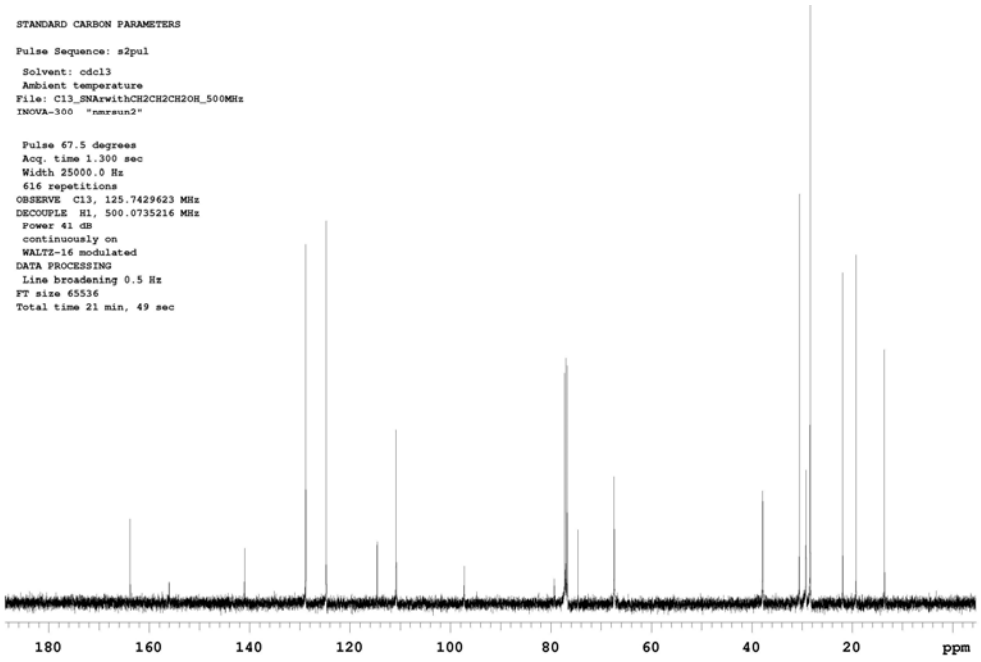


^{13}C NMR of compound **16**

Synthesis of compound 17. The procedure followed that for compound **16** except that in place of compound **20**, *N*-Boc-3-propanolamine was used. The crude product was isolated via column chromatography using 10 % EtOAc/hexanes then 40% EtOAc/hexanes to give the product as light yellow solid (1.08 g, 76 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.21 (d, J = 2.8 Hz, 1H), 8.10 (dd, J = 9.4, 2.8 Hz, 1H), 6.86 (d, J = 9.2 Hz, 1H), 4.87 (s, 1H), 4.14(t, J = 6.0 Hz, 2H), 3.36 (q, J = 6.2 Hz, 2H), 2.46 (t, J = 7.1 Hz, 2H), 2.08-2.02 (m, 2H), 1.63-1.55 (m, 2H), 1.53-1.44 (m, 2H), 1.41 (s, 9H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.8, 166.0, 141.0, 128.8, 124.7, 114.6, 110.8, 97.3, 79.3, 74.6, 67.4, 37.8, 30.5, 29.2, 28.4, 21.9, 19.2, 13.6.

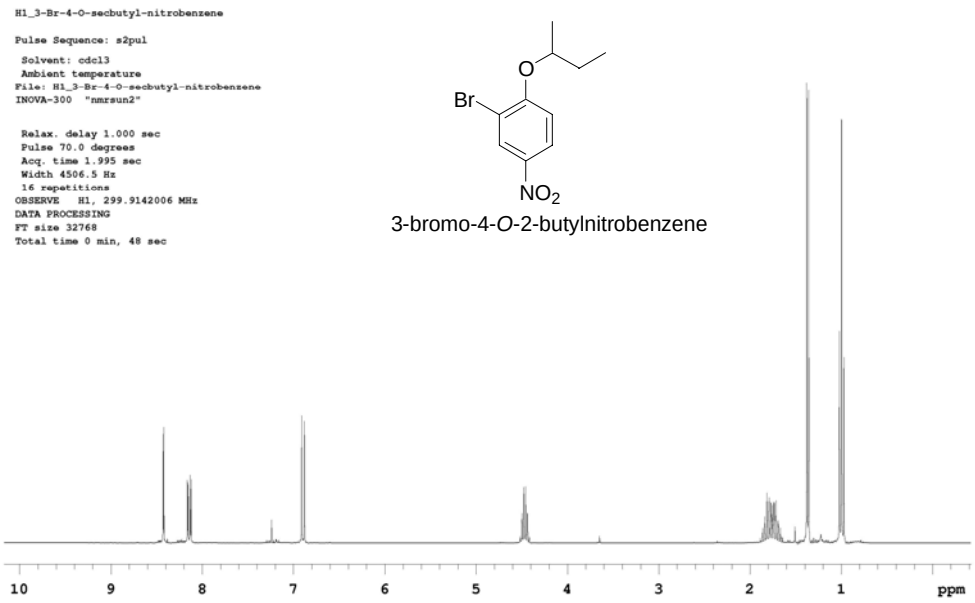


^{1}H NMR of compound **17**

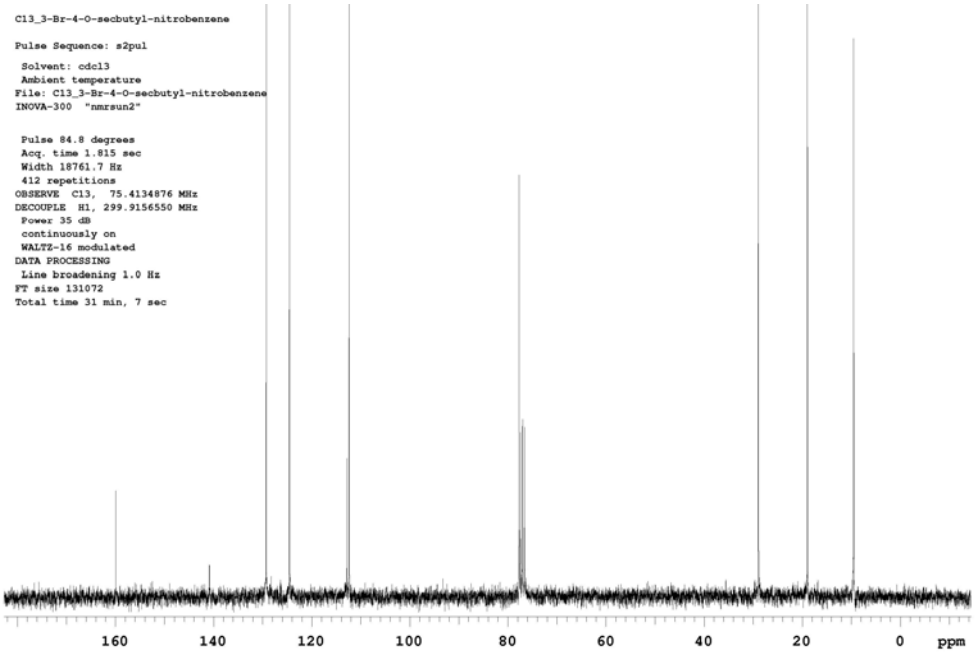


^{13}C NMR of compound **17**

Synthesis of compound 18. In a dry 50 mL round bottom flask, *rac*-2-butanol (0.224 mL, 2.5 mmol) and 10 mL dry THF were added. The solution was cooled to 0 °C and then KO^{t}Bu (0.280 g, 2.5 mmol) was added. The mixture was stirred for 5 min then compound **21** (0.50 g, 2.23 mmol) was added (the color became dark brown after addition). The mixture was stirred for 5 min in ice-bath after which it was removed and allowed to warm to 25 °C and stirring was continued for 5 h. After this time, 5 mL water and 15 mL EtOAc were added. The layers were separated and the water layer was extracted 3 times with 10 mL EtOAc. The organic extracts were combined, dried over Na_2SO_4, concd and dried in vacuum to give 3-bromo-4-*O*-2-butylnitrobenzene as yellow brown oil (0.581 g, 95 %).^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.42 (d, J = 2.8 Hz, 1H), 8.14 (dd, J= 9.1, 2.7 Hz, 1H), 6.89 (d, J = 9.1 Hz, 1H), 4.52-4.42 (m, 1H), 1.89-1.64 (m, 2H), 1.37 (d, J = 6.4 Hz, 3H), 1.00 (t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 159.9, 140.1, 129.3, 124.5, 112.8, 112.4, 77.7, 28.9, 18.9, 9.5.



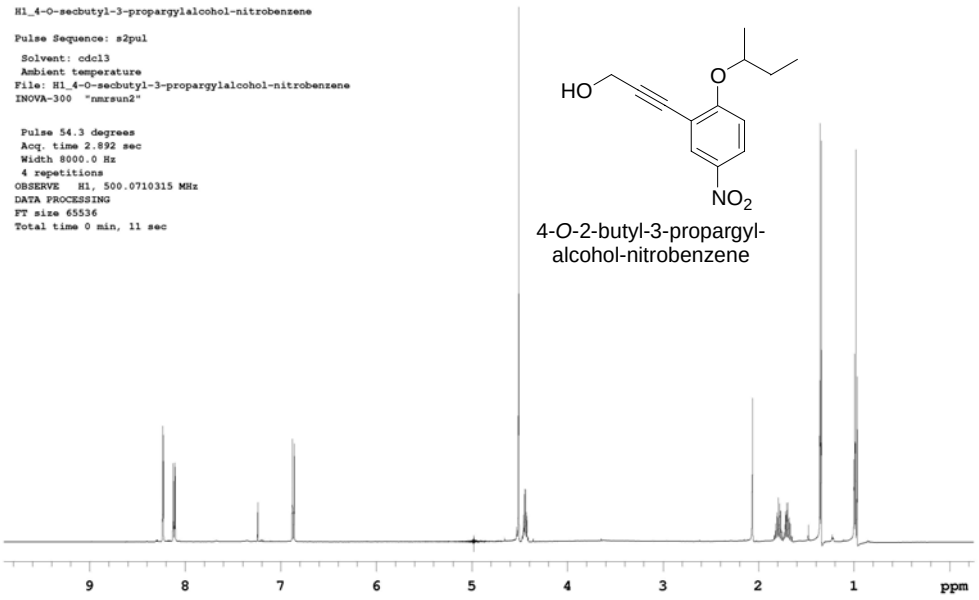
^{1}H NMR of compound 3-bromo-4-*O*-2-butylnitrobenzene



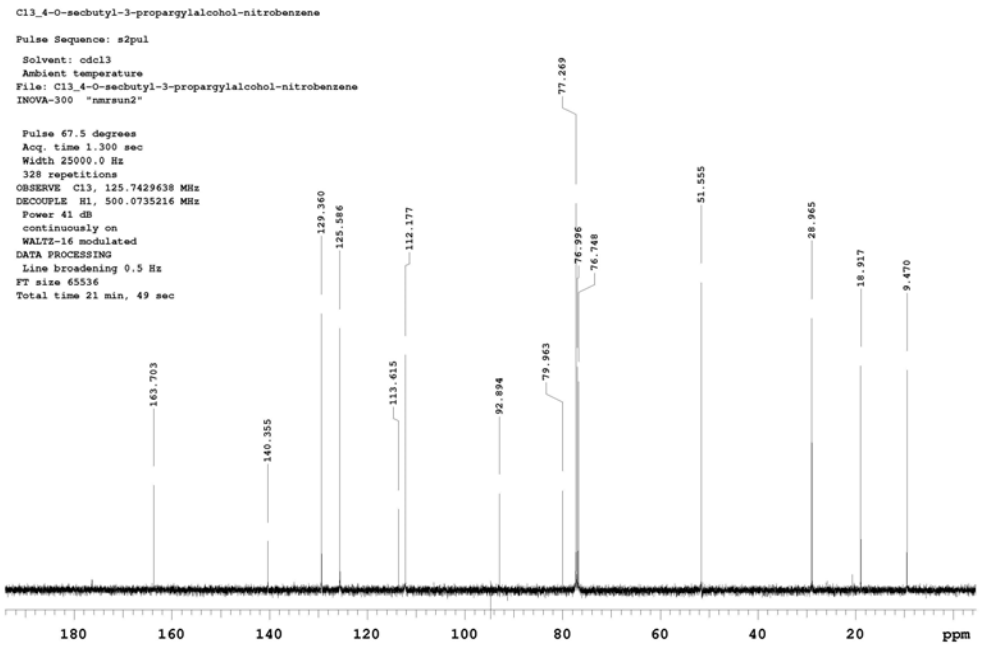
^{13}C NMR of compound 3-bromo-4-*O*-2-butylnitrobenzene

The above product (0.581 g, 2.12 mmol) was placed in a dry 50 mL round bottom flask and was dissolved in 15 mL dry Et_3N. Propargyl alcohol (0.123 mL, 2.12 mmol)

was added and the solution was deaerated by bubbling nitrogen through for 20 min. The resulting solution was transferred to a two-neck 50 mL round bottom flask containing CuI (20 mg, 5 mol %) and $Pd(PPh_3)_4$ (0.122 g, 5 mol %). After the transfer, the two-neck flask was quickly fitted with a reflux condenser and the mixture stirred and heated to reflux (*ca* 95 °C) for 10 h. After this time, 10 mL saturated NH_4Cl was added followed by 20 mL Et_2O. The layers were separated and the aqueous layer extracted further with 10 mL Et_2O (3x). The organic extracts were combined, dried over $MgSO_4$ concentrated and purified via column chromatography using 30% EtOAc/hexanes with 1 % HOAc. The fractions corresponding to the product were collected, concentrated and dried in vacuum to give the Sonogashira product (0.321 g, 61 %).^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.23 (d, J = 2.8 Hz, 1H), 8.11 (dd, J = 9.1, 2.8 Hz, 1H), 6.87 (d, J = 9.3 Hz, 1H), 4.51 (s, 2H), 4.47-4.41 (m, 1H), 2.06 (s, 1H), 1.84-1.75 (m, 2H), 1.73-1.65 (m, 2H), 1.35 (d, J = 6.1 Hz, 3H), 0.98 (t, J = 7.5 Hz, 3H); ^{13}H NMR (125 MHz, $CDCl_3$): δ= 163.7, 140.4, 129.4, 125.6, 113.6, 112.1, 92.9, 80.0, 51.6, 29.0, 18.9, 9.5 (one carbon is missing).



^{1}H NMR of compound 4-*O*-2-butyl-3-propargylalcohol-nitrobenzene

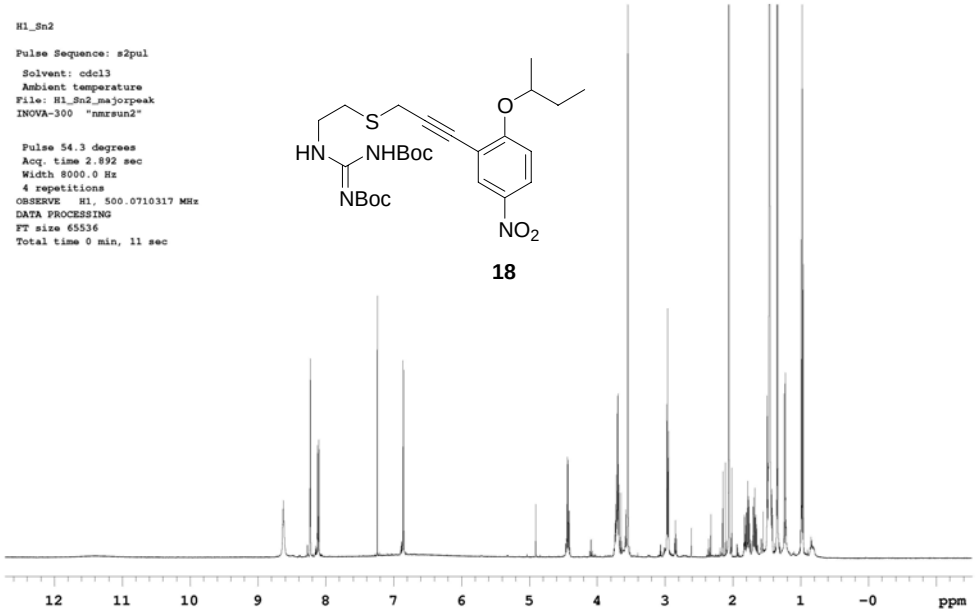


^{13}C NMR of compound 4-*O*-2-butyl-3-propargylalcohol-nitrobenzene

The above product (0.321 g, 1.3 mmol) was placed in a 50 mL dry round bottom flask and dissolved in 10 mL dry CH_2Cl_2. Et_3N was added and the solution was cooled to 0 °C. Mesyl chloride (0.118 mL, 1.52 mmol) was then added slowly and the solution was stirred for 45 min. A TLC indicated consumption of the starting material. The solution was washed with 10 mL cold water, 10 mL cold 5 % HCl, and saturated $NaHCO_3$ and then dried over Na_2SO_4. The solution was concentrated and dried in vacuum to give the mesylated product which was used in the next step without purification.

The crude mesylated product from the above procedure was placed in a 50 mL dry round bottom flask. Dry THF (15 mL) was added to dissolve the compound after which Et_3N (0.725 mL, 5.2 mmol) was added. To this solution was added compound **20** (0.415 g, 1.3 mmol) and the solution was stirred for 10 h. After this time, the solution was evaporated and the residue dissolved in 20 mL Et_2O and washed with 10 mL water (3x), 10 mL brine, and dried over Na_2SO_4. The solution was concentrated and purified via column chromatography using 10% EtOAc/hexanes as solvent to give compound **18** as a yellow solid (200 mg, 27 %). ^{1}H NMR (500 MHz, $CDCl_3$*): δ* = 11.44 (bs, 1H), 8.63 (t, *J* = 5.7 Hz, 1H), 8.23 (d, *J* = 2.9 Hz, 1H), 8.11 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.85 (d, *J* =

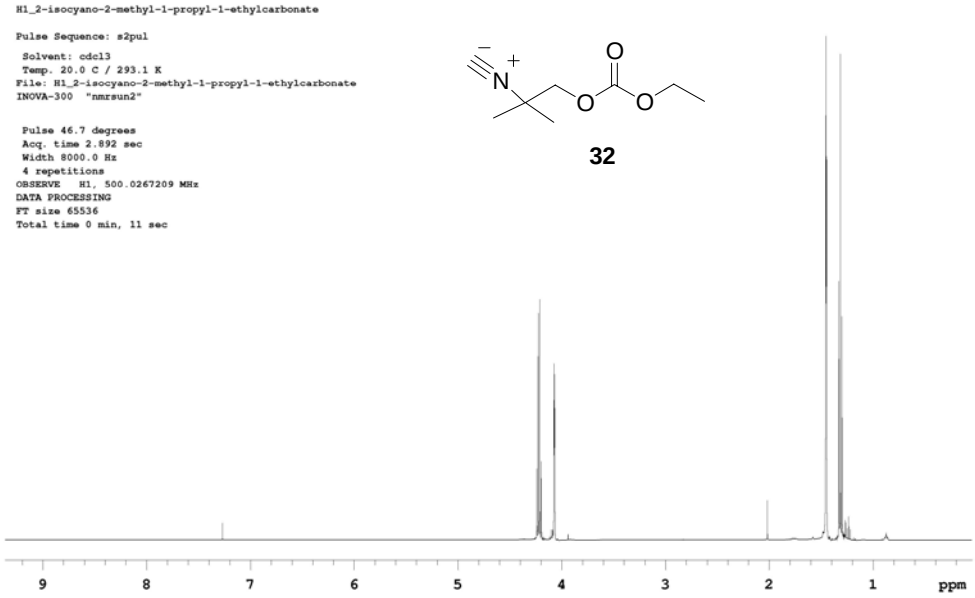
9.3 Hz, 1H), 4.46-4.40 (m, 1H), 3.74-3.68 (m, 2H), 3.55 (s, 2H), 2.96 (t, *J* = 6.5 Hz, 2H), 1.84-1.74 (m, 1H), 1.72-1.62 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.34 (d, *J* = 6.1 Hz, 3H), 0.98 (t, *J* = 7.6 Hz, 3H). MS (ESI) = 551.3 $(M + H)^+$.



^{1}H NMR of compound **18**

Synthesis of convertible isonitrile 32. A dry 100 mL two-neck round bottom flask was charged with 4,4-dimethyloxazoline **31** (5.0 g, 50.4 mmol) and the flask was then purged with nitrogen for 5 min. After this time, 15 mL dry THF was added and the solution was cooled to -78 °C. After equilibration for 10 min, n-BuLi (as 2.5 M/hexane; 22 mL, 55 mmol) was added dropwise via syringe pump for 15 min. The solution turned from clear colorless to clear yellow after addition is completed. The yellow solution was stirred for 1 h at -78 °C then ethyl chloroformate (5 mL, 50.4 mmol) was added dropwise via syringe pump for 5 min. The temperature was kept at -78 °C for 15 min after which the flask was removed from the acetone/dry ice bath. The reaction mixture was allowed to warm to room temperature with continued stirring. This took about 25 min with a change in color from yellow to beige. Then 20 mL Et_2O was added followed by 20 mL water and an additional 20 mL Et_2O. The layers were separated and the Et_2O layer was washed with 20 mL brine and dried over Na_2SO_4. The solution was filtered, concentrated

and dried in vacuum to give isonitrile **32** as a colorless viscous liquid (6.63 g, 77 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.22, (q, J = 7.2 Hz, 2H), 4.07 (m, 2H), 1.46-1.45 (m, 6H), 1.31 (t, J = 7.2 Hz, 3H).

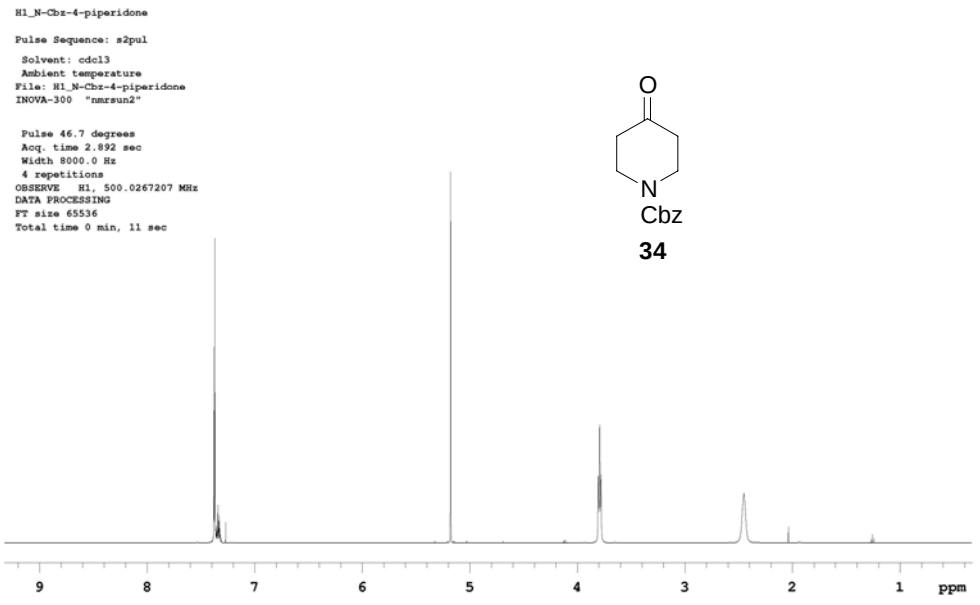


32

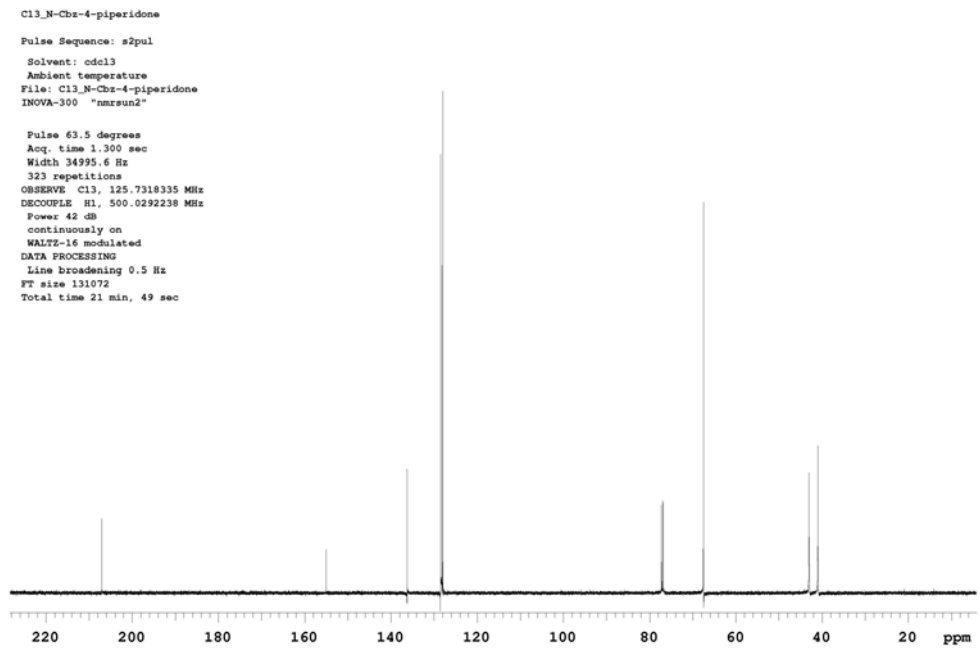
^{1}H NMR of compound **32**

Synthesis of Cbz-protected 4-piperidone 34. A 250 mL round bottom flask was charged with 4-piperidone monohydrate (98%; 5.0 g, 32.5 mmol) and K_2CO_3 (6.7 g, 48.8 mmol). Water (25 mL) was added and the solution was cooled to 5 °C via ice-water bath. Benzyl chloroformate (7.33 mL, 48.8 mmol) was then added dropwise and the mixture was stirred for 1 h at 5 °C. After this time, the flask was removed from the ice-water bath and stirring was continued for 1 h. EtOAc (40 mL) and water (20 mL) were added and the layers were separated. The water layer was extracted further with 20 mL EtOAc (4x). The EtOAc layers were combined and washed with 25 mL 1N HCl then with 25 mL brine and dried over Na_2SO_4. The product was purified via column chromatography first by using 20 % EtOAc/hexanes as solvent then 50 % EtOAc/hexanes. The fractions corresponding to the product were combined and the solvent evaporated in vacuum to give the product as clear colorless oil (7.45 g, 98 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.38-7.37 (m, 4H), 7.35-7.31 (m, 1H), 5.18 (s, 2H), 3.79 (t, J = 6.1 Hz, 4H), 2.45 (s, 4

H); ^{13}C NMR (125 MHz, $CDCl_3$*):* δ = 207.1, 155.0, 136.2, 128.5, 128.5, 127.9, 67.5, 43.0, 40.9.

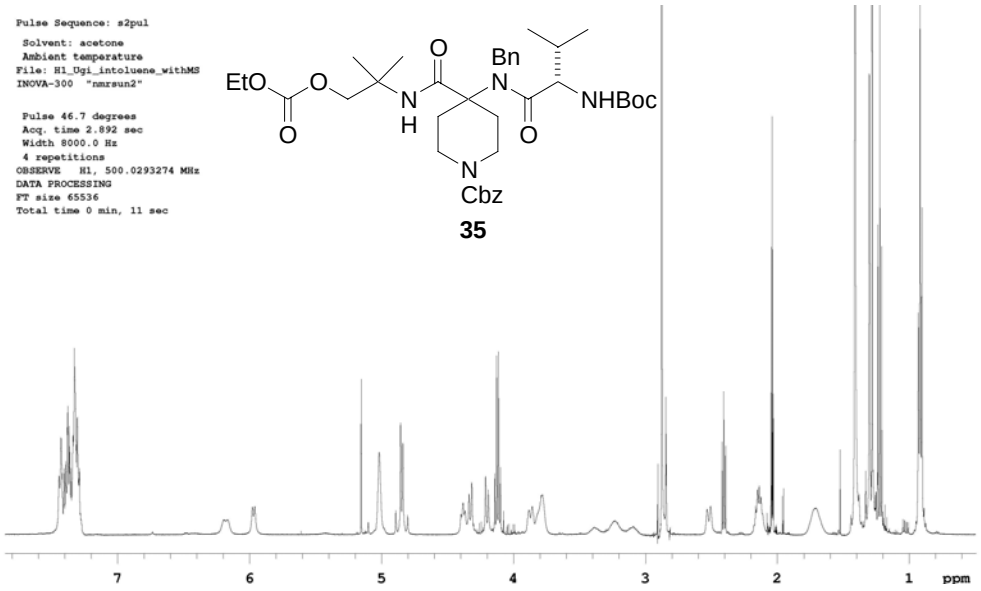


^{1}H NMR of compound **34**

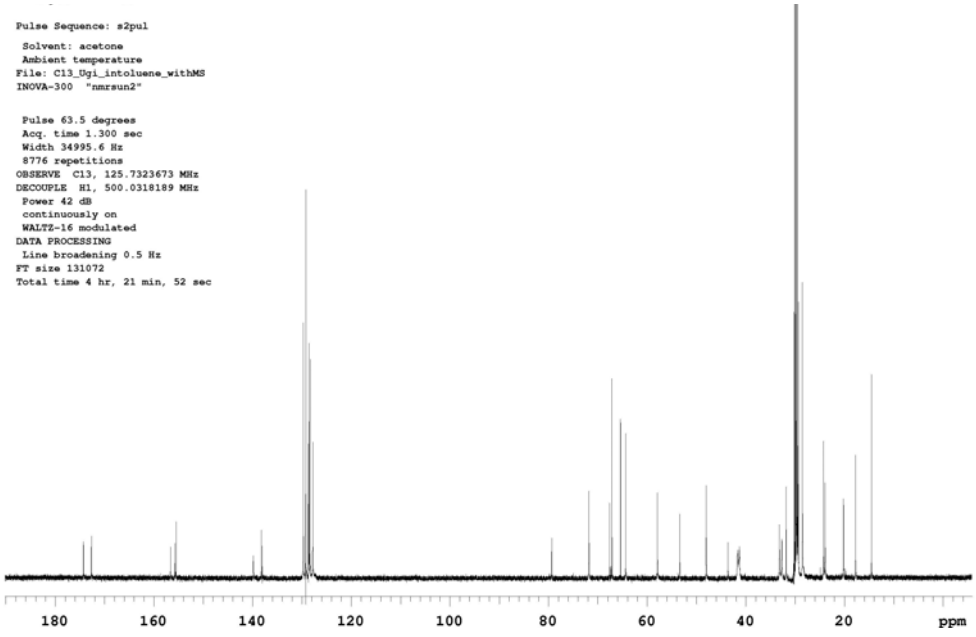


^{13}C NMR of compound **34**

Synthesis of Ugi product 35. A 50 mL dry round bottom flask was charged with compound **34** (0.233 g, 1.0 mmol) and benzyl amine (0.109 mL, 1 mmol). Dry MeOH (10 mL) was added and the solution was stirred for 2h. After this time, isonitrile **32** (0.171 g, 1.0 mmol) was added followed by BocVal-OH (0.217 g, 1.0 mmol). The mixture was stirred for 24 h after which the solution was concentrated to <0.5 mL and the product was isolated via column chromatography first using 10 % EtOAc/hexanes then 50 % EtOAc/hexanes as solvent systems (0.45 g, 64 %). ^{1}H NMR (500 MHz, acetone-d_6): δ = 7.45-7.29 (m, 10H), 6.18 (bs, 1H), 5.97 (d, J = 8.9 Hz, 1H), 5.02, (s, 2H), 4.89-4.80 (m, 2H), 4.40-4.32 (m, 2H), 4.21-4.19 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.88-3.78 (m, 2H), 3.39-3.09 (m, 2H), 2.53-2.50 (m, 1H), 2.42-2.39 (m, 1H), 2.18-2.14 (m, 1H), 1.71 (bs, 2H), 1.41 (s, 9H), 1.30 (s, 3H), 1.28 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.92 (t, J = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, acetone-d_6): δ = 174.3, 172.7, 156.6, 155.7, 155.5, 139.8, 138.2, 138.0, 129.7, 129.2, 128.6, 127.8, 79.3, 71.8, 67.6, 67.1, 65.4, 64.4, 57.9, 53.4, 48.0, 41.4, 32.8, 31.8, 24.1, 20.2, 17.7, 14.5. MS (ESI): $(M + Li)^+$ 717.4.

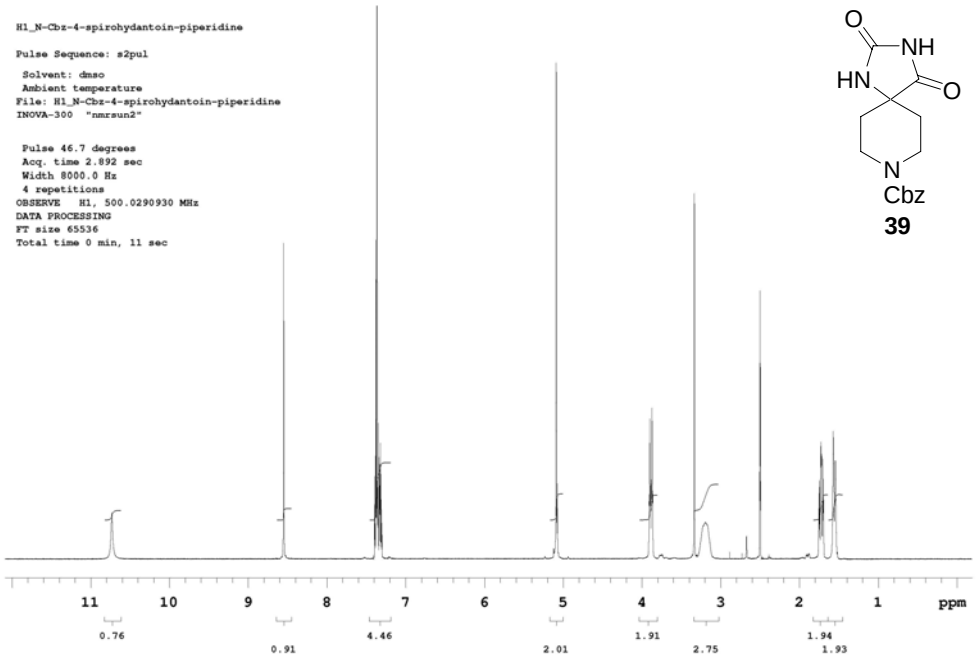


^{1}H NMR of compound **35**

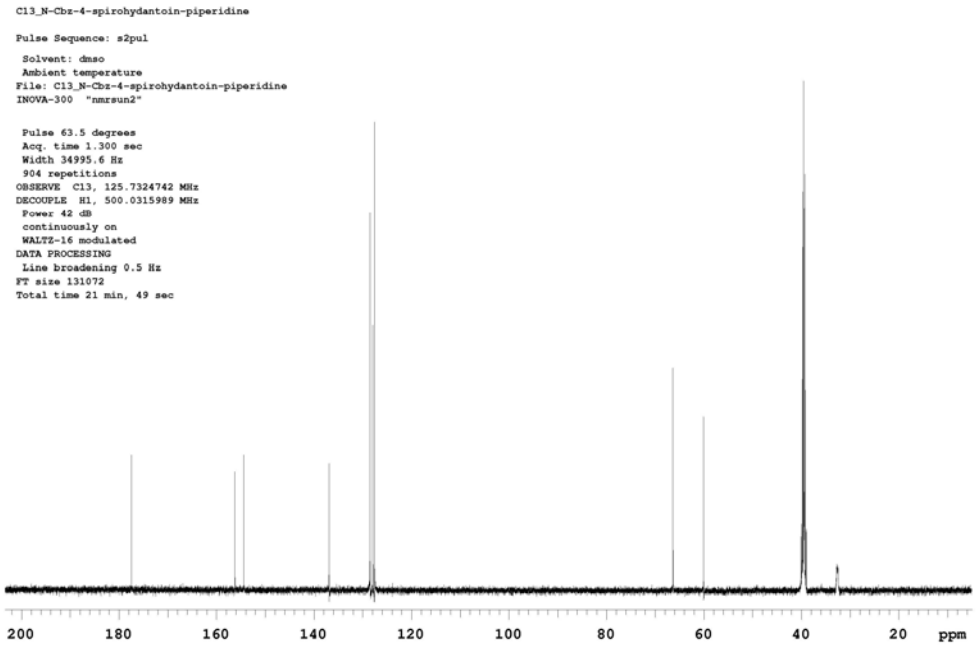


^{13}C NMR of compound **35**

Synthesis of spirocyclic hydantoin 39. A solution of $(NH_4)_2CO_3$ (6.15 g, 64 mmol) in 25 mL deionized water was added into a150 mL pressure flask containing a solution of KCN (1.73 g, 26 mmol) dissolved in 7 mL deionized water. Compound **34** (4.0 g, 17.1 mmol) was added to the reaction flask as a solution in 6 mL DMF. Solids appear to form as **34** is being added dropwise. The flask was sealed and heated to 60 °C with vigorous stirring. Chunks of white solid begin to appear as the mixture was stirred for 4 h. After this time, the mixture was removed from the oil bath and was stirred at room temperature for 12 h. The white precipitate was then filtered and washed with copious amount of cold water followed by 5 mL CH_2Cl_2. The white solid was dried in vacuum overnight and then in a vacuum oven (80 °C) for 8 h (4.85 g, 93 %). ^{1}H NMR (500 MHz, dmso-d_6): δ = 10.73 (s, 1H), 8.55 (s, 1H), 7.39-7.30 (m, 5H), 5.09 (s, 2H), 3.90-3.87 (m, 2H), 3.19 (bs, 2H), 1.75-1.69 (m, 2H), 1.57-1.54 (m, 2H); ^{13}C NMR (125 MHz, dmso-d_6): δ = 177.4, 156.2, 154.4, 136.9, 128.4, 127.9, 127.6, 66.3, 60.0, 40.0, 32.7, 32.4.



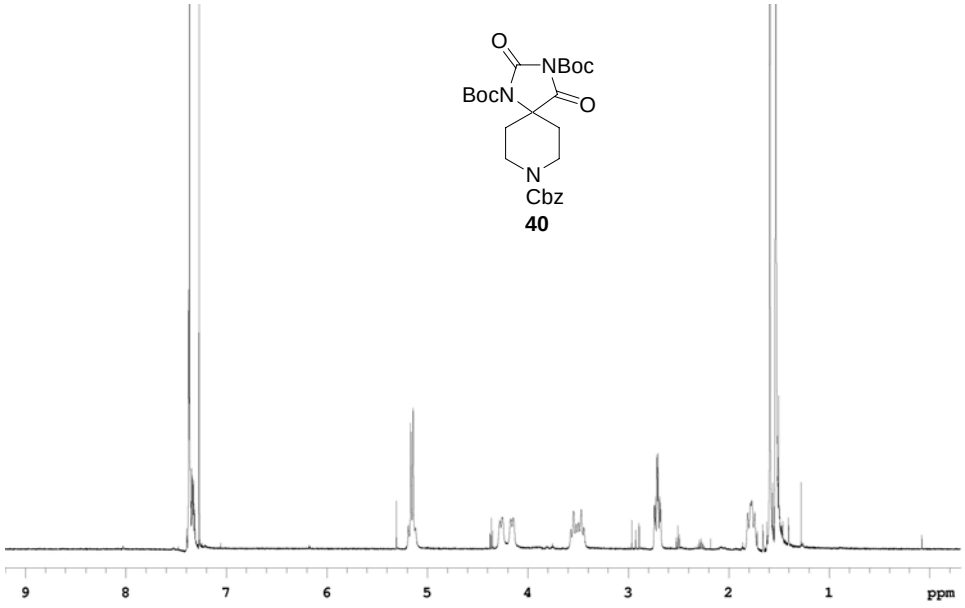
^{1}H NMR of compound **39**



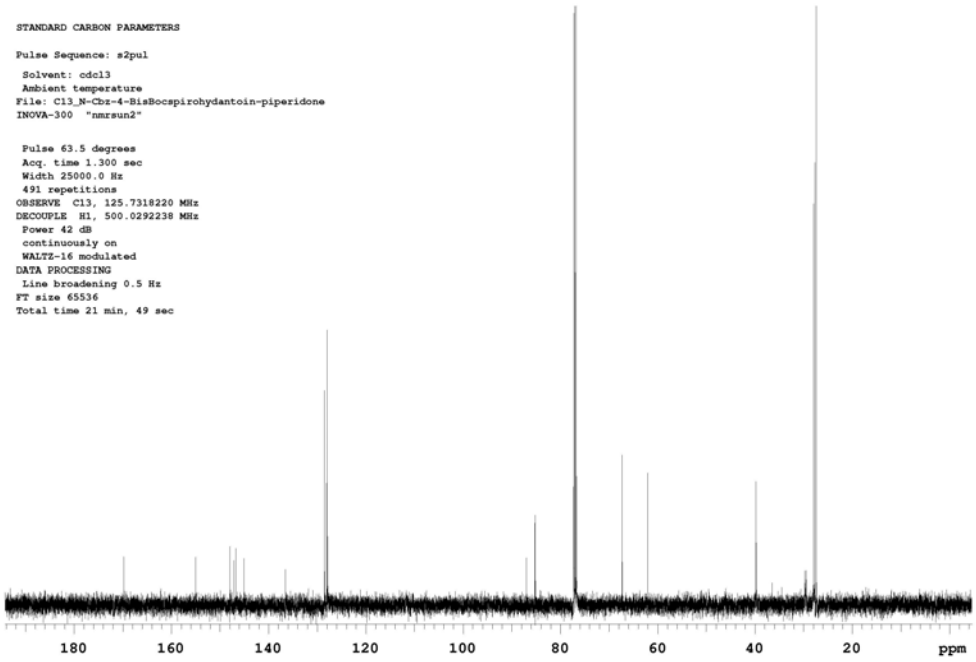
^{13}C NMR of compound **39**

Synthesis of BisBocspirocyclic hydantoin 40. A250 mL three-neck flask was charged with compound **39** (3 g, 9.9 mmol) and DMAP (60 mg, 5 mol %). Anhydrous

DMF (8 mL) and dry THF (35 mL) were then added. To the resulting white mixture was added Boc_2O (as a solution in 35 mL dry THF) dropwise via syringe pump for 13 min. Halfway through the addition, all solids dissolved. The clear colorless solution was stirred at room temperature for 3 h. After this time, the solution was evaporated in vacuum and to the light yellow residue, 80 mL CH_2Cl_2 was added. The resulting solution was washed with 50 mL 1N HCl (2x), saturated 35 mL $NaHCO_3$ (1x) and 25 mL brine (1x) then dried over Na_2SO_4. The solution was filtered, concentrated and dried in vacuum to give the product as white solid (5.0 g, 100 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.38-7.31 (m, 5H), 5.19-5.11 (m, 2H), 4.28-4.14 (m, 2H), 3.57-3.44 (m, 2H), 2.74-2.68 (m, 2H), 1.81-1.74 (m, 2H), 1.59 (s, 9H), 1.53 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 169.7, 155.1, 150.0, 147.2, 146.7, 145.1, 136.6, 128.5, 127.9, 87.0, 85.2, 67.3, 62.1, 39.8, 29.7, 29.5, 27.9, 27.7, 27.4. MS (ESI): 510.2 $(M + Li)^+$.

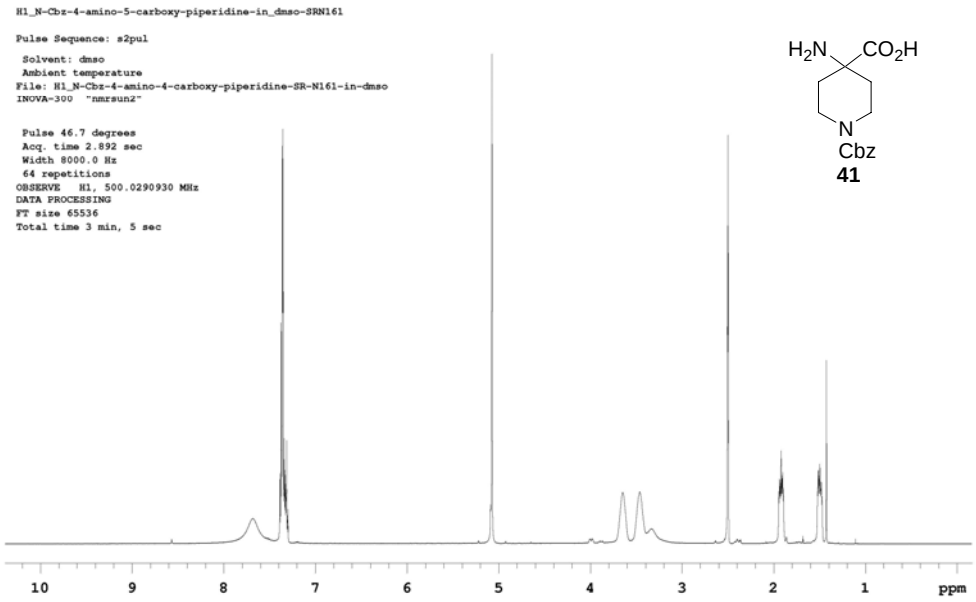


^{1}H NMR of compound **40**



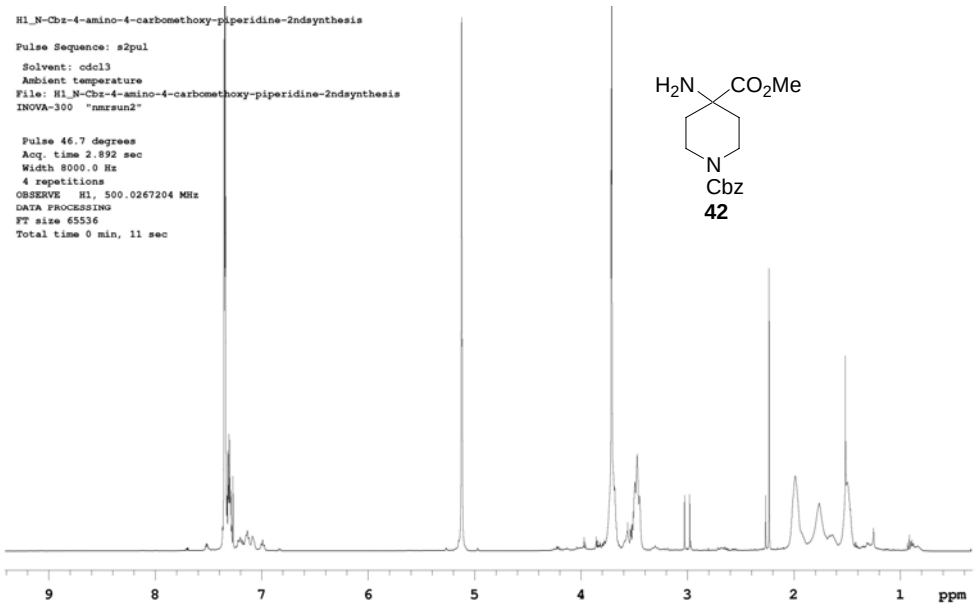
^{13}C NMR of compound **40**

Synthesis of amino acid 41. In a 250 mL round bottom flask, compound **40** (5.0 g, 9.9 mmol) was dissolved in 80 mL THF. Complete dissolution took 10 min. After this time, KOH (5.53 g, 98.5 mmol) dissolved in 60 mL water was added slowly. The mixture was stirred vigorously for 1.5 h. Et_2O (70 mL) and water (30 mL) were then added and the layers were separated. The aqueous layer was extracted once with 50 mL Et_2O and the combined ether layers extracted with 50 mL 1 % KCl solution. The layers were separated and the KCl layer was combined with the original aqueous layer. This layer was cooled to 0 °C via ice-bath and the pH was adjusted to 8 with 6 N HCl, then to 6.5 with 2 N HCl. White precipitate appeared as pH nears to 6.5. Addition of a few drops of 2 N HCl was needed to stabilize the pH at 6.5. The resulting white precipitated was filtered and washed with copious amount of cold water. The filtrate was concentrated to give a second crop of solid which was combined with the first. The combined solids were dried in vacuum at 80 °C for 10 h to give the product as white solid (2.2 g, 80 % based on compound **39**). ^{1}H NMR (500 MHz, dmso-d_6): δ = 7.68 (bs, 2H), 7.39-7.30 (m, 5H), 5.07 (s, 2H), 3.65 (bs, 2H), 3.46 (bs, 2H), 1.95-1.89 (m, 2H), 1.52-1.47 (m, 2H). MS (ESI): 277.1 $(M - H)^-$.

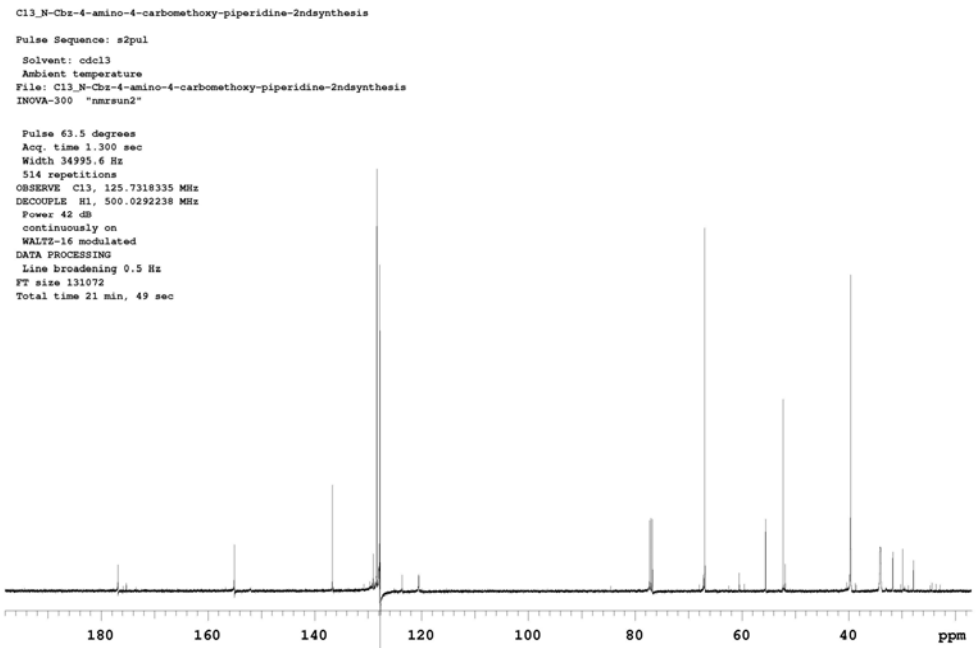


^{1}H NMR of compound **41**

Synthesis of methyl ester 42. A dry 25 mL round bottom flask was charged with amino acid **39** (0.10 g, 0.359 mmol). The solid was dissolved in 5 mL dry MeOH and 5 mL dry CH_2Cl_2 and the solution was cooled to 0 °C. After 5 minutes at this temperature, $TMSCHN_2$ (2M in hexane; 0.54 mL, 1.08 mmol) was added dropwise for 3 min. The yellow green solution was stirred at 0 °C for 15 min. then the ice-bath was removed and stirring was continued for 1.5 h. After this time, 15 mL CH_2Cl_2 and 10 mL saturated $NaHCO_3$ were added to the reaction mixture. The layers were separated and the organic layers was washed with 10 mL brine (2x) and dried over Na_2SO_4. The solution was then filtered, concentrated and dried in vacuum to give the product (93 mg, 88 %). ^{1}HNMR (500 MHz, $CDCl_3$): δ= 7.35-7.34 (m, 5H), 5.12 (s, 2H), 3.71 (s, 3H), 3.51-3.45 (2H), 1.99 (bs, 2H). 1.76 (bs, 2H), 1.50 (bs, 2H) N*H*$_2$ is missing; ^{13}C NMR (125 MHz, $CDCl_3$): δ= 176.8, 155.0, 136.7 128.4, 127.9, 127.7, 66.9, 55.6, 52.3, 39.6, 34.1, 31.7, 28.9, 27.9. MS (ESI): 293.1 $(M + H)^+$.



^{1}H NMR of compound **42**

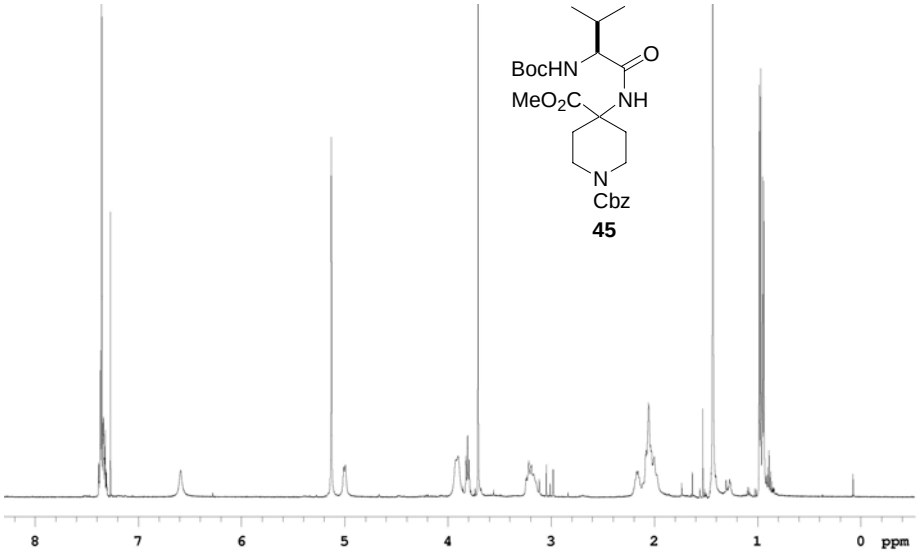


^{13}C NMR of compound **42**

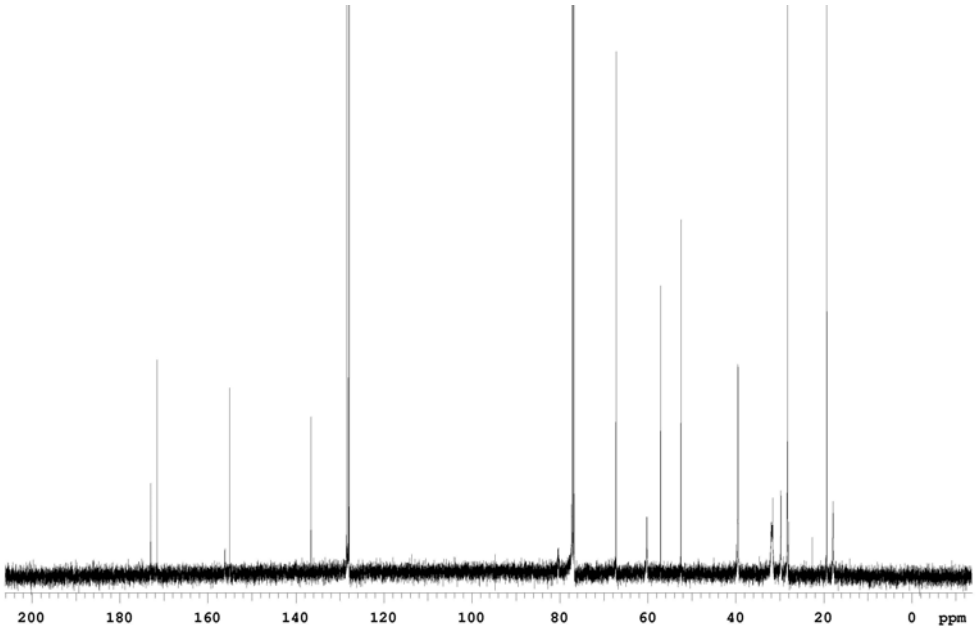
General procedure for dipeptide synthesis using amino methyl ester 42.

Synthesis of dipeptide 45. A 10 mL dry round bottom flask was charged with BocVal-OH (0.35 g, 1.6 mmol) and HOAt (98 %; 0.22 g, 1.60 mmol). The solids were dissolved

in 1 mL anhydrous DMF and then a solution of compound **42** in 0.5 mL anhydrous DMF was added. The reaction mixture was cooled to 0 °C via ice-bath and then EDCI (0.34 g, 1.79 mmol) was added all at once. The yellow solution was stirred at 0 °C for 1 h, then removed from ice-bath and stirred for 24 h at room temperature. After this time, the solution was transferred to a separatory funnel and the reaction flask washed with 20 mL EtOAc. The washings were also transferred to the separatory funnel. Water (100 mL) was then added and the layers were separated. The water layer was extracted with 15 mL CH_2Cl_2 (4x) and the combined organic extracts were washed with saturated 20 mL $NaHCO_3$ (1x), 20 mL water (1x), 15 mL 1N HCl (1x) and 20 mL brine (1x) before being dried over Na_2SO_4. The solution was then filtered, concentrated and dried in vacuum to give the product as white solid (0.74 g, 94 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36-7.30 (m, 5H), 6.55 (bs, 1H), 5.12 (s, 2H), 4.98 (d, J = 8.3 Hz, 1H), 3.94-3.88 (m, 2H), 3.82-3.76 (m, 1H), 3.69 (s, 3H), 3.23-3.15 (m, 2H), 2.21-2.11 (m, 1H), 2.10-1.95 (m, 4H), 0.97-0.92 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.0, 171.6, 156.2, 155.1, 136.6, 128.5, 128.1, 127.9, 67.2, 60.2, 57.1, 52.5, 39.5, 31.9, 31.6, 29.8, 28.3, 28.2, 19.3, 17.9.

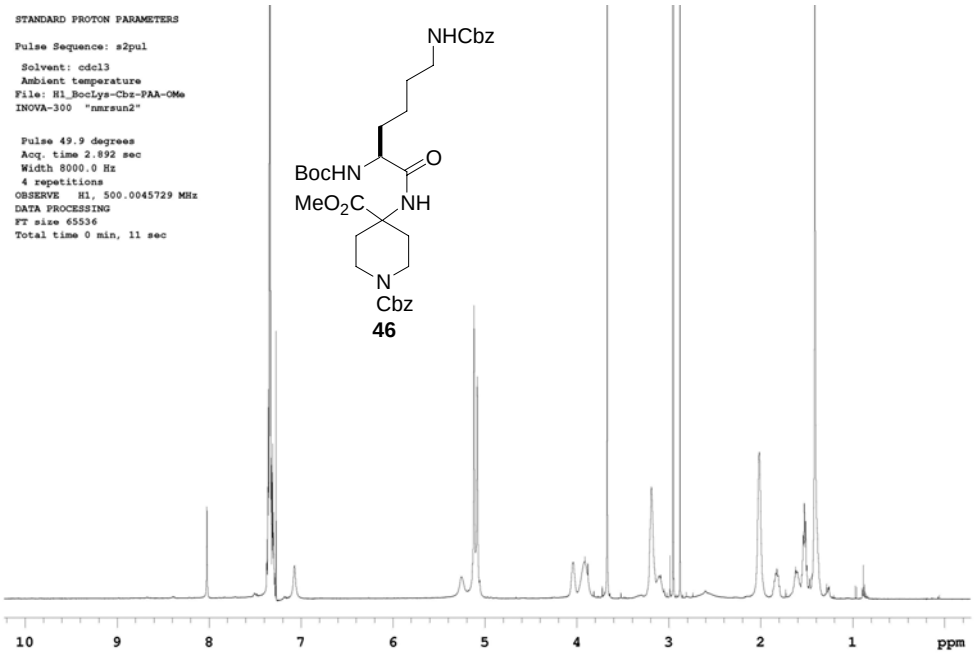


^{1}H NMR of compound **45**

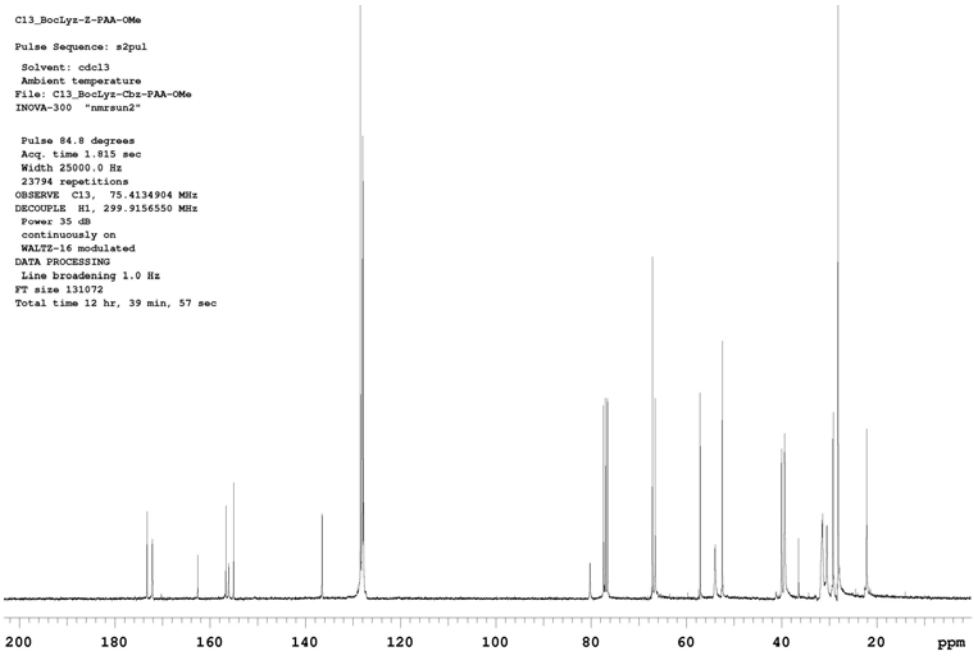


^{13}C NMR of compound **45**

Synthesis of dipeptide 46. The procedure follows that for compound **45** except that for BocVal-OH, BocLys(Cbz)-OH was used. (0.42 g, 96 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ= 7.37-7.32 (m, 9H), 2.31-2.28 (m, 2H), 7.07 (s, 1H). 5.25 (bs, 1H), 5.12 (s, 2H), 5.08 (s, 2H), 4.04 (bs, 1H), 3.91 (bs, 2H), 3.67 (s, 3H), 3.19 (s, 2H), 3.10 (bs, 2H), 2.02 (s, 4H), 1.87-1.79 (m, 1H), 1.67-1.57 (m, 1H), 1.56-1.50 (m, 2H), 1.41 (s, 11H); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 173.2, 172.1, 162.6, 156.7, 156.1, 155.0, 136.5, 136.4, 128.4, 128.2, 128.02, 127.97, 127.8, 80.2, 67.1, 66.6, 57.1, 54.0, 52.5, 40.0, 39.4, 36.5, 31.5, 31.4, 30.5, 29.2, 28.2, 22.2.



^{1}H NMR of compound **46**

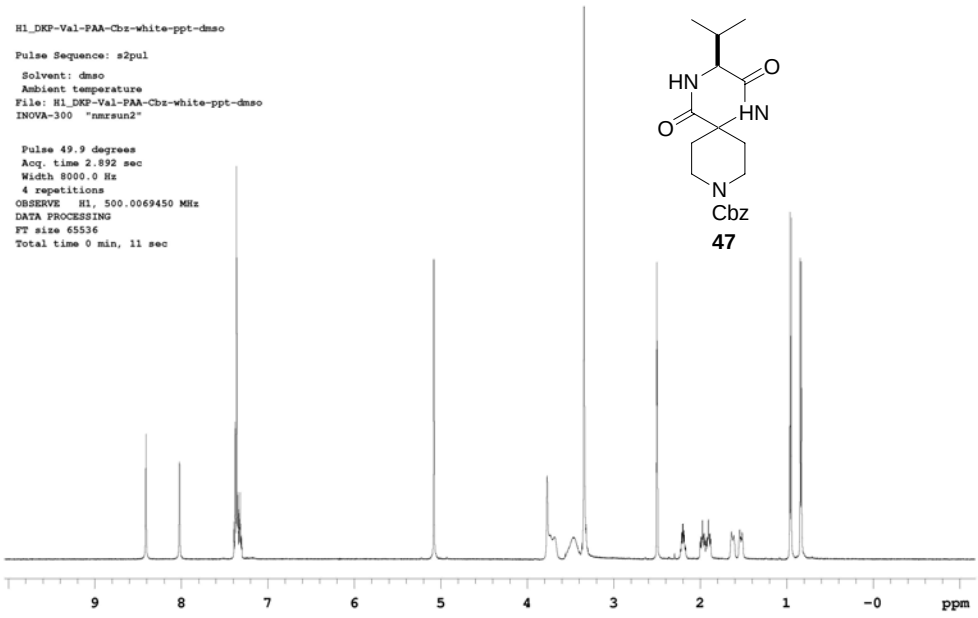


^{13}C NMR of compound **46**

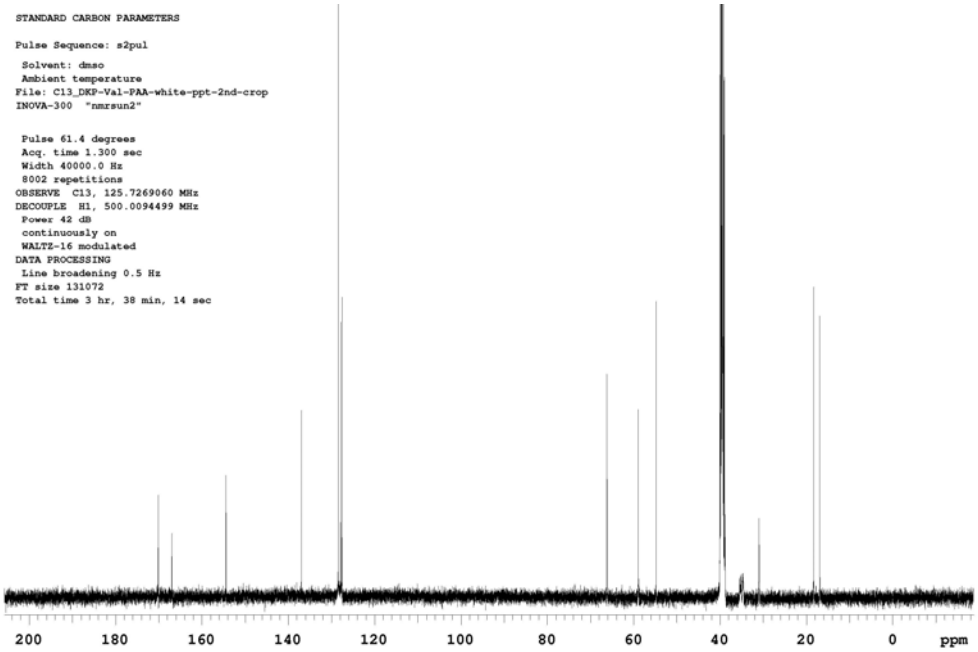
General procedure for the synthesis of spirocyclic diketopiperazines.

Synthesis of DKP 47. In a 10 mL round bottom flask, compound **45** (0.10 g, 0.203 mmol) was dissolved in 1 mL CH_2Cl_2. The solution was cooled to 0 °C and then TFA (0.5 mL, 6.5 mmol) was added. The solution was stirred at the same temperature for 5 min then at 25 °C for 3 h. After this time, saturated $NaHCO_3$ solution was added to make the solution basic (pH 8-9). This was followed by 20 mL CH_2Cl_2 and 10 mL brine. The layers were separated and the aqueous layer was further extracted with 10 mL CH_2Cl_2 (3x). The combined organic extracts were washed with 15 mL brine and dried over Na_2SO_4. The solution was filtered and concentrated in vacuum to give the free amine which was used in the next step without purification.

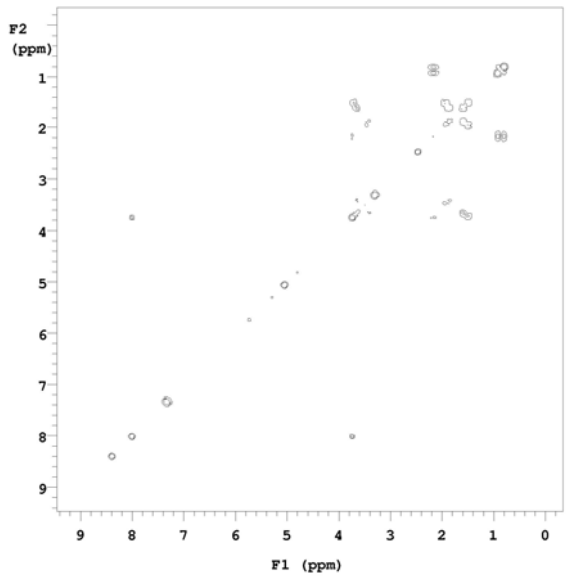
The resulting oily residue from the above procedure was dissolved in 15 mL toluene containing HOAc (resulting concentration is 0.2 %) and was transferred in a Schlenk tube. The solution was then refluxed for 15 h after which white precipitate began to appear. At this time, the reaction was cooled to room temperature and the precipitate was filtered and washed with CH_2Cl_2. The filtrate was recovered, concentrated to 5 mL and was refluxed a second time for 15 h. A second crop of precipitate formed which was filtered, washed with CH_2Cl_2 and was combined with the original crop. The DKP appear as a white solid and was dried in vacuum (46 mg, 63 %). ^{1}H NMR (500 MHz, dmso-d_6): δ = 8.41 (s, 1H), 8.02 (s, 1H). 7.39-7.30 (m, 5H), 5.08 (s, 2H), 3.77-3.68 (m, 3H), 3.46 (bs, 2H), 2.23-2.17 (m, 1H), 2.00-1.88 (m, 2H). 1.65-1.51 (m, 2H), 0.96 (d, J = 7.1 Hz, 3H), 0.84d, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, dmso-d_6): δ = 170.1, 166.9, 154.4, 136.9, 128.4, 127.8, 127.5, 66.2, 58.9, 54.8, 38.8, 35.2, 34.7, 30.9, 18.3, 16.9. MS (ESI): 360.2 $(M + H)^+$.



^{1}H NMR of compound **47**

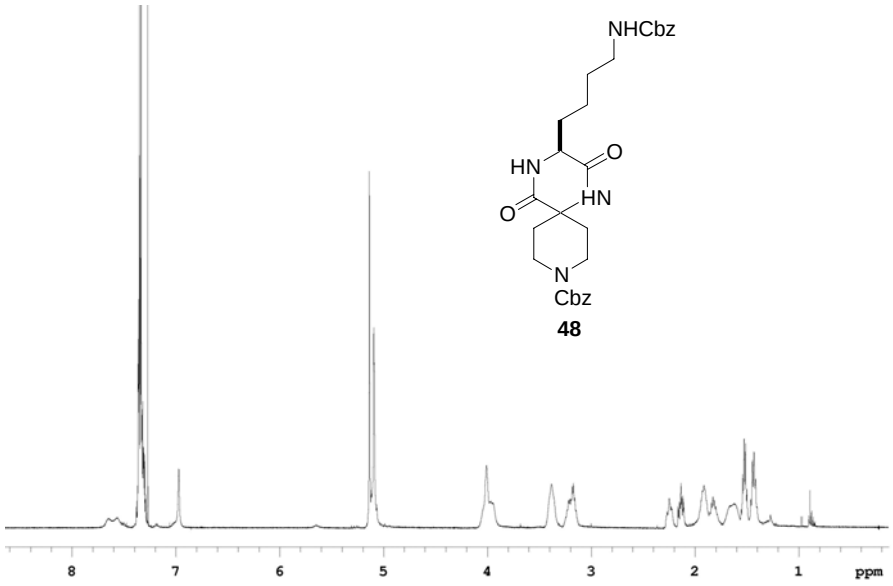


^{13}C NMR of compound **47**

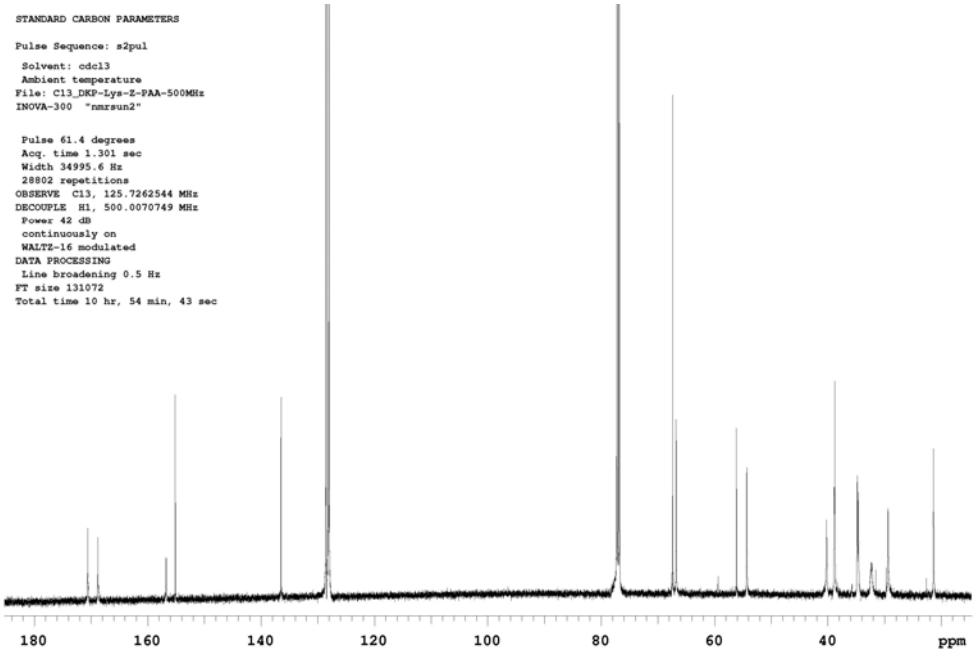


COSY of compound **47**

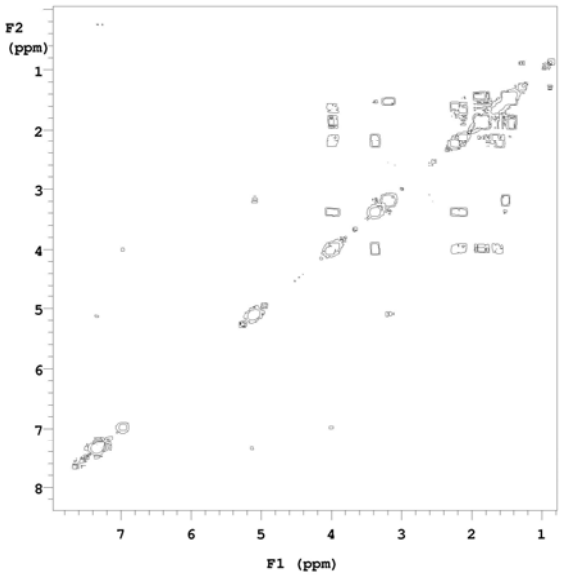
Synthesis of DKP 48. The procedure for this compound followed that for compound **47** except that the dipeptide used was compound **46** (96 mg, 31 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.59 (bs, 1H), 7.37-7.28 (m, 10H), 7.27 (s, 1H), 5.13 (s, 2H), 5.11 (s, 1H), 5.09 (s, 2H), 4.01 (s, 2H), 3.97-3.94 (m, 1H), 3.38 (bs, 2H), 3.22-3.13 (m, 2H), 2.27-2.22 (m, 1H), 2.16-2.10 (m, 1H), 1.97-1.89 (m, 2H), 1.85-1.78 (m, 1H), 1.66-1.62 (m, 1H), 1.55-1.50 (m, 2H), 1.46-1.42 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.6, 168.8, 156.7, 155.1, 136.5, 136.4, 128.55, 128.51, 128.49, 128.1, 128.0, 127.9, 67.4, 56.1, 54.3, 40.2, 38.84, 38.75, 34.8, 34.6, 32.3, 29.4, 21.4. MS (ESI): 523.0 $(M + H)^{+}$.



^{1}H NMR of compound **48**



^{13}C NMR of compound **48**



COSY of compound **48**

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