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Development And Applications Of Thioacids In Peptide Chemistry And Exploration Of Methods Toward The Stereochemical Elucidation And Synthesis Of Virgineone

Kasinath Sana
Wayne State University

DEVELOPMENT AND APPLICATIONS OF THIOACIDS IN PEPTIDE CHEMISTRY AND EXPLORATION OF METHODS TOWARD THE STEREOCHEMICAL ELUCIDATION AND SYNTHESIS OF VIRGINEONE

by

KASINATH SANA

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Approved by:

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Advisor Date

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DEDICATION

This dissertation is dedicated to my parents
for their endless love and support.

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I would like to express my sincere gratitude to my graduate advisor, Professor David Crich for his guidance, encouragement and continuous support throughout my doctoral study in his laboratories at the University of Illinois at Chicago and at Wayne State University. He is a great teacher and an excellent mentor and without his help, this dissertation would not have been completed.

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LIST OF ABBREVIATIONS

AA	Amino acid
Ac	Acetyl
All	Allyl
Alloc	Allyloxycarbonyl
aq	Aqueous
Ar	Aryl
BAL	Backbone amide linkage
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
BSP	1-Benzenesulfinyl piperidine
Bu	Butyl
Bz	Benzoyl
Calcd	Calculated
CAN	Ceric ammonium nitrate
Cbz	Benzyloxycarbonyl
CDI	1,1'-Carbonyldiimidazole
DCM	Dichloromethane
DCC	*N,N'*-Dicyclohexylcarbodiimide
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIC	*N,N'*-Diisopropylcarbodiimide
DIEA	*N,N*-Diisopropylethylamine

DMAP	4-(Dimethylamino)pyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DVB	Divinylbenzene
EI-HRMS	Electron impact high resolution mass spectroscopy
ESI-HRMS	Electrospray ionization high resolution mass spectroscopy
equiv.	Equivalent
Et	Ethyl
Fm	9-Fluorenylmethyl
Fmoc	9-Fluorenylmethoxycarbonyl
h	Hour
HATU	*O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
Hz	Hertz
im	Imidazole
i-Pr	Isopropyl
IR	Infrared
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilazide
Me	Methyl

min	Minutes
mmol	Millimole
Mp	Melting point
MS	Molecular sieves
NaHMDS	Sodium hexamethyldisilazide
NBS	*N*-Bromosuccinimide
NCL	Native chemical ligation
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
p	para
PEG	Polyethylene glycol
PG	Protecting group
Ph	Phenyl
PMB	*p*-Methoxybenzyl
ppm	Parts per million
PTSA	*p*-Toluenesulfonic acid
Py	Pyridine
PyBOP	Benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate
quant.	Quantitative
RP-HPLC	Reverse phase high performance liquid chromatography
r.t.	Room temperature
sat.	Saturated
SPPS	Solid phase peptide synthesis

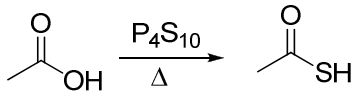
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
temp.	Temperature
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TFE	Trifluoroethanol
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMB	2,4,6-trimethoxybenzyl
TMS	Trimethylsilyl
Trt	Trityl
Ts	*p*-Toluenesulfonyl
TTBP	2,4,6-Tri-*tert*-butylpyrimidine
UV/Vis	Ultraviolate-visible

CHAPTER 1

INTRODUCTION

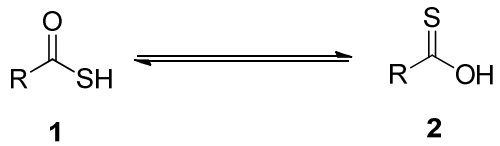
1.1 Importance of Thioacids and Thioesters

Thioacids have been known since the first example, thioacetic acid, was synthesized from the reaction of acetic acid with tetraphosphorous decasulfide by Kekule in 1854 (Scheme 1).[1-2]



Scheme 1. First preparation of thioacetic acid.

The structures of thioacids have been studied extensively using IR, UV/Vis and NMR spectroscopy, and molecular orbital calculations for many decades, all of which indicated that thioacids exist as fast tautomeric equilibrium mixtures of thiol (**1**) and thioxo (**2**) forms (Figure 1).[3-6]



1 **2**

Figure 1. Tautomers of thioacids.

In 1996, IR, UV/Vis and NMR spectroscopic observations were reported which suggested that the thioxo form (**2**) of thioacids predominates in polar solvents at low temperature.[7] Since then, several theoretical studies of tautomerism in thioacids have been reported.[8] Spectroscopic experiments have shown that thioacids exist in the thiol form (**1**) in nonpolar solvents and in the solid state.[7] This change in the equilibrium position with solvent polarity can be

explained by the notion that the thioxo form (**2**) is a better hydrogen bond donor toward polar solvents like THF, ether, acetone, and methanol (Figure 2).[7]

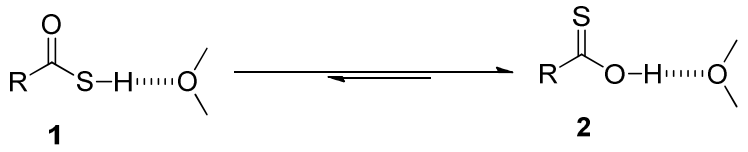
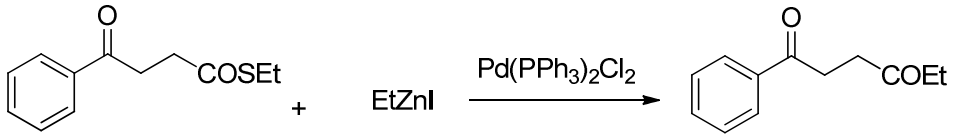


Figure 2. Hydrogen bonding of thiol and thioxo forms of thioacids in polar solvent.

An important difference between thioacids and carboxylic acids is their acidity; thioacids are stronger acids than the corresponding carboxylic acids. This is mainly due to the larger size of sulfur relative to oxygen, which stabilizes the negative charge in the conjugate anion of the acid, and thereby increases the acidity of sulfur compounds.[3,8] For example, the p*K*a of thioacetic acid in water at 25 ℃ is 3.33,[2] about 25-30 times stronger than that of acetic acid (p*K*a: 4.76 in water at 25 ℃);[2] therefore it can be selectively deprotonated even at acidic pH. Hence, a key feature of the thioacid moiety lies in its ability to act as a nucleophile at pH 3–6 with a unique reactivity profile,[9-12] when all other nucleophiles present are unreactive. Accordingly, most applications of thioacids have been in the field of peptide chemistry where they have been employed as key intermediates for the synthesis of peptides and proteins. The application range of thioacids in amide bond formation reactions extends from fragment coupling[13-15] to enzyme-mediated condensation,[16-17] synthesis of proteins with backbone-engineered[18-20] or non-native architectures,[21-23] peptide dendrimers[24] and cyclic peptides.[25]

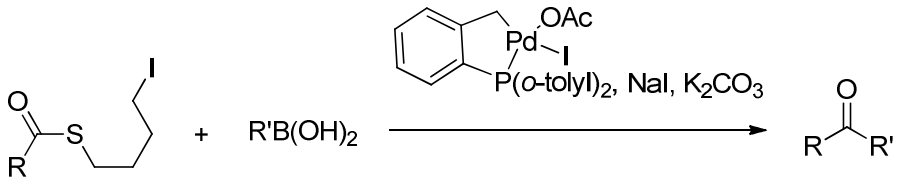
However, until the 1980s the study of thioacids was a comparatively unexplored field in organic chemistry. In contrast, thioacid derivatives such as thioesters, which could easily be prepared from thioacids by simple nucleophilic substitution, have found more applications.[11,26-34] For example, Fukuyama and co-workers disclosed the palladium-catalyzed synthesis of ketones by the reaction of thioesters **3** with organozinc reagents as exemplified in Scheme 2.[33]



3 **4**

Scheme 2. Palladium-catalyzed synthesis of ketone from a thioester.

Likewise, Srogel, Liebeskind and co-workers reported the palladium-catalyzed cross-coupling of the thioesters **5** with boronic acids to give the corresponding ketones **6** (Scheme 3).[35]



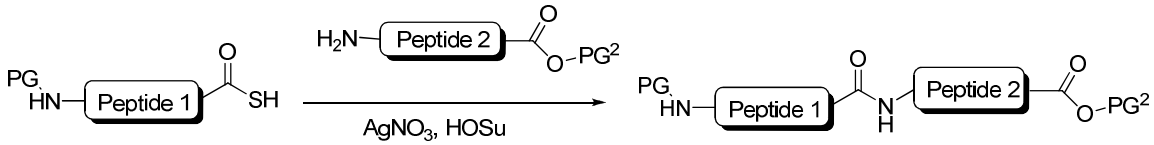
5 **6**

Scheme 3. Palladium-catalyzed cross coupling of the thioesters.

1.1.1 Activation of Thioacids and Amide Bond Formation

Like carboxylic acids, thioacids are also activated with common carbodiimides and can be use for direct amide bond forming reactions with amines.[13] The activation of thioacids with excess silver ion in presence of *N*-

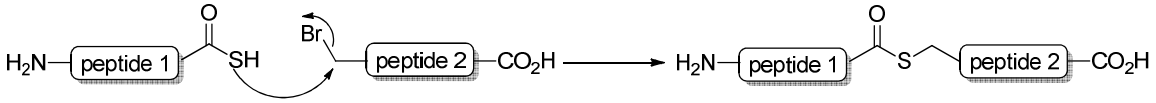
hydroxysuccinimide has been used in the coupling of protected peptide fragments as shown in Scheme 4.[36-38]



Scheme 4. Activation of thioacids with silver nitrate.

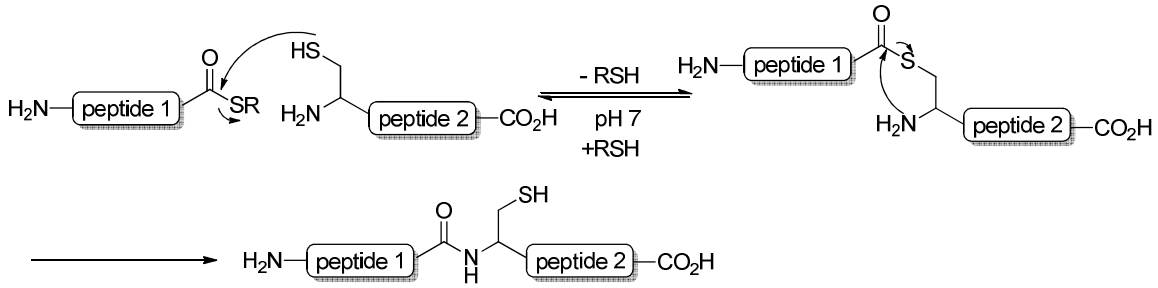
1.1.2 Native Chemical Ligation

The usefulness of thioacids greatly increased with the description of native chemical ligation, by Schnölzer and Kent in 1992.[18] These authors came up with a novel strategy for the coupling of unprotected peptide fragments in aqueous solution. The basis for this new approach is the presence in each peptide fragment of a unique, mutually reactive functionality which enables a chemoselective reaction between the two components. This approach is called 'native chemical ligation'. This chemistry was initially used for a nucleophilic substitution reaction between an SH group of a thioacid attached to the C-terminus of one peptide, and an alkyl bromide attached to the N-terminus of the other fragment, leading to the formation of a thioester at the ligation site (Scheme 5).[18] This reaction can be performed in aqueous solution, and the selectivity of the reaction allows the use of unprotected peptide fragments.



Scheme 5. Ligation of unprotected peptidyl thioacids.

The major disadvantage of the initial chemical ligation approach was that the reaction needed an unnatural structure at the ligation site, which leads to the formation of a non-native peptide. A second generation of ligation chemistry, referred as 'the native chemical ligation' (NCL), was introduced in 1994 by Dawson *et al.*[39] In this new methodology the unnatural alkyl bromide was replaced by a cysteine residue from N-terminus of the second fragment, allowing the trans thioesterification with the C-terminus thioester of first peptide fragments in aqueous solution, with the formation of a 'native' amide bond at the ligation site (Scheme 6).[39] The characteristics of the native chemical ligation methodology enable all the limitations of the traditional convergent approach for the synthesis of large peptides or proteins (i.e., poor solubility and difficulty in purifying the fully protected peptide fragments) to be overcome, and established it as a method for reproducible and practical total chemical synthesis of small-to-medium size peptides and proteins.[40]

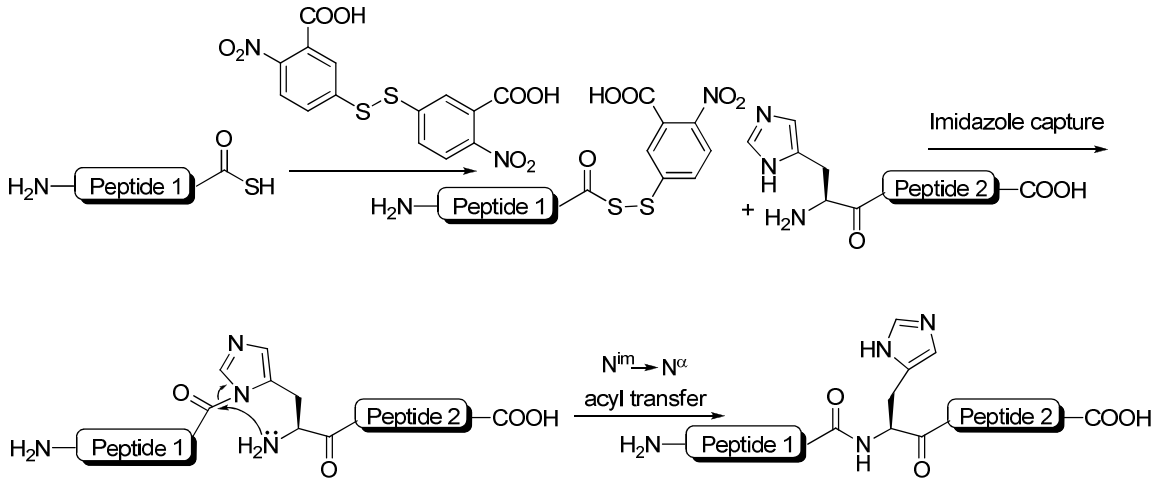


Scheme 6. Native chemical ligation of unprotected peptidyl thioesters.

1.1.2.1 Native Chemical Ligation without Cysteine: Other Thioligations

The native chemical ligation methodology has proven very useful in the synthesis of large peptides and proteins. However, this strategy has some limitations, because of the fact that a cysteine is required at the ligation site, and

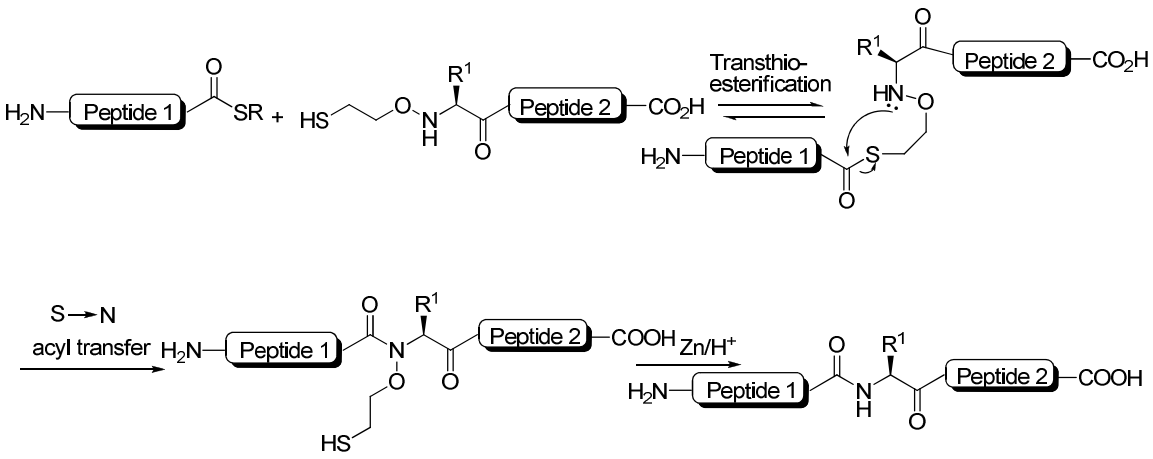
that naturally occurring proteins do not always contain a cysteine residue in the right position of their sequences, making them difficult to synthesize using this methodology. Therefore, several modifications of the initial methods have been introduced to overcome this drawback. In this respect, introduction of homocysteine,[41-42] selenocysteine,[43-45] homoselenocysteine[46] and histidine[47] in the ligation site as a replacement of cysteine, have considerable potential. In the case of histidine, located at the N-terminus of a peptide, the imidazole side chain nitrogen acts as a nucleophile in the ligation reaction (Scheme 7).[47]



Scheme 7. Histidine-mediated ligation of peptidyl thioacids.

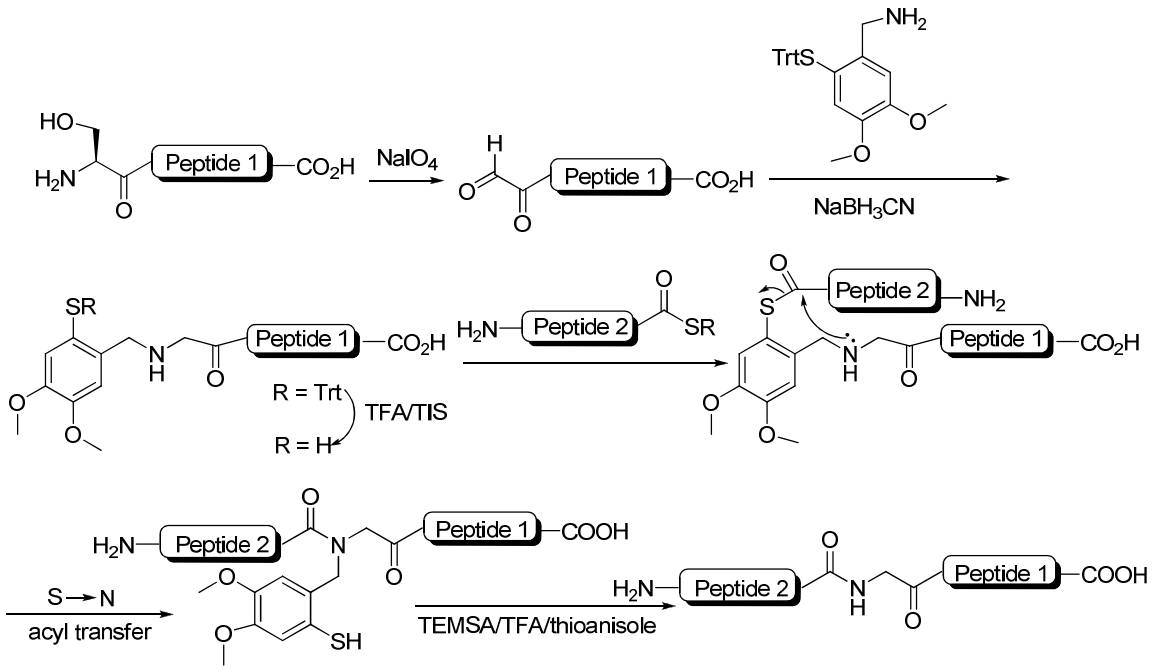
1.1.2.2 Thioligation with a Removable Auxiliary

Another way to overcome the N-terminal cysteine residue requirement in the native chemical ligation methodology is to mimic the characteristic of this cysteine by the use of a removable auxiliary. For this purpose, Canne *et al.* replaced the N-terminal cysteine by an oxyethanethiol-substituted N-terminal group (Scheme 8).[48]



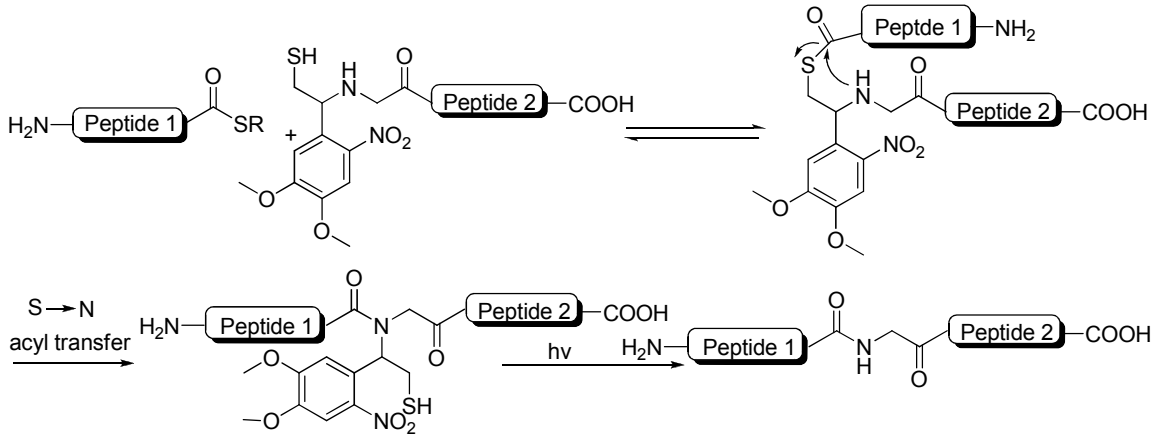
Scheme 8. Peptidyl thioesters ligation through N^{α}-(oxyethanethiol).

Several groups have shown that the N-terminal cysteine can also be replaced by a 2-mercaptobenzylamine linker.[49-51] In this case, the intramolecular acyl shift proceeds through a six-membered ring with the linker, which was subsequently removed by treatment with an acid, leading to the native peptides (Scheme 9).[49]



Scheme 9. Peptidyl thioesters ligation through a mercaptobenzylamine linker.

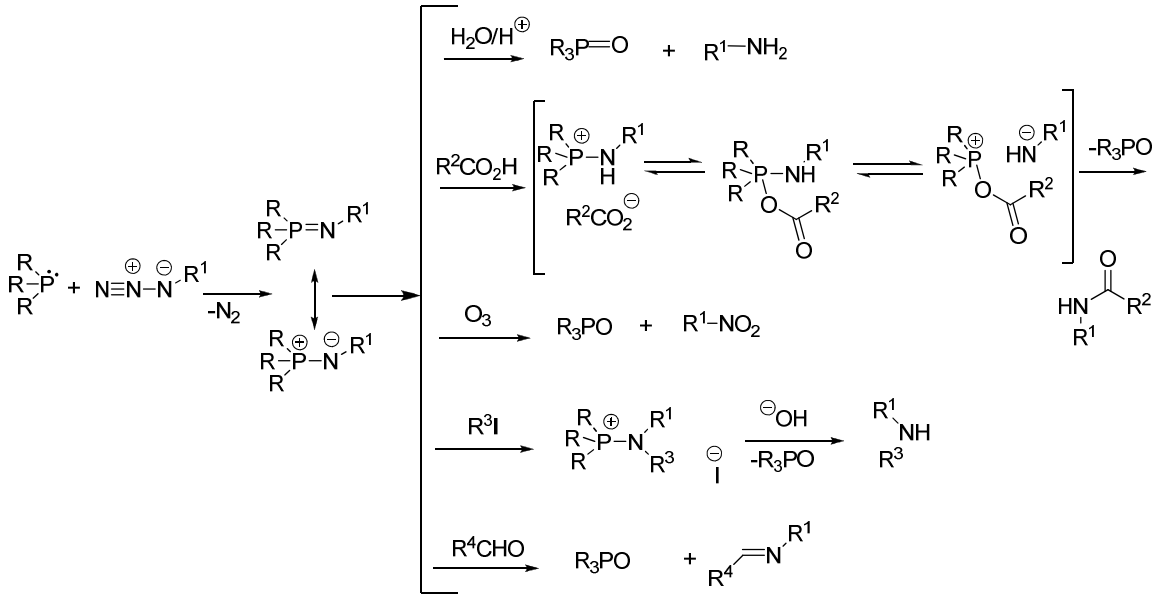
A third type of auxiliary for the thioester ligation of unprotected peptide fragments was introduced by Kawakami and Aimoto[52] and shortly after by Marinzi *et al*.[53] The auxiliary attached at the N-terminus of one peptide fragment is based on an *O*-nitrobenzyl scaffold, with which the thioester present in the second peptide fragment can undergo trans thioesterification as shown in Scheme 10.[52]



Scheme 10. Ligation of peptidyl thioesters through a photoremovable auxiliary.

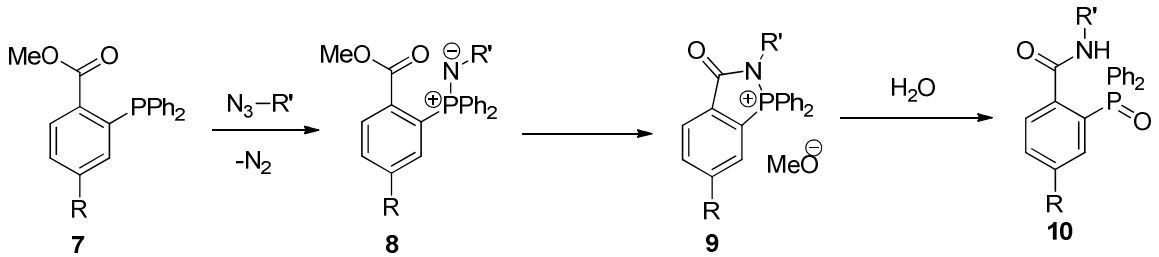
1.1.3 Thioester and Azide Coupling: "Traceless" Staudinger Ligation

The Staudinger reaction[54] is the reaction of a phosphine with an azide to produce an iminophosphorane. This iminophosphorane intermediate can then be trapped by different electrophiles (Scheme 11).[55]



Scheme 11. Staudinger reaction.

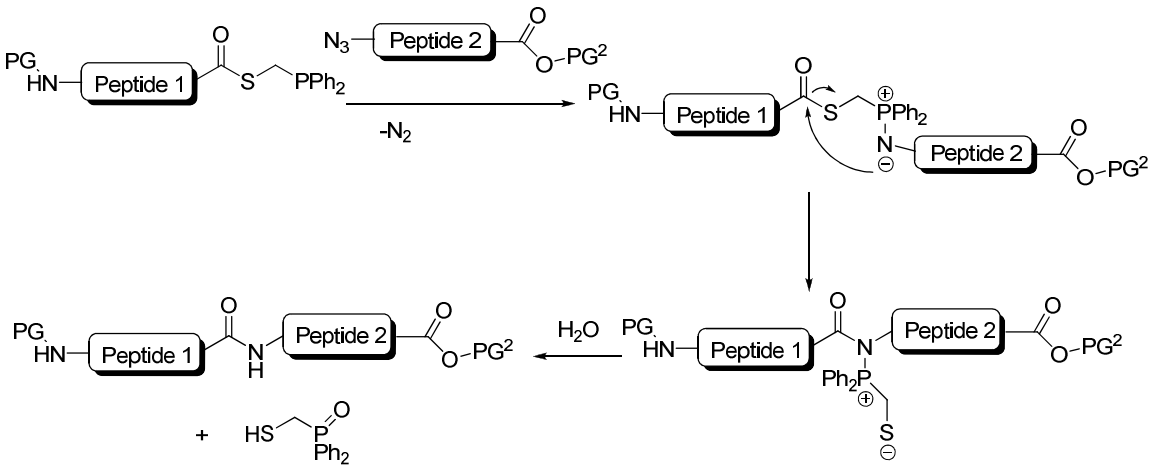
Based on the Staudinger reaction, Raines[56-59] and Bertozzi[60-61] independently developed a methodology, known as the Staudinger Ligation, in which, the iminophosphorane intermediate was intramolecularly trapped by an ester leading to an amidophosphonium salt. The amidophosphonium salt is then hydrolyzed to produce an amide bond attached to a phosphine oxide moiety (Scheme 12).[60]



Scheme 12. Staudinger ligation.

A modification of Staudinger ligation, in which an amide bond is formed between the two coupling partners to give a product without a triarylphosphane oxide moiety, appears even more attractive. Shortly after their first report,

Bertozzi *et al.*[61] and Raines *et al.*[56-58] reported a traceless Staudinger ligation, in which the phosphine oxide moiety is cleaved during the hydrolysis step. This ligation requires two important starting materials; one is a C-terminal phosphinothioester[58] and the other is a N-terminal azide.[62] In the first step of this Staudinger ligation, the phosphinothioester reacts with the azide to give an iminophosphorane, which then undergoes an intramolecular *S* to *N* acyl shift leading to an amidophosphonium salt. The amidophosphonium salt is then hydrolyzed to produce the amide product and a phosphine oxide. The high reactivity of the aza-ylide does not, however, permit the presence of unprotected side-chain functionalities and therefore the Staudinger ligation is limited to the coupling of fully protected peptide fragments (Scheme 13).[63]

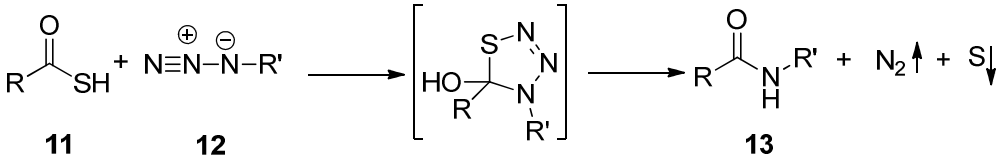


Scheme 13. Ligation of protected peptidyl phosphinothioester.

1.1.4 Thioacid and Azide Coupling

In 2003 Williams and co-workers developed a coupling strategy based on a fundamental mechanistic revision of the reaction of thioacids and organic azides.[64-68] Contrary to the conventional methods for the chemical synthesis of amides this method does not need active esters and amines as precursors for

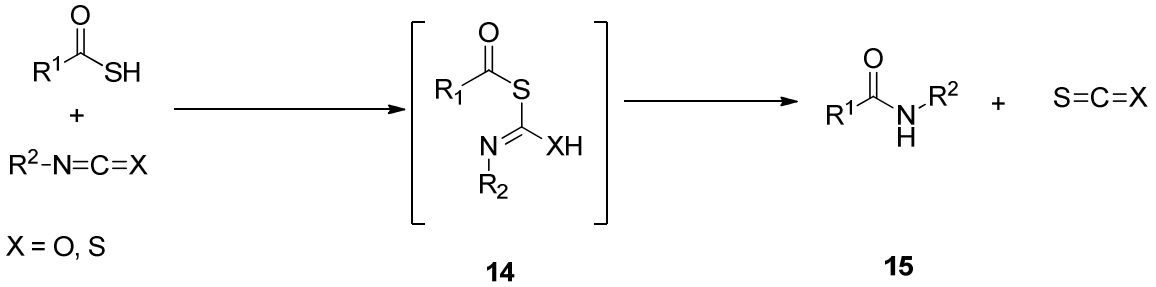
amide synthesis. Moreover the application of this methodology for the preparation of several classes of complex amides in nonpolar and polar solvents including water was reported (Scheme 14).[64]



Scheme 14. Reaction of thioacids and azides to form amides.

1.1.5 Coupling of Thioacids with Isocyanates and Isothiocyanates

Although, the above chemical ligations have proven to be very useful methods for peptide coupling, they are not without limitations, chief among which are the need for a cysteine at the ligation site in NCL, and the need to prepare an N-terminus azide group in Staudinger ligation. In this regard, Crich and Sasaki have developed a new methodology based on the reaction of thioacids with more widely available isocyanates and isothiocyanates, which results in the formation of amides (Scheme 15).[69]



Scheme 15. Reaction of thioacids with isocyanates and isothiocyanates.

1.2 Synthesis of C-Terminal Peptidyl Thioacids and Thioesters

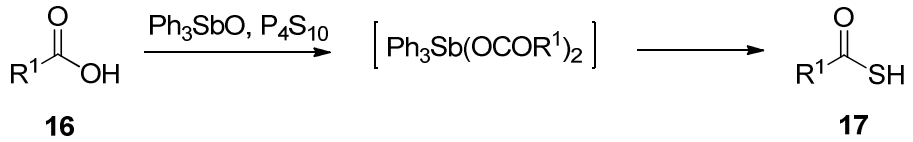
Self-evidently, the synthesis of C-terminal peptidyl thioacids and thioesters is an area of considerable relevance to the amide bond formation reaction by the

native chemical ligation and its variants. For such purposes the synthesis of thioacids and thioesters may be approached 1) in solution and 2) on solid-support.

1.2.1 Synthesis of Thioacids in Solution

1.2.1.1 Direct Thiation of Carboxylic Acids

Direct conversion of carboxylic acids **16** into thioacids **17** using a thiating agent such as tetraphosphorous decasulfide in the presence of catalytic triphenylantimony oxide is high yielding and proceeds under mild conditions (Scheme 16).[70] However without catalytic triphenylantimony oxide, this conversion is not generally an efficient way to make thioacids, and moreover it requires high temperature.[71] More recently, Danishefsky *et al.* have developed a direct conversion of acid to thioacid under microwave irradiation of carboxylic acids and Lawesson's reagent.[72]

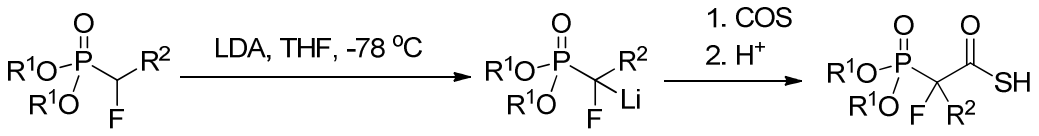


16 **17**

Scheme 16. Direct synthesis of thioacids from carboxylic acids.

1.2.1.2 Nucleophilic Attack on Carbonyl Sulfide

Nucleophilic carbon species such as Grignard reagents[73] or stabilized carbanions react with carbonyl sulfide to give thioacids. For example deprotonation of α-fluoroalkylphosphonates **18** with lithium diisopropylamide followed by quenching with carbonyl sulfide gives the corresponding α-fluoroalkanyl thioacids **19** (Scheme 17).[73]

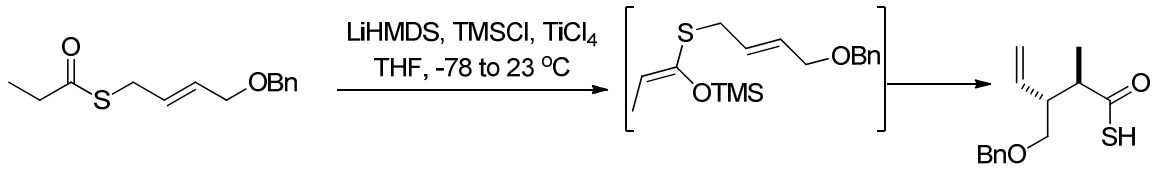


18 **19**

Scheme 17. Preparation of thioacids from carbonyl sulfide.

1.2.1.3 Synthesis of Thioacids by Pericyclic Rearrangement

Several methods are available for preparation of thioacids from thioesters. One of them is a highly diastereoselective Lewis acid promoted Ireland-Claisen rearrangement of a thioester **20** to the corresponding thioacid **21** (Scheme 18).[74]



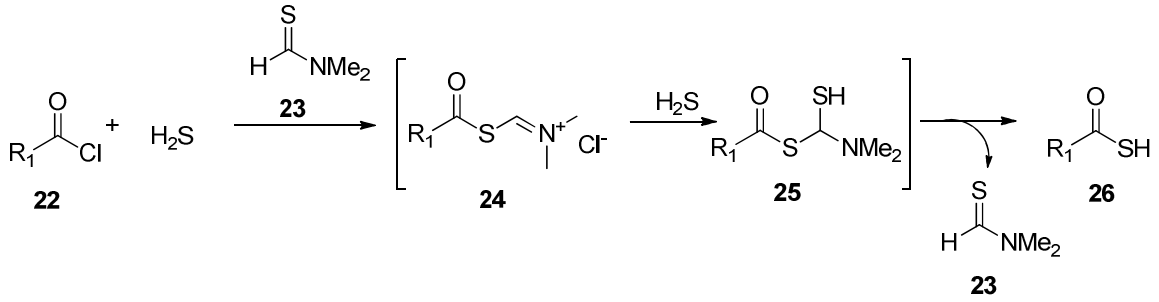
20 **21**

Scheme 18. Synthesis of a thioacid by an Ireland-Claisen rearrangement of a thioester.

1.2.1.4 Acylation with Sulfur Sources

Direct acylation of hydrogen sulfide at high pressure with esters, acid halides and anhydrides has been a common method for thioacid preparation.[75-76] Acid chlorides are generally used as the acylating agents and, when reacted with hydrogen sulfide in pyridine followed by addition to a solution of aqueous potassium hydroxide saturated with hydrogen sulfide, provide the thioacids.[77-78] A more versatile approach to the acylation of a sulfur source involves the treatment of acid halides **22** with *N,N*-dimethylthioformamide (**23**) to give the intermediates

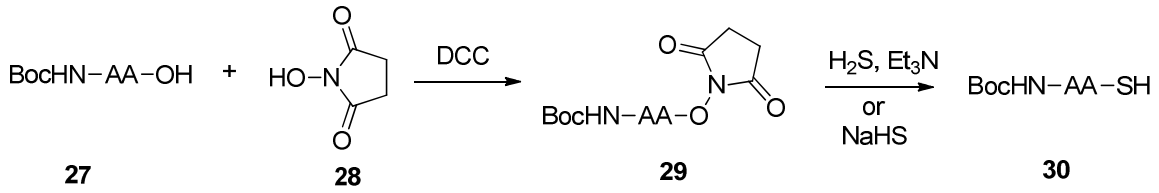
25, which then decompose to the thioacids with regeneration of the thioformamide (Scheme 19).[75]



Scheme 19. Synthesis of thioacids from acid chlorides and dimethylthioformamide.

1.2.1.5 Synthesis of Amino Thioacids

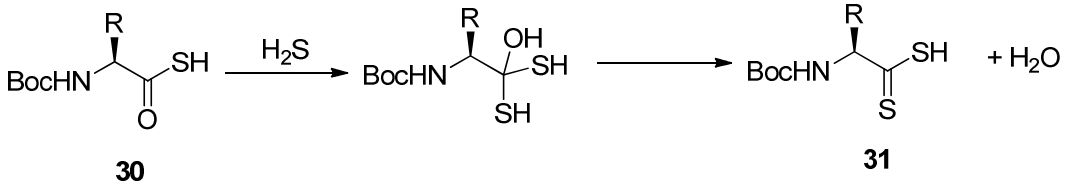
Several methods have been developed for the synthesis of small C-terminal peptidyl thioacids in the solution phase.[13,16] In most of the cases the formation of an active ester of *N*-Boc protected amino acids is invoked, followed by nucleophilic attack on the carbonyl carbon of the active ester by hydrogen sulfide gas or sodium hydrogen sulfide (Scheme 20).[13]



Scheme 20. Synthesis of amino thioacids.

However, this method has limitations which prevent it from being used extensively in synthesis of thioacids. Chief among these limitations is the formation of the symmetrical thioanhydride by the reaction of unreacted active ester **29** and the thioacid salt of **30**.[77,79] The protocol also typically generates a

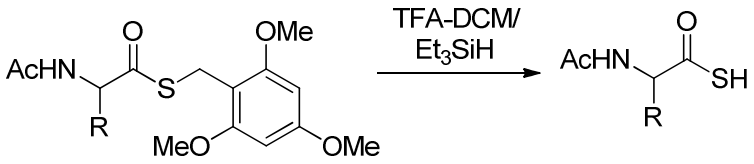
di-thioacid **31** by the reaction of excess hydrogen sulfide with the thioacid **30** (Scheme 21).[79]



30 **31**

Scheme 21. Formaton of di-thioacid.

In this regard, a 2nd protocol for amino thioacids synthesis was developed by Williams and co-workers in 2003, wherein a trimethoxybenzyl (TMB) thioester **32** was deprotected in mild acidic conditions to obtain thioacid **33** (Scheme 22).[64]



32 **33**

Scheme 22. Synthesis of thioacids through TMB-thioesters.

However, due to potential difficulties in the solution-phase methodology, in which the product of each individual reaction step has to be isolated and purified before the next step, alternatives have been sought. In contrast to the solution-phase methodology, SPPS methodology has been employed more prominently for the synthesis of peptidyl thioacids. In which, the growing peptide is linked to an insoluble support and therefore, after each reaction step, the byproducts are simply removed by filtration and washing. Furthermore, because of the repetitive nature of peptide synthesis (deprotection, washing, coupling, washing, deprotection,…), the use of an insoluble support in a single reaction vessel allows for automatization of the processes.

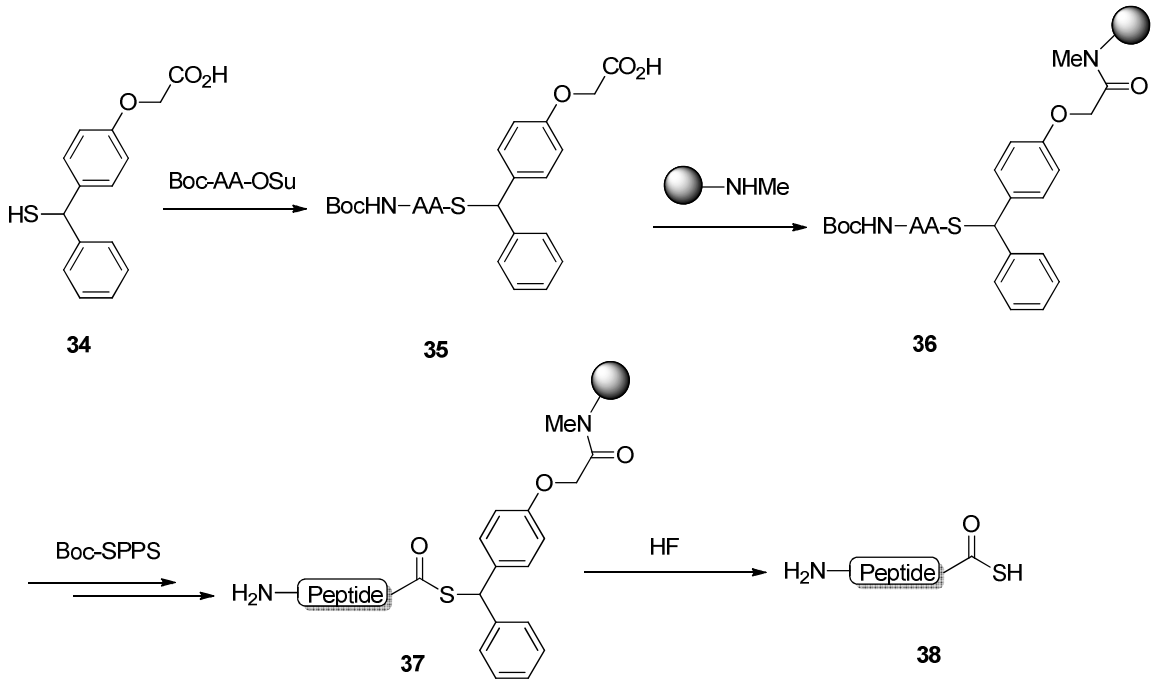
1.2.2 Synthesis of C-Terminal Peptidyl Thioacids and Thioesters on Solid Support

Since Merrifield introduced SPPS,[80] new synthetic methods have continually been reported with the aim of improving efficiency in peptide assembly.[81] Although the vast majority of synthetic peptides are required with C-terminal carboxylic acids or primary carboxamide end groups, many other C-terminal modified peptides are often found in nature and have potential interest as therapeutic agents.[82] For example, peptides in which the C-termini are modified to other functionalities have been extensively used as substrates and inhibitors for a variety of proteolytic enzymes. In addition, C-terminal modified peptide fragments like amides,[83-84] hydrazides,[85] hydroxamic acids,[86] alcohols,[87-88] aldehydes[89-90] and ketones[91] can be readily engaged in segment condensations for the assembly of larger peptides or small proteins.[92-93] In a similar vein, an important goal in C-terminal modified peptide synthesis is the inclusion of peptidyl thioacids and thioesters synthesis on a solid support using both Fmoc and Boc chemistries.

1.2.2.1 Synthesis of C-Terminal Peptidyl Thioacids and Thioesters on Solid Support using Boc Chemistry

The lability of the carbonyl-sulfur bond of thioesters to repeated exposure to the nucleophilic base piperidine in standard Fmoc-SPPS protocols, led to an initial reliance on Boc-SPPS chemistry for the synthesis of peptidyl thioacids and thioesters. In this respect, solid-phase preparation of unprotected peptides with a thiocarboxylic acid functionality at the C-terminus was first reported by Blake *et*

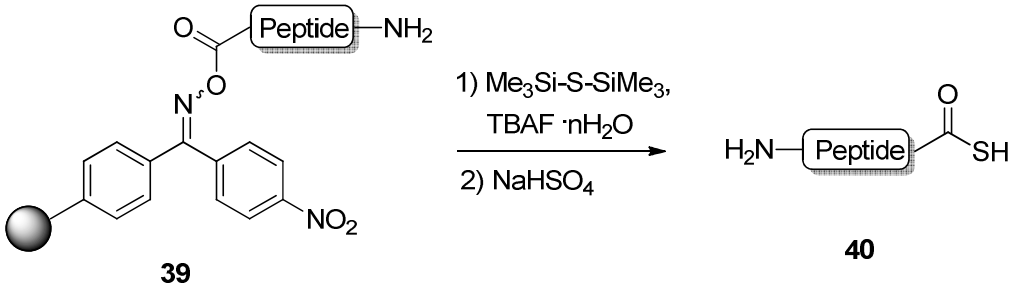
al., who used a preformed benzhydryl thioester handle 4-[α-(Boc–Gly–*S*)benzyl]phenylacetic acid.[36-37] Subsequently, several methods have been developed for the preparation of a modified handle, 2-[4-(mercapto(phenyl)methyl)phenoxy]acetic acid.[15,94-95] The first amino acid is derivatized with this linker, and the resulting thioester **35** is finally attached to an aminomethyl polystyrene resin. Standard Boc-SPPS[96] is then applied for preparation of the desired peptide sequence. Finally, cleavage of the peptide from the resin with HF leads to the formation of the peptidyl thioacid **38** (Scheme 23).[94]



Scheme 23. Peptidyl thioacid synthesis using a handle on solid support.

Schwabacher and Maynard reported a strategy, wherein the target peptide was assembled by standard Boc chemistry on 4-nitrobenzophenone oxime resin.[97-98] The thioacid **40** is obtained after cleavage of active *O*-acyl oxime

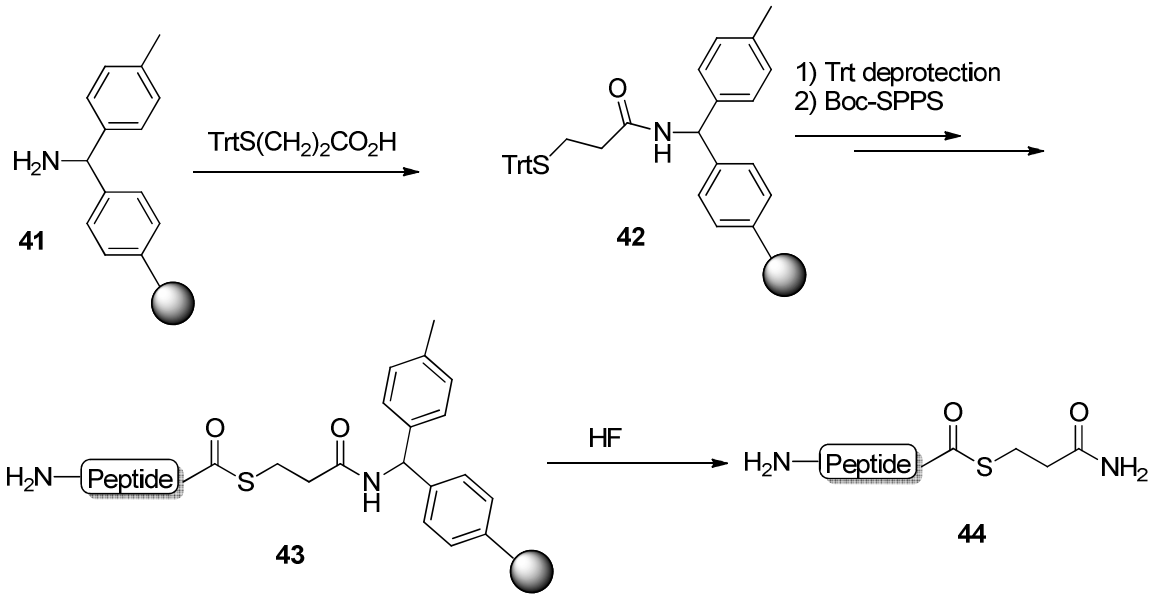
peptidyl ester **39** with hexamethyldisilathiane in presence of TBAF (Scheme 24).[99]



39 **40**

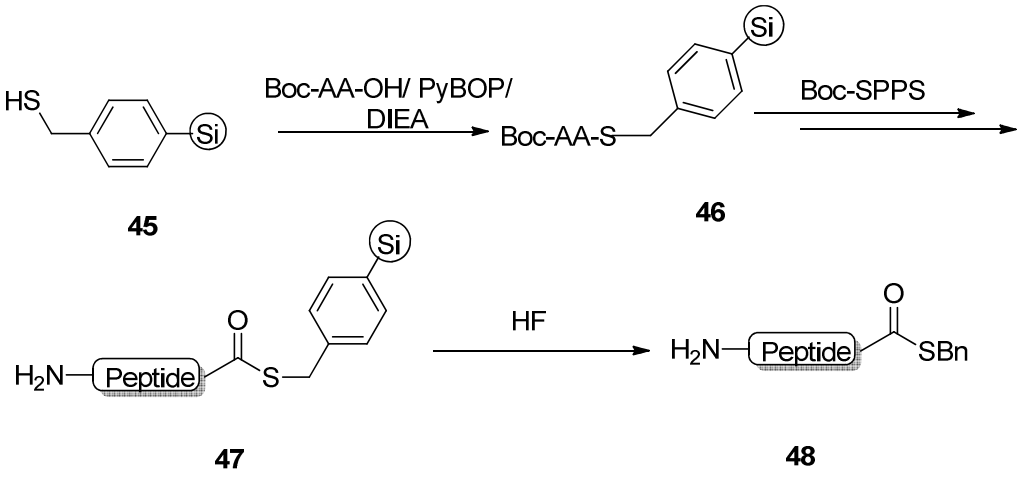
Scheme 24. Synthesis of peptidyl thioacid on oxime resin.

Partially protected peptide *S*-alkyl thioesters were first prepared by Aimoto *et al*. using the 3-sulfanylpropanoic acid handle[100] or its *tert*-butylsulfanyl derivative.[101] These systems were coupled to a *p*-methylbenzhydrylamine (MBHA) resin (**41**) and Boc protocols were employed. In a similar vein, a third protocol for SPPS containing a C-terminal thioester by the Boc strategy was developed by Tam and co-workers.[24,102-103] The method is based on the use of a MBHA resin, which is first loaded with *S*-trityl mercaptopropionic acid. After removal of the trityl-protecting group, the desired polypeptide chain is assembled using standard Boc strategy. Finally, the thioester **44** is obtained after cleavage with HF (Scheme 25).[24]



Scheme 25. Synthesis of a peptide possessing a C-terminal thioester.

Recently, Yangmei *et al*. reported an efficient synthesis of a fully deprotected peptide *S*-benzyl thioester using a volatilizable mercaptomethylphenyl-functionalized silica support (**45**).[104] The first amino acid is derivatized with this linker, and the target peptide was assembled by standard Boc chemistry. Finally, cleavage of the peptide from the resin with HF leads to the formation of the peptidyl thioester **48** (Scheme 26).[104] However, the hazardous HF[105-106] conditions typically required for cleavage from the resin following Boc-SPPS, limit the use of this chemistry.



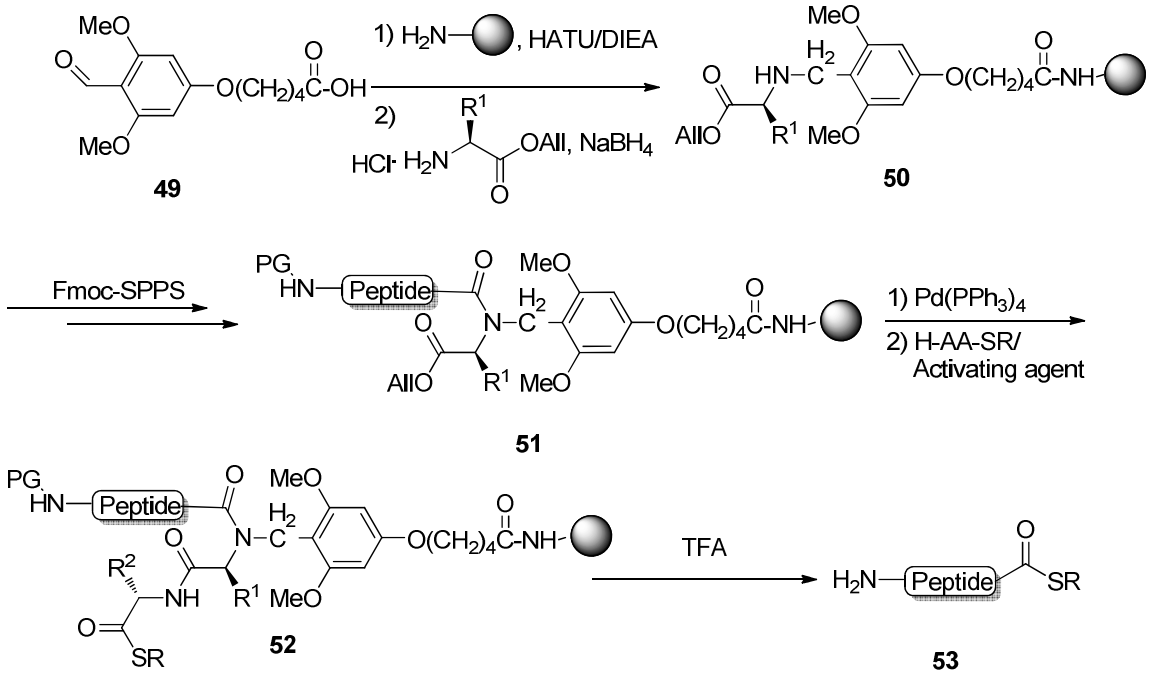
Scheme 26. Synthesis of peptidyl *S*-benzyl thioester on silica support.

1.2.2.2 Synthesis of C-Terminal Peptidyl Thioacids and Thioesters on Solid Support using Fmoc Chemistry

Despite the instability of thioesters to the basic conditions required to remove the widely used Fmoc protecting group in Fmoc-SPPS methodology, several Fmoc chemistry-based methods have been developed. These include the replacement of piperidine by new cleavage cocktails of 1-methylpyrrolidine, hexamethylenimine, and 1-hydroxybenzotriazole for the Fmoc removal steps, but it has been found that these methods tend to epimerize the stereogenic center at the thioester position.[107-108]

In 1999, Barany *et al.* was the first to report the synthesis of peptidyl thioesters following Fmoc-SPPS with BAL strategy, where the growing peptide is anchored through backbone nitrogen instead of through a terminal C^{α}-carboxyl group.[109-111] In the BAL strategy, an acid labile 4-[formyl-3,5-dimethoxyphenoxy]butyric acid-based handle (**49**) was linked with an amino-functionalized PEG-resin. This was followed by on-resin reductive amination with

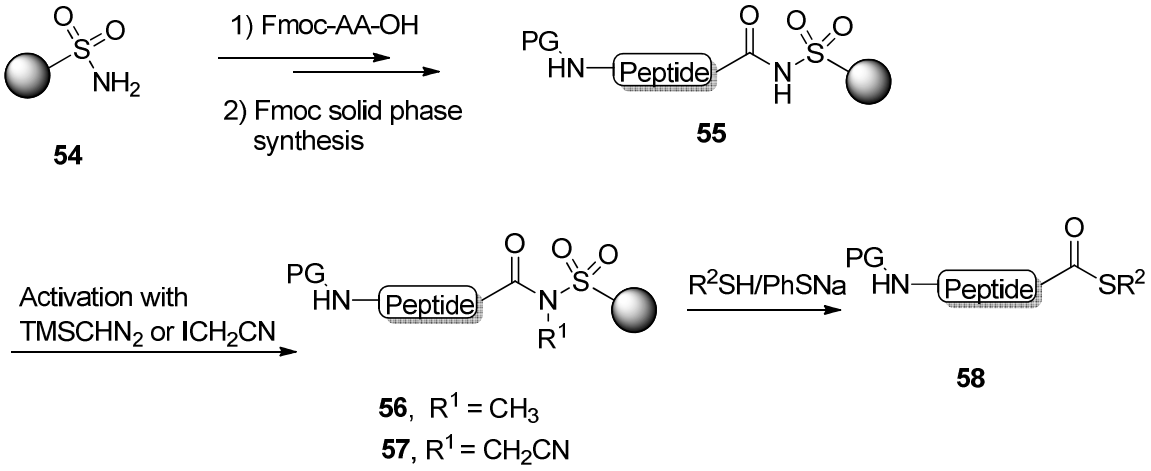
an allyl ester of amino acid hydrochloride salt and the target peptide was assembled by standard Fmoc chemistry on this support. Then selective removal of the C-terminal allyl ester followed by coupling of amino acid thioester leads to the backbone amide anchored C-terminal peptidyl thioester **52**, which was cleaved by TFA to obtain the thioester **53** (Scheme 27).[109]



Scheme 27. Preparation of unprotected peptidyl thioesters using backbone amide linker approach.

Although this approach was successful in many cases, its scope is somewhat limited by the need for special precautions required when coupling the second amino acid to prevent diketopiperazine formation. However, the major drawback to this method is the need for careful control in the activation of backbone peptide carboxylic acid to avoid epimerization on introduction of the thioester to the C-terminal end of the peptide chain.[112-113] To circumvent these problems, numerous methods have been developed using Fmoc chemistry

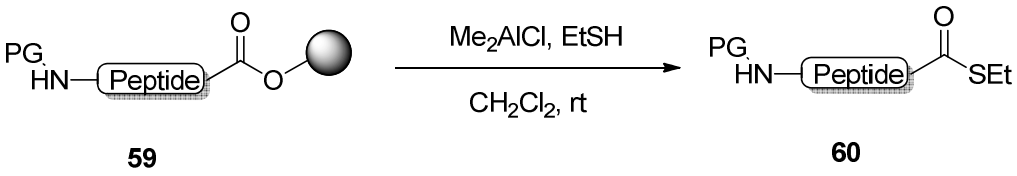
wherein, after completion of the peptide synthesis, the linker to the resin is activated and it permits the displacement by a thiol or thiolate resulting in the liberation of peptidyl thioesters. In this regard, Ingenito *et al.*[114] developed the use of an acylsulfonamide 'safety-catch linker' **54** for SPPS containing a C-terminal thioester by the Fmoc strategy. This linker, which is stable to both strongly basic and acidic conditions, was first introduced by Kenner *et al.*[115] and later modified by Backes and Ellman.[116] The peptide is assembled using the standard Fmoc protocol to afford solid-phase bound peptides of such as **55**. After the final peptide coupling, the resin is activated for cleavage by treatment with diazomethane to give an *N,N*-acylmethylsulfonamide **56**, or by treatment with iodoacetonitrile to give an *N,N*-acylcyanomethylsulfonamide **57**. The peptide is then released from the activated resin by nucleophilic displacement involving a thiol group to yield **58** (Scheme 28).[116]



Scheme 28. Synthesis of a peptidyl thioester on 'Safety-Catch' linker.

Sewing and Hilvert reported a second useful strategy,[117-118] in which the target peptide is assembled by standard Fmoc chemistry on a 4-hydroxymethyl-

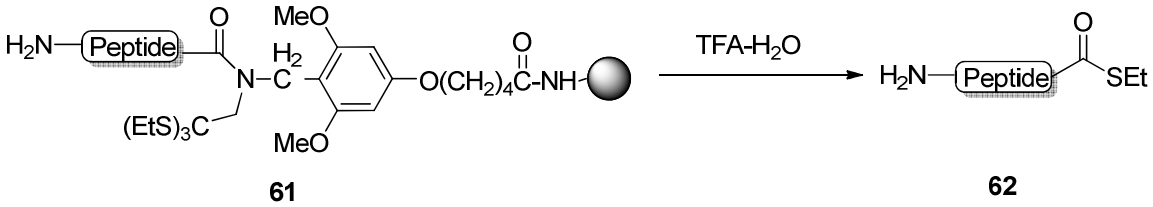
phenylacetamidomethane (PAM) or 4-hydroxymethylbenzoic acid (HMBA) resin, and the cleavage was performed by activation of the ester linkage with $AlMe_2Cl$ in the presence of a large excess of a nucleophilic thiol to yield peptide thioester **60** (Scheme 29).[118]



59 **60**

Scheme 29. Formation of a C-terminal peptidyl thioester by dimethylaluminum mediated cleavage.

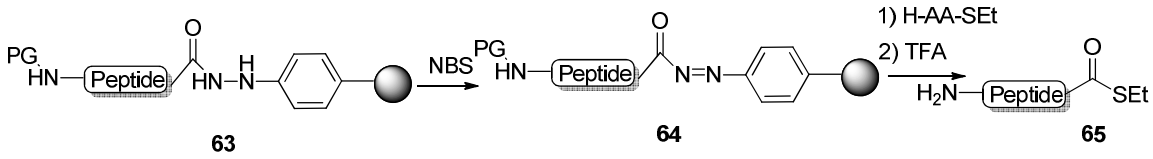
In this method, Sewing and Hilvert observed that treatment with a large excess of oxophilic reagent favored formation of trithioortho esters and ketene dithioacetals, which on acidic workup yielded peptidyl thioesters, with the risk of racemization of C-terminal amino acid. However, the need to expose the whole assembled peptide to harsh reagents caused side-reactions to varying degrees, including conversion of side-chain ester groups to their thioesters and formation of aspartimide. In this regard, Albericio and Jensen *et al.* reported a modification of the BAL approach to the synthesis of peptidyl thioesters, where they linked a masked thioester i.e., trithioortho esters with 4-[formyl-3,5-dimethoxyphenoxy]butyric acid (**49**) handle and assembled the peptide using Fmoc chemistry (Scheme 30).[119] Although the concept of the masked thioester in this strategy can solve the problem of formation of diketopiperazine, its practical utility at the present is restricted mainly to the preparation of glycine thioester peptides.



61 **62**

Scheme 30. Synthesis of peptidiyl thioesters by masking of thioesters.

A third protocol for the synthesis of thioesters along with Fmoc-SPPS and activation of linker was reported by Camarero *et al.*[120] using an aryl hydrazine support, which is totally stable to the conditions of Fmoc- and Boc-SPPS and which yields a peptide hydrazide resin. At the end of the synthesis, the fully protected peptide resin is activated by oxidation with *N*-bromosuccinimide to peptide diazene **64**. The reactive acyl diazene is then cleaved with an α-amino acid *S*-alkyl thioester followed by treatment with TFA to give the peptidyl thioesters **65** (Scheme 31).[120]

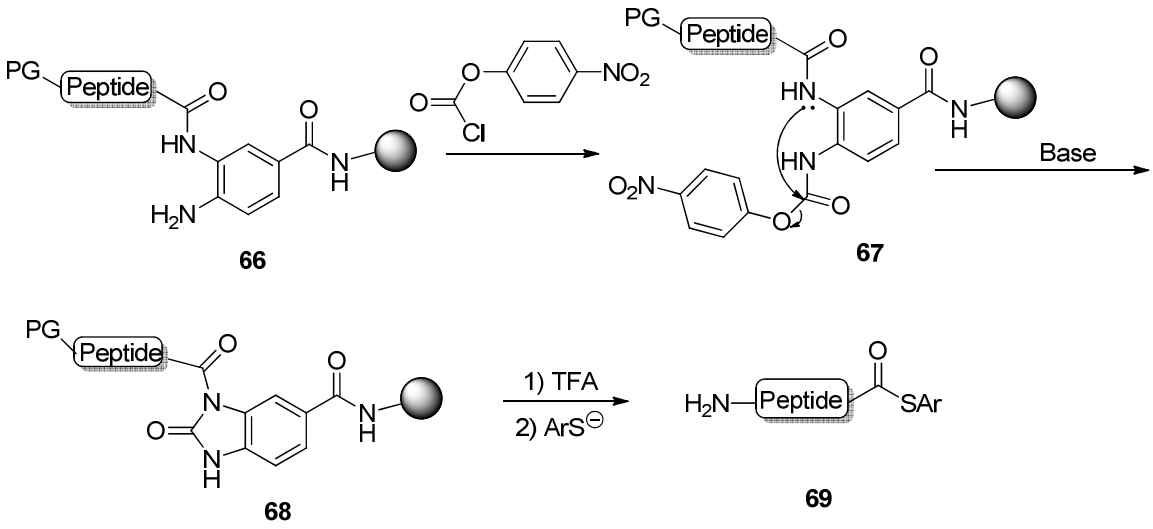


63 **64** **65**

Scheme 31. Synthesis of petidyl thioesters using an aryl hydrazine support.

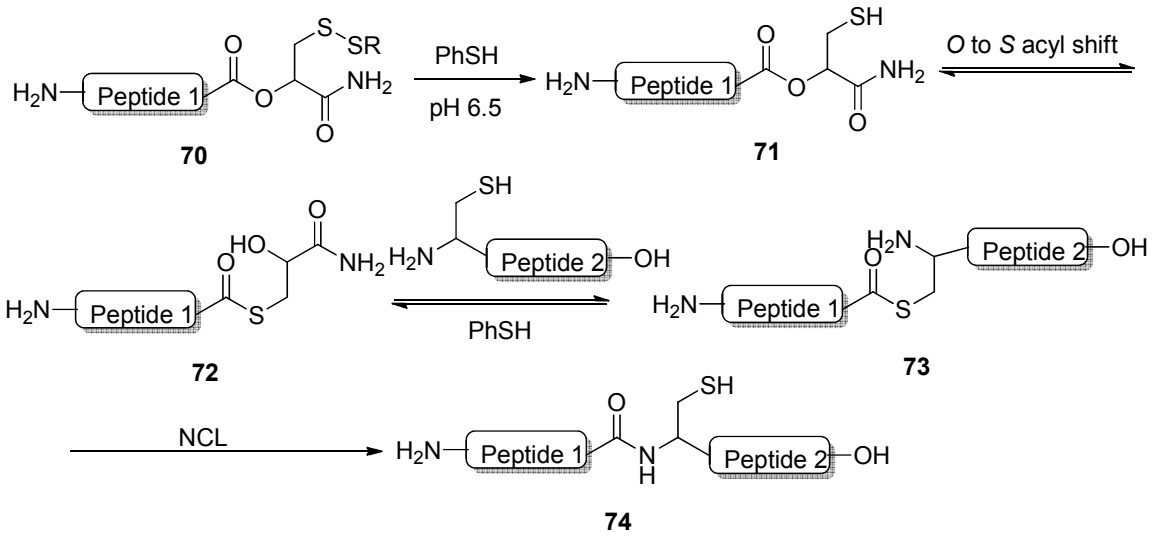
Another elegant way to synthesize petidyl thioesters based on activation of linker, by formation of active C-terminal aromatic *N*-acylurea functionality, was reported by Dawson and co-workers.[121] The linker attached with the C-terminal carboxylic group is 3,4 diamino benzoic acid, which was first coupled with PEG amine, and the peptide was synthesized following Fmoc chemistry to obtain resin bound peptide **66**. After which, **66** was efficiently transformed into an aromatic *N*-

acylurea moiety **68**, followed by deprotection and thiolysis to yield peptidyl thioesters **69** (Scheme 32).[121]



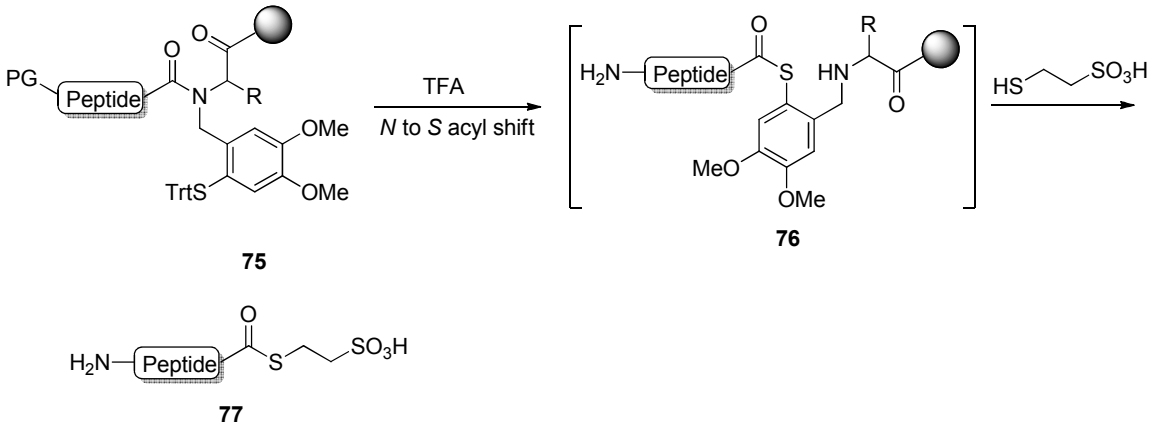
Scheme 32. Synthesis of peptidiyl thioesters through *N*-acylurea.

Although methods based on the activation of a linker followed by thiolysis leading to the synthesis of peptidyl thioesters have had noticeable success, they also have disadvantages, most pertinently the risk of epimerization on introduction of the thioester to the active C-terminal end of the peptide chain, and the need for additional steps after completion of synthesis of peptide chain on the linker. In this regard, Botti *et al.,*[122] and Danishefsky *et al.*[123] individually developed a novel methodology based on the in situ *O* to *S* acyl shift, which leads to the formation of a thioesters **72** (Scheme 33).[122] More recently Muir *et al.* synthesized a number of naturally occurring cyclic peptidyl thioesters i.e., peptidyl thiolactones following the *O* to *S* acyl shift protocol.[124]



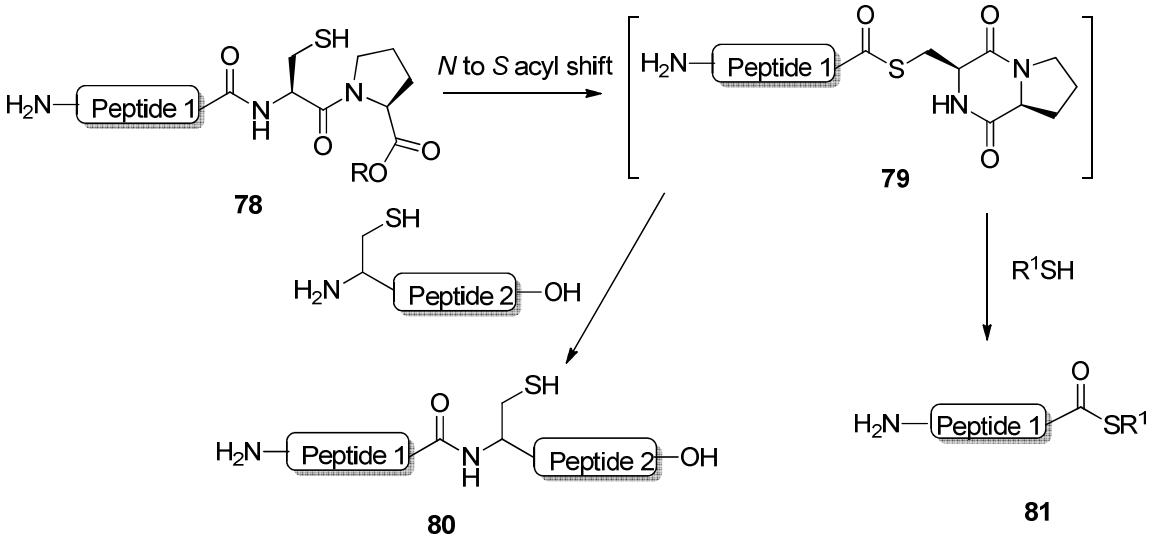
Scheme 33. In situ formation of thioesters through *O* to *S* acyl shift and native chemical ligation.

A second protocol for the direct synthesis of peptidyl thioesters along with Fmoc-SPPS is based on the in situ *N* to *S* acyl shift reaction.[125-130] The methodology was first reported by Aimoto *et al.*, where 4,5-dimethoxy-2-mercaptobenzyl protecting group mediated peptidyl thioester **77** was synthesized via an *N* to *S* acyl shift as shown in scheme 34.[125]



Scheme 34. Formation of thioesters through *N* to *S* acyl shift.

More recently Kawakami and Aimoto reported a peptide containing a cysteinyl prolyl ester (CPE) moiety at the C-terminus is spontaneously transformed into a diketopiperazine thioester **79** via an intramolecular *N* to *S* acyl shift reaction, followed by diketopiperazine formation.[130] Moreover, due to the autoactivating function of the CPE peptide can be ligated with a Cys-peptide in a one-pot procedure and the peptide diketopiperazine thioester can also be transformed into a peptide thioesters **81** by intermolecular thiol–thioester exchange with external thiols (Scheme 35).[130]

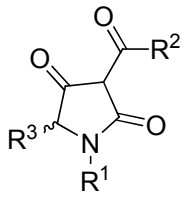


Scheme 35. Thioester formation and ligation of CPE peptide via *N* to *S* acyl shift.

1.3 Toward the Stereochemical Elucidation and Synthesis of Virgineone

The pyrrolidin-2,4-dione ring system carrying a 3-acyl substituent (tetramic acid) **82** is a key structural unit in many natural products (Figure 3).[131-132] The spectrum of biological activity displayed by these natural products is remarkable in its diversity; ranging from potent antibiotic, antiviral and antifungal properties,

cytotoxicity and mycotoxicity as well as the inhibition of tumor growth.[133-137] In addition, certain members of this class are responsible for the pigmentation of certain sponges and molds.[131] Self-evidently, the synthesis of this class of natural products has been and continues to be an area of considerable interest to synthetic organic chemists.[132]



82

Figure 3. Tetramic acid skeleton.

A novel member of this group of natural products, a glycosylated tetramic acid, virgineone (**83**), was isolated and characterized by Singh *et al.* in 2009 from saprotrophic *Lachnum virgineum* (Figure 4).[138] Virgineone was identified in a number of Lachnum strains collected from diverse geographies and habitats, and displays a broad-spectrum of antifungal activity against *Candida spp.* and *Aspergillus fumigatus*.[138] Structurally related compounds include militarinone C (**84**) isolated from the fungus *Cordyceps militaris*,[139] epicoccamide A (**85**) from jellyfish-derived strains of *Epicoccum purpurascens*,[140] and epicoccamide D (**86**) from an *Epicoccum* sp. associated with the wood-decay fungus *Pholiota squarrosa* (Figure 4).[141]

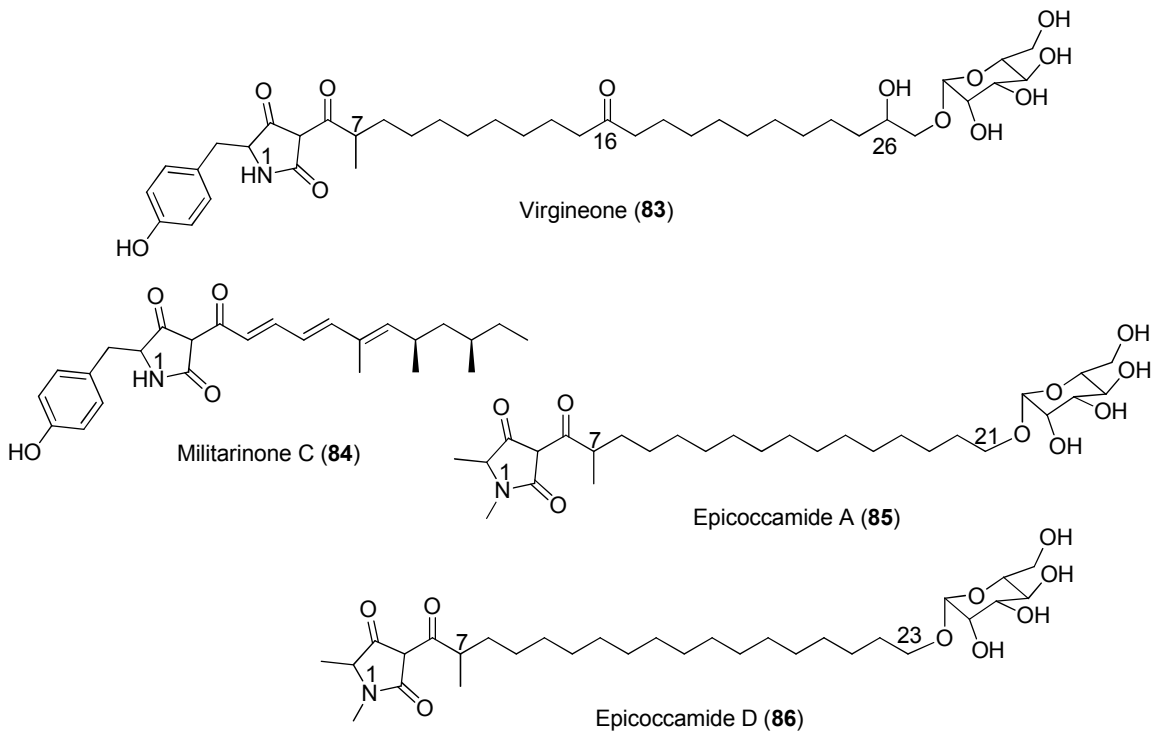
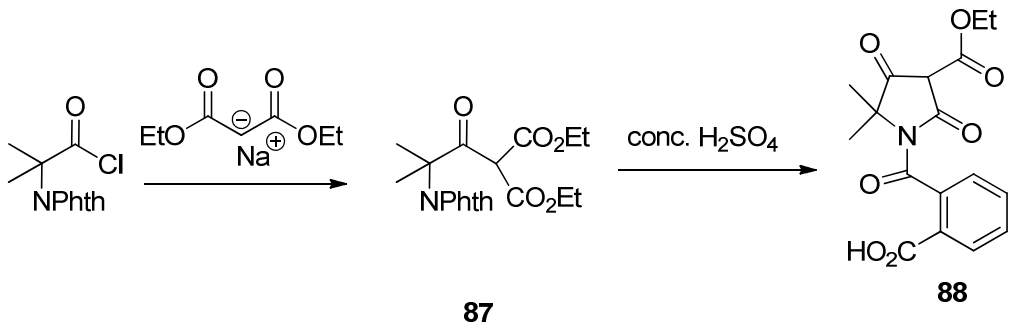


Figure 4. Virgineone and structurally related natural products.

In addition to a tyrosine derived acyl-tetramic acid terminus, virgineone contains a central C-22 oxygenated chain attached at its terminus to β-mannopyranoside. Unfortunately, other than the β-configuration of the mannoside unit, neither the absolute nor the relative configuration of virgineone was established by the original investigators, making it an ideal target for total synthesis. The synthesis of virgineone can be considered to revolve around two key steps: 1) the formation of tetramic acid moiety, and 2) the stereoselective β-mannosylation. In this regard a succinct overview of the currently optimal methods for the synthesis of these two units is given.

1.3.1 Synthesis of Tetramic Acids: Overview

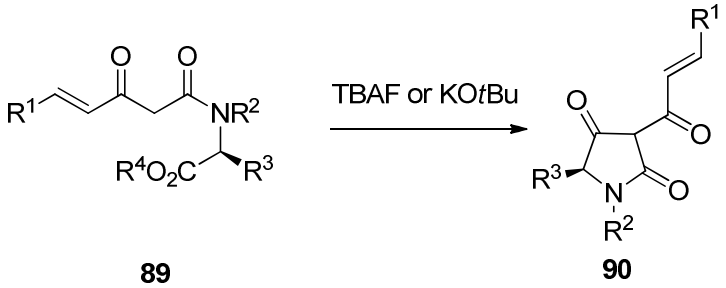
In 1914, Gabriel was the first to synthesize a tetramic acid derivative **88** by cyclization of phthalimidomalonate derivative **87** with concentrated sulfuric acid (Scheme 36).[142-143]



87 **88**

Scheme 36. First synthesis of tetramic acid.

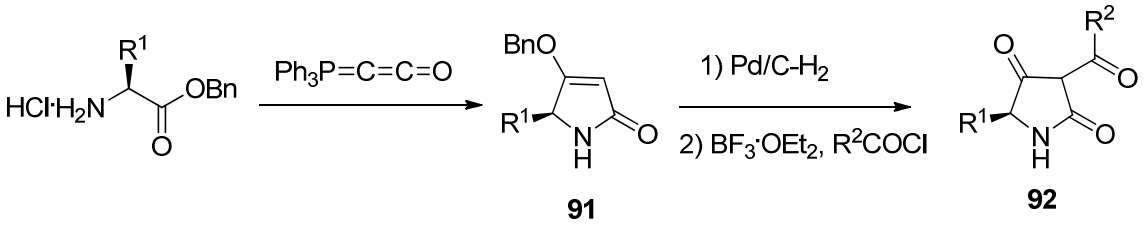
Subsequently and especially during the last few decades tremendous advances in the synthesis of chiral tetramic acids have been reported.[144-146] In most cases, the synthesis is based on the use of amino acid derived precursors whose stereochemical integrity remains more or less conserved in the structure of products. In this context, the most appreciated studies on the synthesis of such optically active compounds have been reported by Ley *et al.*, where a series of enantiomerically pure tetramic acids were prepared by the cyclization of β-keto amides **89** with TBAF or KO*t*Bu as base (Scheme 37).[147]



89 **90**

Scheme 37. Synthesis of enantiomerically pure tetramic acids.

More recently Schobert *et al.* reported a multistep process for the synthesis of enantiomerically pure tetramic acids.[148-149] The method is based on the high temperature Wittig type reaction of excess (triphenylphosphoranylidene)ketene with α-amino acid benzyl esters to obtain tetramates **91**. Hydrogenolysis of **91**, followed by C-acylation with an excess acyl chloride gave the tetramic acid **92** (Scheme 38).[149]



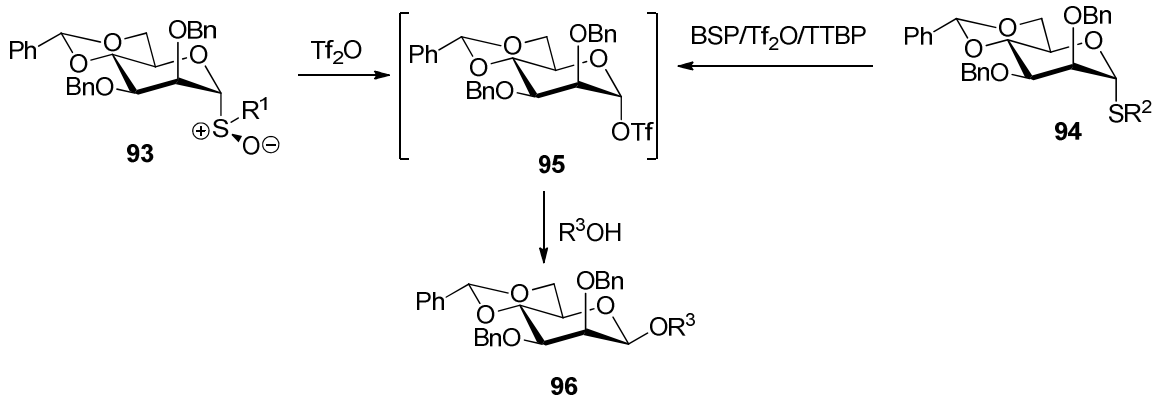
Scheme 38. Synthesis of tetramic acids by Wittig type reaction.

1.3.2 Stereoselective β-Mannosylations: Overview[150]

Due to a formidable combination of steric and stereoelectronic factors, the formation of the equatorial β-mannoside bond has long been considered one of the most challenging linkages to prepare in the field of carbohydrate synthesis.[151-152]

In this regard, as developed and demonstrated by Crich *et al.,* the use of 4,6-*O*-benzylidene protected mannosyl donors bearing ether-type protecting groups on O-2 and O-3, is well-proven to be the most efficient general approach for the construction of β-mannosides with high yield and excellent stereoselectivity. Crich and Sun initially demonstrated this chemistry with mannosyl sulfoxide donors,[153] wherein the donor was activated with Tf_2O and the so-generated covalently bound α-mannosyl triflate was subjected to

nucleophilic displacement by the glycosyl acceptor to give the β-mannoside.[153] Subsequently, Crich and Smith demonstrated the use of thiomannoside donors to generate α-mannosyl triflates with the help of an electrophilic promoter combination of BSP/Tf_2O[154] in presence of a non-participating hindered base, TTBP[155] (Scheme 39).[150] This methodology is highly appreciated and has been extended to the stereocontrolled synthesis of a number of complex β-mannoside-containing oligosaccharides by numerous groups worldwide.[150]



Scheme 39. 4,6-*O*-Benzylidene directed β-mannosylations.

1.4 Goals of This Thesis

The work described in this dissertation was undertaken with two different goals in mind. Firstly, the development of the chemistry of thioacids with particular emphasis on applications in peptide chemistry was envisaged. Thus, as described in Chapter 2, investigations were conducted towards the development of methodologies for the synthesis of thioacids, and their coupling with sulfonamides to synthesize peptide bonds, while Chapter 3 deals with the synthesis of peptidyl thioacids on a solid-support. Continuing this theme, Chapter 4 describes the in situ generation of thioacids by the regioselective ring opening

of β-thiolactones and the application of this chemistry to the formation of amide bonds. A second goal was the exploration of methods toward the synthesis of virgineone and the establishment of its relative and absolute configuration. Work directed toward this end is presented in Chapter 5.

CHAPTER 2

SYNTHESIS OF AMINO THIOACIDS: APPLICATIONS TO THE FORMATION OF AMIDE BONDS AND SYNTHESIS OF PEPTIDES BY THEIR REACTION WITH 2,4-DINITROBENZENESULFONAMIDES

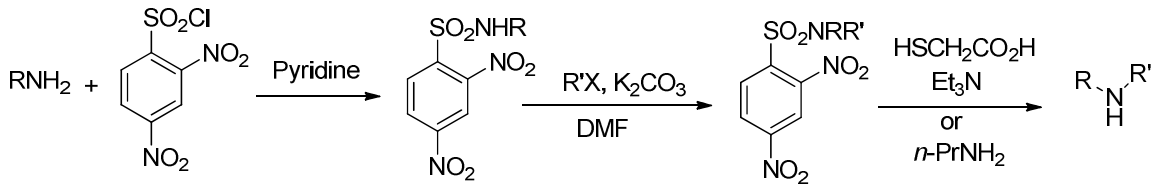
2.1 Background and Significance

The amide function is a common feature in both small and complex synthetic or natural molecules. For example, peptides and proteins are heteropolymers composed of amino acid residues linked by amide bonds between the carboxyl group of one amino acid residue and the α-amino group of the next one, and proteins play a crucial role in virtually all biological processes such as enzymatic catalysis,[156] transport and storage, immune protection[157-158] and mechanical support.[159] Understanding the mechanisms and principles governing the structural and functional properties of bioactive proteins is an important objective in biological and medical research. The first requirement for the study of proteins is to assess their ease of availability in terms of purity and quantity. There are three main routes to consider: (i) native protein isolation,[160-162] (ii) recombinant techniques for the expression of proteins in microorganisms,[163-164] and (iii) chemical synthesis.[165-166] Each of these methods has its advantages and disadvantages, but only chemical peptide synthesis permits the production of large quantities of pure peptides.

As discussed in section **1.1**, a number of elegant approaches have been investigated to meet the challenges present in the synthesis of peptides and proteins, however, these are not without limitations. In this context, the development of a new method for amide and peptide bond formation is envisaged in this chapter.

2.2 Amide Bond Formation by the Tomkinson Reaction

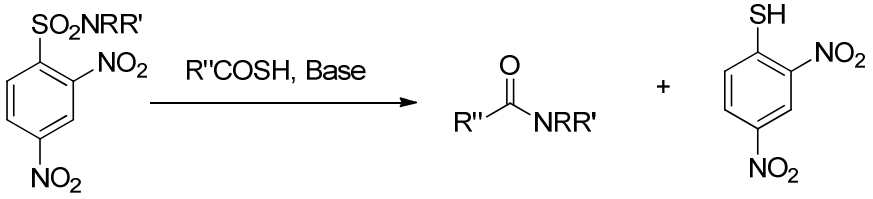
As demonstrated by Fukuyama,[167-168] the 2-nitro- and 2,4-dinitrobenzenesulfonamides function as excellent protecting groups for amines and enable their alkylation on nitrogen under mild reaction conditions, because of acidity of their N-H bond. The sulfonamides are also deprotected under very mild conditions by nucleophilic aromatic substitution with a thiol or amine (Scheme 40).[167]



97 **98** **99** **100** **101**

Scheme 40. Formation of sulfonamides and synthesis of secondary amines.

However, as reported by Tomkinson and co-workers,[169-170] when the nucleophilic thiol is replaced by a simple organic thioacid as in Scheme 35, the product is the amide (Scheme 41).



100 **102** **103**

Scheme 41. Formation of an amide bond by Tomkinson's reaction.

Accordingly, when the simple organic thioacid is replaced by an amino thioacid as in Scheme 41, the product is a peptide. Therefore, an amino acid bearing a C-terminal thioacid group is the key starting material in the synthesis of

peptide bond by the Tomkinson reaction. As discussed in the introduction (section **1.2.1.5**), several methods have been developed for the preparation of thioacids in solution, however, a general or largely accepted method has yet to be accomplished.

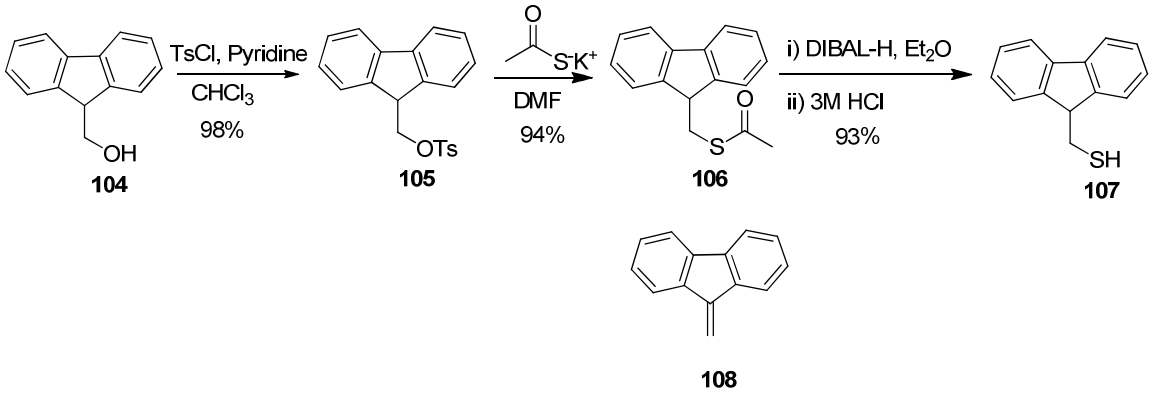
2.3 A Process for the Synthesis of Amino Thioacids

As demonstrated by Giralt *et al.*[171-172] the 9-fluorenylmethyl (Fm) group is an acid stable protecting group for the side chain thiol of cysteine as a cysteine thioether, and can be deprotected under standard Fmoc deprotection conditions leaving the side chain thiol of cysteine. Bearing this fact in mind, the synthesis of 9-fluorenylmethylthiol (FmSH) and the formation of 9-fluorenlylmethylthioester of *N*-Boc protected amino acids, was considered to have significant potential for the synthesis of amino thioacids in solution.

2.3.1 Synthesis of 9-Fluorenylmethylthiol (FmSH)

The synthesis of FmSH (**107**) began with the commercially available starting material, 9-fluorenylmethanol (**104**) (Scheme 42). First, **104** was converted to corresponding tosylate **105**,[173] followed by displacement with potassium thioacetate to provide compound **106**. However, attempted hydrolysis of thioacetate **106** with commonly used basic conditions, such as hydrazine,[174] methanolic ammonia,[175] lithium hydroxide[176] or even mild carbonate base[177] in methanol were unsuccessful, leading to exclusive formation of the eliminated product **108**. Subsequently, the low temperature reduction of **106** with DIBAL-

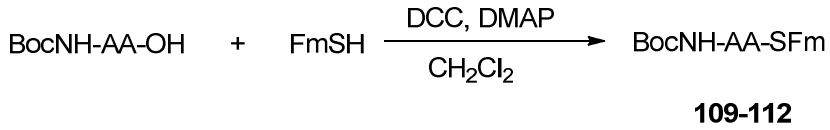
H[178] was found to achieve the desired deprotection and is the method of choice to afford FmSH (Scheme 42).

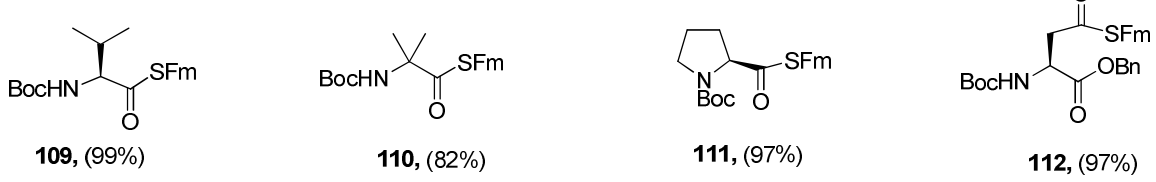


Scheme 42. Synthesis of FmSH.

2.3.2 Synthesis of 9-Fluorenylmethylthioesters of *N-tert*-Butoxycarbonyl-α-amino Acids

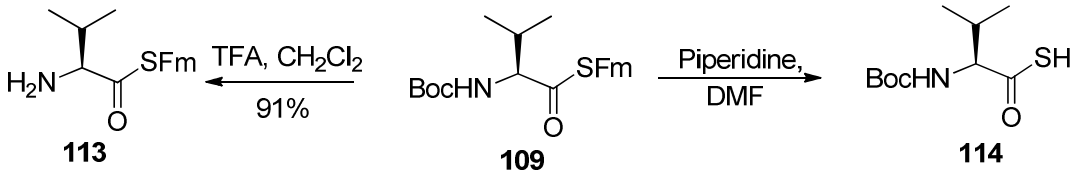
Several *N-tert*-butoxycarbonyl-α-amino acids were coupled with FmSH under standard carbodiimide conditions to give the thioesters in good yield (Scheme 43). The thioesters obtained by the coupling, were purified by column chromatography over silica and were found to be stable at room temperature.





Scheme 43. Synthesis of various Fm thioesters.

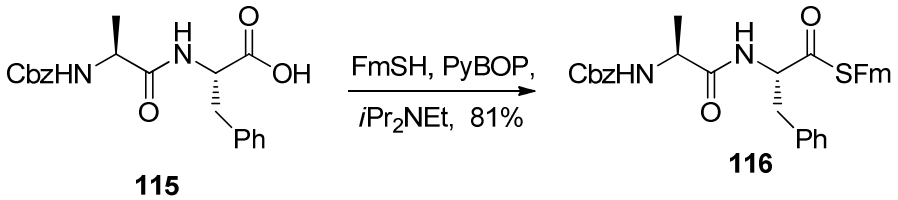
The Fm group is compatible with Boc deprotection conditions, but was selectively cleaved by piperidine in DMF to afford the *N*-Boc protected amino thioacid from the corresponding thioester in good yield. These thioacids were taken on to the coupling reaction without any purification (Scheme 44).



113 **109** **114**

Scheme 44. Deprotection of 9-fluorenylmethylthioester.

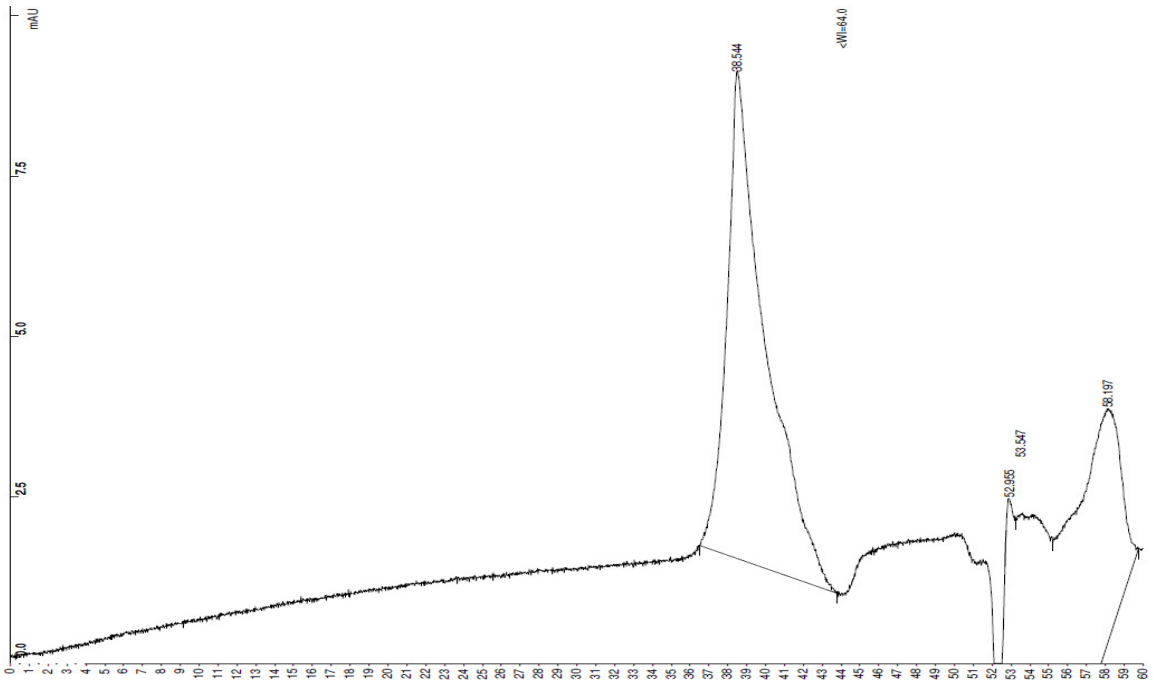
N-Benzyloxycarbonyl-L-alanyl-L-phenylalanine 9-fluorenylmethyl thioester (**116**) was synthesized following the PyBOP coupling method, recommended by Kajihara and coworkers[179] from the corresponding dipeptide **115,** to suppress any racemization of **115** (Scheme 45).



115 **116**

Scheme 45. Synthesis of a thioester under the Kajihara conditions.

The analytical RP-HPLC trace of thioester **116** is shown in Figure 5. Only a single peak with retention time of 38.54 min was seen in the trace with a molecular weight corresponding to that of the thioester, which argues strongly against the possibility of racemization of **115** during thioesterification.

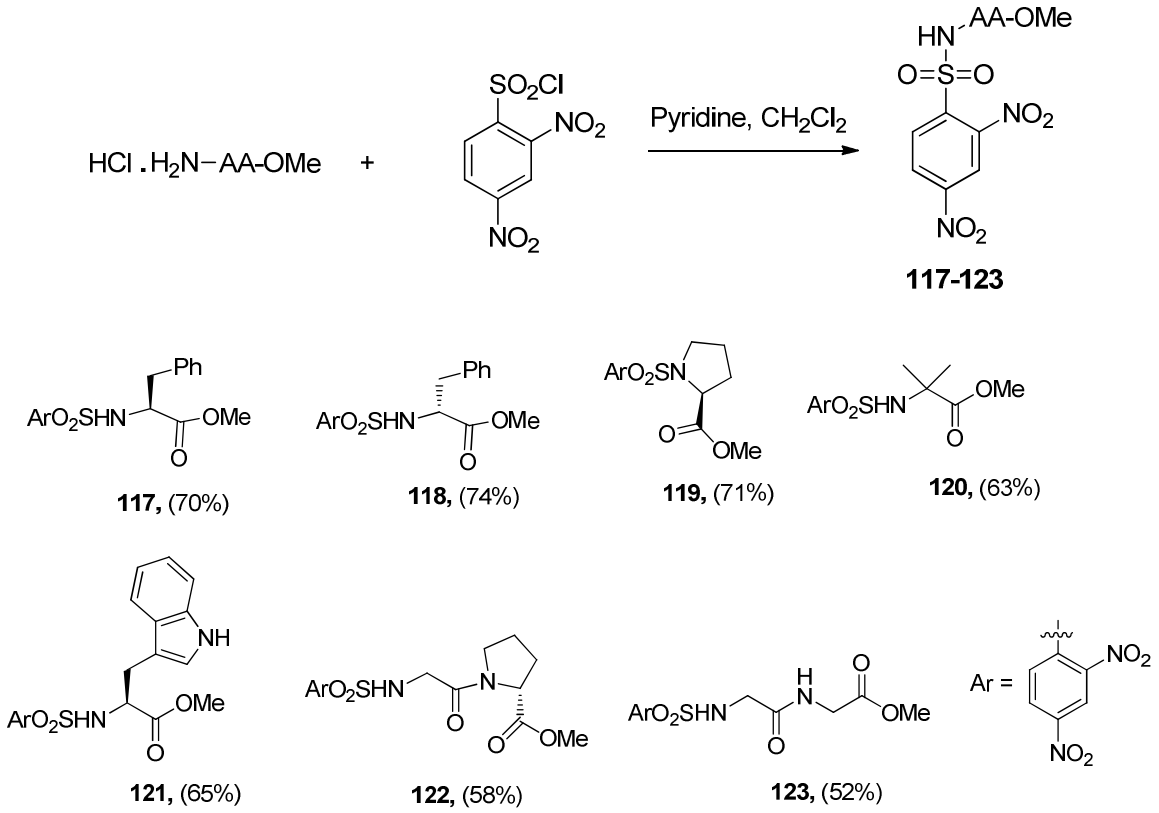


5 - 50% acetonitrile in water over 60 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 38.54 min.

Figure 5. RP-HPLC trace of thioester 116.

2.4 Synthesis of *N*-[(2,4-Dinitrobenzene)sulfonyl]-α-amino Acid Methyl Esters

Following the literature,[167] several *N*-[(2,4-dinitrobenzene)sulfonyl]-α-amino acid methyl esters were prepared from the reaction of the corresponding hydrochloride salts of α-amino acid methyl esters and 2,4-dinitrobenzenesulfonyl chloride in presence of pyridine (Scheme 46). *N*-[(2,4-Dinitrobenzene)sulfonyl]-glycyl-glycine methyl ester (**123**) was prepared from the corresponding water soluble dipeptide in H_2O/THF solvent system using a carbonate base.

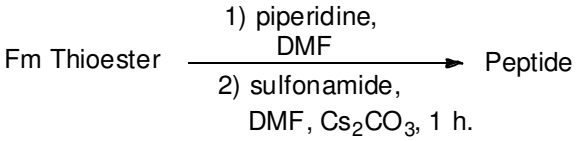


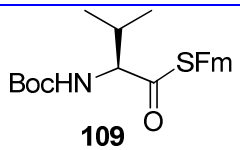
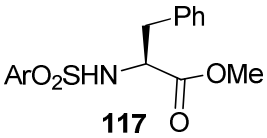
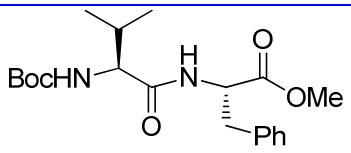
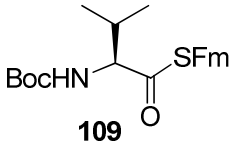
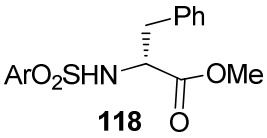
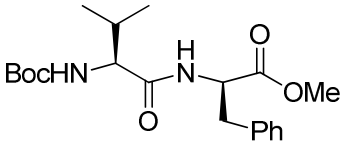
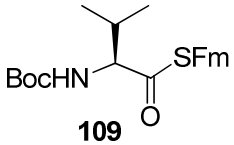
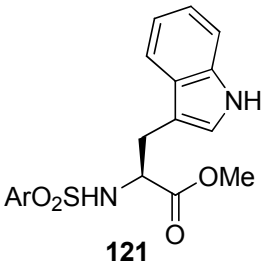
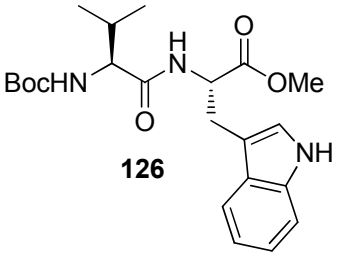
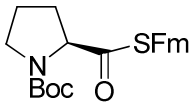
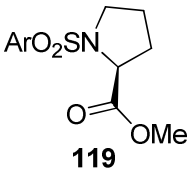
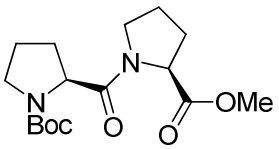
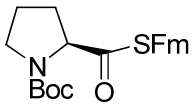
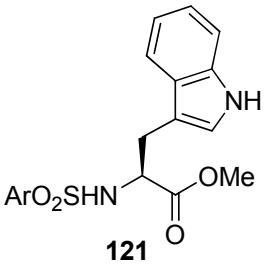
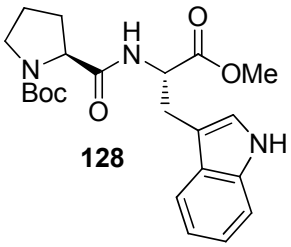
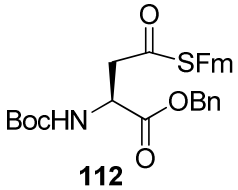
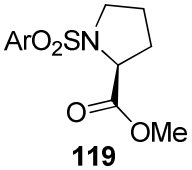
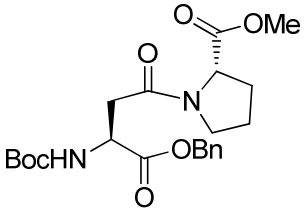
Scheme 46. Synthesis of sulfonamides.

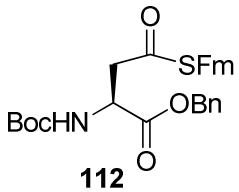
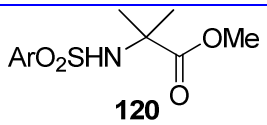
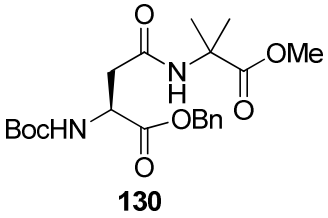
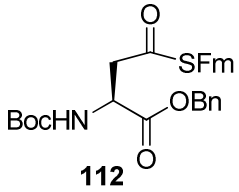
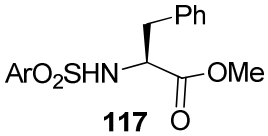
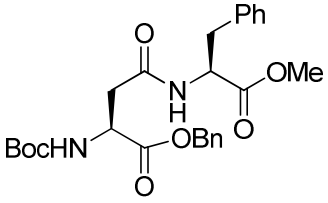
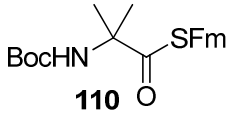
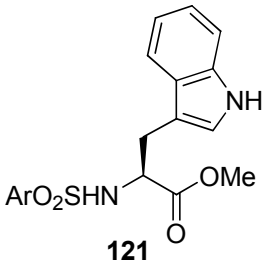
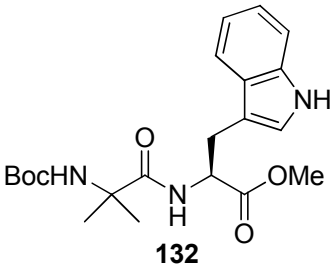
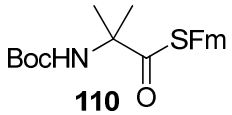
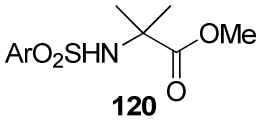
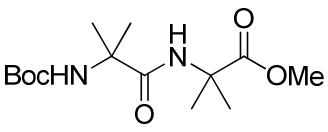
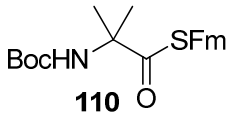
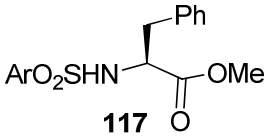
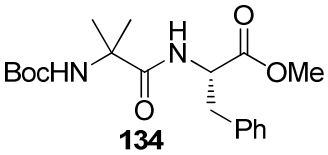
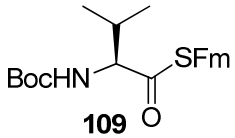
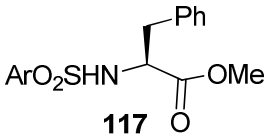
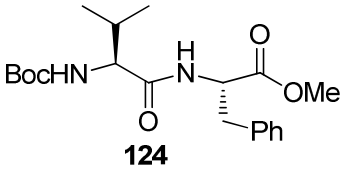
2.5 Coupling between Thioacids and Sulfonamides: Synthesis of Dipeptides

Treatment of the 9-fluorenylmethyl thioesters with piperidine in DMF at room temperature gave the corresponding thioacids, which were coupled to the sulfonamides in the presence of cesium carbonate, at room temperature, resulting in the formation of dipeptides in good yield (Table 1).

Table 1.Synthesis of Dipeptides from Thioesters and Sulfonamides.



Entry	Thioester	Sulfonamide	Peptide	Yield (%)
1	 **109**	 **117**	 **124**	81
2	 **109**	 **118**	 **125**	82
3	 **109**	 **121**	 **126**	82
4	 **111**	 **119**	 **127**	78
5	 **111**	 **121**	 **128**	77
6	 **112**	 **119**	 **129**	75

7	 **112**	 **120**	 **130**	79
8	 **112**	 **117**	 **131**	82
9	 **110**	 **121**	 **132**	80
10	 **110**	 **120**	 **133**	80
11	 **110**	 **117**	 **134**	82
[a,b]12	 **109**	 **117**	 **124**	51

[a] Reaction conducted in methanol as solvent. [b] 20% of **57** was recovered after the reaction.

All reactions in Table 1 were conducted with 1.2:1 ratios of the thioacid and the sulfonamide at ambient temperature. In particular, attention is drawn to

entries 1 and 2 of Table 1 in which diastereomeric products were obtained, enabling the possibility of racemization in the course of thioester deprotection or in the coupling process to be ruled out, by a qualitative comparison of the 500 MHz ^{1}H NMR spectra of both diastereomers as shown in Figure 6. A careful review of the proton NMR spectra of both isomers indicates, each spectrum corresponds to a single diasteromer. The characteristic chemical shifts of the α and β protons of the phenylalanine unit in the diastereomeric dipeptides **124** and **125**, which identify the two diastereomers, are furnished in Table 2 to facilitate comparison. The obtention of the **124** and **125** as single diastereomers also enables it to be stated with confidence that the Fm thioesters themselves were initially formed without racemization.

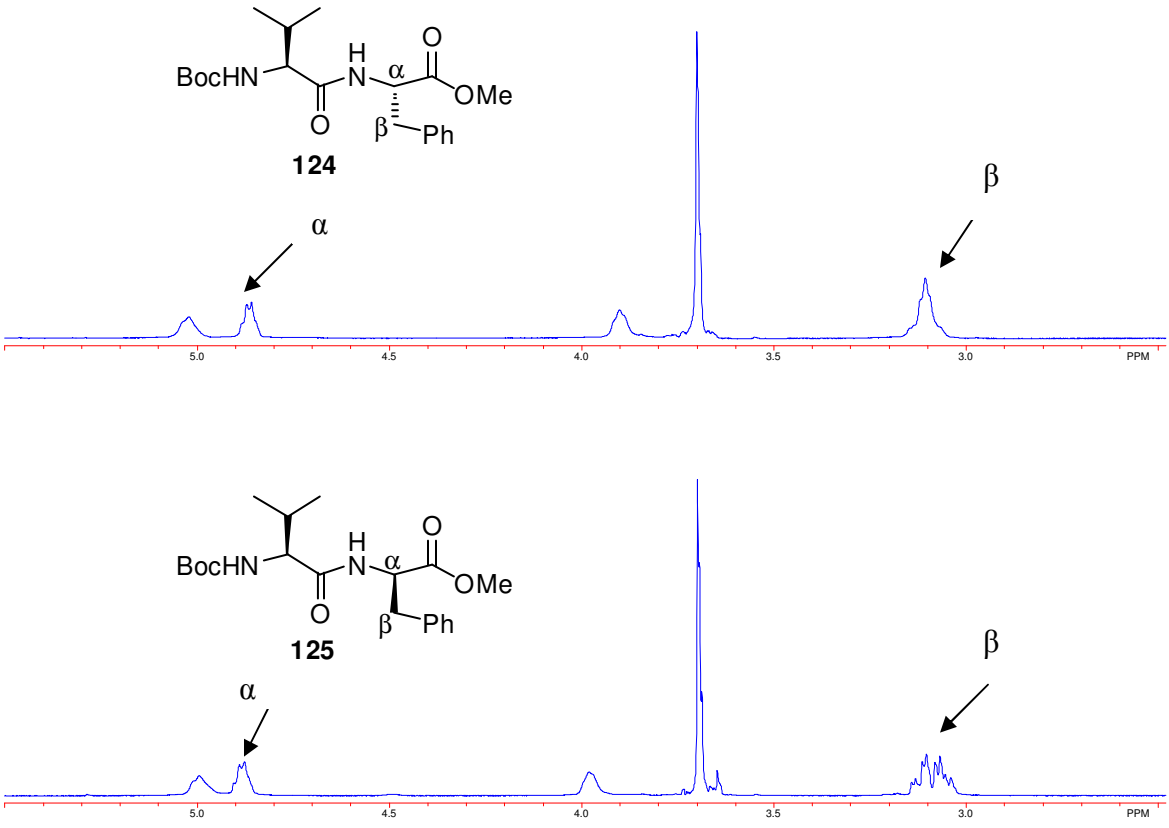


Figure 6. ^{1}H spectra of dipeptides 124 and 125 in $CDCl_3$.

Table 2. Selected ^{1}H Chemical Shifts (δ in ppm) and Coupling Constants (*J*) of Dipeptides 124 and 125.

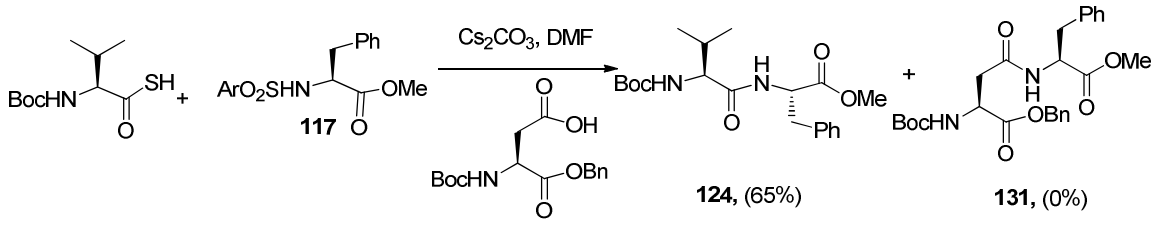
Dipeptide	δ of α-proton (multiplicity, *J*, proton counts)	δ of β- proton (multiplicity, *J*, proton counts)
124	4.87-4.86 (m, 1H)	3.12-3.06 (m, 2H)
125	4.91-4.87 (m, 1H)	3.14-3.10 (dd, *J* = 5.5, 13.7 Hz, 1H) 3.08-3.04 (dd, *J* = 5.5, 13.7 Hz, 1H)

The examples employing proline or aminoisobutyric acid derivatives (Table 1, entries 4-7 and 9-11) are particularly interesting. A successful synthesis of the corresponding dipeptides indicates that the protocol is equally applicable to coupling of hindered amino acids, whichever side of the nascent peptide bond they occupy.

In particular, comparison of entries 1 and 12 in Table 1 indicates that the coupling reaction is compatible with the presence of alcohols, and that methanol may even be employed as solvent. However, a slight loss of yield was recorded, which was due to incompletion of coupling between thioacids and sulfonamides in methanol. In a polar protic solvent like methanol, the decreased acidity of thioacids and the increased solvation of thiocarboxylates, results in a slower reaction.[180-181]

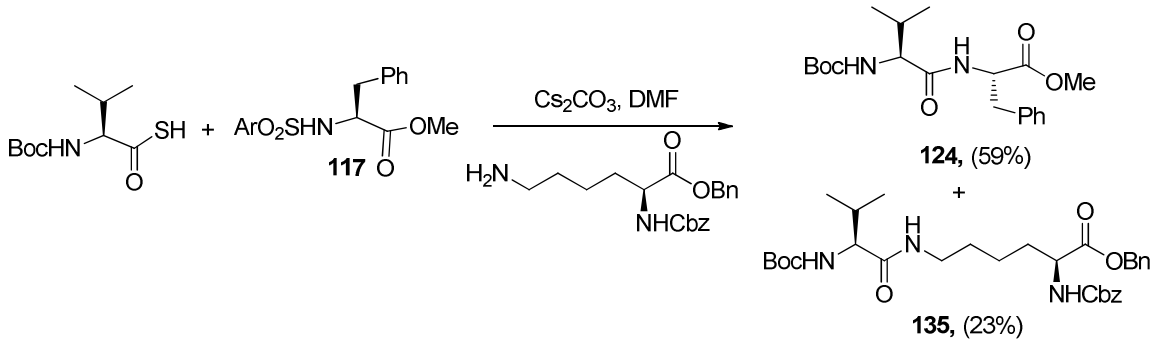
Accordingly, when a standard reaction of thioacid obtained from **109** and the sulfonamide **117** was conducted in the presence of Boc-L-Asp-α-OBn, a comparable yield of the desired dipeptide **124** was recorded but no coupling was observed to the spectator acid as shown in Scheme 47. The absence of side-

chain aspartic acid derivatized amide **131** in the coupling indicates the carboxylic acid compatibility of the reaction.



Scheme 47. Coupling of thioacid and sulfonamide in presence of a carboxylic acid.

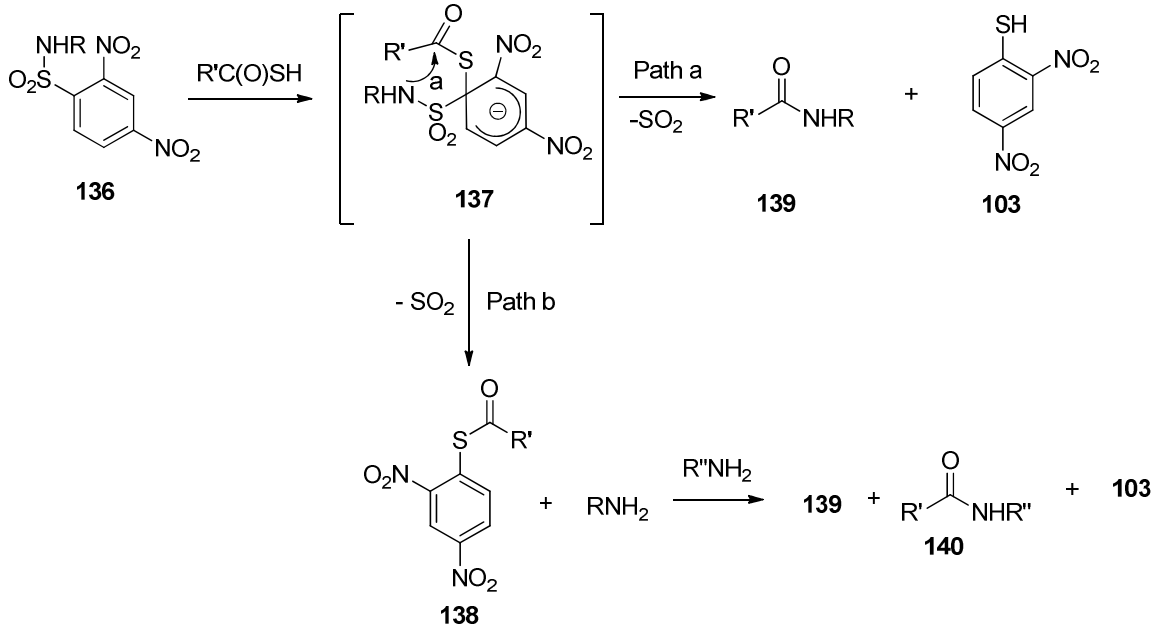
Interestingly, coupling of amino thioacids with the sulfonamide of tryptophan containing unprotected indole amine gave only the desired dipeptides in good yield (Table 1, entries 3, 5 and 9); no indole amine functionalized products were observed. However, the inclusion of *N*-α-Cbz-L-Lys-OBn in the reaction mixture resulted in some loss of yield accompanied by some amidation of the free side chain amine group (Scheme 48).



Scheme 48. Involvement of free amine in a thioacid and sulfonamide coupling.

The interference of unprotected alkyl amines in the peptide bond forming reactions is common to most such reactions. In this case it suggests that the reaction proceeds, at least in part, by decomposition of the intermediate

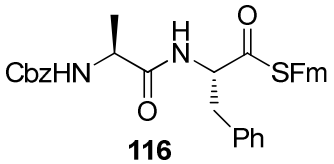
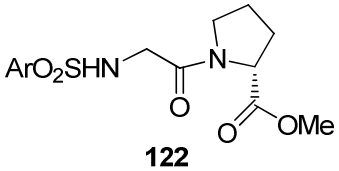
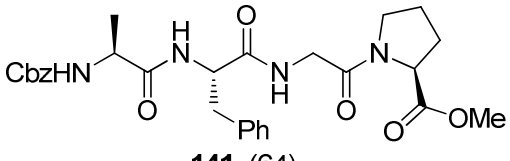
Meisenheimer complex **137** to a 2,4-dinitrophenyl thioester **138** that subsequently captures the nucleophilic amine in an intermolecular fashion (Scheme 49, path b).

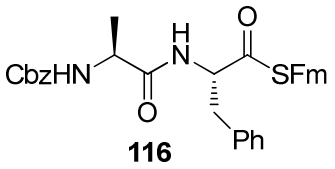


Scheme 49. Mechanism of Tomkinson reaction.

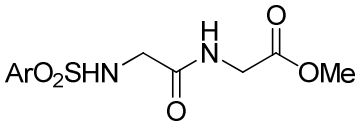
Finally, this methodology was also applied to the synthesis of model tetrapeptides (Table 3) by the segment coupling of two dipeptides with moderate isolated yield. The tetrapeptides obtained by the coupling, are sparingly soluble in common organic solvents like, chloroform, methanol or ethyl acetate, resulting in some loss of products during column chromatographic purification.

Table 3. Synthesis of Tetrapeptides from Thioesters and Sulfonamides.

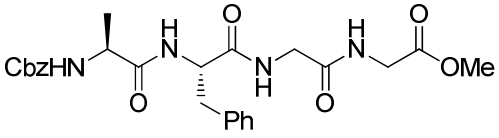
Thioester	Sulfonamide	Peptide (% Yield)
 **116**	 **122**	 **141**, (64)



116

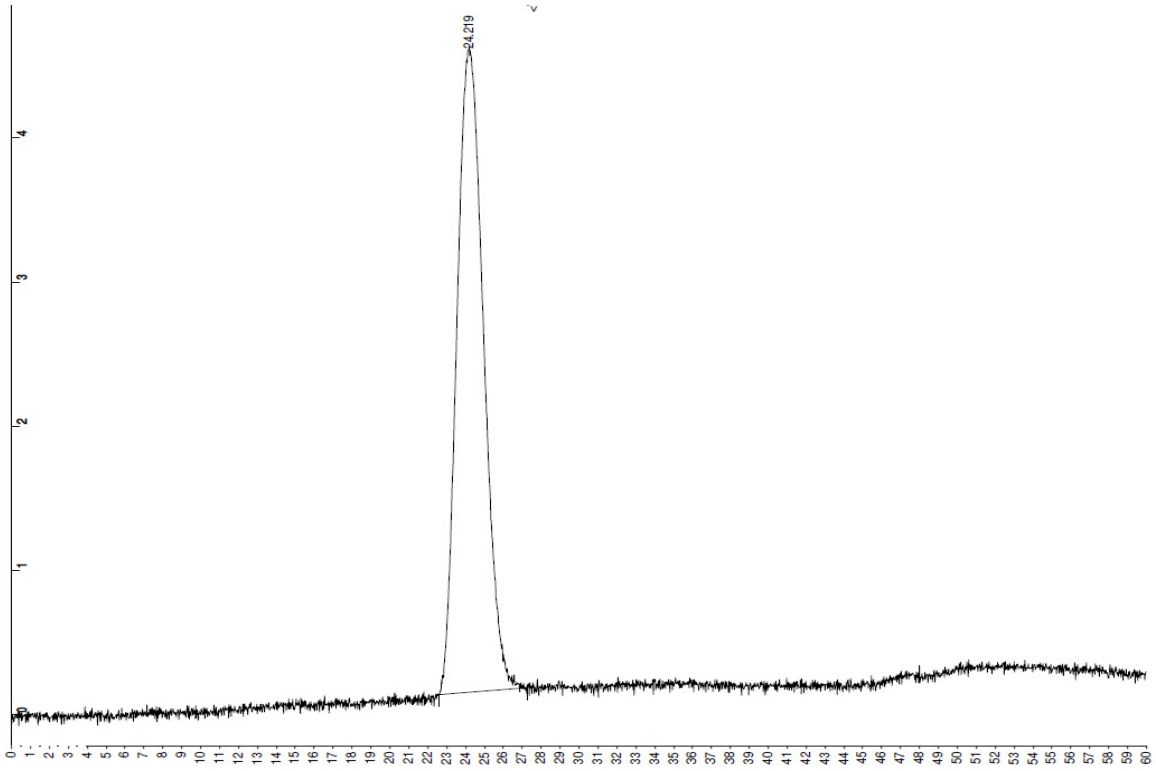


123



142, (62)

The analytical RP-HPLC trace of the tetrapeptide **142** is shown in Figure 7. In the HPLC trace, a single symmetrical retention peak at 24.22 min corresponding to the desired tetrapeptide was observed, which argues against the possibility of racemization of **116** during piperidine deprotection or segment coupling of two dipeptides.



0 - 50% acetonitrile in water over 60 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 24.22 min.

Figure 7. RP-HPLC trace of tetrapeptide 142.

2.6 Conclusions

The above results demonstrate that the methodology based on the reaction of thioacids with readily available 2,4-dinitrobenzenesulfonamides is suitable for the formation of peptide bonds. Unlike native chemical ligation and its variants, this methodology is not limited to the use of any particular amino acid, and importantly is epimerization free. The precursors are easy to prepare, and the coupling reaction takes place under mild conditions at room temperature and is compatible with typical amino acid protecting groups.

CHAPTER 3

SOLID-PHASE SYNTHESIS OF PEPTIDYL THIOACIDS EMPLOYING A 9-FLUORENYLMETHYLTHIOL-LINKED RESIN IN CONJUNCTION WITH Boc CHEMISTRY

3.1 Background and Significance

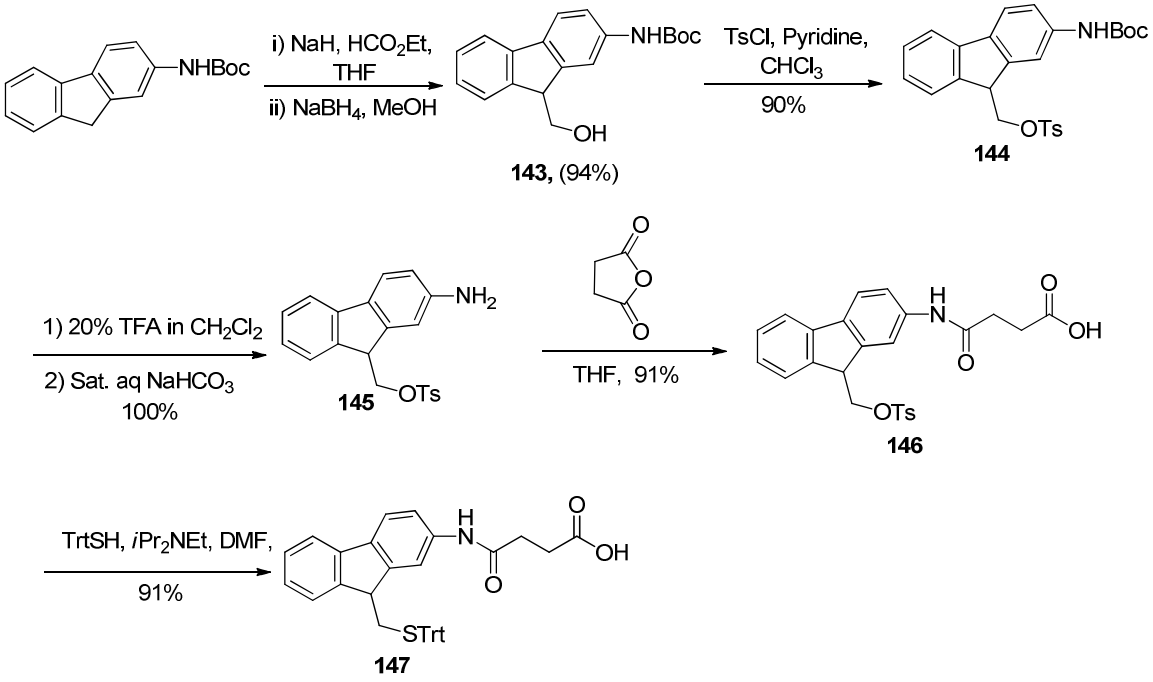
As discussed in Chapter One, over the years several useful and elegant strategies have been developed for the synthesis of C-terminal peptidyl thioacids and thioesters by SPPS. Despite these notable advances, the synthesis of peptidyl thioacids remains significantly more challenging than the synthesis of the corresponding peptidyl acids or amides. In this regard, the development of an appropriate linker and a general method for solid-phase peptidyl α-thioacids and thioesters synthesis was undertaken and forms the basis of this chapter.

In a new approach to the synthesis of thioacids, as presented in section **2.2.2**, the FmSH method was introduced to make 9-fluorenylmethyl thioesters, from which thioacids are liberated by simple treatment with piperidine, i.e., under the conditions usually employed for the cleavage of Fmoc groups in Fmoc-SPPS as shown in Scheme 44. These results prompted the design of a linker based on the 9-fluorenylmethyl thioester, *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**147**), which can be attached to an amino functional resin and a peptide sequence constructed on it.

3.2 Synthesis of *N*-[9-(Tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (147)

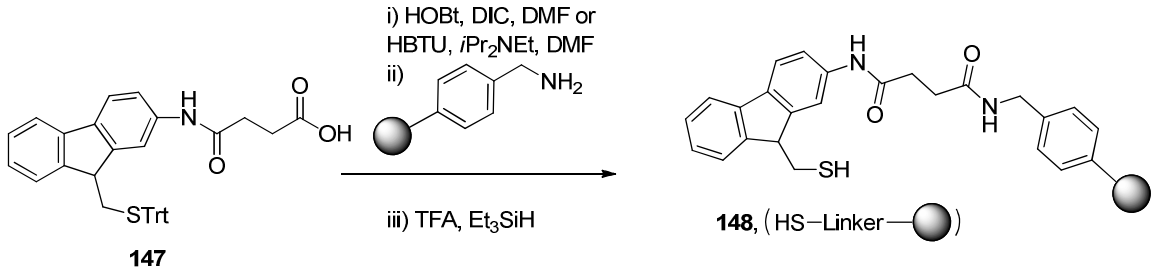
The mercapto functionalized linker, *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**147**), was synthesized starting from commercially available

9*H*-fluoren-2-amine as shown in Scheme 50. The synthesis began with the conversion of 9*H*-fluoren-2-amine to corresponding hydroxyl functional compound **143** following a known literature[182] procedure, which involved formylation of 9*H*-fluorenyl amine derivative by treatment with NaH and ethyl formate, followed by reduction of the resulting aldehyde. Tosylation of the hydroxyl functionality of compound **143** using tosyl chloride and pyridine provided the tosylate **144**, from which the amine **145** was liberated by treatment with trifluoroacetic acid. Then the succinamic acid **146** was obtained by nucleophilic ring opening of succinic anhydride by the free amine group of **145**. Finally, the nucleophilic displacement of tosylate group from compound **146** by tritylmercaptan in presence of Hunig's base led to the protected linker **147** in excellent yield (Scheme 50).



Scheme 50. Synthesis of *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid.

Treatment of linker **147** with DIC and HOBt in DMF gave an activated intermediate, which was allowed to react with 1% divinylbenzene cross linked aminomethylpolystyrene resin. After washing with DMF, the trityl group was removed by treatment with TFA to yield the resin-bound 9-fluorenylmethylthiol derivative **148** as shown in Scheme 51. The attachment of linker **147** to the aminomethylpolystyrene resin was also accomplished in a satisfactory manner with other commonly used activating reagents such as HBTU,[183-186] with the aid of Hunig's base.



Scheme 51. Preparation of a mercapto functionalized resin.

3.3 Amino Acid Side-Chain Functional Group Protection and Orthogonal Protection Strategies

More than half of the amino acids commonly encountered in proteins have side chains that contain reactive functional groups. In solid-phase synthesis it is necessary for all of these potentially reactive groups to be masked because of rather harsh conditions employed and the need to achieve the highest level of efficiency in all chemical reactions.

The concept of orthogonal protection was defined by Baranay and Merrifield in 1977 as "a set of completely independent classes of protection groups, such that each class can be removed in any order and in the presence of

all other classes".[187-188] Due to the multifunctional nature of amino acids, orthogonal protecting group manipulations are widely employed in peptide chemistry and the orthogonality of protecting groups is a key issue in the planning and experimental execution of any given peptide synthesis. For example, the most commonly used Fmoc-SPPS approach based on an orthogonal protecting group strategy, known as Fmoc/*t*-Butyl, involves the use of base-labile N^{α}-Fmoc group and acid-labile side chain protecting groups. In contrast, the most commonly used Boc-SPPS technique, i.e., the Boc/Benzyl strategy, uses the acid-labile N^{α}-Boc group for protection of the α-amino group, which is removed selectively by the treatment with TFA, and the benzyl type side chain protecting groups, with cleavage from the resin by treatment with hazardous HF.

As discussed earlier in the Chapter, the linker **147** for the synthesis of peptidyl thioacids on solid support is labile to base, which permits the synthesis employing Boc-SPPS strategy. In this context, the manipulations of protecting groups of amino acids side chain functionalities were considered. Accordingly, the protecting groups were selected based on their orthogonality to the Boc-SPPS and the groups can be deprotected during the final cleavage of peptidyl thioacids from the linker. For example, side-chain alcohols and amines were protected as Fmoc- carbonates and carbamates respectively, where as acids and thiols were protected as Fm- esters and thioethers respectively. In addition, a third orthogonal side-chain protecting strategy, i.e. Alloc/allyl was also employed with Boc chemistry. This protection strategy was extensively used in

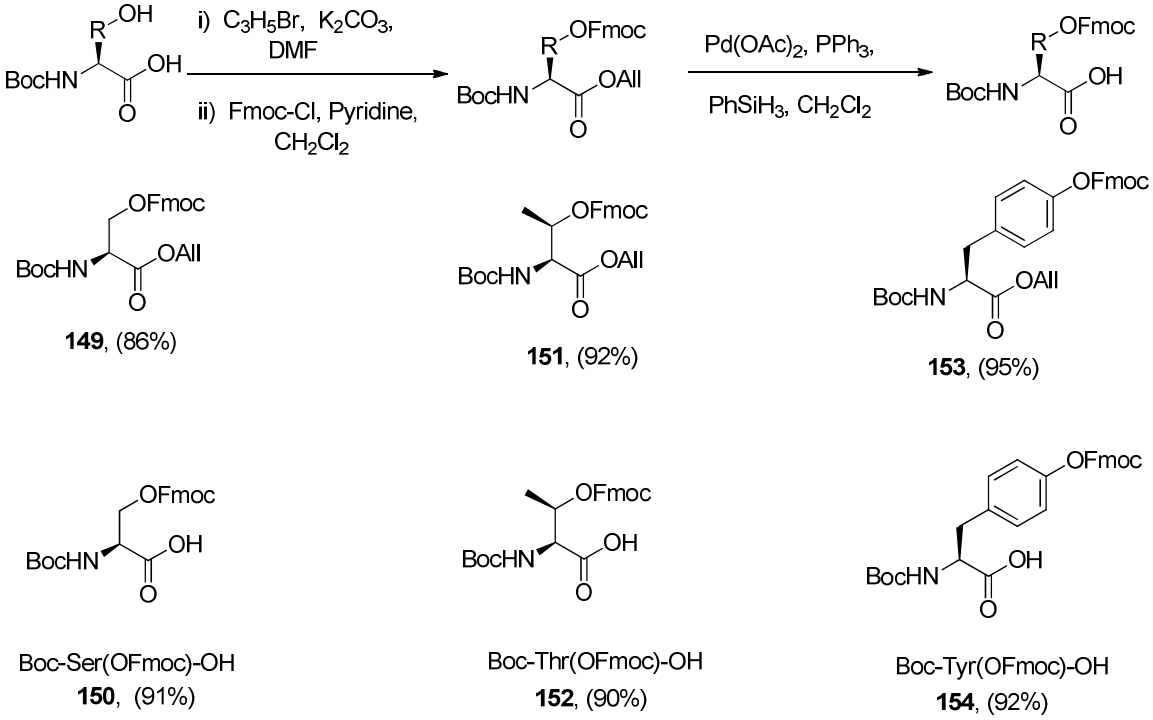
synthesis of cyclic peptides in conjunction with Fmoc/*t*-Butyl methodology,[189-192] and the protecting groups were selectively removed using a palladium(0) catalyst in presence of a nucleophilic scavenger.[190,193] In this regard, the preparation of a series of suitably side-chain protected amino acids was then undertaken.

3.3.1 Preparation of *O*-Fmoc Protected Serine, Threonine and Tyrosine Derivatives

Although the protected derivatives are the safest way to incorporate serine and threonine into the peptide sequence, these amino acids can also be used with the free hydroxyl functionality.[194-195] But unprotected hydroxyl functionalities of serine and threonine can undergo side reactions such as dehydration or *O*-acylation followed by *O* to *N* acyl migration.[196-197] However, protection is more necessary in case of serine and tyrosine in SPPS. In case of serine, the primary alcohol of which is more prone to acylation than the secondary alcohols of threonine, and the high nucleophilicity of unprotected tyrosine can lead to acylation of the phenol group with excess acylating agents used in SPPS. In addition, the electron-rich aromatic ring can undergo alkylation at the *ortho* position of the unprotected tyrosine aromatic ring. In this respect, the Fmoc group, which is orthogonal to Boc chemistry, was selected as the choice of protecting group for hydroxyl functionality.

Thus, N^{α}-Boc-L-serine and the analogous L-threonine and L-tyrosine derivatives were converted to their allyl esters by the reaction of allyl bromide in presence of potassium carbonate and then to the 9-fluorenylmethyl carbonates with Fmoc chloride and pyridine. Subsequently, the removal of the allyl groups

was carried out with a combination of palladium(II) acetate, triphenylphosphine and phenylsilane (Scheme 52).



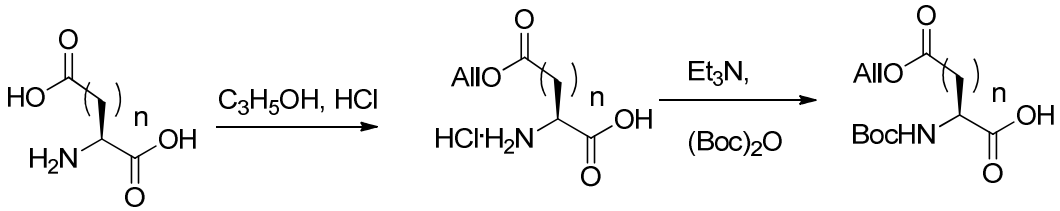
Scheme 52. Preparation of *O*-Fmoc protected hydroxyl amino acids.

3.3.2 Preparation of Aspartic and Glutamic Acid Derivatives

The side chain carboxylic groups of aspartic acid and glutamic acid must be protected in order to prevent their activation during peptide synthesis, which would lead to undesired branched peptides. Furthermore, in the case of aspartic acid, the protecting groups used must also prevent or at least minimize the formation of aspartimide.[198] However, the protection of the side-chain carboxylic acid can be achieved using several methods. The simplest one is the acid catalyzed esterification of the free amino acid, where protonation of the α-amino group makes the α-carboxylic acid less reactive, thereby allowing the selective

protection of the side-chain.[199-200] Copper(II) and boron chelates are also applied for the selective protection of the side-chain of aspartic and glutamic acids.[201-203]

Accordingly, aspartic and glutamic acids were esterified by treatment with HCl in allyl alcohol to give the corresponding allyl ester hydrochlorides. The amino groups were protected as their N^{α}-Boc derivatives by treatment with $(Boc)_2O$ and triethylamine to give N^{α}-Boc-L-aspartic acid β-allyl ester (**157**) and N^{α}-Boc-L-glutamic acid γ-allyl ester (**158**) (Scheme 53).[204]

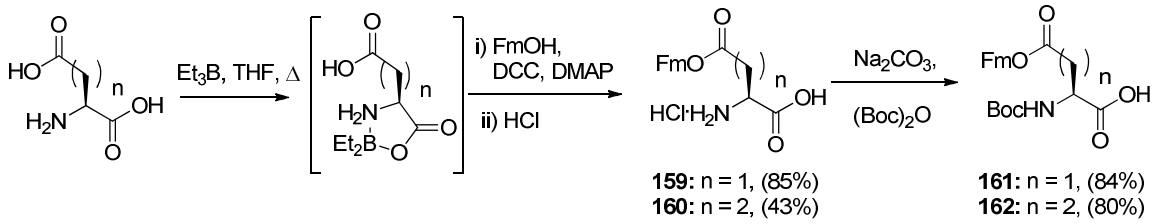


155: n = 1, (85%)
156: n = 2, (80%)

157: n = 1, (80%)
158: n = 2, (80%)

Scheme 53. Preparation of mono allyl esters of aspartic and glutamic acid.

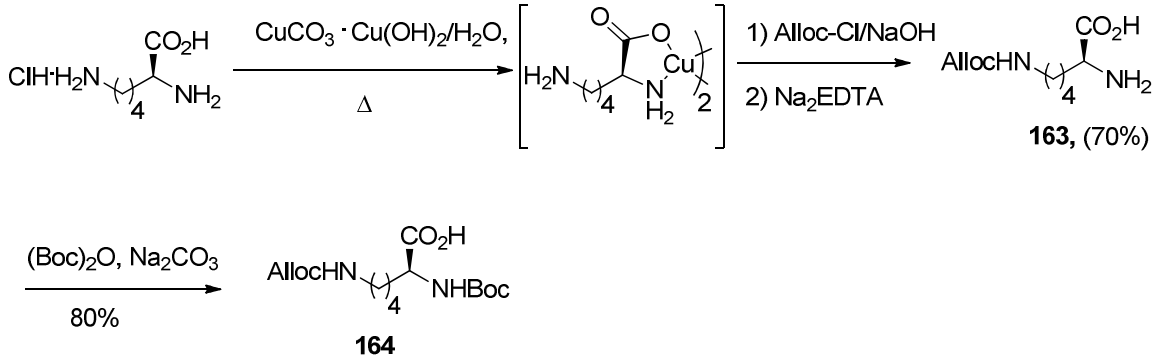
Following a literature protocol,[203] treatment of powdered L-aspartic and L-glutamic acids with triethylborane in THF at reflux afforded the oxazaborolidinone derivatives. Then the side chain carboxylic acid groups were esterified by the activation with DCC and DMAP in the presence of 9-fluorenylmethanol, and finally removal of the chelates with gaseous hydrogen chloride gave the mono esters **159** and **160**. These HCl salts were then converted to the N^{α}-Boc derivatives by the treatment with $(Boc)_2O$ in aqueous sodium carbonate (Scheme 54).[203]



Scheme 54. Preparation of mono 9-fluorenylmethyl esters of aspartic and glutamic acid.

3.3.3 Preparation of an Alloc Protected Lysine Derivative

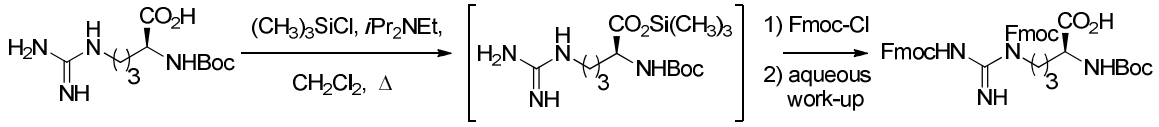
The protection of the side chain amine group of lysine is essential in peptide synthesis to prevent its acylation, which would lead to the formation of undesired branched peptides. Like aspartic and glutamic acids, selective protection of lysine can be achieved by the formation of copper(II) or boron complexes between the α-amino and α-carboxylate groups.[203,205-206] Accordingly, refluxing L-lysine hydrochloride with basic copper carbonate afforded the copper(II) complex, which was reacted with Alloc chloride before decomplexation with ethylenediamine tetraacetic acid disodium salt to give N^{ε}-Alloc-L-lysine (**163**). Reaction of **163** with $(Boc)_2O$ in aqueous sodium carbonate gave N^{α}-Boc-N^{ε}-Alloc-L-lysine (**164**) (Scheme 55).[207-208]



Scheme 55. Preparation of an *N*$^{\varepsilon}$-Alloc protected lysine derivative.

3.3.4 Preparation of a Guanidino Protected Arginine Derivative

Owing to the nucleophilicity of the guanidino group, protection of the guanidino group of arginine is required to prevent deguanidination[209] and δ-lactam formation.[210] Therefore in principle, protection of all the nitrogens of the guanidino group is required to fully mask its nucleophilicity. However, mono- and diprotection are easier to achieve and are usually sufficient to minimize side reactions if bulky and electron withdrawing protecting groups are used. In this regard, a guanidine protected arginine derivative **166** was synthesized following a literature protocol (Scheme 56).[211] The synthesis began with the generation of arginine silyl ester by the treatment of commercially available *N*$^{\alpha}$-Boc-L-arginine (**165**) and chlorotrimethylsilane in presence of Hunig's base, followed by installation of the Fmoc group. Finally, hydrolysis of the silyl ester by a simple aqueous work-up afforded the desire arginine derivative **166** (Scheme 56).[211]



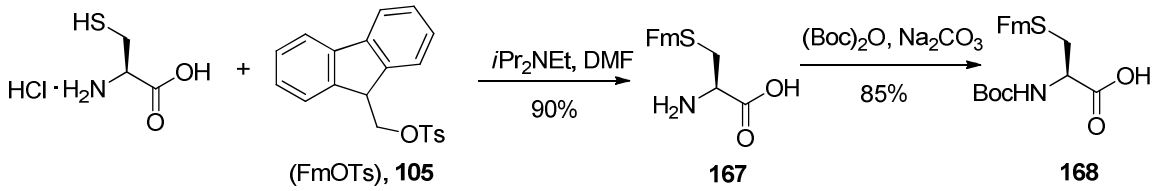
165 **166,** (70%)

Scheme 56. Preparation of a guanidine protected arginine.

3.3.5 Preparation of *S*-Fm Protected Cysteine Derivative

Protection of the side chain of cysteine is mandatory in peptide synthesis to avoid acylation, alkylation, or aerial oxidation to the disulfide of the highly nucleophilic thiol. Cysteine thiol protection is usually carried out on free cysteine itself. Therefore, treatment of cysteine with 9-fluorenylmethyl tosylate (**105**) in

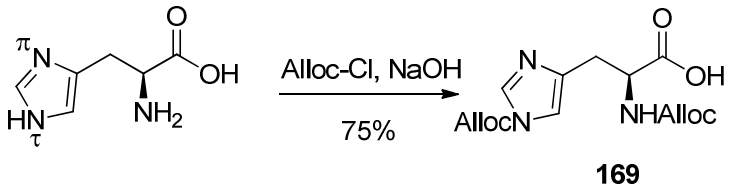
presence of Hunig's base led to the thiol protected cysteine **167**. Subsequently, protection of α-amino function of **167** with $(Boc)_2O$ in aqueous sodium carbonate gave N^{α}-Boc derivative **168** (Scheme 57).[203]



Scheme 57. Preparation of a 9-fluorenylmethyl thioether of cysteine.

3.3.6 Preparation of Alloc Protected Histidine

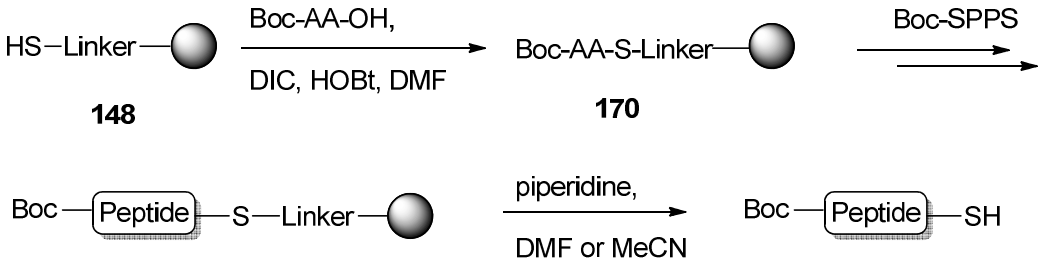
The imidazole ring of histidine has two nucleophilic points; the *π*- and *τ*-nitrogens.[212] Unprotected histidine is highly prone to racemization during the coupling and acylation during peptide synthesis.[213-214] The basic and nucleophilic *π*-nitrogen is involved in racemization and can be masked in two ways: (i) direct protection and (ii) *τ*-nitrogen protection with bulky or electron-withdrawing protecting groups, which reduce the basicity of the *π*-nitrogen. In this respect, both α-amino and *τ*-nitrogen were protected with the Alloc group, as it was intended that this Alloc protected histidine residue would be used at the N-terminus of a target peptidyl thioacid. Accordingly, the Alloc protected histidine derivative **169** was synthesized by the treatment of histidine with Alloc chloride in ice-cold aqueous sodium hydroxide (Scheme 58).[193]



Scheme 58. Preparation of an Alloc protected histidine.

3.4 Solid-Phase Synthesis of Peptidyl Thioacids on the Mercapto Functionalized Resin 148

With all building blocks in hand attention was turned to manual solid-phase synthesis of peptidyl thioacids on the mercapto functionalized resin **148** (Scheme 59). Accordingly, the first N^{α}-Boc amino acid was activated with DIC and HOBt in DMF and the pre-activated amino acid was then allowed to react with resin **148** to yield the resin bound thioester derivative **170**. Subsequently, the desired peptide chain was elongated on the resin **170** using standard Boc techniques,[186,215] which consist of the following steps in each cycle: (i) removal of the N^{α}-Boc protecting group of the last coupled amino acid by treatment with TFA; (ii) neutralization of the $CF_3CO_2^- \cdot {}^+NH_3$-peptide-resin salt with Hunig's base; and, (iii) coupling of the next pre-activated appropriate side-chain protected N^{α}-Boc amino acid. Between each of these steps a thorough flow wash with DMF was carried out to remove excess reagents, and few of peptide-resin beads were removed to monitor the individual reaction steps by a qualitative Kaiser ninhydrin color test.[216] In this test, a dark blue color corresponds to the free amine group before the coupling and changes to a light yellow after the coupling step. In addition, after each coupling step, formation of the desired peptidyl thioacid was confirmed by cleavage of a small amount (~ 5 mg) of resin using a solution of piperidine in DMF, followed by examination by ESI-TOF mass spectrometry. Finally after completion of the on-resin procedure, the resin-bound peptide was treated with a solution of piperidine in DMF or acetonitrile to liberate the desired peptidyl thioacids as shown in Scheme 59.



Scheme 59. Solid-phase synthesis of peptidyl thioacids.

A number of peptidyl thioacids were synthesized in this manner as set out in Table 4 (Entries 1-5). As with the preparation of the resin-bound thiol **148** (Scheme 51), this methodology is adaptable to the use of other coupling reagents as evidenced by the application of a combination of HBTU and Hunig's base activation of the amino acids (Table 4, entry 6).

The thioacid **171** is a simple model tetrapeptidyl thioacid (Table 4, entry 1), the peptidyl thioacids **172**, **173**, **174** and **176** are all natural sequences (Table 4, entries 2-5). The sequences of thioacids **172** and **173** (Table 4, entries 2 and 3) were selected from the Glucagon-like peptide-1 (GLP-1)[217] and represent segments 7–16 and 17–26 respectively. Peptide **174** (Table 4, entry 4) represents the 94–101 segment of Human Secretory Phospholipase A_2 (hsPLAA_2),[218] and peptide **176** (Table 4, entry 5) is the 65-84 unit of Human Parathyroid Hormone (hPTH).[219] Whatever their origins or activities, the sequences were selected based-on their compositions with a wide variety of amino acid residues in different positions of the sequences. The successful synthesis of all sequences indicates that the methodology is not limited to any particular amino acid residue of the sequence, during coupling or in final cleavage as shown in Table 4 (Entries 1-5).

Table 4. Solid-Phase Synthesis of Peptidyl Thioacids

Entry	Resin-Bound Peptide	Peptidyl Thioacid (%Yield)
1	Boc-Met-Ala-Val-Ala-S-linker-	Boc-Met-Ala-Val-Ala-SH **171**, (95)
2	Alloc-His(Alloc)-Ala-Glu(OAll)-Gly-Thr(OFmoc)-Phe-Thr(OFmoc)-Ser(OFmoc)-Asp(OAll)-Val-S-linker-	Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH **172,** (80)
3	Boc-Ser(OFmoc)-Ser(OFmoc)-Tyr(OFmoc)-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-S-linker-	Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH **173**, (78)
4	Boc-Ala-Ala-Thr(OFmoc)-Cys(Fm)-Phe-Ala-Arg(Fmoc)2-Asn-S-Linker-	Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH **174**, (57) + Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH S 2 **175,** (15)
5	Boc-Lys(Fmoc)-Ser(OFmoc)-Leu-Gly-Glu(OFm)-Ala-Asp(OFm)-Lys(Fmoc)-Ala-Asp(OFm)-Val-Asp(OFm)-Val[8]-Leu-Thr(OFmoc)-Lys(Fmoc)-Ala-Lys(Fmoc)-Ser(OFmoc)-Gln-S-Linker-	Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val[8]-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH **176,** (55)
6[a]	Boc-Met-Ala-Val-Ala-S-linker-	Boc-Met-Ala-Val-Ala-SH **171**, (88)

[a] This reaction sequence employed HBTU and Hunig's base for the coupling reactions in place of DIC/HOBt.

The peptidyl thioacids obtained by this methodology, were typically achieved with a high degree of purity as determined by either NMR or ESI-TOF and HPLC methods. For example, the 500 MHz ^{1}H NMR spectrum of the crude tetrapeptidyl thioacid **171** as obtained on simple release from the resin, followed by aqueous HCl work-up, extraction with ethyl acetate and drying is presented in Figure 8. A careful review of the proton NMR spectrum indicates, the spectrum corresponds to a single peptidyl thioacid **171**, which required no further purification. Therefore, with the exception of thioacid **171** (Table 4, entry 1), yields of the other thioacids (Table 4, entries 2-5) were for compounds isolated

and purified by RP-HPLC and were based on the substitution level of the aminomethyl polystyrene resin. The thioacids obtained in this manner were unstable and were found to decompose even at 0 ℃ after few weeks.

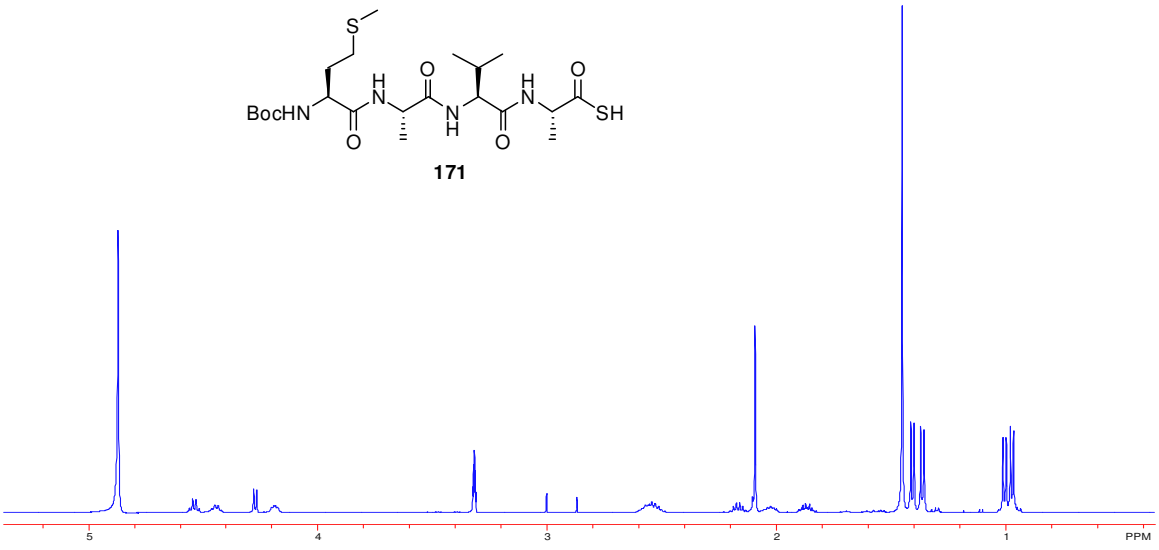
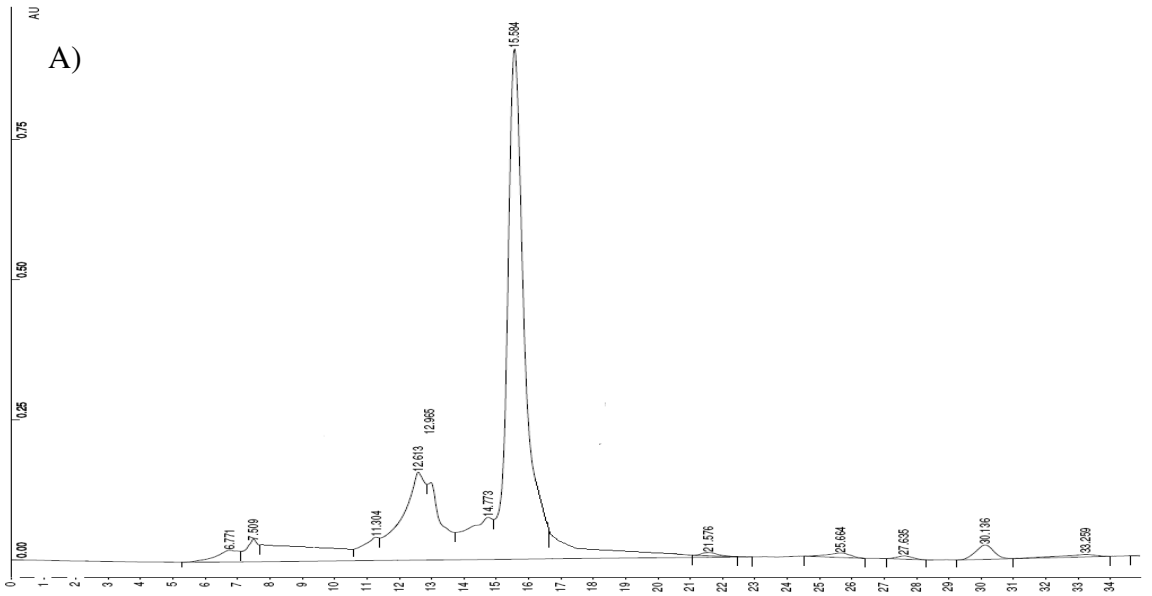


Figure 8. ^{1}H spectrum of thioacid 171 in CD_3OD.

In a similar vein the crude analytical RP-HPLC trace and ESI-TOF mass spectrum of the icosamer peptidyl thioacid **176**, immediately after release from the resin and prior to purification by HPLC are presented in Figure 9. Only a single peak with retention time of 15.58 min is seen in this trace with a molecular weight corresponding to that of the thioacid **176**. Accordingly, m/z = 2220 in the mass spectrum is corresponds to the desired thioacid **176** and a careful review of the spectrum indicates, the absence of any other partially formed peptidyl thioacids or incomplete thioacids of the same sequence. These results also illustrate the high degree of purity of peptidyl thioacids obtained by this method.



0 - 50% acetonitrile in water over 35 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 15.58 min.

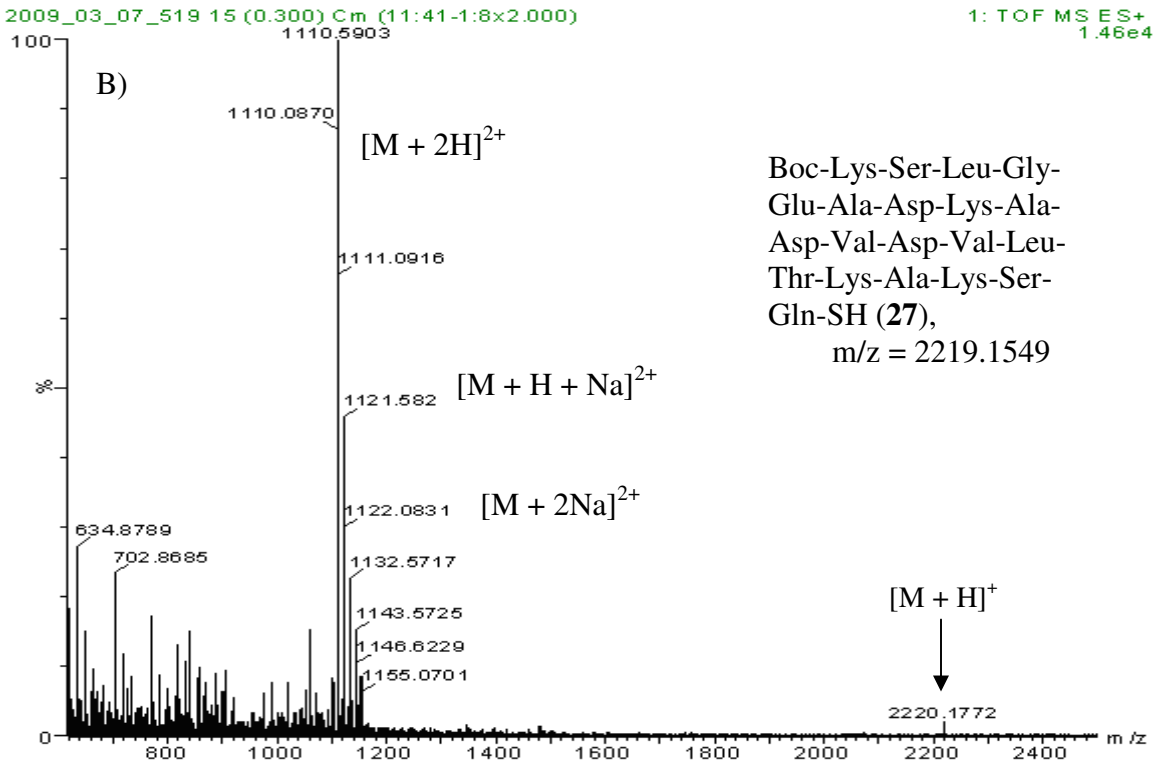
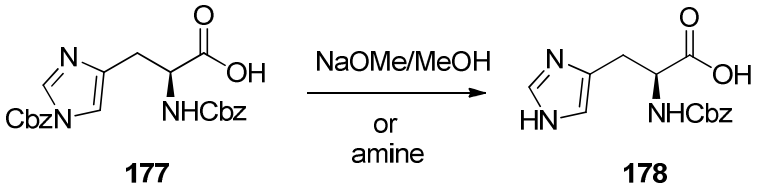


Figure 9. A) RP-HPLC trace of thioacid 176; B) ESI-TOF mass spectrum of thioacid 176.

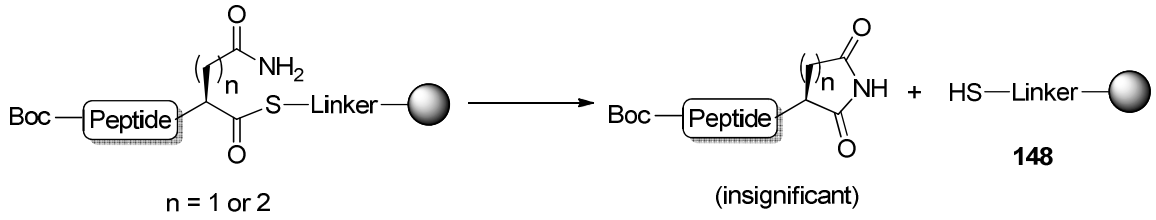
The Alloc group was cleaved from the side chain of the histidine residue in the course of the treatment of corresponding resin-bound peptide with piperidine to obtain the unprotected histidine side chain peptidyl thioacid **172** (Table 4, entry 2). The similar deprotection of histidine side chain is known in literature,[220] where N^{α},N^{π}-di-Cbz-L-histidine (**177**) is readily decarbobenzoxylated by nucleophilic reagents such as sodium methoxide and amines, yielding N^{α}-Cbz-L-histidine (**178**) (Scheme 60).[220] The better leaving group ability of imidazole favors the nucleophilic deprotection of imidazole carbamate group.



177 **178**

Scheme 60. Decarbobenzoxylation from histidine imidazole side chain.

Particular attention is drawn to entries 4 and 5 of Table 4, where the C-terminal amino acid is asparagine and glutamine respectively. The amino acid building blocks for these residues were employed with trityl protecting group for the side chain amide functionality. The acid labile trityl group was removed during first TFA deprotection of N^{α}-Boc and the subsequent peptide chain was elongated with the free side chain amide functionality of asparagine or glutamine. It is noteworthy that the possibility of cyclization of the free side chain amides of asparagine and glutamine onto the resin-bound thioester with premature release of peptidyl imide did not occur to any significant extent during N^{α}-Boc deprotection or in coupling steps (Scheme 61).



Scheme 61. Possibility of formation of peptidyl imide.

The insignificant formation of peptidyl imides is reasoned simply from the calculation of yields of the isolated thioacids. For example, the combined yield of octamer thioacid **174** with the corresponding disulfide **175** is 72%, and is 55% for the icosamer thioacid **176,** which requires average minimum coupling yields of ~96% and ~97% respectively, for each coupling and N^{α}-Boc deprotection cycle.

Attention is drawn to entry 4 of Table 4 in which the disulfide thioacid **175** of the corresponding oxidative dimerization of thioacid **174** was isolated after global deprotection of resin-bound peptide, followed by HPLC purification. This oxidative dimerization is even more rapid, when the deprotection is carried out in alkaline medium.[198,221-222]

The attempted synthesis of icosamer peptidyl thioacid **176** following the standard protocol was unsuccessful (Table 4, entry 5). The problem was encountered during the coupling of 9th amino acid in the sequence, which led incomplete coupling of the corresponding activated amino acid residue. Further efforts of the particular coupling were unproductive, which presumably is due to formation of secondary structure by association of the growing peptide chain, known as aggregation.[223] This association leads to the incomplete solvation of the peptide-resin complex, and reduced reagent penetration, resulting in failure of either the acylation or deprotection or both. The tendency of aggregation

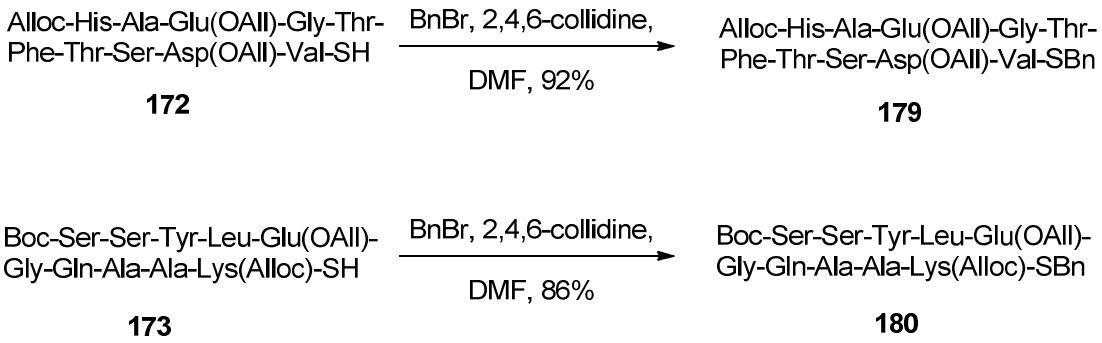
depends on the nature of the peptide and side chain protecting groups.[224] However, numerous modifications have been developed to avoid the aggregation of peptide chains on SPPS. Accordingly, several new polymer supports have been introduced to improve the solvation during the synthesis of peptides.[225-227] Alternatively, improved solvation has been described upon the addition of solvents like, NMP,[228] TFE,[229] hexafluoroisopropanol,[230] and DMSO.[231] In this context, in order to avoid possibility of aggregation during the synthesis of peptidyl thioacid **176**, NMP was employed as solvent from the 9th amino acid of the sequence and the synthesis was completed without finding any other difficulties. This can be explained by the presence of polar and nonpolar functionalities of NMP solvent, which can interact simultaneously with polar amide backbone and nonpolar side chain of the resin-bound peptide, thus preventing aggregation by better solvation.

Finally, the strategy of manipulation of protecting groups of side chain amines and hydroxyls functionalities to Fmoc- carbamates and carbonates, together with the protection of side chain carboxylates and thiols to Fm- esters and thioethers ensures the clean synthesis. In addition, the protecting groups were deprotected during the final cleavage of the resin-bound peptide, which leads to the corresponding fully unprotected peptidyl thioacid (Table 4, entries 4 and 5). Nevertheless, the methodology is also applicable for the synthesis of peptidyl thioacids, in which the retention of side chain protection is required on cleavage from the resin. Accordingly, the side chains protected peptidyl thioacids were conveniently accomplished through the use of building blocks whose side

chain carboxylates and amines were covered with allyl esters and Alloc carbamates respectively (Table 4, entries 2 and 3).

3.5 Application of Peptidyl Thioacids to the Synthesis of Thioesters and Sulfonamide

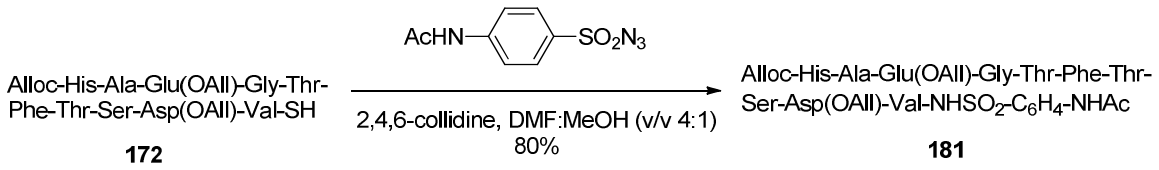
As discussed in the Chapter One, the synthesis of peptidyl thioacids and thioesters are closely associated with each other, but the latter have found more applications in the synthesis of peptides through chemical ligation. Owing to unique reactivity of thioacids as discussed in Chapter One, unprotected peptidyl thioesters could easily be prepared from the corresponding thioacids by simple nucleophilic substitution in an aqueous buffer solution.[232] In contrast, the unprotected side chains hydroxyl functionalities peptidyl thioacids **172** and **173** were converted to the corresponding *S*-benzyl thioesters by simple alkylation with benzyl bromide and 2,4,6-collidine in an organic solvent (Scheme 62). The thioesters obtained by this method were purified by RP-HPLC and were found to decompose even at 0 ℃.



Scheme 62. Synthesis of peptidyl thioesters.

Finally as a discussed in the introduction (section **1.1.4**), thioacids were reacted with organic azides to form amide bonds. Accordingly, peptidyl thioacid

172 was subjected to reaction with an electron deficient sulfonyl azide in presence of 2,4,6-collidine to give corresponding peptidyl sulfonamide **181** (Scheme 63). The acyl sulfonamide **181** was purified by RP-HPLC and was found to be stable at 0 ℃ for few weeks.



Scheme 63. Formation of an acyl sulfonamide.

3.6 Conclusions

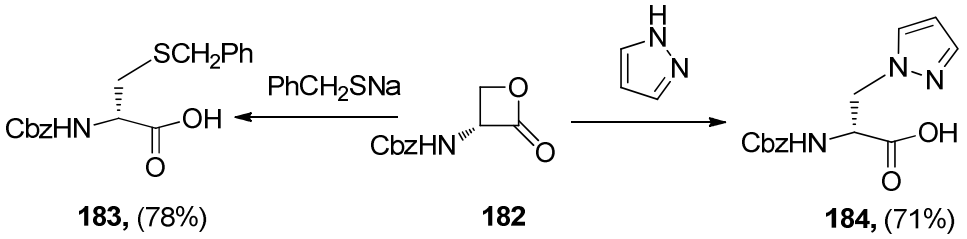
The above results demonstrate that this novel approach for the solid-phase synthesis of peptidyl thioacids on a mercapto functionalized 9-fluorenylmethyl linker in conjunction with standard Boc chemistry is a straightforward and efficient process. By way of protecting group manipulation of amino acids side chain functionalities, this chemistry provides a very convenient and mild means to access protected or unprotected peptidyl thioacids. Additionally, this new approach eliminates the use of HF, which is a very common deprotecting reagent in final cleavage from solid support and side chain functional protecting groups of the peptide in most solid-phase Boc chemistry.

CHAPTER 4

S_N2-TYPE NUCLEOPHILIC RING OPENING OF β-THIOLACTONES AS A SOURCE OF THIOACIDS: APPLICATION TO THE FORMATION OF AMIDE BONDS

4.1 Background and Significance

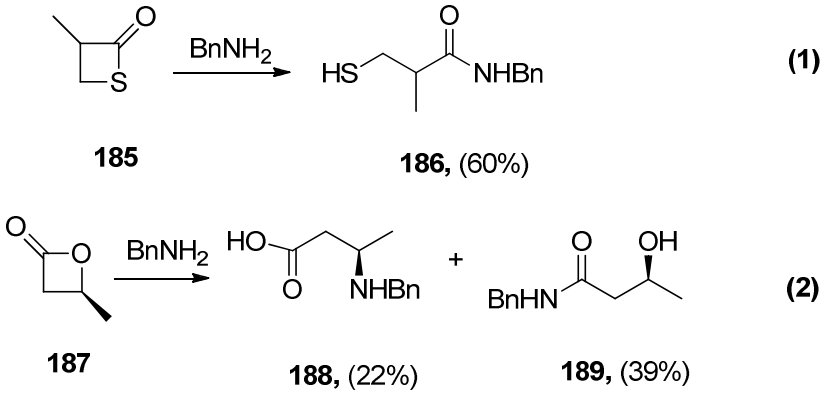
β-Thiolactones (2-oxothietanes) are sulfur analogues of β-lactones,[233-237] and their structural resemblance to β-lactones is reflected in some of the methods of preparing these compounds as well as in their chemistry. The few structural differences between the two series of lactones are reflected in their IR and ^{13}C NMR spectra. For example, for the parent β-propiolactone the IR carbonyl absorption is C=O ν_{max}^{CHCl3} 1845 cm^{-1} and the ^{13}C NMR carbonyl C-2 and C-4 chemical shifts are 169.4 and 59.1 ppm, respectively[238-239] while those for β-propiothiolactone are 1776 cm^{-1} and 191.1 and 15.0 ppm, respectively.[240-241] However, unlike the widely prized β-lactones, β-thiolactones have seen little use in synthesis. It was envisaged that one of the lesser employed features of β-lactone chemistry, ring opening by S_N2-type attack at the 4-position by a series of hard and soft nucleophiles (Scheme 64),[242] might be extended to the β-thiolactones, thereby providing a novel means of generation of thioacids for subsequent exploitation in a one pot reaction.



183, (78%) **182** **184,** (71%)

Scheme 64. S_N2-Type ring opening of a β-lactone.

Although the reactivity of β-thiolactones has been relatively little explored, it was known that one of the simplest, 3-methyl-2-oxothietane (**185**) reacts with benzylamine to give amide **186** exclusively via addition to the carbonyl carbon of the thiolactone (Scheme 65, eq. 1).[241] In contrast, the close oxygen analog, 4-methyl-2-oxetane (**187**), displays both modes of reactivity, despite the more highly substituted nature of its 4-position, and affords **188** via S_N2 attack at secondary β-carbon accompanied by formation of the amide **189** (Scheme 65, eq. 2).[243-244]



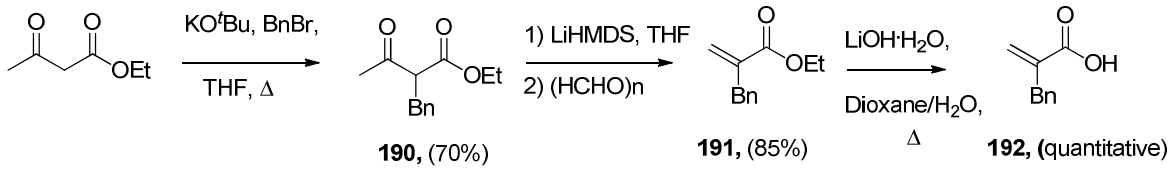
Scheme 65. Modes of reactivity of β-thiolactones and β-lactones toward benzylamine.

Despite the regiochemistry of the reaction of benzylamine with the β-thiolactone **185**, it was considered that nucleophiles softer than amines might still allow access to the alternate mode of S_N2-ring opening, thereby providing a novel entry to thioacids and possible amide bond forming reactions.

4.2 Preparation of Substrates

4.2.1 Synthesis of Simple Mono-Alkyl Substituted β-Thiolactones

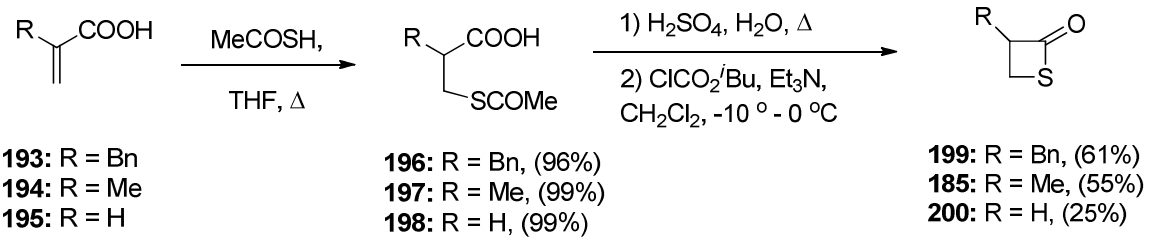
In general, small ring thiolactones can be prepared by the addition of thioacetic acid to simple β-, γ- or δ-unsaturated acids[241,245] or by reaction of ω-halo acid chlorides with sulfur based nucleophiles such as benzyltriethylammonium tetrathiomolybdate, followed by ring closure.[246] Accordingly, β-thiolactones were accessed starting from 2-alkylacrylic acids. For this purpose acrylic acid and 2-methylacrylic acid were obtained from commercial sources, while 2-benzylacrylic acid was synthesized following a literature procedure[247] starting from ethyl acetoacetate as shown in Scheme 66.



Scheme 66. Synthesis of 2-benzylacrylic acid.

Following a literature protocol,[241] conjugate addition of thioacetic acid to the various 2-alkylacrylic acids in THF at reflux afforded the thioacetate derivatives. Acidic hydrolysis of these thioacetates followed by ring closure with isobutyl chloroformate in presence of triethylamine led to the formation of corresponding β-thiolactones (Scheme 67).[241] While the thiolactones **185** and **200** were isolated by Kugelrohr distillation, the thiolactone **199** was purified by column chromatography over silica. The low isolated yield reported for parent thiolactone **200** is mainly due to the volatility of this substance. All of the thiolactones obtained in this manner were found to be stable at room

temperature. The C=O ν_{max}^{CHCl3} of **185, 199** and **200** are 1749, 1747 and 1771 cm^{-1} and the ^{13}C chemical shifts of C-2 of the thiolactones are 195.0, 194.3 and 191.2 ppm, respectively.



193: R = Bn
194: R = Me
195: R = H

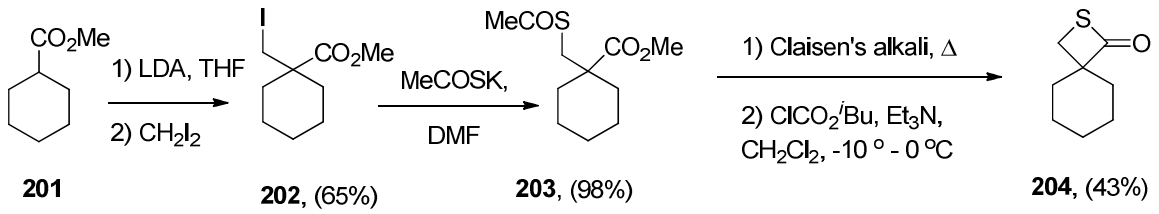
196: R = Bn, (96%)
197: R = Me, (99%)
198: R = H, (99%)

199: R = Bn, (61%)
185: R = Me, (55%)
200: R = H, (25%)

Scheme 67. Synthesis of simple β-thiolactones.

4.2.2 Synthesis of a Spirocyclic β-Thiolactone

The spirocyclic thiolactone, 2-thiaspiro[3.5]nonan-1-one (**204**), was prepared from methyl 1-(acetylthiomethyl)cyclohexanecarboxylate (**203**), which was synthesized from methyl cyclohexanecarboxylate (**201**)**,** following a literature procedure[248] as shown in Scheme 68. The synthesis started with the deprotonation of methyl ester **201** with LDA followed by quenching of the enolate with methylene iodide to give the iodo derivative **202**. The nucleophilic displacement of iodide group from compound **202** by potassium thioacetate led to the thioacetate **203**. Finally, the strong alkaline hydrolysis of hindered ester and thioacetate groups of compound **203** followed by ring closure with isobutyl chloroformate in presence of triethylamine afforded the desired thiolactone **204** (Scheme 68). The spirocyclic thiolactone **204** was purified by column chromatography over silica and was found to be stable at room temperature.

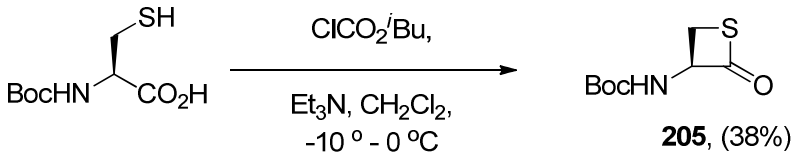


201 **202**, (65%) **203**, (98%) **204**, (43%)

Scheme 68. Synthesis of 2-thiaspiro[3.5]nonan-1-one.

4.2.3 Synthesis of a Cysteine Derived β-Thiolactone

L-*O*-(*tert*-Butyl) *N*-(2-oxothietan-3-yl) carbamate (**205**) was prepared from *N*-Boc-L-cysteine by cyclization with isobutyl chloroformate in presence of triethylamine (Scheme 69). The thiolactone **205** was obtained as a white solid after purification by column chromatography over silica and was found to be stable at room temperature.



205, (38%)

Scheme 69. Synthesis of L-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate.

In order to investigate the stereochemical integrity of L-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate (**205**), a racemic modification of the same β-thiolactone was synthesized from commercially available *N*-Boc-DL-cysteine following an identical cyclization protocol. The enantiomeric purity of L-isomer of the carbamate **205** was determined by 500 MHz NMR spectroscopy with the aid of a chiral shift reagent,[249] tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), [Eu(hfc)$_3$].[250]

Thus, a series of 500 MHz ^{1}H NMR spectra of the DL- and L-isomers of the carbamate were recorded with 0.1 equiv. increments of the Eu(hfc)$_3$ in $CDCl_3$,

leading to the plots presented in Figure 10, which clearly reveal the ability of this chiral shift reagent to distinguish the enantiomers of **205** and enable it to be stated with confidence that no racemization occurred in the ring closure. In particular, attention is drawn to the ^{1}H NMR spectra **b** and **c** of Figure 10 in which signals of the two enantiomers of DL mixture of the carbamate are resolved. In contrast, under identical conditions the L-isomer of the carbamate gave ^{1}H NMR spectra **d** and **e** (Figure 10), which indicates the presence of only one enantiomer.

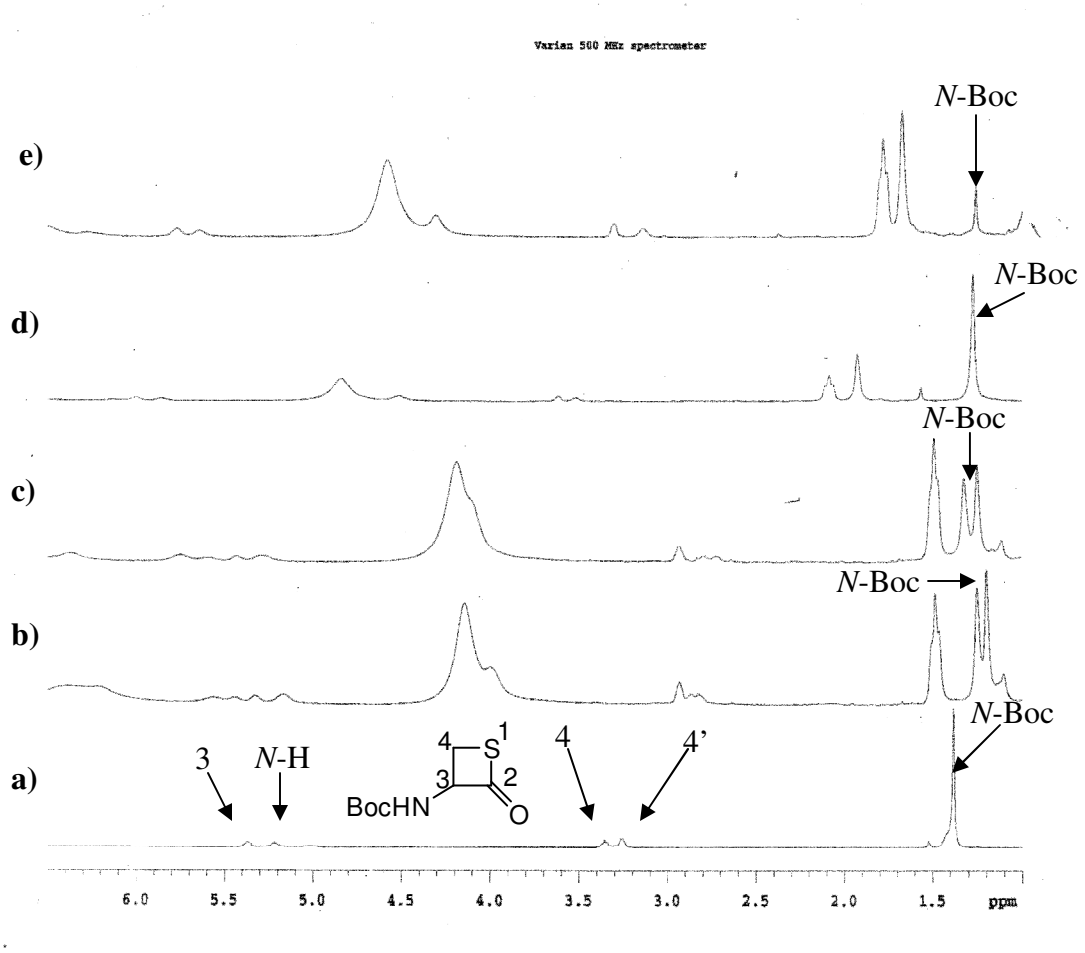
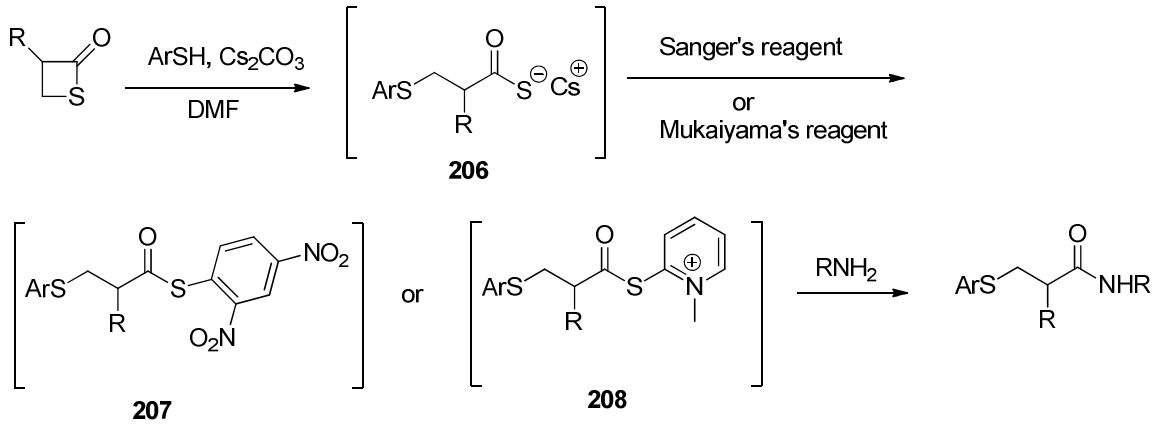


Figure 10. Stack plot of DL- and L-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate with Eu(hfc)$_3$: a) ^{1}H spectrum of DL-*O*-(*tert*-butyl) *N*-(2-

oxothietan-3-yl) carbamate; b) ^{1}H spectrum of DL-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate with 0.7 equiv. of Eu(hfc)$_3$ and c) with 0.8 equiv. of Eu(hfc)$_3$; d) ^{1}H spectrum of L-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate with 0.7 equiv. of Eu(hfc)$_3$ and e) with 0.8 equiv. of Eu(hfc)$_3$.

4.3 S_N2-Type Ring Opening of β-Thiolactones: Sanger or Mukaiyama Reagent Promoted Multicomponent Coupling

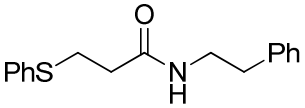
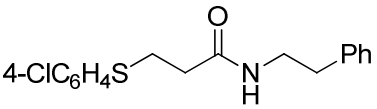
With several β-thiolactones in hand attention was turned to investigation of a reaction sequence involving reaction with an aromatic thiolate followed by trapping of the intended thiocarboxylate **206** (Scheme 70). As discussed in section **2.5**, the condensation of thioacids with 2,4-dinitrobenezensulfonamides goes through 2,4-dinitrophenylthioester **138** intermediates, which are formed by nucleophilic aromatic substitution. The liberated amines then react with the thioester to give the resulting amide (Scheme 49). On the basis of this mechanistic rationale, Crich and Sharma[251] demonstrated that the sulfonamide could be efficiently replaced by a simple amine and Sanger's reagent (2,4-dinitrofluorobenzene).[252-253] In this chemistry the thiocarboxylate **206** replaced the fluoride of the reagent by nucleophilic aromatic substitution to give the active thioester **207**, that was then subjected to reaction with an amine, resulting overall in a one-pot, three-component coupling process to give amides (Scheme 70). A directly analogous protocol employed Mukaiyama's reagent (2-chloro-*N*-methylpyridinium iodide)[254] in the place of Sanger's reagent and involved the alternative intermediate thioester **208** (Scheme 70).[251]

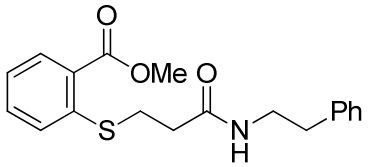
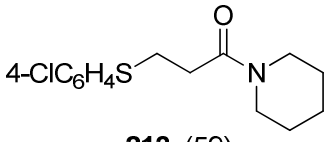
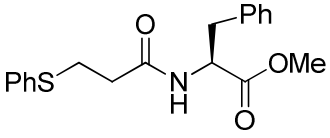
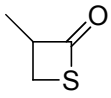
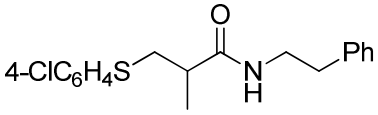
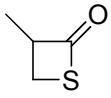
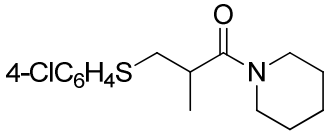
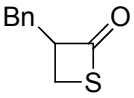
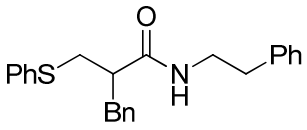
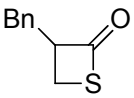
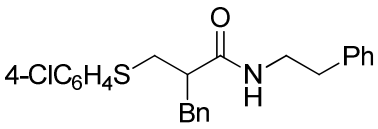
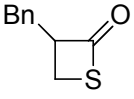
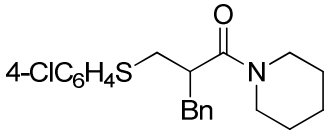


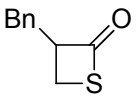
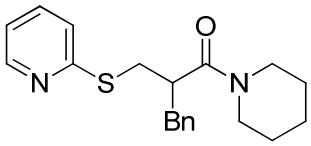
Scheme 70. Mechanism of three component coupling of β-thiolactones.

S_N2-Type ring opening of simple β-thiolactones (**185**, **199** and **200**) with a series of aromatic thiols as nucleophile in presence of cesium carbonate as base followed the anticipated course, resulting in situ generation of thioacids. Subsequent addition of Mukaiyama's reagent or Sanger's reagent and an amine, in the reaction mixture gave a range of 3-arylthiocarboxamides as illustrated in Table 5.

Table 5. Sanger's or Mukaiyama's Reagents Promoted Three-Component Coupling of β-Thiolactones (185, 199 and 200)

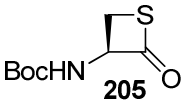
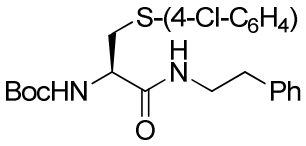
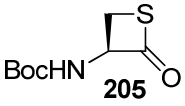
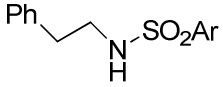
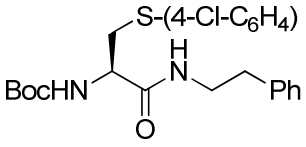
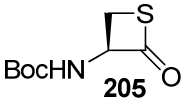
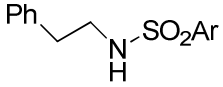
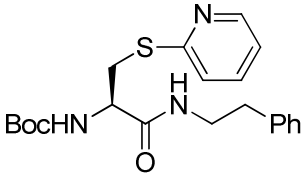
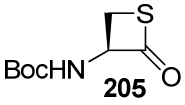
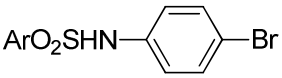
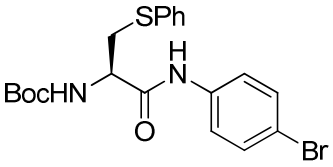
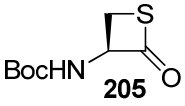
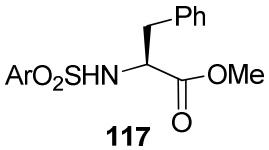
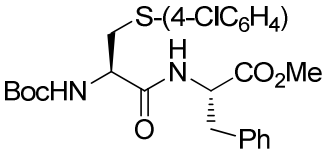
Entry	Thiolactone	Nucleophile	Reagent for Amide Bond Formation	Product (% Yield)
1	 **200**	thiophenol	2-phenethylamine, 2-chloro-*N*-methylpyridinium iodide	 **209**, (66)
2	 **200**	4-chlorothiophenol	2-phenethylamine, 2-chloro-*N*-methylpyridinium iodide	 **210**, (67)

3	 **200**	methyl 2-mercaptobenzoate	2-phenethylamine, 2-chloro-*N*-methylpyridinium iodide	 **211**, (56)
4	 **200**	4-chlorothiophenol	piperidine, 2-chloro-*N*-methylpyridinium iodide	 **212**, (59)
5	 **200**	thiophenol	L-PheOMe·HCl, 2-chloro-*N*-methylpyridinium iodide	 **213**, (61)
6	 **185**	4-chlorothiophenol	2-phenethylamine, 2,4-dinitrofluorobenzene	 **214**, (67)
7	 **185**	4-chlorothiophenol	piperidine, 2,4-dinitrofluorobenzene	 **215**, (58)
8	 **199**	thiophenol	2-phenethylamine, 2-chloro-*N*-methylpyridinium iodide	 **216**, (64)
9	 **199**	4-chlorothiophenol	2-phenethylamine, 2-chloro-*N*-methylpyridinium iodide	 **217**, (68)
10	 **199**	4-chlorothiophenol	piperidine, 2,4-dinitrofluorobenzene	 **218**, (59)

11	 **199**	2-mercaptopyridine	piperidine, 2,4-dinitrofluorobenzene	 **220**, (57)

All reactions in Table 5 were conducted under ambient temperature conditions by addition of the nucleophilic aromatic thiol and cesium carbonate to the β-thiolactone followed by capture by an electron-deficient arene and subsequent reaction of the resulting thioester with an amine, enabling the one-pot formation of both secondary or tertiary 3-arylmercaptocarboxamides in a straightforward manner. In particular, attention is drawn to Table 5 (entries 1-11), where no significant difference in overall yield between the uses of Mukaiyama's or Sanger's reagent was observed. On the other hand, minor differences were seen with the nature of the nucleophilic thiolate and the ultimate amine in the reaction mixture. For example, comparison of entries 1 and 2 of Table 5 in which the ring opening of thiolactone **200** with thiophenol or 4-chlorothiophenol, followed by addition of Mukaiyama's reagent and 2-phenethylamine reveals the 3-arylmercaptocarboxamides **209** and **210** to be obtained in comparable yields. When a weaker nucleophilic methyl 2-mercaptobenzoate was employed for the ring opening of **200** (Table 5, entry 3), a lower yield of corresponding amide **211** was observed. From a comparison of entries 2 and 4 of Table 5, it is clear that lower yield of the final product was observed with a secondary rather than a primary amine.

Table 6. Three-Component Coupling Process with Cysteine Derived β-Thiolactone

Entry	Thiolactone	Nucleophile	Reagent for Amide Bond Formation	Product (% Yield)
1	 **205**	4-chlorothiophenol	2-phenethylamine, 2,4-dinitrofluorobenzene	 **221**, (64)
2[a]	 **205**	4-chlorothiophenol	 **222**	 **221**, (70)
3[a]	 **205**	2-mercaptopyridine	 **222**	 **223**, (61)
4[a]	 **205**	thiophenol	 **224**	 **225**, (57)
5[a]	 **205**	4-chlorothiophenol	 **117**	 **226**, (62)

[a] Ar = 2,4-dinitrophenyl

The examples employing the β-thiolactone **205** (Table 6) are particularly interesting in so far as the products were obtained in highly enantiomerically enriched form when a simple amine was used as nucleophile and as single

diastereomers when a chiral amine was employed. In particular, attention was drawn to entry 5 of Table 6 in which a pure diastereomeric peptide **226** was obtained in 62% yield. The formation of pure **226** was confirmed by the 500 MHz ^{1}H NMR spectrum, which clearly indicates the single diastereomer **226** (Figure 11).

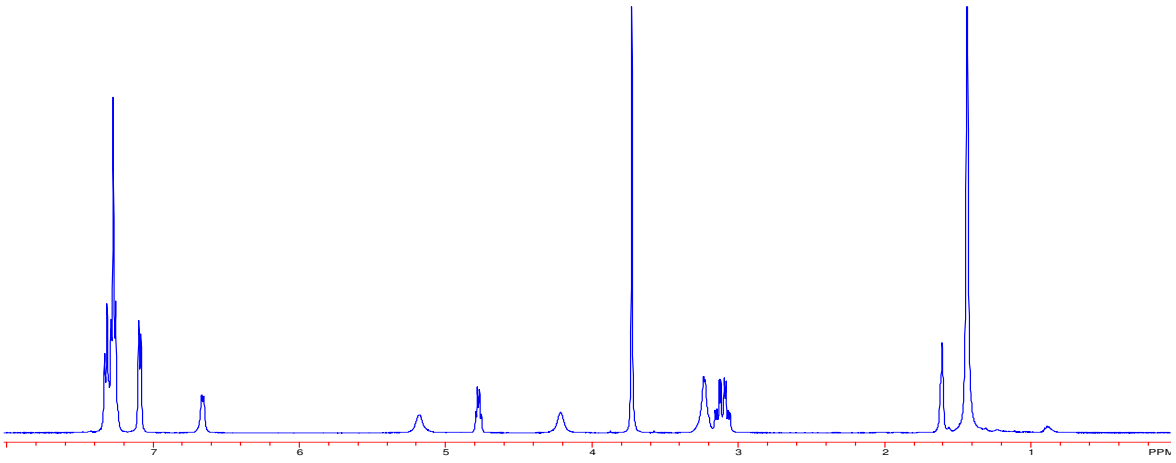
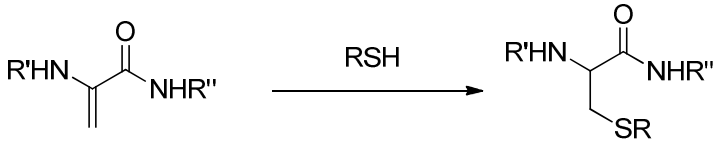


Figure 11. ^{1}H spectrum of 226 in $CDCl_3$.

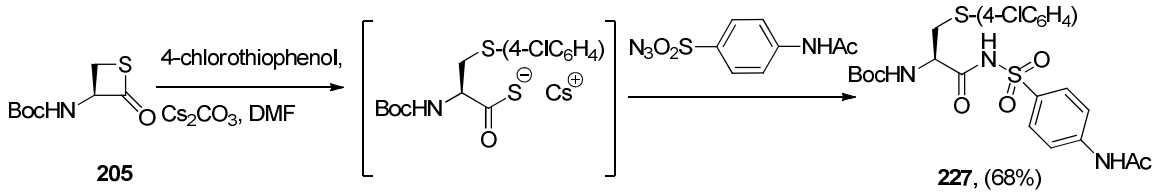
This result clearly sets this chemistry apart from the literature preparation of such compounds by conjugate addition to dehydroalanine derivatives when mixtures of DL isomers are obtained (Scheme 71).[255-256]



Scheme 71. Formation of DL-alanine derivatives via conjugate addition.

As expected, the final coupling using amine with Sanger's reagent (Table 6, entry 1) or corresponding amine sulfonamide (Table 6, entry 2) gave comparable yields of the final products. An especially noteworthy example is that involving the cysteine β-thiolactone **205** with capture by the 2,4-

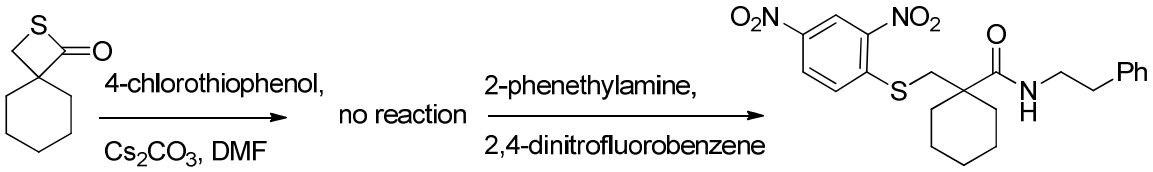
dinitrobenzenesulfonamide of L-phenylalanine methyl ester that couples the nucleophilic ring opening of the thiolactone, with a peptide bond forming process resulting in a dipeptide containing alanine derivative (Table 6, entry 5). In a variant on the general theme the thioacid generated from the ring opening of cysteine β-thiolactone **205** also was captured with an electron deficient azide, resulting in an *N*-sulfonyl amide (Scheme 72).



Scheme 72. A sulfonyl azide-based three-component coupling of cysteine β-thiolactone.

In this chemistry the overall reaction time generally was dependent upon the first step, the S_N2-type ring opening of the β-thiolactones. This is particularly clear from the reaction of 2-thiaspiro[3.5]nonan-1-one (**204**) with 4-chlorothiophenol when no reaction was seen at room temperature even after 24 h unlike the other β-thiolactones which reacted in 2 h. Heating the reaction mixture on an oil-bath at 50 °C for 5 h or in a microwave oven at 120 °C for 30 min resulted in complex reaction mixtures. Several attempts at the ring opening of β-thiolactone **204** with other aromatic thiolates were unsuccessful. Clearly, the highly sterically hindered neopentyl-like nature of the 4-position effectively prevented the S_N2 mode of ring opening. When an amine was introduced into the reaction mixture a product **228** was obtained in high yield but it resulted from

a simple ring opening by attack of the amine on the carbonyl carbon followed by capture of the thiolate by the coupling reagent (Scheme 73).

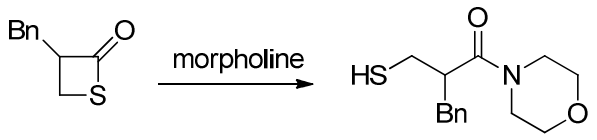


204 **228**, (80%)

Scheme 73. Formation an amide via carbonyl addition of an amine to spirocyclic β-thiolactone.

The attempted use of alkanethiols and/or thiolates as nucleophiles in this chemistry was unproductive under a variety of conditions resulting only in the formation of complex reaction mixtures. Ring opening in methanol as solvent or using 2-aminobenzenethiol as nucleophile gave complex reaction mixtures, indicating the incompatibility of alcohol or amine functional groups with the thiolactone reaction.

Finally, the possibility of replacing the thiolate nucleophile by an amine was investigated for the S_N2-type ring opening of β-thiolactone **199** (Scheme 74), but it proved impossible to divert the chemistry away from attack at the β-thiolactone carbonyl carbon as had been described previously in the literature for this class of nucleophiles (Scheme 65, eq. 1).



199 **229**, (67%)

Scheme 74. Nucleophilic addition at carbonyl carbon of β-thiolactone with an amine nucleophile.

From the literature[242] and from the above results, the reactivity of nucleophiles towards ring opening of β-lactones and β-thiolactones is seen to be quite different, with ring opening of β-thiolactones being more strongly dependent on the nature of the nucleophile. The differing reactivity between the β-thiolactones and their oxygen counterparts apparent from the literature (Scheme 65, eq. 1) and highlighted by the contrast of Table 5 and Table 6 with Scheme 73 and Scheme 74 is likely at least in part, due to the reduced resonance of the ring heteroatom with the carbonyl group and to the less strained nature of the thiolactone. In the case of thiolactones, due to the less efficient overlap between the C=O π and sulfur 3*p* orbitals,[8,241] the reduced resonance may make the 4-position softer and less reactive toward harder nucleophiles (Figure 12).

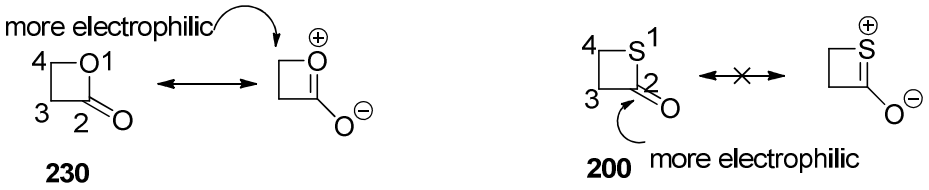


Figure 12. Reduced resonance delocalization in the thiolactones.

4.4 Conclusions

The above results make it obvious that the regioselective ring opening of β-thiolactones by aromatic thiolates is a novel way to generate in situ thioacids for use in amidation reactions. The precursors are easy to prepare, and the coupling reactions take place under mild conditions at room temperature. More importantly, the amino acid derived β-thiolactone has been shown to be compatible with conditions of the three-component coupling reaction to provide peptides containing unnatural alanine derivatives.

CHAPTER 5

TOWARD THE STEREOCHEMICAL ELUCIDATION AND SYNTHETIC STUDIES OF VIRGINEONE

5.1 Synthesis of Models: Approaches to the Assignment of Configuration

As discussed in the introduction (section **1.3**), the original investigators established the stereochemistry of the pyranose moiety to be that of a β-*O*-mannosidic linkage but neither the absolute nor the relative configuration of the remaining stereogenic centers of virgineone were established (Figure 13). Hence, a total synthesis program was initiated towards this challenging molecule with the goal of defining the relative and absolute configurations at the respective positions of this molecule.

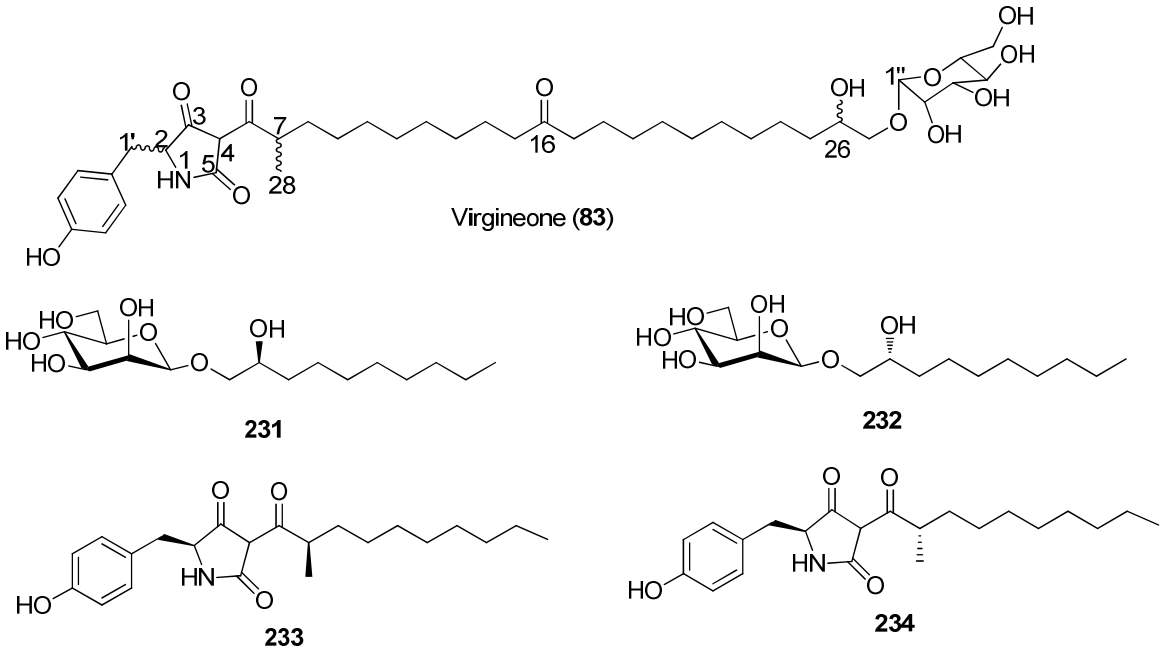


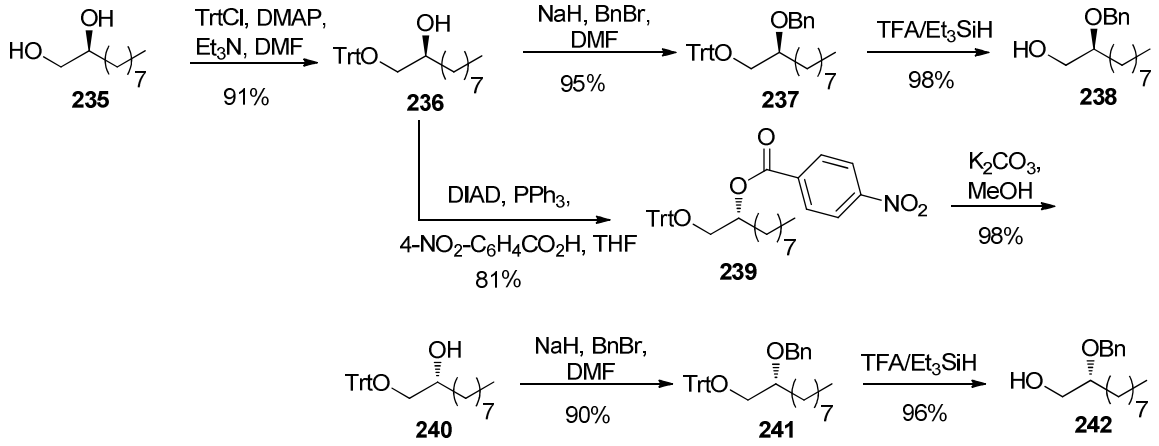
Figure 13. Virgineone and related models.

In this first instance the molecule was dissected into two portions, for each of which two stereoisomers were possible. On this basis the synthesis of the four

model compounds, **231-234** were undertaken with a view to establishing the relative stereochemistry of the stereocenters at the two extremes of the global structure by comparison of NMR chemical shifts with the literature data. The assumption was also made at this stage that the tetramic acid moiety was derived from L-tyrosine and that the sugar moiety was the D-enantiomer on the basis of the predominance of these forms in nature (Figure 13).

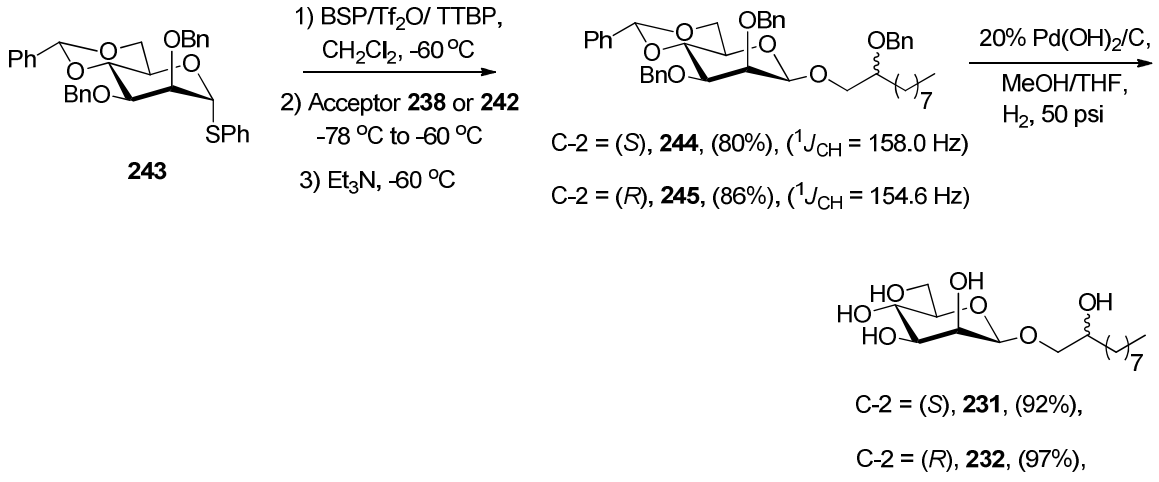
5.1.1 Synthesis of Models 231 and 232

As discussed in section **1.3.2,** the models **231** and **232** were considered to be achievable through Crich's protocol of β-mannosylation with mannopyranoside donor **243**[257] using **238** and **242** as glycosyl acceptors (Scheme 76). Accordingly, the acceptors **238** and **242** were synthesized starting from commercially available (2*S*)-decane-1,2-diol (**235**). Thus, the primary hydroxyl group of **235** was selectively protected as the trityl-ether **236** using trityl chloride and Et_3N in presence of catalytic DMAP. Benzylation of secondary hydroxyl group of **236** using benzyl bromide and sodium hydride provided **237**, from which the acceptor **238** was obtained by treatment with trifluoroacetic acid. In parallel, a portion of **236** was subjected to the Mitsunobu protocol,[258] the inverted ester **239** was obtained. Hydrolysis of the ester **239** with K_2CO_3 in methanol, followed by an identical procedure as established for the synthesis of acceptor **238**, the enantiomeric acceptor **242** was obtained in good overall yield (Scheme 75).



Scheme 75. Synthesis of acceptors 238 and 242.

With two acceptors in hand, attention was turned to mannosylation of donor **243** which was first activated with a combination of BSP/Tf_2O in presence of TTBP, followed by addition of the respective acceptor to the reaction mixture to obtain the fully protected β-mannosides **244** and **245** in good yields, whose stereochemistry was confirmed by measurement of the anomeric $^1J_{CH}$ coupling constants.[259] There was no indication of the formation of the corresponding α-isomers in this reaction. Finally, hydrogenolysis of **244** and **245** gave the models **231** and **232** respectively (Scheme 76).



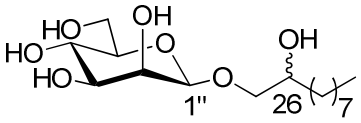
Scheme 76. β-Mannosylations and completion of synthesis of models 231 and 232.

The ^{1}H and ^{13}C NMR spectra of models **231** and **232** were recorded and are set out in Table 7 for comparison with those of the natural product.

Table 7. [a] Comparison of NMR Data of 83 and 231 / 232

Position [b,c,d]	$\Delta\delta_c$ (**83** & **231**)	$\%\Delta\delta_c$ (**83** & **231**)	$\Delta\delta_H$ (**83** & **231**)	$\Delta\delta_c$ (**83** & **232**)	$\%\Delta\delta_c$ (**83** & **232**)	$\Delta\delta_H$ (**83** & **232**)
18	-9.0	-38	-0.52	-9.0	-38	-0.52
19-24	-	-	-	-	-	-
25	0.2	0.6	0.21	0	0	0.21
26	**0.1**	**0.14**	**0.04**	**-0.1**	**-0.14**	**0.06**
27	0.06	0.1	0.09, 0.03	0	0	0.1, 0.01
1”	0	0	0.05	-0.3	-0.3	0.05
2”	0.1	0.14	0.05	0.2	0.3	0.04
3”	0.1	0.14	0.05	0.1	0.14	0.05
4”	0	0	0.05	0	0	0.05
5”	0	0	0.04	0	0	0.04
6”	-0.1	-0.02	0.06 0.05	0	0	0.05 0.05

[a] All NMR were recorded in DMSO-d_6 using 500 MHz NMR spectrometry. [b] For ease of comparison a numbering scheme corresponding to that of the natural product is used for compounds in this Table. [c] Assignments are made on the basis of DEPT, HMQC, HMBC experiments. [d] $\%\Delta\delta_c$ = (δ_c of model - δ_c of natural isolate)/ (δ_c of natural isolate) × 100.



C-26 = (*S*), **231**

C-26 = (*R*), **232**

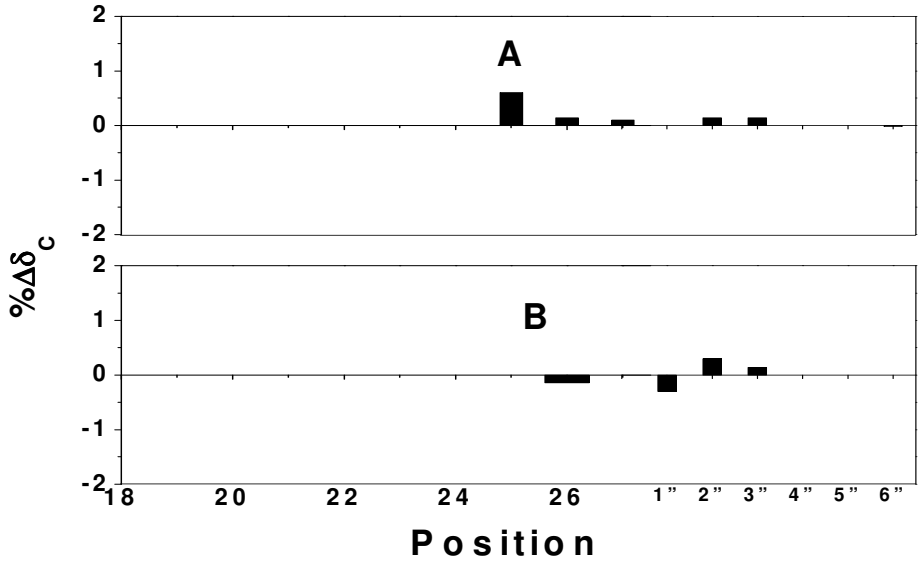
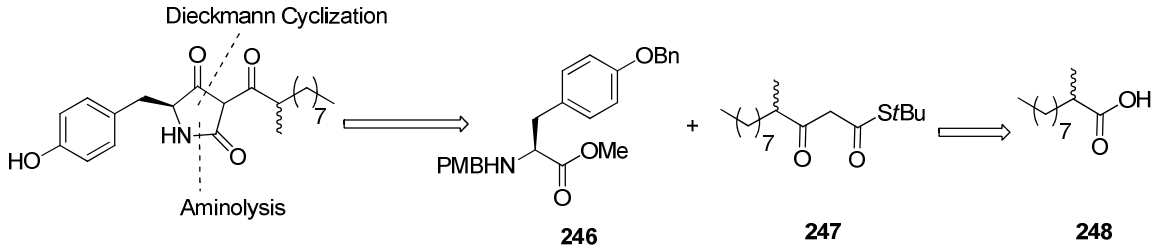


Figure 14. Plot of carbon numbers and %$\Delta\delta_c$ [%$\Delta\delta_c$ = (δ_c of model - δ_c of natural isolate)/ (δ_c of natural isolate) × 100]. A) %$\Delta\delta_c$ of model 231 and B) %$\Delta\delta_c$ of model 232.

Unfortunately, from Table 7 and Figure 14, it is clear that the chemical shift differences between the two models and the natural product are insufficient to permit conclusion to be drawn about the configuration at C-26. However, the excellent agreement of the NMR values in the sugar moiety of **83** with both models indicates that the sugar is indeed a β-mannoside.

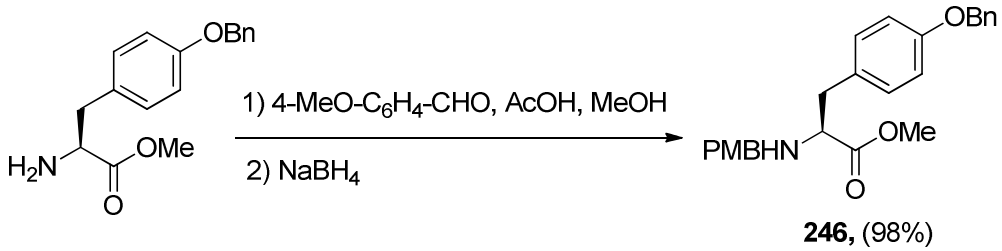
5.1.2 Synthesis of Models 233 and 234

The synthetic strategy for the models **233** and **234** was based on a retrosynthetic approach involving two main fragments, namely the amino ester **246** and a β-keto thioester fragment **247** (Scheme 77). Fragment **246** was readily available from *O*-benzyl-L-tyrosine methyl ester, while the fragment **247** could be obtained from 2-methyldecanoic acid following a literature protocol.[260]



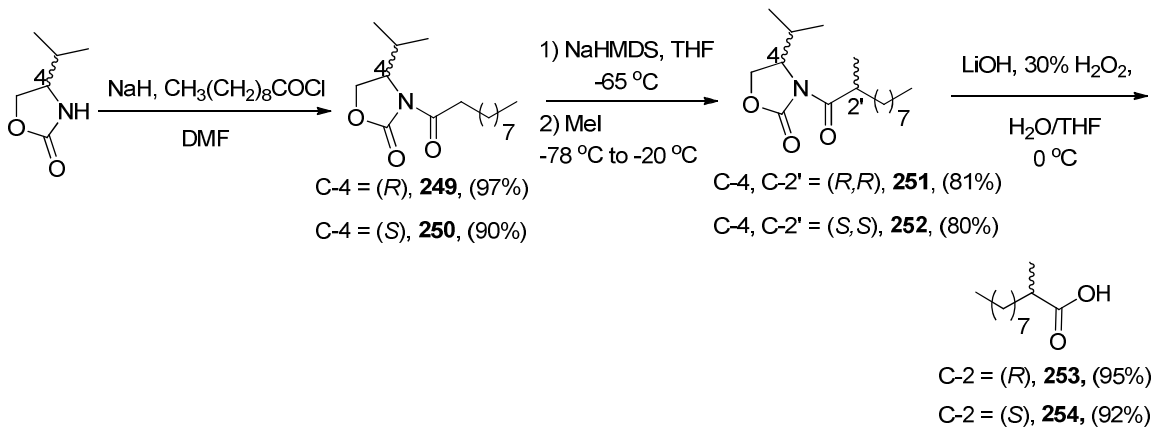
Scheme 77. Retrosynthetic analysis of models 233 and 234.

In this regard, the synthesis of amino ester **246** was readily achieved by the reductive *N*-alkylation of commercially available *O*-benzyl-L-tyrosine methyl ester. Thus, the amino ester was treated with 4-methoxybenzaldehyde in presence of acetic acid, followed by addition of $NaBH_4$ to give *N*-PMB-L-tyrosine derivative **246** (Scheme 78).



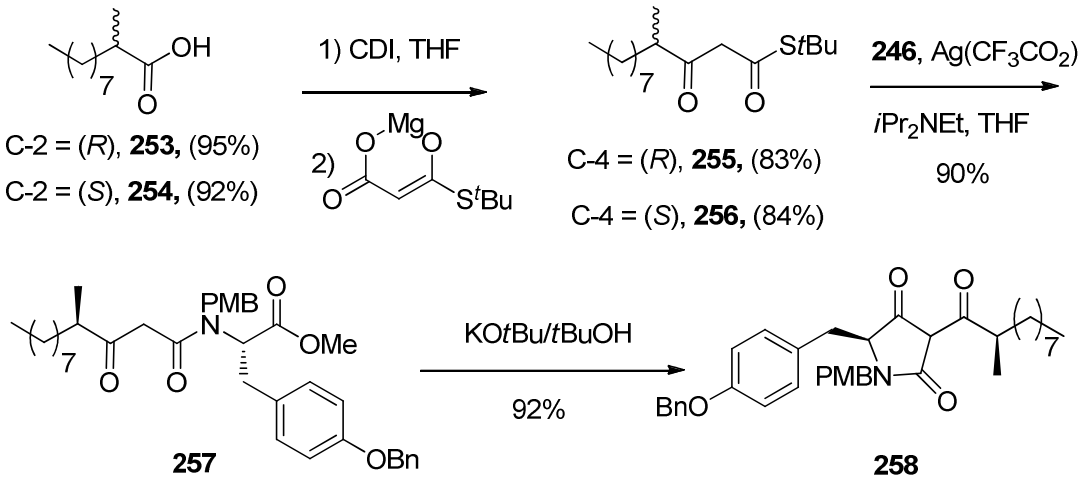
Scheme 78. Synthesis of *N*-(4-methoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester

To access acids **253** and **254**, first (4*R*)- and (4*S*)-4-isopropyloxazolidin-2-one[261] were acylated with decanoyl chloride to give the corresponding imides **249** and **250**. Enolization of respective imides with NaHMDS and methylation of the derived enolate following the literature protocol[262] provided a mixture of diastereomers (dr = 9:1). Chromatographic purification of these mixtures afforded pure diastereomers **251** and **252** in good yield. Alkaline hydrolysis of **251** and **252** in the presence of H_2O_2 gave the corresponding acids **253** and **254** (Scheme 79).



Scheme 79. Synthesis of (2*R*)- and (2*S*)-2-methyldecanoic acid.

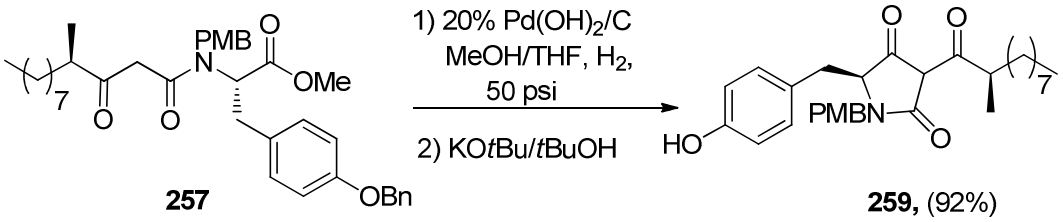
Having both enantiomers of the acid and L-tyrosine methyl ester derivative **246** in hand, the preparation of the *tert*-butyl thioester fragments **255** and **256** were carried out following a literature protocol.[260] Accordingly, the activation of the acids with CDI followed by addition of magnesium enolate of 3-(*tert*-butylthio)-3-oxopropanoic acid was provided the thioesters **255** and **256**. Aminolysis[147] of the thioester **255** with L-tyrosine amine **246** mediated by silver trifluoroacetate afforded the β-keto amide **257**. Finally, the fully protected tetramic acid derivative **258** was obtained by the Dieckmann cyclization of **257** with KO*t*Bu over 30 min (Scheme 80).



Scheme 80. Synthesis of protected tetramic acid.

The attempted hydrogenolysis of **258** over Pd/C or $Pd(OH)_2/C$ as catalyst was unsuccessful and resulted only in a complex reaction mixture within 1 h of shaking under 50 psi of hydrogen. Similarly, various other attempts at benzyl and amide *N*-PMB group removal from **258** using CAN,[263-264] anhydrous $FeCl_3$[265] or anhydrous $AlCl_3$[266] were unproductive and afforded only complex reaction mixtures. In addition, the treatment of **258** with 50% aqueous HBr or 33% HBr in acetic acid, resulted in no cleavage of the benzyl or PMB groups.

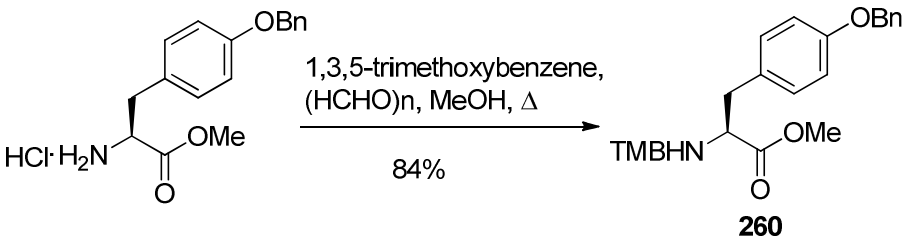
Attention, therefore, was turned to the synthesis of the tetramic acid moiety by simple modifications of the reaction sequences, in which the benzyl group was successfully deprotected from the β-keto amide **257** before the Dieckmann cyclization. Subsequently, the treatment of benzyl-free β-keto amide with KO*t*Bu gave the tetramic acid **259** in 92% yield (Scheme 81).



257 **259,** (92%)

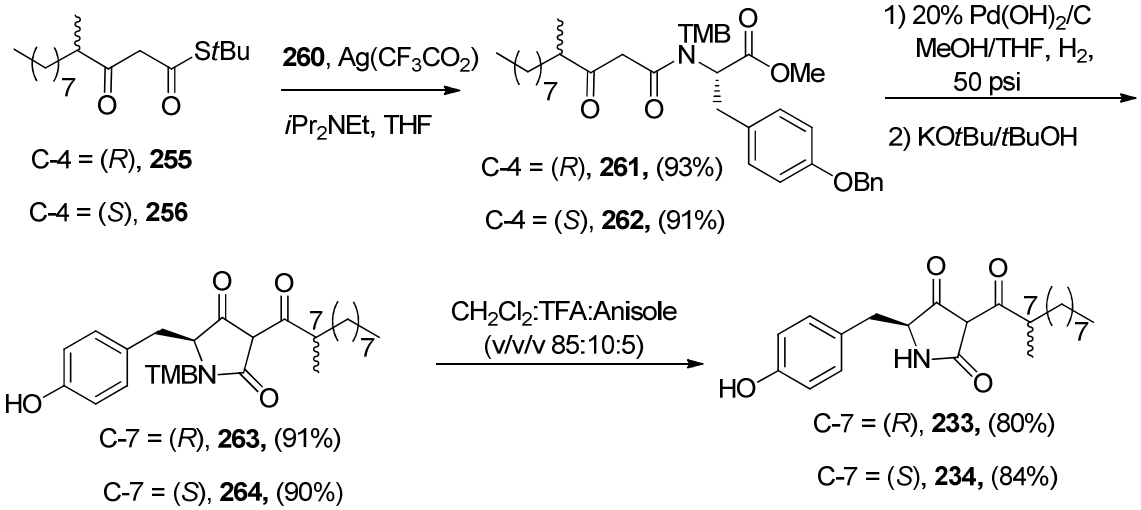
Scheme 81. Cyclization of a phenolic β-keto amide.

In addition to modification of the reaction sequences, the *N*-PMB group was replaced by the more acid labile *N*-TMB group from the *N*-TMB amino ester **260**, whose synthesis was readily achieved by the condensation of a methanolic solution of *O*-benzyl-L-tyrosine methyl ester hydrochloride with 1,3,5-trimethoxybenzene and paraformaldehyde (Scheme 82).



Scheme 82. Synthesis of *N*-(2,4,6-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester.

Finally, combining the chemistry of Schemes 80 and 81, the *N*-TMB derived tetramic acids **263** and **264** were obtained in excellent yields. Subsequently, removal of the TMB was achieved by the treatment with trifluoroacetic acid in presence of anisole, and purification by column chromatography of the crude reaction mixture over silica gel afforded the models **233** and **234** as light yellow foams, which were found to be stable at room temperature (Scheme 83).



Scheme 83. Completion of the synthesis of model tetramic acids.

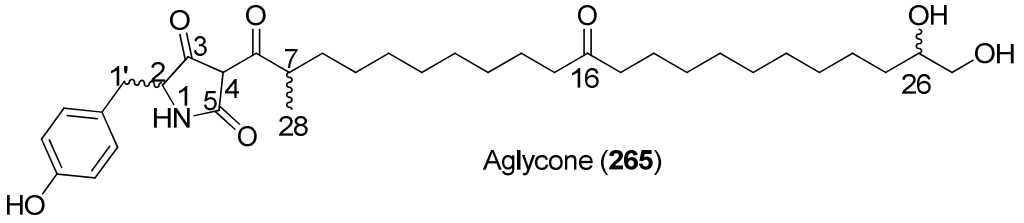
The ^{1}H and ^{13}C chemical shifts of natural product **83** along with those of **233** and **234** are summarized in Table 8 for comparison. In addition, Table 9

furnishes the comparisons of the spectral data of models **233** and **234** with that of the aglycone **265**.[138]

Table 8. [a] Comparisons of NMR Data of 83 and 233 / 234

Position [b,c,d]	$\Delta\delta_c$ (**83** & **233**)	$\%\Delta\delta_c$ (**83** & **233**)	$\Delta\delta_H$ (**83** & **233**)	$\Delta\delta_c$ (**83** & **234**)	$\%\Delta\delta_c$ (**83** & **234**)	$\Delta\delta_H$ (**83** & **234**)
1	-	-	1.62	-	-	1.6
2	1.6	2.6	0.52	1.7	2.8	0.53
3	-1.9	-1.0	-	-1.6	-0.8	-
4	0.7	0.7	-	0.7	0.7	-
5	-1.7	-1.0	-	-1.6	-0.9	-
6	-3.0	-1.5	-	-2.9	-1.5	-
7	**-2.9**	**-7.5**	**-0.2**	**-2.6**	**-6.8**	**-0.22**
8	0	0	0.02, 0.30	0.5	1.5	0.07, 0.32
9-15	-	-	-	-	-	-
28	**-1.1**	**-6.2**	**0.23**	**0.6**	**3.4**	**0.16**
1’	-1.4	-3.8	0.24 0.10	-1.1	-3.0	-
2’	-2.2	-1.7	-	-2.1	-1.6	-
3’/7’	0.2	0.1	-0.01	0.3	0.23	-0.01
4’/6’	0.1	0.1	0.05	0.2	0.2	0.07
5’	0.4	0.3	-	0.5	0.3	-
5’-OH	-	-	-0.77	-	-	-0.75

[a] All NMR were recorded in DMSO-d_6 using 500 MHz NMR spectrometry. [b] For ease of comparison a numbering scheme corresponding to that of the natural product is used for compounds in this Table. [c] Assignments are made on the basis of DEPT, HMQC, HMBC experiments. [d] $\%\Delta\delta_c = (\delta_c \text{ of model} - \delta_c \text{ of natural isolate})/ (\delta_c \text{ of natural isolate}) \times 100$.



Aglycone (**265**)

Table 9.[a] Comparisons of NMR Data of 265 and 233 / 234

Position [b,c,d]	$\Delta\delta_c$ (**265** & **233**)	$\%\Delta\delta_c$ (**265** & **233**)	$\Delta\delta_H$ (**265** & **233**)	$\Delta\delta_c$ (**265** & **234**)	$\%\Delta\delta_c$ (**265** & **234**)	$\Delta\delta_H$ (**265** & **234**)
1	-	-	-	-	-	-
2	0.3	0.5	0.05	0.4	0.6	0.06
3	0	0	-	0.3	0.2	-

4	-0.1	-0.1	-	-0.1	-0.1	-
5	-0.1	-0.05	-	0	0	-
6	0	0	-	0.1	0.05	-
7	**-0.3**	**-0.8**	**0.03**	**0**	**0**	**-0.01**
8	0	0	-0.02 -0.05	-0.5	1.5	0.09, -0.02
9-15	-	-	-	-	-	-
28	**-0.5**	**-3.0**	**0.01**	**0**	**0**	**0.05**
1’	-0.4	-1.1	-	-0.1	-0.3	0.26
2’	-0.2	-0.2	-	0	0	-
3’/7’	0	0	-0.06	0.1	0.1	-0.04
4’/6’	0	0	-0.04	0.1	0.1	-0.02
5’	0	0	-	0.1	0.75	-
5’-OH	-	-	-	-	-	

[a] All NMR were recorded in DMSO-d_6 using 500 MHz NMR spectrometry. [b] For ease of comparison a numbering scheme corresponding to that of the natural product is used for compounds in this Table. [c] Assignments are made on the basis of DEPT, HMQC, HMBC experiments. [d] $\%\Delta\delta_c$ = (δ_c of model - δ_c of natural isolate)/ (δ_c of natural isolate) × 100.

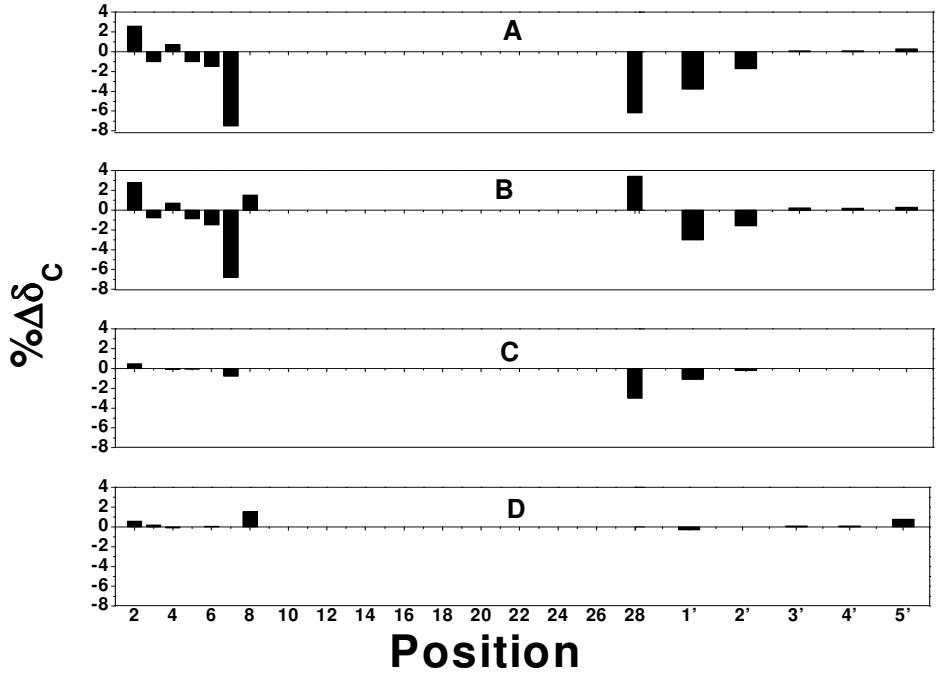


Figure 15. Plot of carbon numbers and $\%\Delta\delta_c$ [$\%\Delta\delta_c$ = (δ_c of model - δ_c of natural isolate)/ (δ_c of natural isolate) × 100]. A) $\%\Delta\delta_c$ between model 233 and virgineone (83); B) $\%\Delta\delta_c$ between model 234 and virgineone (83); C)

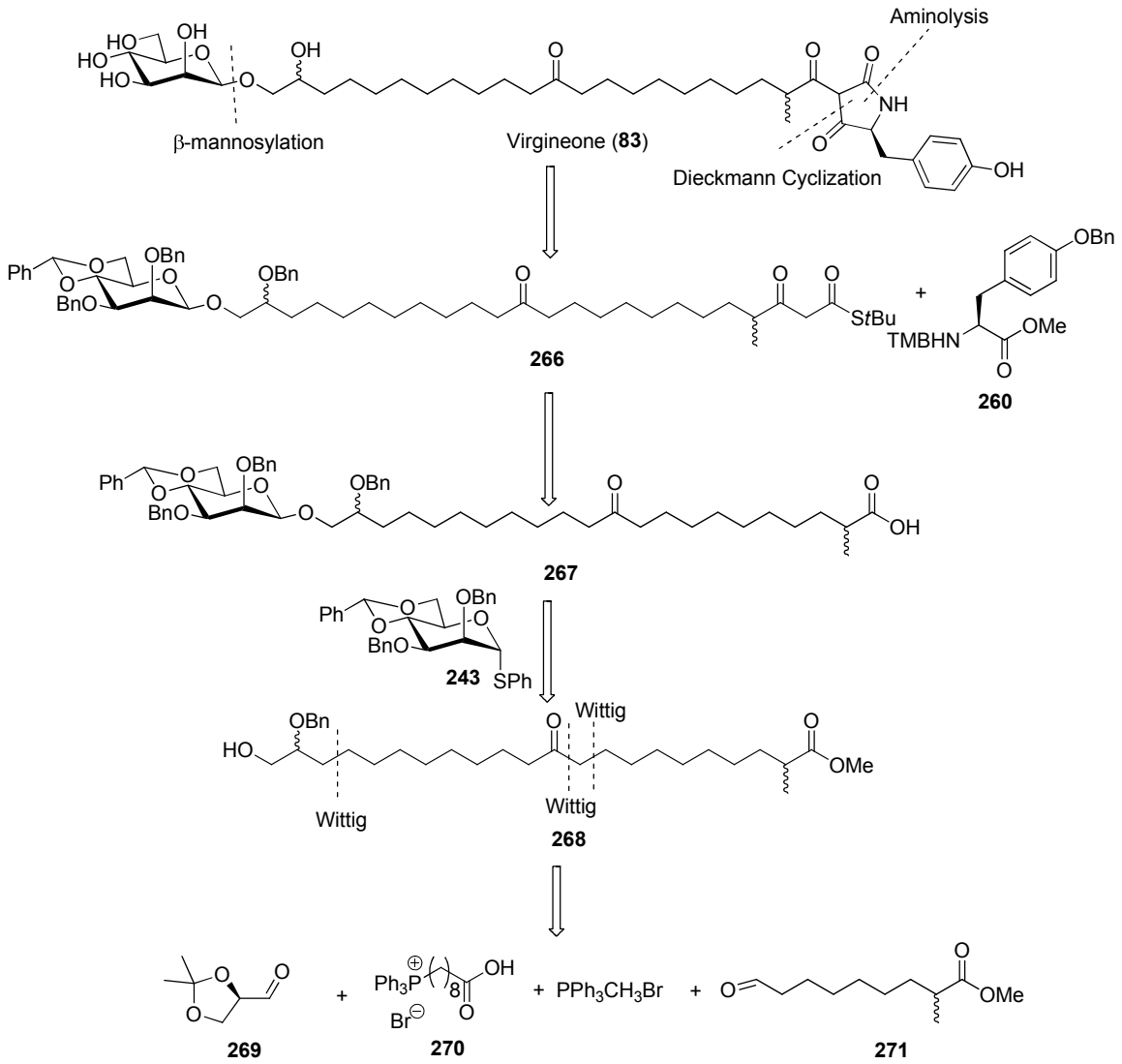
%$\Delta\delta_c$ between model 233 and aglycone (265); D) %$\Delta\delta_c$ between model 232 and aglycone (265).

As is clear from a comparison of the data in Tables 8 and 9 and as reported in the litureature,[138] the ^{1}H and ^{13}C chemical shifts of the tetramic acid moiety of virgineone (**83**) and aglycone (**265**) depend on the presence or absence of the β-mannosidic linkage at C-27. Therefore, the most appropriate conclusions are those from the comparison of the aglycone **265** with models **233** and **234**. Although the differences between the two models are negligible, it is clear from both Tables 8 and 9 and Figure 15 that, particularly at positions C-7 and C-28, the differences are smaller in case of model **234** (C-7 = *S*) than the case of model **233** (C-7 = *R*), which tends to suggest that the aglycone **265** and hence virgineone itself should have the relative stereochemistry of model **234** for the tetramic acid portion. Nevertheless, and in view of the uncertainty regarding the stereochemistry at C-7, it was concluded that the complete synthesis of four stereoisomers of virgineone itself was necessary in order to come to any firm conclusions about the relative and absolute stereochemistry of this intriguing molecule. In this regard, a plan for the total synthesis of four such isomers of virgineone (**83**), available from L-tyrosine was devised that takes advantage of the lessons learnt in the course of the preparation of the various models.

5.2 Retrosynthetic Analysis

As shown in Scheme 77, the synthesis of virgineone was envisioned to involve aminolysis of the β-keto thioester fragment **266** with *N*-TMB-L-tyrosine derivative **260**. The intermediate thioester **266** could be derived as before from

the acid **267**. The β-mannosidic acid **267** could be obtained from acceptor **268** via 4,6-*O*-benzylidine directed β-mannosylation of donor **243**. The C-22 oxygenated chain acceptor **268** could be generated by a series of three Wittig reactions from aldehydes **269** and **271** (Scheme 84).

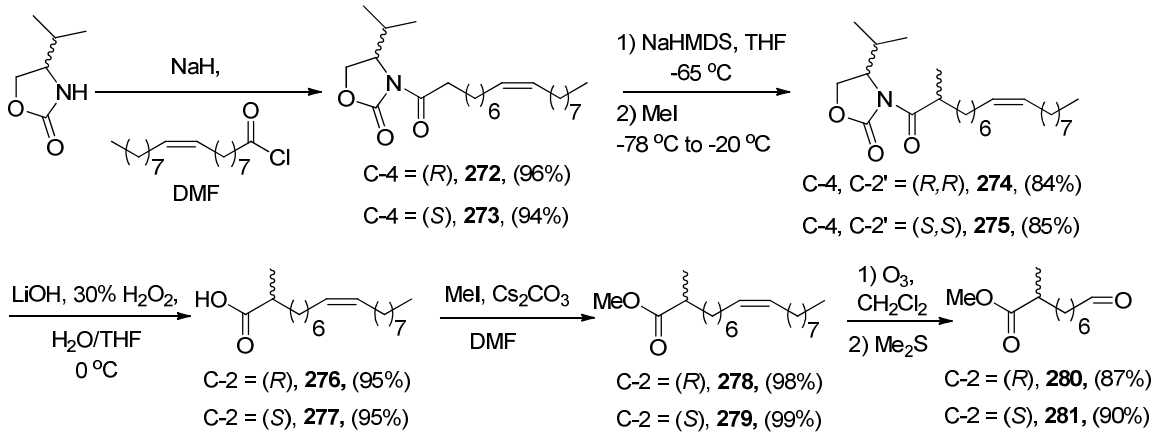


Scheme 84. Retrosynthetic analysis of virgineone.

5.3 Synthesis of Precursors

5.3.1 Synthesis of (2*R*)- and (2*S*)-Methyl 2-Methyl-9-oxononanoate

Following the synthetic Scheme 79 as established for the synthesis of acids **253** and **254**, the enantiomerically pure acids **276** and **277** were synthesized over three steps from oleanoyl chloride, and were converted to their methyl esters **278** and **279** by treatment with methyl iodide in presence of Cs_2CO_3. Subsequently, ozonolysis of **278** and **279** gave the corresponding aldehydes **280** and **281**, which were purified by column chromatography over silica gel (Scheme 85). These aldehydes were found to be stable to polymerization if stored in the freezer at -20 °C.

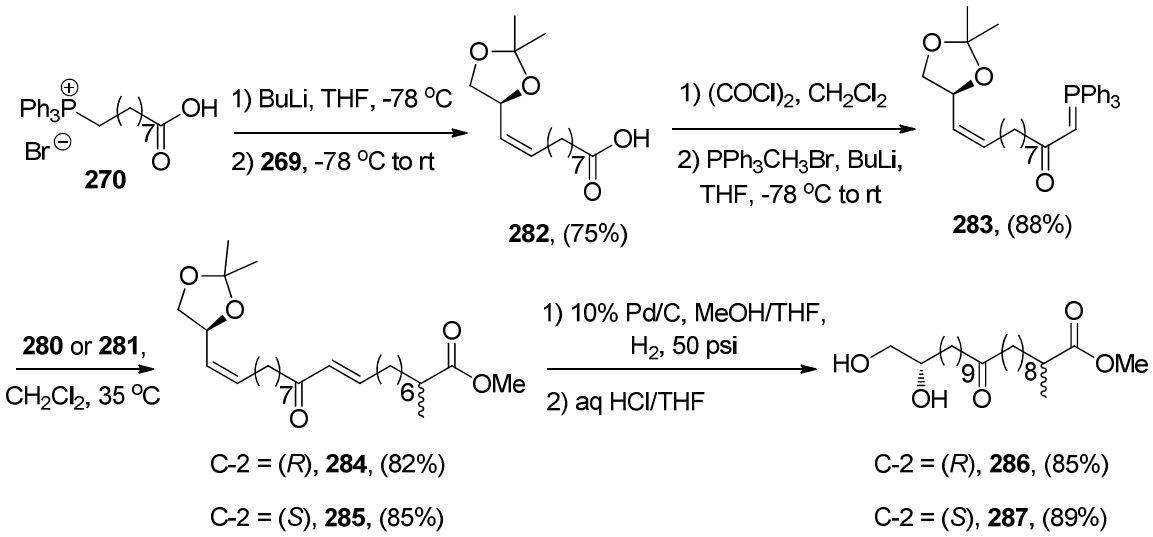


Scheme 85. Synthesis of (2*R*)- and (2*S*)-methyl 2-methyl-9-oxononanoate.

5.3.2 Synthesis of Acceptors

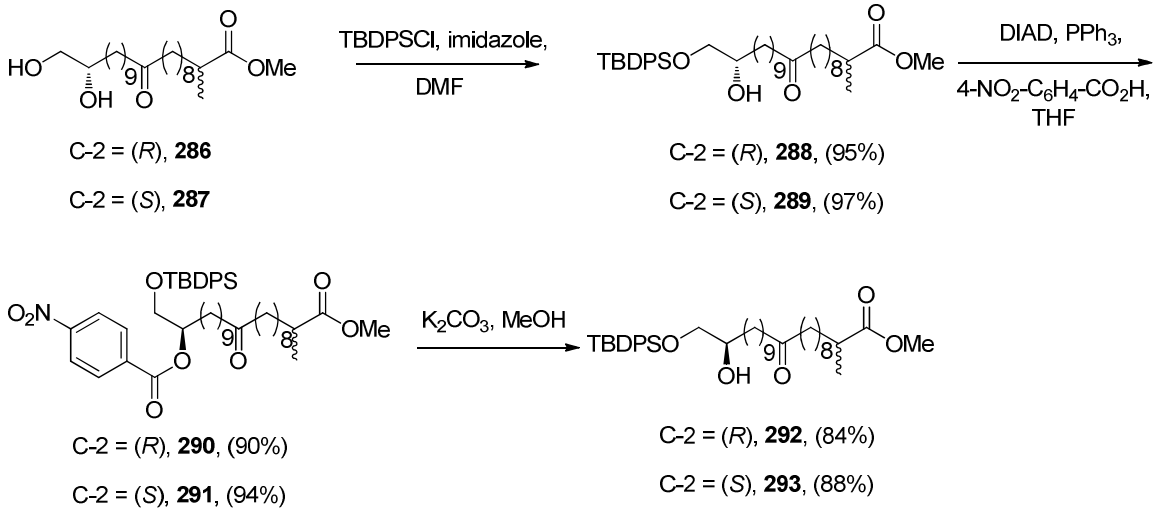
The synthesis of this portion of the target began with Wittig olefination of ylide generated from the carboxyalkyl Wittig salt **270**[267] and aldehyde **269**[268] to give acid **282**. The stable phosphoranylidene reagent **283** was prepared by addition of the acid chloride of **282**, prepared from **282** by the treatment with

oxalyl chloride, into a freshly prepared solution of ylide resulting from *n*-BuLi-mediated deprotonation of methyltriphenylphosphonium bromide. Elongation of stable ylide **283** by a Wittig-type reaction with aldehydes **280** and **281** gave rise the corresponding unsaturated C-22 oxygenated chains **284** and **285** respectively, which upon reduction followed by acid-mediated cleavage of the acetonide group gave the dihydroxy esters **286** and **287** (Scheme 86).



Scheme 86. Synthesis of C-22 dihydroxy esters.

Modifying slightly the chemistry developed in Scheme 75, the primary hydroxyl groups of **286** and **287** were selectively silylated using TBDPS-chloride in presence of imidazole, and a portion of the products were subjected to the Mitsunobu protocol to give the inverted esters **290** and **291**. Saponification of the nitro-esters **290** and **291** with K_2CO_3 in methanol then afforded **292** and **293** (Scheme 87).



C-2 = (*R*), **286**

C-2 = (*S*), **287**

C-2 = (*R*), **288**, (95%)

C-2 = (*S*), **289**, (97%)

C-2 = (*R*), **290**, (90%)

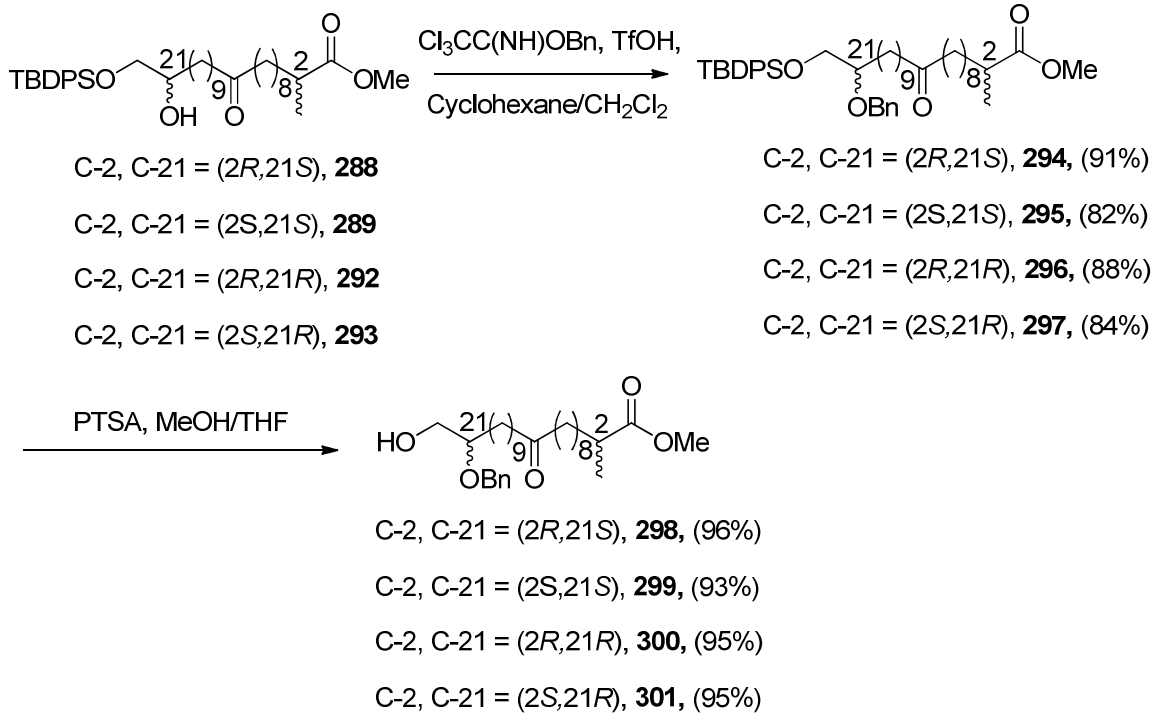
C-2 = (*S*), **291**, (94%)

C-2 = (*R*), **292**, (84%)

C-2 = (*S*), **293**, (88%)

Scheme 87. Synthesis of glycosyl acceptors.

The acid-mediated benzylation of the secondary hydroxyl groups of **288, 289, 292** and **293** with benzyl 2,2,2-trichloroacetimidate and TfOH next provided the fully protected acceptors **294**-**297**. Finally, the C-22 oxygenated chain acceptors **298**-**301** were accessed by exposure to PTSA (Scheme 88).



C-2, C-21 = (2*R*,21*S*), **288**

C-2, C-21 = (2S,21*S*), **289**

C-2, C-21 = (2*R*,21*R*), **292**

C-2, C-21 = (2*S*,21*R*), **293**

C-2, C-21 = (2*R*,21*S*), **294,** (91%)

C-2, C-21 = (2S,21*S*), **295,** (82%)

C-2, C-21 = (2*R*,21*R*), **296,** (88%)

C-2, C-21 = (2*S*,21*R*), **297,** (84%)

C-2, C-21 = (2*R*,21*S*), **298,** (96%)

C-2, C-21 = (2S,21*S*), **299,** (93%)

C-2, C-21 = (2*R*,21*R*), **300,** (95%)

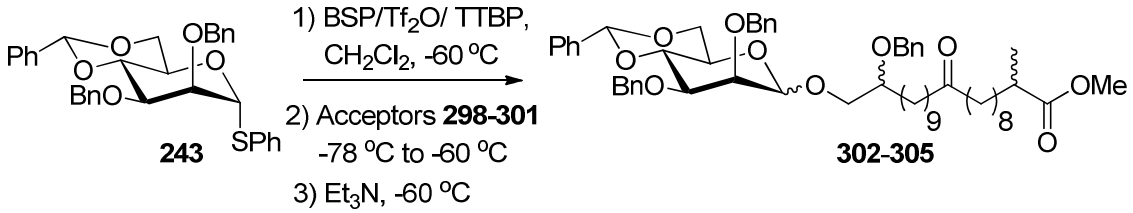
C-2, C-21 = (2*S*,21*R*), **301,** (95%)

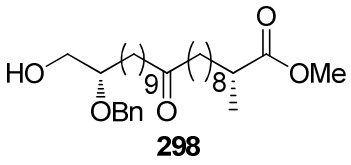
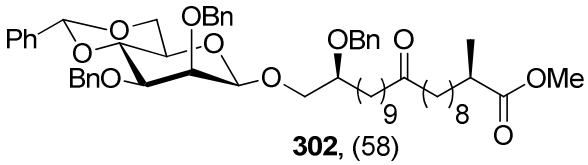
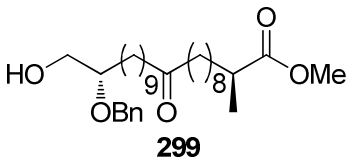
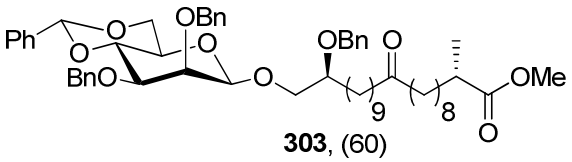
Scheme 88. Completion of the acceptor synthesis.

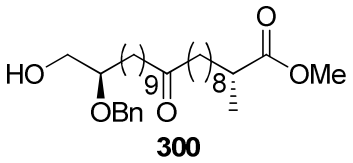
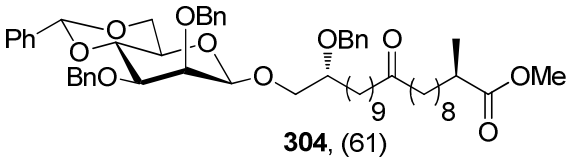
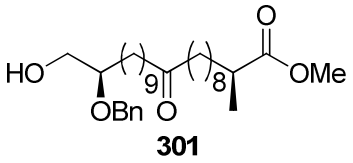
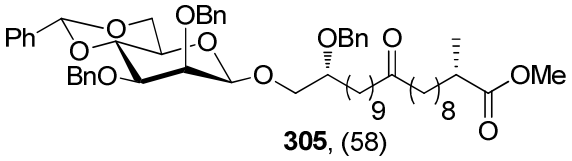
5.3.3 Mannosylation and Completion of Synthesis of Isomers of Virgineone

Adapting the method of Scheme 76, mannosylation of acceptors **298**-**301** with donor **243** was conducted leading to the formation of the mannosides **302**-**305**. Curiously, unlike the mannosylation of the model acceptors **238** and **242** with donor **243** which provided only the β-mannosides, the acceptors **298**-**301** gave the desired β-isomers accompanied by the minor α-isomers. Nevertheless, subsequent chromatographic purification of the mixtures over silica gel provided the pure β-mannosides **302**-**305**, whose stereochemistry was confirmed as before by measurement of the $^1J_{CH}$ coupling constants (Table 10).

Table 10. Mannosylation of Acceptors 298-301 with Donor 243

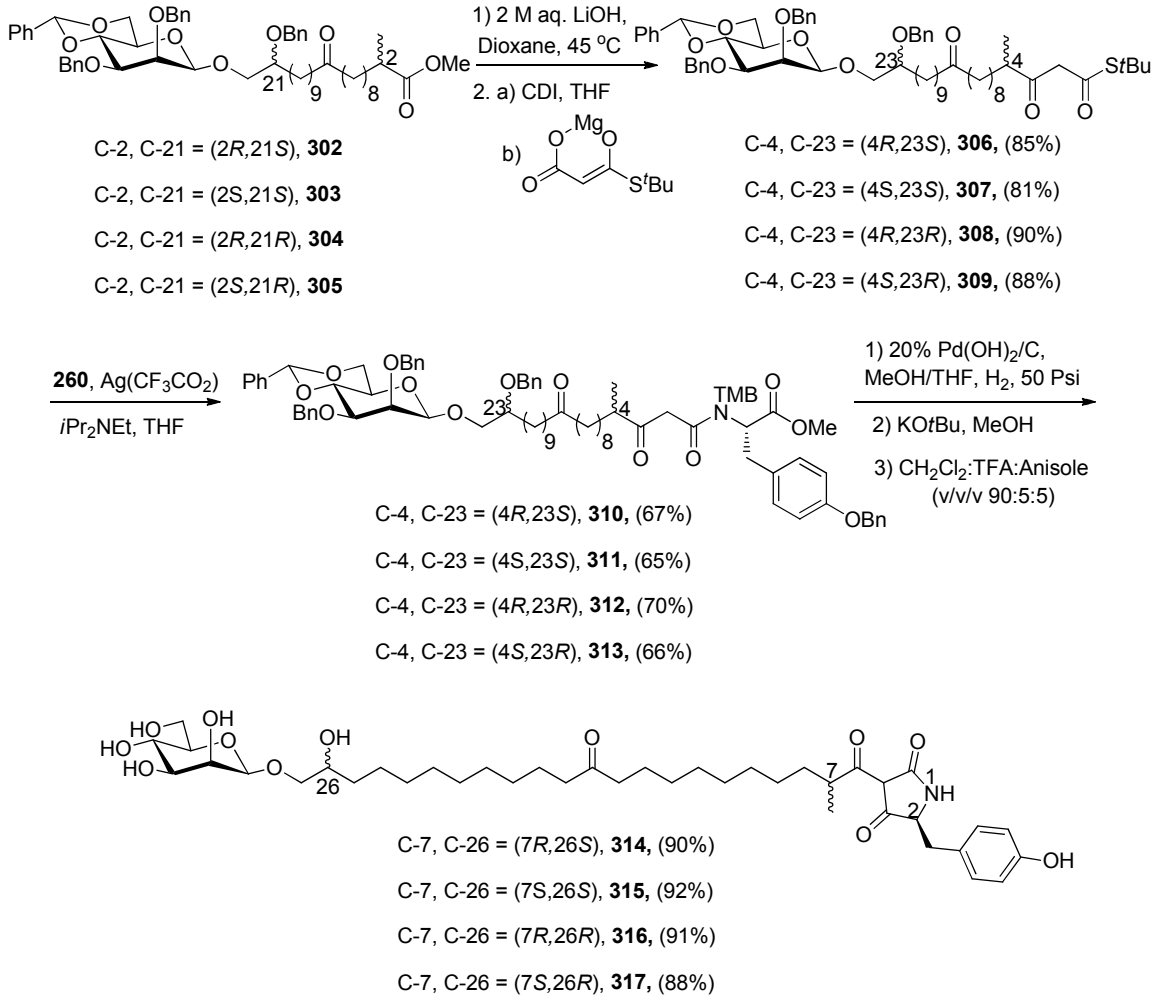


Acceptor	(α/β)[a]	β-Mannoside (%Yield)	$^1J_{CH}$ (in Hz)
 **298**	1/5.8	 **302**, (58)	160.0
 **299**	1/6.2	 **303**, (60)	158.1

 **300**	1/6.5	 **304**, (61)	158.6
 **301**	1/5.4	 **305**, (58)	157.6

[a] Determined by ^{1}H-NMR spectroscopy on the crude reaction mixture.

Saponification of the methyl esters **302**-**305** with LiOH provided the corresponding acids, which required no further purification. Accordingly, combining the chemistry of Schemes 80 and 83, the fully protected β-keto amides **310**-**313** were then synthesized from the acids by activation with CDI followed by the addition of magnesium enolate of 3-(*tert*-butylthio)-3-oxopropanoic acid and then silver-mediated coupling to the amino acid moiety (Scheme 89). Removal of the benzyl and benzylidine groups was carried out by brief, controlled $Pd(OH)_2$/C-mediated hydrogenolysis of **310**-**313**, as it was found that hydrogenolysis over longer reaction times resulted in premature cleavage of the TMB group. Subsequently, Dieckmann cyclization of homogenous methanolic solutions of the resulting β-keto amides with KO*t*Bu, followed by removal of the TMB group with trifluoroacetic acid gave four isomers of virgineone **314**-**317** (Scheme 89). These substances were obtained pure as light orange foams by washing the final reaction mixtures with chloroform followed by decantation of the solvent; they were found to be stable in the freezer at -20 °C.



Scheme 89. Completion of the syntheses of four stereoisomers of virgineone.

The ^{1}H and ^{13}C NMR and ESI-HRMS spectra of these compounds were in full agreement with the structures assigned. However, the ^{13}C NMR spectra showed broadening of the carbon signals corresponding to the tetramic acid moieties, indicating the presence of keto-enol tautomers in the 1,3-dicarbonyl functionality, which is a readily understood feature of compounds of this type.[269]

With four isomers in hand, attention was turned to the comparison of their ^{1}H and ^{13}C chemical shift data with that of the natural product **83** as summarized in Table 11.

Table 11.[a] Comparisons of NMR Data of 83 with 314 / 315 / 316 / 317

Position [b,c,d]	%Δδ$_C$ (**83** & **314**)	%Δδ$_H$ (**83** & **314**)	%Δδ$_C$ (**83** & **315**)	%Δδ$_H$ (**83** & **315**)	%Δδ$_C$ (**83** & **316**)	%Δδ$_H$ (**83** & **316**)	%Δδ$_C$ (**83** & **317**)	%Δδ$_H$ (**83** & **317**)
1	-	22.0	-	-	-	-	-	13.9
2	2.6	14.9	2.1	12.6	-	6.7	3.4	3.9
3	-1.0	-	-1.0	-	-	-	-	-
4	0.4	-	0.3	-	-	-	-	-
5	-1.0	-	-0.9	-	-0.7	-	-0.1	-
6	-1.5	-	-1.4	-	-1.5	-	-1.5	-
7	**-7.5**	**-4.9**	**-6.2**	**-5.2**	**-4.4**	**-5.5**	**-5.2**	**-5.2**
8	0.3	- -2.0	-0.3	- -3.3	-	- -2.7	-0.6	- 0.7
9-14 & 18-24	-	-	-	-	-	-	-	-
15/17	0	2.6	0	2.1	0	2.1	0	2.1
16	0.3	-	0.3	-	0.3	-	0.3	-
25	0.6	- 3.7	0.6	- 4.4	0	- 6.7	0	- 5.2
26	**0.1**	**1.1**	**0.1**	**1.1**	**0.3**	**2.0**	**-0.2**	**1.7**
27	0	1.5, 0.8	0.1	1.8, 1.1	0.2	3.3, 0.3	0	3.0, 0
28	**-5.7**	**29.0**	**-2.8**	**17.0**	**-2.3**	**15.9**	**1.1**	**13.4**
1’	-3.5	-	-2.2	-	-0.3	2.7, 2.5	-1.1	5.4, 2.5
2’	-1.6	-	-2.0	-	-	-	-1.2	-
3’/7’	0.2	-0.1	0.2	0.1	0	0.4	0.1	0.3
4’/6’	0.1	0.6	0.1	0.9	0.1	0.8	0.1	0.9
5’	0.3	-	0.3	-	0.1	-	0.2	-
1”	0	0.9	0	1.2	-0.4	1.4	-0.3	1.2
2”	0.1	1.1	0.1	1.4	0.1	1.4	0.1	1.1
3”	0	0.9	0.1	1.2	0	1.2	0	1.2
4”	0	1.2	0	1.8	-0.1	2.4	0	1.8
5”	0	1.0	0	1.3	-0.1	1.7	-0.1	1.3
6”	0	1.5, 0.8	0	2.0, 1.4	-0.2	2.0, 1.4	-0.1	2.3, 0.8

5'-OH		-7.5		-7.3		-7.1		-7.5

[a] All NMR were recorded in DMSO-d_6 using 500 MHz NMR spectrometry. [b] For ease of comparison a numbering scheme corresponding to that of the natural product is used for compounds in this Table. [c] Assignments are made on the basis of DEPT, HMQC, HMBC experiments. [d] $\%\Delta\delta_c = (\delta_c$ of synthetic isomer - δ_c of natural isolate)/ (δ_c of natural isolate) × 100.

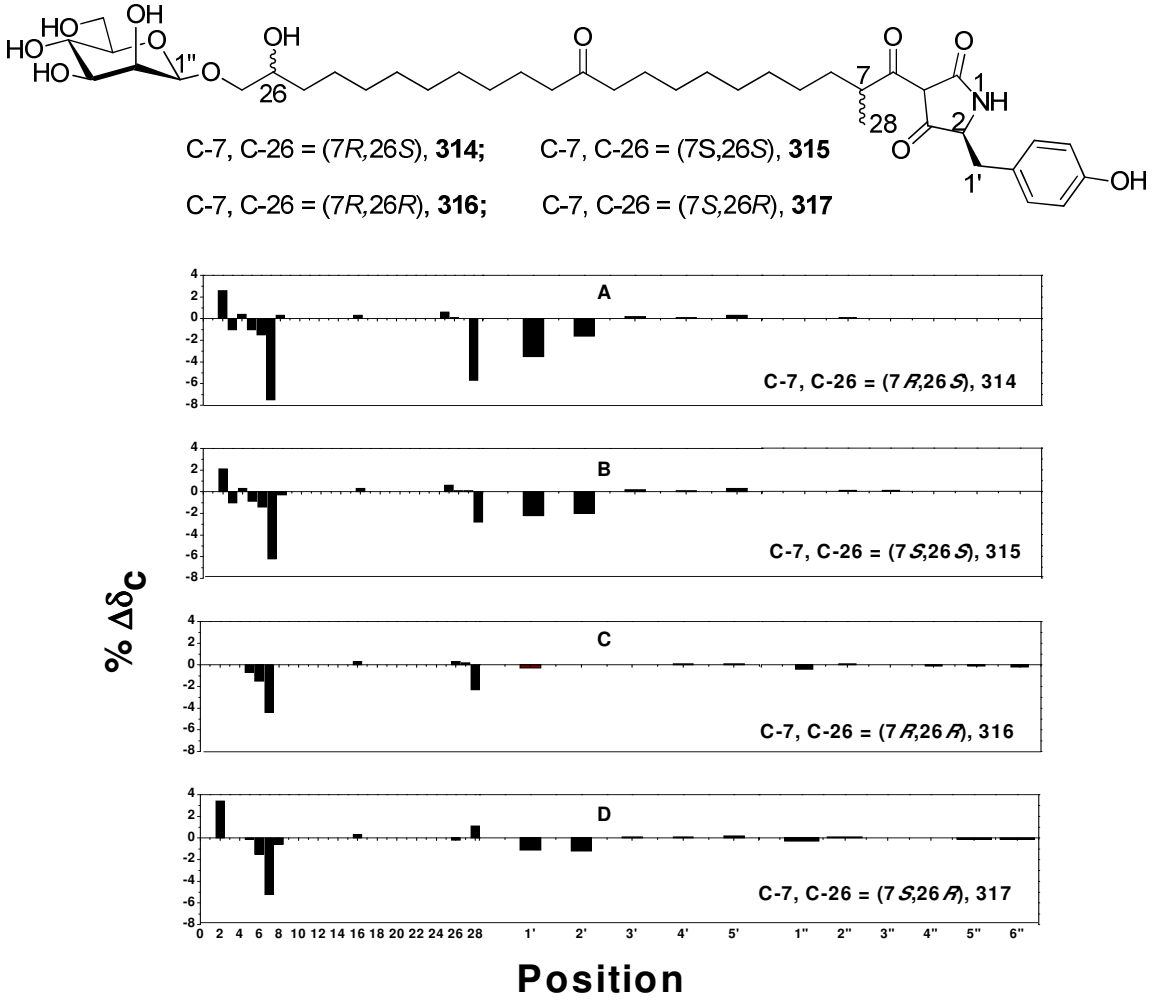


Figure 16. Plot of carbon numbers and $\%\Delta\delta_c$ [$\%\Delta\delta_c = (\delta_c$ of synthetic isomer - δ_c of natural isolate)/ (δ_c of natural isolate) × 100]. A) $\%\Delta\delta_c$ of isomer 314; B) $\%\Delta\delta_c$ of isomer 315; C) $\%\Delta\delta_c$ of isomer 316; D) $\%\Delta\delta_c$ of isomer 317.

From the Table 11 and Figure 16, it is clear that the chemical shifts of none of the four isomers enable a clear distinction to be made among the various isomers just as it is clear that none of these isomers corresponds exactly to the

natural isolate. In addition the specific rotation of each of the four isomers is distinctly different from that of the natural compound (Table 12).

Table 12. [a] Specific Rotation of Synthetic Isomers of Virgineone

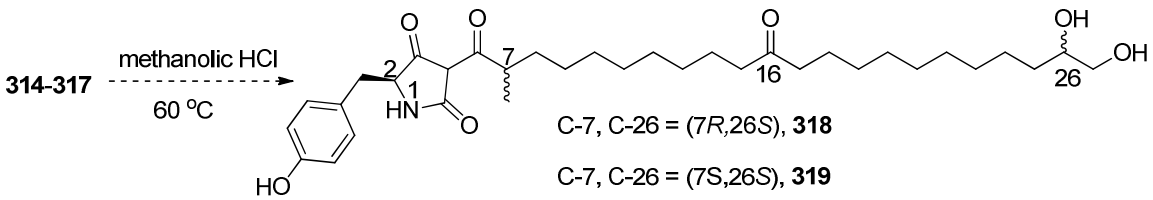
Isomer	**164**	**165**	**166**	**167**
$[\alpha]^{24}_{D}$ (*c* = 0.3 in methanol)	-54.0	-71.7	-47.3	-63.4

[a] $[\alpha]^{23}_{D}$ (isolated virgineone) -95.7 (*c* = 0.3 in methanol)

In spite of the general differences in the data two trends are evident. Thus, the chemical shifts differences of the natural product with the isomers **314** and **315** in the sugar half of the molecule (C-26 = *S*) are smaller than for the isomers **316** and **317** (C-26 = *R*), which indicates that the configuration at C-26 of the natural isolate is likely to be *S*. In the tetramic acid half of the molecule, on the other hand the best correlation is with isomers **315** and **316**. Notably, the differences of chemical shifts between the isolated product and all isomers are quite high in tetramic acid moiety, which suggests that the teramic acid moiety is likely to be derived from the non-proteinogenic amino acid D-tyrosine. Tetramic acids derived from D-amino acids in natural products are known. For example, Fuligorubin A, isolated from yellow slime mould *Fuligo septica* (L) Wiggers, is derived from D-glutamic acid.[270-271] D-Amino acids in general are relatively common in bacterial natural products and are generally synthesized post-translationally by the bacteria from the L-isomer by means of racemase enzymes.[272]

5.4 Conclusions and Feature Direction to the Synthesis of Virgineone

Although it has not been possible to assign the configuration of virgineone, a viable synthetic route has been developed whose applications to the preparation of the remaining four isomers, employing D- rather than L-tyrosine, should enable identification of the correct isomer. In principle, however, the complete synthesis of all four remaining isomers should not be necessary as hydrolysis of the four isomers in hand to the aglycones **318**-**321**, as described for the natural product, will provide all four possible diastereomers of the aglycone for comparison of their spectra data with the one from the natural isolate. Given that the natural compound is likely to be derived from the D-isomers of tyrosine one of these four diastereomers should also give a specific rotation of equal magnitude but opposite sign to that of the natural aglycone, thereby further assisting the identification (Scheme 90). Unfortunately time constraints do not enable those syntheses to be carried out in the context of this thesis. At this point the synthesis of single diastereomers from D-tyrosine should confirm the identity of natural virgineone. Such work is currently underway in the Crich laboratories.



C-7, C-26 = (7*R*,26*S*), **318**

C-7, C-26 = (7S,26*S*), **319**

C-7, C-26 = (7*R*,26*R*), **320**

C-7, C-26 = (7*S*,26*R*), **321**

Scheme 90. Possible hydrolysis of virgineone isomers to the aglycones.

CHAPTER 6

CONCLUSIONS

The potential use of an acid stable 9-fluorenylmethyl (Fm) group to the formation of 9-fluorenylmethylthioesters followed by base-mediated cleavage of the Fm group in the synthesis of amino and peptidyl thioacids has been explored. Thioacids obtained by this methodology were employed successfully for the synthesis of peptides by coupling with electron deficient N-terminal 2,4-dinitrobenzenesulfonamides in an epimerization-free process.

Subsequent of the solution-phase synthesis of amino and peptidyl thioacids, a novel 9-fluorenylmethylthioester-based linker has been developed for the synthesis of peptidyl thioacids on a solid support employing Boc chemistry. The methodology was successfully applied to the synthesis of both partially protected and completely unprotected peptidyl thioacids, by means of modifications of appropriate side chain protection strategies of the amino acids.

The utility of β-thiolactones as convenient synthons for thioacids in three component coupling processes was investigated. The β-thiolactones were shown to undergo nucleophilic ring opening in an S_N2 fashion on treatment with thiolate nucleophiles. The ensuing thioacids were then employed in amide bond forming reactions. In particular, a cysteine-derived β-thiolactone was demonstrated to have the potential to serve as precursor for the synthesis of highly diastereomerically enriched cysteine derived dipeptide in a three component coupling process.

A synthetic route was established for the synthesis of various stereoisomers of the novel glycosylated tetramic acid, virgineone. Although the nature isomer was not one of those synthesized, the route developed should permit access to all other isomers and thus the eventual resolution of this problem.

CHAPTER 7

EXPERIMENTAL SECTION

General: All solvents were dried and distilled by standard protocols. All reactions were conducted under an inert atmosphere of argon or nitrogen unless otherwise stated. All organic extracts were dried over sodium sulfate, and concentrated under aspirator vacuum. Chromatographic purifications were carried out over silica gel. All peptide thioacid syntheses were carried out on a 0.1 mmol scale employing 1% DVB cross linked aminomethyl polystyrene resin (244 mg, resin loading 0.41 mmol/g) in a 10 mL manual synthesizer glass reaction vessel with a Teflon-lined screw cap. The peptide resin was shaken during the both N^{α}-*tert*-butoxycarbonyl deprotection and coupling steps.

Unless otherwise stated optical rotations were recorded on an Autopol® III automatic polarimeter in $CHCl_3$ solution and ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ solution. Melting points were measured on a Barnstead electrothermal (9100) instrument and are uncorrected. Elemental analysis was carried out by Midwest Microlabs, Indianapolis, IN. Mass spectra were recorded by the Research Resources Center at the University of Illinois at Chicago and Central Instrumentation Facility at Wayne State University. Reverse phase HPLC (RP-HPLC) purification was performed with 215 and 254 nm UV detection, using a C-18 analytical and preparative columns (250 × 4.6) and (250 × 21.4), respectively. All runs used linear gradients of A in B (A: CH_3CN and B: Water).

9-Fluorenylmethyl thioacetate (106): A solution of 9-fluorenylmethyl *p*-toluenesulfonate (1.6 g, 4.6 mmol),[173] potassium thioacetate (0.62 g, 5.5 mmol)

and 18-crown-6 (0.12 g, 0.46 mmol) in DMF (15 mL) was stirred at room temperature for 2 h. Then the reaction mixture was poured into ethyl acetate and washed with water and brine. The organic layer was dried and concentrated. Chromatographic purification using 4% ethyl acetate in hexane afforded **106** (1.09 g, 94%). Mp: 72-73 °C (ethyl acetate/ hexane 9:1); ^{1}H NMR (500 MHz) δ 7.77-7.76 (d, J = 7.5 Hz, 2H), 7.68-7.66 (d, J = 7.5 Hz, 2H), 7.43-7.40 (t, J = 7.3 Hz, 2H), 7.36-7.33 (t, J = 7.8 Hz, 2H), 4.20-4.18 (t, J = 5.7 Hz, 1H), 3.55-3.54 (d, J = 6.0 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (125 MHz) δ 195.5, 145.5, 141.1, 127.8, 127.2, 124.7, 120.0, 46.7, 32.5, 30.7; Anal. Calcd for $C_{16}H_{14}OS$ (254.35): C, 75.55; H, 5.55. Found: C, 75.35; H, 5.58.

9-Fluorenylmethylthiol (107): To a stirred solution of **106** (1.0 g, 3.9 mmol) in Et_2O (40 mL), was added DIBAL-H (5.72 mL, 8.6 mmol, 1.5 M solution in toluene) dropwise under nitrogen at -78 °C. After the mixture was warmed up to 0 °C over a period of 30 min, the reaction was quenched by 2 N HCl, and the mixture was vigorously stirred for 10 min. The aqueous layer was extracted with ether, and the combined organic layers were washed with brine. The organic layer was dried and concentrated. Chromatographic purification using 2% ethyl acetate in hexane afforded **107** (0.77 g, 93%). ^{1}H NMR (500 MHz) δ 7.80-7.77 (t, J = 8.0 Hz, 2H), 7.64-7.61 (t, J = 8.5 Hz, 2H), 7.45-7.33 (m, 4H), 4.20-4.16 (m, 1H), 3.22-3.17 (m, 2H), 1.25-1.20 (m, 1H); ^{13}C NMR (125 MHz) δ 145.2, 141.56, 127.7, 127.2, 124.5, 120.1, 49.3, 28.0; ESI-HRMS Calcd for $C_{14}H_{12}S$ M^{+} : 212.0659. Found: 212.0650.

General procedure 1. Synthesis of 9-fluorenylmethyl thiolesters of *N-tert*-butoxycarbonyl-α-amino acids (109-112): To a ~2.0 M solution of *N-tert*-butoxycarbonyl α-amino acid, 9-fluorenylmethylthiol (1.2 equiv.) and DMAP (0.1 equiv.) in methylene chloride, was added a solution of DCC (1.1 equiv.) in methylene chloride (c = 2.0 M) at 0 ℃**.** The suspension was stirred for 1 h at 0 ℃ and overnight at room temperature. The suspension was filtered to remove the resulting white solid which was washed with methylene chloride (10 mL) repeatedly. The filtrate was concentrated. Chromatographic purification afforded the *N-tert*-butoxycarbonyl-α-amino acid 9-fluorenylmethyl thioesters.

***N-tert*-Butoxycarbonyl-L-valine 9-fluorenylmethyl thiolester (109):** Following the general procedure 1, and eluting with 7% ethyl acetate in hexane, **109** was obtained in 99% yield. $[\alpha]^{23}_{D}$ -37.6 (*c* 1.0); ^{1}H NMR (400 MHz) δ: 7.74-7.72 (d, *J* = 7.2 Hz, 2H), 7.64-7.63 (d, *J* = 7.2 Hz, 2H), 7.40-7.28 (m, 4H), 4.91-4.89 (d, *J* = 9.2 Hz, 1H), 4.22-4.16 (m, 2H), 3.56-3.54 (d, *J* = 5.2, 2H), 2.15-2.05 (m, 1H), 1.44 (s, 9H), 0.88-0.86 (d, *J* = 6.8 Hz, 3H), 0.68-0.66 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz) δ: 200.7, 155.5, 145.3, 141.2, 127.7, 127.1, 124.7, 119.8, 80.2, 65.4, 46.7, 31.9, 31.0, 28.3, 19.3, 16.7; ESI-HRMS Calcd for $C_{24}H_{29}NO_3S$ $[M + Na]^+$: 434.1761. Found: 434.1760.

***N-tert*-Butoxycarbonyl-α-aminoisobutyryl 9-fluorenylmethyl thiolester (110):** Following the general procedure 1, and eluting with 10% ethyl acetate in hexane, **110** was obtained in 82% yield. ^{1}H NMR (500 MHz) *δ* 7.74-7.73 (d, *J* = 6.5 Hz, 2H), 7.68-7.67 (d, *J* = 6.0 Hz, 2H), 7.38-7.30 (m, 4H), 5.04 (s, 1H), 4.18 (s, 1H), 3.51 (s, 2H), 1.43 (s, 9H), 1.35 (s, 6H); ^{13}C NMR (125 MHz) *δ* 203.9,

154.1, 145.6, 141.2, 127.6, 127.0, 124.9, 119.8, 80.0, 62.1, 47.0, 32.3, 28.4, 25.5; ESI-HRMS Calcd for $C_{23}H_{27}NO_3S$ [M + Na]$^+$: 420.1604. Found: 420.1616.

***N-tert*-Butoxycarbonyl-L-proline 9-fluorenylmethyl thiolester (111):** Following the general procedure 1, and eluting with 15% ethyl acetate in hexane, **111** was obtained in 97% yield as a mixture of two rotomers. $[\alpha]^{20}_D$ -72.9 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.73-7.71 (d, *J* = 8.0 Hz, 2H), 7.65-7.60 (m, 2H), 7.39-7.36 (t, *J* = 7.5 Hz, 2H), 7.31-7.28 (t, *J* = 6.5 Hz, 2H), 4.40-4.38, 4.27-4.25 (d, *J* = 8.5 Hz, 1H), 4.18-4.16 (t, *J* = 5.5 Hz, 1H), 3.60-3.58 (t, *J* = 5.0, 1H), 3.56-3.3 (m, 3H), 2.06-2.02 (m, 1H), 1.73-1.64 (m, 3H), 1.47, 1.33 (s, 9H); ^{13}C NMR (125 MHz) *δ* 202.3, 201.7, 154.5, 153.9, 145.6, 145.4, 145.3, 145.2, 141.3, 127.7, 127.6, 127.2, 127.1, 127.0, 125.0, 124.9, 124.7, 124.6, 119.9, 119.8, 80.4, 80.2, 66.2, 65.9, 47.0, 46.9, 46.5, 31.7, 31.5, 31.4, 30.6, 28.5, 28.3, 23.9, 23.1; ESI-HRMS Calcd for $C_{24}H_{27}NO_3S$ [M + Na]$^+$: 432.1604. Found: 432.1601.

***α*-Benzyl-*N-tert*-butoxycarbonyl-L-β-aspartyl 9-fluorenylmethyl thiolester (112):** Following the general procedure 1, and eluting with 15% ethyl acetate in hexane, **112** was obtained in 97% yield. $[\alpha]^{20}_D$ +11.4 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.76-7.75 (d, *J* = 7.5 Hz, 2H), 7.63-7.62 (d, *J* = 7.0 Hz, 2H), 7.42-7.39 (m, 4H), 7.35-7.32 (m, 5H), 5.43-5.41 (d, *J* = 8.0 Hz, 1H), 5.15 (s, 2H), 4.58-4.57 (d, *J* = 4.0, 1H), 4.12 (s, 1H), 3.52–3.50 (d, *J* = 5.5 Hz, 2H), 3.22–3.18 (dd, *J* = 4.5, 16.5 Hz, 1H), 3.08–3.03 (dd, *J* = 4.0, 16.5 Hz, 1H), 1.47 (s, 1H); ^{13}C NMR (125 MHz) *δ* 196.5, 170.7, 155.3, 145.3, 145.2, 141.1, 135.3, 128.6, 128.5, 128.4, 127.9, 127.2, 124.8, 124.7, 120.0, 80.2, 67.5, 50.5, 46.6, 45.4, 32.4, 28.4; ESI-HRMS Calcd for $C_{30}H_{31}NO_5S$ [M + Na]$^+$: 540.1816. Found: 540.1812.

***N*-Benzyloxycarbonyl-L-alanyl-L-phenylalanine 9-fluorenylmethyl thioester (116):** To a solution of *N*-benzyloxycarbonyl-L-alanyl-L-phenylalanine (0.228 g, 0.615 mmol) in DMF (10 mL), was added 4Å molecular sieves (0.35 g) and 9-fluorenylmethylthiol (0.18 g, 0.8 mmol), and the mixture was stirred at -20 ℃. After 15 min., PyBOP (0.8 g, 1.54 mmol) and DIEA (0.27 mL, 1.54 mmol) were added and the reaction mixture was stirred at -20 ℃ for 4h, before it was filtered, quenched with saturated solution of NH_4Cl and diluted with methylene chloride. The organic layer was washed with water and brine, dried and concentrated. Chromatographic purification using 30% ethyl acetate in hexane afforded **116** (0.28 g, 81%). $[\alpha]^{22}_D$ -64.0 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.76-7.74 (d, *J* = 7.5 Hz, 2H), 7.61-7.58 (t, *J* = 7.3 Hz, 2H), 7.41-7.18 (m, 12H), 7.05-7.04 (d, *J* = 6 Hz, 2H), 6.61–6.59 (d, *J* = 6 Hz, 1H), 5.31–5.30 (d, *J* = 5.5 Hz, 1H), 5.12–5.02 (m, 2H), 4.91-4.86 (q, *J* = 7.5 Hz, 1H), 4.22–4.21 (m, 1H), 4.16–4.14 (t, *J* = 5.5 Hz, 1H), 3.56–3.46 (m, 2H), 3.05–3.02 (dd, *J* = 5.5, 14.0 Hz, 1H), 2.90–2.86 (t, *J* = 10.3 Hz, 1H), 1.30–1.29 (d, *J* = 5.5 Hz, 3H); ^{13}C NMR (125 MHz) δ 199.1, 171.9, 155.9, 145.2, 141.1, 136.1, 135.4, 129.2, 128.6, 128.5, 128.2, 128.0, 127.8, 127.1, 124.7, 119.9, 67.1, 59.8, 50.3, 46.6, 38.1, 32.4, 18.0; ESI-HRMS Calcd for $C_{34}H_{32}N_2O_4S$ $[M + Na]^+$: 587.1975. Found: 587.1973.

General procedure 2. Synthesis of amino thioacids: A solution of *N*-*tert*-butoxycarbonyl-α-amino acid 9-fluorenylmethyl thioester in 40% piperidine in DMF (c = 0.1 M) was stirred for 1.5h. Then the reaction mixture was concentrated. The concentrate was diluted with ethyl acetate and washed with 1

M HCl solution, water and brine. The organic layer was dried and concentrated. The crude thioacid was taken further without purification.

General procedure 3. Synthesis of *N*-[(2,4-dinitrophenyl)sulfonyl]-α-amino acid methyl esters (117-122): To a ~2 M solution of α-amino acid methyl ester hydrochloride and 2,4-dinitrophenylsulfonyl chloride (1.2 equiv.) in methylene chloride was added a solution of pyridine (4 equiv.) in methylene chloride (c = 4.0 M) at 0 °C. The reaction mixture was stirred at 0 °C for 1h and 4h at room temperature. Then the organic layer was washed with 1M HCl, water, brine, dried and concentrated. Chromatographic purification afforded the *N*-[(2,4-dinitrophenyl)sulfonyl]-α-amino acid methyl esters.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–L-phenylalanine methyl ester (117):**[167] Following the general procedure 3, and eluting with 20% ethyl acetate in hexane, **117** was obtained in 70% yield. Mp: 117-118 °C (ethyl acetate/ hexane 9:1); $[\alpha]^{23}_{D}$ -38.2 (*c* 0.1); ^{1}H NMR (500 MHz) δ 8.61–8.60 (d, *J* = 2.0 Hz, 1H), 8.40–8.38 (dd, *J* = 2, 8.5 Hz, 1H), 8.05–8.04 (d, *J* = 8.5 Hz, 1H), 7.19–7.08 (m, 5H), 6.06 (s, 1H), 4.53–4.51 (dd, *J* = 5.0, 7.5 Hz, 1H), 3.66 (s, 3H), 3.23–3.19 (dd, *J* = 4.5, 14.0 Hz, 1H), 3.07–3.02 (dd, *J* = 8.0, 14 Hz, 1H); ^{13}C NMR (125 MHz) δ 170.8, 149.5, 147.4, 139.6, 134.8, 131.8, 129.3, 128.8, 127.5, 127.2, 120.8, 58.3, 52.9, 38.9.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–D-phenylalanine methyl ester (118):**[273] Following the general procedure 3, and eluting with 20% ethyl acetate in hexane, **118** was obtained in 74% yield. Mp: 119-120 °C (ethyl acetate/ hexane 9:1); $[\alpha]^{23}_{D}$ +38.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 8.60–8.59 (d, *J* = 2.5 Hz, 1H), 8.39–

8.37 (dd, *J* = 2.5, 8.5 Hz, 1H), 8.05–8.03 (d, *J* = 8.5 Hz, 1H), 7.18–7.08 (m, 5H), 6.08 (s, 1H), 4.52 (s, 1H), 3.66 (s, 3H), 3.23–3.19 (dd, *J* = 4.5, 14.0 Hz, 1H), 3.06–3.02 (dd, *J* = 8.0, 14 Hz, 1H); ^{13}C NMR (125 MHz) *δ* 170.8, 149.5, 147.4, 139.6, 134.9, 131.8, 129.3, 128.8, 127.5, 127.2, 120.8, 58.3, 52.9, 38.9.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–L-proline methyl ester (119):** Following the general procedure 3, and eluting with 25% ethyl acetate in hexane, **119** was obtained in 71% yield. $[\alpha]^{23}_{D}$ -69.1 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 8.51–8.46 (m, 2H), 8.33–8.31 (d, *J* = 8.5 Hz, 1H), 4.64–4.62 (m, 1H), 3.68 (s, 3H), 3.65–3.64 (d, *J* = 6.0 Hz, 1H), 2.36-2.29 (m, 1H), 2.12–2.01 (m, 3H); ^{13}C NMR (125 MHz) *δ* 171.9, 149.6, 148.1, 138.5, 132.7, 126.0, 119.5, 61.3, 52.6, 49.0, 31.0, 24.5; ESI-HRMS Calcd for $C_{12}H_{13}N_3O_8S$ $[M + Na]^+$: 382.0316. Found: 382.0312.

***N*-[(2,4-Dinitrophenyl)sulfonyl]-α-aminoisobutyrate methyl ester (120):** Following the general procedure 3, and eluting with 25% ethyl acetate in hexane, **120** was obtained in 63% yield. Mp: 149-150 ℃ (ethyl acetate/ hexane = 9:1); ^{1}H NMR (500 MHz) *δ* 8.71–8.70 (d, *J* = 2 Hz, 1H), 8.56–8.54 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.18 (s, 1H), 3.69 (s, 1H), 1.60 (s, 1H); ^{13}C NMR (125 MHz) *δ* 173.7, 149.5, 147.8, 141.6, 132.0, 127.2, 120.8, 61.0, 53.2, 26.7; Anal. Calcd for $C_{11}H_{13}N_3O_8S$ (374.30): C, 38.04; H, 3.77; N, 12.10. Found: C, 38.16; H, 3.77; N, 11.97.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–L-tryptophan methyl ester (121):[274]** Following the general procedure 3, and eluting with 30% acetone in hexane, **121** was obtained in 65% yield. Mp: 236-237 ℃; literature[274] Mp: 232-234 ℃.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–glycyl-L-proline methyl ester (122):** Following the general procedure 3, and eluting with 3% MeOH in CH_2Cl_2, **122** was obtained in 58% yield as a mixture of two rotomers. $[\alpha]^{22}_D$ -21.5 (*c* 1.0); ^{1}H NMR (500 MHz) δ 8.67–8.66 (d, *J* = 2.0 Hz, 1H), 8.52–8.50 (dd, *J* = 2.0, 8.5 Hz, 1H), 8.27–8.25 (d, *J* = 8.5 Hz, 1H), 6.52 (s, 1H), 4.36–4.29 (m, 1H), 4.17–4.12 (dd, *J* = 6.0, 17.5 Hz, 1H), 4.02–3.98, 3.94–3.90 (dd, *J* = 2.5 Hz, 17.5 Hz, 1H), 3.80, 3.58 (s, 3H), 3.45–3.37 (m, 1H), 2.26–2.23, 2.18–2.10 (m, 1H), 2.06–1.88 (m, 4H); ^{13}C NMR (125 MHz) δ 171.7, 166.0, 165.5, 150.0, 147.8, 139.4, 139.2, 132.3, 127.3, 127.0, 120.8, 58.9, 58.5, 53.1, 52.2, 46.8, 46.0, 45.6, 45.5, 31.2, 28.9, 24.6, 22.1; ESI-HRMS Calcd for $C_{14}H_{16}N_4O_9S$ $[M + H]^+$: 417.0711. Found: 417.0709.

***N*-[(2,4-Dinitrophenyl)sulfonyl]–glycyl-glycine methyl ester (123).** To a stirred solution of glycyl-glycine methyl ester hydrochloride (0.15 g, 0.8 mmol) and $NaHCO_3$ (0.27 g, 3.2 mmol) in THF/ H_2O (1:1, 5mL), was added a solution of 2,4-dinitrophenylsulfonyl chloride (0.21 g, 0.8 mmol) in THF (2 mL) at 0 ℃. The reaction mixture was stirred at 0 ℃ for 30 min. and 2h at room temperature. Then the reaction was poured into 50 mL ethyl acetate and washed with 1M HCl solution, water and brine. The organic layer was dried and concentrated. Chromatographic purification using 4% MeOH in CH_2Cl_2 afforded **123** (0.16 g, 52%). Mp: 167-168 ℃ (acetone/ hexane = 2:1); ^{1}H NMR (400 MHz, DMSO – d_6) δ 8.85–8.84 (d, *J* = 2.4 Hz, 1H), 8.76 (s, 1H), 8.61–8.58 (dd, *J* = 2.4, 8.4 Hz, 1H), 8.44-8.41 (t, *J* = 6.0 Hz, 1H), 8.26–8.23 (d, *J* = 8.4 Hz, 1H), 3.79–3.77 (d, *J* = 6.0 Hz, 2H), 3.75 (s, 2H), 3.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO – d_6) δ 170.4,

168.5, 148.3, 135.8, 130.7, 130.3, 123.7, 116.2, 52.2, 46.0, 41.1; Anal. Calcd for $C_{11}H_{12}N_4O_9S$ (376.30): C, 35.11; H, 3.21; N, 14.89. Found: C, 35.09; H, 3.23; N, 14.66.

General procedure 4. Synthesis of di- and tetra peptides: To a stirred solution of *N-tert*-butoxycarbonyl-α-amino thioacid (1.2 equiv.) in DMF (c = 0.5 M) under nitrogen was added Cs_2CO_3 (1.5 equiv.). After the solution was stirred for 10 min. at room temperature, a solution of *N*-[(2,4-dinitrophenyl)sulfonyl]-α-amino acid methyl ester (1 equiv) in DMF (c = 0.5 M) was added to the above solution and stirring was continued for 1h. Then the reaction mixture was poured into ethyl acetate (50 mL) and washed with an ice cold 1M NaOH solution, water, and brine. The organic layer was dried and concentrated. Chromatographic purification afforded the di- and tetra peptides.

***N-tert*-Butoxycarbonyl-L-valyl-L-phenylalanine methyl ester (124):**[275] Following the general procedure 4, and eluting with 20% ethyl acetate in hexane, **124** was obtained in 81% yield. Mp: 100-101 °C; literature[275] Mp: 101 °C.

***N-tert*-Butoxycarbonyl-L-valyl-D-phenylalanine methyl ester (125):** Following the general procedure 4, and eluting with 22% ethyl acetate in hexane, **125** was obtained in 82% yield. Mp: 104-105 °C (ethanol/ water = 2:1); $[\alpha]^{23}_D$ 37.8 (*c* 1.0); ^{1}H NMR (400 MHz) δ 7.30–7.17 (m, 3H), 7.12–7.10 (d, *J* = 6.8 Hz, 2H), 6.41-6.38 (d, *J* = 8.4 Hz, 1H), 4.95–4.87 (m, 2H), 3.98–3.95 (m, 1H), 3.71 (s, 3H), 3.15–3.05 (m, 2H), 2.16–2.08 (m, 1H), 1.43 (s, 9H), 0.88–0.87 (d, *J* = 7.2 Hz, 3H), 0.81–0.79 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz) δ 171.8, 171.3, 155.8,

135.8, 129.2, 128.7, 127.2, 79.9, 59.6, 53.0, 52.3, 38.0, 30.7, 28.3, 19.3, 17.2; ESI-HRMS Calcd for $C_{20}H_{30}N_2O_5$ $[M + H]^+$: 379.2227. Found: 379.2225.

***N-tert*-Butoxycarbonyl-L-valyl-L-tryptophan methyl ester (126):**[276] Following the general procedure 4, and eluting with 45% ethyl acetate in hexane, **126** was obtained in 82% yield. Mp: 137-138 °C; literature[276] Mp: 136-138 °C.

***N-tert*-Butoxycarbonyl-L-prolyl-L-proline methyl ester (127):**[277] Following the general procedure 4, and eluting with 65% ethyl acetate in hexane, **127** was obtained in 78% yield, whose spectral data corresponded with the literature values.[277]

***N-tert*-Butoxycarbonyl-L-prolyl-L-tryptophan methyl ester (128):**[278] Following the general procedure 4, and eluting with 60% ethyl acetate in hexane, **128** was obtained in 77% yield. Mp: 73-74 °C; literature[278] Mp: 72-74 °C.

***α*-Benzyl-*N-tert*-butoxycarbonyl-L-β-aspartyl-L-proline methyl ester (129):** Following the general procedure 4, and eluting with 60% ethyl acetate in hexane, **129** was obtained in 75% yield as a mixture of two rotomers. $[\alpha]^{19}_D$ -29.2 (*c* 1.0); ^{1}H NMR (400 MHz) *δ* 7.34–7.27 (m, 5H), 5.93–5.90 (d, *J* = 9.6 Hz, 1H), 5.22–5.07 (m, 2H), 4.63–4.59 (m, 1H), 4.43–4.40 (dd, *J* = 2.8, 8.2 Hz, 1H), 3.71, 3.66 (2s, 3H), 3.60–3.53 (m, 1H), 3.44–3.38 (m, 1H), 3.14–3.09 (dd, *J* = 4.4, 17.0 Hz, 1H), 2.77–2.72 (dd, *J* = 4.2 Hz, 16.6 Hz, 1H), 2.14–1.87 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (100 MHz) *δ* 172.4, 171.5, 169.3, 155.9, 135.7, 128.4, 128.1, 128.0, 79.7, 67.2, 67.0, 59.3, 58.5, 52.5, 52.2, 50.2, 47.0, 46.3, 36.7, 36.3, 31.3, 29.2, 28.3, 24.6, 22.5; ESI-HRMS Calcd for $C_{22}H_{30}N_2O_7$ $[M + Na]^+$: 457.1945. Found: 457.1939.

***α*-Benzyl-*N*-*tert*-butoxycarbonyl-L-β-aspartyl-α-aminoisobutyrate methyl ester (130):** Following the general procedure 4, and eluting with 45% ethyl acetate in hexane, **130** was obtained in 79% yield. $[\alpha]^{22}_{D}$ +7.1 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.33-7.29 (m, 5H), 6.36 (s, 1H), 5.80–5.79 (d, *J* = 8.0 Hz, 1H), 5.19–5.12 (q, *J* = 12.5 Hz, 2H), 4.53 (s, 1H), 3.69 (s, 3H), 2.88–2.84 (dd, *J* = 4.0, 15.0 Hz, 1H), 2.70–2.66 (dd, *J* = 4.0, 15.0 Hz, 1H), 1.47 (s, 3H), 1.46 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (125 MHz) *δ* 174.7, 171.2, 169.2, 155.7, 135.5, 128.5, 128.2, 128.1, 79.9, 67.2, 56.6, 52.6, 50.6, 38.0, 28.3, 24.7, 24.6; ESI-HRMS Calcd for $C_{21}H_{30}N_2O_7$ $[M + Na]^+$: 445.1945. Found: 445.1941.

***α*-Benzyl-*N*-*tert*-butoxycarbonyl-L-β-aspartyl-L-phenylalanine methyl ester (131):** Following the general procedure 4, and eluting with 40% ethyl acetate in hexane, **131** was obtained in 82% yield. Mp: 116-117 ℃ (ethanol/ water = 4:1); $[\alpha]^{20}_{D}$ +82.4 (*c* 1.0); ^{1}H NMR (400 MHz) *δ* 7.35–7.23 (m, 8H), 7.03–7.02 (d, *J* = 5.6 Hz, 2H), 6.02–6.00 (d, *J* = 7.6 Hz, 1H), 5.72–5.70 (d, *J* = 8.8 Hz, 1H), 5.18 (s, 2H), 4.84–4.80 (m, 1H), 4.57–4.55 (m, 1H), 3.71 (s, 3H), 3.06–3.05 (d, *J* = 5.6 Hz, 1H), 2.94-2.89 (dd, *J* = 4.4, 16.2 Hz, 1H), 2.73–2.68 (dd, *J* = 4.4, 16.2 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) *δ* 171.6, 171.2, 155.6, 135.6, 135.5, 129.2, 128.6, 128.5, 128.3, 128.1, 127.2, 80.0, 67.3, 53.2, 52.3, 50.4, 37.8, 28.3; Anal. Calcd for $C_{26}H_{32}N_2O_7$ (485.54): C, 64.45; H, 6.54; N, 5.78. Found: C, 64.33; H, 6.54; N, 5.87.

***N*-*tert*-Butoxycarbonyl-α-aminoisobutyryl-L-tryptophan methyl ester (132):** Following the general procedure 4, and eluting with 50% ethyl acetate in hexane, **132** was obtained in 80% yield. $[\alpha]^{22}_{D}$ +37.2 (*c* 1.0); ^{1}H NMR (500 MHz)

δ 8.43 (s, 1H), 7.55-7.53 (d, J = 8.5 Hz, 1H), 7.34–7.32 (d, J = 8.0 Hz, 1H), 7.18–7.15 (t, J = 7.5 Hz, 1H), 7.11–7.08 (t, J = 8 Hz, 1H), 7.03 (s, 1H), 6.91 (s, 1H), 4.99 (s, 1H), 4.90–4.87 (q, J = 5.0 Hz, 1H), 3.63 (s, 3H), 3.35–3.26 (m, 2H), 1.43, 1.39 (2s, 15H); ^{13}C NMR (125 MHz) δ 174.5, 172.4, 154.6, 136.1, 127.6, 123.0, 122.1, 119.4, 118.5, 111.3, 109.9, 56.7, 53.1, 52.2, 28.3, 27.8, 25.5; ESI-HRMS Calcd for $C_{21}H_{29}N_3O_5$ $[M + Na]^+$: 426.1999. Found: 426.1997.

***N-tert*-Butoxycarbonyl-α-aminoisobutyryl-α-aminoisobutyrate methyl ester (133):**[279] Following the general procedure 4, and eluting with 50% ethyl acetate in hexane, **133** was obtained in 80% yield. Mp: 88-89 °C; literature[279] Mp: 91-92 °C.

***N-tert*-Butoxycarbonyl-α-aminoisobutyryl-L-phenylalanine methyl ester (134):**[280] Following the general procedure 4, and eluting with 20% ethyl acetate in hexane, **134** was obtained in 82% yield. Mp: 141-142 °C; $[\alpha]^{20}_D$ +48.9 (*c* 1.0); ^{1}H NMR (400 MHz) δ 7.27-7.20 (m, 3H), 7.12-7.10 (m, 2H), 6.86 (s, 1H), 4.87-4.82 (m, 2H), 3.69 (s, 3H), 3.16-3.07 (m, 2H), 1.44 (s, 3H), 1.41 (s, 12H); ^{13}C NMR (100 MHz) δ 174.2, 171.9, 154.5, 136.1, 129.3, 128.5, 127.0, 56.7, 53.3, 52.1, 38.1, 28.3, 25.7, 25.4.

***N-tert*-Butoxycarbonyl-L-valyl-α-*N*-benzyloxycarbonyl-ε-L-lysine benzyl ester (135):** To a stirred solution of *N-tert*-butoxycarbonyl-L-valyl thioacid (105 mg, 0.5 mmol) in DMF (3 mL) under nitrogen was added Cs_2CO_3 (189 mg, 0.58 mmol). After the solution was stirred for 10 min. at room temperature, successively α-*N*-benzyloxycarbonyl-L-lysine benzyl ester (70 mg, 0.19 mmol) and a solution of *N*-[(2,4-dinitrophenyl)sulfonyl]- L-phenylalanine methyl ester (77

mg, 0.19 mmol) in DMF (3 mL) was added to the above solution and stirring was continued for 2h. Then the reaction mixture was poured into ethyl acetate (50 mL) and washed with an ice cold 1M NaOH solution, water, and brine. The organic layer was dried and concentrated. Chromatographic purification using 22% ethyl acetate in hexane afforded **124** (42 mg, 59%), and 50% ethyl acetate in hexane afforded **135** (25 mg, 23%). Characterizations data of **135**: $[\alpha]^{22}_{D}$ -29.8 (*c* 1.0); ^{1}H NMR (400 MHz) δ 7.36-7.34 (m, 10H), 6.22 (s, 1H), 5.78 (s, 1H), 5.20-5.07 (m, 4H), 4.34-4.33 (d, *J* = 3.2 Hz, 1H), 3.84-3.81 (t, *J* = 6.4 Hz, 1H), 3.29-3.26 (m, 1H), 3.12-3.08 (m, 1H), 2.04-2.01 (m, 1H), 1.82-1.80 (m, 1H), 1.79-1.64 (m, 1H), 1.49-1.46 (m, 1H), 1.42 (s, 9H), 1.35-1.25 (m, 4H), 0.90-0.88 (d, *J* = 6.8 Hz, 3H), 0.82-0.80 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz) δ 172.5, 172.2, 156.3, 136.4, 135.6, 128.8, 128.7, 128.6, 128.5, 128.4, 80.1, 67.3, 60.4, 54.1, 38.7, 31.9, 30.9, 29.9, 29.2, 28.6, 22.7, 19.5, 18.1; EI-HRMS Calcd for $C_{26}H_{35}N_3O_5$ [M – Boc + H]$^{\cdot}$: 469.2577. Found: 469.2589.

***N*-Benzyloxycarbonyl-L-alanyl-L-phenylalanyl-glycyl-L-proline methyl ester (141):** Following the general procedure 4, and eluting with 4% MeOH in CH_2Cl_2, **141** was obtained in 62% yield as a mixture of two rotomers. Mp: 188-189 ℃ (ethanol/ water = 9:1); $[\alpha]^{23}_{D}$ -33.8 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO – d_6) δ 8.14–8.12 (m, 1H), 7.95–7.93 (d, *J* = 8.5 Hz, 1H), 7.40–7.19 (m, 10 H), 7.16–7.15 (d, *J* = 6.5 Hz, 1H), 5.02–4.96 (m, 2H), 4.57–4.55 (m, 1H), 4.32–4.30 (dd, *J* = 4.0, 8.5 Hz, 1H), 4.04–3.97 (m, 2H), 3.89–3.85 (dd, *J* = 4.5, 17.0 Hz, 1H), 3.68, 3.60 (2s, 3H), 3.57–3.49 (m, 2H), 3.06–3.02 (dd, *J* = 4.0, 13.8 Hz, 1H), 2.82–2.77 (m, 1H), 2.16–2.10 (m, 1H), 1.92–1.80 (m, 3H), 1.11-1.10 (d,

J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO – d_6) *δ* 172.8, 172.7, 171.5, 171.4, 167.5, 167.3, 156.0, 138.1, 137.4, 129.7, 129.5, 128.8, 128.6, 128.4, 128.2, 127.7, 127.4, 126.6, 126.5, 65.9, 65.7, 58.9, 58.3, 54.0, 52.9, 52.3, 50.6, 46.7, 46.1, 41.6, 41.4, 38.1, 31.2, 29.1, 24.9, 22.3, 18.9; Anal. Calcd for $C_{28}H_{34}N_4O_7$ (538.59): C, 62.44; H, 6.36; N, 10.40. Found: C, 62.42; H, 6.43; N, 10.36.

***N*-Benzyloxycarbonyl-L-alanyl-L-phenylalanyl-glycyl-glycine methyl ester (142):** Following the general procedure 4, and eluting with 6% MeOH in CH_2Cl_2, **142** was obtained in 64% yield. Mp: 182-183 ℃ (ethanol/ water = 9:1); $[\alpha]^{23}_D$ -7.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO – d_6) *δ* 8.29 (s, 1H), 8.19 (s, 1H), 7.99–7.97 (d, *J* = 7.5 Hz, 1H), 7.43–7.42 (d, *J* = 7.0 Hz, 1H), 7.34– 7.30 (m, 5H), 7.21-7.16 (m, 5H), 5.03–4.96 (m, 2H), 4.50 (s, 1H), 4.02–3.99 (t, *J* = 6.5 Hz, 1H), 3.86–3.85 (d, *J* = 4.0 Hz, 2H), 3.80–3.68 (m, 2H), 3.61 (s, 3H), 3.05–3.03 (d, *J* = 9.5, 1H), 2.85–2.81 (m, 1H), 1.11–1.10 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO – d_6) *δ* 172.8, 171.6, 170.6, 169.6, 156.1, 138.1, 137.4, 129.7, 128.8, 128.4, 128.2, 126.7, 65.9, 54.3, 52.1, 50.7, 42.2, 41.0, 40.9, 37.8, 18.5; Anal. Calcd for $C_{25}H_{30}N_4O_7$ (498.53): C, 60.23; H, 6.07; N, 11.24. Found: C, 60.33; H, 6.08; N, 11.25.

[2-(*tert*-Butoxycarbonylamino)-9*H*-fluoren-9-yl]methyl 4-methylbenzenesulfonate (144): To a stirred solution of [2-(*tert*-butoxycarbonylamino)-9*H*-fluoren-9-yl]methanol[182] (1.8 g, 5.8 mmol) and 4-methylbenzenesulfonyl chloride (1.65 g, 8.7 mmol) in $CHCl_3$ (20 mL) was added pyridine (0.9 mL, 11.6 mmol) at 0 ℃. The reaction mixture was stirred at room temperature for 6 h. Then the organic layer was washed with 1M HCl, water,

brine, dried and concentrated. Chromatographic purification using 30% ethyl acetate in hexane afforded **144** (2.42 g, 90%). Yellowish syrup; ^{1}H NMR (500 MHz) δ 7.78-7.76 (d, J = 8.0 Hz, 2H), 7.66-7.61 (dd, J = 8.5, 12.8 Hz, 2H), 7.57 (s, 1H), 7.51-7.50 (d, J = 7.5 Hz, 1H), 7.38-7.35 (t, J = 7.0 Hz, 2H), 7.31-7.29 (d, J = 8 Hz, 2H), 7.25-7.22 (t, J = 7.5 Hz, 1H), 6.61 (s, 1H), 4.31-4.24 (m, 2H), 4.20-4.17 (t, J = 7.5 Hz, 1H), 2.43 (s, 3H), 1.56 (s, 9H); ^{13}C NMR (125 MHz) δ 153.0, 145.1, 143.7, 142.5, 141.3, 138.0, 136.5, 133.0, 130.1, 128.3, 128.2, 126.7, 125.3, 120.7, 119.8, 118.8, 115.6, 80.9, 72.0, 46.9, 28.6, 21.8; ESI-HRMS Calcd for $C_{26}H_{27}NO_5S$ $[M + Na]^+$: 488.1508. Found: 488.1486.

(2-Amino-9*H*-fluoren-9-yl)methyl 4-methylbenzenesulfonate (145): To a stirred solution of **144** (2.4 g, 5.2 mmol) in CH_2Cl_2 (16 mL), was added TFA (4 mL) dropwise at 0 ℃. The reaction mixture was stirred at same temperature for 20 min before it was neutralized at 0 ℃ by saturated aqueous $NaHCO_3$. Then the organic layer was washed with water, and brine, and dried and concentrated. Chromatographic purification using 40% ethyl acetate in hexane afforded **145** (1.9 g, 100%). Light yellow syrup; ^{1}H NMR (400 MHz) δ 7.78-7.76 (d, J = 8.4 Hz, 2H), 7.57-7.55 (d, J = 7.2 Hz, 1H), 7.50-7.48 (d, J = 8.0 Hz, 1H), 7.44-7.42 (d, J = 7.2 Hz, 1H), 7.34-7.29 (m, 3H), 7.17-7.13 (t, J = 6.4 Hz, 1H), 6.86 (s, 1H), 6.71-6.68 (dd, J = 1.6, 8.4 Hz, 1H), 4.26-4.18 (m, 2H), 4.13-4.09 (t, J = 7.2 Hz, 1H), 3.67 (br s, 2H), 2.41 (s, 3H); ^{13}C NMR (100 MHz) δ 146.4, 145.1, 144.7, 142.0, 141.7, 133.1, 132.3, 130.1, 128.2, 128.1, 125.7, 125.1, 121.1, 119.0, 115.2, 112.2, 72.5, 46.8, 21.9; ESI-HRMS Calcd for $C_{21}H_{19}NO_3S$ $[M + H]^+$: 366.1164. Found: 366.1152.

***N*-[9-(Tosyloxymethyl)-9*H*-fluoren-2-yl]succinamic acid (146):** To a stirred solution of **145** (1.8 g, 4.9 mmol) in THF (10 mL) was added solid succinic anhydride (590 mg, 5.9 mmol) portion wise over a period of 10 min at room temperature. The reaction mixture was stirred at room temperature for 1 h. Then the reaction mixture was concentrated and subjected to chromatographic purification using 5% methanol in dichloromethane when it afforded **146** (2.1 g, 91%). White solid, crystallized from chloroform/hexane, Mp: 165.8-166.2 °C. ^{1}H NMR (400 MHz) δ 7.65-7.63 (m, 3H), 7.55-7.51 (m, 3H), 7.37-7.7.36 (d, J = 7.2 Hz, 1H), 7.26-7.2 (m, 4H), 7.14-7.11 (t, J = 7.2 Hz, 1H), 4.20-4.13 (m, 2H), 4.07-4.04 (t, J = 7.2 Hz, 1H), 2.67-2.62 (m, 4H), 2.32 (s, 3H); ^{13}C NMR (100 MHz) δ 175.6, 171.1, 145.3, 143.3, 142.4, 141.1, 137.7, 137.3, 132.5, 130.1, 128.2, 128.0, 126.8, 125.1, 120.5, 120.1, 120.0, 119.8, 116.8, 72.0, 46.8, 31.7, 29.4, 21.7; ESI-HRMS Calcd for $C_{25}H_{23}NO_6S$ $[M + Na]^+$: 488.1144. Found: 488.1120.

***N*-[9-(Tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (147):** To a stirred solution of **146** (2.0 g, 4.3 mmol) and triphenylmethanethiol (1.5 g, 5.4 mmol) in DMF (15 mL) was added diisopropylethylamine (1.8 mL, 10.8 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 15 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, and brine, and dried and concentrated. Chromatographic purification of the residue using 4% methanol in dichloromethane afforded **147** (2.23 g, 91%). Light brown solid, crystallized from chloroform/hexane, Mp: 84-85 °C. ^{1}H NMR (500 MHz) δ 7.80 (br s, 1H), 7.57-7.53 (m, 4H), 7.43-7.41 (m, 6H), 7.31-7.24 (m, 8H), 7.21-7.7.17 (m, 4H), 3.57-

3.54 (t, *J* = 7.0 Hz, 1H), 2.74-2.70 (m, 3H), 2.67-2.62 (m, 3H); ^{13}C NMR (125 MHz) δ 177.5, 170.5, 147.2, 146.1, 144.9, 140.5, 137.5, 136.8, 130.0, 128.2, 127.7, 127.0, 126.7, 124.9, 120.4, 119.9, 119.7, 116.9, 67.6, 47.2, 36.1, 32.0, 29.7; ESI-HRMS Calcd for $C_{37}H_{31}NO_3S$ $[M + Na]^+$: 592.1922. Found: 592.1892.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (149):** To a stirred solution of *N-tert*-butoxycarbonyl-L-serine (2.0 g, 9.8 mmol) and K_2CO_3 (1.35 g, 9.8 mmol) in DMF (10 mL) was added allyl bromide (1.2 mL, 14.7 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of the solvent, afforded crude *N-tert*-butoxycarbonyl-L-serine allyl ester, which was taken forward without purification.

To a stirred solution of the crude *N-tert*-butoxycarbonyl-L-serine allyl ester (2.3 g, 9.8 mmol) and Fmoc-Cl (3.8 g, 14.7 mmol) in dichloromethane (10 mL) at 0 °C was added dropwise pyridine (1.2 mL, 14.7 mmol). After the addition was complete, the reaction mixture was allowed to warm room temperature and stirred at room temperature for 3 h. Then the reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water, brine, dried and concentrated. Chromatographic purification using 10% ethyl acetate in hexane afforded **149** (3.9 g, 86%). Light yellow syrup; $[\alpha]^{23}_D$ +9.9 (*c* 0.75); ^{1}H NMR (500 MHz) δ: 7.79-7.78 (d, *J* = 7.5 Hz, 2H), 7.62-7.60 (d, *J* = 7.5 Hz, 2H), 7.44-7.41 (t, *J* = 7.5 Hz, 2H), 7.36-7.32 (dt, *J* = 2.5, 7.5 Hz, 2H), 5.97-5.90 (m, 1H), 5.41-5.33 (m, 2H), 5.27-5.25 (d, *J* = 10.5 Hz, 1H), 4.71-4.69 (d, *J* = 7.0 Hz,

1H), 4.66-4.58 (m, 2H), 4.49-4.46 (dd, *J* = 3.5, 11.0 Hz, 1H), 4.42-4.41 (d, *J* = 8.0 Hz, 2H), 4.28-4.25 (t, *J* = 7.5 Hz, 1H), 1.48 (s, 9H); ^{13}C NMR (125 MHz) δ: 169.4, 155.4, 155.0, 143.4, 141.5, 131.5, 128.2, 127.4, 125.4, 120.3, 119.3, 80.7, 70.5, 67.9, 66.7, 53.3, 46.9, 28.5; ESI-HRMS Calcd for $C_{26}H_{29}NO_7$ $[M + Na]^+$: 490.1842. Found: 490.1824.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (150):** A mixture of $Pd(OAc)_2$ (170 mg, 0.8 mmol) and PPh_3 (1.67 g, 6.4 mmol) was stirred in dichloromethane (10 mL) at room temperature for 5 min until a clear yellow solution of tetrakis(triphenylphosphine)palladium(0) was obtained. This solution was transferred *via* cannula to a stirred solution of *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**149**) (3.0 g, 6.4 mmol) and phenylsilane (1.6 mL, 12.8 mmol) in dichloromethane (20 mL) at room temperature and the stirring was continued at same temperature for 1.5 h. Then the reaction mixture was diluted with dichloromethane (20 mL) and was washed with water, brine, dried and concentrated. Chromatographic purification using 50% ethyl acetate in hexane afforded **150** (2.5 g, 91%). White solid, crystallized from ethyl acetate/hexane, Mp: 65-66 °C. $[\alpha]^{22}_D$ +19.4 (*c* 1.7); ^{1}H NMR (500 MHz) δ: 7.77-7.75 (d, *J* = 7.5 Hz, 2H), 7.60-7.59 (d, *J* = 7.0 Hz, 2H), 7.42-7.39 (t, *J* = 7.5 Hz, 2H), 7.33-7.04 (t, *J* = 7.0 Hz, 2H), 5.44-5.43 (d, *J* = 7.5 Hz, 1H), 4.68 (br s, 1H), 4.61-4.59 (d, *J* = 8.5 Hz, 1H), 4.52-4.51 (d, *J* = 8.5 Hz, 1H), 4.42-4.40 (d, *J* = 7.5 Hz, 2H), 4.26-4.23 (t, *J* = 7.5 Hz, 1H), 1.47 (s, 9H); ^{13}C NMR (125 MHz) δ: 173.7, 155.7, 155.0, 143.4, 141.5, 128.2, 127.4, 125.4, 120.3,

81.1, 70.5, 67.6, 53.2, 46.9, 28.5; ESI-HRMS Calcd for $C_{23}H_{25}NO_7$ [M + Na]$^+$: 450.1529. Found: 450.1526.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (151):** Following the same procedure as for the synthesis of *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**149**), compound **151** was synthesized from *N-tert*-butoxycarbonyl-L-threonine in 92% yield. Light yellow syrup; $[\alpha]^{23}_D$ +14.0 (*c* 1.0); ^{1}H NMR (500 MHz) δ: 7.79-7.77 (d, *J* = 7.0 Hz, 2H), 7.61-7.59 (d, *J* = 7.5 Hz, 2H), 7.44-7.41 (t, *J* = 7.5 Hz, 2H), 7.36-7.33 (tt, *J* = 1.5, 7.5 Hz, 2H), 5.93-5.85 (m, 1H), 5.39-5.30 (m, 3H), 5.21-5.19 (d, *J* = 10.5 Hz, 1H), 4.68-4.63 (m, 2H), 4.56-4.54 (dd, *J* = 2.5, 9.7 Hz, 1H), 4.43-4.35 (m, 2H), 4.26-4.23 (t, *J* = 7.5 Hz, 1H), 1.50 (s, 9H), 1.42-1.41 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz) δ: 169.8, 156.1, 154.4, 143.5, 141.5, 131.6, 128.2, 127.4, 125.4, 120.3, 119.3, 80.6, 75.0, 70.3, 66.6, 57.4, 46.9, 28.5, 17.1; ESI-HRMS Calcd for $C_{27}H_{31}NO_7$ [M + Na]$^+$: 504.1998. Found: 504.1974.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine (152):** Following the same procedure as for the synthesis of *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (**150**), compound **152** was synthesized from *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (**151**), in 90% yield. White solid, crystallized from ethyl acetate/hexane, mp: 69-70 °C. $[\alpha]^{23}_D$ +19.6 (*c* 1.34); ^{1}H NMR (500 MHz) δ: 7.75-7.73 (dd, *J* = 3.0, 7.5 Hz, 2H), 7.58-7.55 (t, *J* = 8.5 Hz, 2H, 7.41-7.38 (dt, *J* = 3.0, 7.5 Hz, 2H), 7.32-7.29 (t, *J* = 7.5 Hz, 2H), 5.36-5.33 (m, 2H), 4.50-4.48 (d, *J* = 8.0 Hz, 1H), 4.39-4.34 (m, 2H), 4.22-4.19 (t, *J* = 7.5 Hz, 1H), 1.47 (s, 9H), 1.38-1.37

(d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz) δ: 174.4, 156.3, 154.4, 143.6, 143.4, 141.5, 128.1, 127.4, 125.4, 120.3, 80.8, 74.9, 70.2, 57.4, 46.9, 28.5, 17.2; ESI-HRMS Calcd for $C_{24}H_{27}NO_7$ [M + Na]$^+$: 464.1685. Found: 464.1688.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (153):** Following the same procedure as for the synthesis of *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**149**), compound **153** was synthesized from *N-tert*-butoxycarbonyl-L-tyrosine in 95% yield. Light yellow syrup; [α]$^{23}_D$ +14.7 (*c* 1.5); ^{1}H NMR (500 MHz) δ: 7.82-7.80 (d, *J* = 7.5 Hz, 2H), 7.67-7.66 (d, *J* = 7.5 Hz, 2H), 7.47-7.33 (t, *J* = 7.5 Hz, 2H), 7.38-7.35 (t, *J* = 7.5 Hz, 2H), 7.20-7.18 (d, *J* = 8.5 Hz, 2H), 7.14-7.13 (d, *J* = 8.5 Hz, 2H), 5.60-5.82 (m, 1H), 5.34-5.26 (dd, *J* = 14.0, 25.0 Hz, 2H), 5.04-5.03 (d, *J* = 7.0 Hz, 1H), 4.63-4.62 (t, *J* = 3.0 Hz, 3H), 4.55-4.4.54 (d, *J* = 7.0 Hz, 2H), 4.36-4.33 (t, *J* = 7.0 Hz, 1H), 3.18-3.14 (dd, *J* = 5.5, 13.5 Hz, 1H), 3.12-3.07 (dd, *J* = 5.5, 13.5 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (125 MHz) δ: 171.6, 155.3, 153.8, 150.4, 143.4, 141.6, 134.2, 131.7, 130.7, 128.3, 127.5, 127.3, 125.4, 121.3, 120.4, 120.3, 119.3, 80.3, 70.7, 66.3, 54.6, 47.0, 37.9, 28.6; ESI-HRMS Calcd for $C_{32}H_{33}NO_7$ [M + Na]$^+$: 566.2155. Found: 566.2128.

***N-tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine (154):** Following the same procedure as for the synthesis of *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (**150**), compound **154** was synthesized from *N-tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (**153**), in 92% yield. White solid, crystallized from ethyl acetate/hexane, Mp: 84-85 ℃. [α]$^{22}_D$ -9.3 (*c* 0.6); ^{1}H NMR (500 MHz) δ: 7.81-7.79

(d, *J* = 7.5 Hz, 2H), 7.66-7.65 (d, *J* = 7.5 Hz, 2H), 7.46-7.43 (t, *J* = 7.5 Hz, 2H), 7.37-7.34 (t, *J* = 7.5 Hz, 2H), 7.26-7.24 (d, *J* = 8.5 Hz, 2H), 7.14-7.12 (d, *J* = 8.5 Hz, 2H), 4.77-4.76 (d, *J* = 7.5 Hz, 1H), 4.55-4.53 (d, *J* = 7.5 Hz, 2H), 4.36-4.33 (t, *J* = 7.5 Hz, 1H), 3.87 (br s, 1H), 3.69-3.67 (dd, *J* = 3.5, 11 Hz, 1H), 3.59-3.56 (dd, *J* = 5.0, 10.8 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (125 MHz) δ: 173.7, 156.3, 153.9, 150.0, 143.4, 141.6, 136.0, 130.6, 128.2, 127.5, 125.4, 121.3, 120.4, 80.1, 70.7, 64.4, 53.9, 47.0, 28.6; ESI-HRMS Calcd for $C_{29}H_{29}NO_7$ $[M + Na]^+$: 526.1842. Found: 526.1844.

Synthesis of Boc-L-Met-L-Ala-L-Val-L-Ala-SH (171) employing DIC/HOBt activation method:

(a) Derivatization of aminomethyl polystyrene resin with *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**147**). In a 10 mL glass reaction vessel, aminomethyl polystyrene resin (244 mg, 0.1 mmol) was swelled in DMF (2 mL) for 30 min, after which the solvent was removed by filtration. To a stirred solution of **147** (114 mg, 0.2 mmol) and HOBt (27 mg, 0.2 mmol) in DMF (1 mL) was added DIC (31 µL, 0.2 mmol) at room temperature. The reaction mixture was stirred for 30 min before the activated HOBt ester of **147** was added to the reaction vessel with an additional DMF (1 mL) which was then shaken for 2 h before the resin was washed thoroughly using DMF (3 × 2 mL) and CH_2Cl_2 (3 × 2 mL).

(b) Deprotection of trityl group from derivatized aminomethyl polystyrene resin. To the reaction vessel containing the derivatized resin was added dichloromethane /TFA [v/v 1:1, 1.5 mL, with Et_3SiH (65 µL, 0.4 mmol)]. After

shaking for 1 h the thiol derivatized resin was washed thoroughly using dichloromethane (3 × 2 mL) and a 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(c) Coupling reaction. A stirred solution of Boc-Ala-OH (75 mg, 0.4 mmol) and HOBt (54 mg, 0.4 mmol) in DMF (1 mL) was treated with DIC (62 µL, 0.4 mmol) and then stirred for 30 min before it was added to the thiol derivatized resin with additional DMF (1 mL) and the resulting mixture shaken for 3 h, after which the derivatized resin was washed with DMF (3 × 2 mL) and dichloromethane (3 × 2 mL).

(d) TFA deprotection of N^{α}-Boc group. The derivatized resin was treated with TFA in dichloromethane (25%, 1.5 mL), and shaken for 30 min. The deprotection step was repeated with fresh TFA in dichloromethane (25%, 1.5 mL) for additional 30 min, after which the derivatized resin was washed thoroughly using dichloromethane (3 × 2 mL) and 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(e) The subsequent coupling and N^{α}-Boc deprotection steps were carried out as described in steps (c) and (d) respectively using an appropriate Boc-protected amino acid.

(f) Isolation of Boc-peptide thioacid form the derivatized resin. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin was transferred to a round bottom flask (25 mL) equipped with a magnetic stir bar. A solution of piperidine in DMF (20%, 10 mL) was added into the flask and the mixture was stirred for 20 min, after which the solid was filtered off and the

solution was diluted with EtOAc (20 mL). Then the organic layer was washed successively with 0.5 N aq HCl (2 × 10 mL), brine and dried. Evaporation of the solvent in a rotovapor afforded the peptide thioacid **171** (48 mg, 95%) as white amorphous solid that decomposed prior to melting. ^{1}H NMR (500 MHz, CD_3OD) δ: 4.56-4.52 (q, *J* = 7.0 Hz, 1H), 4.46-4.42 (q, *J* = 7.0 Hz, 1H), 4.28-4.27 (d, *J* = 6.0 Hz, 1H), 2.57-2.51(m, 2H), 2.19-2.15 (q, *J* = 6.5 Hz, 1H), 2.09 (s, 3H), 2.06-2.00 (m, 1H), 1.90-1.84 (m, 1H), 1.45 (s, 9H) 1.41-1.40 (d, *J* = 7.5 Hz, 3H), 1.37-1.36 (d, *J* = 7.0 Hz, 3H), 1.01-1.00 (d, *J* = 6.5 Hz, 3H), 0.98-0.96 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 201.3, 172.9, 172.3, 171.7, 156.1, 80.5, 58.5, 56.3, 53.7, 49.4, 32.1, 31.0, 30.2, 28.4, 19.4, 18.0, 17.5, 15.4; ESI-HRMS Calcd for $C_{21}H_{37}N_4O_6S_2$ $[M - H]^-$: 505.2155. Found: 505.2136.

Synthesis of Boc-L-Met-L-Ala-L-Val-L-Ala-SH (171) employing HBTU and *i*Pr$_2$NEt activation method:

(a) Derivatization of aminomethyl polystyrene resin with *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**147**). In a 10 mL glass reaction vessel, aminomethyl polystyrene resin (244 mg, 0.1 mmol) was swelled in DMF (2 mL) for 30 min, after which the solvent was removed by filtration. To a stirred solution of **147** (114 mg, 0.2 mmol) and HBTU (75 mg, 0.2 mmol) in DMF (1 mL) was added DIPEA (50 µL, 0.3 mmol) at room temperature. The reaction mixture was stirred for 20 min before the activated HOBt ester of **147** was added to the reaction vessel with an additional DMF (1 mL) which was then shaken for 2 h before the resin was washed thoroughly using DMF (3 × 2 mL) and DCM (3 × 2 mL).

(b) Deprotection of trityl group from derivatized aminomethyl polystyrene resin. To the reaction vessel containing the derivatized resin was added DCM/TFA [v/v 1:1, 1.5 mL, with Et_3SiH (65 µL, 0.4 mmol)]. After shaking for 1 h the thiol derivatized resin was washed thoroughly using DCM (3 × 2 mL) and a 5% solution of DIPEA in DCM (2 × 2 mL).

(c) Coupling reaction. A stirred solution of Boc-Ala-OH (75 mg, 0.4 mmol) and HBTU (150 mg, 0.4 mmol) in DMF (1 mL) was treated with DIPEA (100 µL, 0.6 mmol) and then stirred for 20 min before it was added to the thiol derivatized resin with additional DMF (1 mL) and the resulting mixture shaken for 3 h, after which the derivatized resin was washed with DMF (3 × 2 mL) and DCM (3 × 2 mL).

(d) TFA deprotection of N^{α}-Boc group. The derivatized resin was treated with TFA in DCM (25%, 1.5 mL), and shaken for 30 min. The deprotection step was repeated with fresh TFA in DCM (25%, 1.5 mL) for additional 30 min, after which the derivatized resin was washed thoroughly using DCM (3 × 2 mL) and 5% solution of DIPEA in DCM (2 × 2 mL).

(e) The subsequent coupling and N^{α}-Boc deprotection steps were carried out as described in steps (c) and (d) respectively using an appropriate Boc-protected amino acid.

(f) Isolation of Boc-peptide thioacid form the derivatized resin. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin was transferred to a round bottom flask (25 mL) equipped with a magnetic stir bar. A solution of piperidine in DMF (20%, 10 mL) was added into the flask and the

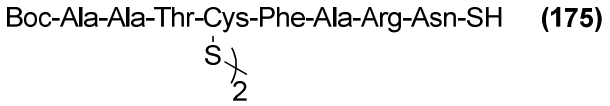
mixture was stirred for 20 min, after which the solid was filtered off and the solution was diluted with EtOAc (20 mL). Then the organic layer was washed successively with 0.5 N aq HCl (2 × 10 mL), brine and dried. Evaporation of the solvent in a rotovapor afforded the peptide thioacid **171** (45 mg, 88%).

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (172): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**171**), Alloc-peptide derivatized resin (substitution level of Alloc-peptide derivatized resin = 0.2161 mmol/g) was synthesized. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (150 mg, 0.0324 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in DMF (20%, 3 mL) was added into the flask and the reaction mixture was stirred for 20 min, after which the solid was filtered off and the solvent was removed at room temperature under vacuum. The residue was washed with dichloromethane (2 × 2 mL) to removed the *N*-[(9*H*-fluoren-9-yl)methyl]piperidine by-product from the crude peptide thioacid, which was subsequently dissolved in acetonitrile/water (v/v 1:1, 3 mL) and subjected to RP-HPLC purification (20 - 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 17 min) to afford the peptide thioacid **172** (37 mg, 80%) as white amorphous solid that decomposed prior to melting.

Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (173): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**171**), Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.2137 mmol/g) was synthesized. Following the same procedure as for

the isolation of **172,** and RP-HPLC purification of crude mixture (20- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 18 min) of Boc-peptide derivatized resin (150 mg, 0.0321 mmol) provided the peptide thioacid **173** (32 mg, 78%) as white amorphous solid that decomposed prior to melting.

Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (174) and



Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**171**), a Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.2208 mmol/g) was synthesized with the following modification of N^{α}-Boc deprotection step. After the cysteine was attached in the sequence, Et_3SiH (0.5%) was added to the solution of 25% TFA in dichloromethane during all N^{α}-Boc deprotection steps. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (50 mg, 0.011 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in acetonitrile (50%, 0.5 mL) was added into the flask and the reaction mixture was stirred for 30 min, after which the solid was filtered off. RP-HPLC purification of the filtrate (0- 50% A in B with a flow rate of 8 mL/min over 72 min and 215 nm UV detection, retention time = 38 min and 41 min) provided the Boc-peptide thioacid **174** (6 mg, 57%) as a white amorphous solid that decomposed prior to melting and the disulfide Boc-peptide thioacid **175** (1 mg, 15%) also as a white amorphous solid that decomposed prior to melting, respectively.

Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val^{8}-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (176): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**171**), a Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.141 mmol/g) was synthesized with the following modification on N^{α}-Boc deprotection and in coupling steps. After N^{α}-Boc deprotection from 8th aminoacid in the sequence, neutralization of TFA from solid was done with a 5% solution of DIPEA in NMP solvent and also NMP was used as coupling solvent. These modifications were followed till up to end of synthesis. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (50 mg, 0.0071 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in acetonitrile (50%, 0.5 mL) was added into the flask and the reaction mixture was stirred for 30 min. Then water (1 mL) was added in to the reaction mixture, which was subsequently stirred for 1 h before the solids which included the insoluble by-product *N*-[(9*H*-fluoren-9-yl)methyl]piperidine and the resin were filtered off. RP-HPLC purification of the filtrate (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 20 min) provided the Boc-peptide thioacid **176** (9 mg, 55%) as white amorphous solid that decomposed prior to melting.

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SBn (179): To a stirred solution of **172** (12 mg, 0.0097 mmol) in DMF (50 µL) were added successively a solution of 2,4,6-collidine/DMF (v/v 15:85, 10 µL, 0.012 mmol) and benzyl bromide (6 µL, 0.048 mmol) at room temperature. The reaction mixture

was stirred for 30 min, after which the solvent was removed at room temperature under vacuum. The crude solid was washed with dichloromethane (2 × 2 mL). The crude peptide thioester was dissolved in acetonitrile/water (v/v 1:1, 1 mL) and RP-HPLC purification (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 29 min) provided the peptide thioester **179** (12 mg, 92%) as a white amorphous solid that decomposed prior to melting.

Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SBn (180): Following the same procedure as for the synthesis of **179,** using Boc-peptide thioacid **173** and RP-HPLC purification of crude mixture (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 34 min) provided the peptide thioester **180** in 86% yield.

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-$NHSO_2$-C_6H_4-NHAc (181): To a stirred solution of **172** (12 mg, 0.0097 mmol) in a mixture of DMF/MeOH (v/v 4:1, 125 µL) was added a solution of 2,4,6-collidine/DMF (v/v 15:85, 10 µL, 0.0116 mmol) followed by stirring for 5 min. 4-Acetamidobenzenesulfonyl azide (4 mg, 0.015 mmol) then was added at room temperature and the reaction mixture stirred for 1 h, after which the solvent was removed at room temperature under vacuum. The crude residue was washed with dichloromethane (2 × 2 mL), then crude peptide thioester was dissolved in acetonitrile/water (v/v 1:1, 1 mL) and RP-HPLC purification of crude mixture (0-50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection,

retention time = 26 min) provided **181** (11 mg, 80%) as white amorphous solid that decomposed prior to melting.

3-Methylthietan-2-one (185), 3-benzylthietan-2-one (199) and thietan-2-one (200) were prepared according to the literature protocols and had spectra data consistent with that given in the literature.[241]

Methyl 1-iodomethylcyclohexane carboxylate (202): Prepared according to the literature procedure,[248] from methyl cyclohexane carboxylate in 65% yield. Light brown liquid; ^{1}H NMR (500 MHz) δ: 3.72 (s, 3H), 3.32 (s, 2H), 2.14-2.11 (m, 2H), 1.60-1.28 (m, 8H); ^{13}C NMR (125 MHz) δ: 174.7, 52.3, 47.7, 34.5, 25.8, 23.2, 15.9; ESI-HRMS Calcd for $C_9H_{15}O_2I$ $[M + Na]^+$: 305.0015. Found: 305.0031.

Methyl 1-acetylsulfanylmethylcyclohexane carboxylate (203): Prepared according to the literature Procedure,[248] from methyl 1-iodomethylcyclohexane carboxylate in 98% yield. Brown syrup; ^{1}H NMR (500 MHz) δ: 3.67 (s, 3H), 3.13 (s, 2H), 2.32 (s, 3H), 2.03-1.59 (m, 2H), 1.59-1.57 (m, 2H), 1.53-1.50 (m, 1H), 1.39-1.28 (m, 5H); ^{13}C NMR (125 MHz) δ: 195.2, 175.8, 52.1, 47.5, 37.4, 33.4, 30.8, 25.7, 23.0; ESI-HRMS Calcd for $C_{11}H_{18}O_3S$ $[M + Na]^+$: 253.0874. Found: 253.0888.

2-Thiaspiro[3.5]nonan-1-one (204): Methyl 1-acetylsulfanylmethylcyclohexane carboxylate (3.92 g, 17 mmol) was dissolved in 22 mL of Claisen's alkali (6.25 mol/L KOH in a mixture of CH_3OH and H_2O (v/v: 3/1)) and heated to reflux for 3 h, and then cooled to 0 °C. The reaction mixture was acidified with 1 N HCl to pH = 1, dichloromethane was added and the

organic layer was extracted, washed with water, and brine, and dried. Evaporation of the solvent, afforded crude 1-(mercaptomethyl) cyclohexane carboxylic acid, which was taken forward for cyclization without purification.

To a stirred solution of the crude 1-(mercaptomethyl) cyclohexane carboxylic acid (2.8 g, 16.1 mmol) and triethylamine (2.6 mL, 17.6 mmol) in dichloromethane (70 mL) at -10 ℃ was added dropwise *iso*-butyl chloroformate (2.4 mL, 17.6 mmol). After the addition was complete, the reaction mixture was allowed to come 0 ℃ over a period of 45 min. The resulting mixture was neutralized at 0 ℃, with 1 N HCl to pH ~ 4 and the organic layer was extracted, dried and concentrated. Chromatographic purification using 2% ethyl acetate in hexane afforded **204** (1.08 g, 43%). Colorless liquid; IR ($CHCl_3$) 2931, 2854, 1771, 1745 cm^{-1}; ^1H NMR (500 MHz) δ: 2.81 (s, 2H), 1.88-1.79 (m, 4H), 1.74-1.70 (m, 2H), 1.52-1.50 (m, 1H), 1.41-1.30 (m, 1H); ^{13}C NMR (125 MHz) δ: 199.2, 77.1, 32.9, 29.5, 25.0, 22.2; EI-HRMS Calcd for $C_8H_{12}OS.[M^{\cdot}]$: 156.0609. Found: 156.0615.

(*S*)-*O*-(*tert*-Butyl) *N*-(2-oxothietan-3-yl) carbamate (205): Following the same procedure as for the preparation of 2-thiaspiro[3.5]nonan-1-one, using *N*-*tert*-butoxycarbonyl-L-cysteine as substrate, and eluting with 20% ethyl acetate in hexane, **205** was obtained in 38% yield. White solid, crystallized from ethyl acetate/hexane, Mp: 140.5-141.5 ℃. $[\alpha]^{22}_D$ -40.3 (*c* 1.2); IR ($CHCl_3$) 3352, 2927, 1747, 1716, 1682 cm^{-1}; ^1H NMR (500 MHz) δ: 5.44 (s, 1H), 5.35 (bs, 1H), 3.44-3.41 (t, *J* = 7.5 Hz, 1H), 3.34-3.31 (t, *J* = 7.5 Hz), 1.46 (s, 9H); ^{13}C NMR (125

MHz) δ: 193.7, 154.4, 81.4, 72.5, 28.4; ESI-HRMS Calcd for $C_8H_{13}NO_3S$ [M + Na]$^+$: 226.0514. Found: 226.0502.

NMR investigation of 205 with a chiral shift reagent: A stock solution of DL-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate (9 mg, 0.044 mmol) in $CDCl_3$ (150 µL) was prepared. 50 µL of the stock solution was introduced into an NMR tube (5 mm i.d.) and diluted with $CDCl_3$ (700 µL), and the ^{1}H NMR spectrum of the sample was recorded at 500 MHz. A stock solution of tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium(III) [Eu(hfc)$_3$] (26 mg, 0.22 mmol) was prepared in $CDCl_3$ (75 µL). Then a 5 µL (~0.1 equiv) increments of the Eu(hfc)$_3$ solution were added to the NMR tube and the ^{1}H NMR spectrum recorded after each addition. The same procedure was repeated for the L-isomer of the carbamate.

General procedure 5. Multi-component coupling reactions using 3-methylthietan-2-one (185), 3-benzylthietan-2-one (199) and thietan-2-one (200), and Mukaiyama's reagent: To a stirred solution of 3-substituted-thietan-2-one **(185, 199** or **200)** (1.0 equiv) in DMF (c = 0.15 – 0.2M) were added aromatic thiol (1.5 equiv) and Cs_2CO_3 (1.0 equiv) at room temperature. The reaction mixture was allowed to stir for ~2 h, after which time the color of the reaction had turned a faint yellow. Then 2-chloro-1-methylpyridinium iodide (1.5 equiv) was added, followed immediately by 0.9 equiv of amine. Upon addition of 2-chloro-1-methylpyridinium iodide and amine the reaction became a dark yellow color and further deepened in color as the reaction continued. The reaction mixture was allowed to stir ~5 h, after which the DMF was removed under high

vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of solvent, followed by column chromatography provided the products as described below.

General procedure 6. Multi-component coupling reactions using 3-methylthietan-2-one (185), 3-benzylthietan-2-one (199) and thietan-2-one (200) and Sanger's reagent: To a stirred solution of 3-substituted-thietan-2-one **(185, 199** or **200)** (1.0 equiv) in DMF (c = 0.15 – 0.2M) were added thiol (1.5 equiv) and Cs_2CO_3 (1.0 equiv) at room temperature. The reaction was allowed to stir for ~2 h, after which time the color of the reaction had turned a faint yellow. Then 1-fluoro-2,4-dinitrobenzene (1.5 equiv) was added followed immediately by 0.9 equiv of amines. Upon addition of 2,4-dinitrofluorobenzene and amine the reaction became a dark red color and further deepened in color as the reaction continued. The reaction mixture was allowed to stir ~2 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of solvent, followed by column chromatography provided the coupled products as described below.

***N*-Phenethyl-3-(phenylthio)propanamide (209):** Following general procedure 5, using thietan-2-one and eluting with 30% ethyl acetate in hexane, **209** was obtained in 66% yield. White solid, crystallized from chloroform/hexane, Mp: 74.5-75.0 °C. ^{1}H NMR (300 MHz) δ: 7.34-7.16 (m, 10H), 5.63 (s, 1H), 3.54-3.48 (q, J = 6.6 Hz, 2H), 3.21-3.16 (t, J = 7.5 Hz, 2H), 2.83-2.78 (t, J = 6.6, 2H), 2.43-2.39 (t, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz) δ: 171.0, 139.0, 135.6, 129.8,

129.3, 129.0, 128.9, 126.8, 126.6, 40.9, 36.4, 35.8, 29.6; ESI-HRMS Calcd for $C_{17}H_{19}NOS$ $[M + Na]^+$: 308.1085. Found: 308.1078.

3-(4-Chlorophenylthio)-*N*-phenethylpropanamide (210): Following general procedure 5, using thietan-2-one and eluting with 32% ethyl acetate in hexane, **210** was obtained in 67% yield. White solid, crystallized from chloroform/hexane, Mp: 78.8-79.5 °C. ^{1}H NMR (300 MHz) δ: 7.33-7.17 (m, 9H), 5.62 (s, 1H), 3.54-3.48 (q, *J* = 6.6 Hz, 2H), 3.18-3.13 (t, *J* = 7.2 Hz, 2H), 2.83-2.78 (t, *J* = 6.6, 2H), 2.41-2.37 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (75 MHz) δ: 170.9, 138.9, 134.2, 132.6, 131.1, 129.4, 129.0, 128.9, 126.8, 40.9, 36.2, 35.8, 29.8; ESI-HRMS Calcd for $C_{17}H_{18}NOSCl$ $[M + Na]^+$: 342.0695. Found: 342.0680.

Methyl 2-(3-oxo-3-(phenethylamino)propylthio)benzoate (211): Following general procedure 5, using thietan-2-one and eluting with 40% ethyl acetate in hexane, **211** was obtained in 56% yield. Light yellow syrup; ^{1}H NMR (300 MHz) δ: 7.95-7.92 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.47-7.40 (m, 1H), 7.36-7.29 (m, 4H), 7.26-7.14 (m, 3H), 5.79 (s, 1H), 3.88 (s, 3H), 3.54-3.47 (q, *J* = 6.6 Hz, 2H), 3.24-3.19 (t, *J* = 7.2 Hz, 2H), 2.82-2.78 (t, *J* = 6.6, 2H), 2.52-2.47 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (75 MHz) δ: 171.1, 167.2, 140.8, 139.0, 132.7, 131.5, 129.0, 128.9, 128.2, 126.8, 126.2, 124.5, 52.4, 41.0, 35.8, 35.5, 28.0; ESI-HRMS Calcd for $C_{19}H_{21}NO_3S$ $[M + Na]^+$: 366.1140. Found: 366.1145.

***N*-3-(4-Chlorophenylthio)propanoyl piperidine (212):** Following general procedure 5, using thietan-2-one and eluting with 40% ethyl acetate in hexane, **212** was obtained in 59% yield. Colorless syrup; ^{1}H NMR (500 MHz) δ: 7.30-7.25 (m, 4H), 3.56-3.54 (t, *J* = 5.5 Hz, 2H), 3.34-3.32 (t, *J* = 5.5 Hz, 2H), 3.24-3.21 (t,

J = 7.5 Hz, 2H), 2.64-2.61 (t, *J* = 7.5 Hz, 2H), 1.67-1.62 (m, 2H), 1.56-1.53 (m, 4H); ^{13}C NMR (125 MHz) δ: 169.2, 134.9, 132.2, 130.6, 129.3, 46.7, 43.1, 33.1, 29.5, 26.7, 25.7, 24.7; ESI-HRMS Calcd for $C_{14}H_{18}NOSCl$ $[M + Na]^+$: 306.0695. Found: 306.0694.

Methyl *N*-(3-(phenylthio)-propanoyl)-L-phenylalaninate (213): Following general procedure 5, using thietan-2-one and eluting with 30% ethyl acetate in hexane, **213** was obtained in 61% yield. Colorless syrup; $[\alpha]^{23}_D$ +59.2 (*c* 0.6); ^{1}H NMR (500 MHz) δ: 7.35-7.20 (m, 8H), 7.11-7.09 (d, *J* = 6.5 Hz, 2H), 6.00 (s, 1H), 4.93-4.89 (q, *J* = 6.0 Hz, 1H), 3.74 (s, 3H), 3.20-3.15 (m, 3H), 3.13-3.09 (dd, *J* = 5.5, 13.8 Hz, 1H), 2.54-2.46 (m, 2H); ^{13}C NMR (125 MHz) δ: 172.1, 170.6, 135.9, 135.4, 130.1, 129.5, 129.3, 128.8, 127.4, 126.7, 53.3, 52.6, 38.1, 36.2, 29.5; ESI-HRMS Calcd for $C_{19}H_{21}NO_3S$ $[M + Na]^+$: 366.1140. Found: 366.1133.

3-(4-Chlorophenylthio)-2-methyl-*N*-phenethylpropanamide (214): Following general procedure 6, using 3-methylthietan-2-one and eluting with 25% ethyl acetate in hexane, **214** was obtained in 67% yield. White solid, crystallized from chloroform/hexane, Mp: 93.0-94.0 °C. ^{1}H NMR (500 MHz) δ: 7.33-7.30 (t, *J* = 7.5 Hz, 2H), 7.27-7.19 (m, 7H), 5.47 (s, 1H), 3.58-3.48 (m, 2H), 3.23-3.18 (dd, *J* = 8.0, 13.5 Hz, 1H), 2.92-2.88 (dd, *J* = 6.5, 13.5 Hz, 1H), 2.84-2.81 (t, *J* = 6.5 Hz, 2H), 2.33-2.26 (m, 1H), 1.22-1.20 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ: 174.4, 139.0, 134.8, 132.4, 130.9, 129.4, 129.1, 128.9, 126.8, 41.5, 40.7, 37.8, 35.8, 17.9; ESI-HRMS Calcd for $C_{18}H_{20}NOSCl$ $[M + Na]^+$: 356.0852. Found: 356.0859.

***N*-3-(4-Chlorophenylthio)-2-methylpropanoyl piperidine (215):** Following general procedure 6, using 3-methylthietan-2-one and eluting with 25% ethyl acetate in hexane, **215** was obtained in 58% yield. White solid, crystallized from chloroform/hexane, Mp: 49.3-50.0 °C. ^{1}H NMR (500 MHz) δ: 7.29-7.25 (m, 4H), 3.61-3.53 (m, 2H), 3.34-3.29 (m, 3H), 2.95-2.91 (m, 2H), 1.66-1.61 (m, 2H), 1.57-1.50 (m, 4H), 1.23-1.22 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz) δ: 173.0, 135.3, 132.3, 130.9, 129.3, 46.9, 43.3, 38.2, 35.8, 27.0, 25.9, 24.8, 17.9; ESI-HRMS Calcd for $C_{15}H_{20}NOSCl$ [M + Na]$^{+}$: 320.0852. Found: 320.0839.

2-Benzyl-*N*-phenethyl-3-(phenylthio)propanamide (216): Following general procedure 5, using 3-benzylthietan-2-one and eluting with 20% ethyl acetate in hexane, **216** was obtained in 64% yield. White solid, crystallized from chloroform/hexane, Mp: 78.1-78.7 °C. ^{1}H NMR (500 MHz) δ: 7.30-7.16 (m, 11H), 7.14-7.12 (d, *J* = 7.5 Hz, 2H), 7.03-7.02 (d, *J* = 7.0 Hz, 2H), 5.23 (s, 1H), 3.46-3.34 (m, 2H), 3.25-3.21 (dd, *J* = 9.0, 13.3 Hz, 1H), 3.08-3.04 (dd, *J* = 5.0, 13.8 Hz, 1H), 2.99-2.95 (dd, *J* = 9.0, 13.3 Hz, 1H), 2.92-2.88 (dd, *J* = 6.5, 13.5 Hz, 1H), 2.72-2.67 (m, 1H), 2.60-2.55 (m, 1H), 2.41-2.36 (m, 1H); ^{13}C NMR (125 MHz) δ: 173.1, 139.2, 139.1, 136.0, 129.3, 129.2, 129.0, 128.8, 128.7, 126.8, 126.6, 126.4, 50.0, 40.7, 38.8, 35.8, 35.6; ESI-HRMS Calcd for $C_{24}H_{25}NOS$ [M + Na]$^{+}$: 398.1555. Found: 398.1549.

2-Benzyl-3-(4-chlorophenylthio)-*N*-phenethylpropanamide (217): Following general procedure 5, using 3-benzylthietan-2-one and eluting with 20% ethyl acetate in hexane, **217** was obtained in 68% yield. White solid, crystallized from chloroform/hexane, Mp: 119.8-120.4 °C. ^{1}H NMR (500 MHz) δ: 7.31-7.20

(m, 8H), 7.13-7.10 (t, *J* = 8.5 Hz, 4H), 7.03-7.02 (d, *J* = 7.0 Hz, 2H), 5.22 (s, 1H), 3.46-3.34 (m, 2H), 3.24-3.19 (dd, *J* = 9.0, 13.3 Hz, 1H), 3.03-3.00 (dd, *J* = 5.0, 13.8 Hz, 1H), 2.98-2.93 (dd, *J* = 9.0, 13.3 Hz, 1H), 2.89-2.85 (dd, *J* = 6.5, 13.5 Hz, 1H), 2.73-2.68 (m, 1H), 2.60-2.55 (m, 1H), 2.38-2.31 (m, 1H); ^{13}C NMR (125 MHz) δ: 173.0, 139.1, 139.0, 134.5, 132.3, 130.6, 129.4, 129.3, 129.2, 129.0, 128.8, 126.9, 126.7, 50.0, 40.7, 38.8, 35.8, 35.7; ESI-HRMS Calcd for $C_{24}H_{24}NOSCl$ $[M + Na]^+$: 432.1165. Found: 432.1167.

***N*-[2-Benzyl-3-(4-chlorophenylthio)]propanoyl piperidine (218):** Following general procedure 6, using 3-benzylthietan-2-one and eluting with 20% ethyl acetate in hexane, **218** was obtained in 59% yield. White solid, crystallized from chloroform/hexane, Mp: 89.0-90.0 °C. ^{1}H NMR (500 MHz) δ: 7.29-7.26 (m, 2H), 7.23-7.20 (m, 3H), 7.18-7.13 (m, 4H), 3.60-3.56 (m, 1H), 3.39-3.35 (m, 1H), 3.35-3.31 (dd, *J* = 8.5, 13.3 Hz, 1H), 3.17-3.12 (m, 1H), 3.07-2.99 (m, 2H), 2.97-2.92 (m, 2H), 2.89-2.85 (dd, *J* = 6.5, 13 Hz, 1H), 1.49-1.43 (m, 4H), 1.36-1.26 (m, 2H); ^{13}C NMR (125 MHz) δ: 171.6, 139.1, 135.0, 132.1, 130.4, 129.3, 129.2, 128.7, 126.8, 46.9, 43.3, 43.2, 39.5, 36.4, 26.3, 25.8, 24.6; ESI-HRMS Calcd for $C_{21}H_{24}NOSCl$ $[M + Na]^+$: 396.1165. Found: 396.1159.

***N*-[2-Benzyl-3-(pyridine-2-ylthio)] propanoyl piperidine (220):** Following general procedure 6, using 3-benzylthietan-2-one and eluting with 25% ethyl acetate in hexane, **220** was obtained in 57% yield. White solid, crystallized from chloroform/hexane, Mp: 73.0-74.0 °C. ^{1}H NMR (500 MHz) δ: 8.41-8.40 (m, 1H), 7.48-7.45 (m, 1H), 7.27-7.15 (m, 6H), 6.99-6.96 (m, 1H), 3.59-3.55 (m, 1H), 3.49-3.38 (m, 4H), 3.13-3.08 (m, 1H), 3.07-2.97 (m, 3H), 1.49-1.41 (m, 4H), 1.26-

1.21 (m, 2H); ^{13}C NMR (125 MHz) δ: 172.3, 159.1, 149.6, 139.8, 136.1, 129.3, 128.5, 126.5, 122.5, 119.6, 46.9, 43.3, 43.2, 39.3, 33.3, 26.3, 25.9, 24.7; ESI-HRMS Calcd for $C_{20}H_{24}N_2OS$ $[M + Na]^+$: 363.1507. Found: 363.1497.

General procedure 7. Multi-component coupling reaction using (*S*)-*O*-(*tert*-butyl) *N*-(2-oxothietan-3-yl) carbamate (205): To a stirred solution of **205** (1.0 equiv) in DMF (c = 0.15 - 0.2M) were added aromatic thiol (1.2 equiv) and Cs_2CO_3 (1.0 equiv) at room temperature. The reaction mixture was allowed to stir for ~6 h, after which time the color of the reaction had turned a faint yellow. Then 2,4-dinitobenbenzene sulfonamide of amine (1.2 equiv) was added to the reaction mixture. Upon addition of sulfonamide the reaction became a dark red, and further deepened in color as the reaction continued. The reaction mixture was allowed to stir ~1 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of solvent, followed by column chromatography provided the coupled products as described below.

(*R*)-*O*-(*tert*-Butyl) *N*-(3-(4-chlorophenylthio)-1-(phenethylaminocarbonyl)propan-2-yl) carbamate (221): Following general procedure 7, eluting with 30% ethyl acetate in hexane, **221** was obtained in 70% yield. White solid, crystallized from ethyl acetate/hexane, Mp: 116.5-117.5 °C. $[\alpha]^{22}_D$ -6.0 (*c* 1.2); ^{1}H NMR (500 MHz) δ: 7.32-7.22 (m, 7H), 7.19-7.17 (d, *J* = 7.0 Hz, 2H), 6.25 (s, 1H), 5.25 (s, 1H), 4.20 (bs, 1H), 3.55-3.40 (m, 2H), 3.26-3.21 (m, 2H), 2.80-2.77 (t, *J* = 7.0 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (125 MHz) δ: 170.2, 155.5, 138.7, 133.5, 133.0, 131.4, 129.5, 129.0, 128.9, 126.9, 80.7, 54.1, 41.0,

36.5, 35.7, 28.5; ESI-HRMS Calcd for $C_{22}H_{27}N_2O_3SCl$ [M + Na]$^+$: 457.1329. Found: 457.1313.

2,4-Dinitro-*N*-phenethylbenzenesulfonamide (222) was prepared according to the literature protocols and had spectra data consistent with that given in the literature.[281]

(*R*)-*O*-(*tert*-Butyl) *N*-3-(pyridin-2-ylthio)-1-(phenethylaminocarbonyl)propan-2-yl) carbamate (223): Following general procedure 7, eluting with 30% ethyl acetate in hexane, **223** was obtained in 61% yield. White solid, crystallized from chloroform/hexane, Mp: 97.0-97.5 °C. $[\alpha]^{22}_D$ -4.7 (*c* 0.9); ^{1}H NMR (500 MHz) δ: 8.31 (s, 1H), 7.54-7.50 (m, 1H), 7.29-7.19 (m, 6H), 7.06-7.03 (t, *J* = 6.0 Hz, 1H), 6.92 (s, 1H), 6.74 (s, 1H), 4.39 (b, 1H), 3.56-3.52 (m, 4H), 2.83-2.80 (t, *J* = 7.0 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (125 MHz) δ: 171.0, 158.8, 156.7, 149.2, 139.1, 136.7, 129.0, 128.8, 126.7, 123.1, 120.3, 80.0, 56.5, 40.9, 36.0, 33.2, 28.5; ESI-HRMS Calcd for $C_{21}H_{27}N_3O_3S$ [M + Na]$^+$: 424.1671. Found: 424.1673.

***N*-(4-Bromophenyl)-2,4-dinitrobenzenesulfonamide (224):** To a solution of 4-bromoaniline (500 mg, 2.9 mmol) and 2,4-dinitrophenylsulfonyl chloride (930 mg, 3.5 mmol) in methylene chloride (3 mL) was added pyridine (350 µL, 4.4 mmol) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 15 min. and 4 h at room temperature. Then the organic layer was washed with 1M HCl, water, brine, dried and concentrated. Chromatographic purification using 20% ethyl acetate in hexane as eluent afforded **224** (680 mg, 58%). Brown solid, crystallized from ethyl acetate/hexane, mp: 143.7-144.4 °C. ^{1}H NMR (500 MHz)

δ: 8.69-8.68 (d, *J* = 2.0 Hz, 1H), 8.44-8.42 (dd, *J* = 2.0, 8.8 Hz, 1H), 8.07-8.06 (d, *J* = 8.8 Hz, 1H), 7.45-7.43 (d, *J* = 8.8 Hz, 2H), 7.29 (s, 1H), 7.12-7.10 (d, *J* = 9.0 Hz); ^{13}C NMR (125 MHz) δ:150.4, 148.7, 137.5, 133.8, 133.7, 133.2, 127.2, 125.3, 121.2, 121.0; ESI-HRMS Calcd for $C_{12}H_7N_3O_6SBr$ $[M - H]^-$: 399.9239. Found: 399.9243.

(*R*)-*O*-(*tert*-Butyl) *N*-3-(phenylthio)-1-(4-bromophenylaminocarbonyl)propan-2-yl) carbamate (225): Following general procedure 7, eluting with 20% ethyl acetate in hexane, **225** was obtained in 57% yield. White solid, crystallized from chloroform/hexane, Mp: 128.0-129.0 °C. $[\alpha]^{22}_D$ -3.5 (*c* 0.95); ^{1}H NMR (500 MHz) δ: 8.31 (s, 1H), 7.43-7.41 (m, 4H), 7.36-7.35 (d, *J* = 9.0 Hz, 2H), 7.32-7.29 (t, *J* = 7.5 Hz, 2H), 7.24-7.21 (t, *J* = 7.5 Hz, 1H), 5.34-5.33 (d, *J* = 7.5 Hz, 1H), 4.35-4.33 (d, *J* = 6.5 Hz, 1H), 3.43-3.33 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (125 MHz) δ: 168.8, 156.0, 136.6, 134.4, 132.2, 130.6, 129.5, 127.4, 121.7, 117.4, 81.4, 54.9, 36.0, 28.5; ESI-HRMS Calcd for $C_{20}H_{23}N_2O_3SBr$ $[M + Na]^+$: 473.0510. Found: 473.0500.

***N*-*tert*-Butoxycarbonyl-(4-chlorophenylsulfanyl)-L-alaninyl-L-phenylalanine methyl ester (226):** Following general procedure 7, eluting with 25% ethyl acetate in hexane, **226** was obtained in 62% yield. Colorless syrup; $[\alpha]^{22}_D$ +15.6 (*c* 1.1); ^{1}H NMR (500 MHz) δ: 7.33-7.25 (m, 7H), 7.10-7.08 (d, *J* = 7.0 Hz, 2H), 6.67-6.65 (d, *J* = 7.5 Hz, 1H), 5.18 (s, 1H), 4.79-4.76 (q, *J* = 6.0 Hz, 1H), 4.21 (s, 1H), 3.73 (s, 3H), 3.24 (bs, 2H), 3.16-3.12 (dd, *J* = 6.0, 13.8 Hz, 1H), 3.10-3.06 (dd, *J* = 5.5, 13.5 Hz, 1H), 1.44 (s, 1H); ^{13}C NMR (125 MHz) δ: 171.6, 169.9, 155.4, 135.8, 133.4, 133.2, 131.8, 129.5, 128.8, 127.4, 80.8, 54.0, 53.6,

52.6, 38.1, 36.6, 28.5; ESI-HRMS Calcd for $C_{24}H_{29}N_2O_5SCl$ [M + Na]$^+$: 515.1383. Found: 515.1371.

(*R*)-*O*-(*tert*-Butyl) *N*-3-(4-chlorophenylthio)-1-(4-acetamidophenylsulfonamido)propan-2-yl) carbamate (227): To a stirred solution of **205** (40 mg, 0.2 mmol) in DMF (1 mL) were added 4-chlorothiophenol (34 mg, 0.24 mmol) and Cs_2CO_3 (64 mg, 0.2 mmol) at room temperature. The reaction mixture was allowed to stir for ~6 h, after which time the color of the reaction had turned a faint yellow. 4-Acetamidobenzenesulfonyl azide (38 mg, 0.16 mmol) was added to the reaction mixture. The reaction mixture was allowed to stir ~1 h, after which the DMF was removed under high vacuum and the crude mixture was purified by column chromatography using 5% methanol in dichloromethane to give **227** (57 mg, 68%). White solid, crystallized from ethyl acetate/hexane, Mp: 169.0-170.0 ℃. $[\alpha]^{22}_D$ -13.1 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, MeOH – d_4) δ: 7.92-7.90 (d, *J* = 8.5 Hz, 2H), 7.74-7.73 (d, *J* = 8.0 Hz, 2H), 7.31-7.26 (m, 4H), 4.15 (bs, 1H), 3.23-3.19 (dd, *J* = 5.5, 13.5 Hz, 1H), 3.09-3.05 (dd, *J* = 7.5, 13.0 Hz, 1H), 2.16 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, MeOH – d_4) δ: 170.9, 156.1, 143.7, 134.3, 133.9, 132.5, 132.0, 131.5, 129.2, 129.1, 129.0, 118.8, 79.8, 54.8, 35.5, 27.5, 22.9; ESI-HRMS Calcd for $C_{22}H_{26}N_3O_6S_2Cl$ [M + Na]$^+$: 550.0849. Found: 550.0826.

1-[(2,4-Dinitrophenylsulfanyl)methyl]-*N*-phenethylcyclohexane carboxamide (228): To a stirred solution of 2-thiaspiro[3.5]nonan-1-one (**204**) (50 mg, 0.32 mmol) in DMF (2 mL) were added 4-chlorothiophenol (70 mg, 0.48 mmol) and Cs_2CO_3 (104 mg, 0.32 mmol) at room temperature. The reaction

mixture was allowed to stir for 2 h before 2,4-dinitrofluorobenzene (90 mg, 0.48 mmol) was added, followed immediately by 2-phenethylamine (36 µL, 0.29 mmol). Upon addition of 2,4-dinitrofluorobenzene and 2-phenethylamine the reaction mixture became a dark red color. The reaction mixture was allowed to stir ~2 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of solvent, followed by column chromatographic purification using 30% ethyl acetate in hexane elutant afforded **228** (102 mg, 80%). Yellow solid, crystallized from ethyl acetate/hexane, Mp: 99.0-99.5 °C. ^{1}H NMR (500 MHz) δ: 9.03-9.02 (d, *J* = 2.0 Hz, 1H), 8.36-8.34 (dd, *J* = 2.5, 9.0 Hz, 1H), 7.65-7.63 (d, *J* = 9.0 Hz, 1H), 7.33-7.20 (m, 5H), 5.88 (s, 1H), 3.54-3.51 (q, 7.0 Hz, 2H), 3.23 (s, 2H), 2.84-2.81 (t, *J* = 7.0 Hz, 2H), 2.01-1.98 (m, 2H), 1.65-1.58 (m, 6H), 1.37-1.35 (m, 2H); ^{13}C NMR (125 MHz) δ: 174.0, 146.7, 145.5, 144.1, 138.8, 129.0, 128.9, 127.9, 127.2, 126.9, 121.7, 46.7, 42.0, 41.1, 35.6, 34.0, 25.7, 22.7; ESI-HRMS Calcd for $C_{22}H_{25}N_3O_5S$ $[M + Na]^+$: 466.1413. Found: 466.1409.

2-Mercaptomethyl-3-phenylpropanoyl morpholine (229): To a stirred solution of 3-benzylthietan-2-one (45 mg, 0.25 mmol) in DMF (2 mL) was added morpholine (26 µL, 0.3 mmol) at room temperature. The reaction mixture was allowed to stir for ~4 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed with water, brine and dried. Evaporation of solvent, followed by column chromatographic purification using 30% ethyl acetate in hexane elutant afforded **229** (45 mg, 67%). Colorless syrup; ^{1}H NMR (300 MHz) δ: 7.30-7.13 (m, 5H), 3.67-3.55 (m, 2H), 3.50-3.12 (m,

4H), 3.11-2.96 (m, 3H), 2.94-2.78 (m, 3H), 2.62-2.53 (m, 1H), 1.52-1.47 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (75 MHz) δ: 172.2, 138.9, 129.2, 128.8, 127.0, 67.0, 66.5, 47.7, 46.3, 42.4, 39.7, 27.4; ESI-HRMS Calcd for $C_{14}H_{19}NO_2S$ $[M + Na]^+$: 288.1034. Found: 288.1021.

(2*S*)-1-Trityloxydecan-2-ol (236): To a stirred solution of (2*S*)-decane-1,2-diol (300 mg, 1.7 mmol), trityl chloride (720 mg, 2.6 mmol), and DMAP (20 mg, 0.17 mmol) in DMF (4 mL) was added Et_3N (0.5 mL, 3.4 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. Then the reaction was quenched with water (5 mL) and extracted with ethyl acetate three times. The combined organic phase was washed with brine, dried and concentrated. Chromatographic purification using 5% ethyl acetate in hexanes afforded **236** (650 mg, 91%) as colorless syrup. $[\alpha]^{23}_D$ +5.5 (*c* 1.5); ^{1}H NMR (500 MHz) *δ* 7.50-7.49 (t, *J* = 2.0 Hz, 6H), 7.36-7.33 (m, 6H), 7.30-7.26 (m, 3H), 3.83-3.79 (m, 1H), 3.24-3.22 (dd, *J* = 3.5, 9.5 Hz, 1H), 3.10-3.06 (dd, *J* = 3.5, 9.5 Hz, 1H), 2.39-2.38 (d, J = 3.5 Hz, 1H), 1.55-1.41 (m, 2H), 1.33-1.29 (m, 12H) 0.94-0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 144.2, 128.9, 128.1, 127.3, 86.9, 71.3, 68.1, 33.6, 32.1, 29.9, 29.8, 29.5, 25.8, 22.9, 14.4; ESI-HRMS Calcd for $C_{29}H_{36}O_2$ $[M + Na]^+$: 439.2613. Found: 439.2595.

(2*S*)-2-Benzyloxy-1-trityloxydecane (237): To an ice-cooled solution of **236** (213 mg, 0.51 mmol) in DMF (2 mL) was added sodium hydride (60%, 41 mg, 1.02 mmol) under stirring. After 30 min, benzyl bromide (91 µL, 0.77 mmol) was added and stirring was continued at 0 ℃ for 1 h. The reaction mixture was concentrated, dissolved in ethyl acetate, and washed with saturated aqueous

NH_4Cl. The aqueous phase was extracted with ethyl acetate three times, and the combined organic phase was washed with water and brine, dried and concentrated. Chromatographic purification using 2% ethyl acetate in hexanes afforded **237** (245 mg, 95%) as colorless syrup. $[\alpha]^{23}_D$ -27.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.52-7.51 (d, *J* = 7.5 Hz, 6H), 7.40-7.24 (m, 14H), 4.76-4.74 (d, *J* = 11.5 Hz, 1H), 4.58-4.56 (d, *J* = 11.5 Hz, 1H), 3.59-3.56 (m, 1H), 3.27-3.24 (dd, *J* = 6.0, 10.0 Hz, 1H), 3.19-3.16 (dd, *J* = 6.0, 10.0 Hz, 1H), 1.58-1.55 (m, 2H), 1.35-1.26 (m, 12H) 0.93-0.90 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 144.5, 139.3, 129.0, 128.5, 128.2, 128.1, 127.7, 127.2, 86.8, 78.8, 72.3, 66.4, 32.4, 32.1, 29.9, 29.8, 29.5, 25.7, 22.9, 14.4; ESI-HRMS Calcd for $C_{36}H_{42}O_2$ $[M + Na]^+$: 529.3083. Found: 529.3078.

(2*S*)-2-Benzyloxydecan-1-ol (238): To a stirred solution of **237** (245 mg, 0.48 mmol) and Et_3SiH (156 µL, 1.0 mmol) in CH_2Cl_2 (4 mL) at room temperature was added TFA (1 mL). The reaction mixture was stirred at same temperature for 20 min before it was neutralized by saturated aqueous $NaHCO_3$. Then the organic layer was washed with water and brine, dried, and concentrated. Chromatographic purification using 15% ethyl acetate in hexane afforded **238** (125 mg, 98%) as colorless syrup. $[\alpha]^{24}_D$ +20.6 (*c* 1.7); ^{1}H NMR (500 MHz) δ 7.37-7.35 (t, *J* = 4.5 Hz, 4H), 7.31-7.30 (m, 1H), 4.65-4.63 (d, *J* = 11.5 Hz, 1H), 4.57-4.55 (d, *J* = 11.5 Hz, 1H), 3.72-3.70 (d, *J* = 8.5 Hz, 1H), 3.57-3.51 (m, 2H), 1.98 (br s, 1H), 1.67-1.62 (m, 1H), 1.53-1.48 (m, 1H), 1.37-1.28 (m, 12H) 0.91-0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 138.8, 128.7, 128.1, 128.0, 80.1,

71.7, 64.5, 32.1, 31.1, 30.1, 29.7, 29.5, 25.6, 22.9, 14.4; ESI-HRMS Calcd for $C_{17}H_{28}O_2$ [M + Na]$^+$: 287.1987. Found: 287.1999.

(2*R*)-1-Trityloxydecan-2-yl 4-nitrobenzoate (239): To a stirred solution of **236** (500 mg, 1.2 mmol), *p*-nitrobenzoic acid (600 mg, 3.6 mmol) and triphenylphosphine (945 mg, 3.6 mmol) in THF (12 mL) at 0 ºC was added dropwise diisopropyl azodicarboxylate (700 µL, 3.6 mmol). After the addition was complete, the reaction mixture was stirred at room temperature for 12 h. The resulting mixture was concentrated and chromatographic purification of the crude using 5% ethyl acetate in hexanes afforded **239** (550 mg, 81%) as light yellow syrup. $[\alpha]^{23}_D$ +6.5 (*c* 1.0); ^{1}H NMR (500 MHz) δ 8.34-8.33 (d, *J* = 9.0 Hz, 2H), 8.28-8.26 (d, *J* = 9.0 Hz, 2H), 7.44-7.42 (m, 6H), 7.28-7.23 (m, 9H), 5.41-5.39 (m, 1H), 3.35-3.32 (dd, *J* = 3.5, 10.5 Hz, 1H), 3.30-3.27 (dd, *J* = 3.5, 10.5 Hz), 1.79-1.77 (m, 2H), 1.30-1.25 (m, 12H), 0.90-0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 164.6, 150.8, 144.0, 136.3, 131.0, 128.9, 128.1, 127.3, 123.8, 86.7, 75.5, 65.0, 32.1, 31.1, 29.7, 29.6, 29.4, 25.4, 22.9, 14.3; ESI-HRMS Calcd for $C_{36}H_{39}NO_5$ [M + Na]$^+$: 588.2726. Found: 588.2723.

(2*R*)-1-Trityloxydecan-2-ol (240): To a solution of **239** (550 mg, 0.97 mmol) in MeOH: THF (v/v 3:1, 10 mL) was added K_2CO_3 (70 mg, 0.5 mmol) and the mixture was stirred at room temperature for 1 h. The solvent was removed and the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried and concentrated. Chromatographic purification using 5% ethyl acetate in hexanes afforded **240** (400 mg, 98%) as

colorless syrup. $[\alpha]^{23}_{D}$ -5.9 (*c* 1.4); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*)-1-trityloxydecan-2-ol (**236**).

(2*R*)-2-Benzyloxy-1-trityloxydecane (241): Following the same procedure as for the preparation of **237**, using **240** (315 mg, 0.75 mmol) as substrate, **241** (345 mg, 90%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ +27.1 (*c* 1.3); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*)-2-benzyloxy-1-trityloxydecane (**237**).

(2*R*)-2-Benzyloxydecan-1-ol (242): Following the same procedure as for the trityl deprotection of **237**, using **241** (345 mg, 0.68 mmol) as substrate, **242** (170 mg, 96%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ -19.0 (*c* 1.1); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*)-2-benzyloxydecan-1-ol (**238**).

General Procedure 8. Mannosylation using the BSP/TTBP/Tf_2O system: To a stirred solution of donor, Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thio-α-D- mannopyranoside (**243**)[257] (1 equiv), BSP (1.2 equiv), TTBP (1.5 equiv), and 4 Å molecular sieves (~1 weight equiv. of the donor) in CH_2Cl_2 (0.05 M in substrate) at -60 °C was added Tf_2O (1.1 equiv). After 30 min of stirring at -60 °C, the reaction mixture was cooled to -78 °C and a solution of the glycosyl acceptor (0.7 equiv) in CH_2Cl_2 (0.05 M + 2 × 0.5 mL rinse) was slowly added. The reaction mixture was allowed to come -60 °C over a period of 3 h, before NEt_3 was added to quench the reaction. The reaction mixture was allowed to reach room temperature, filtered through a pad of Celite, and washed with CH_2Cl_2, after which the filtrate was concentrated.

Chromatographic purification using ethyl acetate in hexanes afforded the corresponding coupled products.

(2*S*)-2-Benzyloxydecan-1-yl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (244): Following the general procedure 8, using **238** (45 mg, 0.17 mmol) as acceptor, and eluting with 10% ethyl acetate in hexanes, **244** (90 mg, 80%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ -46.5 (*c* 1.4); ^{1}H NMR (500 MHz) *δ* 7.52-7.50 (d, *J* = 8.0 Hz, 1H), 7.46-7.45 (d, *J* = 8.0 Hz, 1H), 7.39-7.37 (d, *J* = 9.5 Hz, 2H), 7.32-7.27 (m, 16H), 5.63 (s, 1H), 4.99-4.96 (d, *J* = 12.5 Hz, 1H), 4.88-4.86 (d, *J* = 12.5 Hz, 1H), 4.68-4.65 (d, *J* = 12.0 Hz, 1H), 4.62-4.61 (d, *J* = 2Hz, 2H), 4.58-4.55 (d, *J* = 12.5 Hz, 1H), 4.50 (s, 1H), 4.33-4.30 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.23-4.19 (t, *J* = 10 Hz, 1H), 4.0-3.97 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.96-3.94 (d, *J* = 10.5 Hz, 1H), 3.92-3.91 (d, *J* = 3.0 Hz, 1H), 3.70-3.64 (m, 1H), 3.57-3.53 (m, 2H), 3.35-3.10 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 1.55-1.52 (m, 1H), 1.42-1.38 (m, 1H), 1.30-1.28 (m, 12H) 0.91-0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 139.2, 138.7, 138.6, 137.8, 129.1, 128.9, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 126.3, 103.0, 101.7, 79.0, 78.9, 78.2, 77.5, 76.2, 75.1, 73.8, 72.7, 68.8, 67.8, 32.1, 32.0, 30.0, 29.8, 29.5, 25.6, 22.9, 14.4; ESI-HRMS Calcd for $C_{44}H_{54}O_7$ $[M + Na]^+$: 717.3767. Found: 717.3746.

(2*R*)-2-Benzyloxydecan-1-yl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (245): Following the general procedure 8, using **242** (45 mg, 0.17 mmol) as acceptor, and eluting with 10% ethyl acetate in hexanes, **245** (100 mg, 86%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ -41.1 (*c* 1.2); ^{1}H NMR (500 MHz) *δ* 7.55-7.53 (d, *J* = 8.0 Hz, 2H), 7.50-7.49 (d, *J* = 8.0 Hz, 2H), 7.43-7.28 (m,

16H), 5.65 (s, 1H), 5.04-5.01 (d, *J* = 12.5 Hz, 1H), 4.92-4.89 (d, *J* = 12.5 Hz, 1H), 4.72-4.69 (d, *J* = 12.0 Hz, 1H), 4.67-4.65 (d, *J* = 12Hz, 1H), 4.63-4.61 (d, *J* = 11.5 Hz, 1H), 4.60-4.58 (d, *J* = 11.5 Hz, 1H), 4.46 (s, 1H), 4.36-4.33 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.26-4.22 (t, *J* = 9.5 Hz, 1H), 4.05-4.02 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.99-3.97 (d, *J* = 10.0 Hz, 1H), 3.97-3.95 (d, *J* = 9.5 Hz, 1H), 3.61-3.56 (m, 3H), 3.35-3.10 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 1.67-1.63 (m, 2H), 1.50-1.45 (m, 1H), 1.42-1.38 (m, 1H), 1.35-1.32 (m, 10H) 0.94-0.91 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 139.1, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.0, 127.8, 126.3, 102.8, 101.7, 78.9, 78.2, 78.1, 76.0, 75.0, 72.6, 71.9, 71.4, 68.9, 68.0, 32.2, 32.1, 30.0, 29.8, 29.6, 25.7, 23.0, 14.4.

(2*S*)-2-Hydroxydecan-1-yl β-D-mannopyranoside (231): To a solution of **244** (50 mg, 0.072 mmol) in MeOH: THF (v/v 3:1, 6 mL) was added AcOH (100 µL) and 20% $Pd(OH)_2$/C (20 mg). The mixture was purged with H_2 three times and shaken under H_2 (50 psi) for 12 h. Then the reaction mixture was filtered through a Celite pad and washed with MeOH (3 × 5 mL) and concentrated to afford **231** (22 mg, 92%) as white foam. $[\alpha]^{23}_D$ -19.6 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 4.85 (br s, 1H), 4.73 (br s, 1H), 4.53 (br s, 2H), 4.41 (s, 1H), 4.38 (br s, 1H), 3.70-3.68 (m, 2H), 3.67-3.64 (dd, *J* = 4.5, 10.0 Hz, 1H), 3.58-3.56 (m, 1H), 3.49-3.46 (dd, *J* = 5.5, 10.5 Hz, 1H), 3.38-3.71 (m, 1H), 3.35-3.32 (t, *J* = 10.0 Hz, 1H), 3.29-3.27 (dd, *J* = 3.0, 9.0 Hz, 1H), 3.05-3.02 (m, 1H), 1.41-1.39 (m, 2H), 1.31-1.27 (m, 12H) 0.90-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 100.6, 77.5, 73.7, 73.4, 70.5, 69.1, 67.1, 61.3, 33.6, 31.4,

29.3, 29.1, 28.7, 25.2, 22.2, 14.0; ESI-HRMS Calcd for $C_{16}H_{32}O_7$ $[M + Na]^+$: 359.2046. Found: 359.2056.

(2*R*)-2-Hydroxydecan-1-yl β-D-mannopyranoside (232): Following the same procedure as for the benzyl deprotection of **244**, using **245** (100 mg, 0.144 mmol) as substrate, **232** (47 mg, 97%) was obtained as white foam. $[\alpha]^{23}_D$ -37.4 (*c* 0.7 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.75-4.74 (d, *J* = 4.5 Hz, 1H), 4.57-4.56 (d, *J* = 5.5 Hz, 1H), 4.50-4.49 (d, *J* = 4.0 Hz, 1H), 4.48-4.45 (t, *J* = 5.5 Hz, 1H), 4.40 (s, 1H), 4.30-4.29 (d, *J* = 4.0 Hz, 1H), 3.73-3.68 (m, 2H), 3.65-3.61 (dd, *J* = 7.0, 9.0 Hz, 1H), 3.58-3.56 (m, 1H), 3.49-3.44 (m, 1H), 3.39-3.37 (m, 2H), 3.34-3.31 (dd, *J* = 4.0, 9.0 Hz, 1H), 3.28-3.26 (m, 1H), 3.06-3.03 (m, 1H), 1.41-1.39 (m, 2H), 1.31-1.27 (m, 12H) 0.90-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 100.3, 77.5, 73.7, 73.4, 70.6, 68.9, 67.1, 61.4, 33.4, 31.3, 29.3, 29.1, 28.7, 25.1, 22.1, 14.0.

***N*-(4-Methoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (246):** To a solution of *O*-benzyl-L-tyrosine methyl ester (400 mg, 1.4 mmol) in MeOH (8 mL) were added anisaldehyde (255 µL, 2.1 mmol) and acetic acid (80 µL, 1.4 mmol) at room temperature. After stirring for 10 min at this temperature, the solution was cooled with an ice bath and solid $NaBH_4$ (80 mg, 2.1 mmol) was added. The reaction mixture was stirred for 1 h at 0 □C, and then the solvent was evaporated. The residue was dissolved in ethyl acetate and the organic layer was washed with saturated aqueous Na_2CO_3, water and brine, dried and concentrated. Chromatographic purification using 25% ethyl acetate in hexanes afforded **246** (560 mg, 98%) as colorless foam. $[\alpha]^{23}_D$ +10.4 (*c* 1.25); ^{1}H NMR

(500 MHz) δ 7.47-7.46 (d, J = 7.5 Hz, 2H), 7.43-7.40 (t, J = 7.5 Hz, 2H), 7.37-7.7.34 (t, J = 7.5 Hz, 1H), 7.20-7.18 (d, J = 8.5 Hz, 2H), 7.12-7.10 (d, J = 8.5 Hz, 2H), 6.94-6.92 (d, J = 8.5 Hz, 2H), 6.87-6.85 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 3.81 (s, 3H), 3.79-3.377 (d, J = 13.0 Hz, 1H), 3.68 (s, 3H), 3.63-3.60 (d, J = 13.0 Hz, 1H), 3.55-3.53 (t, J = 7.0 Hz, 1H), 2.95-2.94 (d, J = 6.5 Hz, 2H), 1.94 (b rs, 1H); ^{13}C NMR (125 MHz) δ 175.4, 159.0, 157.9, 137.4, 132.0, 130.5, 129.9, 129.6, 128.8, 128.2, 127.7, 115.1, 114.0, 70.2, 62.3, 55.5, 51.9, 51.7, 39.1; ESI-HRMS Calcd for $C_{25}H_{27}NO_4$ $[M + Na]^+$: 428.1838. Found: 428.1849.

(4*R*)-3-Decanoyl-4-isopropyloxazolidin-2-one (249): To an ice-cooled solution of (4*R*)-4-isopropyloxazolidin-2-one[261] (500 mg, 3.87 mmol) in THF (4 mL) was added sodium hydride (60%, 195 mg, 4.84 mmol) under stirring. After 30 min, decanoyl chloride (950 µL, 4.65 mmol) was added and stirring was continued for 2 h at room temperature. The reaction mixture was concentrated, dissolved in ethyl acetate, and washed with saturated aqueous NH_4Cl. The aqueous phase was extracted with ethyl acetate three times, and the combined organic phase was washed with water and brine, dried and concentrated. Chromatographic purification using 10% ethyl acetate in hexanes afforded **249** (1.06 g, 97%) as colorless syrup. $[\alpha]^{23}_D$ -54.2 (*c* 2.05); ^{1}H NMR (500 MHz) δ 4.44-4.41 (m, 1H), 4.27-4.24 (t, J = 9.0 Hz, 1H), 4.20-4.18 (dd, J = 3.0, 9.0 Hz, 1H), 3.0-2.94 (m, 1H), 2.87-2.80 (m, 1H), 2.34-2.31 (m, 1H), 1.68-1.60 (m, 2H), 1.38-1.25 (m, 12H), 0.91-0.90 (d, J = 7.0 Hz, 3H), 0.88-0.85 (t, J = 7.0 Hz, 3H), 0.87-0.85 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 173.7, 154.3, 63.5, 58.6, 35.7,

32.1, 29.7, 29.6, 29.5, 29.2, 28.6, 24.7, 22.9, 18.1, 14.9, 14.3; ESI-HRMS Calcd for $C_{16}H_{29}NO_3$ $[M + Na]^+$: 306.2045. Found: 306.2038.

(4*S*)-3-Decanoyl-4-isopropyloxazolidin-2-one (250): Following the same procedure as for the preparation of **249**, using (4*S*)-4-isopropyloxazolidin-2-one[261] (300 mg, 2.32 mmol) as substrate, **250** (590 mg, 90%) was obtained as colorless syrup. $[\alpha]^{23}_D$ +55.7 (*c* 2.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (4*R*)-3-decanoyl-4-isopropyloxazolidin-2-one (**249**).

(4*R*,2'*R*)-4-Isopropyl-3-(2'-methyldecanoyl)oxazolidin-2-one (251): To a solution of **249** (1.06 g, 3.74 mmol) in THF (7.5 mL) was added dropwise NaHMDS (2.0 M in THF, 2.2 mL, 4.4 mmol) at -65 °C to give a slightly yellow reaction mixture. After being stirred for 30 min at -65 °C, the reaction mixture was cooled to -78 °C and MeI (0.6 mL, 9.4 mmol) was slowly added. The reaction mixture was then warmed to -20 °C over a period of 2 h and subsequently quenched by the addition of saturated aqueous NH_4Cl solution. The mixture was extracted with CH_2Cl_2 three times, and the combined organic was washed with brine, dried and concentrated (^{1}H NMR of the crude mixture indicate, dr = 9:1). Chromatographic purification of the mixture using 5% ethyl acetate in hexanes provided **251** (900 mg, 81%) as colorless syrup. $[\alpha]^{23}_D$ -85.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 4.46-4.43 (m, 1H), 4.28-4.24 (t, *J* = 9.0 Hz, 1H), 4.21-4.18 (dd, *J* = 3.0, 9.0 Hz, 1H), 3.74-3.70 (q, *J* = 7.0 Hz, 1H), 2.37-2.33 (m, 1H), 1.73-1.68 (m, 1H), 1.38-1.34 (m, 1H), 1.30-1.25 (m, 12H), 1.20-1.19 (d, *J* = 7.0 Hz, 3H) 0.92-0.90 (d, *J* = 7.0 Hz, 3H), 0.89-0.86 (t, *J* = 7.0 Hz, 3H), 0.88-0.87 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 177.6, 153.9, 63.4, 58.7, 37.9, 33.3, 32.1, 30.0, 29.7, 29.5,

28.7, 27.5, 22.9, 18.2, 18.1, 14.9, 14.3; ESI-HRMS Calcd for $C_{17}H_{31}NO_3$ [M + Na]$^+$: 320.2202. Found: 320.2191.

(4*S*,2'*S*)-4-Isopropyl-3-(2'-methyldecanoyl)oxazolidin-2-one (252): Following the same procedure as for the preparation of **251**, using **250** (520 mg, 1.83 mmol) as substrate, a mixture of diastereomers (dr = 9:1) was obtained. Chromatographic purification of the mixture using 5% ethyl acetate in hexanes afforded **252** (435 mg, 80%) as colorless syrup. $[\alpha]^{23}_D$ -83.9 (*c* 0.9); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (4*R*,2'*R*)-4-isopropyl-3-(2'-methyldecanoyl)oxazolidin-2-one (**251**).

(2*R*)-2-Methyldecanoic acid (253): To a stirred solution of **251** (690 mg, 2.32 mmol) and lithium hydroxide (975 mg, 23.2 mmol) in THF: H_2O (v/v 2:1, 21 mL) at 0 ℃ was added dropwise hydrogen peroxide (30%, 2.6 mL, 23.2 mmol). The reaction was stirred at 0 ℃ for 3 h before it was quenched by aqueous sodium sulfite (30%, 10 mL). The reaction mixture was stirred at 0 ℃ for 15 min then acidified to pH 1-2 with aqueous hydrochloric acid (1 M). The mixture was extracted with CH_2Cl_2 three times, and the combined organic was washed with brine, dried and concentrated. Chromatographic purification using 10% ethyl acetate in hexanes provided **253** (410 mg, 95%) as clear oil and eluting with 10% MTB ether in CH_2Cl_2 provided (4*R*)-4-isopropyloxazolidin-2-one (275 mg, 92%) as white solid. $[\alpha]^{23}_D$ -15.7 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz) δ 2.48-2.44 (q, *J* = 7.0 Hz, 1H), 1.71-1.65 (m, 1H), 1.46-1.41 (m, 1H), 1.32-1.23 (m, 12H), 1.19-1.18 (d, *J* = 7.0 Hz, 3H), 0.90-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ

183.8, 39.6, 33.8, 32.1, 29.7, 29.6, 29.5, 27.4, 22.9, 17.0, 14.3; ESI-HRMS Calcd for $C_{11}H_{21}O_2$ [M - H]$^-$: 185.1542. Found: 185.1534.

(2*S*)-2-Methyldecanoic acid (254): Following the same procedure as for the hydrolysis of **251**, using **252** (530 mg, 1.78 mmol) as substrate, **254** (300 mg, 92%) was obtained as clear oil. $[\alpha]^{23}_D$ +14.9 (*c* 1.0 in MeOH); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*)-2-methyldecanoic acid (**253**).

3-(*tert*-Butylthio)-3-oxopropanoic acid:[282] To a stirred solution of malonic acid (4.0 g, 38.4 mmol) and ethyl polyphosphate (PPE)[283] (6.0 g) in CH_2Cl_2: THF (v/v 6:1, 42 mL) at room temperature was added dropwise *tert*-butylthiol (1.1 mL, 9.6 mmol). After stirring for 30 h at room temperature, the reaction mixture was diluted with MTB ether (50 mL) and washed the organic layer with saturated aqueous $NaHCO_3$ (3 × 20 mL). The combined aqueous extracts were acidified to pH 1-2 with aqueous hydrochloric acid (1 M) and extracted with CH_2Cl_2 three times. The combined organic was washed with brine, dried and concentrated. Chromatographic purification using 5% MeOH in CH_2Cl_2 provided (1.66 g, 99%) as white solid. Mp 43-43.5 °C, literature[282] mp 44-45 °C

(4*R*)-*S*-*tert*-Butyl 4-methyl-3-oxothiododecanoate (255): To a solution of 3-(*tert*-butylthio)-3-oxopropanoic acid (1.0 g, 5.65 mmol) in THF (20 mL) was added $Mg(OEt)_2$ (325 mg, 2.84 mmol), and the mixture was stirred at room temperature for 24 h. Evaporation of solvent afforded the magnesium enolate of 3-(*tert*-butylthio)-3-oxopropanoic acid (820 mg, quantitative) as a white powder,[260] which was stored in a dry desiccator.

To a stirred solution of acid **253** (185 mg, 1.0 mmol) in THF (7 mL) was added 1,1'-carbonyldiimidazole (325 mg, 2.0 mmol). After 12 h of stirring at room temperature, magnesium enolate of 3-(*tert*-butylthio)-3-oxopropanoic acid (690 mg, 2.0 mmol) was added. The reaction mixture was stirred for a further 15 h, before saturated NH_4Cl was added to quench the reaction. The mixture was extracted with CH_2Cl_2 three times, and the combined organic was washed with brine, dried and concentrated. Chromatographic purification using 3% ethyl acetate in hexanes provided **255** (250 mg, 83%, a 1:1 mixture of keto and enol forms) as light orange oil. $[\alpha]^{24}_D$ -19.4 (*c* 1.25); ^{1}H NMR (500 MHz) δ 5.32 (s, 1/2 H), 3.60 (s, 1H), 2.69-2.65 (q, *J* = 7.0 Hz, 1/2H), 2.15-2.11 (q, *J* = 7.0 Hz, 1/2H), 1.61-1.57 (m, 1H), 1.52 (s, 9/2H), 1.48 (s, 9/2H), 1.36-1.26 (m, 13H), 1.12-1.10 (d, *J* = 7.0 Hz, 3/2H), 1.10-1.08 (d, *J* = 7.0 Hz, 3/2H), 0.90-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 206.3, 196.5, 192.8, 180.7, 98.6, 57.0, 49.2, 48.3, 46.8, 39.7, 34.3, 32.8, 32.1, 30.4, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 27.3, 22.9, 18.2, 16.2, 14.3; ESI-HRMS Calcd for $C_{17}H_{32}O_2S$ $[M + Na]^+$: 323.2021. Found: 323.2016.

(4*S*)-*S*-*tert*-Butyl 4-methyl-3-oxothiododecanoate (256): Following the same procedure as for the preparation of **255**, using **254** (130 mg, 0.7 mmol) as substrate, **256** (175 mg, 84%, a 1:1 mixture of keto and enol forms) was obtained as light orange oil. $[\alpha]^{24}_D$ +20.3 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (4*R*)-*S*-*tert*-butyl 4-methyl-3-oxothiododecanoate (**255**).

***N*-[(4*R*)-4-Methyl-3-oxododecanoyl]-*N*-(4-methoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (257):** To a stirred solution of **255** (190 mg, 0.62 mmol),

246 (240 mg, 0.59 mmol), and 4 Å molecular sieves (~175 mg) in THF (3 mL) was added Et_3N (430 µL, 3.1 mmol). After 10 min of stirring at room temperature, silver trifluoroacetate (165 mg, 0.75 mmol) was added in one portion, and the reaction mixture was stirred for further 1 h at same temperature. The mixture was then filtered through a pad of Celite, and washed with CH_2Cl_2, after which the filtrate was concentrated. Chromatographic purification using 20% ethyl acetate in hexanes afforded **257** (330 mg, 90%, as a mixture of rotomers) as light yellow syrup. $[\alpha]^{24}_D$ -116.9 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.47-7.44 (t, *J* = 7.0 Hz, 2H), 7.41-7.40 (t, *J* = 7.0 Hz, 2H), 7.38-7.35 (d, *J* = 7.0 Hz, 1H), 7.10-7.04 (m, 4H), 6.93-6.88 (d, *J* = 8.5 Hz, 2H), 6.84-6.80 (d, *J* = 8.5 Hz), 5.08 (s, 2H), 5.03 (s, 2H), 4.41-4.38 (d, *J* = 15.0 Hz, 1H), 3.85-3.82 (d, *J* = 15.0 Hz, 1H), 3.79 (s, 3H), 3.66 (s, 3H), 3.39-3.35 (dd, *J* = 6.0, 14.5 Hz, 1H), 3.09-2.05 (dd, *J* = 8.0, 14.5 Hz, 1H), 2.79-2.75 (q, *J* = 7.0 Hz, 1H), 1.74-1.72 (m, 1H), 1.41-1.37 (m, 1H), 1.31-1.27 (m, 12H), 1.09-1.08 (d, *J* = 7.0 Hz, 3H), 0.89-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 208.1, 183.4, 173.2, 171.5, 170.8, 167.8, 159.4, 157.8, 137.3, 131.6, 130.6, 128.8, 128.1, 127.7, 115.2, 114.3, 70.1, 61.0, 55.6, 55.5, 52.1, 48.0, 46.4, 42.8, 34.7, 33.1, 32.1, 30.0, 29.7, 29.5, 27.4, 22.9, 16.1, 14.3; ESI-HRMS Calcd for $C_{38}H_{49}NO_6$ $[M + Na]^+$: 638.3458. Found: 638.3423.

(5*S*,2'*R*)-5-(4-(Benzyloxy)benzyl)-3-(2'-methyldecanoyl)-1-(4''-methoxybenzyl)pyrrolidine-2,4-dione (258): To a stirred solution of **257** (300 mg, 0.49 mmol) in ^{t}BuOH: THF (v/v 5:1, 7.5 mL) at room temperature was added KO^{t}Bu (65 mg, 0.58 mmol) in one portion. The reaction mixture was stirred at same temperature for 30 min before it was neutralized by saturated aqueous

NH_4Cl. Then the reaction mixture was concentrated, dissolved in ethyl acetate, and washed with saturated aqueous NH_4Cl. The aqueous phase was extracted with ethyl acetate three times, and the combined organic phase was washed with water and brine, dried and concentrated. Chromatographic purification using 20% ethyl acetate in hexanes afforded **258** (260 mg, 92%) as colorless syrup. $[\alpha]^{24}_D$ -211.1 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.45-7.41 (t, *J* = 7.0 Hz, 2H), 7.38-7.35 (t, *J* = 7.0 Hz, 2H), 7.30-7.27 (d, *J* = 7.0 Hz, 1H), 7.08-7.04 (d, *J* = 8.0 Hz, 2H), 7.03-7.0 (d, *J* = 8.0 Hz, 2H), 6.88-6.87 (d, *J* = 8.0 Hz, 2H), 6.86-6.85 (d, *J* = 8.0 Hz, 2H), 5.22-5.19 (d, *J* = 15.0 Hz, 1H), 5.05 (s, 2H), 3.91-3.88 (d, *J* = 15.0 Hz, 1H), 3.81 (s, 3H), 3.63-3.60 (q, *J* = 7.0 Hz, 1H), 3.08-3.05 (m, 2H), 1.56-1.52 (m, 1H), 1.43-1.40 (m, 1H), 1.33-1.22 (m, 12H), 1.18-1.16 (d, *J* = 7.0 Hz, 3H), 0.89-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 195.3, 191.9, 173.7, 161.8, 160.1, 155.3, 131.0, 126.7, 115.4, 103.4, 101.5, 90.7, 65.0, 55.9, 55.6, 36.4, 33.9, 33.6, 32.8, 29.9, 29.7, 27.3, 22.9, 17.1, 14.3; ESI-HRMS Calcd for $C_{37}H_{45}NO_5$ $[M + Na]^+$: 606.3195. Found: 606.3190.

***N*-(2,4,6-Trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (260):** A suspension of *O*-benzyl-L-tyrosine methyl ester hydrochloride (1.43 g, 5.0 mmol), 1,3,5-trimethoxybenzene (1.26 g, 7.5 mmol) and paraformaldehyde (225 mg, 7.5 mmol) in MeOH (5 mL) was heated to reflux for 5 h. The reaction mixture was concentrated, dissolved in CH_2Cl_2, and washed with saturated aqueous $NHCO_3$. The aqueous phase was extracted with CH_2Cl_2 three times, and the combined organic phase was washed with water and brine, dried and concentrated. Chromatographic purification using 55% ethyl acetate in hexanes afforded **260**

(1.95 g, 84%) as colorless foam. $[\alpha]^{25}_{D}$ +27.4 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.44-7.43 (d, *J* = 7.5 Hz, 2H), 7.40-7.37 (t, *J* = 7.5 Hz, 2H), 7.34-7.33 (d, *J* = 7.5 Hz, 1H), 7.04-7.03 (d, *J* = 8.5 Hz, 2H), 6.88-6.86 (d, *J* = 8.5 Hz, 2H), 6.05 (s, 2H), 5.04 (s, 2H), 3.80 (s, 3H), 3.77 (s, 2H), 3.67 (s, 6H), 3.60 (s, 3H), 3.45-3.42 (dd, *J* = 6.0, 8.0 Hz, 1H), 2.93-2.89 (dd, *J* = 6.0, 10.0 Hz, 1H), 2.86-2.82 (dd, *J* = 6.0, 10.0 Hz, 1H), 2.09 (br s, 1H); ^{13}C NMR (125 MHz) *δ* 175.3, 160.6, 159.5, 157.7, 137.4, 130.3, 130.2, 128.8, 128.2, 127.7, 114.9, 108.4, 90.4, 77.5, 70.2, 62.0, 55.7, 55.5, 51.7, 39.9, 38.9; ESI-HRMS Calcd for $C_{27}H_{31}NO_6$ $[M + Na]^+$: 488.2049. Found: 488.2040.

***N*-[(4*R*)-4-Methyl-3-oxododecanoyl]-*N*-(2,4,6-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (261):** To a stirred solution of **255** (65 mg, 0.22 mmol), **260** (100 mg, 0.22 mmol), and 4 Å molecular sieves (~60 mg) in THF (2.2 mL) was added Hunig's base (190 µL, 1.1 mmol). After 10 min of stirring at room temperature, silver trifluoroacetate (55 mg, 0.26 mmol) was added in one portion, and the reaction mixture was stirred for further 1 h at same temperature. The mixture was then filtered through a pad of Celite, and washed with CH_2Cl_2, after which the filtrate was concentrated. Chromatographic purification using 35% ethyl acetate in hexanes afforded **261** (135 mg, 93%, as a mixture of rotomers) as light yellow syrup. $[\alpha]^{24}_{D}$ -77.2 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.45-7.44 (d, *J* = 7.5 Hz, 2H), 7.41-7.38 (t, *J* = 7.5 Hz, 2H), 7.34-7.33 (d, *J* = 7.5 Hz, 1H), 6.95-6.94 (d, *J* = 8.5 Hz, 2H), 6.80-6.78 (d, *J* = 8.5 Hz), 6.03 (s, 2H), 5.03 (s, 2H), 4.35-4.33 (d, *J* = 15.0 Hz, 1H), 4.17-4.14 (d, *J* = 15.0 Hz, 1H), 3.89 (s, 2H), 3.81 (s, 3H), 3.70 (s, 6H), 3.61 (s, 3H), 3.39-3.35 (dd, *J* = 6.0, 14.5 Hz, 1H), 3.02-2.97

(dd, *J* = 8.0, 14.5 Hz, 1H), 2.79-2.75 (q, *J* = 7.0 Hz, 1H), 1.74-1.72 (m, 1H), 1.41-1.37 (m, 1H), 1.31-1.27 (m, 12H), 1.16-1.15 (d, *J* = 7.0 Hz, 3H), 0.89-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 209.0, 171.7, 167.5, 161.7, 159.9, 157.3, 137.5, 131.6, 130.6, 128.8, 128.1, 127.7, 114.5, 104.2, 90.5, 70.1, 61.0, 55.6, 55.5, 52.1, 48.0, 46.4, 42.8, 34.7, 33.1, 32.1, 30.0, 29.7, 29.5, 27.4, 22.9, 16.0, 14.3; ESI-HRMS Calcd for $C_{40}H_{53}NO_8$ $[M + Na]^+$: 698.3669. Found: 698.3666.

***N*-[(4*S*)-4-Methyl-3-oxododecanoyl]-*N*-(2,4,6-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (262):** Following the same procedure as for the preparation of **261**, using **256** (85 mg, 0.28 mmol) as substrate, **262** (175 mg, 91%, as a mixture of rotomers) was obtained as light yellow syrup. $[\alpha]^{24}_D$ -98.4 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.45-7.44 (d, *J* = 7.0 Hz, 2H), 7.40-7.37 (t, *J* = 7.0 Hz, 2H), 7.34-7.33 (d, *J* = 7.0 Hz, 1H), 6.96-6.94 (d, *J* = 8.5 Hz, 2H), 6.80-6.78 (d, *J* = 8.5 Hz), 6.03 (s, 2H), 5.03 (s, 2H), 4.35-4.32 (d, *J* = 15.0 Hz, 1H), 4.17-4.14 (d, *J* = 15.0 Hz, 1H), 3.90 (s, 2H), 3.81 (s, 3H), 3.72 (s, 6H), 3.59 (s, 3H), 3.39-3.35 (dd, *J* = 6.0, 14.5 Hz, 1H), 3.02-2.98 (dd, *J* = 8.0, 14.5 Hz, 1H), 2.79-2.75 (q, *J* = 6.5 Hz, 1H), 1.74-1.72 (m, 1H), 1.33-1.27 (m, 13H), 1.15-1.14 (d, *J* = 6.5 Hz, 3H), 0.89-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 209.0, 171.7, 167.6, 161.7, 159.9, 157.3, 137.5, 131.6, 130.6, 128.8, 128.1, 127.7, 114.5, 104.3, 90.6, 70.1, 61.0, 55.6, 52.1, 48.0, 46.4, 42.8, 34.6, 33.1, 32.1, 30.0, 29.8, 29.5, 27.4, 22.9, 16.1, 14.3.

(5*S*,2'*R*)-5-(4-Hydroxybenzyl)-3-(2'-methyldecanoyl)-1-(2'',4'',6''-trimethoxybenzyl)pyrrolidine-2,4-dione (263): To a solution of **261** (100 mg, 0.148 mmol) in MeOH: THF (v/v 3:1, 8 mL) was added AcOH (50 µL) and 20%

$Pd(OH)_2/C$ (20 mg). The mixture was purged with H_2 three times and shaken under H_2 (50 psi) for 3 h. Then the reaction mixture was filtered through a Celite pad and washed with ethyl acetate (3 × 5 mL). Evaporation of the solvent afforded the corresponding debenzylated ketoamide, which was taken forward for cyclization without purification.

To a stirred solution of the ketoamide (65 mg, 0.113 mmol) in ^tBuOH: THF (v/v 5:1, 2 mL) at room temperature was added KO^tBu (20 mg, 0.17 mmol) in one portion. The reaction mixture was stirred at same temperature for 30 min before it was neutralized by saturated aqueous NH_4Cl. Then the reaction mixture was concentrated, dissolved in ethyl acetate, and washed with saturated aqueous NH_4Cl. The aqueous phase was extracted with ethyl acetate three times, and the combined organic phase was washed with water and brine, dried and concentrated. Complete evaporation of the solvent afforded **263** (57 mg, 91%) as colorless syrup, which required no further purification. $[\alpha]^{24}_D$ -182.2 (*c* 1.0); ^1H NMR (500 MHz) δ 6.96-6.94 (d, *J* = 8.0 Hz, 2H), 6.65-6.64 (d, *J* = 8.0 Hz), 6.14 (s, 2H), 5.13-5.10 (d, *J* = 14.0 Hz, 1H), 4.43-4.40 (d, *J* = 14.0 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 6H), 3.65-3.63 (t, *J* = 4.0 Hz, 1H), 3.51-3.47 (q, *J* = 7.0 Hz, 1H), 3.08-3.05 (m, 2H), 1.56-1.52 (m, 1H), 1.33-1.22 (m, 13H), 1.11-1.10 (d, *J* = 7.0 Hz, 3H), 0.89-0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 195.3, 191.9, 173.7, 161.8, 160.1, 155.3, 131.0, 126.7, 115.4, 103.4, 101.5, 90.7, 65.0, 55.9, 55.6, 36.4, 33.9, 33.6, 32.8, 29.9, 29.7, 27.3, 22.9, 17.1, 14.3; ESI-HRMS Calcd for $C_{32}H_{43}NO_7$ $[M + Na]^+$: 576.2937. Found: 576.2894.

(5*S*,2'*S*)-5-(4-Hydroxybenzyl)-3-(2'-methyldecanoyl)-1-(2'',4'',6''-trimethoxybenzyl)pyrrolidine-2,4-dione (264): Following the same reaction sequences as for the preparation of **263**, using **262** (125 mg, 0.184 mmol) as substrate, **264** (92 mg, 90%) was obtained as colorless syrup. $[\alpha]^{24}_{D}$ -243.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 6.95-6.94 (d, *J* = 8.5 Hz, 2H), 6.66-6.64 (d, *J* = 8.5 Hz), 6.14 (s, 2H), 5.13-5.10 (d, *J* = 14.0 Hz, 1H), 4.44-4.42 (d, *J* = 14.0 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 6H), 3.66-3.64 (t, *J* = 4.0 Hz, 1H), 3.51-3.47 (q, *J* = 7.0 Hz, 1H), 3.08-3.05 (m, 2H), 1.62-1.58 (m, 1H), 1.41-1.37 (m, 1H), 1.28-1.22 (m, 12H), 1.0-0.99 (d, *J* = 7.0 Hz, 3H), 0.89-0.85 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 195.4, 192.0, 173.8, 161.8, 160.1, 155.3, 131.1, 126.5, 115.4, 103.5, 101.5, 90.7, 65.0, 55.9, 55.6, 36.4, 33.6, 33.4, 32.8, 32.1, 29.8, 29.7, 27.5, 22.9, 17.5, 14.3.

(5*S*,2'*R*)-5-(4-Hydroxybenzyl)-3-(2'-methyldecanoyl)pyrrolidine-2,4-dione (233): A solution of **263** (50 mg, 0.09 mmol) in CH_2Cl_2: TFA: anisole (v/v/v 85:10:5, 1 mL) was stirred at room temperature for 30 min. After which, the mixture was diluted with toluene (2 mL) and concentrated. Chromatographic purification using 5% MeOH in CH_2Cl_2 afforded **233** (27 mg, 80%) as light yellow foam. $[\alpha]^{24}_{D}$ -170.4 (*c* 0.75); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.18 (br s, 1H), 8.95 (br s, 1H), 6.93-6.91 (d, *J* = 8.0 Hz, 2H), 6.62-6.60 (d, *J* = 8.0 Hz), 4.08-4.07 (m, 1H), 3.48-3.44 (q, *J* = 7.5 Hz, 1H), 2.92-2.81 (m, 2H), 1.51-1.48 (m, 1H), 1.36-1.23 (m, 13H), 1.07-1.06 (d, *J* = 6.0 Hz, 3H), 0.90-0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 194.5, 191.7, 175.4, 155.9, 130.6, 125.4, 114.7,

100.4, 62.4, 35.4, 33.0, 31.3, 31.2, 28.8, 28.7, 28.6, 26.4, 22.1, 16.6, 13.9; ESI-HRMS Calcd for $C_{22}H_{31}NO_4$ $[M + Na]^+$: 396.2151. Found: 396.2132.

(5*S*,2'*S*)-5-(4-Hydroxybenzyl)-3-(2'-methyldecanoyl)pyrrolidine-2,4-dione (234): Following the same procedure as for the *N*-2,4,6-trimethoxybenzyl deprotection of **263**, using **264** (100 mg, 0.18 mmol) as substrate, **234** (57 mg, 84%) was obtained as light yellow foam. $[\alpha]^{24}_D$ -183.2 (*c* 1.0); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (br s, 1H), 8.93 (br s, 1H), 6.94-6.92 (d, *J* = 8.0 Hz, 2H), 6.64-6.62 (d, *J* = 8.0 Hz), 4.01-4.09 (m, 1H), 3.45-3.41 (m, 1H), 2.86-2.85 (d, *J* = 4.5 Hz, 2H), 1.57-1.55 (m, 1H), 1.38-1.37 (m, 1H), 1.28-1.23 (m, 12H), 1.00-0.99 (d, *J* = 6.5 Hz, 3H), 0.89-0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 194.6, 192.0, 175.5, 156.0, 130.7, 125.6, 114.8, 100.4, 62.5, 35.8, 32.5, 31.3, 28.9, 28.8, 28.7, 28.6, 26.7, 22.1, 17.0, 14.0.

(4*R*,9'*Z*)-4-Isopropyl-3-(octadec-9'-enoyl)oxazolidin-2-one (272): Following the same procedure as for the preparation of **249**, using (4*R*)-4-isopropyloxazolidin-2-one (615 mg, 4.77 mmol) and oleoyl chloride (2.3 mL, 7.15 mmol) as substrate, and eluting with 10% ethyl acetate in hexanes, **272** (1.8 g, 96%) was obtained as colorless syrup. $[\alpha]^{24}_D$ -43.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 5.35-5.33 9 (m, 2H), 4.45-4.42 (m, 1H), 4.28-4.24 (t, *J* = 9.0 Hz, 1H), 4.21-4.19 (dd, *J* = 3.0, 9.0 Hz, 1H), 3.01-2.95 (m, 1H), 2.88-2.82 (m, 1H), 2.39-2.35 (m, 1H), 2.04-2.01 (m, 4H), 1.68-1.62 (m, 2H), 1.34-1.27 (m, 20H), 0.92-0.91 (d, *J* = 7.0 Hz, 3H), 0.88-0.85 (t, *J* = 7.0 Hz, 3H), 0.87-0.85 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 173.6, 154.3, 130.2, 130.0, 63.5, 58.6, 35.7, 32.1, 30.0, 29.9, 29.8,

29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 27.4, 24.7, 22.9, 18.2, 14.9, 14.3; ESI-HRMS Calcd for $C_{24}H_{43}NO_3$ [M + Na]$^+$: 416.3141. Found: 416.3126.

(4*S*,9*'Z*)-4-Isopropyl-3-(octadec-9'-enoyl)oxazolidin-2-one (273): Following the same procedure as for the preparation of **249**, using (4*R*)-4-isopropyloxazolidin-2-one (700 mg, 5.42 mmol) and oleoyl chloride (2.7 mL, 8.13 mmol) as substrate, and eluting with 10% ethyl acetate in hexanes, **273** (2.0 g, 94%) was obtained as colorless syrup. $[\alpha]^{24}_D$ +44.2 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (4*R*,9*'Z*)-4-isopropyl-3-(octadec-9'-enoyl)oxazolidin-2-one (**272**).

(4*R*,2'*R*,9'*Z*)-4-Isopropyl-3-(2'-methyloctadec-9'-enoyl)oxazolidin-2-one (274): Following the same procedure as for the preparation of **251**, using **272** (1.5 g, 3.8 mmol) as substrate, a mixture of diastereomers (dr = 8.6:1) was obtained. Chromatographic purification of the mixture using 8% ethyl acetate in hexanes afforded **274** (1.3 g, 84%) as colorless syrup. $[\alpha]^{24}_D$ -57.3 (*c* 1.0); ^{1}H NMR (500 MHz) δ 5.35-5.33 (m, 2H), 4.46-4.43 (m, 1H), 4.28-4.24 (t, *J* = 9.0 Hz, 1H), 4.21-4.18 (dd, *J* = 3.0, 9.0 Hz, 1H), 3.74-3.70 (q, *J* = 7.0 Hz, 1H), 2.37-2.33 (m, 1H), 2.01-1.98 (m, 4H), 1.72-1.69 (m, 1H), 1.39-1.27 (m, 21H), 1.20-1.19 (d, *J* = 7.0 Hz, 3H), 0.92-0.91 (d, *J* = 7.0 Hz, 3H), 0.88-0.85 (t, *J* = 7.0 Hz, 3H), 0.87-0.85 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 177.5, 153.9, 130.2, 130.0, 63.4, 58.6, 37.9, 33.3, 32.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4, 28.7, 27.5, 27.4, 22.9, 18.2, 14.9, 14.3; ESI-HRMS Calcd for $C_{25}H_{45}NO_3$ [M + Na]$^+$: 430.3297. Found: 430.3298.

(4*S*,2'*S*,9'*Z*)-4-Isopropyl-3-(2'-methyloctadec-9'-enoyl)oxazolidin-2-one (275): Following the same procedure as for the preparation of **251**, using **273** (1.98 g, 5.0 mmol) as substrate, a mixture of diastereomers (dr = 8.0:1) was obtained. Chromatographic purification of the mixture using 8% ethyl acetate in hexanes afforded **275** (1.74 g, 85%) as colorless syrup. $[\alpha]^{24}_{D}$ +58.0 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (4*R*,2'*R*,9'*Z*)-4-isopropyl-3-(2'-methyloctadec-9'-enoyl)oxazolidin-2-one (**274**).

(2*R*,9*Z*)-2-Methyloctadec-9-enoic acid (276): Following the same procedure as for the hydrolysis of **249**, using **274** (1.3 g, 3.2 mmol) as substrate, and eluting with 10% ethyl acetate in hexanes, **276** (900 mg, 95%) was obtained as clear oil. $[\alpha]^{24}_{D}$ -7.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 5.37-5.34 (m, 2H), 2.48-2.44 (q, *J* = 7.0 Hz, 1H), 2.06-2.0 (m, 4H), 1.71-1.66 (m, 1H), 1.46-1.42 (m, 1H), 1.34-1.26 (m, 20H), 1.19-1.18 (d, *J* = 7.0 Hz, 3H), 0.91-0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 183.9, 130.2, 130.0, 39.6, 33.7, 32.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4, 27.4, 22.9, 17.0, 14.3; ESI-HRMS Calcd for $C_{19}H_{35}O_2$ $[M - H]^-$: 295.2637. Found: 295.2640.

(2*S*,9*Z*)-2-Methyloctadec-9-enoic acid (277): Following the same procedure as for the hydrolysis of **249**, using **275** (1.45 g, 3.6 mmol) as substrate, and eluting with 10% ethyl acetate in hexanes, **277** (1.0 g, 95%) was obtained as clear oil. $[\alpha]^{24}_{D}$ +7.1 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*,9*Z*)-2-methyloctadec-9-enoic acid (**276**).

(2*R*,9*Z*)-Methyl 2-methyloctadec-9-enoate (278): To a stirred suspension of **276** (810 mg, 2.73 mmol) and Cs_2CO_3 (890 mg, 2.73 mmol) in

DMF (3.0 mL) was added MeI (340 µL, 5.5 mmol) and the mixture was stirred at room temperature for 2 h. The solvent was removed and the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine, dried and concentrated. Chromatographic purification using 2% ethyl acetate in hexanes afforded **278** (840 mg, 98%) as clear oil. $[\alpha]^{24}_D$ -10.2 (*c* 1.0); ^{1}H NMR (500 MHz) δ 5.35-5.31 (m, 2H), 3.65 (s, 3H), 2.44-2.40 (q, *J* = 7.0 Hz, 1H), 2.01-1.97 (m, 4H), 1.71-1.66 (m, 1H), 1.46-1.42 (m, 1H), 1.30-1.26 (m, 20H), 1.13-1.12 (d, *J* = 7.0 Hz, 3H), 0.88-0.85 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 177.5, 130.1, 129.9, 51.5, 39.6, 34.0, 32.1, 30.0, 29.9, 29.8, 29.7, 29.5, 29.3, 27.4, 22.9, 17.2, 14.3; ESI-HRMS Calcd for $C_{20}H_{38}O_2$ $[M + Na]^+$: 333.2770. Found: 333.2761.

(2*S*,9*Z*)-Methyl 2-methyloctadec-9-enoate (279): Following the same procedure as for the preparation of **278**, using **277** (1.0 g, 3.4 mmol) as substrate, and eluting with 2% ethyl acetate in hexanes, **279** (1.04 g, 99%) was obtained as clear oil. $[\alpha]^{24}_D$ +10.6 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*,9*Z*)-methyl 2-methyloctadec-9-enoate (**278**).

(2*R*)-Methyl 2-methyl-9-oxononanoate (280): To a solution of **278** (840 mg, 2.7 mmol) in CH_2Cl_2 (15 mL) at -78 °C ozone was bubbled until a blue color was observed (10 – 15 min). After which the solution was purged with O_2 for 5 min and dropwise dimethylsulfide (1.0 mL) was added. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for 12 h. Excess dimethylsulfide and solvent were then removed. Chromatographic purification using 10% ethyl acetate in hexanes afforded **280** (480 mg, 87%) as

colorless liquid. $[\alpha]^{24}_{D}$ -16.9 (*c* 1); ^{1}H NMR (500 MHz) δ 9.74-9.73 (t, *J* = 1.5 Hz, 1H), 3.64 (s, 3H), 2.41-2.38 (m, 3H), 1.61-1.58 (m, 3H), 1.32-1.28 (m, 1H), 1.30-1.25 (m, 6H), 1.12-1.11 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 203.0, 177.5, 51.6, 44.0, 39.6, 33.9, 29.4, 29.2, 27.2, 22.1, 17.3; ESI-HRMS Calcd for $C_{11}H_{20}O_3$ $[M + Na]^+$: 223.1310. Found: 223.1289.

(2*S*)-Methyl 2-methyl-9-oxononanoate (281): Following the same procedure as for the ozonolysis of **278**, using **279** (1.04 g, 3.3 mmol) as substrate, and eluting with 10% ethyl acetate in hexanes, **281** (600 mg, 90%) was obtained as colorless liquid. $[\alpha]^{24}_{D}$ +16.1 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*)-methyl 2-methyl-9-oxononanoate (**280**).

(9*Z*,4'*S*)-10-(2',2'-Dimethyl-1',3'-dioxolan-4'-yl)dec-9-enoic acid (282): To a stirred suspension of (8-carboxyoctyl)triphenylphosphonium bromide (**270**)[267] (4.8 g, 9.6 mmol) in THF (100 mL) at -78 °C was added dropwise BuLi (1.6 M in cyclohexane, 12.6 mL, 20.1 mmol). After 30 min of stirring at -78 °C, a solution of (4*R*)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (**269**)[268] (1.5 g, 11.5 mmol) in THF (10 mL + 5 mL rinse) was slowly added. After the addition was complete, the reaction mixture was warmed to room temperature over a period of 4 h, before saturated aqueous NH_4Cl was added to quench the reaction. The resulting mixture was partially concentrated, residue was diluted with CH_2Cl_2 and the organic layer was extracted, dried and concentrated. Chromatographic purification using 25% ethyl acetate in hexanes afforded **282** (1.95 g, 75%) as colorless liquid. $[\alpha]^{23}_{D}$ +7.7 (*c* 1.0); ^{1}H NMR (500 MHz) δ 5.65-5.60 (m, 1H), 5.42-5.39 (t, *J* = 10.0 Hz, 1H), 4.87-4.82 (q, *J* = 8.0 Hz, 1H), 4.08-4.05 (dd, *J* = 6.5, 8.0

Hz, 1H), 3.54-3.50 (t, *J* = 8.0 Hz, 1H), 2.36-2.33 (t, *J* = 7.5 Hz, 2H), 2.15-2.04 (m, 2H), 1.66-1.61 (m, 2H), 1.43 (s, 3H), 1.41 (s, 3H), 1.39-1.32 (m, 8H); ^{13}C NMR (125 MHz) *δ* 179.9, 135.4, 127.3, 109.3, 72.2, 69.7, 34.2, 29.7, 29.3, 29.2, 29.1, 27.9, 27.0, 26.2, 24.8; ESI-HRMS Calcd for $C_{15}H_{25}O_4$ $[M - H]^-$: 269.1753. Found: 269.1742.

(9*Z*,4'*S*)-10-(2',2'-Dimethyl-1',3'-dioxolan-4'-yl)-1-(triphenylphosphoranylidene)undec-9-en-2-one (283): To a stirred solution of **282** (1.0 g, 3.7 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added dropwise oxalyl chloride (480 µL, 5.5 mmol). After 30 min of stirring at 0 °C, the reaction mixture was refluxed for 2 h. Evaporation of solvent and excess oxalyl chloride, afforded (9*Z*,4'*S*)-10-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)dec-9-enoyl chloride, which was taken forward without purification.

To a stirred suspension of methyltriphenylphosphonium bromide (1.96 g, 5.5 mmol) in THF (50 mL) at -78 °C was added dropwise BuLi (1.6 M in cyclohexane, 2.8 mL, 4.4 mmol). The mixture was stirred for 30 min at -78 °C, whereupon a solution of (9*Z*,4'*S*)-10-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)dec-9-enoyl chloride (3.7 mmol) in THF (8 mL + 4 mL rinse) was slowly added, and the final mixture mixture was warmed to room temperature over a period of 4 h. After evaporation of THF, the resulting oil was dissolved in ethyl acetate (50 mL) and the organic layer was washed with aqueous NaOH (2 N, 50 mL), dried, and concentrated. Chromatographic purification using 10% *iso*-propanol in ethyl acetate afforded **283** (1.72 g, 88%) as a yellow viscous oil. $[\alpha]^{23}_D$ +5.6 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.69-7.62 (m, 6H), 7.54-7.51 (t, *J* = 7.0 Hz, 3H), 7.48-7.42 (m,

6H), 5.65-5.59 (m, 1H), 5.41-5.37 (t, *J* = 10.0 Hz, 1H), 4.86-4.82 (q, *J* = 8.0 Hz, 1H), 4.06-4.04 (t, *J* = 7.0 Hz, 1H), 3.72-3.67 (d, *J* = 24 Hz, 1H), 3.51-3.48 (t, *J* = 8.0 Hz, 1H), 2.32-2.29 (t, *J* = 7.5 Hz, 2H), 2.12-2.04 (m, 2H), 1.69-1.63 (m, 2H), 1.41 (s, 3H), 1.39 (s, 3H), 1.37-1.31 (m, 8H); ^{13}C NMR (125 MHz) *δ* 194.3, 135.5, 133.3, 132.3, 129.0, 128.7-128.0 (d, *J* = 82.0 Hz), 127.2, 109.2, 72.2, 69.7, 51.6-50.8 (d, *J* = 107.0 Hz), 42.0-41.9 (d, *J* = 15.0 Hz), 29.9, 29.6, 29.4, 28.0, 27.4, 27.0, 26.2; ESI-HRMS Calcd for $C_{34}H_{42}O_3P$ $[M + H]^+$: 529.2872. Found: 529.2838.

(2*R*,9*E*,19*Z*,4'*S*)-Methyl 20-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)-2-methyl-11-oxocos-9,19-dienoate (284): To a stirred solution of **283** (450 mg, 0.85 mmol) in CH_2Cl_2 (5 mL) was added a solution of (2*R*)-methyl 2-methyl-9-oxononanoate (**280**) (190 mg, 0.94 mmol) in CH_2Cl_2 (2 mL) at room temperature. After stirring for 30 min at same temperature, the mixture was warmed to 35 ℃ and stirring was continued for 12 h. The resulting mixture was concentrated and chromatographic purification of the crude using 15% ethyl acetate in hexanes afforded **284** (315 mg, 82%) as colorless syrup. $[\alpha]^{23}_D$ -5.2 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 6.84-6.78 (m, 1H), 6.09-6.06 (d, *J* = 16.0 Hz, 1H), 5.64-5.59 (m, 1H), 5.41-5.37 (t, *J* = 10.0 Hz, 1H), 4.85-4.81 (q, *J* = 8.0 Hz, 1H), 4.07-4.04 (dd, *J* = 6.0, 8.0 Hz, 1H), 3.66 (s, 3H), 3.52-3.49 (t, *J* = 8.0 Hz, 1H), 2.53-2.50 (t, *J* = 7.0 Hz, 2H), 2.45-2.41 (q, *J* = 7.0 Hz, 1H), 2.21-2.17 (q, *J* = 7.0 Hz, 2H), 2.13-2.06 (m, 2H), 1.64-1.58 (m, 4H), 1.42 (s, 3H), 1.39 (s, 3H), 1.38-1.29 (m, 16H), 1.14-1.13 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 201.1, 177.5, 147.4, 135.3, 130.5, 127.3, 109.2, 72.2, 69.7, 51.7, 40.3, 39.6, 33.9, 32.6, 29.8, 29.5, 29.4,

29.3, 29.2, 28.3, 27.9, 27.3, 27.0, 26.2, 24.5, 17.3; ESI-HRMS Calcd for $C_{27}H_{46}O_5$ [M + Na]$^+$: 473.3243. Found: 473.3233.

(2*S*,9*E*,19*Z*,4'*S*)-Methyl 20-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)-2-methyl-11-oxocos-9,19-dienoate (285): Following the same procedure as for the preparation of **284**, using **283** (480 mg, 0.91 mmol) and **281** (200 mg, 1.0 mmol) as substrates, and eluting with 15% ethyl acetate in hexanes, **285** (350 mg, 85%) was obtained as colorless syrup. $[\alpha]^{23}_D$ +13.6 (*c* 2.05); ^{1}H NMR (500 MHz) δ 6.82-6.76 (m, 1H), 6.08-6.04 (d, *J* = 16.0 Hz, 1H), 5.62-5.57 (m, 1H), 5.39-5.35 (t, *J* = 10.0 Hz, 1H), 4.84-4.79 (q, *J* = 8.0 Hz, 1H), 4.05-4.02 (t, *J* = 7.0 Hz, 1H), 3.65 (s, 3H), 3.50-3.42 (t, *J* = 8.0 Hz, 1H), 2.51-2.48 (t, *J* = 7.0 Hz, 2H), 2.44-2.40 (q, *J* = 7.0 Hz, 1H), 2.19-2.15 (q, *J* = 7.0 Hz, 2H), 2.13-2.06 (m, 2H), 1.62-1.56 (m, 3H), 1.40 (s, 3H), 1.37 (s, 3H), 1.36-1.28 (m, 17H), 1.12-1.11 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 201.1, 177.5, 147.4, 135.3, 130.5, 127.3, 109.2, 72.2, 69.7, 51.7, 40.3, 39.6, 33.9, 32.6, 29.8, 29.5, 29.4, 29.3, 29.2, 28.2, 27.9, 27.3, 27.0, 26.2, 24.4, 17.3.

(2*R*,21*S*)-Methyl 21,22-dihydroxy-2-methyl-11-oxodocosanoate (286): To a solution of **284** (300 mg, 0.67 mmol) in MeOH: THF (v/v 3:1, 10 mL) was added AcOH (100 µL) and 10% Pd/C (45 mg). The mixture was purged with H_2 three times and shaken under H_2 (50 psi) for 3 h. Then the reaction mixture was filtered through a Celite pad and washed with ethyl acetate (3 × 5 mL). Evaporation of the solvent afforded (2*R*,4'*S*)-methyl 20-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)-2-methyl-11-oxodocosanoate, which was taken forward without purification.

To a stirred solution of (2*R*,4'*S*)-methyl 20-(2',2'-dimethyl-1',3'-dioxolan-4'-yl)-2-methyl-11-oxodocosanoate (300 mg, 0.67 mmol) in THF (5 mL) was added 20% aqueous HCl (5 mL) at room temperature. The reaction mixture was stirred at same temperature for 30 min before it was neutralized by saturated aqueous $NaHCO_3$. The resulting mixture was partially concentrated, residue was diluted with ethyl acetate and the organic layer was extracted, dried and concentrated. Chromatographic purification using 55% ethyl acetate in hexanes afforded **286** (235 mg, 85%) as white solid. $[\alpha]^{23}_D$ -9.2 (*c* 1.0); ^{1}H NMR (500 MHz) δ 3.71-3.69 (m, 1H), 3.67 (s, 3H), 3.64-3.62 (m, 1H), 3.43-3.41 (dd, *J* = 8.0, 11.0 Hz, 1H), 2.45-2.41 (q, *J* = 7.0 Hz, 1H), 2.39-2.36 (t, *J* = 7.0 Hz, 4H), 2.33-2.25 (m, 2H), 1.64-1.62 (m, 1H), 1.54-1.52 (m, 4H), 1.43-1.40 (m, 4H), 1.28-1.25 (m, 21H), 1.14-1.13 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 212.1, 177.7, 72.5, 67.1, 51.7, 43.0, 39.7, 34.0, 33.4, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 25.7, 24.0, 17.3; ESI-HRMS Calcd for $C_{24}H_{46}O_5$ $[M + Na]^+$: 437.3243. Found: 437.3251.

(2*S*,21*S*)-Methyl 21,22-dihydroxy-2-methyl-11-oxodocosanoate (287): Following the same reaction sequences as for the preparation of **286**, using **285** (350 mg, 0.77 mmol) as substrate, **287** (280 mg, 89%) was obtained as white solid. $[\alpha]^{23}_D$ +9.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 3.70-3.68 (m, 1H), 3.65 (s, 3H), 3.63-3.62 (m, 1H), 3.45-3.41 (t, *J* = 9.5 Hz, 1H), 2.61 (br s, 2H), 2.43-2.39 (q, *J* = 7.0 Hz, 1H), 2.38-2.35 (t, *J* = 7.0 Hz, 4H), 1.63-1.61 (m, 1H), 1.53-1.51 (m, 4H), 1.41-1.39 (m, 4H), 1.28-1.25 (m, 21H), 1.13-1.12 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 212.1, 177.7, 72.5, 67.0, 51.7, 43.0, 39.7, 34.0, 33.4, 29.8, 29.6, 29.5, 29.4, 29.3, 27.4, 25.7, 24.0, 17.2.

(2*R*,21*S*)-Methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (288): To a stirred solution of **286** (230 mg, 0.55 mmol) and imidazole (55 mg, 0.84 mmol) in DMF (1 mL) at room temperature was added TBDPSCl (155 µL, 0.60 mmol) and the stirring was continued at same temperature for 12 h. Then the reaction was quenched with water and extracted with ethyl acetate three times. The combined organic phase was washed with brine, dried and concentrated. Chromatographic purification using 15% ethyl acetate in hexanes afforded **288** (350 mg, 95%) as colorless syrup. $[\alpha]^{23}_{D}$ -3.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.68-7.66 (d, *J* = 7.5 Hz, 4H), 7.44-7.38 (m, 6H), 3.71-3.69 (m, 1H), 3.67 (s, 3H), 3.65-3.63 (m, 1H), 3.51-3.47 (dd, *J* = 8.0, 11.0 Hz, 1H), 2.53-2.52 (d, *J* = 3.0 Hz, 1H), 2.45-2.41 (q, *J* = 7.0 Hz, 1H), 2.39-2.36 (t, *J* = 7.0 Hz, 4H), 1.64-1.62 (m, 1H), 1.57-1.54 (m, 4H), 1.40-1.38 (m, 4H), 1.28-1.25 (m, 21H), 1.15-1.14 (d, *J* = 7.0 Hz, 3H), 1.08 (s, 9H); ^{13}C NMR (125 MHz) δ 211.9, 177.6, 135.8, 133.4, 130.0, 128.0, 72.2, 68.3, 51.7, 43.0, 39.7, 34.0, 33.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.1, 25.7, 24.1, 24.0, 19.5, 17.3; ESI-HRMS Calcd for $C_{40}H_{64}O_5Si$ $[M + Na]^+$: 675.4421. Found: 675.4418.

(2*S*,21*S*)-Methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (289): Following the same procedure as for the preparation of **288**, using **287** (245 mg, 0.59 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **289** (375 mg, 97%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ +8.0 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.68-7.66 (d, *J* = 7.5 Hz, 4H), 7.46-7.40 (m, 6H), 3.71-3.69 (m, 1H), 3.67 (s, 3H), 3.65-3.63 (m, 1H), 3.51-3.47 (dd, *J* = 7.5, 10.5 Hz, 1H), 2.52 (br s, 1H), 2.44-2.41 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, *J* = 7.0

Hz, 4H), 1.64-1.62 (m, 1H), 1.57-1.54 (m, 4H), 1.40-1.38 (m, 4H), 1.28-1.25 (m, 21H), 1.15-1.14 (d, *J* = 7.0 Hz, 3H), 1.08 (s, 9H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 135.8, 133.4, 130.0, 128.0, 72.2, 68.3, 51.7, 43.0, 39.7, 34.0, 33.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.1, 25.7, 24.1, 24.0, 19.5, 17.3.

(2*R*,21*R*)-1-*tert*-Butyldiphenylsilyloxy-22-methoxy-21-methyl-12,22-dioxodocosan-2-yl 4'-nitrobenzoate (290): Following the same procedure as for the preparation of **239**, using **288** (350 mg, 0.54 mmol) as substrate, and eluting with 5% ethyl acetate in hexanes, **290** (385 mg, 90%) was obtained as light yellow syrup. $[\alpha]^{23}_{D}$ -2.0 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 8.30-8.28 (d, *J* = 9.0 Hz, 2H), 8.20-8.18 (d, *J* = 9.0 Hz, 2H), 7.64-7.61 (t, *J* = 7.0 Hz, 4H), 7.41-7.39 (m, 2H), 7.35-7.31 (m, 4H), 5.30-5.28 (m, 1H), 3.85-3.84 (d, J = 4.5 Hz, 2H), 3.67 (s, 3H), 2.45-2.41 (q, *J* = 7.0 Hz, 1H), 2.39-2.36 (t, *J* = 7.0 Hz, 4H), 1.78-1.74 (m, 2H), 1.66-1.64 (m, 1H), 1.56-1.53 (m, 5H), 1.40-1.34 (m, 2H), 1.28-1.25 (m, 20H), 1.15-1.14 (d, *J* = 7.0 Hz, 3H), 1.02 (s, 9H); ^{13}C NMR (125 MHz) *δ* 211.8, 177.6, 164.5, 150.7, 136.3, 135.8, 133.4, 130.9, 130.0, 127.9, 123.7, 76.6, 65.2, 51.7, 43.0, 39.7, 34.0, 30.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 26.9, 25.5, 24.1, 24.1, 19.4, 17.3; ESI-HRMS Calcd for $C_{47}H_{67}NO_8Si$ $[M + Na]^+$: 824.4534. Found: 824.4667.

(2*R*,21*S*)-1-*tert*-Butyldiphenylsilyloxy-22-methoxy-21-methyl-12,22-dioxodocosan-2-yl 4'-nitrobenzoate (291): Following the same procedure as for the preparation of **239**, using **289** (170 mg, 0.26 mmol) as substrate, and eluting with 5% ethyl acetate in hexanes, **291** (195 mg, 94%) was obtained as light yellow syrup. $[\alpha]^{23}_{D}$ +7.8 (*c* 1.0); ^{1}H NMR (400 MHz) *δ* 8.29-8.26 (d, *J* = 8.8

Hz, 2H), 8.18-8.16 (d, *J* = 8.8 Hz, 2H), 7.64-7.61 (t, *J* = 6.8 Hz, 4H), 7.40-7.37 (m, 2H), 7.34-7.30 (m, 4H), 5.30-5.28 (m, 1H), 3.83-3.82 (d, *J* = 4.8 Hz, 2H), 3.66 (s, 3H), 2.44-2.40 (q, *J* = 7.2 Hz, 1H), 2.38-2.34 (t, *J* = 7.2 Hz, 4H), 1.76-1.74 (m, 2H), 1.66-1.64 (m, 1H), 1.56-1.53 (m, 5H), 1.40-1.34 (m, 2H), 1.28-1.25 (m, 20H), 1.14-1.13 (d, *J* = 7.2 Hz, 3H), 1.0 (s, 9H); ^{13}C NMR (100 MHz) δ 211.8, 177.6, 164.5, 150.7, 136.3, 135.8, 133.4, 130.9, 130.0, 127.9, 123.7, 76.6, 65.2, 51.6, 43.0, 39.7, 34.0, 30.6, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 26.9, 25.4, 24.1, 19.4, 17.3.

(2*R*,21*R*)-Methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (292): Following the same procedure as for the preparation of **240**, using **290** (380 mg, 0.47 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **292** (260 mg, 84%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ -7.5 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*,21*S*)-methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (**289**).

(2*S*,21*R*)-Methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (293): Following the same procedure as for the preparation of **240**, using **291** (195 mg, 0.24 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **293** (140 mg, 88%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ +4.8 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*,21*S*)-methyl 22-*tert*-butyldiphenylsilyloxy-21-hydroxy-2-methyl-11-oxodocosanoate (**288**).

(2*R*,21*S*)-Methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (294): To a solution of the alcohol **288** (170 mg, 0.26 mmol) in cyclohexane: CH_2Cl_2 (v/v 2:1, 2.5 mL) at 0 ^oC was added dropwise benzyl 2,2,2-trichloroacetimidate (95 µL, 0.5 mmol). After 10 min of stirring at 0 ^oC, TfOH (7 µL, 0.1 mmol) was added and the resulting light yellow solution was stirred at room temperature for 1 h. The reaction was quenched with the addition of saturated aqueous $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 three times. The combined organic phase was washed with brine, dried and concentrated. Chromatographic purification using 8% ethyl acetate in hexanes afforded **294** (175 mg, 91%) as colorless syrup. $[\alpha]^{23}_D$ -17.0 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.72-7.70 (m, 4H), 7.44-7.42 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.40-7.36 (m, 7H), 7.34-7.33 (d, *J* = 4.5 Hz, 2H), 4.70-4.68 (d, *J* = 11.5 Hz, 1H), 4.55-4.52 (d, *J* = 11.5 Hz, 1H), 3.78-3.74 (dd, *J* = 7.0, 10.0 Hz, 1H), 3.68 (s, 3H), 3.67-3.65 (m, 1H), 3.48-3.44 (m, 1H), 2.47-2.43 (q, *J* = 7.0 Hz, 1H), 2.41-2.38 (t, *J* = 7.5 Hz, 4H), 1.67-1.65 (m, 1H), 1.58-1.53 (m, 5H), 1.40-1.34 (m, 2H), 1.28-1.25 (m, 22H), 1.17-1.15 (d, *J* = 7.0 Hz, 3H), 1.09 (s, 9H); ^{13}C NMR (125 MHz) δ 211.9, 177.6, 139.3, 135.9, 135.1, 133.9, 129.8, 128.5, 128.0, 127.9, 80.1, 72.3, 66.7, 51.7, 43.2, 39.7, 34.0, 31.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 27.1, 26.8, 25.6, 24.1, 19.5, 17.3. ESI-HRMS Calcd for $C_{47}H_{70}O_5Si$ $[M + Na]^+$: 765.4890. Found: 765.4887.

(2*S*,21*S*)-Methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (295): Following the same procedure as for the preparation of **294**, using **289** (215 mg, 0.33 mmol) as substrate, and eluting with 8% ethyl

acetate in hexanes, **295** (200 mg, 82%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ -8.7 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.72-7.69 (m, 4H), 7.44-7.42 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.40-7.36 (m, 7H), 7.34-7.33 (d, *J* = 4.5 Hz, 2H), 4.70-4.67 (d, *J* = 11.5 Hz, 1H), 4.54-4.52 (d, *J* = 11.5 Hz, 1H), 3.78-3.75 (dd, *J* = 6.0, 10.0 Hz, 1H), 3.68 (s, 3H), 3.67-3.64 (m, 1H), 3.47-3.43 (m, 1H), 2.46-2.42 (q, *J* = 7.0 Hz, 1H), 2.41-2.38 (t, *J* = 7.5 Hz, 4H), 1.63-1.60 (m, 1H), 1.58-1.53 (m, 5H), 1.40-1.34 (m, 2H), 1.28-1.25 (m, 22H), 1.16-1.15 (d, *J* = 7.5 Hz, 3H), 1.08 (s, 9H); ^{13}C NMR (125 MHz) δ 211.9, 177.6, 139.3, 135.9, 135.1, 133.9, 129.8, 128.5, 128.0, 127.9, 80.1, 72.3, 66.6, 51.7, 43.1, 39.7, 34.0, 31.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 27.1, 26.8, 25.6, 24.1, 19.5, 17.3.

(2*R*,21*R*)-Methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (296): Following the same procedure as for the preparation of **294**, using **292** (230 mg, 0.35 mmol) as substrate, and eluting with 8% ethyl acetate in hexanes, **296** (230 mg, 88%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ +8.4 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*,21*S*)-methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (**295**).

(2*S*,21*R*)-Methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (297): Following the same procedure as for the preparation of **294**, using **293** (110 mg, 0.17 mmol) as substrate, and eluting with 8% ethyl acetate in hexanes, **297** (105 mg, 84%) was obtained as colorless syrup. $[\alpha]^{23}_{D}$ +17.9 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer

(2*R*,21*S*)-methyl 21-benzyloxy-22-*tert*-butyldiphenylsilyloxy-2-methyl-11-oxodocosanoate (**294**).

(2*R*,21*S*)-Methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (298): To a solution of **294** (135 mg, 0.18 mmol) in MeOH: THF (v/v 3:1, 2 mL) was added PTSA (45 mg, 0.27 mmol) and the mixture was stirred at room temperature for 24 h. After which the reaction mixture was concentrated and chromatographic purification using 25% ethyl acetate in hexanes afforded **298** (88 mg, 96%) as colorless foam. $[\alpha]^{23}_{D}$ +2.6 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.36-7.35 (m, 4H), 7.32-7.28 (q, *J* = 5.0 Hz, 1H), 4.64-4.62 (d, *J* = 11.5 Hz, 1H), 4.56-4.54 (d, *J* = 11.5 Hz, 1H), 3.71-3.68 (m, 1H), 3.67 (s, 3H), 3.55-3.51 (m, 2H), 2.44-2.41 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, *J* = 7.0 Hz, 4H), 1.99 (br s, 1H), 1.66-1.64 (m, 2H), 1.58-1.53 (m, 5H), 1.40-1.33 (m, 3H), 1.28-1.26 (m, 20H), 1.15-1.14 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 211.9, 177.6, 138.8, 128.7, 128.0, 127.9, 80.0, 71.7, 64.5, 51.7, 43.0, 39.7, 34.0, 31.0, 30.0, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 25.6, 24.1, 17.3. ESI-HRMS Calcd for $C_{31}H_{52}O_5$ $[M + Na]^+$: 527.3712. Found: 527.3725.

(2*S*,21*S*)-Methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (299): Following the same procedure as for the preparation of **298**, using **295** (190 mg, 0.26 mmol) as substrate, and eluting with 25% ethyl acetate in hexanes, **299** (120 mg, 93%) was obtained as colorless foam. $[\alpha]^{23}_{D}$ +18.1 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.36-7.35 (m, 4H), 7.32-7.28 (q, *J* = 5.0 Hz, 1H), 4.64-4.62 (d, *J* = 11.5 Hz, 1H), 4.56-4.54 (d, *J* = 11.5 Hz, 1H), 3.71-3.369 (m, 1H), 3.67 (s, 3H), 3.56-3.51 (m, 2H), 2.44-2.40 (q, *J* = 7.0 Hz, 1H), 2.39-2.36

(t, *J* = 7.0 Hz, 4H), 1.97 (br s, 1H), 1.66-1.64 (m, 2H), 1.58-1.53 (m, 5H), 1.40-1.33 (m, 3H), 1.28-1.26 (m, 20H), 1.15-1.13 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 138.8, 128.7, 128.0, 127.9, 80.1, 71.7, 64.5, 51.7, 43.0, 39.7, 34.0, 31.1, 30.0, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27.4, 25.6, 24.1, 17.3.

(2*R*,21*R*)-Methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (300): Following the same procedure as for the preparation of **298**, using **296** (225 mg, 0.3 mmol) as substrate, and eluting with 25% ethyl acetate in hexanes, **300** (145 mg, 95%) was obtained as colorless foam. $[\alpha]^{23}_{D}$ -17.4 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*S*,21*S*)-methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (**299**).

(2*S*,21*R*)-Methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (301): Following the same procedure as for the preparation of **298**, using **297** (100 mg, 0.14 mmol) as substrate, and eluting with 25% ethyl acetate in hexanes, **301** (65 mg, 95%) was obtained as colorless foam. $[\alpha]^{23}_{D}$ -2.0 (*c* 1.0); ^{1}H and ^{13}C NMR spectra data consistent with the enantiomer (2*R*,21*S*)-methyl 21-benzyloxy-22-hydroxy-2-methyl-11-oxodocosanoate (**298**).

(2*R*,21*S*)-Methyl 21-benzyloxy-22-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-2-methyl-11-oxodocosanoate (302): Following the general procedure 8, using **298** (85 mg, 0.17 mmol) as acceptor, a mixture of α/β = 1:5.8 was obtained. Chromatographic purification of the mixture using 12% ethyl acetate in hexanes afforded **302** (90 mg, 58%) as colorless syrup. $[\alpha]^{24}_{D}$ -41.0 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.52-7.50 (d, *J* = 7.0 Hz, 2H),

7.46-7.45 (d, *J* = 6.0 Hz, 2H), 7.39-7.38 (d, *J* = 7.5 Hz, 2H), 7.32-7.29 (m, 14H), 5.63 (s, 1H), 5.0-4.97 (d, *J* = 12.0 Hz, 1H), 4.88-4.86 (d, *J* = 12.0 Hz, 1H), 4.68-4.65 (d, *J* = 12.5 Hz, 1H), 4.65-4.59 (m, 2H), 4.58-4.55 (d, *J* = 12.5 Hz, 1H), 4.50 (s, 1H), 4.33-4.30 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.23-4.19 (t, *J* = 9.5 Hz, 1H), 4.0-3.97 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.96-3.91 (m, 2H), 3.68 (s, 3H), 3.67-3.65 (m, 1H), 3.57-3.53 (m, 2H), 3.35-3.31 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.47-2.43 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, 7.0 Hz, 4H), 1.65-1.56 (m, 4H), 1.43-1.39 (m, 2H), 1.30-1.28 (m, 24H) 1.16-1.14 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 139.2, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 126.3, 103.0, 101.7, 79.0, 78.9, 78.2, 76.2, 75.1, 73.8, 72.7, 72.6, 68.8, 67.8, 51.7, 43.0, 30.7, 34.0, 32.0, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.2, 24.1, 17.3; ESI-HRMS Calcd for $C_{58}H_{78}O_{10}$ $[M + Na]^+$: 957.5493. Found: 957.5488.

(2*S*,21*S*)-Methyl 21-benzyloxy-22-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-2-methyl-11-oxodocosanoate (303): Following the general procedure 8, using **299** (90 mg, 0.18 mmol) as acceptor, a mixture of α/β = 1:6.2 was obtained. Chromatographic purification of the mixture using 12% ethyl acetate in hexanes afforded **303** (100 mg, 60%) as colorless syrup. $[\alpha]^{23}_D$ -38.1 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.53-7.51 (d, *J* = 8.0 Hz, 2H), 7.46-7.45 (d, *J* = 6.0 Hz, 2H), 7.39-7.38 (d, *J* = 7.5 Hz, 2H), 7.32-7.29 (m, 14H), 5.63 (s, 1H), 5.0-4.97 (d, *J* = 12.0 Hz, 1H), 4.88-4.86 (d, *J* = 12.0 Hz, 1H), 4.68-4.66 (d, *J* = 12.0 Hz, 1H), 4.64-4.59 (m, 2H), 4.58-4.56 (d, *J* = 12.5 Hz, 1H), 4.50 (s, 1H), 4.34-4.31 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.24-4.20 (t, *J* = 9.5 Hz, 1H), 4.0-

3.97 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.97-3.91 (m, 2H), 3.68 (s, 3H), 3.67-3.65 (m, 1H), 3.57-3.53 (m, 2H), 3.35-3.31 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.47-2.43 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, 7.0 Hz, 4H), 1.65-1.56 (m, 4H), 1.43-1.39 (m, 2H), 1.30-1.28 (m, 24H) 1.16-1.15 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 139.2, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 126.3, 103.0, 101.7, 79.0, 78.9, 78.2, 76.2, 75.1, 73.8, 72.7, 72.6, 68.8, 67.8, 51.7, 43.1, 40.0, 34.0, 32.0, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.2, 24.1, 17.3.

(2*R*,21*R*)-Methyl 21-benzyloxy-22-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-2-methyl-11-oxodocosanoate (304): Following the general procedure 8, using **300** (130 mg, 0.28 mmol) as acceptor, a mixture of α/β = 1:6.5 was obtained. Chromatographic purification of the mixture using 12% ethyl acetate in hexanes afforded **304** (145 mg, 61%) as colorless syrup. $[\alpha]^{24}_{D}$ -36.8 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.53-7.52 (d, *J* = 7.0 Hz, 2H), 7.48-7.47 (d, *J* = 7.0 Hz, 2H), 7.40-7.38 (d, *J* = 7.5 Hz, 2H), 7.37-7.27 (m, 14H), 5.64 (s, 1H), 5.02-4.99 (d, *J* = 12.0 Hz, 1H), 4.90-4.88 (d, *J* = 12.0 Hz, 1H), 4.70-4.68 (d, *J* = 12.5 Hz, 1H), 4.66-4.64 (d, *J* = 11.5 Hz, 1H), 4.61-4.59 (d, J = 12.0 Hz, 1H), 4.59-4.56 (d, *J* = 11.5 Hz, 1H), 4.45 (s, 1H), 4.34-4.31 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.24-4.21 (t, *J* = 9.5 Hz, 1H), 4.03-4.0 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.97-3.93 (m, 2H), 3.68 (s, 3H), 3.58-3.55 (m, 3H), 3.34-3.29 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.47-2.43 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, 7.0 Hz, 4H), 1.65-1.56 (m, 4H), 1.43-1.39 (m, 2H), 1.30-1.28 (m, 24H) 1.16-1.15 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 139.1, 138.7, 138.6, 137.8, 129.1,

128.9, 128.6, 128.5, 128.4, 128.3, 128.0, 127.8, 126.3, 102.8, 101.6, 78.9, 78.2, 78.1, 76.0, 75.0, 72.6, 71.9, 71.4, 68.8, 67.9, 51.7, 43.0, 40.0, 34.0, 32.2, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.2, 24.0, 17.3.

(2*S*,21*R*)-Methyl 21-benzyloxy-22-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-2-methyl-11-oxodocosanoate (305): Following the general procedure 8, using **301** (60 mg, 0.12 mmol) as acceptor, a mixture of α/β = 1:5.4 was obtained. Chromatographic purification of the mixture using 12% ethyl acetate in hexanes afforded **305** (65 mg, 58%) as colorless syrup. $[\alpha]^{23}_{D}$ -33.8 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.52-7.51 (d, *J* = 6.0 Hz, 2H), 7.47-7.46 (d, *J* = 6.0 Hz, 2H), 7.40-7.38 (d, *J* = 7.5 Hz, 2H), 7.35-7.28 (m, 14H), 5.63 (s, 1H), 5.01-4.99 (d, *J* = 12.5 Hz, 1H), 4.89-4.87 (d, *J* = 12.5 Hz, 1H), 4.70-4.67 (d, *J* = 13.0 Hz, 1H), 4.65-4.63 (d, *J* = 12 Hz, 1H), 4.60-4.58 (d, *J* = 12 Hz, 1H), 4.58-4.56 (d, *J* = 11.5 Hz, 1H), 4.45 (s, 1H), 4.33-4.30 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.24-4.20 (t, *J* = 9.5 Hz, 1H), 4.02-3.99 (dd, *J* = 3.5 Hz, 10.5 Hz, 1H), 3.97-3.93 (m, 2H), 3.68 (s, 3H), 3.58-3.54 (m, 3H), 3.33-3.28 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.46-2.42 (q, *J* = 7.0 Hz, 1H), 2.40-2.37 (t, 7.0 Hz, 4H), 1.65-1.56 (m, 4H), 1.43-1.39 (m, 2H), 1.30-1.28 (m, 24H) 1.16-1.15 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 177.6, 139.0, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.9, 127.7, 126.3, 102.8, 101.6, 78.8, 78.2, 78.1, 76.0, 75.0, 72.5, 71.9, 71.4, 68.8, 67.9, 51.7, 43.0, 40.0, 34.0, 32.2, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.1, 24.0, 17.3.

(4*R*,23*S*)-*S*-*tert*-Butyl 23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-

dioxothiotetracosanoate (306): To a solution of **302** (80 mg, 0.085 mmol) in dioxane (1 mL) at room temperature was added aqueous LiOH (2.0 M, 1 mL). The mixture was warmed to 45 ^{o}C and stirred at same temperature for 12 h. After which the mixture was cooled to room temperature, stirred with saturated aqueous NH_4Cl for 30 min, and extracted with ethyl acetate three times. The combined organic phase was washed with brine and dried. Evaporation of solvent afforded the desired acid (quantitative), which was used directly for the next step.

Following the same procedure as for the preparation of **255**, using (2*R*,21*S*)-21-benzyloxy-22-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-2-methyl-11-oxodocosanoic acid (80 mg, 0.085 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **306** (70 mg, 85%, a 6:5 mixture of keto and enol forms) was obtained as light orange syrup. $[\alpha]^{24}_{D}$ -38.5 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.52-7.51 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.46-7.45 (d, *J* = 7.5 Hz, 2H), 7.39-7.38 (d, *J* = 7.5 Hz, 2H), 7.32-7.27 (m, 14H), 5.63 (s, 1H), 5.33 (s, 5/11H), 4.99-4.97 (d, *J* = 12.5 Hz, 1H), 4.88-4.86 (d, *J* = 12.5 Hz, 1H), 4.68-4.66 (d, *J* = 12.5 Hz, 1H), 4.63-4.62 (m, 2H), 4.58-4.55 (d, *J* = 12.5 Hz, 1H), 4.50 (s, 1H), 4.34-4.31 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.23-4.19 (t, *J* = 9.5 Hz, 1H), 3.99-3.96 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.96-3.92 (m, 2H), 3.68-3.66 (m, 1H), 3.61 (s, 12/11H), 3.57-3.53 (m, 2H), 3.35-3.30 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.69-2.65 (q, *J* = 7.0 Hz, 6/11H), 2.40-2.37 (t, *J* = 7.0 Hz, 4H), 2.14-2.11 (q, *J* = 7.0 Hz, 5/11H), 1.66-1.56 (m, 8H), 1.53 (s, 45/11H), 1.49 (s, 54/11H), 1.47-1.44 (m, 1H), 1.40-1.34 (m, 1H), 1.30-1.28 (m, 20H) 1.13-1.11 (d, *J* = 7.0 Hz, 15/11H), 1.11-

1.09 (d, *J* = 7.0 Hz, 18/11H); ^{13}C NMR (125 MHz) *δ* 211.9, 206.3, 196.5, 192.8, 180.6, 139.2, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 127.6, 126.3, 103.0, 101.7, 98.7, 79.0, 78.9, 78.2, 76.2, 75.1, 73.8, 72.7, 72.6, 68.8, 67.8, 57.1, 49.2, 48.3, 46.8, 43.1, 39.7, 34.3, 32.8, 32.0, 30.4, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 27.3, 25.5, 24.1, 18.2, 16.2; ESI-HRMS Calcd for $C_{63}H_{86}O_{10}S$ $[M + Na]^+$: 1057.5839. Found: 1057.5872.

(4*S*,23*S*)-*S*-*tert*-Butyl 23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxothiotetracosanoate (307): Following the same procedure as for the preparation of **306**, using **303** (70 mg, 0.075 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **307** (63 mg, 81%, a 7:5 mixture of keto and enol forms) was obtained as light orange syrup. $[\alpha]^{23}_D$ -27.5 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.52-7.51 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.46-7.45 (d, *J* = 7.5 Hz, 2H), 7.39-7.38 (d, *J* = 7.5 Hz, 2H), 7.32-7.27 (m, 14H), 5.63 (s, 1H), 5.33 (s, 5/12H), 4.99-4.97 (d, *J* = 12.5 Hz, 1H), 4.88-4.86 (d, *J* = 12.5 Hz, 1H), 4.68-4.66 (d, *J* = 12.5 Hz, 1H), 4.64-4.62 (m, 2H), 4.58-4.56 (d, *J* = 12.5 Hz, 1H), 4.50 (s, 1H), 4.34-4.32 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.23-4.19 (t, *J* = 9.5 Hz, 1H), 4.0-3.96 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.97-3.92 (m, 2H), 3.64-3.60 (m, 1H), 3.61 (s, 14/12H), 3.57-3.53 (m, 2H), 3.35-3.30 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.66-2.65 (q, *J* = 7.0 Hz, 7/12H), 2.40-2.37 (t, *J* = 7.0 Hz, 4H), 2.13-2.2.11 (q, *J* = 7.0 Hz, 5/12H), 1.66-1.56 (m, 8H), 1.53 (s, 45/12H), 1.49 (s, 63/12H), 1.47-1.44 (m, 1H), 1.40-1.34 (m, 1H), 1.30-1.28 (m, 20H) 1.13-1.11 (d, *J* = 7.0 Hz, 15/12H), 1.11-1.09 (d, *J* = 7.0 Hz, 21/12H); ^{13}C NMR (125 MHz) *δ* 211.9, 206.3, 196.5, 192.8, 180.6, 139.2,

138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 127.6, 126.3, 103.0, 101.7, 98.7, 78.9, 78.8, 78.2, 75.1, 73.8, 72.7, 72.6, 68.8, 67.8, 57.0, 49.2, 48.4, 46.8, 43.1, 39.7, 34.3, 32.8, 32.0, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 27.3, 25.5, 24.1, 24.0, 18.2, 16.2.

(4*R*,23*R*)-*S*-*tert*-Butyl 23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxothiotetracosanoate (308): Following the same procedure as for the preparation of **306**, using **304** (128 mg, 0.14 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **308** (130 mg, 90%, a 8:5 mixture of keto and enol forms) was obtained as light orange syrup. $[\alpha]^{24}_{D}$ -36.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.53-7.52 (d, *J* = 6.5 Hz, 2H), 7.48-7.47 (d, *J* = 6.5 Hz, 2H), 7.40-7.38 (d, *J* = 7.0 Hz, 2H), 7.37-7.31 (m, 14H), 5.64 (s, 1H), 5.34 (s, 5/13H), 5.02-4.99 (d, *J* = 12.5 Hz, 1H), 4.90-4.88 (d, *J* = 12.5 Hz, 1H), 4.70-4.68 (d, *J* = 12.5 Hz, 1H), 4.66-4.64 (d, *J* = 12.5 Hz, 1H), 4.61-4.59 (d, *J* = 12.0 Hz, 1H), 4.59-4.57 (d, *J* = 11.5 Hz, 1H), 4.45 (s, 1H), 4.34-4.31 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.25-4.21 (t, *J* = 9.5 Hz, 1H), 4.01-3.99 (m, 1H), 4.0-3.94 (m, 2H), 3.61 (s, 16/13H), 3.58-3.55 (m, 3H), 3.34-3.29 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.67-2.66 (q, *J* = 7.0 Hz, 8/13H), 2.41-2.38 (t, *J* = 7.0 Hz, 4H), 2.15-2.2.12 (q, *J* = 7.0 Hz, 5/13H), 1.66-1.56 (m, 8H), 1.54 (s, 45/13H), 1.50 (s, 72/13H), 1.47-1.44 (m, 1H), 1.40-1.34 (m, 1H), 1.30-1.28 (m, 20H) 1.15-1.13 (d, *J* = 7.0 Hz, 15/13H), 1.11-1.10 (d, *J* = 7.0 Hz, 24/13H); ^{13}C NMR (125 MHz) δ 211.8, 206.2, 196.5, 192.8, 180.6, 139.1, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 126.3, 102.8, 101.6, 98.7, 78.9, 78.2, 78.1, 75.0, 72.6, 71.9, 71.4, 68.8, 67.9, 57.1, 49.2,

48.3, 46.8, 43.1, 39.7, 34.3, 32.8, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 27.3, 25.7, 24.1, 24.0, 18.2, 16.2.

(4*S*,23*R*)-*S*-*tert*-Butyl 23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxothiotetracosanoate (309): Following the same procedure as for the preparation of **306**, using **305** (60 mg, 0.065 mmol) as substrate, and eluting with 15% ethyl acetate in hexanes, **309** (58 mg, 88%, a 5:2 mixture of keto and enol forms) was obtained as light orange syrup. $[\alpha]^{24}_{D}$ -24.3 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.52-7.51 (d, *J* = 6.0 Hz, 2H), 7.47-7.46 (d, *J* = 6.5 Hz, 2H), 7.40-7.38 (d, *J* = 7.5 Hz, 2H), 7.37-7.28 (m, 14H), 5.63 (s, 1H), 5.33 (s, 2/7H), 5.01-4.99 (d, *J* = 12.5 Hz, 1H), 4.90-4.87 (d, *J* = 12.5 Hz, 1H), 4.70-4.67 (d, *J* = 12.5 Hz, 1H), 4.65-4.63 (d, *J* = 11.5 Hz, 1H), 4.61-4.58 (d, *J* = 12.0 Hz, 1H), 4.58-4.56 (d, *J* = 11.5 Hz, 1H), 4.45 (s, 1H), 4.33-4.30 (dd, *J* = 5.0, 10.5 Hz, 1H), 4.24-4.20 (t, *J* = 9.5 Hz, 1H), 4.02-3.99 (dd, *J* = 3.0, 8.5 Hz, 1H), 3.97-3.94 (m, 2H), 3.61 (s, 10/7H), 3.58-3.54 (m, 3H), 3.34-3.29 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 2.69-2.66 (q, *J* = 7.0 Hz, 5/7H), 2.40-2.37 (t, *J* = 7.0 Hz, 4H), 2.15-2.12 (q, *J* = 7.0 Hz, 2/7H), 1.66-1.56 (m, 8H), 1.53 (s, 18/7H), 1.49 (s, 45/7H), 1.47-1.44 (m, 1H), 1.40-1.34 (m, 1H), 1.30-1.28 (m, 20H) 1.13-1.11 (d, *J* = 7.0 Hz, 6/7H), 1.11-1.09 (d, *J* = 7.0 Hz, 15/7H); ^{13}C NMR (125 MHz) δ 211.8, 206.2, 196.5, 192.8, 180.6, 139.0, 138.7, 138.6, 137.8, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 126.3, 102.8, 101.6, 98.7, 78.9, 78.2, 78.1, 76.0, 75.0, 71.9, 71.4, 68.8, 67.9, 57.0, 49.2, 48.3, 46.8, 43.1, 39.7, 34.3, 32.8, 32.2, 30.4, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 27.3, 25.7, 24.5, 24.1, 24.0, 18.2, 16.2.

(4*R*,23*S*)-*N*-[23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxotetracosanoyl]-*N*-(2',4',6'-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (310): Following the same procedure as for the preparation of **261**, using **306** (65 mg, 0.063 mmol) as substrate, and eluting with 35% ethyl acetate in hexanes, **310** (59 mg, 67%, a mixture of rotomers) was obtained as light yellow foam. $[\alpha]^{23}_{D}$ -49.4 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.52-7.51 (d, *J* = 6.0 Hz, 2H), 7.47-7.45 (m, 4H), 7.40-7.28 (m, 19H), 6.96-6.94 (d, *J* = 8.0 Hz, 2H), 6.80-6.78 (d, *J* = 8.0 Hz, 2H), 6.03 (s, 2H), 5.63 (s, 1H), 5.03 (s, 2H), 4.98-4.96 (d, *J* = 12.0 Hz, 1H), 4.88-4.86 (d, *J* = 12.0 Hz, 1H), 4.68-4.65 (d, *J* = 12.5 Hz, 1H), 4.63-4.62 (m, 2H), 4.58-4.55 (d, *J* = 12.0 Hz, 1H), 4.50 (s, 1H), 4.36-4.31 (m, 2H), 4.23-4.20 (t, *J* = 9.5 Hz, 1H), 4.17-4.14 (m, 1H), 4.00-3.97 (dd, *J* = 3.0, 11.0 Hz, 1H), 3.96-3.89 (m, 4H), 3.81 (s, 3H), 3.72 (s, 6H), 3.67-3.65 (m, 1H), 3.59 (s, 3H), 3.56-3.53 (m, 2H), 3.39-3.30 (m, 2H), 3.01-2.97 (dd, *J* = 5.5, 14.0 Hz, 1H), 2.79-2.75 (q, *J* = 7.0 Hz, 1H), 2.39-2.37 (m, 4H), 1.76-1.74 (m, 1H), 1.64-1.58 (m, 9H), 1.34-1.28 (m, 20H) 1.15-1.13 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 211.9, 209.0, 171.7, 167.5, 161.7, 159.9, 157.3, 139.2, 138.7, 138.6, 137.8, 137.5, 131.6, 130.6, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 126.3, 114.5, 104.2, 103.0, 101.6, 90.6, 79.0, 78.9, 78.2, 76.2, 75.1, 73.8, 72.7, 72.6, 70.2, 68.8, 67.8, 61.0, 55.6, 55.5, 52.1, 48.8, 46.3, 43.0, 42.8, 34.7, 33.0, 32.0, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.3, 25.5, 24.1, 16.0; ESI-HRMS Calcd for $C_{86}H_{107}NO_{16}$ $[M + Na]^{+}$: 1432.7488. Found: 1432.7444.

(4*S*,23*S*)-*N*-[23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxotetracosanoyl]-*N*-(2',4',6'-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (311): Following the same procedure as for the preparation of **261**, using **307** (57 mg, 0.038 mmol) as substrate, and eluting with 35% ethyl acetate in hexanes, **311** (50 mg, 65%, a mixture of rotomers) was obtained as light yellow foam. $[\alpha]^{23}_{D}$ -35.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.53-7.52 (d, *J* = 6.0 Hz, 2H), 7.47-7.45 (m, 4H), 7.40-7.28 (m, 19H), 6.97-6.96 (d, *J* = 8.0 Hz, 2H), 6.81-6.80 (d, *J* = 8.0 Hz, 2H), 6.04 (s, 2H), 5.64 (s, 1H), 5.04 (s, 2H), 5.00-4.98 (d, *J* = 12.0 Hz, 1H), 4.89-4.87 (d, *J* = 12.0 Hz, 1H), 4.69-4.66 (d, *J* = 12.5 Hz, 1H), 4.63-4.60 (m, 2H), 4.59-4.56 (d, *J* = 12.0 Hz, 1H), 4.50 (s, 1H), 4.37-4.31 (m, 2H), 4.24-4.20 (t, *J* = 9.5 Hz, 1H), 4.15-4.12 (m, 1H), 4.01-3.98 (dd, *J* = 3.0, 11.0 Hz, 1H), 3.97-3.90 (m, 4H), 3.81 (s, 3H), 3.73 (s, 6H), 3.60 (s, 3H), 3.58-3.54 (m, 2H), 3.40-3.31 (m, 2H), 3.04-3.00 (dd, *J* = 5.5, 14.0 Hz, 1H), 2.78-2.74 (q, *J* = 7.0 Hz, 1H), 2.40-2.38 (m, 4H), 1.76-1.74 (m, 1H), 1.64-1.58 (m, 9H), 1.34-1.28 (m, 20H) 1.18-1.16 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 211.9, 208.9, 171.7, 167.6, 161.7, 159.9, 157.3, 139.2, 138.7, 138.6, 137.8, 137.5, 131.6, 130.7, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 126.3, 114.5, 104.2, 103.0, 101.7, 90.6, 79.0, 78.9, 78.2, 76.2, 75.1, 73.8, 72.7, 72.6, 70.2, 68.9, 67.8, 61.0, 55.6, 55.5, 52.1, 47.7, 46.6, 43.1, 42.8, 34.6, 32.8, 32.0, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.3, 25.5, 24.1, 16.2.

(4*R*,23*R*)-*N*-[23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxotetracosanoyl]-*N*-(2',4',6'-

trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (312): Following the same procedure as for the preparation of **261**, using **308** (130 mg, 0.125 mmol) as substrate, and eluting with 35% ethyl acetate in hexanes, **312** (125 mg, 70%, a mixture of rotomers) was obtained as light yellow foam. $[\alpha]^{23}_{D}$ -42.8 (*c* 1.0); ^{1}H NMR (500 MHz) δ 7.54-7.53 (d, *J* = 6.0 Hz, 2H), 7.49-7.47 (m, 4H), 7.40-7.28 (m, 19H), 6.98-6.96 (d, *J* = 8.0 Hz, 2H), 6.82-6.80 (d, *J* = 8.0 Hz, 2H), 6.05 (s, 2H), 5.65 (s, 1H), 5.05 (s, 2H), 5.03-5.01 (d, *J* = 12.0 Hz, 1H), 4.92-4.89 (d, *J* = 12.0 Hz, 1H), 4.71-4.69 (d, *J* = 12.5 Hz, 1H), 4.67-4.65 (d, *J* = 12.5 Hz, 1H), 4.62-4.60 (d, *J* = 12.0 Hz, 1H), 4.60-4.58 (d, *J* = 12.0 Hz, 1H), 4.46 (s, 1H), 4.38-4.32 (m, 2H), 4.26-4.22 (t, *J* = 9.5 Hz, 1H), 4.19-4.17 (m, 1H), 4.03-4.02 (m, 1H), 3.98-3.92 (m, 4H), 3.82 (s, 3H), 3.74 (s, 6H), 3.61 (s, 3H), 3.60-3.56 (m, 2H), 3.41-3.38 (dd, *J* = 5.5, 14.0 Hz, 1H), 3.35-3.30 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 3.05-3.01 (dd, J = 5.5, 14.0 Hz, 1H), 2.80-2.77 (q, *J* = 7.0 Hz, 1H), 2.41-2.38 (m, 4H), 1.76-1.74 (m, 1H), 1.64-1.58 (m, 8H), 1.47-1.44 (m, 1H), 1.34-1.28 (m, 20H) 1.17-1.16 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 211.9, 209.0, 171.7, 167.5, 161.7, 159.9, 157.4, 139.1, 138.7, 138.6, 137.9, 137.5, 131.6, 130.7, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 126.3, 114.5, 104.2, 102.8, 101.7, 90.6, 78.9, 78.2, 76.0, 75.0, 72.6, 71.9, 71.5, 70.2, 68.9, 67.9, 61.1, 55.6, 55.5, 52.1, 48.0, 46.4, 43.1, 42.8, 34.7, 33.1, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.1, 16.1.

(4*S*,23*R*)-*N*-[23-benzyloxy-24-(2',3'-di-*O*-benzyl-4',6'-*O*-benzylidene-β-D-mannopyranosyloxy)-4-methyl-3,13-dioxotetracosanoyl]-*N*-(2',4',6'-trimethoxybenzyl)-*O*-benzyl-L-tyrosine methyl ester (313): Following the

same procedure as for the preparation of **261**, using **309** (50 mg, 0.048 mmol) as substrate, and eluting with 35% ethyl acetate in hexanes, **313** (42 mg, 66%, a mixture of rotomers) was obtained as light yellow foam. $[\alpha]^{23}_{D}$ -32.0 (*c* 1.0); ^{1}H NMR (500 MHz) *δ* 7.53-7.51 (d, *J* = 6.0 Hz, 2H), 7.48-7.46 (m, 4H), 7.40-7.28 (m, 19H), 6.97-6.95 (d, *J* = 8.0 Hz, 2H), 6.81-6.79 (d, *J* = 8.0 Hz, 2H), 6.03 (s, 2H), 5.63 (s, 1H), 5.04 (s, 2H), 5.01-4.99 (d, *J* = 12.0 Hz, 1H), 4.90-4.87 (d, *J* = 12.0 Hz, 1H), 4.70-4.67 (d, *J* = 12.5 Hz, 1H), 4.65-4.63 (d, *J* = 12.5 Hz, 1H), 4.61-4.58 (d, *J* = 12.0 Hz, 1H), 4.58-4.56 (d, *J* = 12.0 Hz, 1H), 4.45 (s, 1H), 4.37-4.31 (m, 2H), 4.24-4.20 (t, *J* = 9.5 Hz, 1H), 4.15-4.12 (m, 1H), 4.02-4.01 (m, 1H), 3.97-3.92 (m, 4H), 3.81 (s, 3H), 3.73 (s, 6H), 3.59 (s, 3H), 3.58-3.55 (m, 2H), 3.39-3.35 (dd, *J* = 5.5, 14.0 Hz, 1H), 3.33-3.28 (ddd, *J* = 5.0, 9.0, 9.8 Hz, 1H), 3.03-2.99 (dd, *J* = 5.5, 14.0 Hz, 1H), 2.78-2.76 (q, *J* = 7.0 Hz, 1H), 2.40-2.38 (m, 4H), 1.76-1.74 (m, 1H), 1.64-1.58 (m, 8H), 1.47-1.44 (m, 1H), 1.34-1.28 (m, 20H) 1.18-1.16 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) *δ* 211.9, 208.9, 171.7, 167.6, 161.7, 159.9, 157.3, 139.0, 138.7, 138.6, 137.8, 137.5, 131.6, 130.7, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 126.3, 114.5, 104.2, 102.8, 101.6, 90.6, 78.9, 78.1, 76.0, 75.0, 72.6, 71.9, 71.4, 70.2, 68.8, 67.9, 61.0, 55.6, 55.5, 52.1, 47.7, 46.6, 43.1, 42.8, 34.6, 32.8, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.4, 25.7, 24.1, 16.2.

(5*S*,2'*R*,21'*S*)-5-(4''-Hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)pyrrolidin-2,4-dione (314): To a solution of **310** (40 mg, 0.028 mmol) in MeOH: THF (v/v 3:1, 6 mL) was added AcOH (20 µL) and 20% $Pd(OH)_2$/C (20 mg). The mixture was purged

with H_2 three times and shaken under H_2 (50 psi). The progress of the deprotection was carefully monitored in every 1 h by ESI-TOF mass spectrometry and shaking was continued until no starting material or partially benzyl deprotected compounds were observed (3 - 4 h; longer time shaking under H_2 deprotect the TMB group). After which the reaction mixture was filtered through a Celite pad and washed with MeOH (3 × 5 mL). Evaporation of the solvent afforded the corresponding debenzylated ketoamide, which was taken forward for cyclization without purification.

To a stirred solution of the ketoamide (27 mg, 0.028 mmol) in MeOH (2 mL) at room temperature was added KO^{t}Bu (12 mg, 0.112 mmol) in one portion. The reaction mixture was stirred at same temperature for 30 min before it was neutralized to pH 5-6 by Amberlyst-15 ion exchange resin. The solid was filtered off, and evaporation of the filtrate afforded (5*S*,2'*R*,21'*S*)-5-(4''-hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)-1-(2'',4'',6''-trimethoxybenzyl)pyrrolidin-2,4-dione, which was taken forward for TMB deprotection without purification.

A solution of (5*S*,2'*R*,21'*S*)-5-(4''-hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)-1-(2'',4'',6''-trimethoxybenzyl)pyrrolidin-2,4-dione (25 mg, 0.027 mmol) in CH_2Cl_2: TFA: anisole (v/v/v 90:5:5, 1 mL) was stirred at room temperature for 1 h. After which, the mixture was diluted with toluene: MeOH (v/v 1:1, 2 mL) and the mixture was concentrated to dryness. The resulting residue was washed with $CHCl_3$ (2 × 2 mL) and the solvent was decanted. Subsequently, the residue was again

dissolved in MeOH (2 mL) and the mixture was concentrated to dryness followed by washing with $CHCl_3$ (2 × 2 mL) afforded **314** (19 mg, 90% over three steps) as light orange foam. $[\alpha]^{24}_D$ -54.0 (*c* 0.3 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 9.20 (br s, 1H), 8.97 (br s, 1H), 6.92-6.90 (d, *J* = 8.0 Hz, 2H), 6.61-6.59 (d, *J* = 8.0 Hz, 2H), 4.40 (s, 1H), 4.09-4.08 (m, 1H), 3.71-3.68 (m, 2H), 3.67-3.64 (dd, *J* = 4.5, 10.0 Hz, 1H), 3.57-3.55 (m, 1H), 3.48-3.45 (dd, *J* = 6.5, 11.5 Hz, 1H), 3.34-3.29 (m, 2H), 3.27-3.25 (dd, *J* = 3.0, 9.5 Hz, 1H), 3.05-3.01 (m, 1H), 2.89-2.82 (m, 2H), 2.41-2.39 (m, 4H), 1.47-1.40 (m, 8H), 1.28-1.24 (m, 22H) 1.06-1.05 (d, *J* = 2.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 210.7, 194.6, 191.6, 175.4, 155.9, 130.6, 125.5, 114.7, 100.6, 100.4, 77.5, 76.0, 73.6, 73.4, 70.5, 69.1, 67.1, 62.4, 61.4, 41.8, 35.5, 33.6, 33.1, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4, 26.4, 25.1, 23.3, 16.6; ESI-HRMS Calcd for $C_{49}H_{58}NO_7$ $[M + Na]^+$: 772.4213. Found: 772.4199.

(5*S*,2'*S*,21'*S*)-5-(4''-Hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)pyrrolidin-2,4-dione (315): Following the same procedure as for the preparation of **314**, using **311** (50 mg, 0.035 mmol) as substrate, **315** (24 mg, 92%, over three steps) was obtained as light orange foam. $[\alpha]^{24}_D$ -71.7 (*c* 0.3 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 9.22 (br s, 1H), 6.95-6.93 (d, *J* = 8.0 Hz, 2H), 6.63-6.61 (d, *J* = 8.0 Hz, 2H), 4.72-4.67 (br m, 5H), 4.41 (s, 1H), 4.05-4.00 (m, 1H), 3.72-3.69 (m, 2H), 3.67-3.65 (dd, *J* = 4.5, 10.0 Hz, 1H), 3.58-3.54 (m, 1H), 3.50-3.46 (dd, *J* = 6.5, 11.5 Hz, 1H), 3.46-3.44 (m, 1H), 3.35-3.31 (m, 2H), 3.28-3.26 (dd, *J* = 3.0, 9.5 Hz, 1H), 3.06-3.03 (m, 1H), 2.88-2.82 (m, 2H), 2.41-2.39 (m, 4H), 1.47-1.40 (m, 8H),

1.28-1.24 (m, 22H) 0.97-0.96 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 210.7, 194.7, 191.6, 175.6, 156.0, 130.6, 125.1, 114.7, 100.6, 100.0, 77.5, 73.7, 73.5, 70.5, 69.1, 67.1, 62.1, 61.3, 41.8, 36.0, 33.6, 32.6, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4, 26.8, 25.2, 23.3, 17.1; ESI-HRMS Calcd for $C_{49}H_{58}NO_7$ [M + Na]$^+$: 772.4213. Found: 772.4204.

(5*S*,2'*R*,21'*R*)-5-(4''-Hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)pyrrolidin-2,4-dione (316): Following the same procedure as for the preparation of **314**, using **312** (28 mg, 0.02 mmol) as substrate, **316** (13 mg, 91%, over three steps) was obtained as light orange foam. [α]$^{24}_D$ -47.3 (*c* 0.3 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 9.24 (br s, 1H), 6.96-6.94 (d, *J* = 8.0 Hz, 2H), 6.63-6.61 (d, *J* = 8.0 Hz, 2H), 4.42 (s, 1H), 3.82-3.81 (m, 1H), 3.72-3.69 (m, 2H), 3.64-3.60 (m, 2H), 3.58-3.54 (m, 1H), 3.51-3.47 (dd, *J* = 6.5, 11.5 Hz, 1H), 3.40-3.37 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.33-3.28 (m, 2H), 3.06-3.03 (m, 1H), 2.87-2.84 (m, 1H), 2.67-2.64 (m, 1H), 2.41-2.39 (m, 4H), 1.47-1.40 (m, 8H), 1.28-1.24 (m, 22H) 0.96-0.94 (d, *J* = 2.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 210.7, 194.6, 175.8, 155.7, 130.4, 114.7, 100.2, 77.4, 73.6, 73.4, 70.5, 68.8, 67.0, 61.2, 41.8, 36.7, 33.4, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4, 26.8, 25.1, 17.2; ESI-HRMS Calcd for $C_{49}H_{58}NO_7$ [M + Na]$^+$: 772.4213. Found: 772.4201.

(5*S*,2'*S*,21'*R*)-5-(4''-Hydroxybenzyl)-3-(21'-hydroxy-22'-β-D-mannopyranosyloxy-2'-methyl-11'-oxodocosanoyl)pyrrolidin-2,4-dione (317): Following the same procedure as for the preparation of **314**, using **313** (31 mg, 0.022 mmol) as substrate, **317** (14.5 mg, 88%, over three steps) was

obtained as light orange foam. $[\alpha]^{24}_{D}$ -63.4 (*c* 0.3 in MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (br s, 1H), 8.35 (s, 1H), 6.95-6.94 (d, *J* = 8.0 Hz, 2H), 6.63-6.62 (d, *J* = 8.0 Hz, 2H), 4.41 (s, 1H), 3.72-3.70 (m, 1H), 3.69-3.67 (m, 2H), 3.64-3.58 (m, 2H), 3.50-3.46 (dd, *J* = 6.5, 11.5 Hz, 1H), 3.40-3.30 (m, 3H), 3.29-3.26 (dd, *J* = 3.0, 9.5 Hz, 1H), 3.06-3.03 (m, 1H), 2.88-2.84 (m, 1H), 2.75-2.73 (m, 1H), 2.41-2.39 (m, 4H), 1.47-1.40 (m, 8H), 1.28-1.24 (m, 22H) 0.94-0.93 (d, *J* = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.6, 194.6, 175.4, 155.8, 130.5, 125.1, 114.7, 100.3, 77.4, 73.6, 73.4, 70.5, 68.8, 67.1, 62.9, 61.4, 41.8, 36.0, 33.3, 32.8, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4, 26.8, 25.1, 23.3, 17.4; ESI-HRMS Calcd for $C_{49}H_{58}NO_7$ $[M + Na]^+$: 772.4213. Found: 772.4197.

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ABSTRACT

DEVELOPMENT AND APPLICATIONS OF THIOACIDS IN PEPTIDE CHEMISTRY AND EXPLORATION OF METHODS TOWARD THE STEREOCHEMICAL ELUCIDATION AND SYNTHESIS OF VIRGINEONE

by

KASINATH SANA

December 2010

Advisor: David Crich

Major: Chemistry

Degree: Doctor of Philosophy

This dissertation describes investigations toward the development of methods for the synthesis of peptidyl thioacids and thioesters, with particular emphasis on applications in peptide chemistry, and the exploration of methods toward the assignment of the relative and absolute configuration of virgineone and its total synthesis.

The first part of chapter one introduces the general characteristics and reactivity of thioacids, and describes their potential for application to the chemical synthesis of peptides and proteins. The second part of chapter one overviews the solution and solid-phase synthesis of peptidyl thioacids and thioesters. The last section of chapter one reviews the importance of tetramic acid containing natural products, and the various methods for their formation, and continues with an overview of stereoselective methods for β-mannosylation, such as are required for the total synthesis of virgineone, a novel glycosylated tetramic acid containing natural product.

The second chapter describes studies focused on the development of a methodology for the synthesis of amino and peptidyl thioacids based on the cleavage of the 9-fluorenylmethyl (Fm) group from 9-fluorenlylmethyl thioesters. Subsequently, the formation of native amide bonds in the reaction between C-terminal peptidyl thioacids and electron deficient N-terminal 2,4-dinitrobenzenesulfonamides is presented.

In chapter three, building on the method for the solution-phase synthesis of amino and peptidyl thioacids described in chapter two, the development of a 9-fluorenlylmethyl thioester based linker, *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid is described. The use of this linker for the synthesis of peptidyl thioacids on solid support employing Boc-SPPS is then demonstrated.

In chapter four, continuing the theme of the development of the chemistry of thioacids, a method for the in situ generation of thioacids by the regioselective ring opening of β-thiolactones is set out. To further explore this concept, investigations were conducted on the ring-opening of various β-thiolactones with soft aromatic thiolates to form thiocarboxylates, with subsequent trapping in-situ by Mukaiyama's reagent or Sanger's reagent in presence of an amine to form amide bonds.

In chapter five, studies toward the assignment of configuration and the total synthesis of vigineone are described. A practical synthetic route to various stereoisomers of virgineone is elaborated.

In chapter six, overall conclusions of the dissertation are presentated, while in chapter seven, the experimental procedures and characterization data for the synthesized compounds are documented.

AUTOBIOGRAPHICAL STATEMENT

Education

- 2007.07-Present, Candidate for Ph.D. in Organic Chemistry
  Wayne State University, Detroit, Michigan
  Advisor: Professor David Crich
- 2005.08-2007.07, Candidate for Ph.D in Organic Chemistry
  University of Illinois at Chicago, Chicago, Illinois
  Advisor: Professor David Crich
- 2003.07-2005.06, Master of Science in Chemistry
  Indian Institute of Technology (I.I.T.), Kanpur, India.

Publications

1. Crich, D.; Sana, K. "Solid-Phase Synthesis of Peptidyl Thioacids Employing a 9-Fluorenylmethyl Thioester-Based Linker in Conjunction with Boc Chemistry." *J. Org. Chem.*, **2009**, *74*, 7383-7388. Highlighted in *Synfacts,* **2009**, *12*, 1417-1417.
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Invited Publications

1. Sana, K. "Bis(trimethylsilylphenyl) Disulfide and Bis(trimethylsilylphenyl) Diselenide." *Electronic Encyclopedia of Reagents for Organic Synthesis*, ed. L. A. Paquette, John Wiley & Sons. Ltd.
2. Sana, K. "Dodecyl Methyl Sulfide." *Electronic Encyclopedia of Reagents for Organic Synthesis*, ed. L. A. Paquette, John Wiley & Sons. Ltd.