The Synthesis and Characterization of Water Soluble Hydroindole-Based Nanostructures of Designed Three-Dimensional Architectures

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This dissertation explores the design and synthesis of *bis*-amino acid molecular building blocks and their assembly into small oligomers of designed shape. Specifically, we investigated a class of monomer known as the hydroindole *bis*-amino acids. One member of this family, hin(2*S*4*R*7*R*9*R*), provided a sharp turn in three-dimensional space when assembled into oligomers. We employed various strategies to synthesize the hydroindole monomers, including a Diels-Alder pathway and an oxidative rearrangement of (*L*)-tyrosine.

Larger oligomers containing this building block were modeled and found to have distinct compact tertiary structures. We then carried out the solid phase synthesis of these oligomers, composed of the hin(2*S*4*R*7*R*9*R*) and other monomers, and optimized their rigidification into spiro-ladder scaffolds of well defined architectures. Efforts towards full structural characterization of these molecules are also presented here.

The hydroindole-based *bis*-amino acid building blocks are an integral part of a larger library of monomers that are used to construct water soluble macromolecules of designed shape. The ultimate goal of this new technology is to develop these macromolecules into functional nano-scale devices that display compact tertiary structures and present chemical functionality for use in a wide range of biomimetic and nanotechnology applications.

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"Among proteins, the term 'compact' is associated with tertiary structure, and there is as yet no synthetic polymer that displays a specific tertiary structure."

- Samuel H. Gellman

"It's supposed to be hard. If it wasn't hard, everyone would do it. The hard…is what makes it great."

- Tom Hanks

1. Introduction

1.1. An Introduction to Molecular Nanotechnology

A revolutionary new field of study is currently emerging that promises to combine the imagination, creativity and technical expertise of scientists worldwide. This new field focuses the areas of chemistry, physics, biology, materials science and engineering toward one singular goal; manipulating matter and arranging atoms with ultimate precision.

Although in its infancy, molecular nanotechnology has been placed at the top of the list of promising fields of research and development in the United States. This new field, because of its broad interdisciplinary nature, has far-reaching applications and societal implications. Envisioned breakthroughs include, but are not limited to, orders-of-magnitude increases in computer efficiency, human organ restoration using engineered tissue, "designer" materials created from directed assembly of atoms and molecules and renewable and efficient energy sources.[1]

The properties of all materials are determined predominantly by the arrangement of the atoms from which they are composed. Thus, complete control over the positioning and properties of each and every atom would give the scientist great power to develop materials and machinery on the nanometer length (one billionth of a meter) scale.[2] In essence, the ultimate goal of molecular nanotechnology is the creation of useful materials, devices and systems through the control of matter on the nano-scale and the exploitation of novel properties and

phenomena developed at that scale.[1] The only current useful examples of these nano-scale devices are those found within biology in the form of biological proteins and catalytic RNAs.

Nature's general solution to constructing nano-scale molecular devices lies in biological proteins. These macromolecules have very powerful catalytic, energy transduction and information processing capabilities that arise because of their ability to both encapsulate smaller molecules and position functional groups in three-dimensional space with sub-angstrom precision. To apply these aspects of proteins to molecular nanotechnology, science must develop the ability to study and construct systematically macromolecules with these capabilities.

De novo design of unnatural proteins is an active field of research[3,4] and has yielded many advances in the production of simple α-helical[5] and β-sheet proteins, however the synthesis of proteins with new functions is only recently just beginning to yield results.[6] The overwhelming protein folding problem has been the prohibitive factor in these accomplishments. One of the great unsolved problems of science is the prediction of the three-dimensional structure of a protein from its amino acid sequence.[7] Both experimental and theoretical approaches[8] have been used in the search for a solution, with many promising results[9], but no general resolution. Even with all the above mentioned strategies, there is still currently no one universal method of building functional macromolecules. Thus, the question remains: Can large, highly functional macromolecules be synthesized in an efficient and engineerable manner that approach biological proteins in their function?

1.2. Understanding and Mimicking the Macromolecules of Nature

To develop unnatural macromolecules that mimic protein structure/function, it is first necessary to understand how nature constructs its own oligomers. Proteins truly are "oligomers" that nature constructs from the combination of a limited number of building blocks (~20 amino acids). The amino acid building blocks are connected to one another through single amide (peptide) bonds. The peptide or protein is then a flexible oligomer that must fold into a particular conformation before it can perform a biological function (Figure 1). The essential functions of proteins are intrinsically linked to their structure and dynamic switching among different conformational states.[1] This phenomenon is demonstrated in the open and closed forms of ion channel proteins in a membrane. However, accurately predicting the manner in which a protein will fold, from only its amino acid sequence, is currently impossible.

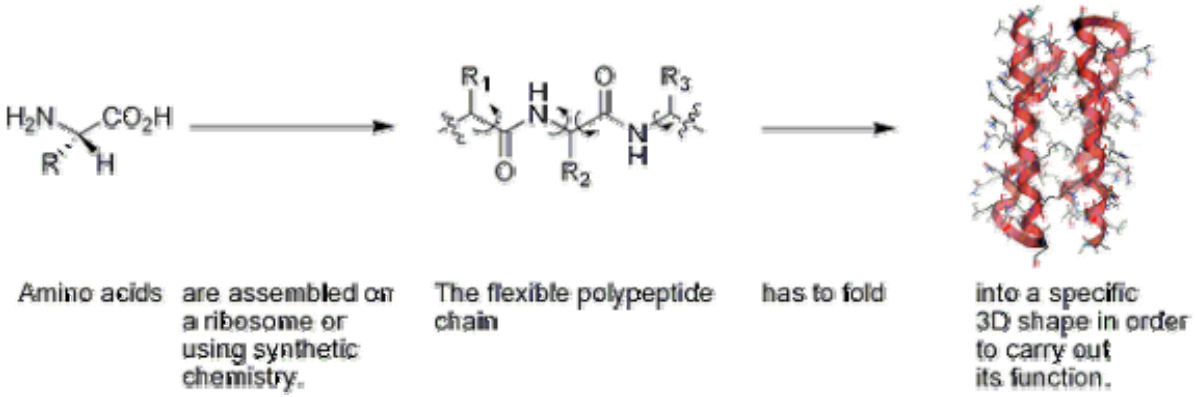


Figure 1: Nature's assembly of functional macromolecules

Numerous researchers have undertaken the challenge of creating synthetic oligomers to study the relationship between structure and function.[15,22,24] Oligomer synthesis is an efficient approach to macromolecules because it is modular and allows the rapid assembly of large structures from a collection of small monomers. This research has spawned an entirely new field of inquiry known as "foldamers." A foldamer is defined as "any oligomer that folds into a

conformationally ordered state in solution, the structures of which are stabilized by a collection of noncovalent interactions between nonadjacent monomer units."[10] Many groups[11,12,13] are currently developing unnatural monomers that are assembled through single bonds to form foldamers (Figure 2). These foldamers have strong tendencies to form well-defined secondary structures through the influence of weak noncovalent interactions.[10,14,15,16,17]

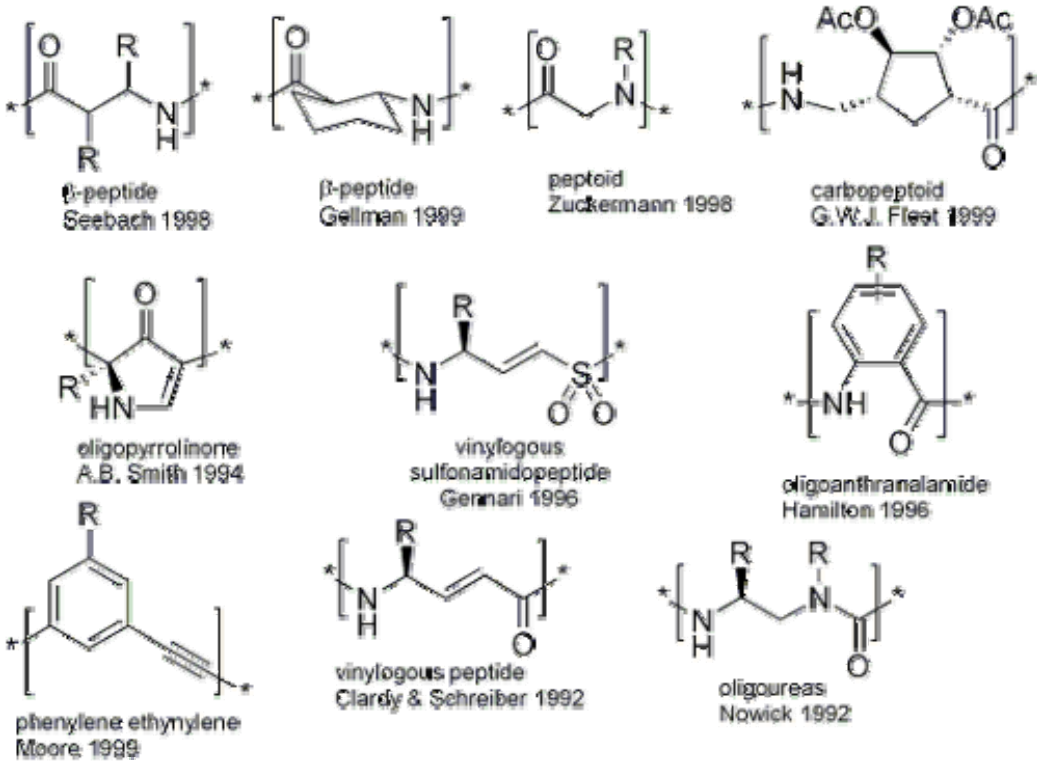


Figure 2: Examples of monomer subunits of foldamers

One type of foldamer that has been extensively studied[18,19,15] and has a potential for applications in nanotechnology is that of the β-peptide. β-peptides have a particular appeal for extending the understanding of protein structure and stabilization into the realm of folded, nonbiological polymers, because β-amino acids represent the smallest step away from α-amino acids in "backbone space."[18] Like α-peptides (i.e., peptides composed of α-amino acids), β-peptides contain amide bonds capable of forming stabilizing, intramolecular hydrogen bonds.[18]

As such, various types of β-peptides will have certain secondary structural characteristics that can be pre-determined in a "molecular engineering" manner.

Gellman and coworkers[20] are pioneers in the area of β-peptides and have observed the α-helical nature of these molecules both in the liquid and solid state. β-peptides have also been found to display useful biological functions.[20,21] In fact, it was discovered by Gellman's group that certain helical, cationic and amphiphilic β-oligomers had antibiotic function.[21]

From these β-peptides and other molecules, Gellman wishes to design foldamers that may mimic certain secondary structures, and eventually tertiary structures, of proteins in an engineerable fashion. Because the folding patterns of proteins generate "active sites" via precise three-dimensional arrangements of functional groups, it is proposed by Gellman that control over the secondary structural units in a folding polymer will lead to tertiary structural control.[22] Thus, it should be possible for the chemist to design functional polymers with biological, catalytic and organizational properties not precedented in nature.[20] However, there are currently no experimental results that support Gellman's hypothesis of secondary structural control leading to tertiary structural control. Designable, functional oligomers would be a tremendous advance for molecular nanotechnology, but one would still need to identify new polymeric backbones with suitable folding propensities, functionalize the backbones and be able to generate these materials efficiently; a series of tasks that even Gellman admits is daunting at best.[22]

There has been some success at generating folding oligomers that contain small cavities. Moore and coworkers have demonstrated that their folding phenylene ethynylene oligomers form helical secondary structural motifs[23] in certain solvents that can bind a variety of small molecule guests, including methyl iodide[24] and piperazinium dihydrochloride salts.[25] These intriguing,

cavity-containing oligomers have enormous potential to develop into supramolecular catalysts[26], nonetheless, they have yet to demonstrate any catalytic activity.

An obvious next step in the evolution of foldamer research, then would be to design and build "larger, more complex chain molecules capable of folding into truly tertiary structures or structures having long-range intrachain energetic interactions."[10] These tertiary structures must be able to bind small molecule guests to promote biomimetic applications. Clearly much intensive work on this problem will be necessary for any future applications to be developed. A new set of folding rules will have to be elucidated for each new oligomer designed and a more efficient method of assembling macromolecules with designed tertiary structures needs to be developed. This method must provide oligomers with three-dimensional architectures that are predictable and designable without the necessity of deducing how the structure will fold. The design and construction of such tertiary structures is the focus of this dissertation.

1.3. A Novel Method for the Synthesis of Macromolecules with Designed Structures

To circumvent the protein folding problem, oligomers can be constructed from monomers that are assembled through *pairs* of bonds (Figure 3). The structures of these molecular architectures would depend predominantly upon the intrinsic rigidity and absolute stereochemistry of each building block from which they are composed. These structures will not fold but, instead, adopt complex conformations that are fixed and predictable.

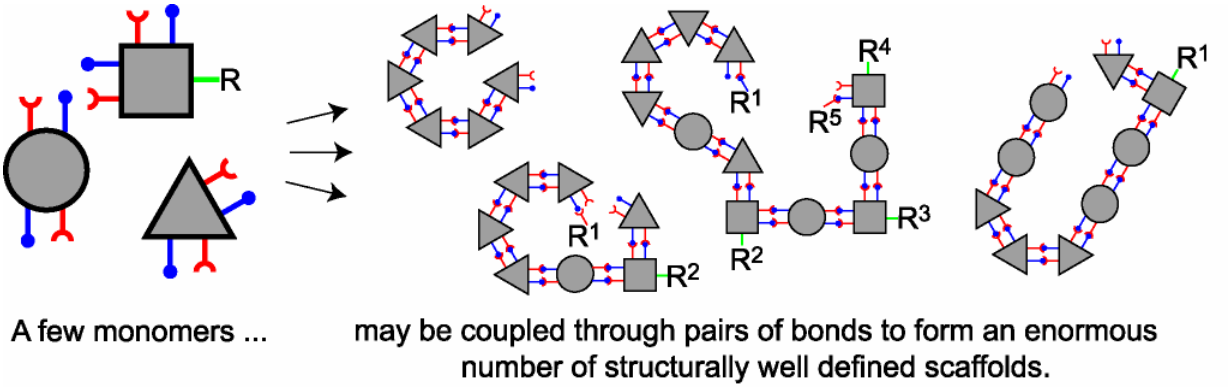


Figure 3: Cartoon of our approach to the construction of macromolecules with designed shapes

The molecular building blocks necessary to accomplish this task must be stereochemically pure, cyclic, and easy to synthesize from inexpensive commercially available materials. For biomimetic and nanotechnological applications, it is also imperative to mimic some of the properties of proteins (e.g., water solubility, compact structure, etc.). Whereas proteins are assembled from a sequence of amino acid building blocks coupled through *one* amide bond, these oligomers are constructed from a sequence of *bis*-amino acid building blocks coupled through *two* amide bonds. As a result, these monomers hold each of its two adjacent partners in a particular three-dimensional conformation connected through a rigid diketopiperazine linkage (Figure 4).

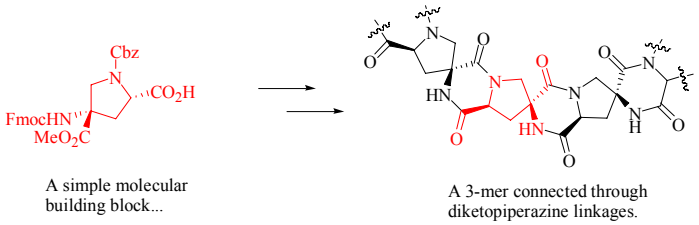
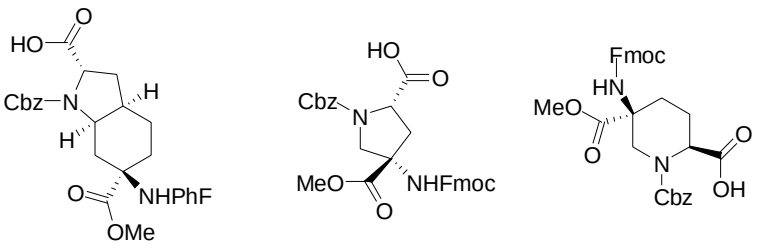


Figure 4: A proline based monomer coupled to two adjacent partners through pairs of amide bonds

The diketopiperazine moiety is the result of a well known side reaction in both solid phase and solution phase peptide chemistry.[27,28] They are formed by the intramolecular cyclization of the

growing peptide chain. The reaction is thermodynamically favored[27] with a half life that depends on the molecular context and conditions, but can range from seconds to days.[28]

A variety of building blocks have been synthesized[29,30,31] (Figure 5) that provide numerous structural features to the rigidified oligomers, such as extending rods, sharp turns, and helical pitch. As the number of monomers in our library increases, the number of oligomers that can be synthesized from those building blocks grows exponentially. Thus, for example, if we were to synthesize sequences of ten monomers from just four molecular building blocks, we could construct 4^{10} (~1,000,000) different shapes. From this enormous collection of potential structures, sequences that have interesting properties could be selected. The overall method is comparable to foldamer construction and de novo protein design, but without having to solve the complex folding problem.



hin(2*S*4*R*7*R*9*R*) "sharp turn"

pro4(2*S*4*S*) "extending rod"

pip5(2*S*5*S*) "helical pitch"

Figure 5: A small library of *bis*-amino acid monomers

The oligomers can be designed *a priori* on software developed in our laboratory (Figure 6). The Computer Aided Nanostructure Design and Optimization (CANDO) program[32] is used in conjunction with the molecular mechanics package MOE[33] to design and predict various oligomer conformations of interest. The synthesis of the modeled oligomer is then carried out in the laboratory and the solution structure determined by NMR spectroscopy or, perhaps, the solid

state structure can be obtained by X-ray crystal diffraction. The structural predictions are then compared with the experimental data to better improve the computer software precision.

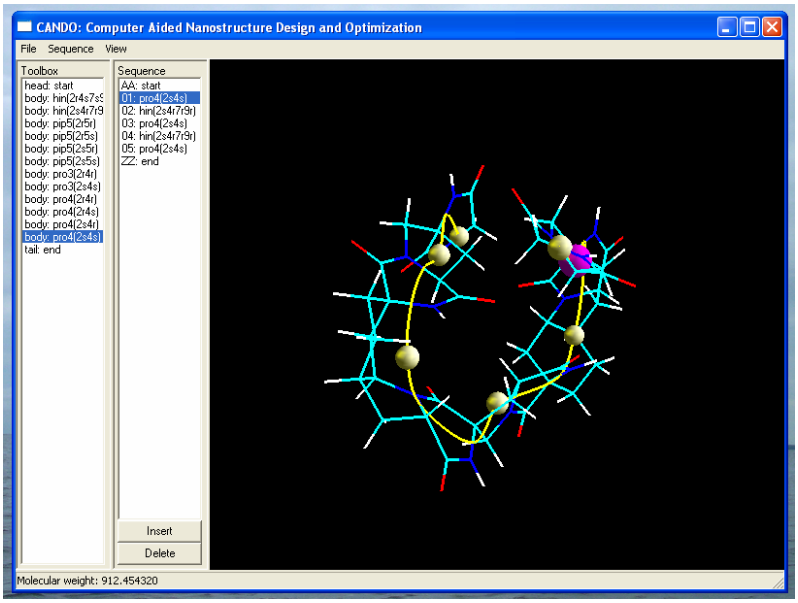


Figure 6: CANDO software

Our long term goal is to rapidly design, synthesize, and study macromolecules that have compact tertiary structures and contain small-molecule sized cavities. There are essentially limitless applications for these oligomers. For instance, in the area of biomimetic chemistry, the efficient catalysis carried out by nature's enzymes has influenced researchers to imitate it in the laboratory.[34] Enzymes tend to bind small molecule substrates and then catalyze a chemical reaction performed on that substrate in the binding pocket. The ability to accurately position catalytically active functional groups inside of a cavity-containing macromolecule will greatly facilitate enzyme mimetics. To meet this goal our oligomers must be able to form designed cavity structures for binding small molecules and be constructed from a sequence of molecular building blocks that contain functional groups properly positioned to perform the catalytic function. We believe that this can be accomplished using our synthetic and computational methods.

In the realm of molecular manufacturing applications, i.e. nanotechnology, there is an opportunity for the development of molecular machinery, nano-scale tools, and devices. To manufacture products on the nano-scale, precise control over the shape and rigidity of the macromolecules is essential. Positional assembly, as opposed to self assembly, is a relatively unexplored method of molecular manufacturing with organic materials.[35] Positional assembly requires stiff materials that maintain a shape-persistent architecture, unlike foldamers and supramolecular complexes. By connecting our molecular building blocks through pairs of covalent bonds, we obtain a level of rigidity unlike almost any other organic macromolecule.

2. A Diels-Alder Approach to the Synthesis of Molecular Building Blocks

2.1. Introduction

To construct rigid oligomers that can display chemical functionality in three-dimensional space, it is first necessary to have the component building blocks in hand. Unlike target directed natural product synthesis, there is an inherent flexibility in what can be chosen as a molecular building block. The ideal properties of a monomer are synthetic accessibility (can be synthesized in fewer than 10-12 steps), stereochemical purity, and synthetic flexibility (easily functionalized). Most importantly, however, is the monomer's ability to provide a unique architectural shape when coupled to its two adjacent partners.

Because the syntheses are short, it allows for the quick testing of synthetic pathways. If separation methods can be developed, weakly diastereoselective reactions and racemic syntheses can be used because each stereoisomer produced leads to a new building block. As part of this methodology for the synthesis of molecular building blocks, the Diels-Alder reaction can be considered a potentially valuable reaction.

The Diels-Alder [4+2π] cycloaddition reaction (DA reaction) is particularly useful, due to its high regio- and stereo-selectivity and for the wide variety of functional groups that can be tolerated.[36] This reaction also allows the construction of molecular building blocks through a convergent method rather than a stepwise synthesis. Although, the reaction could conceivably give rise to a number of structural or stereo isomers, usually one isomer is formed exclusively or at least in predominant amount.[37] Nonetheless, any and all stereo isomers generated from these syntheses would be utilized.

For the purposes of designing *bis*-amino acid monomers, this particularly useful ring-forming reaction allows the generation of rigid carbo- and heterocycles by the reaction of a protected amino acid diene with a masked amino acid dienophile (Figure 7). Of course, the success of this reaction depends largely upon the proper matching of the frontier molecular orbital energies of the diene and dienophile. Initially, it will be difficult to decipher such energies because there is little precedent for DA reactions between these types of DA components.

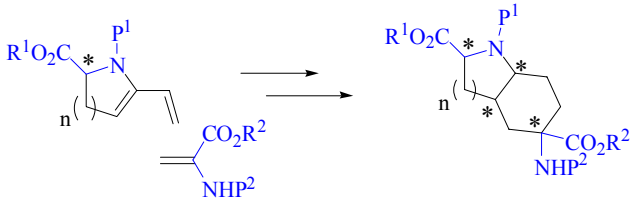


Figure 7: *bis*-Amino acid DA example.

Two initially desired types of building blocks were conceived. The first, "hydroindole" class of monomer (Figure 8), provides a gentle three-dimensional turn, in the *cis* configuration, and has multiple points of possible substitution for adding masked functional groups on the oligomer. This monomer is perfectly well suited for construction via the Diels-Alder method. Lending confidence to this method, similar ring systems were synthesized by Speckamp[38] and Cha[39,40] utilizing DA reactions under mild conditions in good yields.

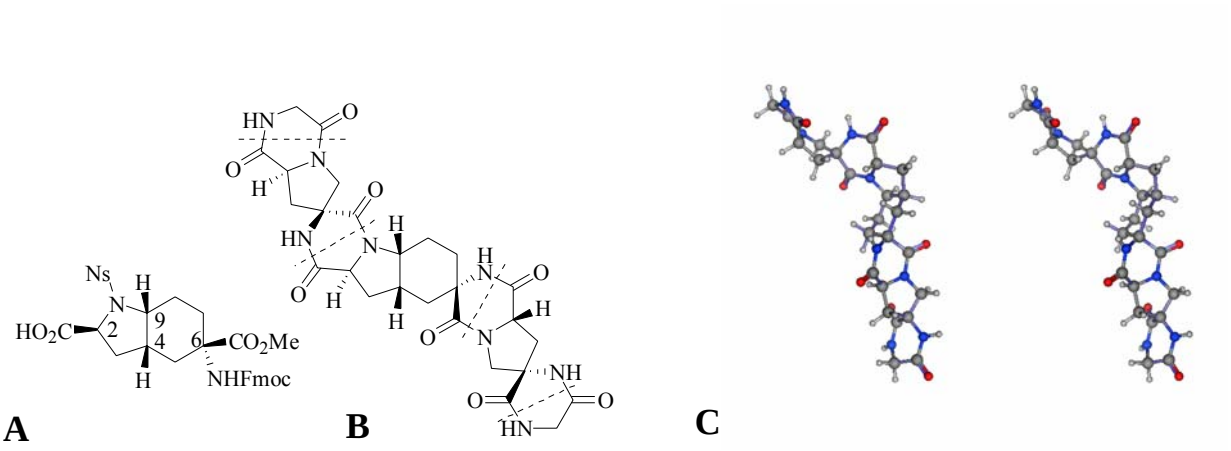


Figure 8: (A) One isomer of the hydroindole monomer. (B) An oligomer composed of the sequence pro4(2*S*4*S*)-hin(2*S*4*R*6*R*9*R*)-pro4(2*S*4*S*). (C) An energy minimized stereo view of the turn induced by the hin(2*S*4*R*6*R*9*R*) monomer.

The "azabicyclooctane" based molecular building block is a second desirable monomer (Figure 9). The substructure of the monomer contains a very rigid, fused aza-bicycle that provides excellent structural support for any oligomer from which it is synthesized and induces a sharp turn in three-dimensional space. Moreover, several points of further functionalization are accessible on the molecule.

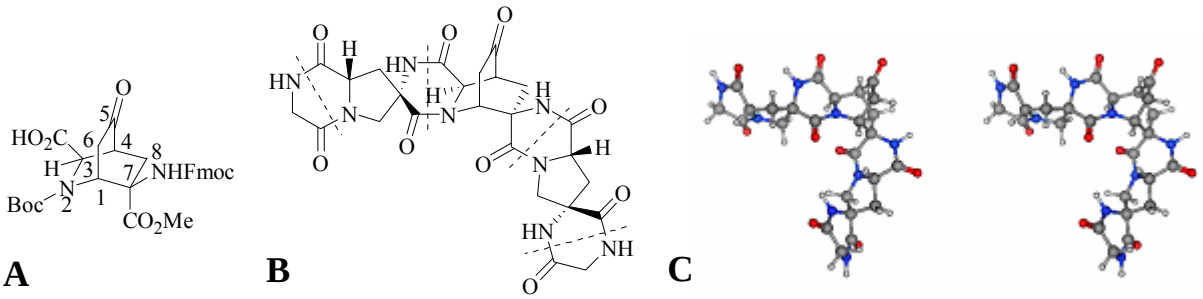


Figure 9: (A) One isomer of the bicyclooctane monomer. (B) An oligomer composed of the sequence pro4(2*S*4*S*)-2abco(1*S*3*R*4*S*7*R*)-pro4(2*S*4*S*). (C) An energy minimized stereo view of the sharp turn induced by the 2abco(1*S*3*R*4*S*7*R*) monomer.

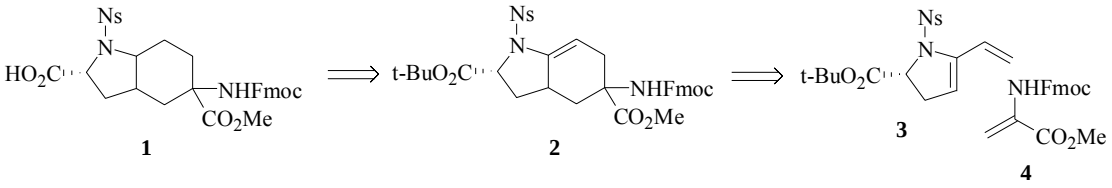
Similar isonuclidine ring structures have been synthesized, utilizing a dihydropyridine moiety as the diene component of the DA reaction with various dienophiles, by both Raucher[41] and Matsumura.[42] Considering these methods as precedent, it should be possible to carry out the DA reaction of an electron rich, chiral dihydropyridine with a dehydroamino acid with excellent

regioselectivity. If the dehydroamino acid does not undergo DA reaction there are alternatives, such as methylene Meldrum's acid, that may be more reactive. The endo/exo selectivity, however, is undetermined. Regardless, all potential isomeric products are useful. Most importantly, the precedent of the DA reaction applied to the synthesis of rigid *bis*-amino acid compounds would facilitate the production of other *bis*-amino acid monomers.

2.2. Hydroindole Monomer

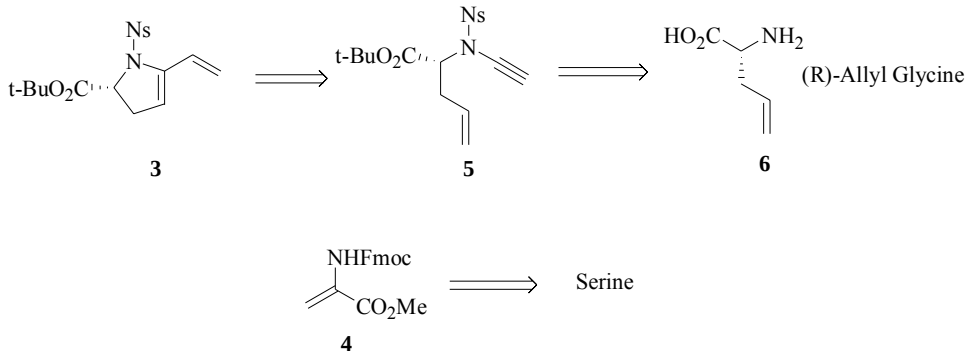
2.2.1. Retrosynthetic Analysis

In light of the Diels-Alder approach to constructing molecular building blocks, a retrosynthetic analysis of the hydroindole monomer **1** (Scheme 1) reveals that it can be derived from enamide **2**, resulting from a DA reaction of the appropriate diene **3**, and dienophile **4**. Although both fragments undergo DA reactions separately, and under various conditions, the DA reaction here is unprecedented.



Scheme 1: Retrosynthesis of hydroindole monomer 1

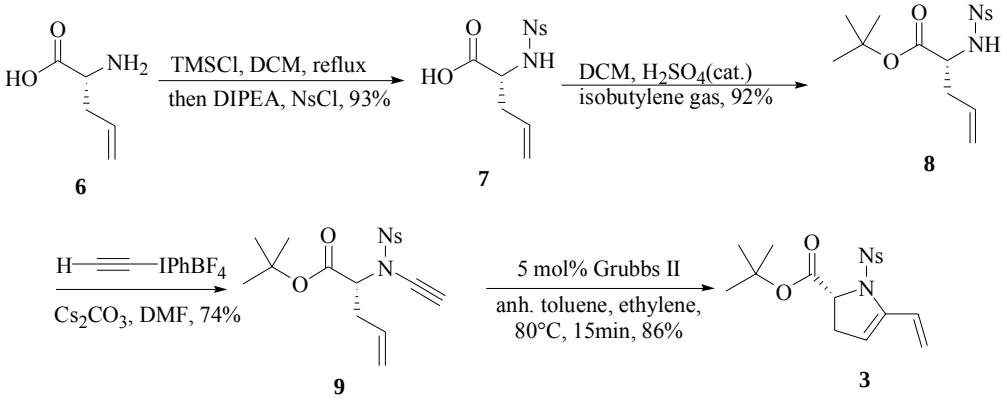
Accordingly, the next step in the retrosynthetic analysis leads to alkynamide intermediate **5** (Scheme 2). Witulski[43] and Mori[44] have both shown that such N-ethynylamides are readily available from the corresponding sulfonamides (derived from (R)-allyl glycine **6**) via alkylation with ethynyl(phenyl)iodonium triflate. The dienophilic dehydroalanine **4** is easily prepared from commercially available serine.



Scheme 2: Retrosynthesis of diene 3 and dienophile 4

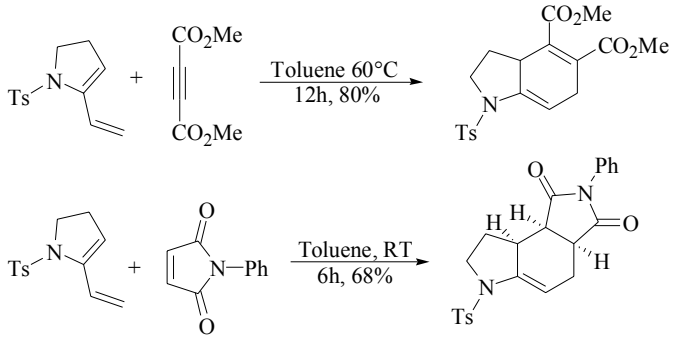
2.2.2. Results and Discussion

The diene component of the hydroindole monomer was synthesized from (*R*)-allyl glycine, prepared in large quantities by the method of Myers.[45] With the appropriate starting material in hand, the synthesis began (Scheme 3) with the protection of the amino end of (*R*)-allyl glycine with Fukuyama's[46] 2-nitrobenzene sulfonyl (Ns = nosyl) protecting group.[47] The reaction proceeded smoothly to give the nosyl protected allyl glycine **7** in excellent yield (93 %). This material was then protected as the t-butyl ester using the isobutylene method of Anderson[48] to yield **8** in excellent yield (92 %). The fully protected amino acid was then N-alkynylated[43] to compound **9** with commercially available ethynyl(phenyl)iodonium tetrafluoroborate.[49]



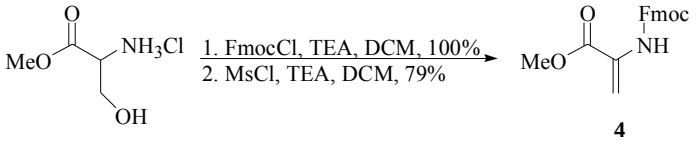
Scheme 3: Diene synthesis

Ruthenium catalyzed enyne metathesis[44] of compound **9** was induced by treatment with five mole percent of Grubbs second generation catalyst in anhydrous toluene at 80 °C. The ene-ynamide was smoothly converted to the corresponding diene **3** in good yield (86 %) in only 15 minutes. The diene was then crystallized to yellow florets from ethyl acetate/hexanes. This dienamide moiety **3** is analogous to Mori's[44] substrate which was utilized for DA reactions with a variety of dienophiles (Scheme 4).



Scheme 4: DA reactions of Mori's dienamide substrate

The dienophilic component **4** (Scheme 5) was subsequently synthesized from serine methyl ester hydrochloride by 9-fluorenylmethyloxy carbonyl (Fmoc) protection[47] of the amino group followed by elimination of the corresponding mesylate intermediate[50] to give the dehydroamino acid in good yield.

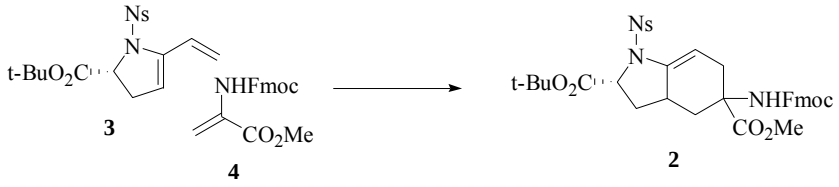


4

Scheme 5: Preparation of the dienophile

Dehydroamino acids such as **4** can undergo DA reactions under various conditions.[51,52] With these facts in mind, it was hoped that a Diels-Alder reaction could be accomplished under similar conditions. However, certain forcing circumstances may be necessary.

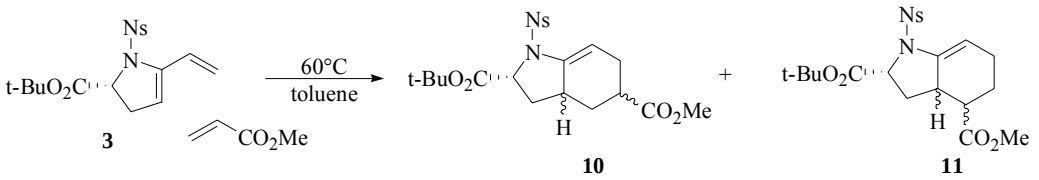
Thus, with the two requisite fragments available in a straight-forward manner, a short series of DA reactions were attempted between diene **3** and dienophile **4** (Table 1).



SOLVENT	REAGENTS	TEMP(°C)	TIME(h)	RESULT
Toluene	---	60	24	NR
Toluene	---	110	6	decomposition
Toluene	---	80	6	decomposition
Toluene	$TiCl_4$, $Ti(Oi\text{-}Pr)_4$	RT	6	decomposition

Table 1: Attempted Diels-Alder reactions

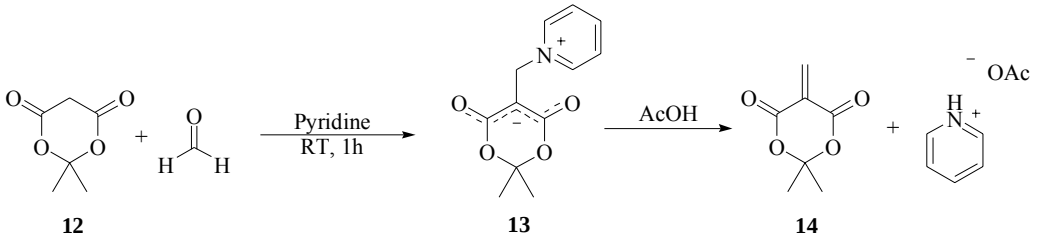
Upon heating of the reaction mixture to any temperature above 60 °C, only decomposition of the diene component was observed. Conversely, at lower temperatures, no reaction was detected. Titanium Lewis Acid activation of the dienophile[52] failed to produce any cycloadduct, with only decomposition of the diene apparent. Apparently the HOMO-LUMO energy gap was too large to facilitate reaction, so a model reaction was performed to verify that the dienamide was indeed a reactive DA diene. Reaction of the dienamide **3** with methyl acrylate, did indeed result in cycloadduct formation (Scheme 6), however only in modest yield (44 %). It was clear by mass spectral and ^1H NMR analysis that at least three regio- or stereoisomers were formed. This result is in accord with the work of Cha[39] who observed reversed regioselectivity and low exo/endo selectivity in DA reactions with carbamate derivatives and ethyl acrylate. Because the objective of this experiment was to test the reactivity of the dienamide **3** and not the selectivity of the reaction, no further attempt was made at separating or fully characterizing the mixture of cycloadducts **10** and **11**.



Scheme 6: DA reaction with methyl acrylate

With this encouraging result it was apparent that a more electron deficient dienophile was necessary to undergo DA reaction with dienamide **3**. Therefore a method was needed to synthesize a different masked amino acid dienophile. Because of the positive result seen with methyl acrylate, it was expected that a methylene malonate derivative should undergo DA reaction just as readily. Moreover, the malonate can be converted to a protected amino acid moiety in just two steps after the DA reaction.

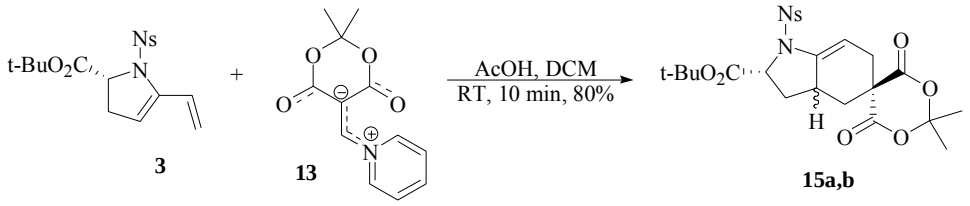
Methylene malonates are very useful intermediates for organic synthesis[53], however, their high instability to anionic polymerization does not allow for easy handling or purification of these compounds. Nonetheless, a one step preparation[54] from the Eli Lilly Corporation of a methylene Meldrum's acid precursor was discovered and proved to be a very efficient method of generating an electron deficient dienophile for DA reactions (Scheme 7).



Scheme 7: Synthesis of methylene Meldrum's Acid

Aqueous formaldehyde was added to Meldrum's acid **12,** dissolved in pyridine, to form the stable solid betaine compound **13**. Treatment with mild acid *in situ* generated the methylene Meldrum's acid dienophile **14**. Dienamide **3** rapidly underwent DA reaction (Scheme 8) with

methylene Meldrum's acid **14** at room temperature to give cycloadducts **15a,b** as a 1:1 mixture of diastereomers.



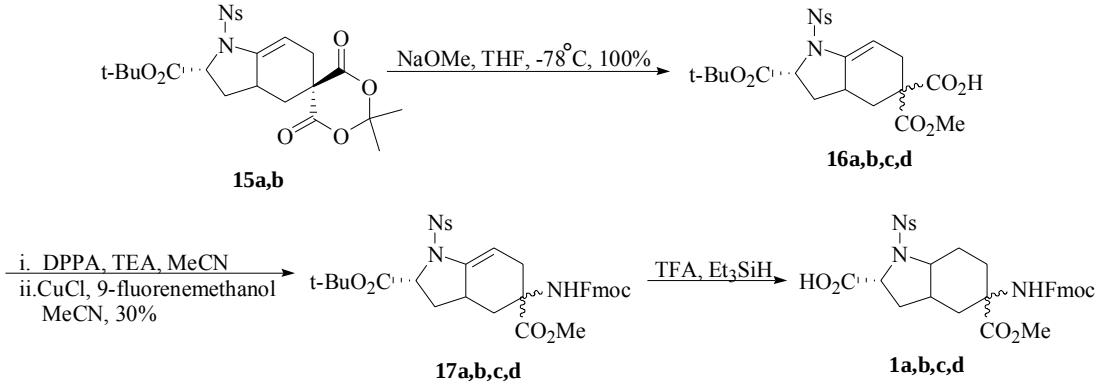
Scheme 8: Diels-Alder reaction with methylene Meldrum's acid

No directing effect was observed from the t-butyl ester of the diene to give any diastereofacial selectivity. Additionally, lowering the reaction temperature to -80 °C failed to change the diastereomeric ratio. This lack of stereoselectivity may be due to a stepwise mechanism rather than a concerted route.

Separation of the diastereomers **15a,b** was accomplished on activated silica gel using a 95:5 benzene:ether mobile phase, however the diastereomers surprisingly isomerized, quite readily, to the thermodynamically more stable tetra-substituted enamide, such as compound **20** (Scheme 11). The structure was verified by ^{1}H NMR, ^{1}H-^{1}H homodecoupling, and mass spectral analysis. Disappearance of the amino acid α-protons (4.37, 4.63 ppm) of diastereomers **15a,b** and appearance of a single α-proton signal (4.62 ppm) suggested an isomerization of the olefin to the ring junction of the hydroindole. It was discovered that similar isomerizations were observed by Mori[44] and Merck Laboratories.[55] As a result, the diastereomers were carried on through the synthesis as a mixture (Scheme 9).

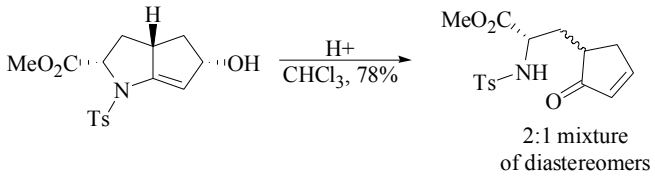
Quantitative ring opening of the Meldrum's acid ring of **15a,b** was accomplished[56] by treatment with sodium methoxide at low temperature. When the reaction was conducted at room temperature, decarboxylation accompanied to a great extent, resulting in low yield. However, decarboxylation could be avoided completely at -78 °C. The result was a mixture of four

diastereomers of the mono methyl ester **16a,b,c,d** visible by NMR. A two step one pot Curtius rearrangement/Fmoc protection[57] provided enamide **17** in low yield presumably due to the inefficiency of the trapping of the isocyanate intermediate by 9-fluorenemethanol.[58] Increasing the amount of copper (I) chloride catalyst did not affect the yield to any great extent. Alternative methods[59,60] are available to optimize this reaction.



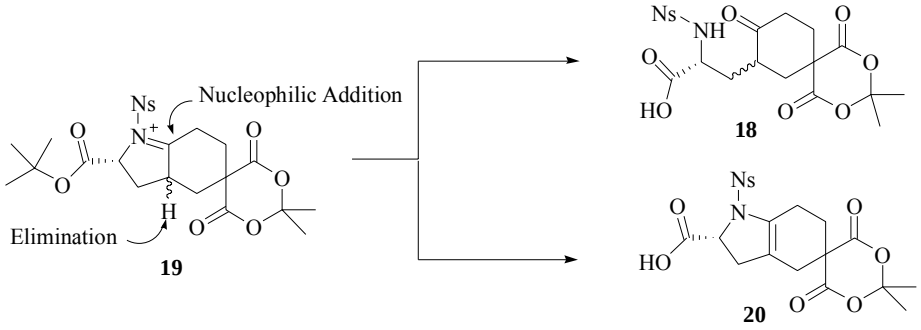
Scheme 9: Completion of the hydroindole monomer synthesis

Reduction of enamide[61] **17** was accomplished with triethyl silane in trifluoroacetic acid to give the hydroindole molecular building block **1** (Scheme 9). Disappointingly, a large amount of unexpected ring cleaved product was discovered by LC-MS analysis. The iminium ion intermediate was prone to attack by water. Interestingly, a similar hydrolysis was observed by Witulski[43] on a separate enamide substrate containing a more acid labile allylic alcohol (Scheme 10).



Scheme 10: Witulski's ring cleavage and rearrangement

Based on these results, an attempt to remove the enamide olefin at an earlier stage was made. Ionic hydrogenation conditions were applied to DA adduct **15**, but an analogous partial ring cleavage reaction, identical to that of compound **17,** was observed to yield **18**. This was verified by the appearance of two characteristic sulfonamide nitrogen proton doublets (6.11, 6.22 ppm) and two diastereomeric α-proton ddd signals (4.03, 4.22 ppm). The iminium ion intermediate **19** (Scheme 11) was prone not only to nucleophilic attack, but also α-elimination of a proton allowing ring opening or isomerization of the enamide olefin.



Scheme 11: Iminium ion transformations

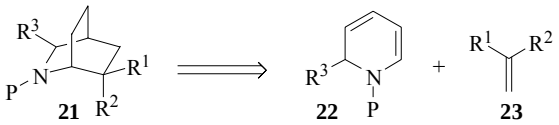
2.2.3. Conclusions

It has been proven that a chiral amino acid diene, synthesized from (*R*)-allyl glycine, can undergo a DA reaction with a sufficiently electron deficient, masked amino acid dienophile to generate a monomer for potential use in constructing rigidified oligomers. The hydroindole molecular building block **1** was synthesized as a mixture of stereoisomers, however, due to the propensity of the enamide intermediate to isomerize and the acid sensitivity of the molecular building block, partial ring cleavage occurred. The instability of the enamide olefin required that it be absent to produce a useful hydroindole monomer. It was now apparent that this monomer will not be accessible by a Diels-Alder pathway due to the resultant unstable cyclohexene ring. An alternate path must be explored.

2.3. Azabicyclo[2.2.2]octane Monomer

2.3.1. Retrosynthetic Analysis

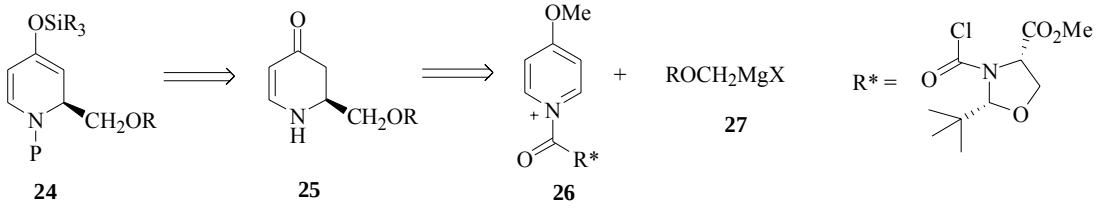
The azabicyclo[2.2.2]octane ring structure **21** (Scheme 12) has been synthesized by the Diels-Alder method by numerous researchers.[41,42,62] The ring structure itself is a very rigid three dimensional molecule that has great potential for use in rigidified oligomers. Complications in the synthesis of an azabicyclo[2.2.2]octane monomer (2ABCO) are invoked by the need to incorporate two amino acid functionalities into the ring system. 1,2-dihydropyridine **22** derivatives can act as sufficiently good DA dienes[41,62] for the construction of an 2ABCO ring system both in an inter- and intramolecular fashion. Here it was hoped that a similar chiral 1,2-dihydropyridine would undergo DA reaction with a dehydroamino acid dienophile, yielding an appropriate monomer for use in oligomer synthesis.



Scheme 12: Construction of 2ABCO ring system via DA reaction

Synthesis of the dienophilic dehydroalanine was already accomplished (*vide infra*) thus, only work toward construction of the 1,2-dihydropyridine moiety will be presented here. Comins[63] has devised a very efficient method of synthesizing chiral dihydropyridines via enantioselective Grignard addition into a chiral acyl pyridinium salt followed by reduction of the resultant dihydropyridone and elimination. Streith[64] then adapted the method of Comins to the development of a simplified procedure for the synthesis of dihydropyridones that does not require an overly expensive chiral auxiliary or installation/deprotection of bulky silyl groups.

Here, the method of Streith (Scheme 13) was used to attempt the Grignard addition of a masked carboxylic acid derivative **27** into a chiral 4-methoxy acyl pyridinium salt **26**.



Scheme 13: Retrosynthesis of chiral 1,2-dihydropyridine

The requisite dihydropyridine **24** could then be obtained by N-protection and trapping of the enolate of dihydropyridone **25**. The method of asymmetric induction for this Grignard reaction, in this case, was believed to originate from chelation controlled Grignard addition into the 2-position of the chiral pyridinium salt. Streith proposed the following model (Figure 10) displaying the minimum energy conformation of the pyridinium complex where a dihdedral angle of 60° exists between the urea carbonyl and the pyridine ring. The magnesium of the Grignard chelates to the urea carbonyl of the chiral auxiliary, holding the Grignard above the two position of the pyridine ring, allowing *re*-face attack to the less hindered β-side.

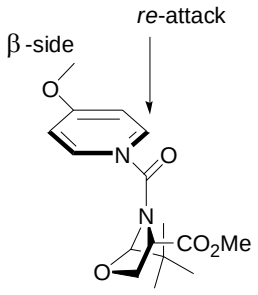
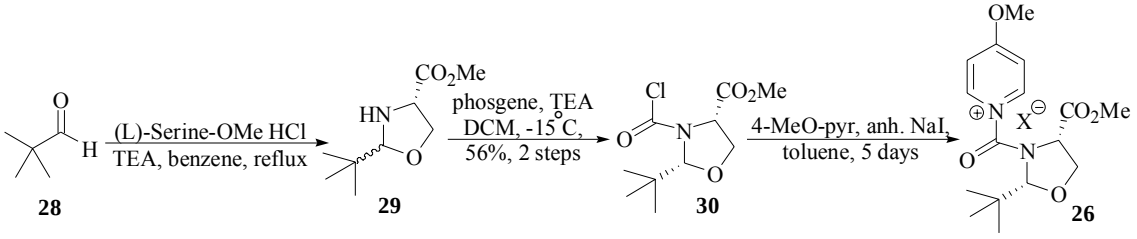


Figure 10: Streith's proposed model for asymmetric induction

2.3.2. Results and Discussion

The chiral pyridinium salt **26** was prepared via literature conditions[65] with commercially available 4-methoxy pyridine and the Seebach auxiliary **30**[66], available from the condensation of

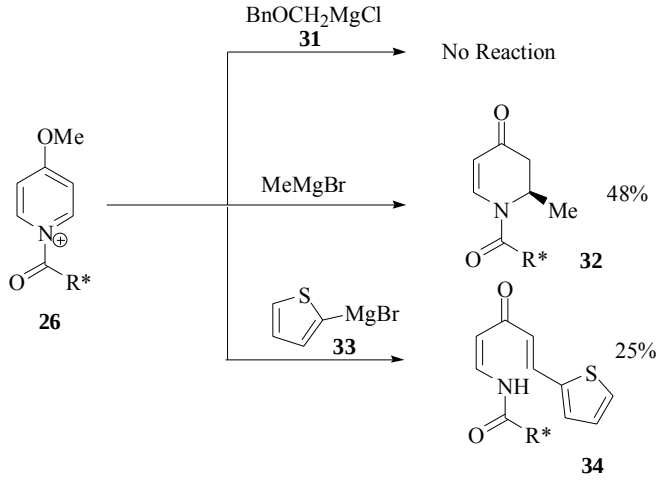
serine methyl ester hydrochloride with pivaldehyde **28** followed by acylation with a solution of phosgene (Scheme 14). The condensation reaction of pivaldehyde with serine methyl ester provided the oxazolidine as a *ca*. 1:1 mixture of diastereomers, visible by NMR. Acylation with phosgene and crystallization led to the diastereomerically pure chiral auxiliary **30** in 56% yield over two steps. Treatment of **30** with 4-methoxypyridine in the presence of sodium iodide led to gradual formation of the pyridinium ion after 5 days, the progress of which, was followed by NMR.



Scheme 14: Preparation of Seebach's auxiliary

A suitable masked carboxylic acid Grignard was found in benzyloxymethyl magnesium chloride **31**. After addition to the substrate, subsequent debenzylation and oxidation of the resultant alcohol to the carboxylic acid would afford the amino acid moiety. Preparation of the Grignard[67], however, proved nontrivial. The reproducibility of Grignard formation was poor due to its great instability. Grignard formation took place, nonetheless, at 0 °C in the presence of magnesium metal and freshly sublimed mercury (II) chloride in THF. Great care was taken in preparation of materials and purification of reagents, but it appeared side reactions repeatedly prevented addition to the pyridinium salt, as only benzyl alcohol and chiral auxiliary were recovered from the reaction mixture. As a result, Streith's method was tested (Scheme 15) by addition of methyl magnesium bromide into his chiral pyridinium salt **26**. The reaction

proceeded in moderate yield (48 %) giving the chiral methylated dihydropyridone **32**, with spectral data matching the literature values[64].

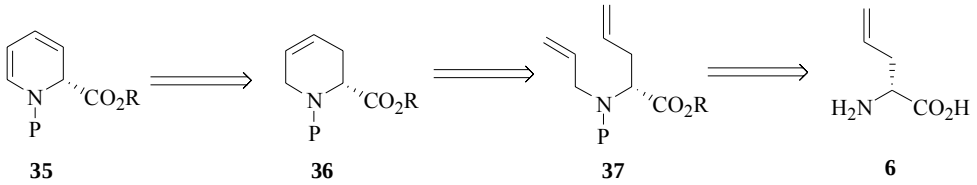


Scheme 15: Grignard reactions

With the knowledge that addition into the pyridinium salt was possible, another masked carboxylic acid Grignard reagent was prepared in hope of generating the amino acid containing dihydropyridine. 2-Thiophene magnesium bromide **33** was prepared via a published procedure.[68] The thiophene moiety can be oxidized readily[69] to the carboxylic acid by treatment with sodium periodate and ruthenium trichloride.

Upon addition of the thiophene Grignard and subsequent hydrolysis with 10% HCl, compound **34** was isolated in 25 % yield as a single product of the reaction. Ring opening most likely occurred during the hydrolysis of the intermediate methyl enol ether with acid. Perhaps the ring opening is favored due to the extended conjugation generated by the bis-enone with the aromatic thiophene. With repeatedly low yielding and/or failed attempts at accomplishing the desired Grignard reaction, it was decided that an alternate route to the 1,2-dihydropyridine DA diene was needed.

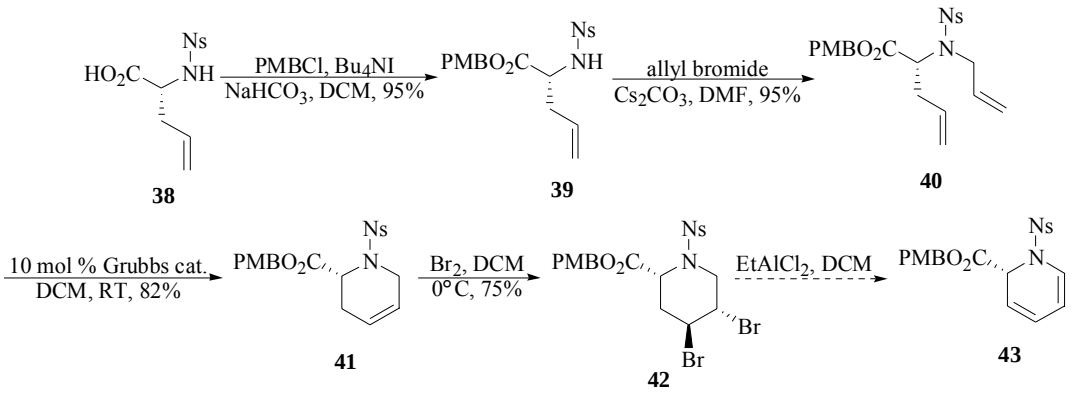
Entry into the 1,2-dihydropyridine moiety might be accomplished through the use of a ruthenium catalyzed ring-closing metathesis (RCM) strategy.



Scheme 16: Alternative retrosynthesis of 1,2-dihydropyridine

The target diene **35** could be obtained from monoene **36** by a bromination/double dehydrobromination sequence.[70] The requisite monoene is derived from N-allylated-(R)-allyl glycine **37** through an RCM[71] reaction. The unnatural amino acid precursor **6** is then prepared from known chemistry (*vide infra*).

The synthesis began (Scheme 17) from Ns-protected (*R*)-allyl glycine **38**. PMB protection[72] of the carboxyl functionality was accomplished under biphasic reaction conditions to yield the fully protected allyl glycine **39** in excellent yield. Compound **39** then underwent allylation of the sulfonamide nitrogen[73] in DMF to give the bis-allyl amino acid **40** which then was subjected to ring closing metathesis conditions[71] to afford monoene **41** in 82 % yield. Bromination[70] of the monoene was done by dropwise addition of liquid bromine at 0 °C, yielding the dibromide **42** as one diastereomer, the absolute configuration of which, was determined by the relative coupling constants of the ring protons, revealing that both bromines occupy axial positions on the piperidine ring.

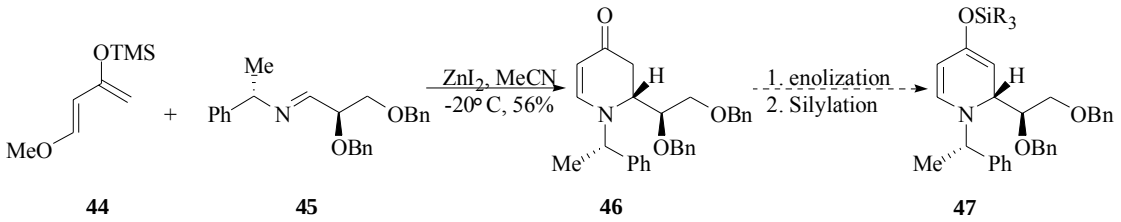


Scheme 17: Alternate synthesis of 1,2-dihydropyridine

Double dehydrobromination was attempted using modified Raucher conditions[70] with ethyl aluminum dichloride in dichloromethane at room temperature. These nonbasic conditions would allow dehydrobromination without epimerization of the amino acid α-proton, however no elimination was observed after 24 hours. More polar solvents such as HMPA and DMPU were also tried, but no reaction was observed. The PMB ester was removed and various non-nucleophilic bases such as triethylamine and DBU were also investigated, along with aqueous solutions of KOH, but to no avail. Even in refluxing KOH, no product was obtained. Finally, a set of conditions was found that did result in dehydrobromination, however over-elimination accompanied, resulting in pyridine formation with loss of the nosyl protecting group. After exhaustively exploring various conditions for this reaction it was decided, disappointingly so, that yet another approach to the 1,2-dihydropyridine was necessary.

Galvez[74] and coworkers have developed a very convergent method (Scheme 18) to chiral dihydropyridone compound **46** through Lewis acid catalyzed tandem Mannich-Michael reaction of chiral imine **45**, derived from (*R*)-glyceraldehyde, with Danishefsky's diene **44**. This enone **46** could then be converted to the corresponding dihydropyridine **47** by enolization and trapping

with a trialkylsilyl halide. The reaction was repeated here to give the desired compound **46** in 56 % yield as one diastereomer.



44 **45** **46** **47**

Scheme 18: Aza Diels-Alder reaction

With enaminone **46** in hand, the desired dihydropyridine was now accessible in just one step by silyl enol ether formation. The methods of Rawal[75] and Comins[62] were tested, however, no reaction was observed. Silyl enol ether formation was rather difficult and, as of yet, unsuccessful.

2.3.3. Conclusions

The 1,2-dihydropyridine moiety can be difficult to synthesize when containing a chiral amino acid subunit. Three approaches to this molecule were attempted with limited success. If further methods can be discovered to complete the synthesis of the requisite DA diene, subsequent Diels-Alder testing could begin. The 2-azabicyclo[2.2.2]octane *bis*-amino acid monomer is still considered a valuable molecular building block for use in our rigidified oligomers. It is hoped that further work in this area will be fruitful in the future.

2.4. Experimental Section

General: Tetrahydrofuran and diethyl ether were distilled from Na/benzophenone ketyl. Acetonitrile and dichloromethane were distilled from CaH_2. Pyridine and triethylamine were dried by storage over KOH pellets. All other reagents were used as received unless otherwise

noted. Column chromatography was performed using ICN Silitech 32-63 D (60 A) grade silica gel. Thin layer chromatography was performed using plates from EM Science (silica gel 60 F_{254} /250μm thickness). NMR spectroscopy was performed using Bruker 300 and 500 MHz instruments. IR spectra were obtained on a Nicolet FT-IR. Mass Spectra were done using a VG high resolution mass spectrometer. HPLC analysis was performed using a Hewlett Packard Series 1050 HPLC. LCMS analysis was performed using a Hewlett Packard Series 1100 LC-MS. Chemical shifts were reported in parts per million downfield relative to trimethylsilane and categorized as s = singlet, d = doublet, dd = double doublet, ddd = doublet of doublet of doublet, etc., t = triplet, q = quartet, m = multiplet. All reactions were carried out under inert atmosphere of nitrogen or argon in oven or flamed dried glassware unless otherwise noted.

(2*R*)-2-(2-nitrobenzenesulfonyl amino)-pent-4-enoic acid (7, 38).

(*R*)-allyl glycine (5.10 g, 44.3 mmol) was ground to a fine powder using a mortar and pestle and suspended in dry DCM in a flame-dried 250 mL round bottom flask under N_2 atmosphere. Chlorotrimethylsilane (11.2 mL, 88.6 mmol) was added and the mixture refluxed for 1 h. The reaction flask was then cooled in an ice bath and N,N-diisopropyl ethyl amine (13.4 mL, 76.7 mmol) added dropwise followed by 2-nitrobenzenesulfonyl chloride (6.50 g, 29.5 mmol) in one portion. The mixture was stirred on ice for 20 min and then warmed to room temperature. After stirring for two additional hours, the reaction mixture was concentrated *in vacuo* and distributed between 100 mL diethyl ether and 100 mL of 2.5 % aqueous $NaHCO_3$. The phases were separated and the aqueous portion extracted twice with 20 mL portions of ether. The organic layers were washed with water (2 x 30 mL) and the combined aqueous layers were then acidified to pH 2 with 1 M aqueous HCl. The cloudy aqueous layer was then further extracted with EtOAc (3 x 40 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in*

vacuo to yield 8.30 g (93 %) of **7** as a yellow oil from which EtOAc was difficult to remove. IR (neat): 3317, 1727, 1540, 1355, 1169 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07-8.10 (m, 1H), 7.90-7.94 (m, 1H), 7.74-7.77 (m, 2H), 6.05 (d, 1H, J = 8.5 Hz), 5.63 (dddd, 1H, J = 17.0, 9.7, 7.1, 7.1 Hz), 5.16 (dd, 1H, J = 17.1, 1.4 Hz), 5.15 (d, 1H, J = 11.4 Hz), 4.30 (ddd, 1H, J = 11.6, 8.5, 5.9 Hz), 2.57 (br t, 2H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.9, 147.7, 134.5, 133.9, 133.1, 130.7, 130.5, 125.7, 120.8, 55.8, 37.16; HR-MS (EI) calcd for $C_{11}H_{12}N_2O_6S$ (M+) 300.0416, found 300.0416.

(2*R*)-2-(2-nitrobenzenesulfonyl amino)-pent-4-enoic acid *tert*-butyl ester (8).

To an oven dried 250 mL round-bottom flask with a magnetic stirring bar, was added (*R*)-2-nitrobenzenesulfonyl protected allyl glycine **7** (8.00 g, 26.6 mmol) in dry DCM (80 mL) under N_2 atmosphere. Conc. sulfuric acid (266 μL) was then added and the reaction flask placed on ice. Isobutylene gas was bubbled through the solution until the point of saturation (when the volume of solvent increased by half). The reaction mixture was then warmed to room temperature and stirred for three hours. Sufficient, solid anhydrous Na_2CO_3 (approx. 1 g) was added to the mixture to neutralize the acid (verified by pH paper). The mixture was poured into a 250 mL separatory funnel containing 50 mL of water. The phases were separated and the organic phase washed once with a 20 mL portion of water. The organic layer was dried over Na_2SO_4, filtered and concentrated by rotary evaporation to yield **8** (8.70 g, 92 %) as a yellow oil. The oil was recrystallized in diethyl ether to yield pure **8** (7.30 g, 77 %, mp 80.0-80.1 °C) as a highly crystalline, yellow solid. The mother liquor was concentrated and dissolved in hot ether to yield a second crop (0.80 g, 8 %) for a total yield of 8.1 g (85 %); IR(neat): 3295, 1707, 1643, 1592, 1542, 1343, 1311, 1261, 1145, 856, 742 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07-8.10 (m, 1H), 7.90-7.93 (m, 1H), 7.70-7.73 (m, 2H), 6.10 (1H, d, J = 9.1 Hz), 5.71 (dddd, 1H, J =

17.1, 9.2, 7.0, 7.0 Hz), 5.16 (dd, 1H, J = 11.2, 0.9 Hz), 5.15 (dd, 1H, J = 15.9, 0.9 Hz), 4.17 (ddd, 1H, J = 11.6, 9.0, 5.8 Hz), 2.55 (dd, 1H, J = 5.9, 5.9 Hz), 2.55 (dd, 1H, J = 8.0, 8.0 Hz), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.3, 147.7, 134.6, 133.4, 132.8, 131.2, 130.4, 125.5, 119.8, 82.7, 56.6, 37.7, 27.7; HR-MS (EI) calcd for $C_{12}H_{15}N_2O_6S$ (M-C_3H_5) 315.0651, found 315.0639.

(2*R*)-2-[ethynyl-(2-nitro-benzenesulfonyl)-amino]-pent-4-enoic acid *tert*-butyl ester (9).

To a dry, 100 mL, round-bottom flask, under N_2, was added 2.50 g (7.00 mmol) of **8** and anhydrous DMF (21 mL). Solid anhydrous Cs_2CO_3 (3.40 g, 10.5 mmol) was added in one portion to the clear slightly yellow solution. The mixture was allowed to stir for approximately 20 min, at which time the solution turned a turbid bright yellow color. The ethynyl(phenyl) iodonium tetrafluoroborate (2.80 g, 9.10 mmol) was added in 6 portions over a period of 15 min, after which the solution turned a dark brown color. After stirring for three more hours at room temperature, the bulk of the DMF was removed by rotary evaporation at 80 °C. The resulting solution was partitioned between sat. aq. NH_4Cl and DCM (20 mL each). The organic layer was removed and the aqueous layer extracted with three 30 mL portions of DCM. The combined organic layers were washed once with 1 M HCl (30 mL) and three times with sat. aq. NaCl. The organic layers were dried, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by flash chromatography (1:2 EtOAc:hexane) to yield 2.10 g of **9** as an oil that solidified under high vacuum (1 mmHg). The solid was recrystallized in diethyl ether/hexanes to yield 1.90 g (74 %, mp 77.0-78.0°C) as rod-like crystals. IR(neat): 3293, 2136, 1742 cm^{-1}; ^{1}H NMR(300 MHz, $CDCl_3$): δ 8.22-8.25 (m, 1H), 7.75-7.79 (m, 2H), 7.66-7.69 (m, 1H), 5.81 (dddd, 1H, J = 17.7, 10.0, 7.7, 6.1 Hz), 7.24 (dd, 1H, J = 17.0, 1.4 Hz), 5.14 (dd, 1H, J = 9.9, 0.8, Hz), 4.60 (dd, 1H, J = 10.3, 4.7 Hz), 2.90 (s, 1H), 2.75-2.85 (m, 1H), 2.58-2.71 (m, 1H), 1.36 (s,

9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.4, 147.8, 134.5, 132.2, 132.0, 131.8, 131.0, 124.1, 119.2, 82.8, 72.2, 62.0, 61.5, 34.3, 27.7; HR-MS (EI) calcd for $C_{12}H_{11}N_2O_4S$ (M-$C_5H_9O_2$) 279.0440, found 279.0447.

(2*R*)-1-(2-Nitro-benzenesulfonyl)-5-vinyl-2,3-dihydro-1H-pyrrole-2-carboxylic acid *tert*-butyl ester (3).

The alkynamide **9** (690 mg, 1.80 mmol) was dissolved in anh. toluene (90 mL) in a 250 mL Schlenk flask. To the solution was also added Grubbs second generation catalyst (76 mg, 0.09 mmol, 5 mol %). The solvent was then degassed by the freeze-pump-thaw method. Ethylene gas was then bubbled through the solution and the mixture heated to 80 °C for 15 min. The solution was cooled to room temperature and a few drops of ethyl vinyl ether added to the flask. The toluene was removed *in vacuo* and the residue was chromatographed on silica gel (1:2 EtOAc:hexane, Rf = 0.30). The resulting brown oil was recrystallized from EtOAc/hex to yield 596 mg (86 %) of product as yellow florets. IR (neat): 3096, 1741, 1629, 1547, 1167, 990, 852 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02-8.06 (m, 1H), 7.61-7.74 (m, 3H), 6.52 (ddd, 1H, J = 17.4, 11.0, 1.0 Hz), 5.55 (d, 1H, J = 17.4 Hz), 5.35 (dd, 1H, J = 2.5, 2.5 Hz), 5.23 (d, 1H, J = 11.0 Hz), 4.92 (dd, 1H, J = 10.39, 2.90 Hz), 2.90 (m, 1H), 2.62 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 148.2, 141.4, 133.8, 132.4, 131.7, 131.3, 127.3, 124.0, 118.6, 110.7, 82.3, 63.4, 32.3, 27.8; HR-MS (EI) calcd for $C_{17}H_{20}N_2O_6S$ (M+) 380.1042, found 380.1052.

2-(9H-Fluoren-9-ylmethoxycarbonylamino)-acrylic acid methyl ester (4).

Commercially available serine methyl ester hydrochloride (2.00 g, 12.8 mmol) was suspended in dry DCM and triethylamine (3.50 mL, 25.7 mmol) was added. The now homogeneous solution was cooled on ice and 9-fluorenylmethyl chloroformate (4.00 g, 15.4 mmol) was added in one portion. The reaction was warmed to room temperature and allowed to stir for one hour. The

reaction mixture was then quenched with sat. aq. $NaHCO_3$ and poured into water. The mixture was extracted three times with DCM, dried (Na_2SO_4), filtered and concentrated to yield crude Fmoc-Ser-OMe (4.40 g, 100 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (m, 2H), 7.62 (m, 2H), 7.42 (m, 2H), 7.33 (m, 2H), 5.70 (d, 1H, J = 7.6 Hz), 4.47 (m, 1H) 4.44 (d, 2H, J = 6.8 Hz), 4.24 (t, 1H, J = 6.7 Hz), 3.98 (m, 2H), 3.81 (s, 3H), 2.57 (br s, 1H) in addition to minor impurities. Crude Fmoc-Ser-OMe (4.40 g, 12.8 mmol) was dissolved in DCM and cooled to 0 °C and mesyl chloride (1.00 mL, 14.1 mmol) added dropwise. The mixture was stirred for 10 min and triethylamine (3.90 mL, 28.2 mmol) was added in one portion. The reaction was allowed to warm to room temperature overnight. The reaction was diluted with DCM, washed with sat. aq. NH_4Cl, $NaHCO_3$, and brine. The organic layer was dried and concentrated. The product can be isolated by column chromatography (15 % EtOAc:hexane) or by precipitation in MeOH (although the product must be further dried by this method) to yield 3.30 g (79 %) of pure **4** as a fluffy white powder. IR (neat): 1715, 1636 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (m, 2H), 7.61 (m, 2H), 7.42 (m, 2H), 7.33 (m, 2H), 6.24 (s(br), 1H), 5.81 (s, 1H), 4.46 (d, 2H, J = 6.9 Hz), 4.26 (t, 1H, J = 6.8 Hz), 3.87 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.2, 153.1, 143.6, 141.3, 130.8, 127.8, 127.1, 124.9, 120.0, 67.1, 53.0, 46.9. HR-MS (EI) calcd for $C_{19}H_{17}NO_4$ (M+) 323.1158, found 323.1149.

Diels-Alder Reaction of Diene (3) with Methyl Acrylate; (10, 11).

The diene **3** (20 mg, 0.05 mmol) was dissolved in dry toluene (160 μL) and methyl acrylate (24 μL, 0.26 mmol) was added. The mixture was stirred at 60 °C for 24 h. The solvent was removed by rotary evaporation and the residue was purified by preparative TLC (1:1 EtOAc:hexane). The product band (Rf = 0.52) was soaked in EtOAc, filtered and the solvent removed to yield 11 mg

(44 %) of product as a mixture of multiple diastereomers and regioisomers. HR-MS (EI) calcd for $C_{21}H_{26}N_2O_8S$ (M+) 466.1410, found 466.1404.

Diels-Alder Reaction of Diene (3) with Methylene Meldrum's Acid; (15a,b).

The diene (1.57 g, 4.10 mmol) was dissolved in dry DCM (20 mL) and acetic acid (589 μL, 8.20 mmol) was added. A DCM (20 mL) solution of the dienophile precursor **13** (1.94 g, 8.20 mmol) was added dropwise to the stirring solution. After 10 min, the yellow reaction was washed with water and brine then dried and the solvent removed. The residue was purified on silica gel (30% EtOAc:hexane) to yield 1.97 g (89 %) of the Diels-Alder adduct **15** as a 1:1 mixture of diastereomers (t_r = 24.8, 25.0 min; 5-95 % MeCN:water, 0.1 % TFA). MS (ES): calcd for $C_{24}H_{28}N_2O_{10}SNa$ (M+Na), 559.1, found 559.1.

N-(2-Nitro-benzenesulfonyl)-octahydro-indole-2,5,5-tricarboxylic acid 2-tert-butyl ester 5-methyl ester (16a,b,c,d).

Compounds **15a,b** (140 mg, 0.28 mmol) were dissolved in THF (14 mL) and cooled to -78 °C. A solution of 25 % NaOMe in MeOH (77.2 μL, 0.300 mmol) was added dropwise over 20 min. After 15 min, the solution was quenched with 1 N HCl. The reaction was poured into a separatory funnel and extracted three times with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to yield the crude half malonic ester in 100 % yield (132 mg). Analysis of the mixture by ^{1}H NMR revealed a mixture of four diastereomers in nearly equal amounts. MS (ES): calcd for $C_{22}H_{26}N_2O_{10}SNa$ (M+Na) 533.1, found 533.1.

5-(9H-Fluoren-9-ylmethoxycarbonylamino)-1-(2-nitro-benzenesulfonyl)-octahydro-indole-2,5-dicarboxylic acid 2-tert-butyl ester 5-methyl ester (17a,b,c,d).

The free carboxylic acid (132 mg, 0.300 mmol) was dissolved in MeCN (1.4 mL) and triethylamine (432 μL, 0.300 mmol) was added. Diphenylphosphoryl azide (668 μL, 0.300

mmol) was added directly and the mixture refluxed for 30 min. The reaction was cooled to room temperature and 25 mol % CuCl (7.0 mg, 0.07 mmol) was added along with 9-fluorenemethanol (166 mg, 0.800 mmol). The cloudy mixture was stirred for 2 h and quenched with sat. aq. $NaHCO_3$. Extraction three times with EtOAc followed by drying (Na_2SO_4) and removal of the solvent *in vacuo* led to a brown residue that was purified by column chromatography (1:2 EtOAc:hexane, Rf = 0.23) to give 55 mg (30 %) of product as a dark yellow oil. MS (ES): calcd for $C_{36}H_{37}N_3O_{10}S$+H (M+1) 704.2, found 704.2.

5-(9H-Fluoren-9-ylmethoxycarbonylamino)-1-(2-nitro-benzenesulfonyl)-octahydro-indole-2,5-dicarboxylic acid 5-methyl ester (1a,b,c,d).

Compound **17** (150 mg, 0.20 mmol) was dissolved in DCM (577 μL) and triethylsilane (109 μL, 0.700 mmol) added. The mixture was cooled on ice and trifluoroacetic acid (295 μL, 3.80 mmol) added dropwise. The reaction was warmed to room temperature and the solvent removed. The resulting brown residue (152 mg, 100 %) was analyzed by LC-MS to reveal the ionic hydrogenation product **1** diastereomers (MS (ES) calcd for $C_{32}H_{31}N_3O_{10}S$+H (M+1) 650.1, found 650.2), and the ring opened product (MS (ES) calcd for $C_{32}H_{31}N_3O_{11}S$+H (M+1) 666.1, found 666.2).

(2*R*,4*S*)-2-*tert*-Butyl-3-(3-oxo-5-thiophen-2-yl-penta-1,4-dienylcarbamoyl)-oxazolidine-4-carboxylic acid methyl ester (34).

IR (neat): 1749, 1694, 1601, 1253, 1111, 959, 860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.87, (d, 1H, J = 10.2 Hz), 7.73 (d, 1H, J = 15.6 Hz), 7.54 (dd, 1H, J = 10,5, 8.4 Hz), 7.40 (d, 1H, J = 5.5 Hz), 7.30 (d, 1H, J = 3.5 Hz), 7.07 (dd, 1H, J = 5.0, 3.5 Hz), 6.57 (d, 1H, J = 15.6 Hz), 5.70 (d, 1H, J = 8.4 Hz), 5.34 (s, 1H), 4.67 (dd, 1H, J = 7.0, 2.6 Hz), 4.59 (dd, 1H, J = 8.7, 2.6 Hz), 4.01 (dd, 1H, J = 8.7, 7.1 Hz), 3.95 (s, 3H), 0.94 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.4,

169.9, 154.9, 140.8, 140.2, 134.5, 131.3, 128.6, 128.2, 125.8, 102.6, 96.9, 67.5, 60.1, 53.0, 37.3, 25.2; HR-MS (EI) calcd for $C_{15}H_{15}N_2O_5S$ (M-C_4H_9) 335.0702, found 335.0701.

(2*R*)-2-(2-Nitrobenzenesulfonyl amino)-pent-4-enoic acid 4-methoxybenzyl ester (39). Compound **38** (200 mg, 0.6 mmol) was dissolved in DCM (1.4 mL) and sat. aq. $NaHCO_3$ (937 µL), tetrabutylammonium iodide (269 mg, 0.700 mmol) and 4-methoxybenzyl chloride (91.0 µL, 0.700 mmol) were added sequentially. The reaction was stirred for 24 h at room temperature, after which, the layers were separated and the aqueous portion extracted with three portions (10 mL) of DCM. The combined organic layers were washed with brine, dried, filtered and concentrated. The resulting residue was purified by column chromatography (2:1 EtOAc:hexane, Rf = 0.48) to give 232.00 mg (98 %) of **39** as a dark yellow oil. IR (neat): 3318, 1737, 1612, 827 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.99 (m, 1H), 7.80 (m, 1H), 7.64 (m, 2H), 7.12 (d, 2H, J = 8.5 Hz), 6.82 (d, 2H, J = 8.6 Hz), 6.16 (d, 1H, J = 8.8 Hz), 5.61 (dddd, 1H, J = 17.5, 10.3, 7.1, 7.1 Hz), 5.11 (dd, 1H, J = 8.7, 1.6 Hz), 5.09 (dd, 1H, J = 15.2, 1.5 Hz), 4.87 (s, 2H), 4.28 (ddd, 1H, J = 11.7, 8.9, 5.8 Hz), 3.79 (s, 3H), 2.57 (dd, 1H, J = 6.0, 6.0 Hz), 2.57 (dd, 1H, J = 6.0, 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 159.6, 147.3, 133.8, 133.5, 132.9, 132.7, 130.9, 130.1, 126.6, 125.4, 119.9, 113.7, 67.1, 56.0, 55.1, 37.2; HR-MS (EI) calcd for $C_{19}H_{20}N_2O_7S$ (M+) 420.0991, found 420.0980.

(2*R*)-2-[Allyl-(2-nitrobenzenesulfonyl) amino] pent-4-enoic acid 4-methoxybenzyl ester (40). Dry Cs_2CO_3 (183 mg, 0.600 mmol) was suspended in anhydrous DMF (1.3 mL) and protected amino acid **39** (182 mg, 0.400 mmol) was added, dropwise, as a solution in DMF (0.5 mL). The mixture was cooled on an ice bath and allyl bromide (75.4 µL, 0.800 mmol) was added in one portion. The reaction was warmed to room temperature and stirred an additional 2 h. The bulk of the DMF was removed by rotary evaporation and the resulting residue was partitioned

between sat. aq. NH_4Cl and EtOAc. The aqueous phase was extracted with three, 30 mL portions of EtOAc and the combined organic extracts washed with brine and dried. The solvent was then removed and the residue purified by column chromatography (2:1 EtOAc:hexane, Rf = 0.55) to give 189 mg (95 %) of product **40** as a yellow oil. IR (neat): 1639 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (m, 1H), 7.53 (m, 3H), 7.18 (d, 2H, J = 8.7 Hz), 6.86 (d, 2H, J = 8.6 Hz), 5.81 (m, 2H), 5.14 (m, 4H), 4.96 (d(br), 2H, J = 2.3 Hz), 4.77 (dd, 1H, J = 9.0, 5.9 Hz), 4.06 (m, 1H), 3.94 (m, 1H), 3.82 (s, 3H), 2.77 (m, 1H), 2.54 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 159.9, 146.8, 139.9, 133.7, 132.3, 132.2, 130.3, 128.5, 125.6, 124.8, 118.7, 115.0, 113.8, 75.1, 67.3, 61.2, 55.8, 47.5, 34.9; HR-MS (EI) calcd for $C_{22}H_{24}N_2O_7S$ (M+) 460.1304, found 460.1282.

(2*R*)-1-(2-Nitrobenzenesulfonyl)-1,2,3,6-tetrahydropyridine-2-carboxylic acid 4-methoxybenzyl ester (41).

Compound **40** (1.10 g, 2.50 mmol) was dissolved in dry DCM (43 mL) and the mixture was degassed three times via the freeze-pump-thaw method. When the solution had warmed up to room temperature Grubbs' catalyst (209 mg, 0.200 mmol) was quickly added and the mixture stirred for 30 min. The solvent was then removed and the residue purified on silica gel (2:1 EtOAc:hexane, Rf = 0.38) to give 907 mg (82 %) of **41** as a yellow oil. IR (neat): 1738, 1617 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (m, 1H), 7.65 (m, 3H), 7.17 (d, 2H, J = 8.6 Hz), 6.85 (d, 2H, J = 8.6 Hz), 5.75 (m, 1H), 5.67 (m, 1H), 5.02 (d, 1H, J = 11.9 Hz), 4.94 (m, 1H), 4.91 (d, 1H, J = 11.9 Hz), 4.03 (m, 2H), 3.82 (s, 3H), 2.69 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 159.8, 147.6, 139.9, 133.5, 131.6, 131.0, 130.1, 127.4, 124.4, 122.9, 122.8, 114.0, 37.7, 55.4, 53.7, 42.8, 27.8; HR-MS (EI) calcd for $C_2H_{20}N_2O_7S$ (M+) 432.0991, found 432.1013.

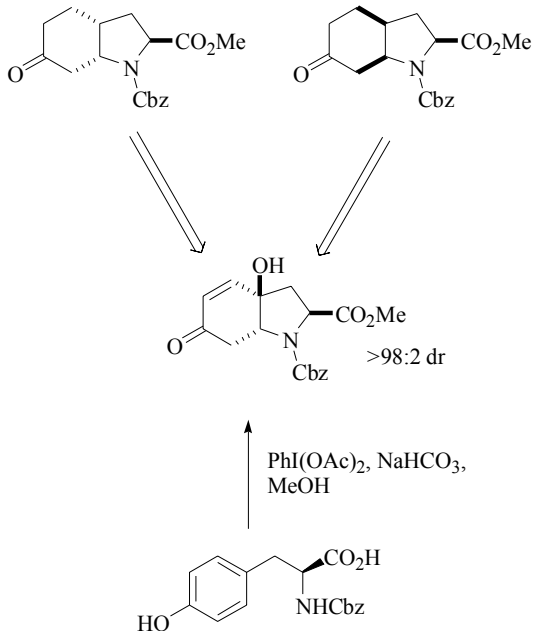
(2*R*,4*S*,5*S*)-4,5-Dibromo-1-(2-nitrobenzenesulfonyl) piperidine-2-carboxylic acid 4-methoxybenzyl ester (42).

Monoene **41** (651 mg, 1.50 mmol) was dissolved in DCM (21 mL) and cooled to 0 °C. Liquid bromine (93.0 μL, 1.80 mmol) was added dropwise over 10 min. The reaction was stirred for 2 h at 0 °C. The solvent was evaporated and the residue purified by column chromatography (1:2 EtOAc:hexane, Rf = 0.57) to give 664 mg (75 %) of pure product **42** as a white foam. IR (neat) 1740, 1542, 1171, 735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (m, 1H), 7.66 (m, 3H), 7.25 (d, 2H, J = 8.5 Hz), 6.86 (d, 2H, J = 8.6 Hz), 5.09 (d, 1H, J = 11.7 Hz), 4.99 (d, 1H, J = 11.7 Hz), 4.86 (br d, 1H, J = 6.8 Hz), 4.46 (m, 2H), 4.37 (br d, 1H, J = 15.2 Hz), 3.93 (br d, 1H, J = 15.2 Hz), 3.82 (s, 3H), 3.15 (ddd, 1H, J = 15.5, 6.8, 3.1 Hz), 2.70 (br d, 1H, J = 15.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 159.8, 147.5, 133.6, 133.4, 131.8, 131.2, 130.6, 126.8, 124.5, 113.8, 67.4, 55.3, 52.6, 47.3, 46.2, 44.8, 28.9; HRMS (EI) calcd for $C_{20}H_{20}Br_2N_2O_7S$ (M+) 589.9358, found 589.9345.

3. Synthesis of a Bis-amino Acid that Creates a Sharp Turn

3.1. Introduction

An alternate approach to the construction of *bis*-amino acid monomers was necessary because the Diels-Alder reaction had not been relatively facile. Wipf and coworkers[76] have shown that hydroindole ring systems of *cis*-fusion can be synthesized stereoselectively through the use of a cyclooxidation-rearrangement sequence of Cbz-*L*-tyrosine (Scheme 19). The ketone functionality can then be elaborated, via a Bucherer-Bergs reaction[77], into an amino acid, resulting in a rigid bis-amino acid building block.



Scheme 19: Wipf's diastereoselective cyclooxidation

Several other groups[78,79,80] have developed synthetic pathways to the hydroindole ring system towards constructing the core of such marine metabolites as Aeruginosin 298A[81] and Dysinosin A.[82] As various methods were available to construct such a ring system, Wipf's process was chosen simply for convenience and facility.

The hydroindole ring acts as a conformationally restricted proline derivative.[76] These types of compounds project peptide chains into defined regions of space, promoting specific turns in peptide folding and bestowing bioactive conformations.[83,84,85] This inherent quality of rigidity represents an important characteristic of a *bis*-amino acid monomer.

The synthesis of a new *bis*-amino acid hydroindole monomer **1** is presented (Figure 11). This monomer is designed to create a tightly curved structure when assembled into oligomers. The monomer is demonstrated to couple to the previously developed monomer **2**[29] through pairs of amide bonds to create a strongly bent spiro-ladder oligomer. The structure of oligomer **3** was determined in aqueous solution using two-dimensional NMR techniques.

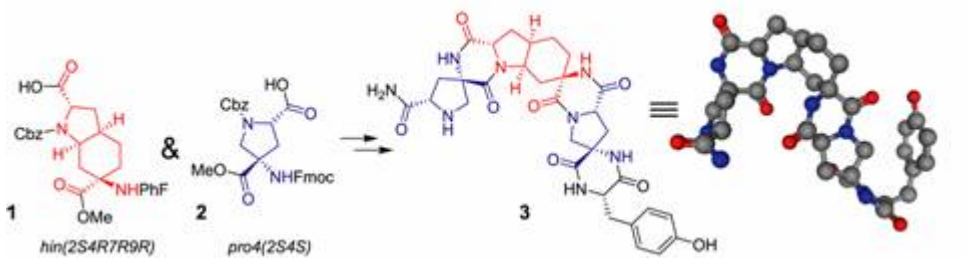
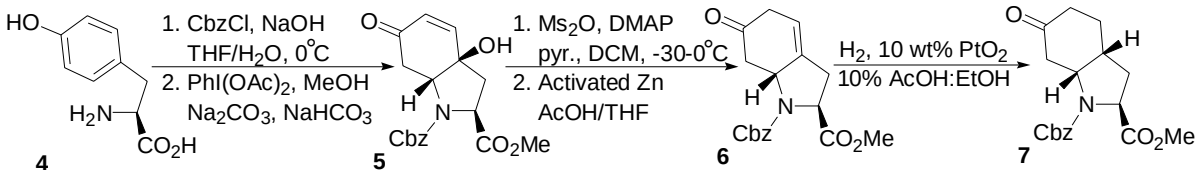


Figure 11: A sharp turn

The hydroindole monomer was given the name hin(2*S*4*R*7*R*9*R*). This abbreviation is intended to be used as shorthand for hydroindole (hin) followed by the absolute stereochemistry of each chiral center contained in the building block. Similarly, monomer **2** was named pro4(2*S*4*S*) because it was derived directly from 4-hydroxyproline (pro4). Thus, the above oligomer **3** is composed of the sequence pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)-(*L*)-tyrosine.

3.2. Results and Discussion

The synthesis of monomer **1** used chemistry developed by Wipf and coworkers[76,86] to convert (*L*)-tyrosine into ketone **7** in a five step sequence (Scheme 20).



Scheme 20: Synthesis of Wipf's ketone

The amino group of (*L*)-tyrosine **4** was protected with the carboxybenzyl (Cbz) group followed by exposure to iodobenzene diacetate in basic methanol. The amino acid underwent an oxidative-rearrangement reaction that results in compound **5** as a single diastereomer. The excellent selectivity of this reaction was based upon destabilizing steric interactions between the amide oxygen and the methyl ester substituent in the transition state. Elimination of the tertiary mesylate of compound **5** with activated zinc gave **6**. Catalytic hydrogenation of enone **6** proceeded with good facial selectivity (8:1), leading to the *cis*-fused hydroindolinone **7**.

Elaboration of the ketone into a hydantoin mixture through the use of a Bucherer-Bergs reaction was first attempted as a possible pathway to an amino acid moiety. Although the reaction proceeded in decent yield (69 %) the resultant mixture of four hydantoin diastereomers proved inseparable after exhaustive chromatography/crystallization trials. As a result, an additional strategy was employed.

Reduction of ketone **7** with the anion of chloroform in tetrahydrofuran at low temperature yielded the trichloromethyl carbinol **8** in moderate yield with 10:1 stereoselectivity (Scheme 21). Fortunately, the major diastereomer was able to be crystallized from ethyl acetate/hexanes and the stereochemistry confirmed using X-ray crystallography (Appendix A). The degree of stereoselectivity is consistent with equatorial attack of the bulky nucleophile to the minimum energy conformation of the ketone (Figure 12).[87] Bonjoch has shown that the Boc derivative of

the ketone has a strong preference for a conformation that avoids $A^{1,3}$ strain between the N-substituent and the C(7) methylene group.[88]

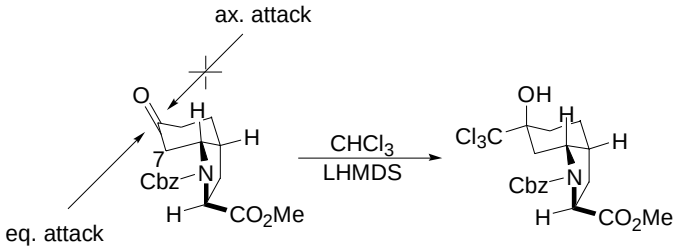
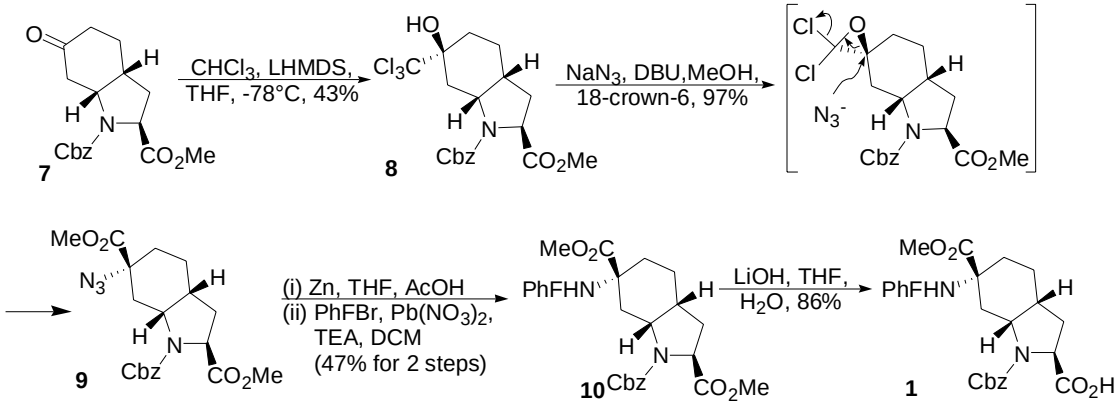


Figure 12: Reduction of ketone 7

The alcohol **8** was then converted in a single step, with inversion of configuration, to the azido methyl ester **9** using a modified Corey-Link[89] reaction developed by Dominguez and coworkers.[90,91] This remarkable reaction is considered to proceed via the *gem*-dichloro-oxirane intermediate shown in Scheme 21.

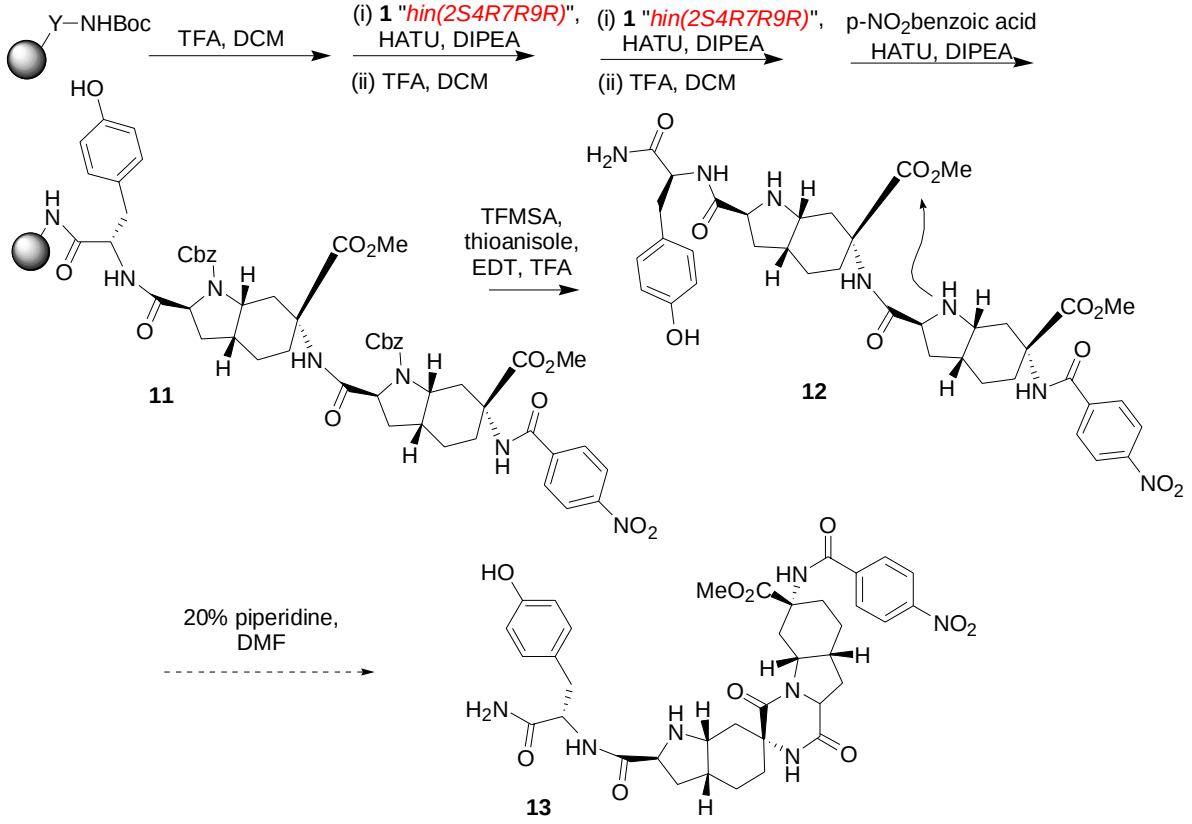


Scheme 21: Synthesis of hin(2*S*4*R*7*R*9*R*)

Several methods were evaluated for reduction of azide **9**, however, only zinc in acetic acid[92] provided the amine in any appreciable yield and purity. Protection of the amine intermediate with the phenylfluorenyl (PhF) group was accomplished under basic conditions in the presence of the bromide scavenger, lead (II) nitrate, to give **10**.[93] Other protecting groups were similarly experimented with (e.g., Nosyl, Boc, Adoc, Trityl), but only the PhF group

proved successful. The PhF group is chemically orthogonal to the Cbz protecting group on the secondary amine of the building block. The steric bulk provided by the PhF group also shields the adjacent methyl ester, allowing for regioselective saponification of the distal methyl ester unit, yielding **1**. Monomer **1** contains two orthogonally protected amino acid moieties suitable for sequential solid-phase coupling (Scheme 22).

The molecular building block **1** was first incorporated into a two-mer structure to test the solid phase coupling chemistry on a 4.45 μmol scale utilizing an MBHA resin preloaded with Boc-*L*-tyrosine (Novabiochem). The resin was deblocked with trifluoroacetic acid, followed by two sequential couplings of monomer **1**, and finally capped with p-nitrobenzoic acid to give **11**.



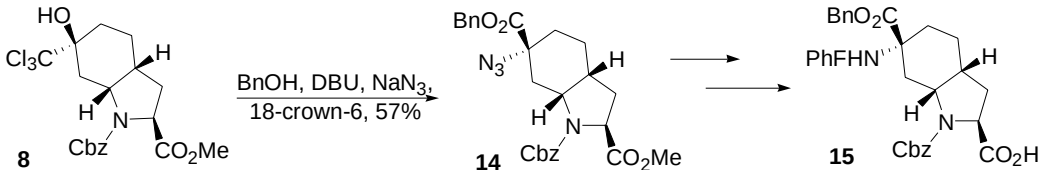
Scheme 22: SPS of (*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)-hin(2*S*4*R*7*R*9*R*)-p-NO_2-benzoate

The roles of the tyrosine and p-nitrobenzyl groups are to provide UV-active chromophores and to enhance the lipophilicity of the oligomer to allow C_{18} reverse-phase purification. It has been

observed[29] previously that oligomers that do not carry lipophilic groups are too water soluble and refuse to bind to a C_{18} reverse-phase column. Each monomer was activated as the 1-hydroxy-7-azabenzotriazole (HOAt) ester.[94] Quantitative coupling to the previous monomer was achieved through double coupling of two equivalents of activated monomer with respect to resin loading. Couplings were carried out at room temperature for 90 minutes. The oligomer was then cleaved from the resin with simultaneous removal of all the carboxybenzoyl (Cbz) groups using trifluoroacetic/trifluoromethane sulfonic acid mixture to produce **12**.

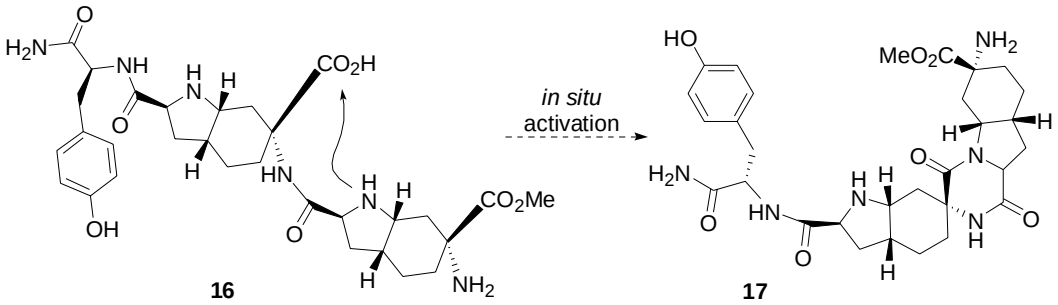
Diketopiperazine (DKP) closure was tested, to rigidify the oligomer, by incubation of **12** in a 20% piperidine solution in dimethylformamide. Pedroso and coworkers have found that piperidine in DMF greatly accelerates DKP formation in peptides.[95] Nonetheless, after multiple days of incubation, no trace of compound **13** was observed by LC-MS. As a result, the two-mer was resynthesized, containing instead, a hin(2*S*4*R*7*R*9*R*) monomer with a benzyl ester, replacing the methyl ester, as in compound **15** (Scheme 23). The expected outcome from solid-phase synthesis was that upon cleavage from the resin, the resulting flexible oligomer would contain both a free secondary amine and a carboxylic acid that could be coupled together via *in situ* activation with a peptide coupling reagent.

Compound **15** was synthesized (Scheme 23) in a similar manner to **1**, differing only by the conditions used in the Corey-Link reaction. Benzyl alcohol was substituted for methanol as the solvent and was removed by Kugelrohr distillation and chromatography. Although not optimized, the synthetic sequence provided enough of compound **15** to test the *in situ* activation strategy.



Scheme 23: Synthesis of a hin(2*S*4*R*7*R*9*R*) derivative

Two-mer **16** (Table 2) of the sequence (*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)(OBn)-hin(2*S*4*R*7*R*9*R*)(OMe) was synthesized as previously (*vide infra).* The first hydroindole monomer contained a benzyl ester, which upon cleavage from the resin unmasked a free carboxylic acid. The second monomer in the sequence was compound **1**, containing a methyl ester. Various peptide coupling reagents were evaluated, but none gave sufficient DKP closed product **17**.

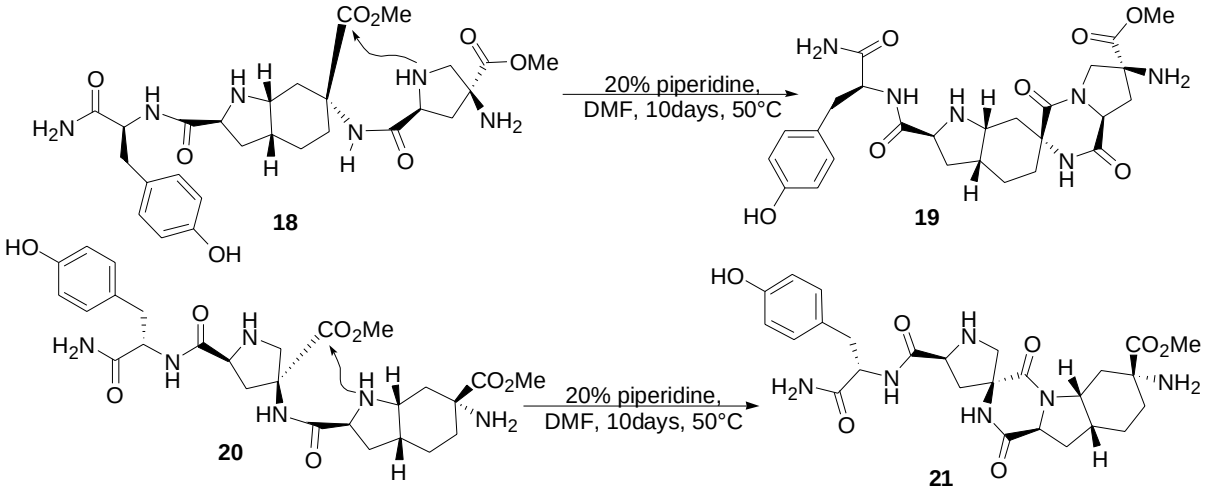


Coupling Reagent	Solvent/Conditioins	DKP Product
PyBOP/HOBt	DMF/DIPEA	Trace
TFAA	DMF	None
PyBrop	DMF/DIPEA	None
HATU	DMF/DIPEA	None

Table 2: *In situ* coupling trials

The inability of the DKP to close may be due to a combination of the sterically hindered nature of the nucleophile and a strained transition state tetrahedral intermediate.[96] Thus, a new design rule must be established that no sequence can contain two hin(2*S*4*R*7*R*9*R*) monomers in a row in order to make rigidified oligomers or better conditions are needed to close the DKPs.

Another option is to synthesize hetero-oligomers containing sequences of different building blocks. Ultimately, the most desirable scenario is one in which larger oligomers are constructed from a library of diverse monomers. This will allow greater range in oligomer architecture. Previously a proline based monomer pro4(2*S*4*S*) was published that is compatible with the hin(2*S*4*R*7*R*9*R*) coupling chemistry. Initially two short sequences were synthesized to test whether DKP closure would be facile enough to construct rigid hetero-oligomers. Two-mers of the sequence (*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*) **18** and (*L*)-tyrosine-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*) **20** were constructed as previously (Scheme 24).

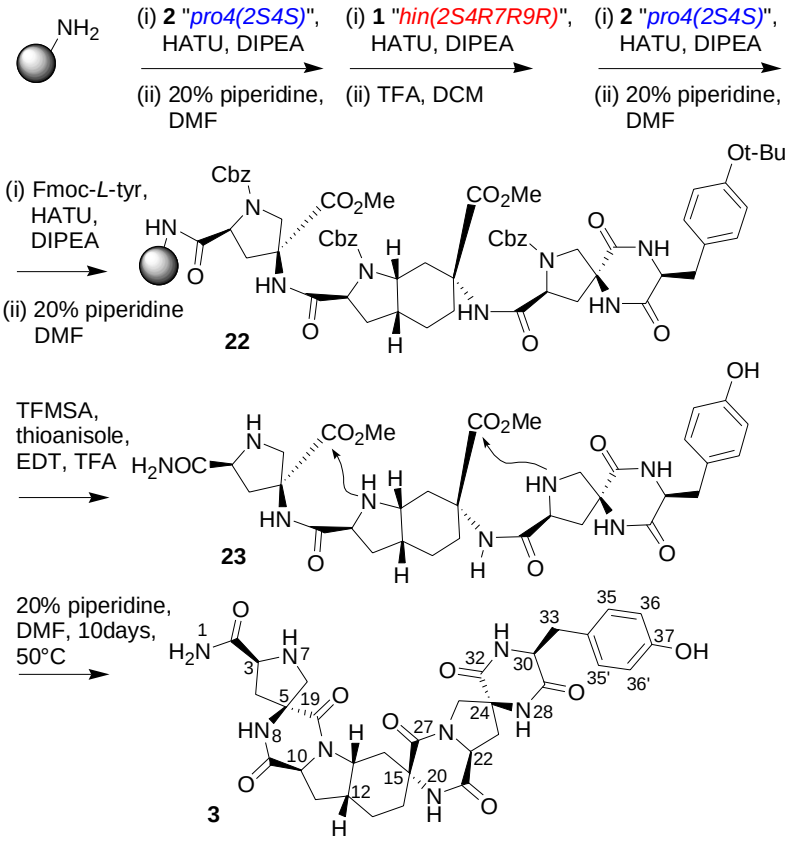


Scheme 24: DKP closure of two-mers

Both compounds **18** and **20** were found to close in 20 % piperidine in DMF at 50 °C. Reaction progress was monitored by LC-MS and was complete after 10 days. With this result it was possible to construct a three-mer and determine its solution structure using NMR spectroscopy.

A three-mer of the sequence pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)-(*L*)-tyrosine was synthesized on the MBHA·LL HCl resin (Novabiochem) using the previous methods (Scheme 25). After the three monomers were coupled, the oligomer was functionalized with an Fmoc-(*L*)-tyrosine residue. An extended, two hour deprotection step was then carried out to remove the N-

terminal Fmoc group and to accelerate the attack of the terminal amine on the methyl ester of the pro4(2*S*4*S*) monomer that precedes it to for diketopiperazine **22**.



Scheme 25: Synthesis of a three-mer containing a sharp turn

The oligomer was then cleaved from the resin with concomitant cleavage of all the Cbz groups and the t-butyl protecting group of the tyrosine side chain, yielding **23**. This flexible oligomer was then subjected to DKP annealing conditions as before to provide the rigidified oligomer **3**.

The molecular mechanics package MOE[97] was then used to carry out a stochastic conformational search[98] of compound **3** to locate the lowest AMBER94[99] energy minima *in vacuo*. The modeled structure of the global energy minimum showed that the molecule was strongly bent, with the cyclohexane ring of the central "hin" monomer adopting a chair conformation that places the amide nitrogen substituents N18 and N20 in a 1,3-diaxial

arrangement (Figure 13). This 1,3-diaxial conformation was reasonable because the planes of the two amides are parallel to one another and are locked in an extended fused ring system. The first lowest energy minimum that displays a different cyclohexane chair conformation is 2.4 kcal/mol higher than the global energy minimum, indicating that molecular mechanics predicted that this oligomer has a four times greater kT preference for this conformation.

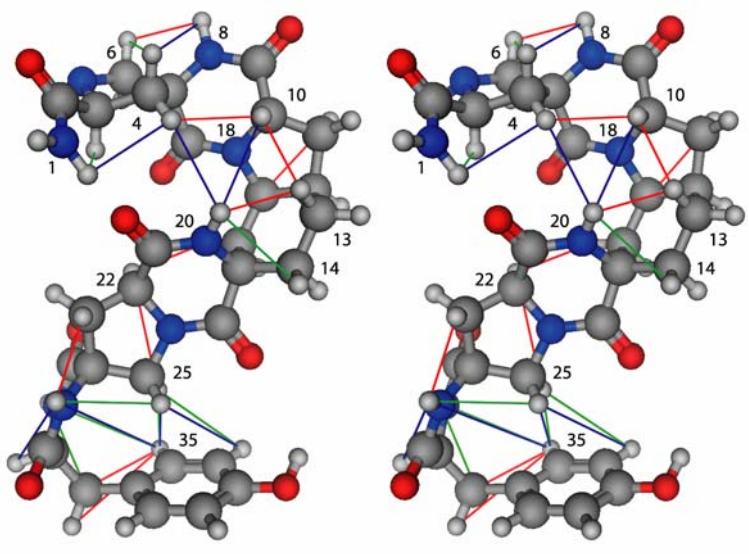


Figure 13: Stereoimage of the lowest energy conformation of 3. Protons that are correlated in the 2D ROESY spectrum are connected with lines (strong, medium, and weak ROESY correlations are colored red, green, and blue, respectively).

To test this molecular mechanics prediction the solution structure of the three-mer was determined using two dimensional NMR. The spectra were taken in $H_2O/D_2O/CD_3CN$ 80:10:10 at a concentration of 4 mM at 4 °C. Low temperature was utilized to allow the residual solvent peak to shift downfield a few ppm so that it did not obscure the α-proton resonances. The ^{1}H and ^{13}C resonances were assigned through the interpretation of a collection of 2D spectra including a COSY, an HMBC, an HMQC and a ROESY. The assignment was carried out using the software package SPARKY.[100] The ROESY spectrum provided 26 cross peaks that correlated non-J-coupled protons. The cross-peaks were ranked as strong, medium, and weak on the basis of their

relative intensity. The ROESY correlations were superimposed on top of the minimum energy structure and were completely consistent with the model (Figure 13). The bent conformation of the hin(2*S*4*R*7*R*9*R*) monomer was supported by eight ROESY correlations, including a web of correlations among the proton on N20 and protons on C4, C10, C13, and C14, as well as strong correlations between the α-proton on C22 and the proton on C16 (Figure 13). The diketopiperazines between monomers formed shallow boats with their substituents C11 and C23 occupying pseudoequatorial orientations. The proline ring-based pro4(2*S*4*S*) monomers adopted an envelope structure that avoids a 1,3-interaction between the two substituents that are *syn* to one another on the ring (C2 and N8, and C21 and N28). These conformations are identical to what was seen in a previous NMR structure that contained pro4(2*S*4*S*).[29] The folded-back conformation of the tyrosine side chain was also a feature seen in a previous NMR structure and was probably due to a hydrophobic interaction between the aryl ring and the methylene of the last pro4(2*S*4*S*) monomer.[29]

3.3. Conclusions

The synthesis of the hin(2*S*4*R*7*R*9*R*) monomer **1** and its assembly into a heterosequence with pro4(2*S*4*S*) monomers **2** have been demonstrated. The solution structure of this monomer in the context of a short oligomer, at 4 °C, has been determined to have a strong conformational preference to form a tight curve. Combining this monomer with others to form longer sequences should enable the construction of compact, water soluble macromolecules that contain cavities with controlled size and shape for a variety of biomimetic and nanotechnology applications.

3.4. Experimental Section

General: THF was distilled from Na/benzophenone under N_2. DCM and $CHCl_3$ were distilled from CaH_2. Anhydrous methanol was purchased from Aldrich Chemical Company and used

directly. $Pb(NO_3)_2$ was dried by heating in a vacuum oven (95 °C, 1 mm Hg) overnight and stored in a sealed container over anhydrous $CaCl_2$. Phenylfluorenyl bromide was prepared according to a previously reported procedure (Jamison, T.F., Lubell, W.D., Dener, J.M., Krische, M.J., Rapoport, H. *Org. Syn.*, *CV9*, 103-106.). All other reagents were used as received unless otherwise noted. All reactions were carried out in flame-dried or oven-dried glassware under N_2 atmosphere unless otherwise noted. Column chromatography was performed using ICN Silitech 32-63 D (60 A) grade silica gel and TLC analysis was performed on EM Science Silica Gel 60 F_{254} plates (250 μm thickness). NMR experiments were performed on either Bruker Avance 300 MHz or Bruker Avance DRX 500 MHz spectrometers. Chemical shifts (δ) are reported relative to $CDCl_3$ or DMSO-d_6 residual solvent peaks, unless otherwise noted. If possible, rotational isomers were resolved by obtaining spectra above 100 °C in DMSO-d_6 at the below indicated temperatures. IR spectra were obtained on a Nicolet Avatar E.S.P. 360 FT-IR. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter at the indicated temperatures. EI-MS was performed on a Micromass Autospec high resolution mass spectrometer (CEBE geometry). High resolution ESI-MS was performed on a Waters LC/Q-TOF instrument. HPLC analysis was performed on a Hewlett-Packard Series1050 instrument with diode array detector, using a Varian Chrompack Microsorb 100 C_{18} column (5 μm packing, 2.6 mm x 250 mm). HPLC-MS analysis was performed on a Hewlett-Packard Series 1100 instrument with diode array detector, HP 1100 MS detector (ESI), using a Waters Xterra MS C_{18} column (3.5 μm packing, 4.6 mm x 100 mm). Analysis of 2D NMR data was performed using Sparky 3, T.D. Goddard and D.G. Kneller, University of California, San Fransisco.

(2*S*,4*R*,7*S*,9*R*)-1-benzyl 2-methyl 7-(trichloromethyl) octahydro-7-hydroxyindole-1,2-dicarboxylate (8).

A solution of ketone **(7)** (2.55 g, 7.70 mmol) in THF (40 mL) was cooled to -78 °C in a dry ice/acetone bath and dry $CHCl_3$ (1.55 mL, 19.2 mmol) was added. A 1 M solution of LHMDS in THF (16.9 mL) was added dropwise over a period of 20 min via a syringe pump. After addition was complete, the reaction was stirred for an additional 1 h at -78 °C. The reaction mixture was then poured into a saturated solution of NH_4Cl and extracted twice with EtOAc (2 x 50 mL). The combined organic fractions were dried (Na_2SO_4), filtered and the solvent removed *in vacuo*. The remaining residue was then sufficiently purified by flash column chromatography (1:2 EtOAc:hexanes, loading in 1:1 EtOAc:hexanes). The resulting foam from purification was then recyrstallized from EtOAc/hexanes to yield 1.47 g (43 %) of diastereomerically pure (determined by NMR) **(8)** as small, white granular crystals (mp = 173.8-174.5 °C). $[\alpha]^{22}_D$ = -32.2 (*c* 0.5, DCM); IR (neat) 3344, 1746, 1685, 1419, 1352, 1291, 1203, 1173, 1024, 773 cm^{-1}; ^1H NMR (300 MHz, 105 °C, DMSO-d_6) δ 7.35-7.27 (m, 5H), 5.68 (br s, 1H), 5.13 (br d, J = 12.5 Hz, 1H), 5.02 (br d, J = 12.5 Hz, 1H), 4.35 (d, J = 8.8 Hz, 1H), 4.22-4.15 (m, 1H), 3.59 (s, 3H), 2.60-2.50 (m, 1H), 2.47-2.40 (m, 1H), 2.36-2.21 (m, 1H), 2.13-1.90 (m, 2H), 1.86-1.76 (m, 2H), 1.70-1.53 (m, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) (~1:1 mixture of rotamers) δ 172.8, 172.7, 154.3, 153.8, 136.5, 136.2, 128.4, 128.3, 127.9, 127.8, 109.0, 82.0, 81.9, 67.1, 58.4, 58.3, 55.5, 55.0, 52.3, 52.1, 33.8, 32.9, 32.5, 32.0, 31.9, 30.7, 25.5, 25.4, 21.0; HR-MS (EI) calculated for $C_{19}H_{22}Cl_3NO_5$ 449.0564, found 449.0569.

(2*S*,4*R*,7*R*,9*R*)-1-benzyl-2,7-dimethyl-7-azido octahydroindole-1,2,7-tricarboxylate (9).

To a solution of alcohol **(8)** (1.05 g, 2.33 mmol) in 46 mL of anhydrous methanol, was added sodium azide (454 mg, 6.99 mmol) and a catalytic amount of 18-crown-6 (10 mg). DBU (1.75 mL, 11.6 mmol) was added slowly and the reaction allowed to stir for 22 h. The reaction mixture was diluted with diethyl ether (100 mL), washed with NH_4Cl (50 mL), dried (Na_2SO_4),

filtered and concentrated. The oily residue was then purified by flash column chromatography (1:2 EtOAc:hexanes) to yield azido ester **(9)** (939 mg, 97 %) as a yellow oil. $[\alpha]^{21}_{D}$ = -16.9 (*c* 5, DCM); IR (neat) 2105, 1726, 763, 697 cm^{-1}; ^{1}H NMR (300 MHz, 105 °C, DMSO-d_6) δ 7.35-7.28 (m, 5H), 5.10 (d, J = 12.2 Hz, 1H), 5.02 (d, J = 12.2 Hz, 1H), 4.35 (br d, J = 7.8 Hz, 1H), 4.13-4.05 (m, 1H), 3.76 (br s, 3H), 3.59 (br s, 3H), 2.58-2.50 (m, 1H), 2.42-2.29 (m, 2H), 1.99-1.92 (m, 1H), 1.85-1.78 (m, 1H), 1.75-1.65 (m, 3H), 1.65-1.58 (m, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) (~1:1 mixture of rotamers) δ 172.7, 172.4, 170.6, 170.3, 154.0, 153.5, 136.1, 128.2, 128.1, 127.7, 127.6, 127.6, 127.3, 66.8, 66.6, 64.8, 58.3, 55.1, 54.7, 52.6, 52.5, 52.0, 51.8, 34.2, 33.6, 33.3, 32.3, 31.0, 27.9, 27.4, 21.5; HR-MS (EI) calculated for $C_{20}H_{24}N_2O_6$ (M-N_2) 388.1634, found 388.1635.

(2*S*,4*R*,7*R*,9*R*)-1-benzyl-2,7-dimethyl-7-amino octahydroindole-1,2,7-tricarboxylate (9a). To a solution of azido ester **(9)** (2.51 g, 6.03 mmol) in 20 mL of 1:1 AcOH:THF was added powdered Zn (250 mg, 10 wt%) in one portion. The suspension was allowed to stir for approximately 45 min. Evolution of bubbles of N_2 gas was observed during this time period. (Note: If the reaction was sluggish, an additional 5 wt% of Zn was added to achieve complete reaction.) The Zn was then filtered from the reaction mixture and washed with a copious amount (100 mL) of ethyl acetate. The diluted solution was transferred to a separatory funnel and carefully washed several times with saturated $NaHCO_3$. The combined bicarbonate portions were washed twice with fresh portions of EtOAc (20 mL). The combined organic fractions were dried (Na_2SO_4), filtered and the solvent removed *in vacuo*. Non- polar impurities were removed by adsorption of the residue onto a small plug of silica and elution first with 1:2 EtOAc:hexanes followed by 9:1 $CHCl_3$:MeOH. Finally, the solvent was removed to provide the amine product **(9a)** (1.80 g, 76 %) as a clear, viscous oil. This compound was used without further purification.

$[\alpha]^{20}_D$ = -5.5 (*c* 0.8, DCM); IR (neat) 3365, 3298, 1705, 1408, 1203, 1122, 1009, 768, 691 cm^{-1}; ^1H NMR (300 MHz, 110 °C, DMSO-d_6) (~3:1 mixture of rotamers) δ 7.33-7.27 (m, 5H), 5.11-5.05 (m, 2H), 4.79 (br s, 2H), 4.32 (br d, J = 8.3 Hz, 1H), 4.25-4.19 (m, 0.25H), 4.19-4.08 (m, 0.75H), 3.68 (br s, 3H), 3.59 (br s, 2.25H), 3.54 (br s, 0.75H), 2.82 (dd, J = 13.0, 5.6 Hz, 0.25H), 2.53-2.49 (m, 0.75H), 2.35-2.25 (m, 2H), 1.90-1.75 (m, 2H), 1.72-1.49 (m, 3H), 1.39 (dd, J = 12.9, 11.1 Hz, 0.75H), 1.23 (dd, J = 12.7, 11.0 Hz, 0.25H); ^{13}C NMR (75.4 MHz, DMSO-d_6) (~1:1 mixture of rotamers) δ 173.7, 173.5, 172.7, 172.4, 153.5, 152.9, 136.7, 136.6, 128.3, 128.2, 127.7, 127.2, 126.9, 65.9, 58.1, 57.8, 57.3, 54.9, 54.7, 52.2, 52.0, 51.9, 35.5, 34.5, 33.6, 32.6, 31.5, 30.5, 29.3, 29.1, 21.3, 21.2; HR-MS (EI) calculated for $C_{20}H_{26}N_2O_6$ 390.1791, found 390.1790.

(2*S*,4*R*,7*R*,9*R*)-1-benzyl-2,7-dimethyl-7-(9-phenyl-9H-fluoren-9-ylamino) octahydroindole-1,2,7-tricarboxylate (10).

To a solution of amine (1.80 g, 4.61 mmol) in dry DCM (10 mL), was added dry $Pb(NO_3)_2$ (1.10 g, 3.32 mmol) and triethylamine (771 μL, 5.53 mmol). A solution of PhFBr (1.48 g, 4.61 mmol) in DCM (5 mL) was added dropwise. The reaction mixture was allowed to stir for 20 h during which time a white cloudy ppt formed. The reaction mixture was filtered through Celite, the solvent removed and the resulting residue purified by flash column chromatography (1:2 EtOAc:hexanes) to give the PhF-protected product **(10)** (1.80 g, 62 %) as a white foam. $[\alpha]^{20}_D$ = -33.3 (*c* 0.6, DCM); IR (neat) 3313, 1700, 1413, 1203, 732, 702 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 7.84-7.13 (m, 18H), 5.03 (d, J = 13.1 Hz, 0.67H), 4.98 (d, J = 12.7 Hz, 0.33H), 4.90 (d, J = 12.7 Hz, 0.33H), 4.70 (d, J = 13.1 Hz, 0.67H), 4.22 (br d, J = 8.9 Hz, 0.33H), 4.13 (br d, J = 8.8 Hz, 0.67H), 3.66-3.55 (m, 1H), 3.55 (s, 2H), 3.47 (s, 1H), 3.09 (s, 1H), 3.04 (s, 2H), 2.31-1.95 (m, 3H), 1.67-1.62 (m, 2H), 1.51-1.08 (m, 4H), 0.93 (br t, J =

11.7 Hz, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 173.6, 173.5, 172.7, 172.3, 153.3, 152.5, 149.9, 149.3, 149.1, 148.4, 147.1, 147.0, 139.7, 139.5, 139.4, 139.1, 136.7, 136.4, 128.2, 128.0, 127.7, 127.5, 127.2, 126.9, 126.8, 126.7, 126.3, 126.0, 125.3, 120.0, 119.9, 71.2, 65.8, 65.7, 60.9, 60.7, 57.8, 55.0, 54.8, 51.8, 51.0, 36.8, 33.4, 32.6, 31.5, 31.1, 30.1, 21.0; HR-MS (EI) calculated for $C_{37}H_{35}N_2O_4$ (M-CO_2CH_3) 571.2597, found 571.2583.

(2*S*,4*R*,7*R*,9*R*)-1-((benzyloxy)carbonyl)-7-(methoxycarbonyl)-7-(9-phenyl-9H-fluoren-9-ylamino)-octahydro-1*H*-indole-2-carboxylic acid (1).

To a solution of **(10)** (1.80 g, 2.85 mmol) in 6 mL THF was added a solution of LiOH•H_2O (533 mg, 7.12 mmol) in 6 mL of H_2O. The reaction was allowed to stir for 24 h. It was then partitioned between DCM and 1 M HCl and the aqueous portion removed. The organic layer was washed with water (20 mL) and the combined aqueous layer washed twice with DCM (20 mL). The combined DCM layers were dried (Na_2SO_4), filtered and concentrated to yield the carboxylic acid **(1)** (1.50 g, 86 %) as a white foam that was used without further purification. $[\alpha]^{20}_D$ = -70.4 (*c* 0.5, DCM); IR (neat) 3062, 1705, 1413, 1347, 1209, 1132, 727, 697 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 7.78-7.10 (m, 18H), 5.02 (d, J = 13.1 Hz, 0.67H), 4.98 (d, J = 12.9 Hz, 0.33H), 4.91 (d, J = 12.9 Hz, 0.33H), 4.69 (d, J = 13.0 Hz, 0.67H), 4.13 (br d, J = 9.3 Hz, 0.33H), 4.02 (br d, J = 8.8 Hz, 0.67H), 3.64-3.54 (m, 1H), 3.42 (br s, 1H), 3.10 (s, 1H), 3.04 (s, 2H), 2.23-2.00 (m, 2H), 1.98-1.94 (m, 1H), 1.66-1.61 (m, 2H), 1.43-1.05 (m, 4H), 0.91 (br t, J = 11.9 Hz, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 173.7, 173.3, 153.3, 152.7, 150.0, 149.3, 149.2, 148.5, 147.1, 147.0, 139.7, 139.4, 139.1, 136.9, 136.5, 129.0, 128.2, 128.0, 127.7, 127.4, 126.9, 126.8, 126.3, 125.9, 125.4, 120.0, 71.2, 65.6, 60.9, 60.7, 57.9, 54.9, 54.7, 51.0, 36.8, 33.4, 31.1, 30.5, 21.0; HR-MS (EI) calculated for $C_{36}H_{33}N_2O_4$ (M-CO_2CH_3) 557.2440, found 557.2461.

Synthesis of Oligomers

General: Solid phase chemistry was performed using a home made manual synthesizer. Dry dichloromethane was obtained from distillation over CaH_2. Anhydrous DMF was purchased from Aldrich Chemical Company. Diisopropylethyl amine (DIPEA) was distilled from ninhydrin and potassium hydroxide and stored over molecular sieves (4 A). O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) was obtained from Acros. MBHA•LL HCl and MHBA-Y-Boc resins were purchased from NovaBiochem. All solid phase reactions were mixed by bubbling Ar through the reaction vessel and continuously kept under Ar atmosphere.

(*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)-hin(2*S*4*R*7*R*9*R*) (12)

To a 5 mL polypropylene solid phase peptide synthesis (SPPS) reactor vessel was added MBHA-Y-Boc Resin (10.6 mg, 4.45 μmol loading). The resin was swelled for 2 h in dry DMF under Ar atmosphere. Boc deprotection was completed by treatment with a 1:1 mixture of TFA:DCM (0.500 mL) for 15 minutes. Kaiser test was used to verify a free amine on the resin. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL).

To a 2.2 mL polypropylene micro centrifuge vial were added monomer **(1)** (5.48 mg, 8.90 μmol) and HATU (3.38 mg, 8.90 μmol). DCM (320 μL) and DMF (80 μL) were added. DIPEA (3.10 μL, 17.8 μmol) was added and the solution was vortexed for 1 min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction

under identical conditions. The resin was washed three times with DMF (1 mL). The terminal PhF protected amine of the building block was deblocked by treatment with 50 % TFA/DCM (500 μL) over 15 min. Release of the PhF chromophore was monitored by UV-vis spectroscopy to ensure complete deprotection. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL). The above mentioned coupling and deprotection sequence for monomer **(1)** was performed again under identical conditions.

To a 2.2 mL polypropylene micro centrifuge vial were added 4-nitrobenzoic acid (7.43 mg, 44.5 μmol) and HATU (16.9 mg, 44.5 μmol). DCM (320 μL) and DMF (80 μL) were added. DIPEA (15.5 μL, 89.0 μmol) was added and the solution was vortexed for 1 min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was washed twice with DMF, twice with DCM, twice with methanol and twice again with DCM. The oligomer-containing resin **11** was then dried under vacuum at room temperature overnight.

Deprotection of the Cbz groups of the oligomer and cleavage of the oligomer from the resin were done by treatment with a cleavage solution containing TFA (500 μL), thioanisole (50 μL), ethane dithiol (25 μL) and trifluoromethane sulfonic acid (50 μL) for 2.5 h at room temperature with magnetic stirring. The cleavage solution was drained into a centrifuge tube containing an excess volume of diethyl ether (35 mL), whereupon the oligomer precipitated as a yellow solid. The resin was washed with an additional portion of TFA (250 μL) and that volume also drained into the ether. The solution was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue washed with an additional portion of ether. The yellow solid was dried *in vacuo* and the

product purified by preparative HPLC on a Waters Delta Prep 4000 instrument with photodiode array detector (10-35 % MeCN:H_2O over 30 min, flow = 43 mL/min, UV detection = 274 nm). The product containing fractions were lyophilized to remove the solvent, yielding a fluffy white powder **12**. HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 13.42 min; LC-MS (ESI) calculated for $C_{38}H_{48}N_7O_{11}$ (M+H) 778.3, found 778.3.

(2*S*,4*R*,7*R*,9*R*)-7-(9-Phenyl-9H-fluoren-9-ylamino)-octahydro-indole-1,2,7-tricarboxylic acid 1,7-dibenzyl ester (15).

$[\alpha]^{20}_D$ = -41.0 (*c* 0.6, DCM); IR (neat) 3060, 3027, 1699, 1450, 1413, 1348, 1151, 1119, 735, 694 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 7.73 (d, 1H, J = 7.4 Hz), 7.62 (d, 1H, J = 7.5 Hz), 7.53 (d, 1H, J = 7.4 Hz), 7.12-7.36 (m, 20H), 5.01 (d, 1H, J = 13.1 Hz), 4.71 (d, 1H, J = 13.1 Hz), 4.56 (d, 1H, J = 12.5 Hz), 4.45 (d, 1H, J = 12.5 Hz), 4.12 (br d, 0.40H, J = 8.9 Hz), 4.00 (br d, 0.60H, J = 8.5 Hz), 3.55-3.65 (m, 1H), 1.90-2.38 (m, 2H), 1.52-1.70 (m, 2H), 1.20-1.50 (m, 4H), 0.75-0.95 (m, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) (~2:1 mixture of rotamers) δ 173.9, 173.4, 173.3, 153.4, 152.8, 150.0, 149.5, 149.2, 148.6, 147.1, 147.0, 139.7, 139.5, 139.4, 139.2, 137.0, 136.8, 135.9, 135.8, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.0, 126.9, 126.8, 126.3, 126.0, 125.4, 120.1, 120.0, 71.3, 65.6, 65.4, 61.1, 60.8, 58.0, 57.8, 54.9, 54.6, 36.9, 36.7, 33.4, 32.5, 31.7, 31.0, 30.4, 21.0; LC-MS (ESI) calculated for $C_{25}H_{28}N_2O_6$ (M+1-PhF) 452.2, found 452.3.

(*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)(OH)-hin(2*S*4*R*7*R*9*R*) (16)

Compound **16** was synthesized in an identical manner to compound **12**. The synthesis was done on an 8.31 μmol scale. Monomer **15** was coupled to the resin first, followed by monomer **1**. HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.36 min; LC-MS (ESI) calculated for $C_{30}H_{43}N_6O_8$ (M+H) 615.3, found 615.3.

(*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*) (18)

To a 5 mL polypropylene solid phase peptide synthesis (SPPS) reactor vessel was added MBHA-Y-Boc Resin (24.6 mg, 10.3 μmol loading). The resin was swelled for 2 h in dry DMF under Ar atmosphere. Boc deprotection was completed by treatment with a 1:1 mixture of TFA:DCM (0.500 mL) for 15 minutes. Kaiser test was used to verify a free amine on the resin. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL)

To a 2.2 mL polypropylene micro centrifuge vial were added monomer **(1)** (12.7 mg, 20.6 μmol) and HATU (7.80 mg, 20.6 μmol). DCM (320 μL) and DMF (80 μL) were added. DIPEA (7.17 μL, 41.2 μmol) was added and the solution was vortexed for 1min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal PhF protected amine of the building block was deblocked by treatment with 50 % TFA/DCM (500 μL) over 15 min. Release of the PhF chromophore was monitored by UV-vis spectroscopy to ensure complete deprotection. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL).

To a 2.2 mL polypropylene micro centrifuge tube were added monomer **(2)** (11.2 mg, 20.6 μmol) and HATU (7.8 mg, 20.6 μmol). DCM (320 μL), DMF (80 μL) and DIPEA (7.17 μL, 41.2 μmol) were added and the solution vortexed for 1 min to initiate activation of the building block.

The homogeneous solution was then immediately pipetted onto the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal Fmoc protected amine of the building block was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 40 min. UV-visible spectroscopic analysis of the piperidine fluorenyl adduct from the deblocking reaction solvent at 301 nm (ε = 7800 $M^{-1}cm^{-1}$) revealed nearly quantitative coupling, relative to initial resin loading. The resin was washed three times with DMF (1 mL). The resin was washed twice with DMF, twice with DCM, twice with methanol and twice again with DCM. The oligomer-containing resin was then dried under vacuum at room temperature overnight.

Deprotection of the Cbz groups of the oligomer and cleavage of the oligomer from the resin were done by treatment with a cleavage solution containing TFA (500 μL), thioanisole (50 μL), ethane dithiol (25 μL) and trifluoromethane sulfonic acid (50 μL) for 2.5 h at room temperature with magnetic stirring. The cleavage solution was drained into a centrifuge tube containing an excess volume of diethyl ether (35 mL), whereupon the oligomer precipitated as a yellow solid. The resin was washed with an additional portion of TFA (250 μL) and that volume also drained into the ether. The solution was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue washed with an additional portion of ether. The yellow solid **18** was dried *in vacuo.* HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.21 min; LC-MS (ESI) calculated for $C_{27}H_{39}N_6O_8$ (M+H) 575.2, found 575.2.

(*L*)-tyrosine-hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*) (19)

A solution of **(18)** in approximately 1 mL of 20 % piperidine/DMF was incubated at 50 °C for 10 days. The reaction progress was monitored by HPLC-MS. The reaction mixture was then added dropwise to a centrifuge vial containing an excess volume of diethyl ether (35 mL) to precipitate the product. The vial was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue dried under vacuum. HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 8.31 min, MS (ESI) calculated for $C_{26}H_{35}N_6O_7$ (M+H) 543.2, found 543.2.

(*L*)-tyrosine-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*) (20)

To a 5 mL polypropylene solid phase peptide synthesis (SPPS) reactor vessel was added MBHA-Y-Boc Resin (20.0 mg, 8.4 μmol loading). The resin was swelled for 2 h in dry DMF under Ar atmosphere. Boc deprotection was completed by treatment with a 1:1 mixture of TFA:DCM (0.500 mL) for 15 minutes. Kaiser test was used to verify a free amine on the resin. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL).

To a 2.2 mL polypropylene micro centrifuge tube were added monomer **(2)** (9.10 mg, 16.8 μmol) and HATU (6.40 mg, 16.8 μmol). DCM (320 μL), DMF (80 μL) and DIPEA (5.85 μL, 33.6 μmol) were added and the solution vortexed for 1 min to initiate activation of the building block. The homogeneous solution was then immediately pipetted onto the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal Fmoc protected amine of the building block was deblocked by treatment with 20 % piperidine/DMF (2

mL) over 40 min. UV-visible spectroscopic analysis of the piperidine fluorenyl adduct from the deblocking reaction solvent at 301 nm (ε = 7800 $M^{-1}cm^{-1}$) revealed nearly quantitative coupling, relative to initial resin loading. The resin was washed three times with DMF (1 mL).

To a 2.2 mL polypropylene micro centrifuge vial were added monomer **(1)** (10.30 mg, 16.8 μmol) and HATU (6.38 mg, 16.8 μmol). DCM (320 μL) and DMF (80 μL) were added. DIPEA (5.85 μL, 33.6 μmol) was added and the solution was vortexed for 1min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal PhF protected amine of the building block was deblocked by treatment with 50 % TFA/DCM (500 μL) over 15 min. Release of the PhF chromophore was monitored by UV-vis spectroscopy to ensure complete deprotection. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL). The resin was washed twice with DMF, twice with DCM, twice with methanol and twice again with DCM. The oligomer-containing resin was then dried under vacuum at room temperature overnight.

Deprotection of the Cbz groups of the oligomer and cleavage of the oligomer from the resin were done by treatment with a cleavage solution containing TFA (500 μL), thioanisole (50 μL), ethane dithiol (25 μL) and trifluoromethane sulfonic acid (50 μL) for 2.5 h at room temperature with magnetic stirring. The cleavage solution was drained into a centrifuge tube containing an excess volume of diethyl ether (35 mL), whereupon the oligomer precipitated as a yellow solid. The

resin was washed with an additional portion of TFA (250 μL) and that volume also drained into the ether. The solution was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue washed with an additional portion of ether. The yellow solid **20** was dried *in vacuo.* HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.44 min; LC-MS (ESI) calculated for $C_{27}H_{39}N_6O_8$ (M+H) 575.2, found 575.2.

(*L*)-tyrosine-pro4(2*S*4*S*)=hin(2*S*4*R*7*R*9*R*) (21)

A solution of **(20)** in approximately 1 mL of 20 % piperidine/DMF was incubated at 50 °C for 10 days. The reaction progress was monitored by HPLC-MS. The reaction mixture was then added dropwise to a centrifuge vial containing an excess volume of diethyl ether (35 mL) to precipitate the product. The vial was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue dried under vacuum. HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 8.00 min, MS (ESI) calculated for $C_{26}H_{35}N_6O_7$ (M+H) 543.2, found 543.2.

pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)=(*L*)-tyrosine (23)

To a 5 mL polypropylene solid phase peptide synthesis (SPPS) reactor vessel was added MBHA·LL·HCl Resin (26.7 mg, 16.5 μmol loading). The resin was swelled for 2 h in dry DMF under Ar atmosphere.

To a 2.2 mL polypropylene micro centrifuge vial were added monomer **(2)** (17.9 mg, 33.0 μmol) and HATU (12.5 mg, 33.0 μmol). DCM (320 μL) and DMF (80 μL) were added. DIPEA (11.5 μL, 660. μmol) was added and the solution was vortexed for 1 min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin

was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal Fmoc protected amine of the building block was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 40 min. UV-visible spectroscopic analysis of the piperidine fluorenyl adduct from the deblocking reaction solvent at 301 nm (ε = 7800 $M^{-1}cm^{-1}$) revealed nearly quantitative coupling, relative to initial resin loading. The resin was washed three times with DMF (1 mL).

To a 2.2 mL polypropylene micro centrifuge tube were added monomer **(1)** (20.3 mg, 33.0 μmol) and HATU (12.5 mg, 33.0 μmol). DCM (320 μL), DMF (80 μL) and DIPEA (11.5 μL, 660 μmol) were added and the solution vortexed for 1min to initiate activation of the building block. The homogeneous solution was then immediately pipetted onto the solid phase resin. The coupling reaction was mixed for 90 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal PhF protected amine of the building block was deblocked by treatment with 50 % TFA/DCM (500 μL) over 15 min. Release of the PhF chromophore was monitored by UV-vis spectroscopy to ensure complete deprotection. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL).

The above mentioned coupling and deprotection sequence for monomer **(2)** was performed again under identical conditions.

To a 2.2 mL polypropylene micro centrifuge tube were added N-α-Fmoc-O-*tert*-butyl-L-tyrosine (15.1 mg, 33.0 μmol), HATU (12.5 mg, 33.0 μmol), DMF (80 μL), DCM (320 μL) and DIPEA (11.5 μL, 660 μmol). The mixture was vortexed for 1min to ensure activation of the amino acid and then immediately transferred to the resin. The coupling reaction was mixed for 90 min and the solution drained from the beads. The terminal Fmoc protected amine of the tyrosine was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 2 h. The resin was washed twice with DMF, twice with DCM, twice with methanol and twice again with DCM. The oligomer-containing resin was then dried under vacuum at room temperature overnight.

Deprotection of the Cbz groups of the oligomer, *tert*-butyl protecting group of the tyrosine hydroxyl and cleavage of the oligomer from the resin were done by treatment with a cleavage solution containing TFA (500 μL), thioanisole (50 μL), ethane dithiol (25 μL) and trifluoromethane sulfonic acid (50 μL) for 2.5 h at room temperature with magnetic stirring. The cleavage solution was drained into a centrifuge tube containing an excess volume of diethyl ether (35 mL), whereupon the oligomer precipitated as a yellow solid. The resin was washed with an additional portion of TFA (250 μL) and that volume also drained into the ether. The solution was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue washed with an additional portion of ether. The yellow solid was dried *in vacuo* and the product purified by preparative HPLC on a Waters Delta Prep 4000 instrument with photodiode array detector (10-35 % MeCN:H_2O over 30 min, flow = 43 mL/min, UV detection = 274 nm). The product containing fractions were lyophilized to remove the solvent, yielding a fluffy white powder (~11 mg). HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.91 min; HR-MS (ESI) calculated for $C_{33}H_{45}N_8O_{10}$ (M+H) 713.3259, found 713.3234.

pro4(2*S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=(*L*)-tyrosine (3)

A solution of **(23)** in approximately 1 mL of 20 % piperidine/DMF was incubated at 48 °C for 10 days. The reaction progress was monitored by HPLC-MS. The reaction mixture was then added dropwise to a centrifuge vial containing an excess volume of diethyl ether (35 mL) to precipitate the product. The vial was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue purified by preparative HPLC on a Waters Delta Prep 4000 instrument with photodiode array detector (10-35 % MeCN:H_2O over 30 min, flow = 43 mL/min, UV detection = 274 nm). The product containing fractions were lyophilized to remove the solvent, yielding a fluffy white powder (~3 mg). HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 8.19 min, MS (ESI) calculated for $C_{31}H_{37}N_8O_8$ (M+H) 649.2, found 649.2.

NMR Sample Preparation

Approximately 1mg of oligomer **(3)** was dissolved in 332 μL of 9:1 H_2O/D_2O, 18 μL of 1 M (9:1 H_2O/D_2O) $ND_4CD_3CO_2$ buffer and 50 μL CD_3CN. The 2-D NMR data was collected at 4 °C. The sample was prepared in an advanced micro tube matched with D_2O purchased from Shigemi, Inc. Spectra were taken on a Bruker 500 MHz instrument.

4. Towards Macromolecules with Compact Tertiary Structures

4.1. Introduction

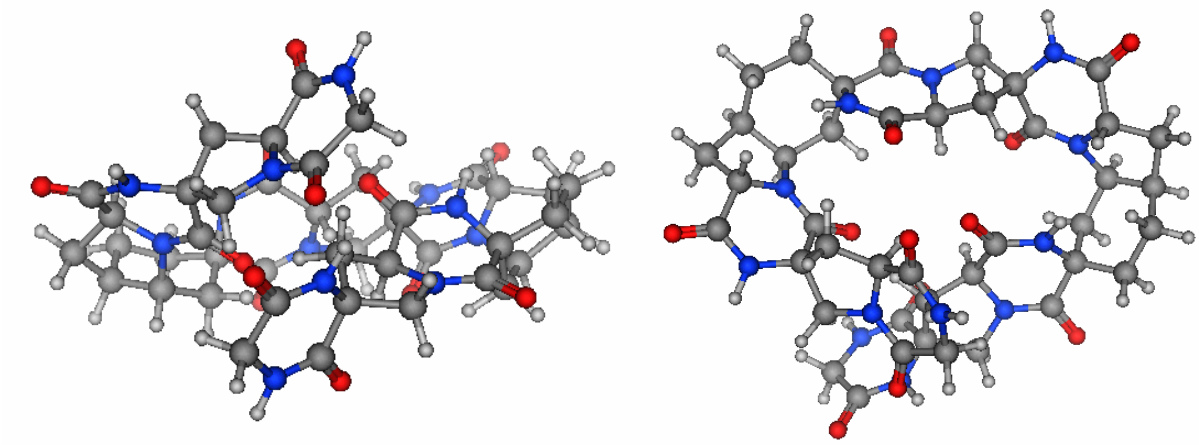
The first step in designing biomimetics[34] or functional nano-scale devices[35] is to construct large molecules with clefts, pockets, loops, and cavities that can bind the small molecule guests upon which the device will act.[101] The next step involves positioning functional or catalytic groups on the concave interior of the macromolecule with sufficient precision in solution to carry out a function.[102] On the other hand, to achieve such high-affinity and high-specificity molecular recognition, it is necessary to control the conformation of the host macromolecule.[25] Thus, controlling structure invariably controls function. This final chapter examines the first step in the creation of protein-like molecular machines, that is, the formation of asymmetric, water-soluble macromolecules containing loops and cavities. Moreover, these molecules will have "compact" tertiary structures; a phenomenon not known to synthetic polymers.

"Primary structure", as in proteins, is defined here as the sequence of building blocks composing the oligomer. Additionally, "secondary structure" is the conformations of the monomer fused ring systems and "tertiary structure" refers to the overall global structure of the rigid oligomer. This "tertiary structure" implies an architecture where the oligomer can fold back on itself, having long-range interactions among monomers on opposite sides of the sequence.

4.2. Results and Discussion

In searching through longer heterooligomer sequences of interest, a particular five-mer stood out as a candidate that contained a compact tertiary structure. Although not functional in design, the modeled structure (Figure 14) did possess a loop-like cavity where the two ends of

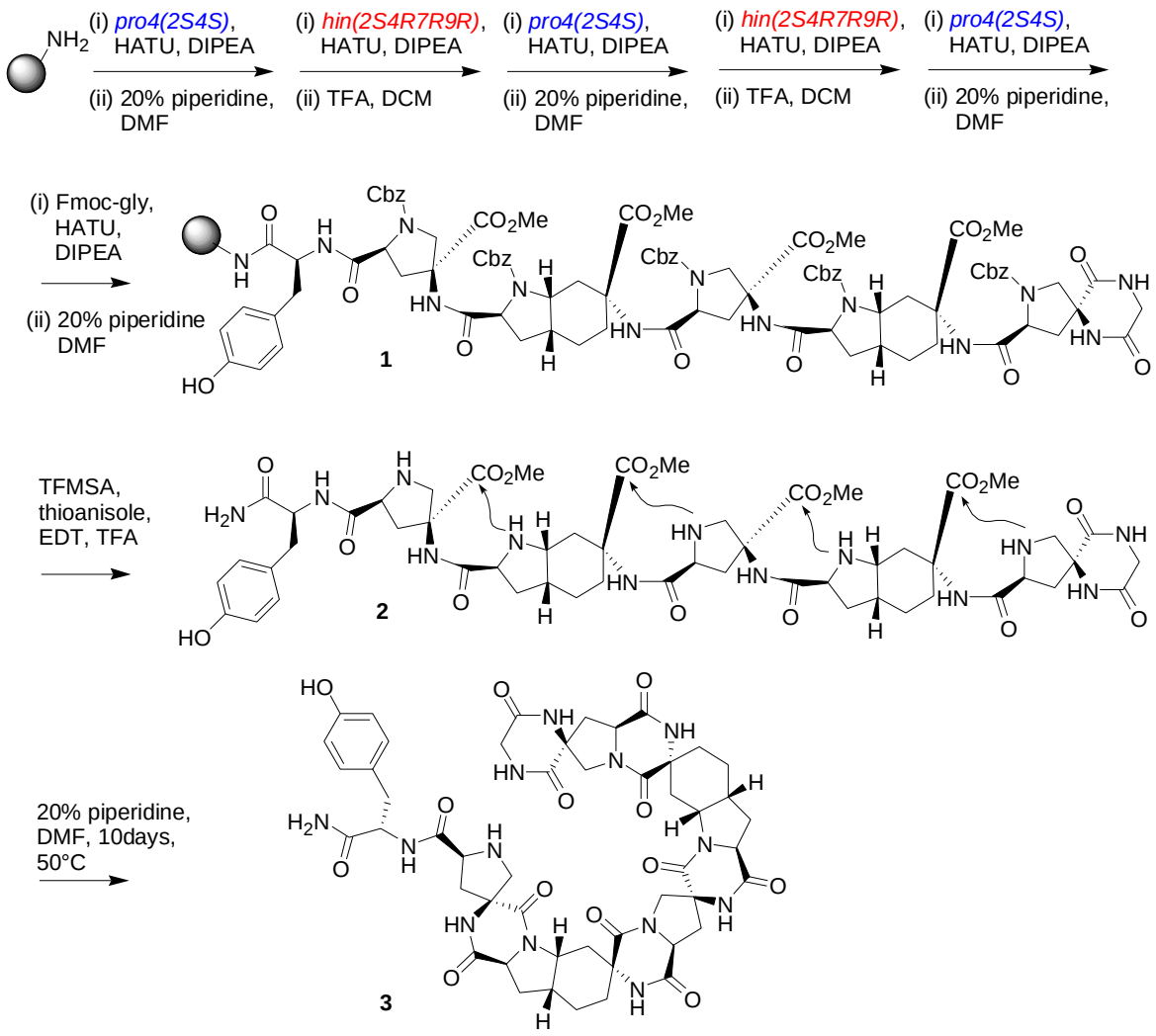
the sequence were projected over one another in space such that ROESY NMR correlations should be seen across the ends of the scaffold. The sequence of this oligomer was pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*). This molecule would be the first known example of a synthetic polymer that adopts a compact tertiary structure.



Front View Top View

Figure 14: A five-mer loop-containing macromolecule

The five-mer was constructed using the same chemistry as described previously (Chapter 3) on the MBHA·LL HCL resin on a 105 μmol scale. Initially an (*L*)-tyrosine residue was attached to the resin as a lipophilic UV chromophore. The alternating sequence of pro4(2*S*4*S*) and hin(2*S*4*R*7*R*9*R*) monomers was followed by a glycine residue to close a diketopiperazine on the leading end, as in **1** (Scheme 26). Cleavage from the resin with simultaneous removal of the Cbz protecting groups yielded crude **2** which was purified via preparative HPLC.



Scheme 26: Synthesis of a five-mer

The cleaved product **2** was then incubated in a 20 % piperidine solution in DMF at elevated temperature for 10 days to affect annealing of the oligomer to give **3**. The rigidified scaffold was then purified via preparative HPLC and characterized by high resolution electrospray mass spectrometry [HR-MS (ESI) calculated for $C_{49}H_{58}N_{13}O_{13}$ (M+H) 1036.4272, found 1036.4301]. Unfortunately, the overall yield for the synthesis of oligomer **3** was <1 %. This did not allow for the accumulation of sufficient material for 2-D NMR analysis. Subsequent attempts to optimize the overall yield failed to provide enough additional material.

There appeared to be two reasons for the low overall yield, the first being inefficient cleavage from the MBHA resin. The resin was allowed to stir in a trifluoroacetic/ trifluoromethane sulfonic acid mixture for approximately two hours. The solution was drained from the resin into a large volume of diethyl ether to precipitate the oligomer and the beads washed thoroughly with additional portions of TFA. Attempts to resubject the resin to another cleavage reaction did not provide any more precipitate. A hydrofluoric acid cleavage was not attempted due to lack of proper equipment.

Several other commercially available resins were tested. Table 3 shows the resin linkers, conditions for the coupling of the first residues to the beads, and cleavage conditions. All this chemistry is outlined in great detail in the Novabiochem catalogue.

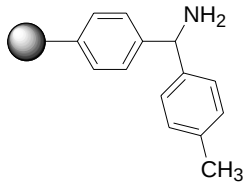
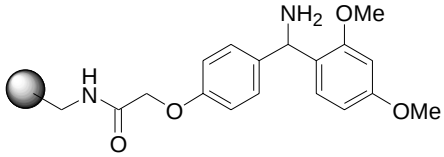
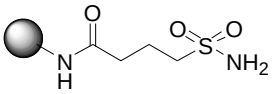
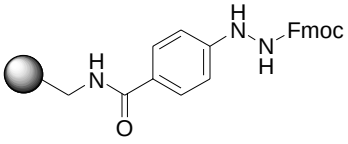
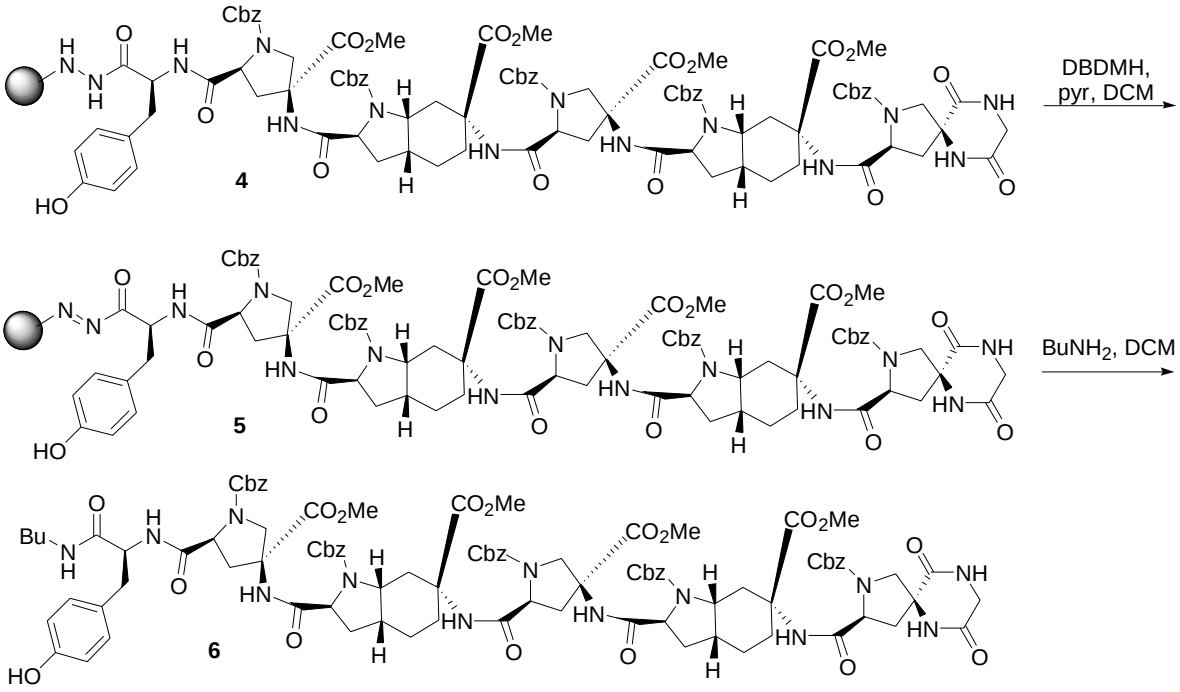
NAME	STRUCTURE	FIRST COUPLING	CLEAVAGE CONDITIONS
MBHA LL HCl		HATU, DIPEA DCM, DMF	TFMSA, EDT thioanisole, TFA or HF
Rink Amide		HATU, DIPEA DCM, DMF	95% TFA scavengers
4-Sulfamyl butyryl AM		DIC, MeIm DMF, DCM	1. ICH_2CN, DIPEA or $TMSCHN_2$ 2. RNH_2, THF
4-Fmoc hydrazino benzoyl AM		HATU, DIPEA DCM, DMF	1. NBS or DBDMH pyridine, DCM 2. RNH_2, DCM

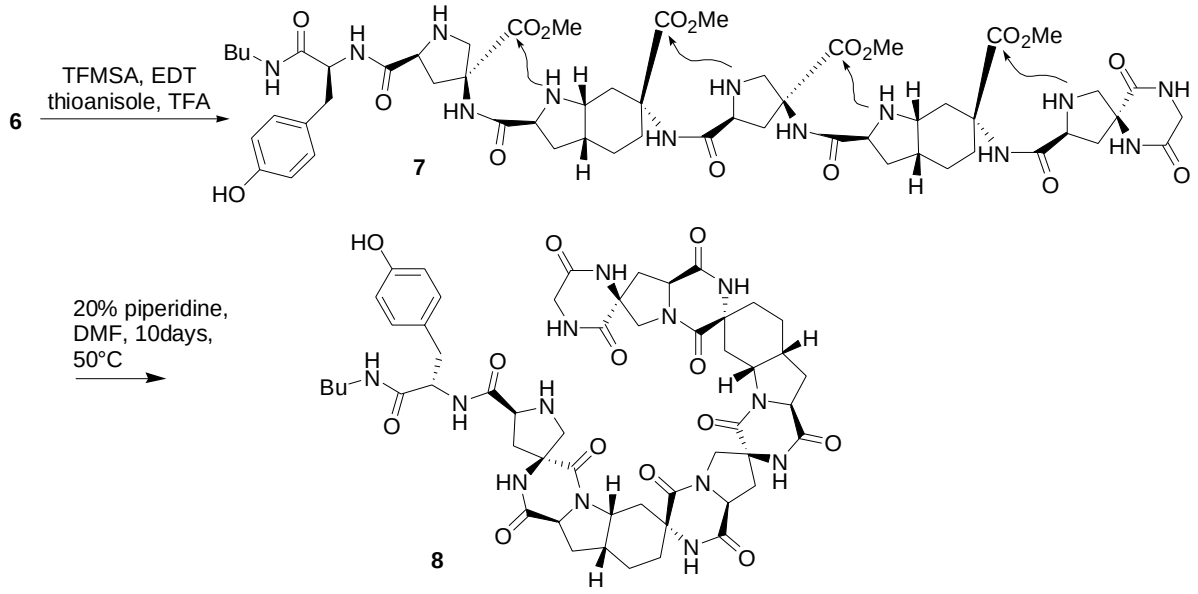
Table 3: Commercially available resins

First, the Rink Amide resin was used. This 95 % TFA cleavable linker was hoped to withstand treatment of a few equivalents of TFA during the PhF deprotection step, however, the linker was only able to withstand one such deprotection, after which, premature degradation of the linker was observed. The 4-sulfamylbutyryl resin was examined also, but it was impossible to couple any residue in significant yield to the resin. Finally the 4-Fmoc hydrazinobenzoyl resin was utilized to synthesize the 5-mer (Scheme 27). Removal of the terminal Fmoc group followed by standard solid phase synthesis revealed this linker was stable to both acidic and basic conditions, yielding **4**. Activation of the linker for cleavage was accomplished by either N-bromosuccinimide or N,N-dibromo-2,2-dimethyl-hydantoin in the presence of pyridine with dichloromethane as the solvent, giving **5**. Cleavage of the peptide from the resin could then be accomplished with various primary amines, in this case, butyl amine.



Scheme 27: Synthesis of 5-mer on the hydrazinobenzoyl resin

The resulting peptide **6** was then subjected to triflic acid to remove the Cbz groups, revealing the free and flexible oligomer **7**, now in 16 % yield (Scheme 28). Although not optimal, this was a substantial increase from 1 %.



Scheme 28: Alternate synthesis of closed 5-mer

Oligomer **7** was again incubated in the standard diketopiperazine annealing conditions to form scaffold **8**. Unfortunately, this DKP closing reaction was not clean, leaving the scaffold contaminated with large amounts of multiple side-products all of the same molecular weight, visible by LC-MS analysis. This suggested that epimerization was taking place during the incubation period and causing diastereomeric mixtures to form.

Epimerization was the second major cause of low yields during oligomer synthesis. The products were always accompanied by a small amount of a diastereomer that must be purified away from the product via preparative HPLC. However, in longer sequences, the amount of epimers present in the finished product was prohibitive to further experiments, particularly NMR analysis.

Base-catalyzed epimerization of the diketopiperazines is common[103,104] and can take place by the removal of the acidic proton adjacent to the amide, generating an enolate anion that reprotonates equally well from either face, creating a mixture of isomers (Figure 15).

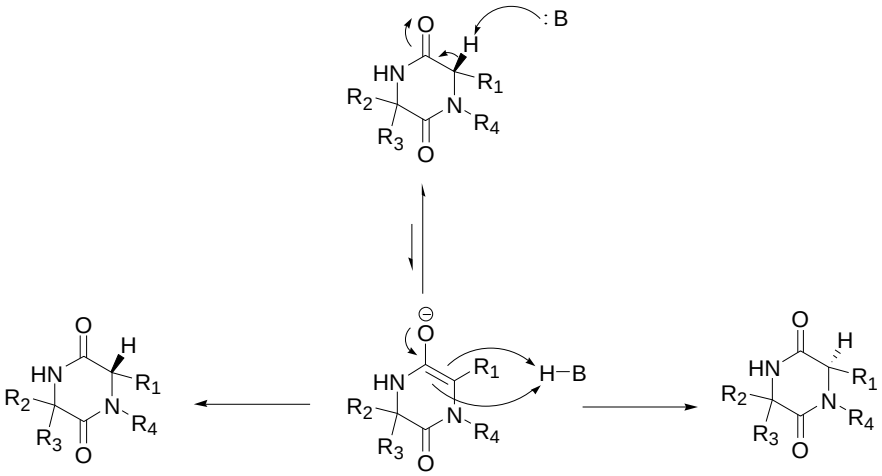


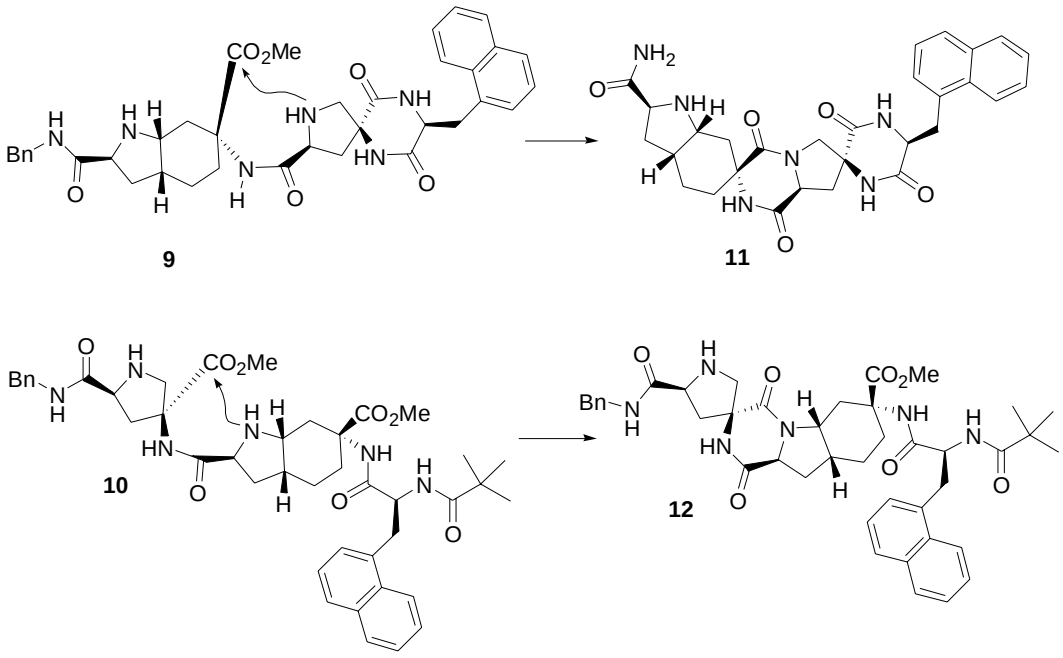
Figure 15: Base-catalyzed epimerization of DKP

As a result, testing was done to speed up the DKP-forming reaction of 5-mer **2**, under basic conditions, such that epimerization might be avoided by less exposure to harsh conditions. Table 4 shows the various conditions sampled. Heating in the microwave reactor for various time periods did produce some DKP product **3**, but also produced epimers as well. The addition of polar aprotic solvents such as propionitrile can accelerate DKP formation[105], but in 80 % propionitrile, the oligomer was not soluble. Solvent mixtures of propionitrile and DMF did not provide rigidified oligomer.

CONDITIONS	TIME	TEMPERATURE	AMOUNT DKP
20% piperidine NMP, microwave	15 min	100°C	none
20% piperidine NMP, microwave	1 h	100°C	trace
20% piperidine 80% propionitrile	10 days	RT	none
20%piperidine 60% propionitrile 20% DMF	10 days	RT	trace

Table 4: Attempted acceleration of DKP formation of compound 2

Because the basic conditions for DKP annealing proved to be too harsh to provide rigidified oligomers cleanly, acidic conditions were investigated as well. Two short two-mer sequences were synthesized on the hydrazino benzoyl resin. The sequences consisted of hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)-(*L*)-napthylalanine and pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-(*L*)-napthylalanine. The two-mers **9** and **10** were tagged with a napthylalanine residue that closed a DKP on the end in **9** but did not close in **10**. The rate of the DKP-closing reaction was much slower with the hin(2*S*4*R*7*R*9*R*) monomer than the pro4(2*S*4*S*). Instead, the free amine was capped with a pivalate group. Both oligomers were then cleaved from the resin with benzyl amine and purified before DKP testing.



Scheme 29: Two-mer DKP annealing

Acid catalysis for DKP formation was first observed by Merrifield.[106] He proposed a bifunctional mechanism (Figure 16) in which the catalytic acid assists in the breakdown of the tetrahedral intermediate. This breakdown is the rate-determining step for aminolysis reactions in aprotic media.[107] Bifunctional catalysis of the aminolysis process has been extensively studied.[108,95,109,110,111]

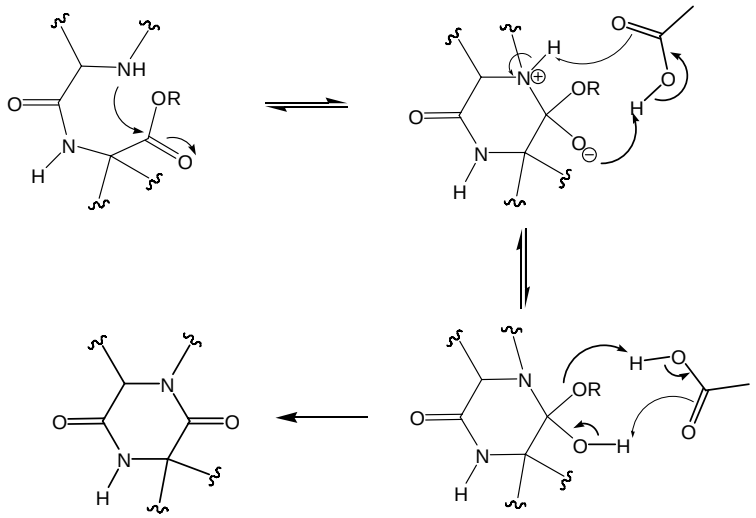


Figure 16: Acetic acid catalyzed DKP formation

Compound **10** was taken through several sets of DKP annealing conditions. The microwave reactor was used to accelerate the DKP-closing reaction. Table 5 displays the annealing results. Previous studies[112] on similar oligomer molecules have shown that the optimal catalytic conditions involved 100 mM acetic acid and 50 mM triethylamine.

REAGENTS	SOLVENT	TEMP (°C)	TIME (h)	% CONVERSION
100 mM AcOH 50 mM TEA	NMP	100	1	- - -
100 mM AcOD 50 mM TEA	o-xylene	100	1	33
100 mM AcOD 50 mM TEA	o-xylene	130	1	64
100 mM AcOH 50 mM TEA	o-xylene	100	1	71
100 mM AcOH 50 mM TEA	o-xylene	100	3	83

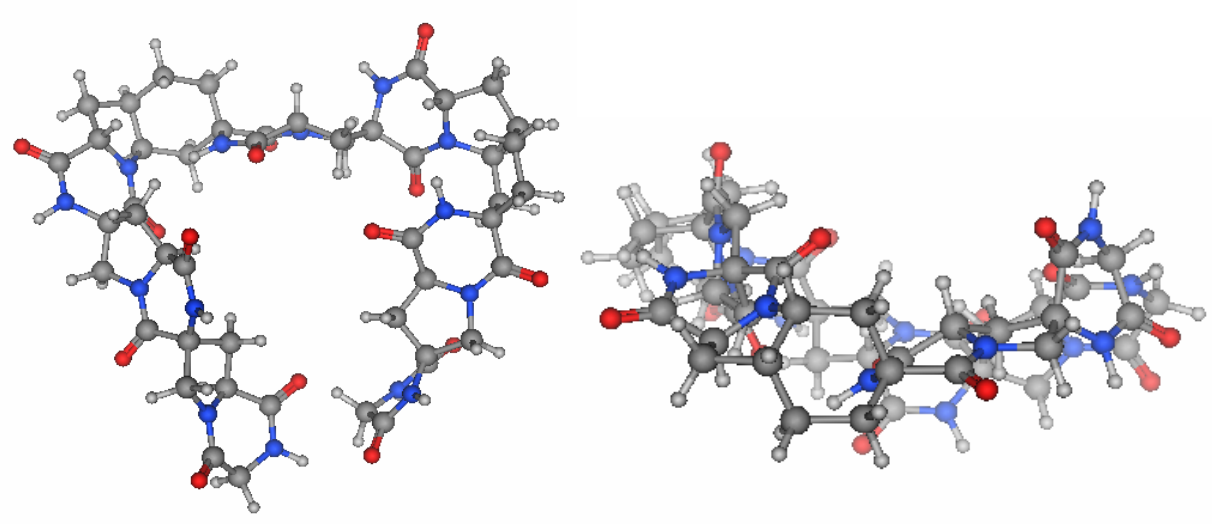
Table 5: Microwave DKP annealing of pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-napala

When the reaction was heated in the microwave in NMP for one hour some conversion to the closed DKP **12** took place, however decomposition of the solvent accompanied the reaction, yielding inadequate results. However, when o-xylene was chosen as the solvent, no

decomposition was observed and very clean conversion took place. Deuterated acetic acid was used in several runs to determine if any epimerization took place. If any epimerization occurred, then the oligomer should pick up a deuteron from solution, which would be visible via mass spectrometry. Nonetheless, no epimerization was detected under these acidic conditions.

Similar results were obtained for the other two-mer **9**. After two hours in the microwave reactor, conversion to the closed DKP **11** reached 79 %. Conversion could be pushed to near completion by running the reactions for longer periods of time; however this was not practical for a microwave reactor. Thus, a high temperature incubator was used to complete the annealing reactions at 100 °C for a period of over six hours.

From these encouraging results, two longer oligomer sequences were synthesized for annealing under these conditions. Again, the same 5-mer sequence (as in Figure 14) was assembled along with another sequence of six monomers that formed an interesting triangular shape (Figure 17). This oligomer, consisted of the sequence pro4(2*S*4*S*)-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*R*4*S*)-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*), including the two previously discussed monomers, in addition to the pro4(2*R*4*S*) diastereomer. The triangular structure of this rigidified oligomer could be used in future applications where two functional or catalytic groups are held in close proximity to one another on the ends of the scaffold.



Top View Side View

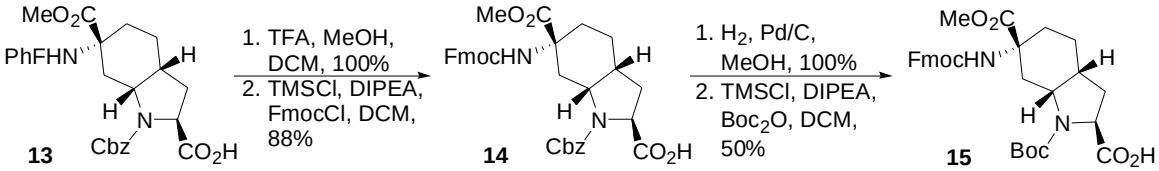
Figure 17: A six-mer triangular macromolecule

Unfortunately, neither oligomer was soluble in o-xylene. Addition of DMF as a co-solvent did improve the solubility of the oligomers, however clean conversion to the DKP closed oligomer did not take place under mixed solvent conditions. As a result, after much difficulty and exhaustive experimentation on improving the solution-phase DKP annealing reaction, a new strategy was employed.

This new strategy had to be fast, high yielding, without any side reactions and be applicable to longer sequences. On-resin DKP closure was very appealing for these reasons. Additionally, there would be no solubility problems, forcing conditions could be used to drive the reaction to completion, and the catalysts and byproducts could be filtered and washed away. However, with the current protecting group scheme used for the hin(2*S*4*R*7*R*9*R*) building block, it was impossible to close the diketopiperazines with the oligomer still bound to the resin. The Cbz protecting group can only be removed via hydrogenolysis or under very acidic conditions, two processes that cannot be done on solid support. Therefore, it was necessary to change the

nitrogen protecting groups of the hin(2*S*4*R*7*R*9*R*) monomer to those more suitable for on-resin DKP closure.

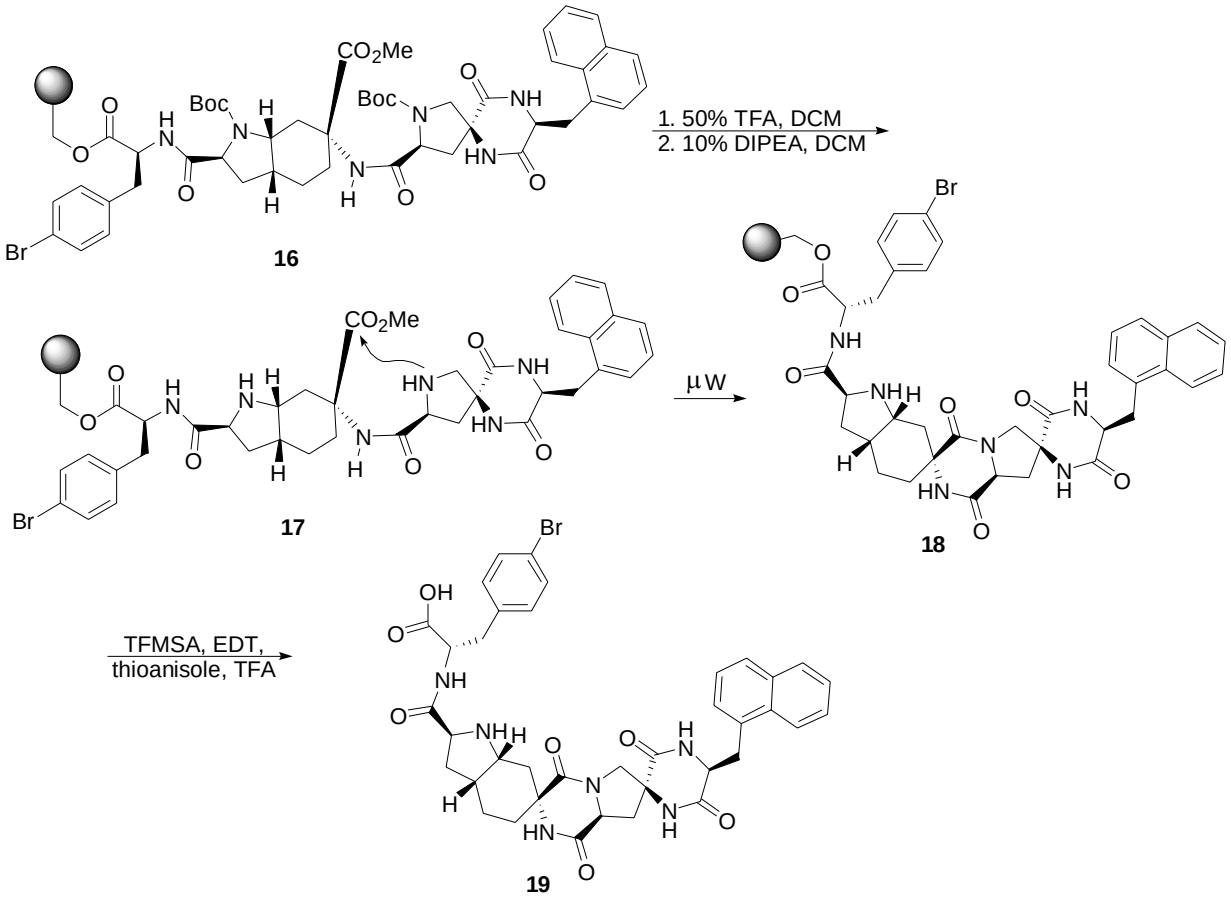
The PhF group was removed by treatment of the hin(2*S*4*R*7*R*9*R*) monomer **13** with five equivalents of TFA in dichloromethane with methanol present as a cation scavenger. The free amine was then protected with an Fmoc group in good yield, resulting in **14**. The Cbz group was then removed via hydrogenolysis over Pd/C. The resulting secondary amine was protected with a Boc group to yield another differentially protected hydroindole monomer **15**.



Scheme 30: Another hin monomer

As done previously, the monomer was then incorporated into a two-mer sequence **16** on solid support (Scheme 31). In this case, a hydroxymethyl polystyrene resin (Novabiochem) was used. This resin proved to be a bit more acid labile during the cleavage reaction with triflic acid, providing increased yields of oligomer. A bromophenyl alanine residue was first coupled to the resin to act as a mass spectral tag for the oligomer. The bromine containing residue allows the tracking of oligomers via the characteristic M and M+2 molecular ions seen in the mass spectrum due to the approximate 1:1 relative abundances of ^{79}Br and ^{81}Br isotopes. In addition, a napthylalanine was coupled to the leading end of the two-mer as a potent UV chromophore. The pro4(2*S*4*S*) monomer, this time containing a Boc group in place of a Cbz protecting group, was also used. After assembly of **16** on resin, the Boc groups were removed by treatment of the resin with TFA for 15 minutes, followed by a neutralization to yield the free amino compound **17**.

The oligomer-containing resin was then placed into the microwave reactor under various conditions to evaluate the closing of the DKP moiety.

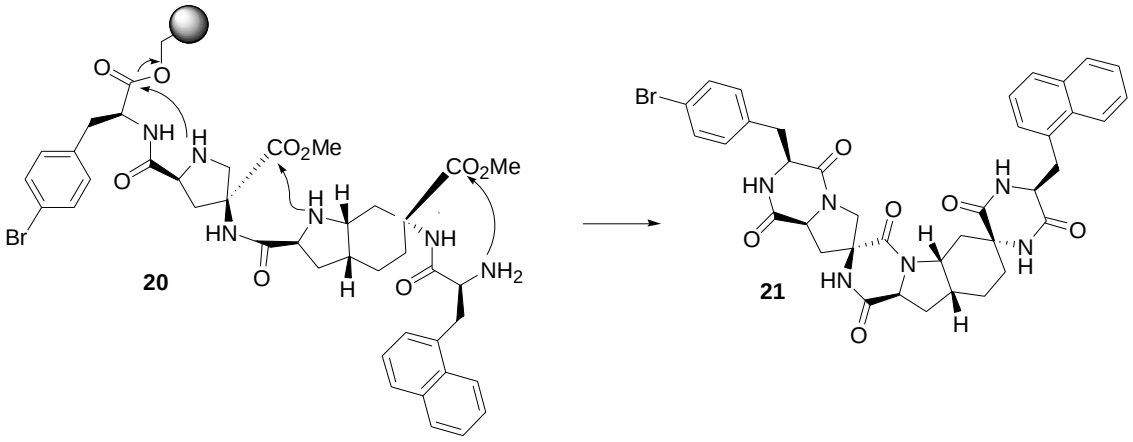


Scheme 31: On resin microwave closure of a two-mer

The standard conditions (100 mM AcOH, 50 mM TEA, o-xylene) were applied and the reaction was heated to 130 °C for 30 minutes. After this time period, a small portion of the resin **18** was treated with triflic acid cleavage conditions and analyzed by LC-MS. The MS revealed 18 % conversion to compound **19**. Raising the temperature to 180 °C for 90 minutes in the microwave pushed conversion to 72 %, but the best solvent in which to perform the microwave reaction was o-dichlorobenzene at a temperature of 250 °C for 60 minutes. The DKP was

completely closed with no visible starting material or epimerization. These astonishing conditions have accelerated the DKP half-life from days to minutes!

The opposite two-mer sequence **20** was synthesized as well, but no starting material or product was obtained after workup. This was probably due to self-cleavage of the two-mer from the resin as shown in Scheme 32. In the case of **18** this self-cleavage does not occur, most likely due to the steric effects of the hin(2*S*4*R*7*R*9*R*) building block. Self-cleavage can be avoided in the future by coupling the monomer directly to the resin without first attaching an amino acid or by using a resin with an amide linker.



Scheme 32: Probable self cleavage of a two-mer from the hydroxymethyl resin

4.3. Conclusions

After much experimentation, the best solid-phase resins for the synthesis of the oligomers have been discovered. The ideal DKP-closing conditions were found that do not give any epimerization and yield very clean products. This knowledge will allow for the synthesis of the longer 5-mer and 6-mer sequences and provide enough rigidified material for NMR and other analyses. In the future, this technology should permit the assembly of longer, more complex and functional macromolecules of well defined architectures.

4.4. Experimental Section

General: THF was distilled from Na/benzophenone under N_2. DCM was distilled from CaH_2. All other reagents were used as received unless otherwise noted. All reactions were carried out in flame-dried or oven-dried glassware under N_2 atmosphere unless otherwise noted. Column chromatography was performed using ICN Silitech 32-63 D (60 A) grade silica gel and TLC analysis was performed on EM Science Silica Gel 60 F_{254} plates (250 μm thickness). NMR experiments were performed on either Bruker Avance 300 MHz or Bruker Avance DRX 500 MHz spectrometers. Chemical shifts (δ) are reported relative to $CDCl_3$ or DMSO-d_6 residual solvent peaks, unless otherwise noted. If possible, rotational isomers were resolved by obtaining spectra above 100 °C in DMSO-d_6 at the below indicated temperatures. IR spectra were obtained on a Nicolet Avatar E.S.P. 360 FT-IR. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter at the indicated temperatures. EI-MS was performed on a Micromass Autospec high resolution mass spectrometer (CEBE geometry). High resolution ESI-MS was performed on a Waters LC/Q-TOF instrument. HPLC analysis was performed on a Hewlett-Packard Series1050 instrument with diode array detector, using a Varian Chrompack Microsorb 100 C_{18} column (5 μm packing, 2.6 mm x 250 mm). HPLC-MS analysis was performed on an Agilent LC/MSD SL instrument (ESI).

Solid phase chemistry was performed using a home made manual synthesizer. Dry dichloromethane was obtained from distillation over CaH_2. Anhydrous DMF was purchased from Aldrich Chemical Company. Diisopropylethyl amine (DIPEA) was distilled from ninhydrin and potassium hydroxide and stored over molecular sieves (4 A). O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) was obtained from Acros. MBHA•LL HCl, MHBA-Y-Boc, Fmoc-hydrazino benzoyl, rink amide,

and 4-sulfamylbutyryl resins were purchased from NovaBiochem. All solid phase reactions were mixed by bubbling Ar through the reaction vessel and continuously kept under Ar atmosphere.

(*L*)-tyrosine-pro4(*2S4S*)-hin(2*S4R7R9R*)-pro4(2*S4S*)-hin(2*S4R7R9R*)-pro4(2*S4S*)=glycine (2).

To a 5 mL polypropylene solid phase peptide synthesis (SPPS) reactor vessel was added MBHA·LL·HCl Resin (169 mg, 104 μmol loading). The resin was swelled for 2 h in dry DMF under Ar atmosphere. To a 15 mL Falcon tube were added N-Boc-2-BrZ-*L*-tyrosine (103.5 mg, 209.4 μmol), HATU (79.6 mg, 209 μmol), DMF (200 μL), DCM (800 μL) and DIPEA (73.0 μL, 418 μmol). The mixture was vortexed for 1 min to ensure activation of the amino acid and then immediately transferred to the resin. The coupling reaction was mixed for 30 min and the solution drained from the beads. The terminal Fmoc protected amine of the tyrosine was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 2 h. The resin was then washed three times with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again three times with DMF (1 mL).

To a 15 mL Falcon tube were added pro4(2*S*4*S*) monomer (114 mg, 209 μmol) and HATU (79.6 mg, 209 μmol). DCM (800 μL) and DMF (200 μL) were added. DIPEA (73.0 μL, 418 μmol) was added and the solution was vortexed for 1min to initiate activation of the building block. The homogeneous solution was then added immediately to the solid phase resin. The coupling reaction was mixed for 30 min and the solution drained from the resin. The resin was then washed three times with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again three times with DMF (1 mL). The terminal

Fmoc protected amine of the building block was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 25 min. The resin was washed three times with DMF (1 mL).

To a 15 mL Falcon tube were added hin(2*S*4*R*7*R*9*R*) monomer (129 mg, 209 μmol) and HATU (79.6 mg, 209 μmol). DCM (800 μL), DMF (200 μL) and DIPEA (73.0 μL, 418 μmol) were added and the solution vortexed for 1 min to initiate activation of the building block. The homogeneous solution was then immediately pipetted onto the solid phase resin. The coupling reaction was mixed for 30 min and the solution drained from the resin. The resin was then washed twice with fresh DMF (1 mL) and subjected to a second coupling reaction under identical conditions. The resin was washed again with DMF (1 mL). The terminal PhF protected amine of the building block was deblocked by treatment with 50 % TFA/DCM (500 μL) over 15 min. The resin was washed twice with DCM (1 mL), neutralized (20 % DIPEA/DCM, 400 μL) and washed twice with DMF (1 mL).

The above mentioned coupling and deprotection sequences were performed again under identical conditions twice for the pro4(2*S*4*S*) and once for hin(2*S*4*R*7*R*9*R*) monomer.

To a 15 mL Falcon tube were added Fmoc-glycine (62.2 mg, 209 μmol), HATU (79.6 mg, 209 μmol), DMF (200 μL), DCM (800 μL) and DIPEA (73.0 μL, 418 μmol). The mixture was vortexed for 1 min to ensure activation of the amino acid and then immediately transferred to the resin. The coupling reaction was mixed for 30 min and the solution drained from the beads. The terminal Fmoc protected amine of the tyrosine was deblocked by treatment with 20 % piperidine/DMF (2 mL) over 2 h. The resin was then washed three times with fresh DMF (1

mL) and subjected to a second coupling reaction under identical conditions. The resin was washed twice with DMF, twice with DCM, twice with methanol and twice again with DCM. The oligomer-containing resin was then dried under vacuum at room temperature overnight.

Deprotection of the Cbz groups of the oligomer, *tert*-butyl protecting group of the tyrosine hydroxyl and cleavage of the oligomer from the resin were done by treatment with a cleavage solution containing TFA (500 μL), thioanisole (50 μL), ethane dithiol (25 μL) and trifluoromethane sulfonic acid (50 μL) for 2.5 h first at 0 °C for one hour and then room temperature with magnetic stirring. The cleavage solution was drained into a centrifuge tube containing an excess volume of diethyl ether (35 mL), whereupon the oligomer precipitated as a white solid. The resin was washed with an additional portion of TFA (250 μL) and that volume also drained into the ether. The solution was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue washed with an additional portion of ether. The white solid was dried *in vacuo* and the product purified by preparative HPLC on a Varian Prostar instrument with photodiode array detector (10-35 % MeCN:H_2O over 30 min, flow = 15 mL/min, UV detection = 274 nm). The product containing fractions were lyophilized to remove the solvent, yielding a fluffy white powder. HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 8.97 min; LC-MS (ESI) calculated for $C_{53}H_{74}N_{13}O_{17}$ (M+H) 1164.3, found 1164.3.

(*L*)-tyrosine-pro4(*2S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=glycine (3).

A solution of **(2)** in approximately 1 mL of 20 % piperidine/DMF was incubated at 50 °C for 11 days. The reaction progress was monitored by HPLC-MS. The reaction mixture was then added

dropwise to a centrifuge vial containing an excess volume of diethyl ether (35 mL) to precipitate the product. The vial was centrifuged (9000 rpm, 30 min), the supernatant decanted and the residue purified by preparative HPLC on a Varian Prostar instrument with photodiode array detector (10-35 % MeCN:H_2O over 30 min, flow = 15 mL/min, UV detection = 274 nm). The product containing fractions were lyophilized to remove the solvent, yielding a fluffy white powder. HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.68 min, HR-MS (ESI) calculated for $C_{49}H_{58}N_{13}O_{13}$ (M+H) 1036.4272, found 1036.4301.

Bu-(*L*)-tyrosine-pro4(*2S4S*)-hin(2*S4R7R9R*)-pro4(2*S4S*)-hin(2*S4R7R9R*)-pro4(2*S4S*)-glycine (7).

The oligomer was synthesized in the exact same manner as **2** on the hydrazino benzoyl resin on a 12.69 μmol scale. Capping between each coupling was done with pivalic anhydride. The flexible oligomer was cleaved from the resin by a two step procedure. The resin was first treated with a solution (DCM, 1 mL) of DBDMH (3.60 mg, 12.7 μmol) and pyridine (2.07 μL, 25.4 μmol) for 10 min at room temperature. The solution was then drained from the resin and the beads washed three times with fresh DCM (1 mL). The beads were then treated to a solution (DCM, 1 mL) of butyl amine (12.5 μL, 127 μmol) for 4 hours with magnetic stirring. The solution was drained from the resin and the DCM removed *in vacuo*. The resulting yellow oil was treated with EDT (25 μL), thioanisole (50 μL), TFA (500 μL), and TFMSA (50 μL). The solution was allowed to stir for 1.5 h and then dripped into a large volume of diethyl ether (35 mL). The ppt (2.45 mg, 15.8 %) was centrifuged, the supernatant decanted, and the residue dried in vacuo. HPLC: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 7.40 min; LC-MS (ESI) calculated for $C_{57}H_{82}N_{13}O_{17}$ (M+H) 1220.1, found 1220.1.

Bu-(*L*)-tyrosine-pro4(*2S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=glycine (8).

A solution of **(7)** in approximately 1 mL of 20 % piperidine/DMF was incubated at 50 °C for 11 days. The reaction progress was monitored by HPLC-MS. The reaction mixture was then added dropwise to a centrifuge vial containing an excess volume of diethyl ether (35 mL) to precipitate the product. The vial was centrifuged (9000 rpm, 30 min), the supernatant decanted. HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 10.97 min, LC-MS (ESI) calculated for $C_{53}H_{66}N_{13}O_{13}$ (M+H) 1092.2, found 1092.2.

Bn-hin(2*S*4*R*7*R*9*R*)-pro4(2*S*4*S*)=(*L*)-napala (9).

Synthesized on hydrazino benzoyl resin (16.17 μmol scale). HPLC-MS: MeCN/H_2O 5-95% over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 9.86 min, LC-MS (ESI) calculated for $C_{37}H_{43}N_6O_6$ (M+H) 667.3, found 667.0.

Bn-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-(*L*)-napala-piv (10).

Synthesized on hydrazino benzoyl resin (19.50 μmol scale). HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 12.97 min, LC-MS (ESI) calculated for $C_{43}H_{55}N_6O_8$ (M+H) 783.4, found 783.2.

Bn-hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=(*L*)-napala (11).

HPLC-MS: MeCN/H_2O 25-55 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274nm, t_R = 4.72 min, LC-MS (ESI) calculated for $C_{36}H_{39}N_6O_5$ (M+H) 635.0, found 635.0.

Bn-pro4(2*S*4*S*)-hin(2*S*4*R*7*R*9*R*)-(*L*)-napala-piv (10).

HPLC-MS: MeCN/H_2O 25-55 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 13.93 min, LC-MS (ESI) calculated for $C_{42}H_{51}N_6O_7$ (M+H) 751.1, found 751.2.

Typical solution phase microwave DKP closing experiment.

A small volume of a solution of the oligomer was placed into a GC vial and the solvent removed *in vacuo*. The dried residue was then placed into a microwave reactor vessel containing another GC vial. The reactor vessel was filled with a few mL (2-3) of reaction solvent and catalysts. Approximately 200 μL of reaction solvent was placed into the GC vial containing the oligomer and the vessel sealed with a pressure cap. The reaction was run in the microwave for the desired length of time and the resulting reaction volume was pipetted directly into another GC vial for LC-MS analysis.

Typical on-resin DKP closing experiment.

The oligomer-containing resin was transferred directly to a microwave reactor vessel with a small magnetic stir bar. Approximately 3 mL of reaction solvent and catalysts were pipetted directly onto the beads. The vessel was sealed and run in the microwave for a specific time length. The beads and reaction solution were pipetted into a SPPS reactor vessel and the solution drained from the resin. The beads were washed 3x with DMF, 3x with iPrOH, and 3x with DCM. The dried resin was then taken onto the appropriate cleavage step and the precipitated oligomer injected onto the LC-MS for analysis.

(2*S*,4*R*,7*R*,9*R*)-7-(9H-Fluoren-9-ylmethoxycarbonylamino)-octahydro-indole-1,2,7-tricarboxylic acid 1-tert-butyl ester 7-methyl ester (15).

To a solution of hin(2*S*4*R*7*R*9*R*) in DCM (10 mL) and MeOH (137 μL, 3.39 mmol) was added TFA (421 μL, 5.65 mmol). The mixture was stirred at RT for 20 min. The solvent was removed *in vacuo* and the crude material taken on directly to the next step. To a suspension of the amine in DCM (10 mL) was added DIPEA (984 μL, 5.65 mmol) and TMSCl (356 μL, 2.82 mmol). The mixture was refluxed for 1.5 h and then transferred to an ice bath. FmocCl (292 mg, 1.13

mmol) was added and the reaction was allowed to warm to RT overnight. The mixture was rotovapped, dissolved in EtOAc, washed 2x with 1 M aq. HCl and the aqueous layers back extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified on SiO_2 (1:1 EtOAc:hex followed by 9:1 $CHCl_3$:MeOH, 1 % AcOH). The product **14** was isolated as a white foam (595 mg, 88 %). To a solution of compound **14** (326 mg, 0.545 mmol) in MeOH (10 mL) was added 32.6 mg of Pd/C. The suspension was purged and flushed 4x with hydrogen. The reaction was allowed to stir under an H_2 balloon for 2 h. The mixture was filtered, rotovapped, and carried on crude to the next step. To a suspension of the amino acid in DCM (10 mL) was added TMSCl (276 μL, 2.18 mmol). The solution was refluxed for 1 h, transferred to an ice bath, and DIPEA (759 μL, 4.36 mmol) and Boc_2O (238 mg, 1.09 mmol) added. The mixture was allowed to warm to RT overnight. The solution was rotovapped, dissolved in EtOAc, and washed 2x with 5 % aq. $NaHSO_4$. The aqueous layer was extracted with EtOAc and the combined organic layers washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified on SiO_2 (5% MeOH:$CHCl_3$ followed by 10 % MeOH:$CHCl_3$). The product **15** was isolated as a white foam (155 mg, 50 %). $[\alpha]^{20}_D$ = -22.8 (*c*= 0.54, DCM); IR (neat) 3415, 2083, 1670, 1409, 1258, 1233, 1168, 1131, 1045 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (~2:1 mixture of rotamers) δ 7.77 (d, 2H, J = 7.4 Hz), 7.58 (d, 2H, J = 7.1 Hz), 7.29-7.43 (m, 4H), 4.30-4.46 (m, 3H), 4.20 (br t, 2H, J = 6.8 Hz), 3.73 (br s, 3H), 2.90-2.97 (m, 1H), 2.32-2.55 (m, 2H), 1.90-2.22 (m, 3H), 1.60-1.82 (m, 2H), 1.45 (s, 4.5H), 1.41 (s, 4.5H), 0.82-0.94 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) (~2:1 mixture of rotamers) δ 178.7, 177.9, 175.6, 172.8, 155.5, 143.7, 141.2, 136.1, 128.3, 127.6, 127.0, 124.9, 119.9, 81.3, 80.8, 67.3, 66.6, 58.9, 58.6, 58.3, 55.2, 55.0, 52.5, 52.4, 47.0, 34.5,

33.6, 32.9, 30.8, 30.5, 29.6, 28.8, 28.2, 28.1, 21.7; LC-MS (ESI) calculated for $C_{26}H_{28}N_2O_6$ (M+1-Boc) 464.2, found 464.8.

BrPhe-hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=(*L*)-napala (19).

The two-mer was synthesized using previously established peptide synthesis chemistry. On resin compound **16** was treated with a 50 % TFA/DCM solution for 15 minutes to remove the Boc protecting groups. The resin was neutralized with 10 % DIPEA/DCM (1 mL), washed 3x with DMF, 3x with iPrOH, and 3x with DCM. The resin was then dried *in vacuo*. The beads were subjected to microwave conditions as previously described and cleaved using standard TFMSA conditions. Compound **19** was then analyzed via LC-MS. HPLC-MS: MeCN/H_2O 5-95 % over 30 min, flow rate = 0.30 mL/min, UV detection = 274 nm, t_R = 12.11 min, LC-MS (ESI) calculated for $C_{38}H_{40}BrN_6O_7$ (M+2) 772.2, found 772.0.

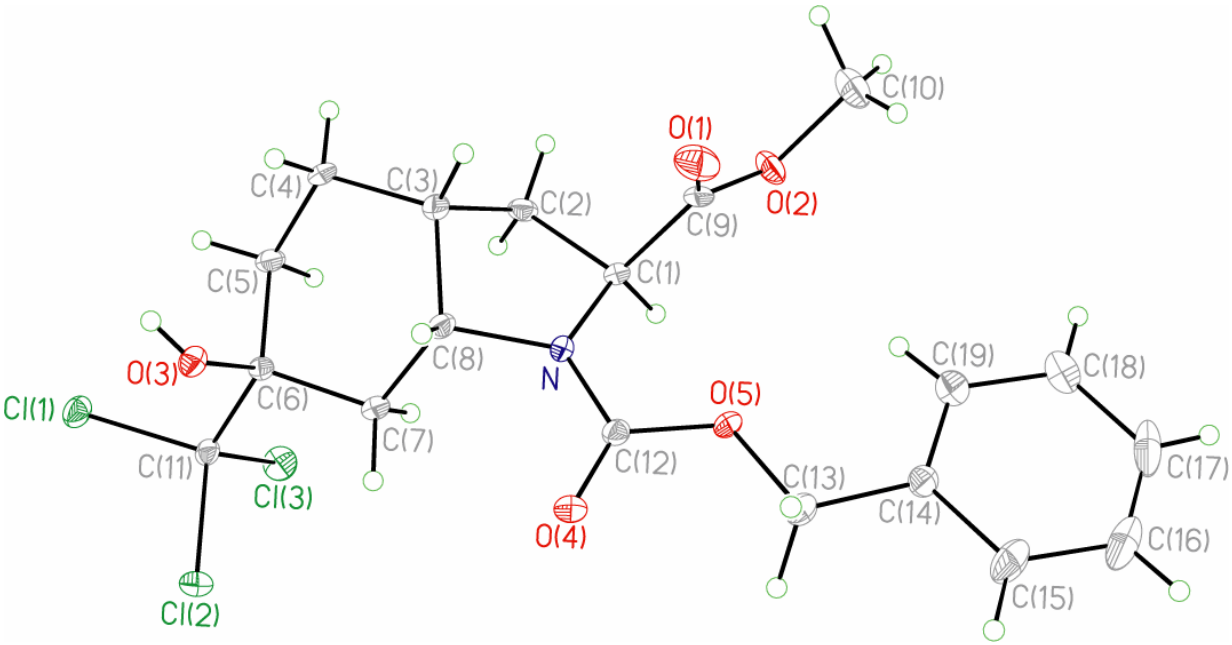
APPENDIX A
Crystallographic Data for Trichloromethyl Carbinol 8

Table 1. Crystal data and structure refinement for steveh.

Identification code	steveh	
Empirical formula	C19 H22 Cl3 N O5	
Formula weight	450.73	
Temperature	100.0(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.0300(12) Å	α= 90°.
	b = 11.333(2) Å	β= 90°.
	c = 29.296(6) Å	γ = 90°.
Volume	2002.0(7) $Å^3$	
Z	4	
Density (calculated)	1.495 Mg/m^3	
Absorption coefficient	0.489 mm^{-1}	
F(000)	936	
Crystal size	0.26 x 0.14 x 0.07 mm^3	
Theta range for data collection	1.93 to 32.61°.	
Index ranges	-9<=h<=9, -17<=k<=17, -43<=l<=42	
Reflections collected	25765	
Independent reflections	7033 [R(int) = 0.0758]	
Completeness to theta = 32.61°	97.8 %	
Absorption correction	Sadabs	
Max. and min. transmission	0.9666 and 0.8834	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7033 / 0 / 342	
Goodness-of-fit on F^2	1.148	
Final R indices [I>2sigma(I)]	R1 = 0.0613, wR2 = 0.1248	
R indices (all data)	R1 = 0.0708, wR2 = 0.1286	
Absolute structure parameter	0.10(6)	
Largest diff. peak and hole	0.823 and -0.385 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for steveh. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N	3502(4)	2623(2)	6835(1)	11(1)
Cl(1)	1121(1)	1780(1)	8981(1)	17(1)
O(1)	4370(4)	1162(2)	6063(1)	23(1)
C(1)	5714(4)	2158(2)	6744(1)	11(1)
Cl(2)	-24(1)	3854(1)	8474(1)	17(1)
O(2)	7988(4)	1618(2)	6137(1)	20(1)
C(2)	5995(4)	1217(2)	7122(1)	13(1)
Cl(3)	4558(1)	3220(1)	8598(1)	19(1)
O(3)	-443(3)	1472(2)	8009(1)	13(1)
C(3)	3613(5)	813(2)	7209(1)	13(1)
O(4)	863(3)	4017(2)	6693(1)	16(1)
C(4)	3253(5)	108(2)	7647(1)	16(1)
O(5)	4012(3)	3873(2)	6268(1)	14(1)
C(5)	3397(5)	859(2)	8079(1)	13(1)
C(6)	1765(4)	1884(2)	8055(1)	10(1)
C(7)	2293(5)	2655(2)	7643(1)	14(1)
C(8)	2297(4)	1975(2)	7192(1)	12(1)
C(9)	5868(5)	1590(2)	6271(1)	15(1)
C(10)	8471(7)	1053(3)	5705(1)	32(1)
C(11)	1856(4)	2644(2)	8501(1)	12(1)
C(12)	2630(4)	3540(2)	6606(1)	12(1)
C(13)	3200(5)	4812(2)	5980(1)	17(1)
C(14)	4792(5)	4991(2)	5592(1)	15(1)
C(15)	4360(6)	5932(3)	5299(1)	24(1)
C(16)	5816(6)	6185(3)	4944(1)	29(1)
C(17)	7667(7)	5512(3)	4877(1)	30(1)
C(18)	8099(6)	4570(3)	5165(1)	27(1)
C(19)	6663(5)	4315(3)	5523(1)	21(1)

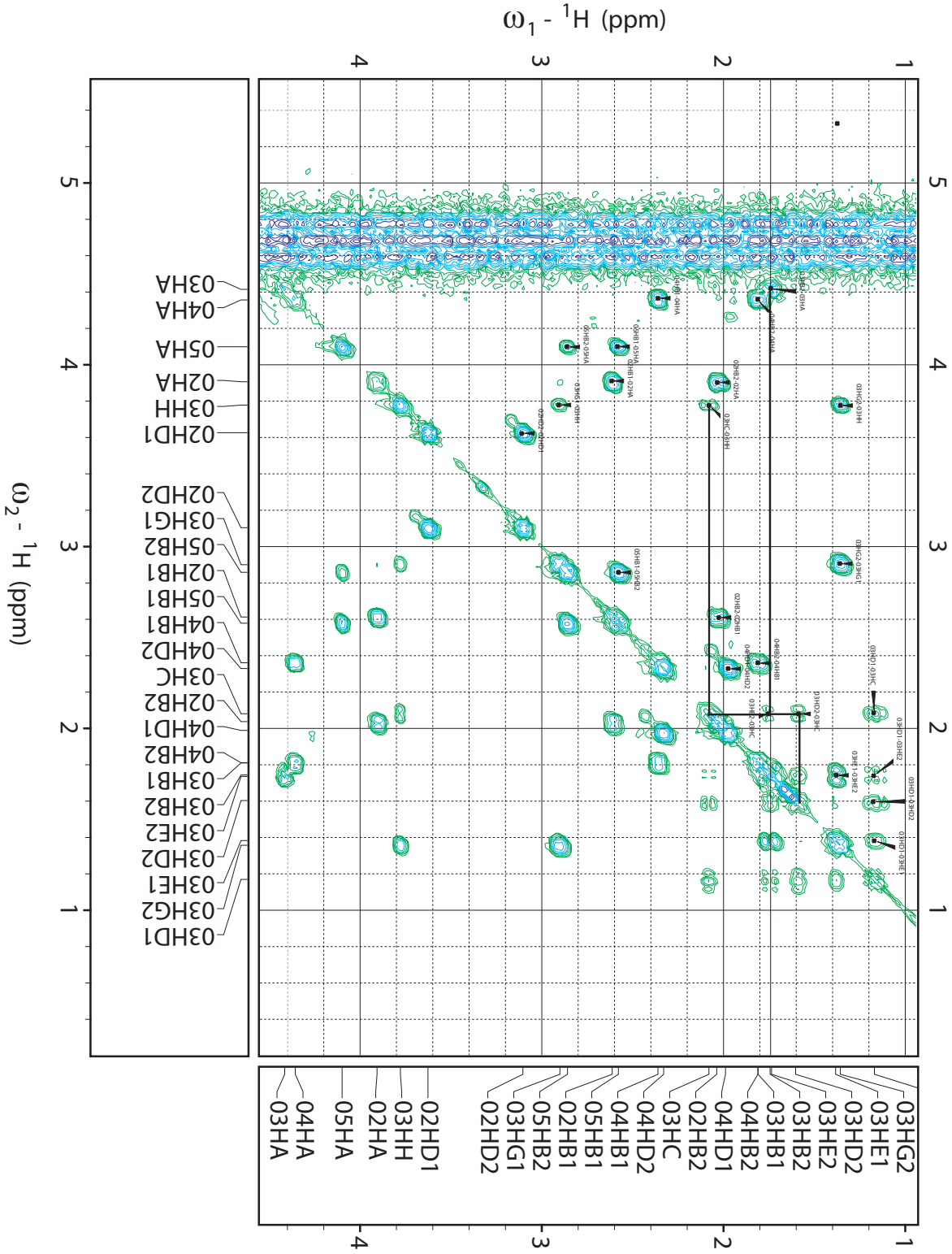


ORTEP Figure from crystal data

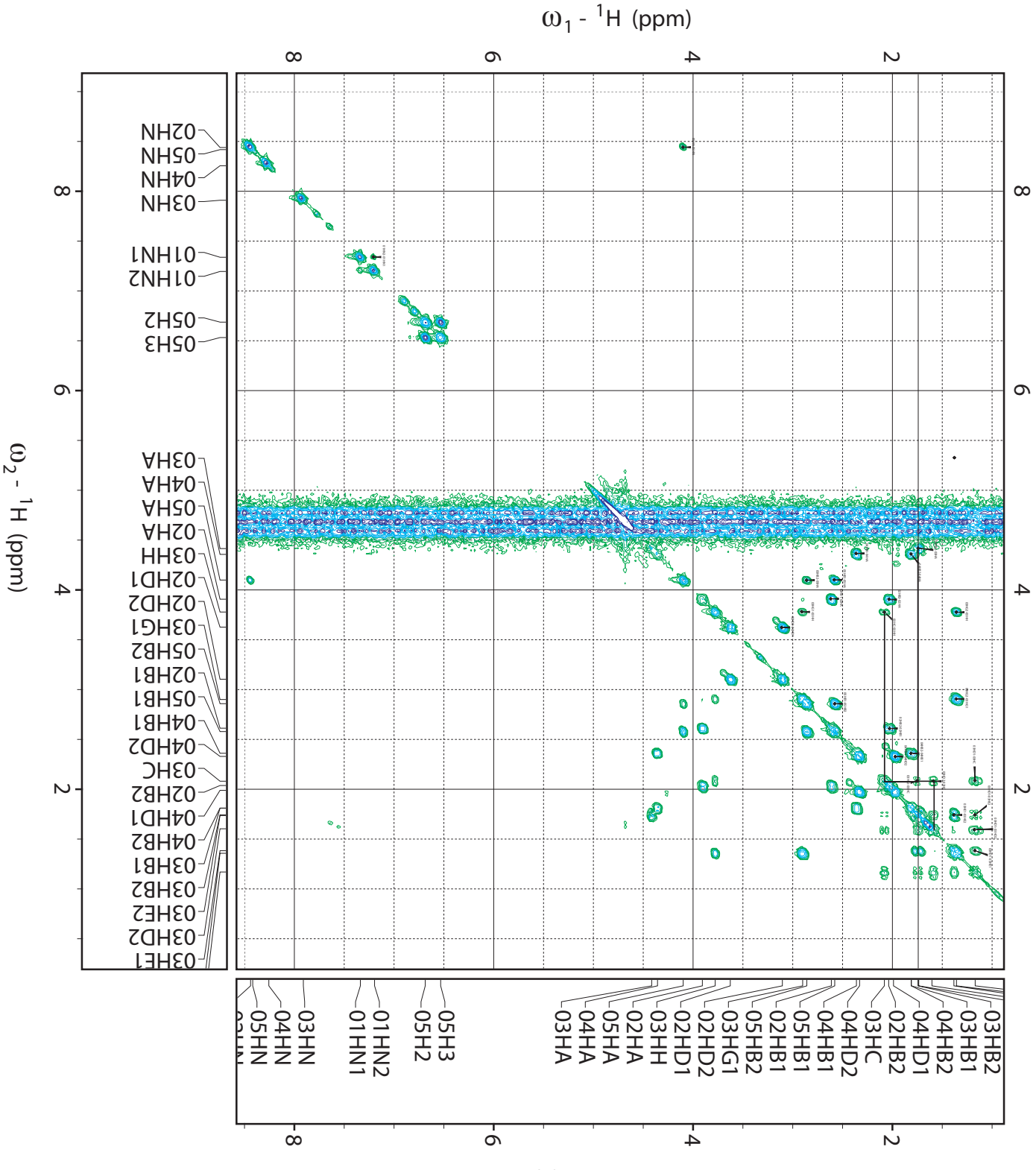
APPENDIX B
2D NMR Data for pro4(2*S*4*S*)=hin(2*S*4*R*7*R*9*R*)=pro4(2*S*4*S*)=(*L*)-tyr

Supplemental figure 12
2D-COSY of compound 3, focused in aliphatic/aliphatic region
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: cosy_12
User: meister Date: Sun Jul 18 17:06:49 2004
Positive contours: low 7.75e+004 levels 11 factor 1.69
Negative contours: low -3.40e+004 levels 1 factor 1.40

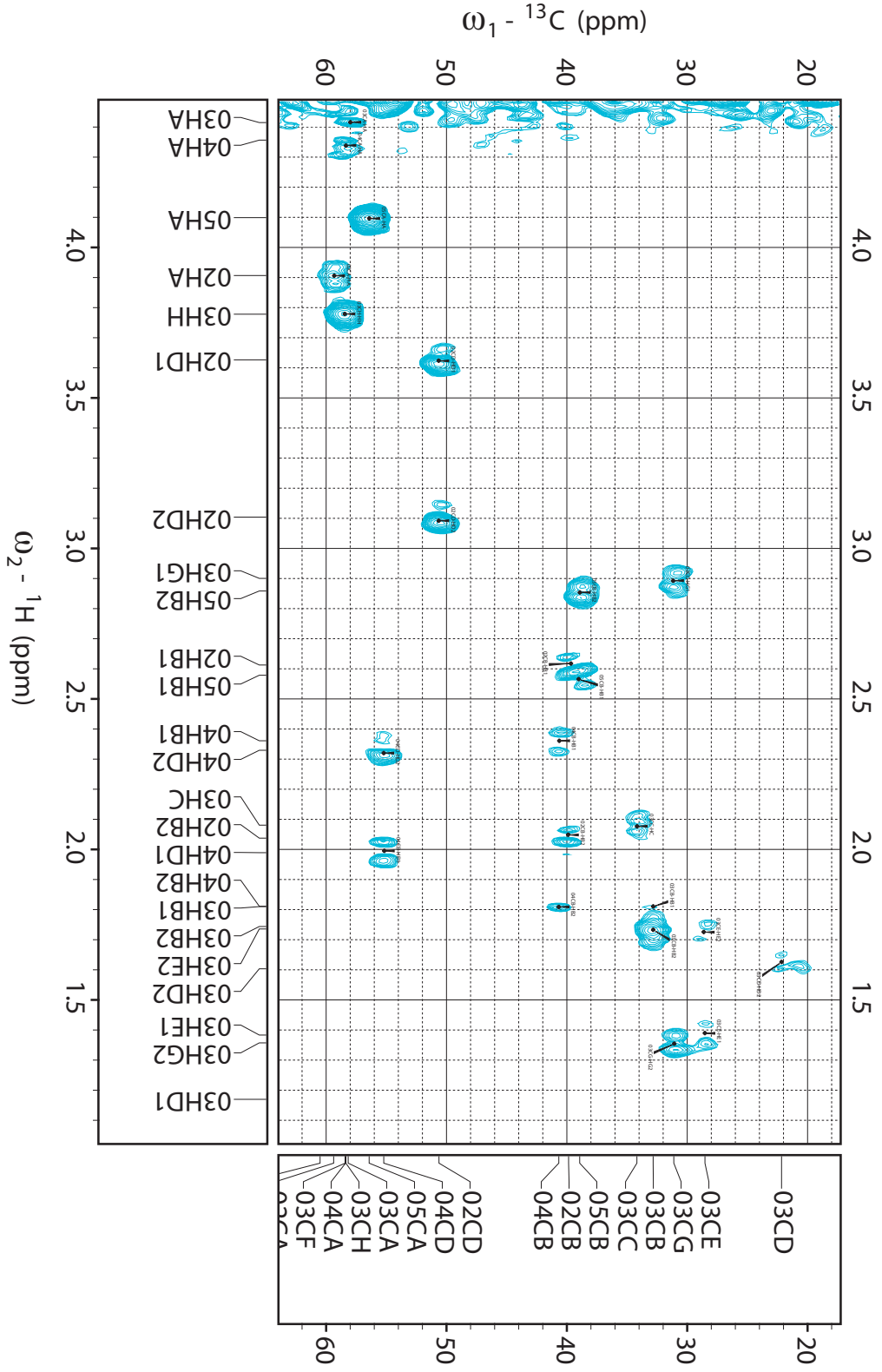


Supplemental figure 13
2D-COSY of compound 3, entire spectrum
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5



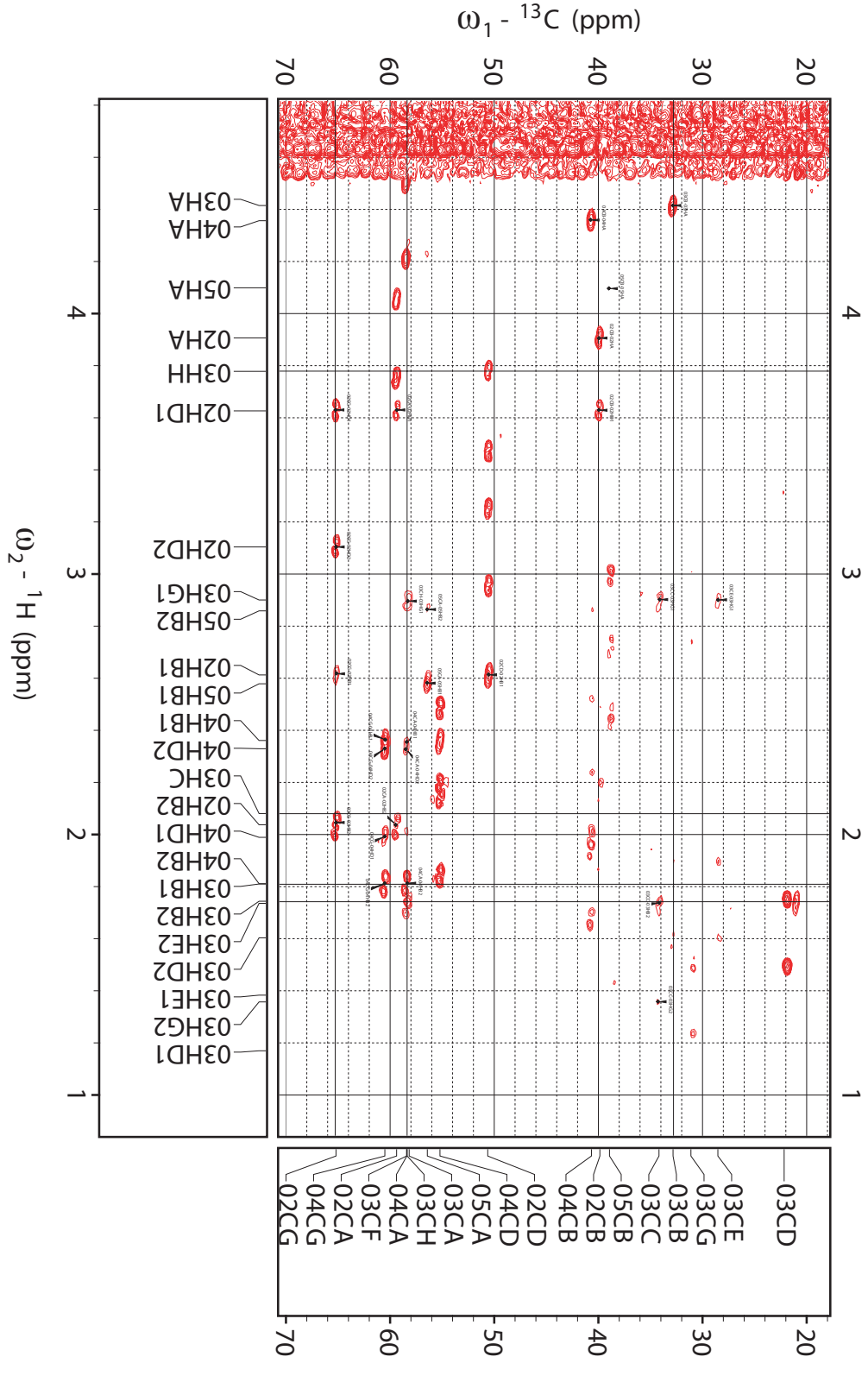
Supplemental figure 14
2D-HMQC of compound 3
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: hmqc_62full
User: meister Date: Sun Jul 18 17:01:43 2004
Positive contours: low 2.85e+005 levels 19 factor 1.12
Negative contours: low -3.12e+005 levels 1 factor 1.40



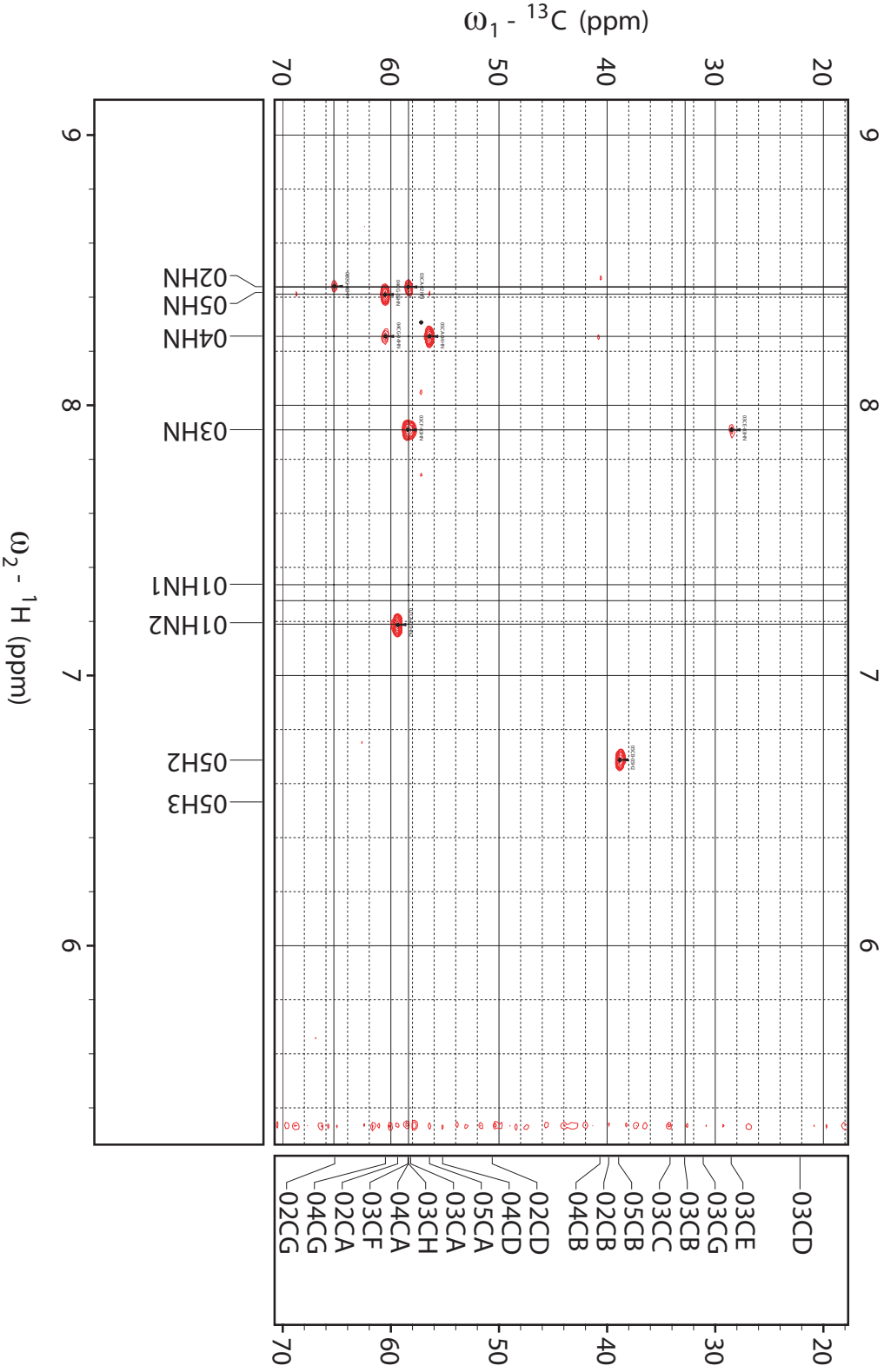
Supplemental figure 15
2D-HMBC of compound 3
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: hmbc_61
User: meister Date: Sun Jul 18 17:14:05 2004
Positive contours: low 1.99e+006 levels 11 factor 1.42
Negative contours: low -3.20e+006 levels 1 factor 1.40



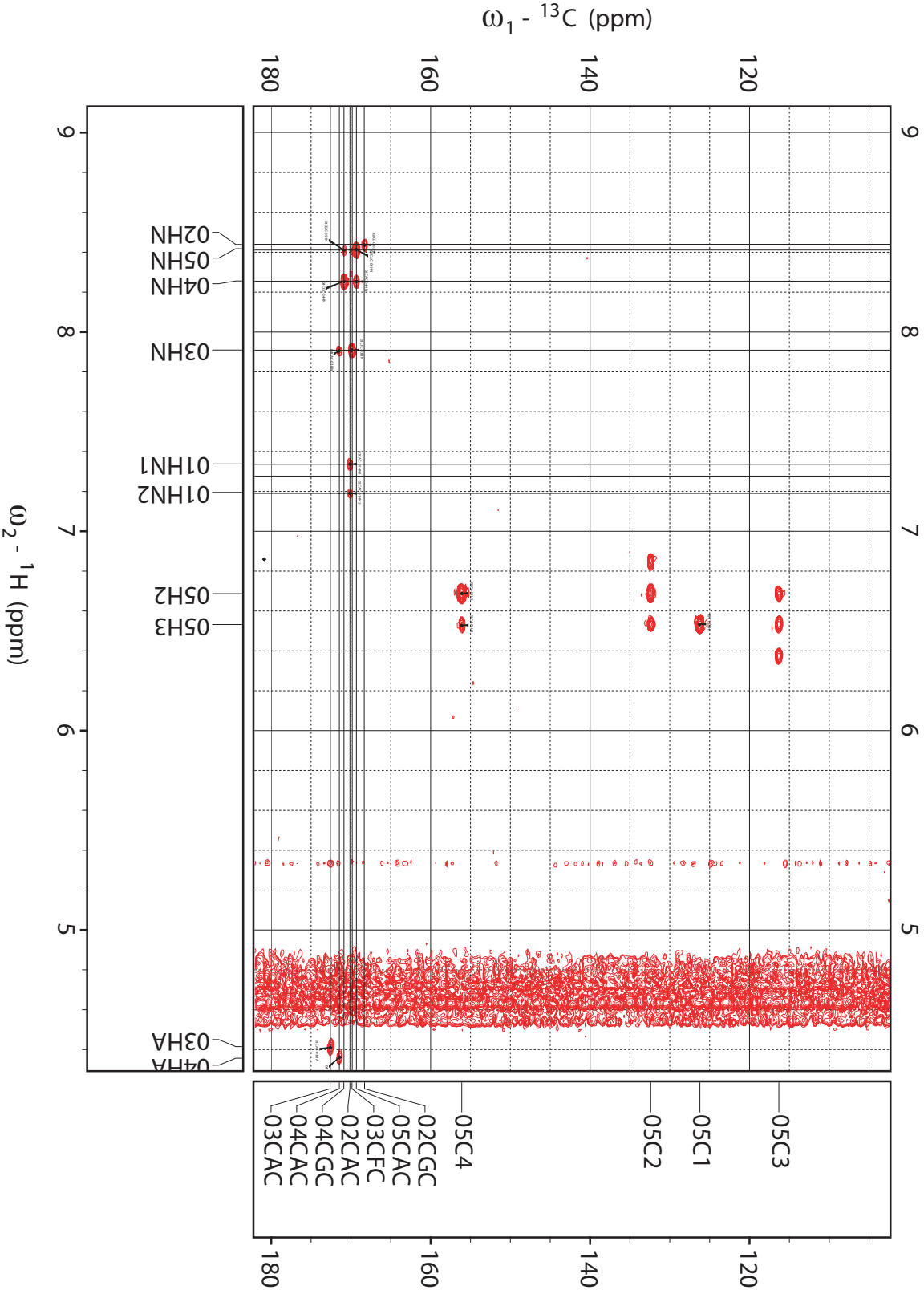
Supplemental figure 16
2D-HMBC of compound 3
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: hmbc_61
User: meister Date: Sun Jul 18 17:14:23 2004
Positive contours: low 1.99e+006 levels 11 factor 1.42
Negative contours: low -3.20e+006 levels 1 factor 1.40



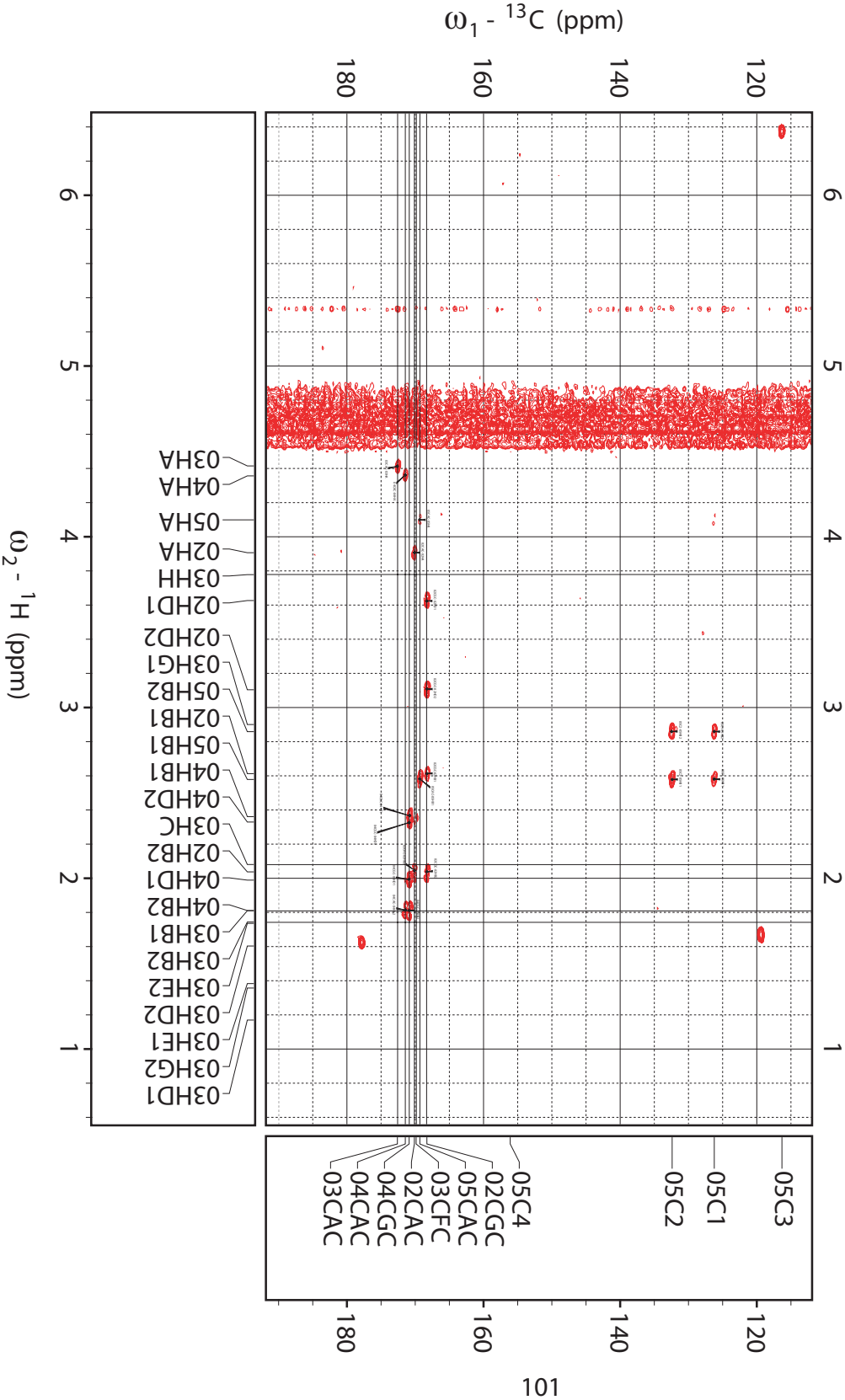
Supplemental figure 17
2D-HMBC of compound 3
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: hmbc_61
User: meister Date: Sun Jul 18 17:14:54 2004
Positive contours: low 1.99e+006 levels 11 factor 1.42
Negative contours: low -3.20e+006 levels 1 factor 1.40



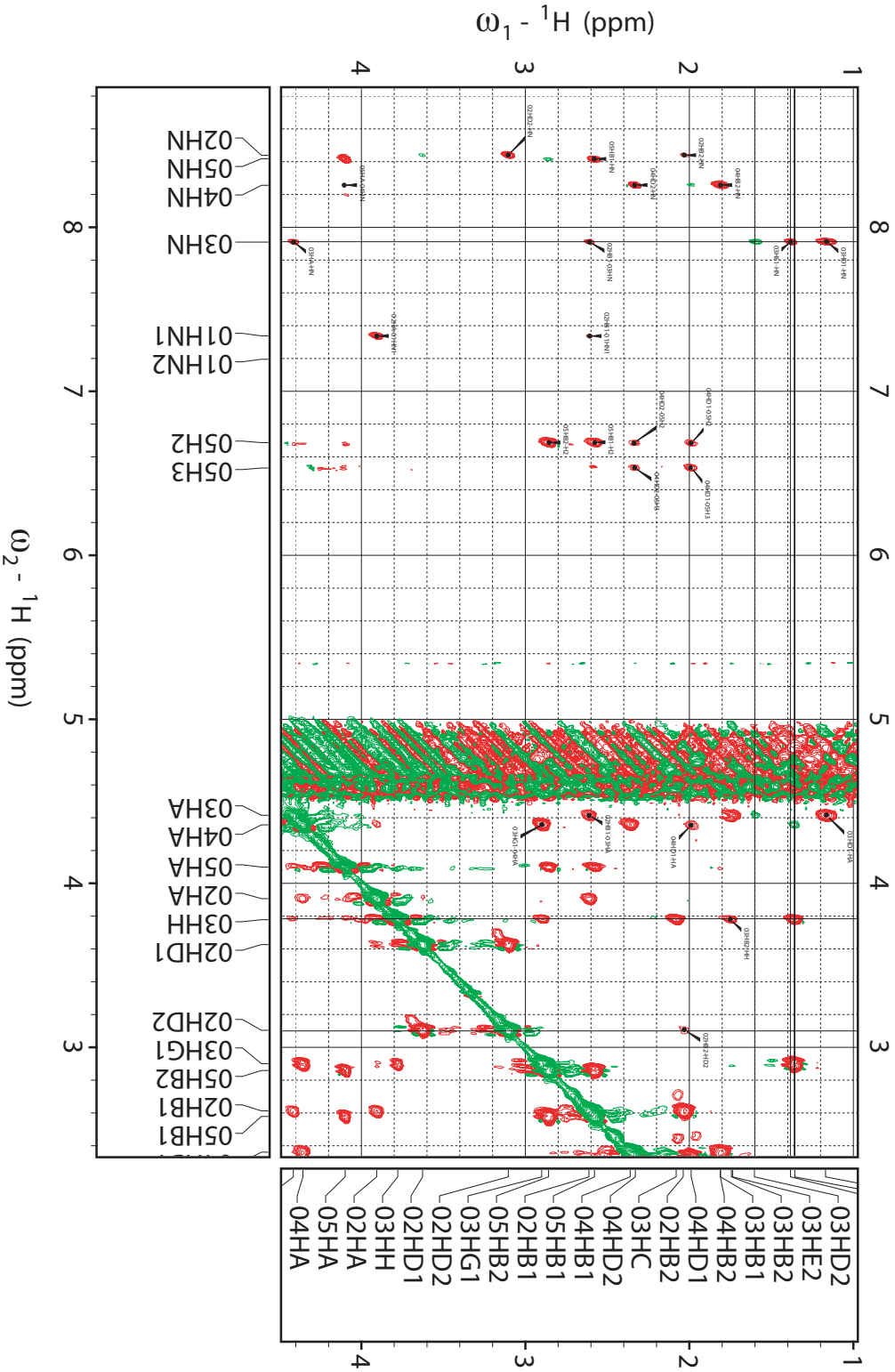
Supplemental figure 18
2D-HMBC of compound 3
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: hmbc_61
User: meister Date: Sun Jul 18 17:15:18 2004
Positive contours: low 1.99e+006 levels 11 factor 1.42
Negative contours: low -3.20e+006 levels 1 factor 1.40



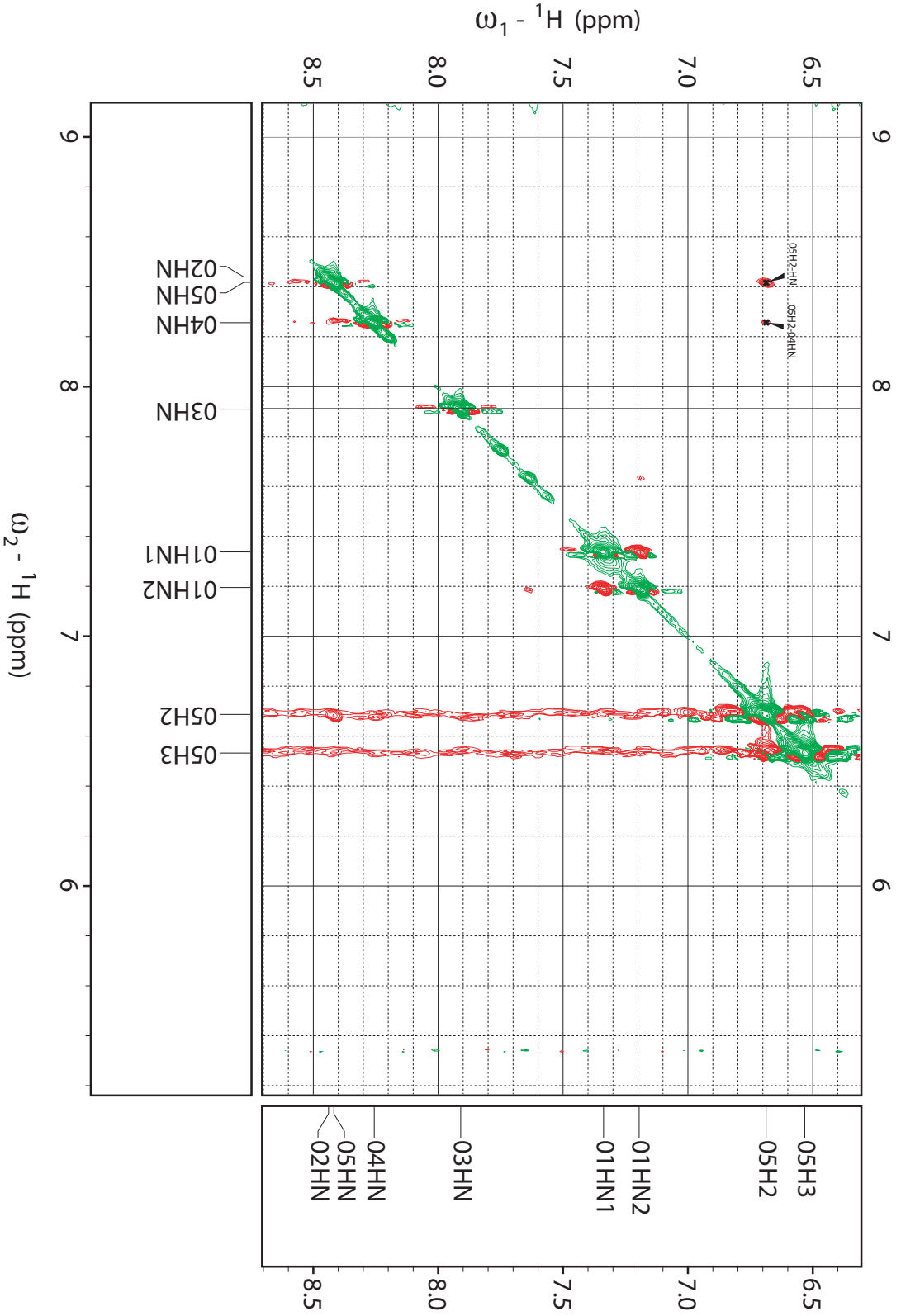
Supplemental figure 19
2D-ROESY of compound 3, positive contours=RED, negative contours=GREEN
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: roesy_71
User: meister Date: Sun Jul 18 17:25:23 2004
Positive contours: low 5.70e+003 levels 25 factor 1.43
Negative contours: low -4.83e+003 levels 24 factor 1.40



Supplemental figure 20
2D-ROESY of compound 3, positive contours=RED, negative contours=GREEN
80% H2O/ 10% D2O/ 10% CD3CN buffered with 45 uM CD3COOD/ND3 pH=~5

Spectrum: roesy_71
User: meister Date: Sun Jul 18 17:26:19 2004
Positive contours: low 5.70e+003 levels 25 factor 1.43
Negative contours: low -4.83e+003 levels 24 factor 1.40



SPARKY naming legend for compound **3**

Red = residue 1
Blue = residue 2
Magenta = residue 3
Black = residue 4
Green = residue 5

**SPARKY NMR requires a residue based atom naming system (e.g., 03HB2 represents the 3rd residue-proton on beta carbon(HB)-anto to alpha proton(2)). Heavy atoms were numbered in the manuscript in a more conventional way.

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