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Functionalized 2-oxopiperazines from amino acids

Ulhas Bhatt

Department of Chemistry

McGill University

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Abstract

This thesis describes the development of four synthetic routes towards the syntheses of N, N' ethylene-bridged dipeptides and their application towards the synthesis of such 2-oxopiperazine derivatives on solid support.

The first route proceeds by the generation of an N-allyl substituted amino ester, its coupling to a second amino acid and subsequent oxidative cleavage of the carbon-carbon double bond to provide the cyclized product. The second route proceeds by the generation of 4-nitrobenzenesulfonamide dipeptides and their alkylation by 1,2-dibromoethane. The third route proceeds by a Mitsunobu reaction between sulfonamide dipeptides and bromoethanol followed by treatment with DBU to generate the desired 2-oxopiperazine derivative. The fourth route again uses the sulfonamide dipeptide esters as intermediates. This time an allyl group is installed and this is then converted to the diol that is subsequently oxidatively cleaved by action of sodium periodate. Alternatively, the allyl group is converted to the epoxide that is treated with periodic acid to effectively carry out the same transformation.

The synthesis of these ethylene-bridged dipeptides was also carried out on solid support in an effort to create a combinatorial library of such constrained peptidomimetics. The partial success of the above-mentioned approaches on solid support is described.

Finally, the synthesis of two new analogs of Thyrotropin Releasing Hormone (TRH) incorporating the 2-oxopiperazine ring is described.

"The most fundamental and lasting objective of synthesis is not the production of new compounds, but of production of properties."

George S. Hammond, Norris Award Lecture, 1968.

Résumé

Cette thèse décrit le développement de quatre voies de synthèse vers des dipeptides N, N' éthylène pontés et leur application pour la synthèse de dérivés 2-oxopipérazine sur support solide.

La première voie procède par la génération d'un amino ester N-allyl substitué, son couplage à un second amino acide et consécutivement le clivage oxydatif de la double liaison carbone-carbone suivi de la cyclisation. La seconde méthode procède par la génération de dipeptides 4-nitrobenzènesulfonamide et leur alkylation par le 1,2-dibromoéthane. La troisième route consiste en une réaction de Mitsunobu entre des dipeptides sulfonamides et le bromoéthanol, suivi par un traitement au DBU afin de produire le dérivé 2-oxopipérazine désiré. La quatrième voie utilise aussi des dipeptides sulfonamides en tant qu'intermédiaires. Cette fois, un groupe allyl est installé et est ensuite converti en diol, avant d' être clivé de manière oxydative par l'action du periodate de sodium. Une méthode alternative consiste en la conversion du groupe allyl en époxyde qui est ensuite traité par de l'acide periodique afin d'effectuer la même transformation.

La synthèse de ces dipeptides N N' éthylène pontés est aussi exécutée sur support solide dans le but de créer une librairie combinatoire de tels peptidomimétiques constraints. Les succès partiels des approches mentionnées ci-dessus sur support solide sont décrits.

Enfin, deux nouveaux analogues du TRH (Thyrotropin Releasing Hormone) incluant le cycle 2-oxopipérazine furent synthétisés.

Acknowledgements

I thank Prof. Just for guiding me throughout this study. I am grateful for all his efforts and timely inputs in pushing this project forward. I also appreciate the enormous amount of freedom he gave me and the patience he displayed when things were not falling into place. Thanks a lot!

I also thank Dr. Edward Roberts for suggesting the research topic and giving me an opportunity to work at Astra Research Centre, Montreal (now AstraZeneca) laboratories and gain valuable experience in solid phase synthesis.

My colleagues in lab 238 were extremely helpful throughout my stay and I really enjoyed working with them all. Thanks to Eric, Costa, Naz, Yi, Marija, Arlene, Jianchao, Toni, Lu and Kevin for helping me inspite of all the troubles I caused them in the lab! I am also grateful to Prof. Gleason and his group for all their suggestions during this study. Thanks to Regis for translating the abstract into French. Merci!

I thank the department for providing me with unending teaching assistantships. I also thank Astra, NSERC and FCAR for financial support. Thank you Renee, for taking care of all the administrative matters. Of course, this work would not have been possible without Dr. Sauriol. Thank you very much for teaching me NMR spectroscopy. Thanks to Mr. Saadeh and Dr. Mamer for performing mass spectra on the tiniest amounts of samples that I always provided. Thanks to Mr. Kopp, for making all the columns and the solid phase synthesis vessels.

Finally, I feel extremely fortunate to have had Bala, not only as my roommate but also as a wonderful friend, and Prasadas for providing me with a family atmosphere.

Table of Contents

EI	electron ionization
eq.	equivalent
ES (±)	electron spray
EtOAc	ethyl acetate
FAB	fast atom bombardment
Fmoc	9-fluorenmethyloxycarbonyl group
g	gram(s)
Glp	pyroglutamic acid
Gly	glycine
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
hr(s)	hour(s)
His	histidine
Hz	Hertz
HRMS	high resolution mass spectrometry
LCMS	liquid chromatography mass spectrometry
m	multiplet
m/z	mass to charge ratio
MBHA	4-methylbenzhydrylamine
min(s)	minute(s)
mmol	millimole
M.P.	melting point
MS	mass spectrometry
N	normality
NBA	nitrobenzyl alcohol

1.1 Combinatorial Chemistry

The single greatest time constraint on the process of drug discovery has been the synthesis and evaluation of candidate molecules one at a time. During the 1970s, a detailed understanding of enzyme mechanisms led to an emphasis on rational drug design. Consequently target molecules contained chemical features tailored to elicit maximum biological response based on information available through crystallographic, NMR, modeling and computational studies. Although these approaches have achieved remarkable success, the steady stream of potential drug targets uncovered by dramatic technological advances in molecular biology made it necessary to generate molecules at an even greater speed.

Combinatorial chemistry has come forward as one response to this challenge.[1] Whereas synthesis in medicinal chemistry conventionally serves the goal of producing a single product of previously specified structure for bioassay, the goal of combinatorial chemistry is to create searchable *populations* of molecules. Combinatorial chemistry is a type of synthetic strategy which leads to a collection of differing molecules i.e. chemical library with *subsets* of molecules exhibiting their own individual properties. This is achieved by generating molecules with distinct structural motifs that display a spectrum of biological response.

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[1] (a) Bunin, B. A. *The Combinatorial Index* **1998**, Academic Press. (b) Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*, 144.

A number of factors may be considered in the selection of compounds for library synthesis. One strategy is to select privileged structures that have shown propensity towards the desired biological target. For example; various libraries based on the 1,4-benzodiazepine ring system have been screened for lead compounds.[2] Another approach relies upon the ease of recognition of certain molecular recognition motifs (e.g. turn mimetics, helices, strands, etc.) by proteins.[3] Yet another plan revolves around generation of a stable mimic of the transition state or intermediate of the reaction catalyzed by an important enzyme classes. In addition, there are various approaches towards generation of completely random molecules with no regard whatsoever to the chemical properties being generated. Regardless of the strategy followed, the library compounds should meet a few prerequisites before they are synthesized. First, several different sets of building blocks should be incorporated to provide access to a large number of diverse compounds. Second, the chemistry should be compatible with the display of as much functionality as possible. Finally, the building blocks used in the synthesis must be commercially available or at least readily accessible.

1.2 Peptide Based Combinatorial Libraries

Peptides and peptidomimetics meet all the prerequisites mentioned above and hence it is not surprising that a large number of libraries have been synthesized using peptides. These peptide libraries have proven to be an excellent source of lead compounds in many drug discovery projects. This is so not only because these are easy to synthesize, but also because a large number of peptides and peptide-like molecules

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[2] (a) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997.
[3] DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M. R.; Pavia, M. R. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *33*, 116.

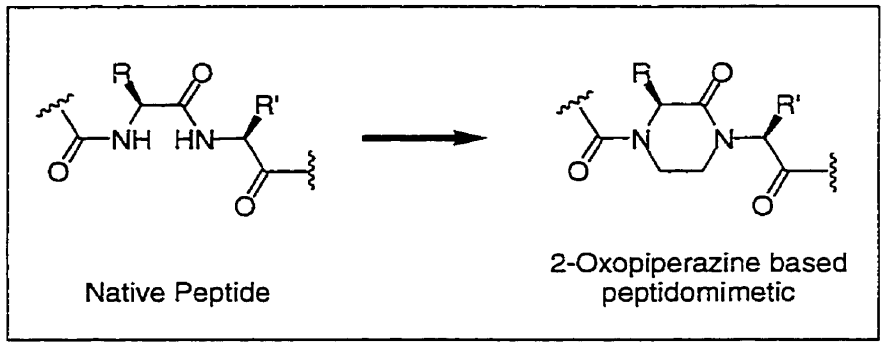
(peptidomimetics) have the ability to interact with biological molecules. The constituent amino acids ($H_2NCH(R)COOH$) of these peptides have varied chemical and physiological properties owing to the presence of a variety of functional groups. In most cases, these peptides interact with their receptors via the functional groups present on the "R" side chains of the amino acids. The amide backbone usually only provides the spatial framework supporting these functional groups. In many of these compounds however, it is found that only two or three amino acids played the critical role in eliciting the desired biological response. Thus it is not necessary to synthesize large peptides to elicit favorable biological response; instead small peptides (or in some cases, small peptide-like molecules) can generate the desired biological response. Thus, even a small peptidomimetic compound can have a high potential to interact with a biological receptor, provided the amino acid side-chains are oriented in the specific desired orientation.

1.3 2-Oxopiperazine Ring As A Peptidomimetic

The 2-oxopiperazine ring, which gets generated by the installation of the ethylene bridge across the two nitrogen atoms of a dipeptide, was chosen to direct the amino acid side chains into specific orientations. The ethylene-bridge imparts conformational restriction on the molecule thereby reducing its degrees of freedom. By doing so the molecule will have predefined spatial orientations and this makes the interaction with a biological receptor favorable. Czaplewski *et al.* have described the effect of installing the ethylene-bridge across a dipeptide.[4] Their computations indicate these molecules have equal preference for only two enantiomeric-twisted chair/boat-like pucker for the 2-

oxopiperazine ring. Further substitutions on these 2-oxopiperazine ring derivatives can lead to compounds that adopt conformations resembling β-turns as has been described in at least two recent reports.[5]

2-Oxopiperazine Ring As A Peptidomimetic



As shown in the section below, these 2-oxopiperazine derivatives have been synthesized in a variety of ways. We wanted to use amino acids for the construction of the 2-oxopiperazine ring and thus utilize the power of functional groups diversity present in amino acid side chains to unearth peptidomimetics with optimum biological response.

1.4 Literature Precedents for 2-Oxopiperazine Ring Synthesis

Interestingly, one of the first reports on the synthesis of oxopiperazine derivatives from using amino acids was by John DiMaio and Bernard Belleau from our own department.[6] They synthesized an oxopiperazine ring derivative using Z-tyrosine and ethyl N-(2, 2-dimethoxy ethyl)glycinate (which was prepared from amino acetaldehyde diethyl acetal and ethyliodoacetate) as shown below. Thus in their synthesis, only one of

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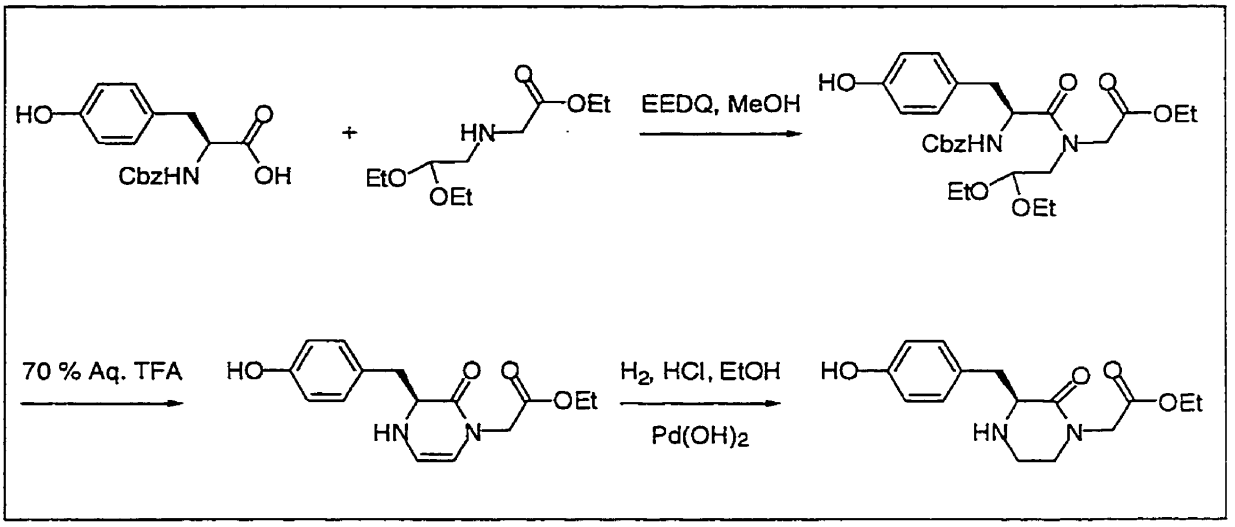
[4] Czaplewski, C.; Lammek, B.; Ciarkowski, J. *Polish J. Chem.* **1994**, *68*, 2589.
[5] (a) Goodfellow, V. S.; Laudeman, C. P.; Gerrity, J. I.; Burkard, M.; Strobel, E.; Zizack, J. S.; McLeod, D. A. *Molecular Diversity*, **1996**, 2, 97. (b) Smith, L. R.; Bartlett, P. A. *Molecules Online* **1998**, 2, 58.

the two nitrogen atoms in the oxopiperazine ring was coming from an amino acid which in this case was tyrosine.

2-Oxopiperazine Ring Synthesis By DiMaio et al.



Thus from the point of synthesizing a library of such compounds using this methodology, two sites of diversity were coming from the two amino acids and another site could be generated by a final acylation of the amine. Their study was limited to the synthesis of only one such compound and no evaluation of its biological activity was reported.

In 1990, Dutta *et al.* reported a short synthesis of the oxopiperazine ring system utilizing diethyl maleate and a substituted ethylenediamine.[7] Thus this route completely bypassed amino acids as potential precursors for oxopiperazine ring synthesis.

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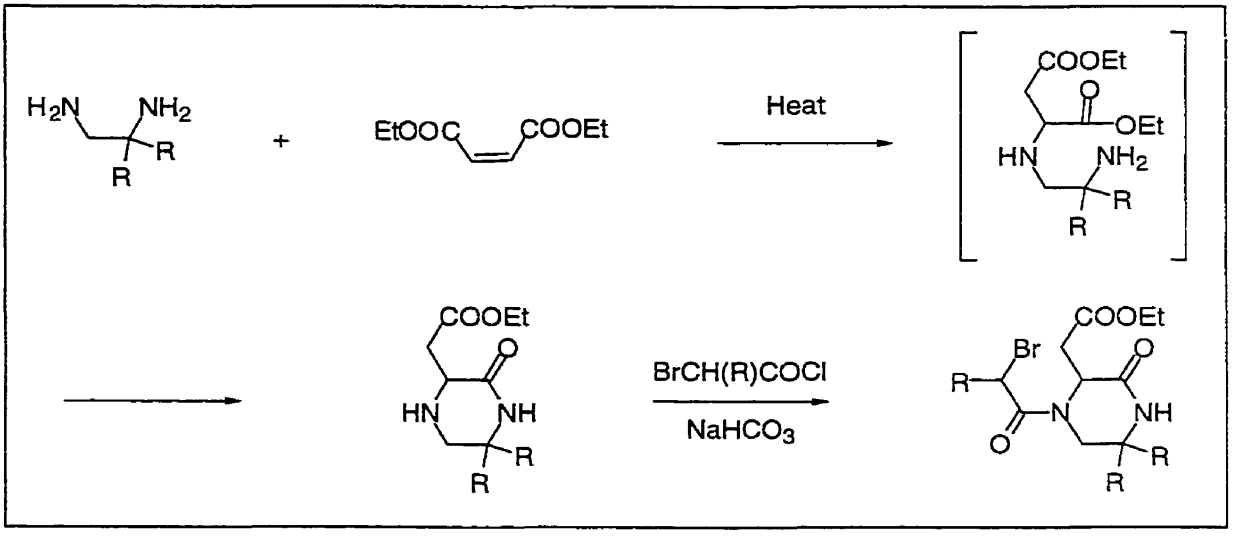
[6] DiMaio, J.; Belleau, B. *J. Chem. Soc. Perkin Trans. I* **1989**, 1687.
[7] Dutta, P. L.; Foye, W. O. *J. Pharm. Sci.* **1990**, *79*, 447.

2-Oxopiperazine Ring Synthesis By Dutta et al.



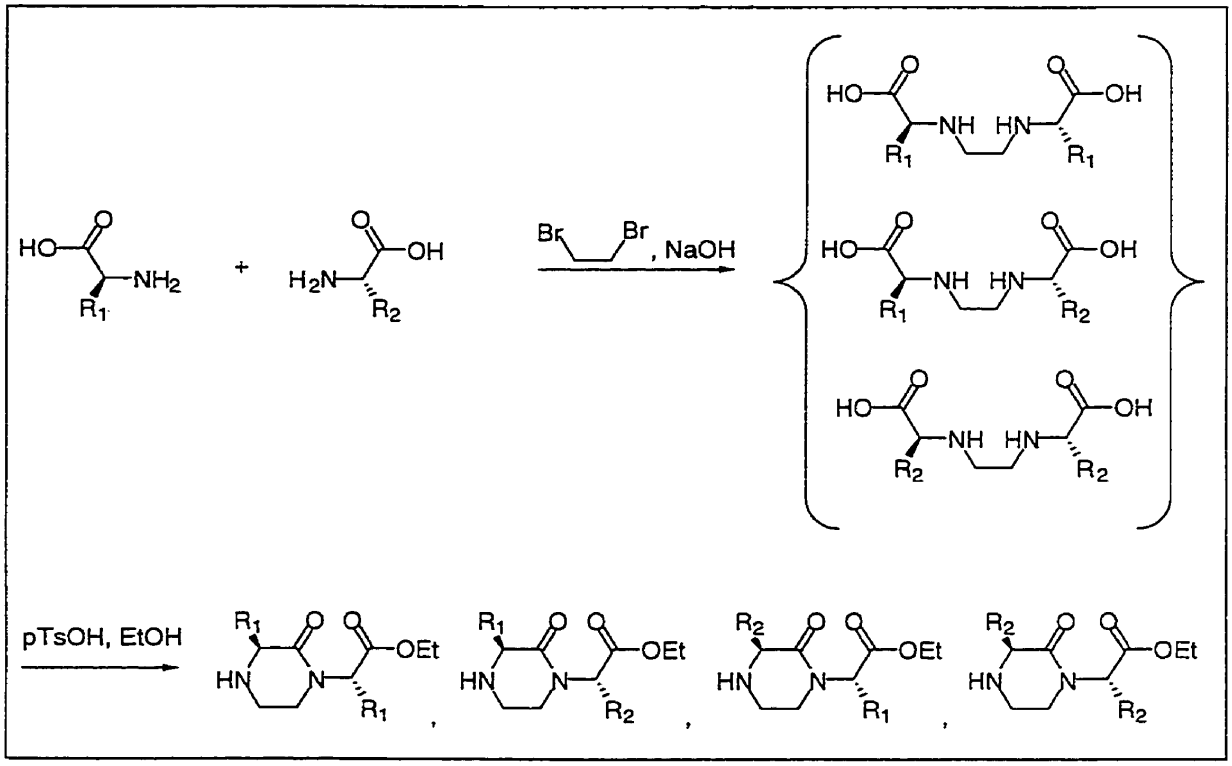
This route of course generates at least two sites of diversity on the ethylene-bridge itself. These compounds showed good inhibition of aspartate transcarbamoylase and also demonstrated inhibition of growth of a few cancer cell lines.

Yamashita *et al.* reported a methodology by which oxopiperazines were synthesized using two amino acids as precursors such that each amino acid contributed its nitrogen atom to the final cyclic product.[8] In this route, an amino acid was reacted with 1,2-dibromoethane in K_2CO_3 to give the linear product that could be subsequently cyclized to generate the oxopiperazine ring. Although this synthesis was short and simple, it only allowed easy synthesis of oxopiperazines with the same amino acid residue at the two positions. When two different amino acids were used, a mixture of four

[8] (a) Takenaka, H.; Miyake, H.; Kojima, Y.; Yasuda, M.; Gemba, M.; Yamashita, T. *J. Chem. Soc. Perkin Trans. I* **1993**, 933. (b) Yamashita, T.; Hatamoto, E.; Takenaka, H.; Kojima, Y.; Inoue, Y.; Gemba, M.; Yasuda, M. *Chem. Pharm. Bull.* **1996**, *44*, 856. (c) Yamashita, T.; Tsuru, E.; Doe, M.; Shibata, K.; Yasuda, M.; Gemba, M. *Chem. Pharm. Bull.* **1997**, *45*, 1940.

compounds was created that were separated using chromatography. Despite this major drawback, this route was the first route that used two amino acid residues to provide the two nitrogen atoms and derive the ethylene bridge from a two-carbon unit. These compounds were prepared as analogs of enkephalin (H-tyrosyl-D-alanyl-glycyl-eXX'-OEt) but failed to show significant improvement in their binding to the enkephalin receptor. This group also reduced the amide bond of the oxopiperazines and evaluated the resulting piperazines for opiate activity in a subsequent study.

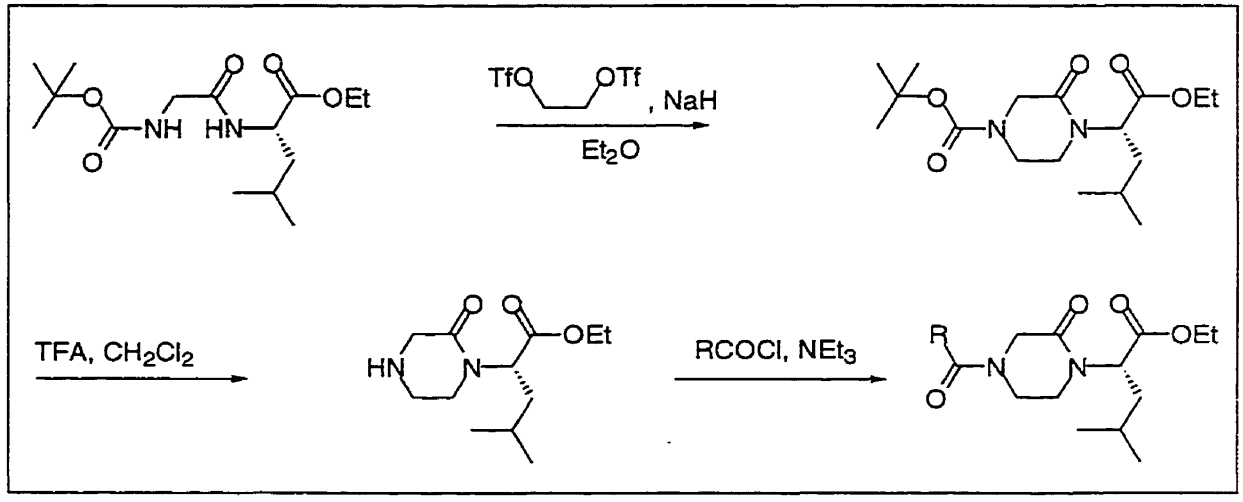
2-Oxopiperazine Ring Synthesis By Yamashita et al.



In 1997 while our work was in progress, Pohlmann *et al.* reported an elegant synthesis of the oxopiperazine ring system using two amino acids and a two-carbon unit

to link the two nitrogen atoms by an ethylene bridge.[9] As shown below, N-(*tert*Boc)GlyLeuOMe was reacted with NaH in ether and then with ethylene glycol bistriflate to give the N-(*tert*Boc)oxopiperazine ring system. This route generated the oxopiperazine ring system from its components using just two simple steps. They did not report any biological evaluation of these compounds. They subsequently cleaved the

2-Oxopiperazine Ring Synthesis By Pohlmann et al.



*tert*Boc group and acylated the amine thereby creating yet another site of diversity.

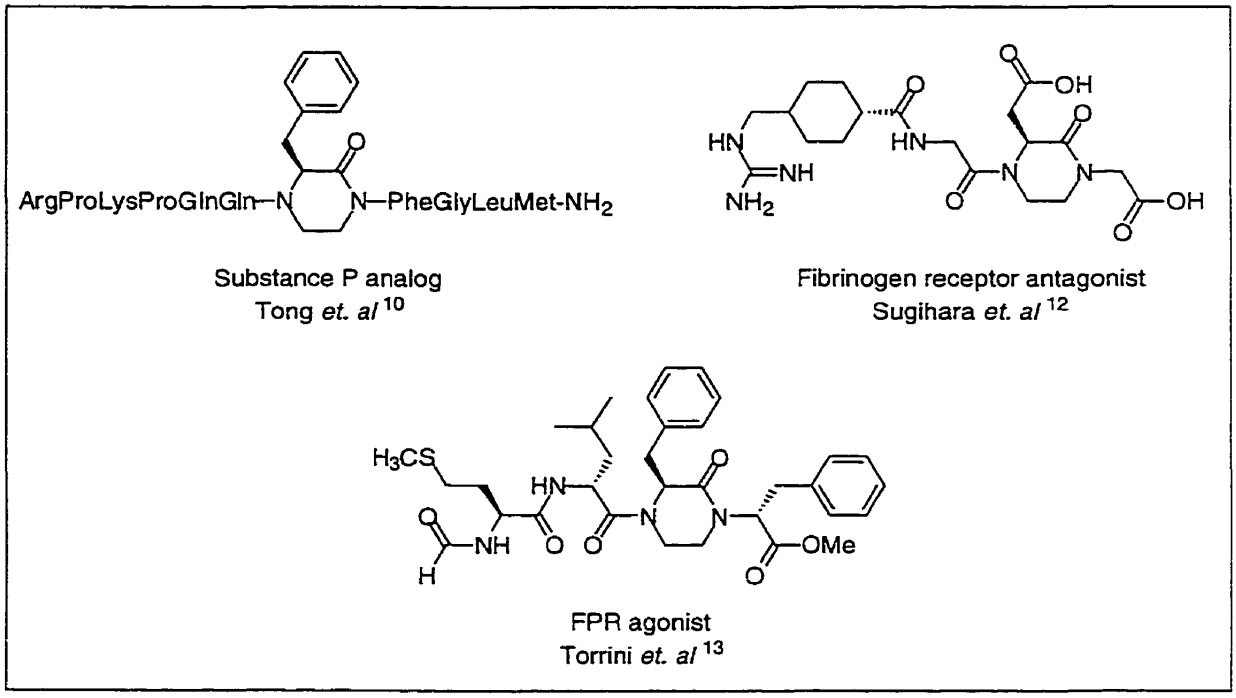
This was precisely what we had in mind too. However, this methodology could not be successfully carried out on dipeptides that had amino acids other that glycine on the N-terminus. Thus, a route that allowed use of any two amino acids and a two-carbon unit for the generation was still not available.

1.5 Biologically Active 2-Oxopiperazines

[9] (a) Pohlmann, A.; Schanen, V.; Guillaume, D.; Quirion, J.-C.; Husson, H.-P. *J. Org. Chem.* **1997**, *62*, 1016. (b) Pohlmann, A.; Guillaume, D.; Quirion, J.-C.; Husson, H.-P. *J. Peptide Res.* **1998**, *51*, 116.

A few reports of the biological activities of peptides incorporporating the 2-oxopiperazine ring have been published recently.

Biologically Active 2-Oxopiperazine Derivatives



Tong *et al.* synthesized a series of Substance P analogs incorporating the 2-oxopiperazine ring.[10] These analogs showed 10^3 to 10^4 times lower binding affinity than Substance P to its receptor. It was conjectured that perhaps these analogs did not adopt the conformation required by the receptor for effective binding.

Sugihara *et al.* synthesized a series of fibrinogen receptor antagonists[11] based on the RGDF tetrapeptide unit in which the N atoms of Asp (D) and Phe (F) were linked

[10] Tong, Y.; Fobian, Y. M.; Wu, M.; Boyd, N. D.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1679.

[11] The final step in the platelet activation leading to aggregation is the cross-linking of dimeric plasma protein fibrinogen between glycoprotein IIb-IIIa (GP IIb-IIIa) receptor complexes exposed on adjacent activated platelets. Antagonism of the fibrinogen-GPIIb-IIIa interaction therefore represents an attractive therapeutical target with potential utility in the treatment and prevention of acute myocardial infraction, unstable angina, or transient ischemic attacks (TIA).

through an ethylene bridge creating the 2-oxopiperazine ring.[12] One of these compounds is currently being developed further towards treatment of arterial thrombotic diseases. The 2-oxopiperazine-ring system was synthesized using the methodology developed by DeMaio *et al.* that was described previously. [6]

Torrini *et al.*[13] have synthesized novel tripeptide HCO-MetLeuPheOMe (fMLF) analogs[14] incorporating the 2-oxopiperazine ring at the C-terminus. They replaced the terminal Phe unit by a 2-oxopiperazine ring comprising two Phe units whose two N atoms were bridged via an ethylene unit. This modified compound was more potent than the parent peptide (fMLF-OMe) in superoxide production but showed lower ability to induce chemotaxis. The 2-oxopiperazine-ring system was synthesized using the methodology developed by Yamashita *et al.* as described previously.[8]

1.6 Project Goals

Thus, it can be seen that although various oxopiperazine derivatives have been prepared, only one relies on the use of amino acids for their synthesis. Our aim was to synthesize the 2-oxopiperazine ring as shown below starting from amino acids in solution phase and then carry out the synthesis on a solid support. If successful, we then

---

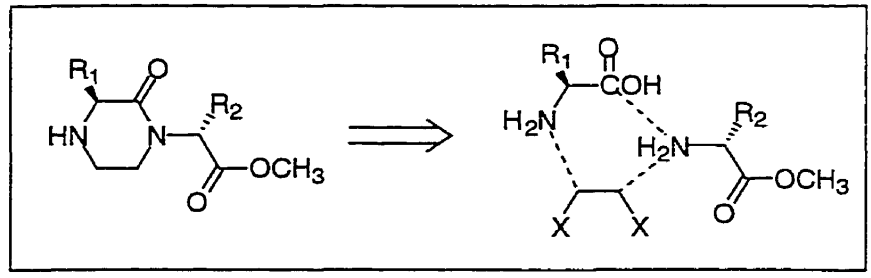
[12] Sugihara, H.; Fukushi, H.; Miyaki, T.; Imai, Y.; Terashita, Z.; Kawamura, M.; Fujisawa, Y.; Kita. S. *J. Med. Chem.* **1998**, *41*, 489.

[13] Torrini, I.; Mastropietro, G.; Zecchini, G. P.; Paradishi, M. P.; Lucente, G.; Spisani, S. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 170.

[14] The tripeptide HCO-MetLeuPheOH (fMLF) is the reference agonist of the G protein-coupled N-formylpeptide receptor (FPR). This represents a specific binding site located on the cell surface of phagocytic leukocytes. Binding of agonists to FPR triggers chemotaxis and a subsequent cascade of biochemical events which is considered one of the primary physiological responses to bacterial invasion and tissue injury.

envisioned generating a library of such peptidomimetics to screen for exciting biologically active compounds.

Project Goal



We also wanted to observe the effect of incorporating the 2-oxopiperazine ring into the biological activity of a small peptide. With this is mind, TRH (Thyrotropin Releasing Hormone) which is a tripeptide (Glp-His-Pro-NH_2) showing a wide range of biological activity was chosen. Our aim therefore was to synthesize analogs of TRH incorporating the 2-oxopiperazine ring and evaluate their biological activity.

Our results on these two aspects of the 2-oxopiperazine ring containing peptidomimetics are described in the chapters that follow.

2.1 Attempted Direct Bis-Alkylation of N-(*tert*Boc)dipeptide esters

Conceptually, the simplest route to 2-oxopiperazines is by reacting an N-(*tert*Boc)dipeptide ester with a base to deprotonate the two nitrogens and generate a dianion. Its reaction with an ethylene derivative having good leaving groups at both carbons should then give the ethylene-bridged dipeptide with the Boc protected nitrogen. Thus, N-(*tert*Boc)ValPheOMe (**1**) was prepared by reaction of N-(*tert*Boc)ValOH and PheOMe with EDC and HOBt in presence of triethylamine. This dipeptide was treated with sodium hydride followed by 1,2-dibromoethane in ether. However after workup, no new product was obtained and the unreacted dipeptide was recovered (**Scheme 1**). Changing the base to potassium hydride also did not lead to the formation of the desired product. In an effort to increase the leaving group ability, ethylene glycol bistriflate[15] (**2**) was used in place of 1,2-dibromoethane as the biselectrophile. However, using NaH as the base in ether, the starting dipeptide was again recovered. Changing the base to KO*t*Bu or LiO*t*Bu also did not lead to the desired cyclized product. Finally, N-(*tert*Boc)AlaPheOMe (**3**) was reacted with nBuLi at - 78 °C in THF followed by addition of 1,2-dibromoethane. Disappointingly none of the cyclized product was isolated again. Pietzonka *et al.* have reported successful alkylation of the carbamate and amide NHs of linear tetrapeptides by using P4-phosphazene base and benzyl bromide.[16] P4-phosphazenebase t-butyl was then used as the base to deprotonate the amide and the carbamate NHs of N-(*tert*Boc)PheAlaOMe (**4**) but none of the desired product was obtained after reaction with 1,2–dibromoethane. This route was therefore given up. These

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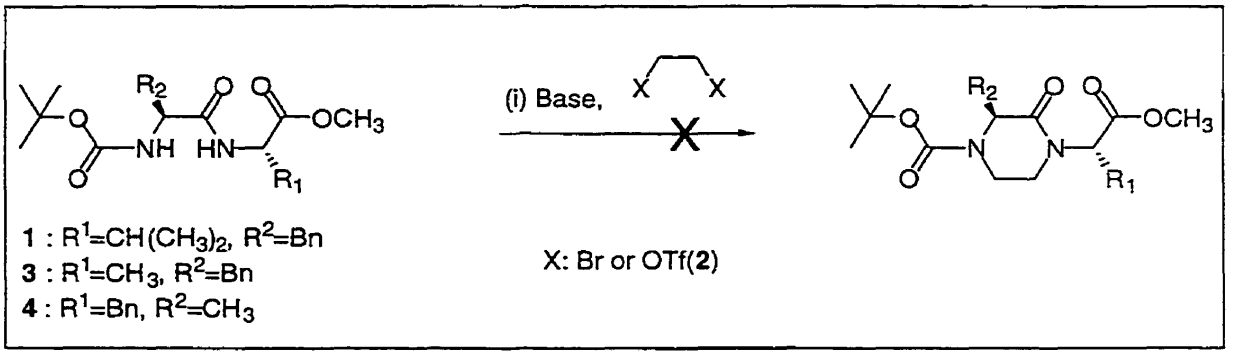
[15] Lindner, E.; von Au, G.; Eberle, H. -J. *Chem. Ber.* **1981**, *114*, 810.

[16] Pietzonka, T.; Seebach, D. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1481. Seebach, D.; Bezencon, O.; Bernhard, J.; Pietzonka, T.; Matthews, J. L.; Kuhnle, F. N. M.; Schweizer, W. B. *Helv. Chim. Acta*, **1996**, *79*, 588.

reactions were unsuccessful probably because the base induced elimination of HBr from 1,2-dibromoethane was more rapid than coupling of the amide with 1,2-dibromoethane.



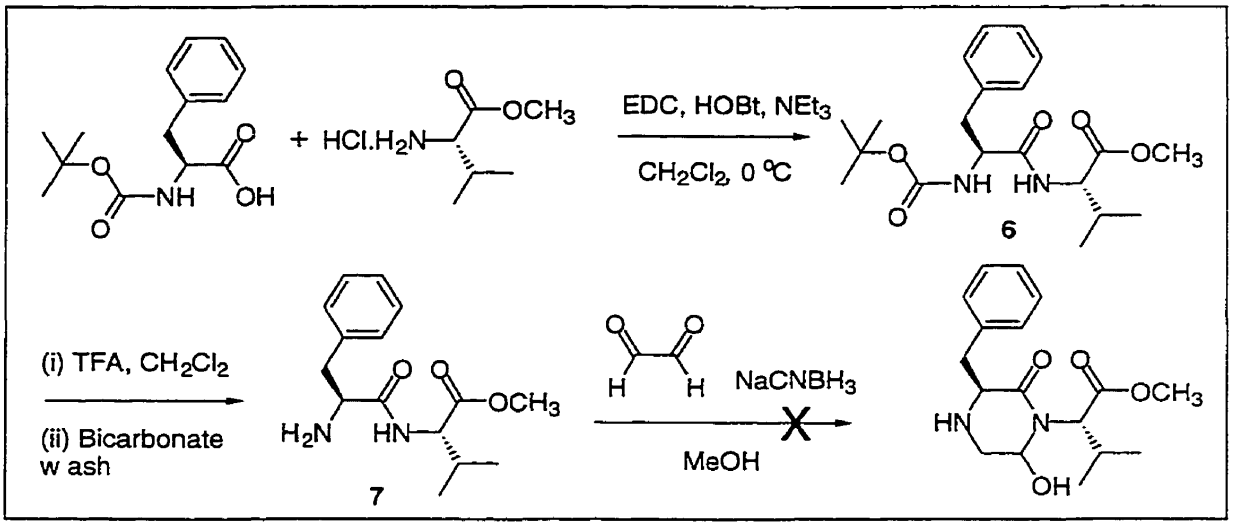
Scheme 1 : Attempted direct bis-alkylation of N-*tert*Boc dipeptide ester

Dorner *et al.* reported the alkylation of the amide bonds in peptides with activated alkyl halides (e.g. methyl iodide, allyl bromide, benzyl bromide) on a solid support using lithium *tert*butoxide as the base,[17] after capping the N-terminus of the growing peptide chain by a trityl group. Although they had unimpressive yields even after repeating the alkylation several times, this method appeared to have the potential to alkylate the amide nitrogen and eventually give the required cyclic oxopiperazine product. The dipeptide ValPheOMe was therefore reacted with trityl chloride and triethylamine to give TrtValPheOMe (**5**). This was taken in THF and LiO^{t}Bu was added followed by 1,2-dibromoethane (**Scheme 2**). Unfortunately, after workup only the racemized dipeptide was observed as the product. There was absolutely no evidence of any alkylated product. These results again pointed at the poor electrophilicity of 1,2-dibromoethane, with elimination of HBr from 1,2-dibromoethane being a likely possibility as well, responsible for the failure of these reactions. After these unsuccessful attempts at synthesizing the 2-

[17] Dorner, B.; Husar, G. M.; Ostresh, J. M.; Houghten, R. A. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 709.

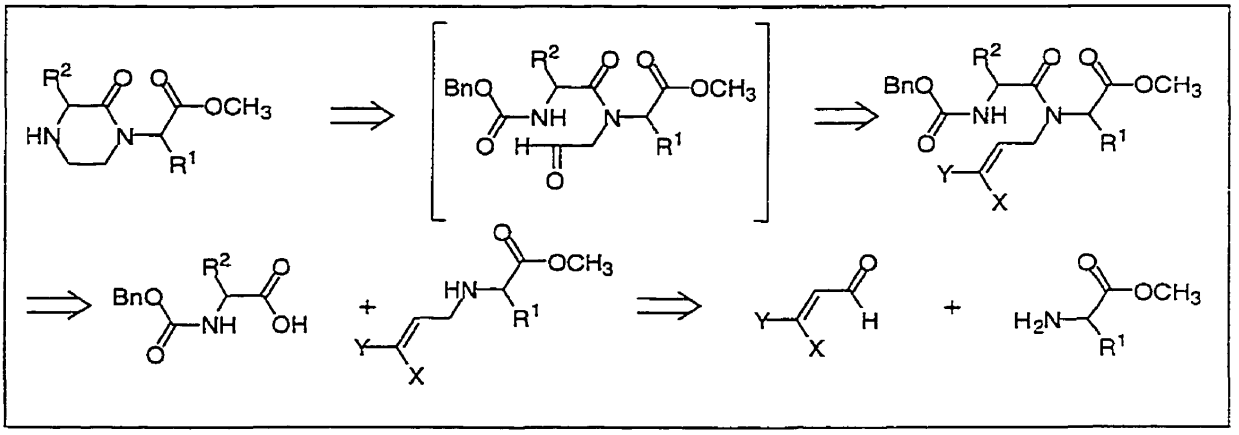
CHAPTER 2

Results and Discussion



Scheme 3 : Attempted reductive amination of dipeptide ester with glyoxal

2.2 N-Allyl Dipeptide Route to 2-Oxopiperazine



Scheme 4 : N-allyl dipeptide route to 2-oxopiperazines

Our next approach towards the synthesis of the 2-oxopiperazine ring involved performing reductive alkylation on the carbamate nitrogen atom of the N-terminus of the dipeptide. It was hoped that an initial reaction between an amino acid ester with an α, β unsaturated aldehyde will give an N-allyl amino acid ester as shown in **Scheme 4**. This

secondary amine would then be coupled to the second amino acid. Oxidative cleavage of the carbon – carbon double bond would give rise to an aldehyde, which would be attacked by the carbamate nitrogen giving us the desired 2-oxopiperazine-ring skeleton. Carbamate deprotection would then generate the 2-oxopiperazine ring. Variations of this approach using an oxidation step followed by a cyclization step have now been successfully used by others as well.[18]

2.2.1 Attempted Reductive Amination of α, β Unsaturated Aldehydes

It was felt that a classical reductive amination of α, β-unsaturated aldehydes with amino esters could be utilized to generate the N-allyl compounds. Accordingly acrolein, phenylalanine methyl ester and sodium cyanoborohydride were reacted with sodium cyanoborohydride and acetic acid in methanol. However, a complex mixture was obtained, containing the desired product along with dialkylated and over reduced side products (i.e. both 1,4 and 1,2 reduction had taken place). In order to prevent dialkylation, bulkier aldehydes like cinnamaldehyde and 4-methoxycinnamaldehyde were then used but again a complex mixture of products was obtained as before. It was reasoned that dialkylation of the amine could be prevented by performing the reductive amination in two separate steps: i.e. an initial formation of the α, β-unsaturated imine and then selective reduction of the imine.

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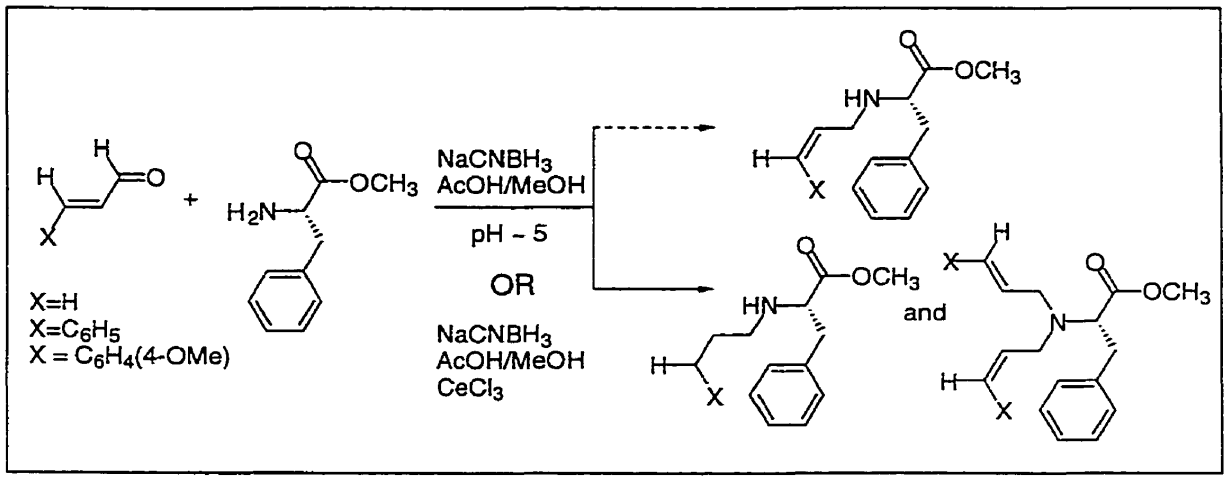
[18] (a) Fobian, Y. M.; d'Avignon, D. A.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 315. (b) Vojkovsky, T.; Weichse, A.; Patek, M. *J. Org. Chem.* **1998**, *63*, 3162. (c) Horwell, D. C.; Lewthwaite, R. A.; Pritchard, M. C.; Ratcliffe, G. S.; Rubin, J. R. *Tetrahedron*, **1998**, *54*, 4591. (d) Uchida, H.; Achiwa, K. *Synlett* **1996**, 969. (e) Smith, L. R.; Bartlett, P. A. *Molecules Online* **1998**, *2*, 58.



Scheme 5 : Reductive amination of α,β unsaturated aldehydes

The imine was formed by reaction of an α, β unsaturated aldehyde with phenylalanine methyl ester. Phenylalanine methyl ester hydrochloride was neutralized and the free amine was reacted with cinnamaldehyde in methylene chloride with constant distillation to remove the water that was formed.[19] There is literature precedence for selective reduction of the aldehyde functionality in α, β-unsaturated aldehydes by adding $CeCl_3$ along with $NaBH_4$.[20] However, when the reduction of the α, β-unsaturated imines was performed in presence of $CeCl_3$ and $NaCNBH_3$ in acidic conditions, both 1,2 and 1,4 reduction was observed, with the desired 1,2 reduced product being the major product. In an effort to improve the yields the desired allyl substituted amino acid ester, we decided to optimize both the formation of the α, β-unsaturated imine and its selective 1,2 reduction.

2.2.2 **Optimized Synthesis of N-allyl Amino Esters**

[19] Just, G.; Liak, T. –J. *Can J. Chem.* **1977**, *56*, 211.
[20] Gemal, A. L.; Luche, J.-L. *J. Am.Chem. Soc.* **1981**, *103*, 5454.

Look *et al.* published a report describing the use of trimethyl orthoformate as the solvent in imine forming reactions.[21] Trimethyl orthoformate acts as dehydrating reagent in the reaction and drives the equilibrium towards imine formation. Using this literature procedure, amino acid esters (valine, phenylalanine, and tryptophan), which were obtained by neutralizing the commercially available amino ester hydrochloride salts with bicarbonate, were reacted with α, β-unsaturated aldehydes (4-methoxycinnamaldehyde or 3-methyl-2-butenal) in trimethyl orthoformate and the corresponding imines were isolated in nearly quantitative yield. Later on, amino acid ester hydrochloride salts were used in presence of triethylamine with no loss in yields of the recovered imines as shown in **Scheme 6**. The selective 1,2 reduction of these α, β unsaturated imines was achieved by using $NaBH_4$ as the reducing agent.[22] This generated the corresponding amines (**8, 9, 10, 11**). Initially this reduction was carried out in presence of $CeCl_3.7H_2O$, but later on it was found to be unnecessary to add $CeCl_3.7H_2O$ as the reduction proceeded in excellent regioselectivity and yield without it. Chromatography was not required to purify these N-allyl amino acid ester derivatives at this stage and this procedure could be carried out efficiently on a variety of amino acids in high yields.

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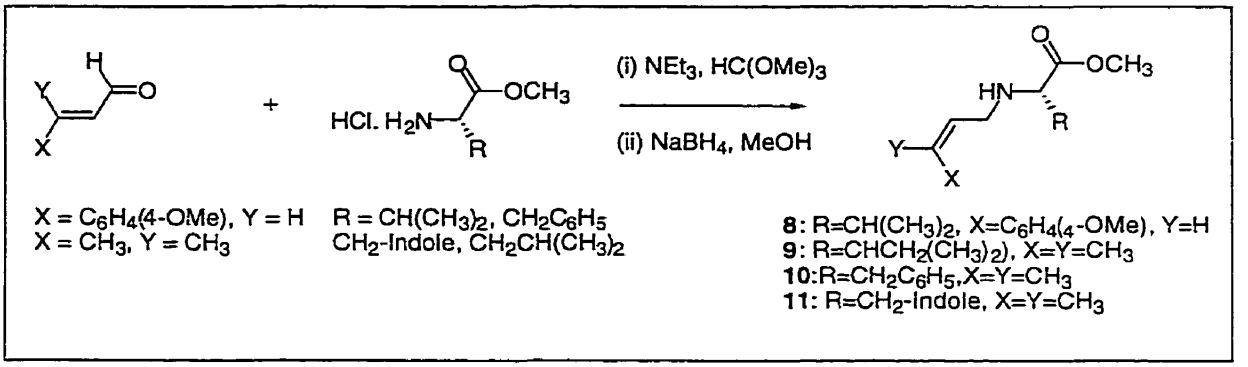
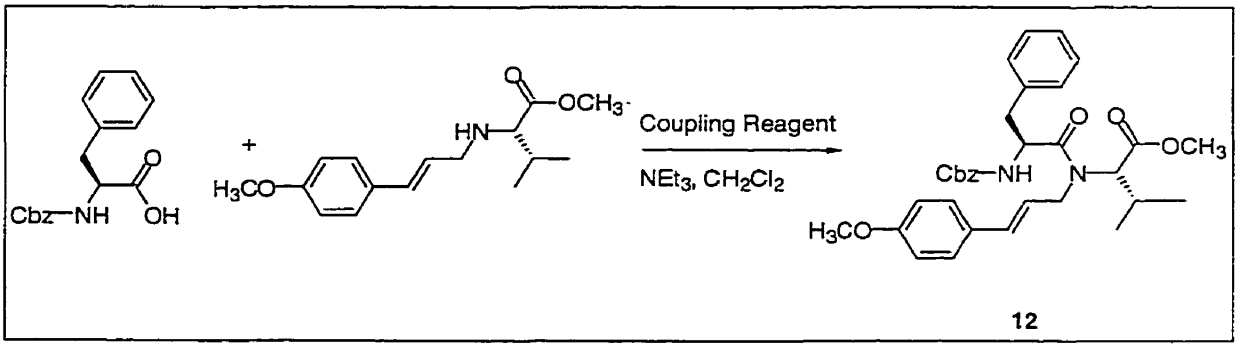
[21] Look, G. C.; Murphy, M. M.; Campbell, D. A.; Gallop, M. A. *Tetrahedron Lett.* **1995**, *36*, 2937.
[22] Similar reduction has been performed using $NaBH_4$ by others. Harrison, D. M.; Sharma, R. B. *Tetrahedron* **1993**, *49*, 3165.



Scheme 6 : Optimized synthesis of N-allyl substituted amino esters

2.2.3 Peptide Coupling of Hindered Secondary Amines

The next step was the coupling of these N-(allyl)amino acid ester derivatives with another amino acid. Peptide coupling reactions proceed by an initial activation of the acid followed by the nucleophilic attack of the amine to form the amide bond. The acid can be activated by reaction with carbodiimides, anhydrides, phosphonium salts, halides etc.[23]



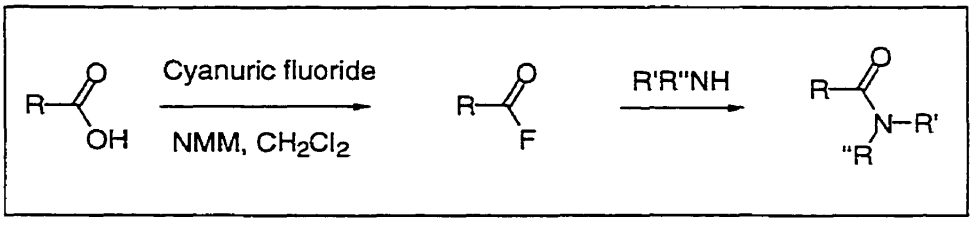
Scheme 7 : Peptide coupling using various coupling reagents

The first attempts at coupling the N-allyl amino acid ester derivatives with the second amino acid using DCC or water-soluble carbodiimide EDC as the coupling reagents gave extremely low yields (only 5 %) of the desired product even after 3 days. Use of diisopropyl carbodiimide as the coupling reagent in presence of HOBt gave better

[23] Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis* , Springer Verlag, **1994**.

yields as did the use of PyBroP. Finally, HATU[24], which was only recently described as the coupling reagent of choice for difficult couplings, was tried. Still, the product could not be obtained in greater than 50 % yield. All these reaction required chromatography to isolate the coupled dipeptide derivative. We then turned our attention to the use of acid fluorides, which had then only recently been described as excellent activating groups for troublesome peptide couplings.

2.2.4 Peptide Coupling With Acid Fluorides



Scheme 8 : Activation of carboxylic acids as acid fluorides

Carpino *et al.* have recently described the use of acid fluorides as excellent acid activators for peptide coupling.[25] Acid fluorides can be prepared by reaction of the corresponding acid with cyanuric fluoride in presence of a tertiary amine.[26] They are nice crystalline compounds and couple in excellent yield with highly hindered amines. We therefore decided to use acid fluorides for coupling the second amino acid to our highly hindered secondary allylic amine. To our relief, the coupling went in over 75 % yield! We therefore decided to continue using this methodology for our coupling reactions. As

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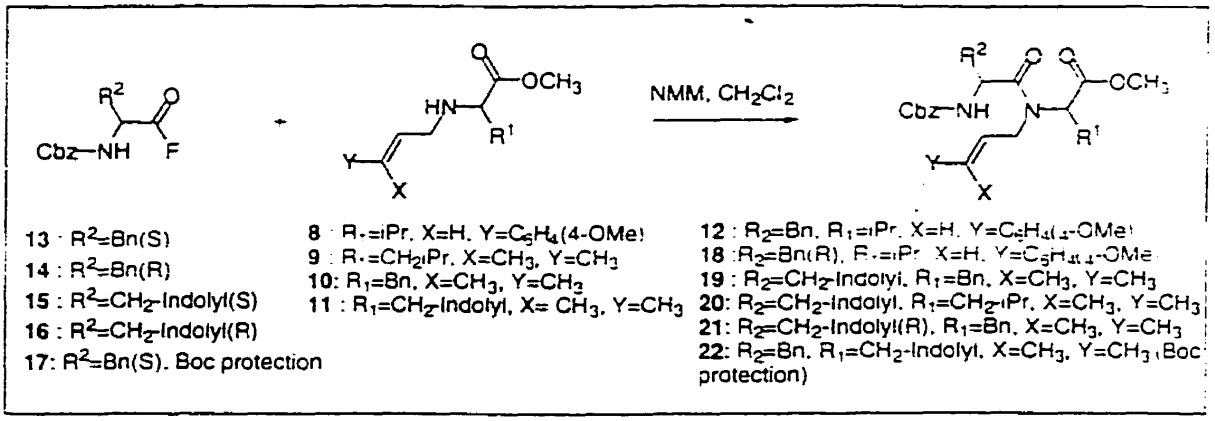
[24] (a) Carpino, L. A *J. Am. Chem. Soc.* **1993**, *115*, 4397.

[25] (a) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H.; *J. Am. Chem. Soc.*, **1990**, *112*, 9651. (b) Wenschuh, H.; Beyermann, M.; Winter, R.; Bienert, M., Ionescu, D.; Carpino, L. A. *Tetrahedron Lett.* **1996**, *37*, 5483.

[26] Protected amino acid fluorides can also be prepared *in situ* with tetramethylfluoroformadium hexafluorophosphate (TFFH). Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401.

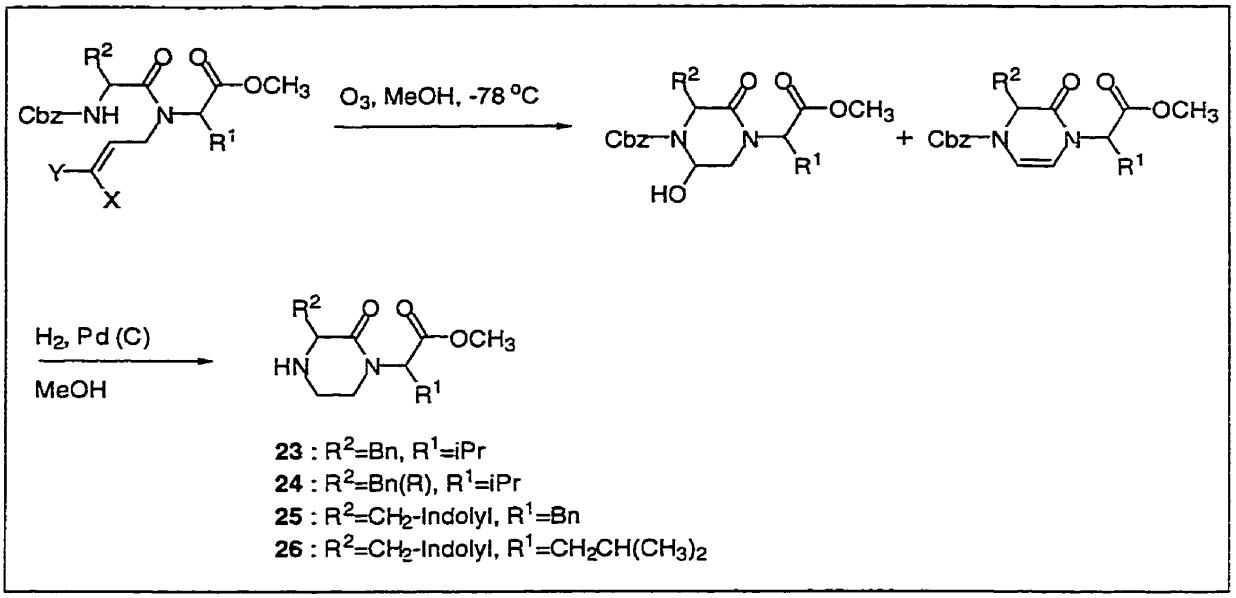
can be seen from **Scheme 9**, this methodology was successful with a variety of amino acid derivatives.



Scheme 9 : Peptide Coupling Using Acid Fluorides

2.2.5 Ozonolysis and Protecting Group Cleavage

The N-(Cbz), N'-(allyl)dipeptide esters (**12, 18, 19 and 20**) were dissolved in methylene chloride or methanol and ozone was bubbled through the solution at – 78 °C. The ozonides were reduced by reaction with dimethyl sulfide to generate the aldehyde, which reacted with the amide intramolecularly to generate the cyclic alcohol. This process also produced the corresponding dehydrated product. Initially these reactions were performed in methylene chloride but later on, it was found that use of methanol as the solvent gave better yields. It was also found that keeping the ozone bubbling for over an hour (even after the solution had turned blue) gave better yields of the cyclized products.



Scheme 10 : Ozonolysis and Cbz group deprotection

These compounds were difficult to purify by chromatography and hence the crude products were subjected directly to hydrogenation to cleave the Cbz group and reduce the alkene thus generating the desired 2-oxopiperazines (**23 - 26**). Use of $Pd(OH)_2$ gave better results than Pd(C) and using larger amounts of the Pd catalyst led to higher yields.

This procedure for synthesizing the 2-oxopiperazine ring compounds from amino acids was unfortunately not amenable to solid phase synthesis on synthesizers. Ozonolysis and catalytic hydrogenation are not routinely carried out on these machines.[27] Therefore an alternate synthetic route was required which could be adapted to efficient solid phase synthesis.

2.3 Sulfonamide Alkylation Route to 2-Oxopiperazines

In 1995, Fukuyama *et al.* described the advantages of using 2- or 4-nitrobenzene sulfonamide as an activating/protecting group for amines.[28] This group can be easily attached to primary amines, and the sulfonamide is easily alkylated, either by direct alkylation or under Mitsunobu conditions. Subsequent removal of the 2- or 4-nitrobenzene sulfonamide group with thiophenolate produces the secondary amine in excellent yield. Moreover, these reactions can also be carried out on a solid support. Two years later, Fukuyama's group also reported the use of 2,4-dinitrobenzene sulfonamides, which can be cleaved with n-propylamine after alkylation.[29] This strategy seemed to be an interesting strategy for synthesizing intermediate secondary N-allyl amine. Thus in a scheme parallel to the one described earlier, Dr. Mohamed studied the application of Fukuyama's strategy to the synthesis of 2-oxopiperazines as part of his graduate studies in our lab.[30]

2.3.1 Mohamed's Route to 2-Oxopiperazines Using Sulfonamides

Reaction of amino acid ester hydrochlorides with 4-nitrobenzenesulfonyl chloride in the presence of triethylamine gave the corresponding sulfonamides. Their reaction with allyl bromide and K_2CO_3 in DMF at 60 °C gave the allyl derivatized sulfonamide amino esters. Cleavage of the sulfonamide group with thiophenol and K_2CO_3 in DMF produced the desired N-allyl amino ester as expected. This allyl compound was then coupled with another N-Cbz amino acid using the acid fluoride activation methodology. Subsequent ozonolysis and reduction with dimethylsulfide generated the cyclic intermediates as

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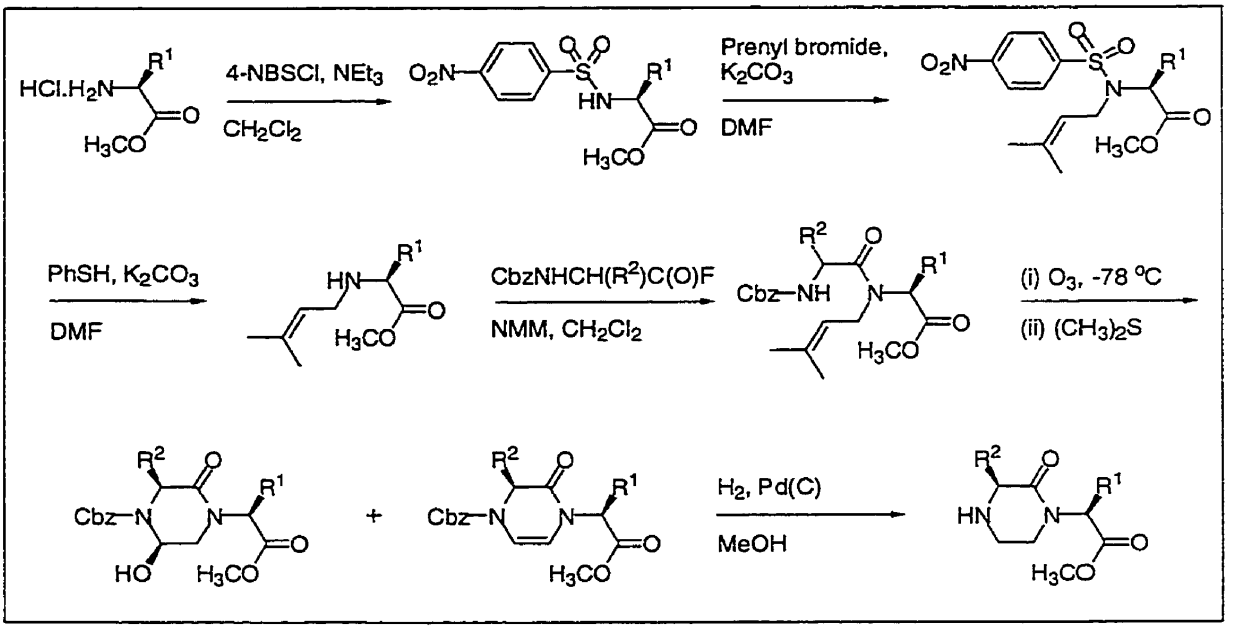
[27] Ozonolysis has been performed on solid phase. Sylvain, C.; Wagner, A.; Mioskowski, C. *Tetrahedron Lett.* **1997**, 1043. Unfortunately the apparatus at Astra was not compatible with ozonolysis.
[28] Fukuyama, T.; Jow, C. -K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.
[29] Fukuyama, T.; Cheung, M.; Jow, C. -K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831.
[30] Mohamed, N. *Ph.D. Thesis, Department of Chemistry, McGill University*, **1998**, Section 2.2

before whose hydrogenation produced the desired 2-oxopiperazines as before (**Scheme 11**).



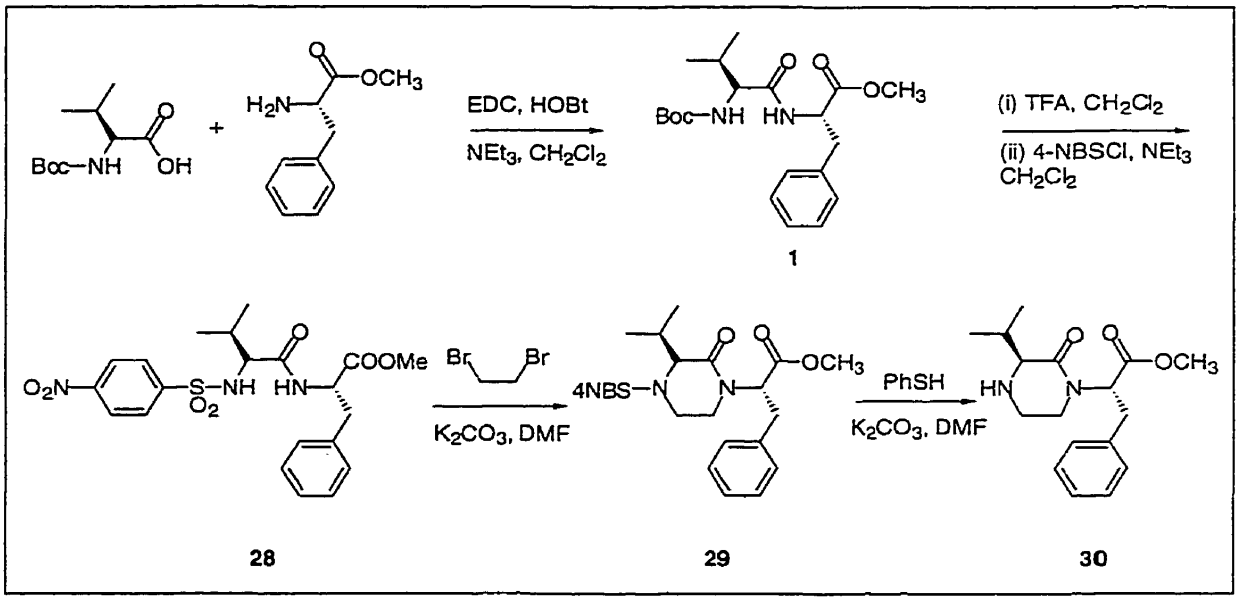
Scheme 11 : Mohamed's route to 2-oxopiperazines using 4-nitrobenzenesulfonamides

This route, which was independently carried out by Dr. Mohamed in our lab, was conceptually similar to the one described earlier in **Section 2.2** and thus suffered from the same drawbacks for application to solid phase combinatorial library synthesis of 2-oxopiperazines (i.e. inability to perform ozonolysis and hydrogenation on synthesizers).

2.3.2 Direct Alkylation of Sulfonamide Dipeptides

Although the previous approach could not be carried out on solid support, the successful use of the 4-nitrobenzenesulfonamide group prompted us to use it further. Its ease of installation, successful substitution of the nitrogen atom and a subsequent facile removal proved to be of great value.

We have described earlier (**Section 2.1**) that direct alkylation of N-(*tert*Boc)-dipeptides with 1,2-dibromoethane failed to provide the cyclized 2-oxopiperazine derivatives. Since we had been successfully prenylating the nitrogen terminus using Fukuyama's sulfonamide strategy, we decided to use N-(4-nitrobenzenesulfonamide)-dipeptides as substrates for alkylation with 1,2-dibromoethane. Nazim Mohamed carried out these studies and found that reaction of 1,2-dibromoethane with 4-



Scheme 12 : Direct alkylation of 4-nitrobenzenesulfonamide dipeptides with 1,2-dibromoethane

nitrobenzenesulfonamide dipeptides (in contrast with Boc dipeptides) and K_2CO_3 as the base in DMF did indeed produce the cyclized product (i.e. the 4-nitrobenzenesulfonamide-2-oxopiperazine derivative).

Optimized reaction conditions for this reaction involved heating the dipeptide sulfonamide with powdered K_2CO_3 in DMF at 60 °C for 30 minutes, before adding excess 1,2-dibromoethane and then further heating for 24 hours. An acidic work-up followed by

simple chromatography yielded the cyclized products. The 4-nitrobenzenesulfonamide protecting group could then be removed as before with thiophenol and K_2CO_3 in DMF as described earlier to give the desired 2-oxopiperazine derivatives. I repeated this synthesis on ValPhe oxopiperazyl derivative as shown in **Scheme 12.** This route was later used in the synthesis of TRH analogs as described in later sections.

2.3.3 Failure of Direct Alkylation Route on Solid Support

Dr. Mohamed tried to utilize this route on a solid support (crossed-linked polystyrene resin) in an effort to synthesize a library of such compounds.[31] The first four steps in the synthesis (i.e. deprotection of the Fmoc protection from the first amino acid attached to the resin; coupling of the second Fmoc protected amino acid; deprotection of the Fmoc; and addition of the 2-nitrobenzenesulfonamide group) could be carried out in near quantitative yields very quickly. However the dialkylation of the sulfonamide dipeptide coupled to the resin could not be carried out successfully in sufficiently high yield. Despite attempting various bases (K_2CO_3, tetramethylguanidine, and DBU), solvents and reaction conditions, this alkylation could not be carried out in high yields. In each case, substantial amount of the unreacted starting material was observed. Subsequent deprotection of the 2-nitrobenzenesulfonamide group and acylation of the free amine were carried out successfully. Finally the compounds were cleaved from the resin to yield the 2-oxopiperazine derivative. However this study was carried out on a single compound only on solid support and was not carried out combinatorially due to the low yield of the sulfonamide alkylation step. A new strategy was therefore required.

2.4 Mitsunobu Reaction Route to Oxopiperazines

The 4-nitrobenzenesulfonamide activation methodology seemed to have a lot of potential for easy functionalization of nitrogen atoms on a peptide. I therefore decided to search for an alternate route towards the synthesis of 2-oxopiperazine-ring system using this methodology. The sulfonamide NH in the 4-nitrobenzenesulfonamides is acidic enough that a Mitsunobu reaction could be carried out on it. In fact, the original publication by Fukuyama *et al.* also described the synthesis of secondary amines via the Mitsunobu reaction of these sulfonamides with simple alcohols and subsequent deprotection of the sulfonamide.[15]

It was reasoned that the Mitsunobu reaction of these sulfonamides with 2-bromoethanol would generate a compound that could be easily cyclized. Furthermore, this could potentially be a nice route for carrying out solid phase studies and subsequent library synthesis. Moreover, it was felt that this procedure could perhaps also lead to a variety of larger sized rings by using appropriately long halo-alcohols.

2.4.1 **Mitsunobu Coupling Reaction and Optimizations**

This route began with the synthesis of N(-*tert*Boc)dipeptide esters **31(a-c)** by using EDC and HOBt as the peptide coupling reagents. Deprotection of the *tert*Boc group was performed as usual with TFA in methylene chloride and the TFA salts so obtained were then converted to the 2-nitrobenzenesulfonamides **32(a-c)**.[32] The Mitsunobu reaction between 2-nitrobenzenesulfonamides and bromoethanol was carried out using

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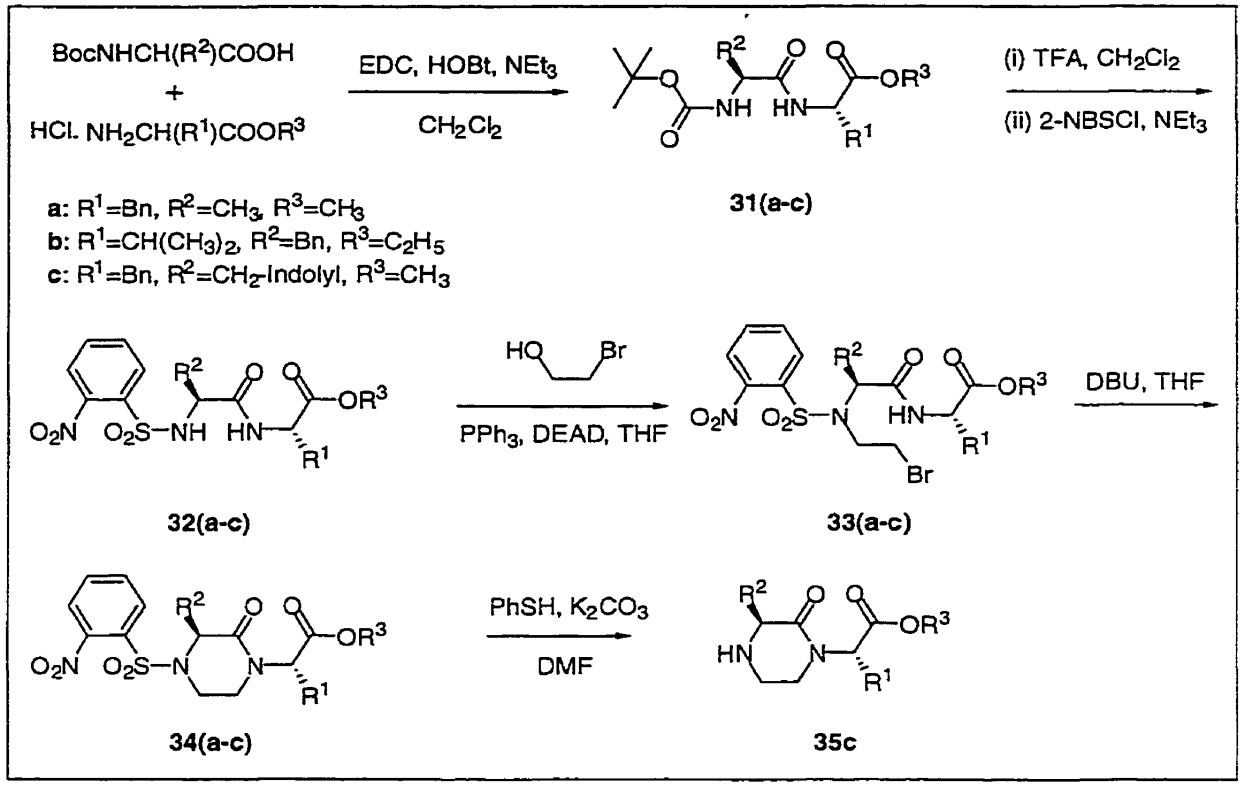
[31] Mohamed, N. *Ph.D. Thesis, Department of Chemistry, McGill University*, **1998**, Section 2.6.

[32] 2-nitrobenzenesulfonamides are reported to be cleaved more easily than the 4-nitrobenzenesulfonamides. Miller, S. C.; Scalan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301.

triphenylphosphine and diethyl azodicarboxylate (DEAD) in THF.[33] After a few trials, optimized conditions for performing this reaction were achieved by using 1.5 equivalents of PPh_3, 1.6 equivalents of DEAD, 1.7 equivalents of bromoethanol with 1 equivalent of 2-nitrobenzene sulfonamide dipeptides in THF at 0 °C. It was essential that DEAD be added last dropwise at 0 °C and the solution maintained at 0 °C for about an hour after the addition was complete. The products **33(a-c)** were obtained in good yields (> 75 %) after chromatography. These compounds were then cyclized by reaction with DBU in THF under dilute conditions (approx. 0.05 M) to give **34(a-c)**. This step also proceeded in good yields (> 70 %) and there was no need for chromatographic purification after an acidic workup (**Scheme 13**). Sulfonamide deprotection with thiophenol was carried out for only PheTrp 2-oxopiperazine derivative to generate **35c**.

[33] For a review, see : Mitsunobu, O. *Synthesis*, **1981**, 1.

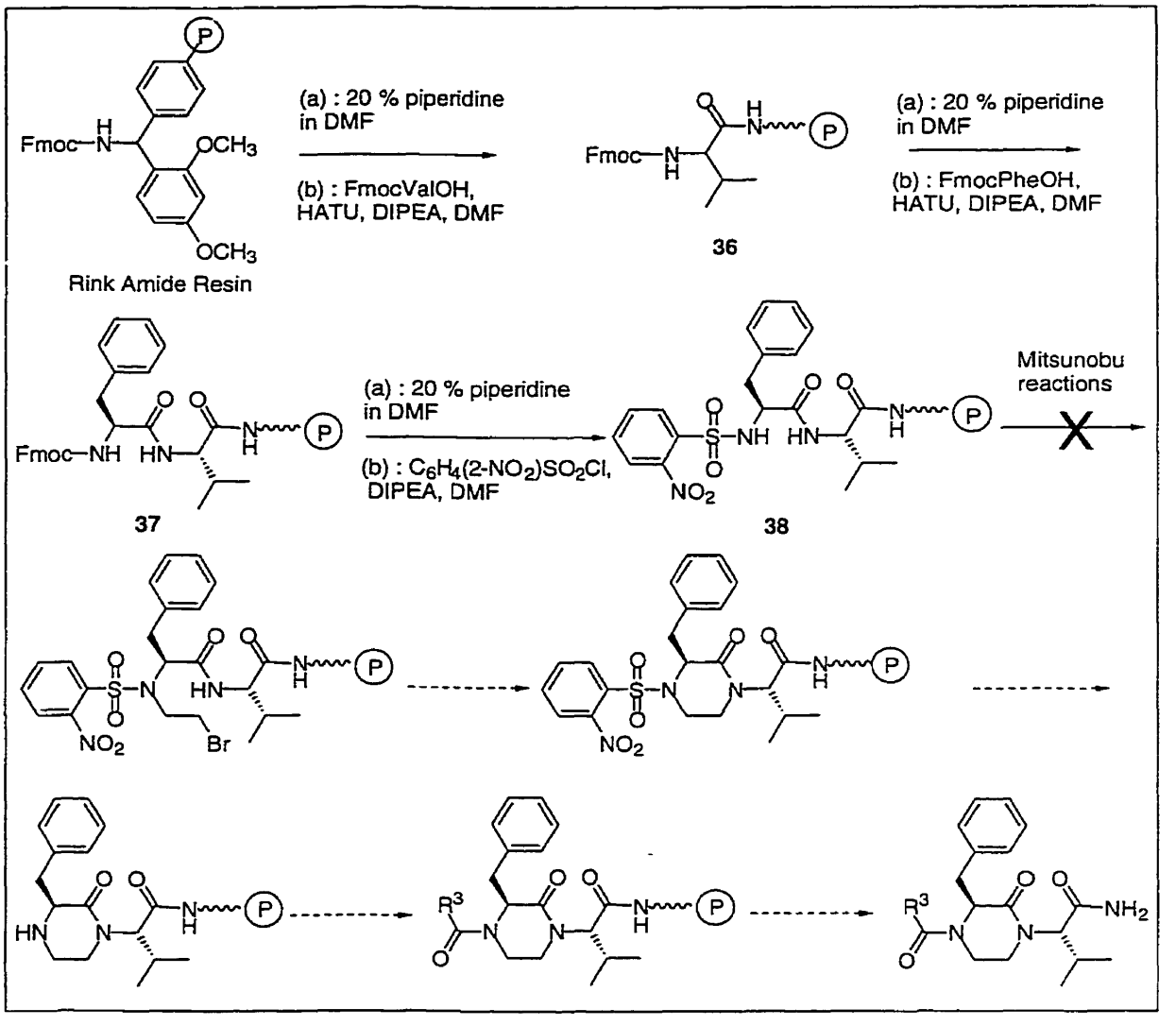


Scheme 13 : Mitsunobu Reaction route to 2-oxopiperazines

2.4.2 Mitsunobu Reactions on Solid Support

The success of this route prompted us to go ahead and attempt to carry out this route on solid phase. We decided to use the Rink amide MBHA resin which is sensitive to strong acid, and the products are cleaved from the resin by treatment with dilute trifluoroacetic acid (TFA). The Fmoc group on the linker was removed by reaction with 20 % piperidine in DMF. After washing with DMF, CH_2Cl_2 and methanol, the free amine was coupled with N-Fmoc valine using HATU as the peptide-coupling reagent in DMF and diisopropylamine (DIPEA) as the base. The completeness of this reaction was checked by taking a few representative beads and reacting them with ninhydrin solution (Kaiser test). A blue color indicated the presence of unreacted amine group on these

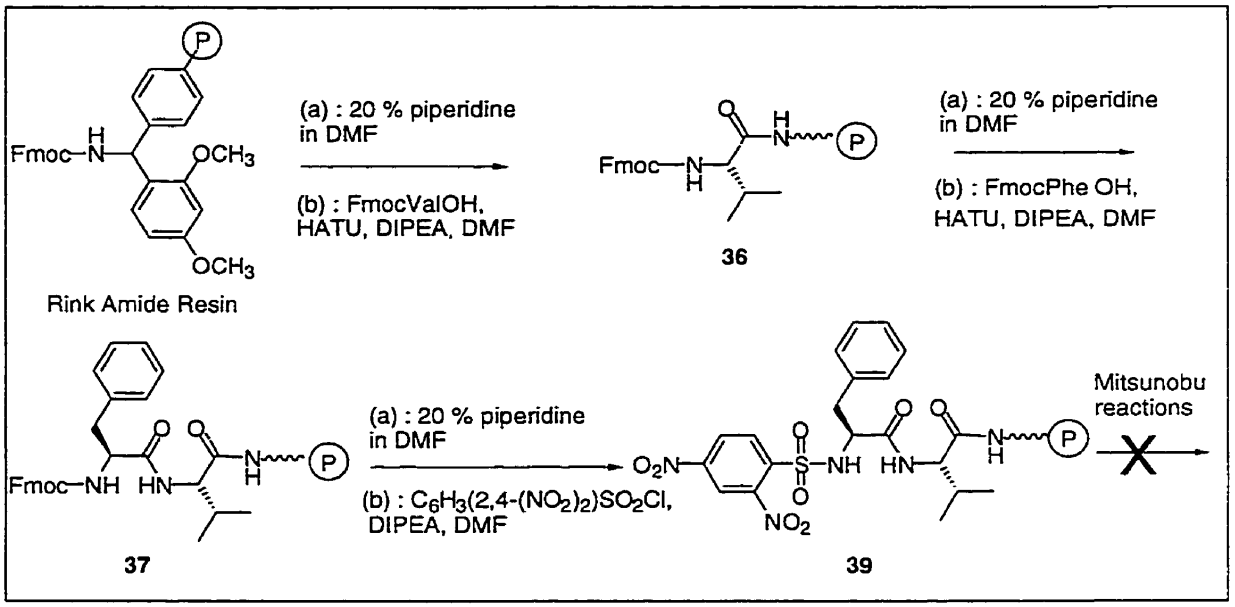
beads and when this occurred, the coupling was repeated. After making sure that all amino groups on the resin beads had reacted, the terminal Fmoc group on **36** was deprotected by washing the resin with 20 % piperidine solution. After this the resin was washed with DMF (3 times), methylene chloride (3 times) methanol (3 times) and then dried. N-(Fmoc)phenylalanine was then coupled using 3 fold excess of the N-(Fmoc) phenylalanine and coupling reagent (HATU) in presence of 6 equivalents diisopropylethylamine (DIPEA) for 2 hours to give **37**. Subsequent washing and deprotection of Fmoc as before gave the free dipeptide, which was then reacted with 3 fold excess of 2-nitrobenzenesulfonyl chloride in presence of 6 fold excess of DIPEA to produce the resin bound sulfonamide **38**. Mitsunobu reactions were usually carried out on this sulfonamide dipeptide resin **38** using a minimum 5-fold excess each of DEAD and triphenylphosphine (TPP) and 10-fold excess of bromoethanol in DMF or THF for 24 hours. Mass Spectrum (MS) of the product obtained after cleavage from the resin using dilute TFA showed no indication of any of the desired product. Instead the MS only showed peaks corresponding to the starting sulfonamide dipeptide.



Scheme 14 : Unsuccessful Mitsunobu reactions on solid support using 2-NBS dipeptides

Tsunoda *et al.* have described the use of tetramethyl azodicarboxamide (TMAD)/ tributylphosphine (TBP) in place of DEAD/triphenylphosphine to increase the yields in Mitsunobu reactions.[34] Mitsunobu reactions were then carried out with the TMAD/TBP reagents on **38** but again none of the desired product was obtained. These reactions were also carried out with minimum 5-fold excess each of TMAD and TBP and 10-fold excess

of bromoethanol. After this setback, we felt that perhaps a more acidic hydrogen atom at the sulfonamide group was required for these Mitsunobu reactions. We therefore decided to activate the hydrogen atom attached to the sulfonamide nitrogen atom by modifying the nature of the sulfonamide group.



Scheme 15 : Unsuccessful Mitsunobu reactions on solid support using 2,4 DNBS dipeptides

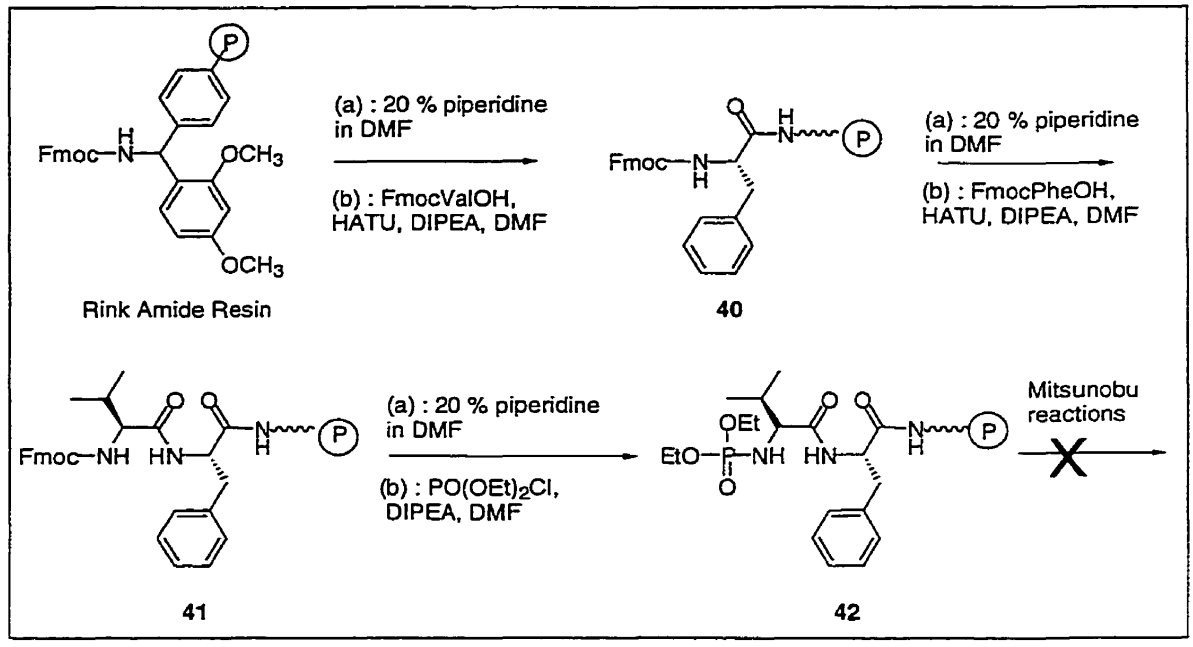
In an effort to increase the acidity of the sulfonamide hydrogen atom by incorporating two strongly electron withdrawing nitro groups on the aromatic ring, the 2,4-dinitrobenzenesulfonamide dipeptide derivative **39** was prepared by reacting the resin supported dipeptide with 2,4-dinitrobenzenesulfonyl chloride. The sulfonamide dipeptides were then subjected to Mitsunobu reactions with bromoethanol using DEAD and triphenylphosphine. Changing solvents (DMF, THF and methylene chloride) and temperature (0 °C and room temperature) had no beneficial effect. TMAD and tributylphosphine were then used for carrying out these Mitsunobu reactions. However,

[34] (a) Tsunoda, T.; Otsuka, J.; Yamamiya, Y.; Ito, S. *Chem. Lett.*, **1994**, 539. (b) Tsunoda, T.; Ozaki, F.; Ito,

we were disappointed not to observe any product again. MS of the compounds cleaved from the resin after performing these Mitsunobu reactions showed the peaks corresponding to the starting 2,4-dinitrobenzenesulfonamide dipeptide only.

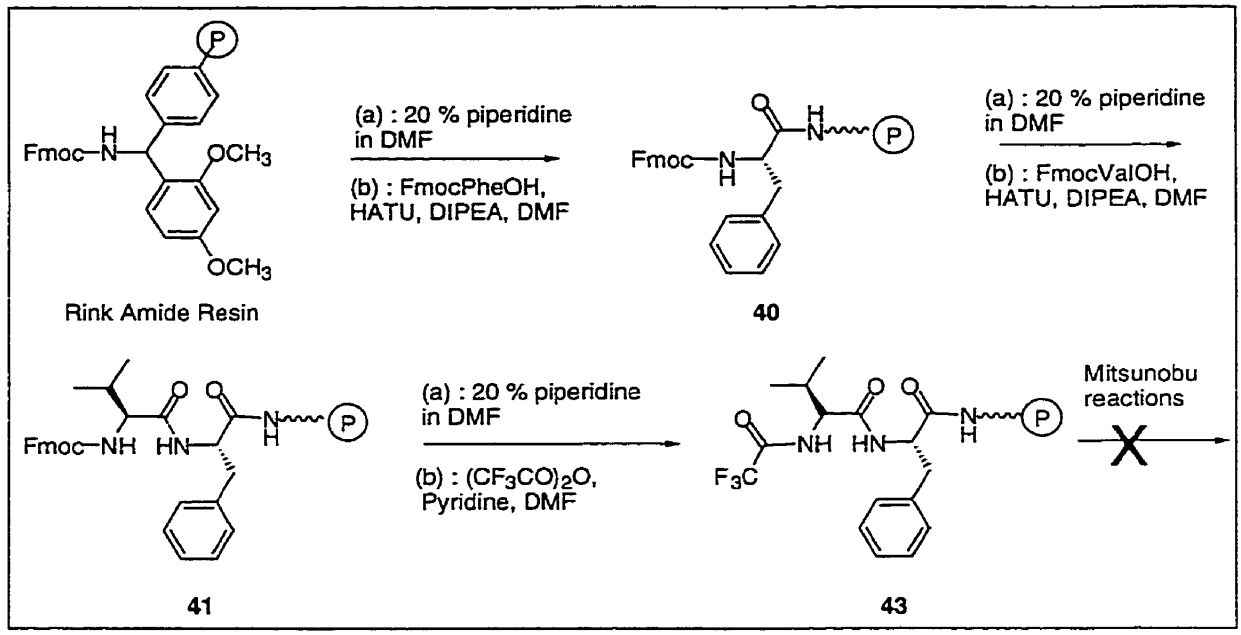
Since the sulfonamides proved to be completely ineffective in alkylating the nitrogen terminus of the dipeptide by a Mitsunobu reaction, it was decided to activate the nitrogen by converting it to a phosphoramidate. The dipeptide was reacted with 3 equivalents of diethyl chlorophosphate in presence of 3 equivalents diisopropylethylamine (DIPEA) to furnish the diethyl phosphoramidate dipeptide resin **42**. This was treated with 5-fold excess of tributylphosphine and TMAD in presence of 10-fold excess of bromoethanol but, again to our disappointment, only the peaks corresponding to the starting phosphoramidate were observed in the mass spectrum and no reaction had occurred at the phosphoramidate nitrogen.

S. *Tetrahedron Lett.* **1994**, *35*, 5081.



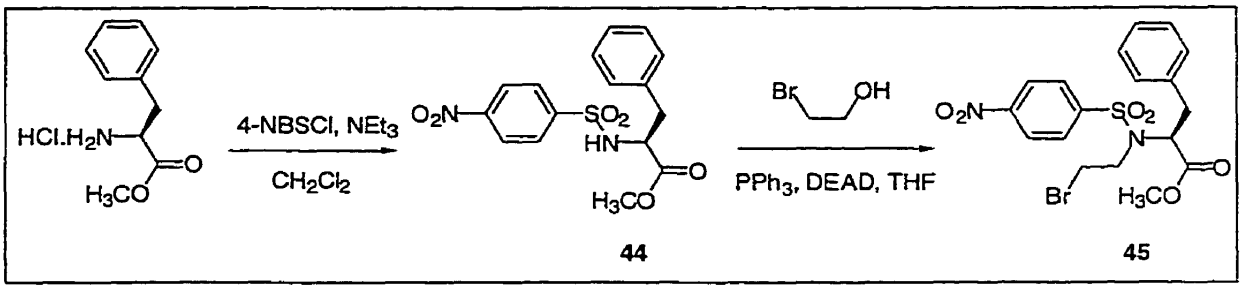
Scheme 16 : Unsuccessful Mitsunobu reactions on solid support using phosphoramidate dipeptides

We next turned our attention to the possibility of using the trifluoroacetyl group, with its strong electron withdrawing ability, to activate the NH group. The trifluoroacetamide group had the added advantage of being cleaved under mild basic conditions (aqueous $NaHCO_3$). Hence, the resin-supported dipeptide was reacted with 3-fold excess each of trifluoroacetic anhydride and pyridine at 0 °C to produce the trifluoroacetyl dipeptide **43**. This was reacted with excess tributylphosphine, tetramethyl azodicarbonamide (TMAD) and bromoethanol in DMF, but unfortunately no product formation was observed again.



Scheme 17 : Unsuccessful Mitsunobu reactions using trifluoroacetamide dipeptides

At this stage, we decided to carry out a control experiment to check the purity of the reagents that were being used in the above Mitsunobu reactions. N-(2-Nitrobenzenesulfonyl)phenylalanine methyl ester **44** was prepared by reaction of phenylalanine methyl ester hydrochloride with 2-nitrobenzenesulfonyl chloride and triethylamine in methylene chloride. This sulfonamide was reacted with triphenylphosphine (1.5 equivalents), bromoethanol (1.7 equivalents) and DEAD (1.6 equivalents) in THF, and after usual work-up and chromatography, the desired N-alkylated product **45** was obtained in 53 % yield.



Scheme 18 : Successful Mitsunobu reaction in solution phase

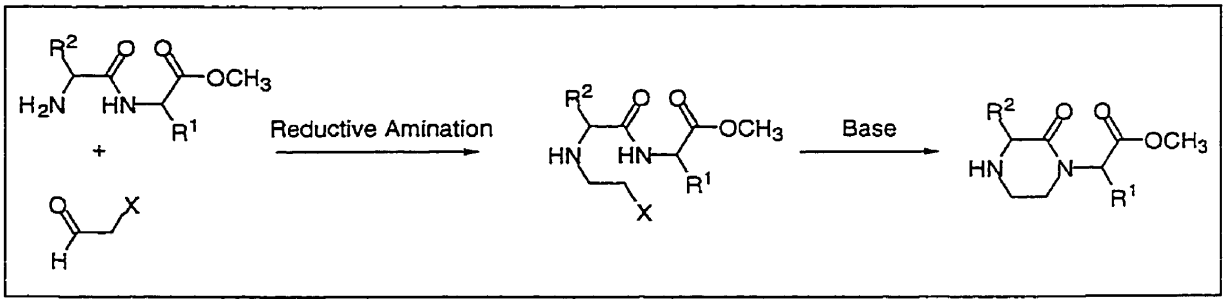
The success of this reaction showed that the reagents and solvents used in the Mitsunobu reactions on solid support were pure. Thus the failure of the Mitsunobu reaction on solid support was either a result of steric hindrance imposed by the bulky aromatic side chains of the polystyrene resin or due to the non-polar nature of the large number of aromatic rings in the micro environment in the reactions sites.

In order to overcome these limitations of the Rink-amide polystyrene resin, we decided to use the Tentagel resin instead. Tentagel resin is a copolymer of polystyrene and polyethyleneglycol. The presence of polyethyleneglycol makes the reaction sites less non-polar and less sterically hindered. Moreover it has excellent swelling properties. The Fmoc group on commercially available N-(Fmoc)-L-valine-tentagel resin was deprotected by reaction with 20 % piperidine and after washings, the free amine coupled to N-Fmoc alanine with HATU and DIPEA in DMF. Fmoc deprotection followed by reaction with 4-nitrobenzenesulfonyl chloride produced the desired sulfonamide. This was then reacted with triphenylphosphine (5 equivalents), bromoethanol (10 equivalents) and DEAD (5 equivalents) in DMF. However, none of the desired product could be observed by the mass spectrum of the product. It showed peaks corresponding to the unreacted sulfonamide dipeptide instead.

After the failure of these attempts to perform the Mitsunobu reaction, we decided to stop and instead concentrated on coming up with a new route better suited to the unique needs of the solid phase organic synthesis.

2.5 Reductive Amination Using Haloaldehydes

At this stage in our quest for a simple synthesis of derivatized 2-oxopiperazines from amino acids, it was felt that reductive amination reaction between a dipeptide with free amine and a two-carbon halo-aldehyde compound (i.e. bromo or chloro acetaldehyde) could generate a good leaving group such that attack by the amide nitrogen atom should result in a facile cyclization step. This would also eliminate the amine activation/deprotection steps from the previous approach. More importantly, it was felt that these reactions would be more suitable for solid phase since reductive amination reactions are well documented on solid support.

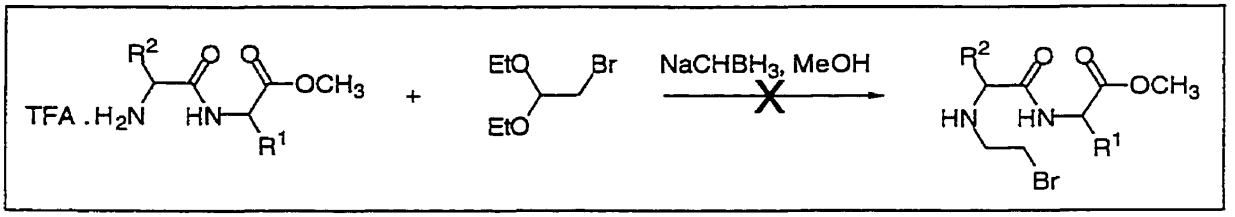


Scheme 19 : Reductive amination of dipeptide esters using haloaldehydes

2.5.1 Initial Attempts with Bromoacetaldehyde Dimethylacetal

Bromoacetaldehyde is available commercially in its protected version as bromoacetaldehyde dimethyacetal. On reacting this with free amino dipeptide in presence of $NaCNBH_3$ and acetic acid in methanol, no product formation was observe. Reasoning

that acetic acid was perhaps not a strong enough acid to generate the aldehyde from the acetal, this was then carried out with trifluoroacetic acid instead of acetic acid. Unfortunately, again no product was observed.

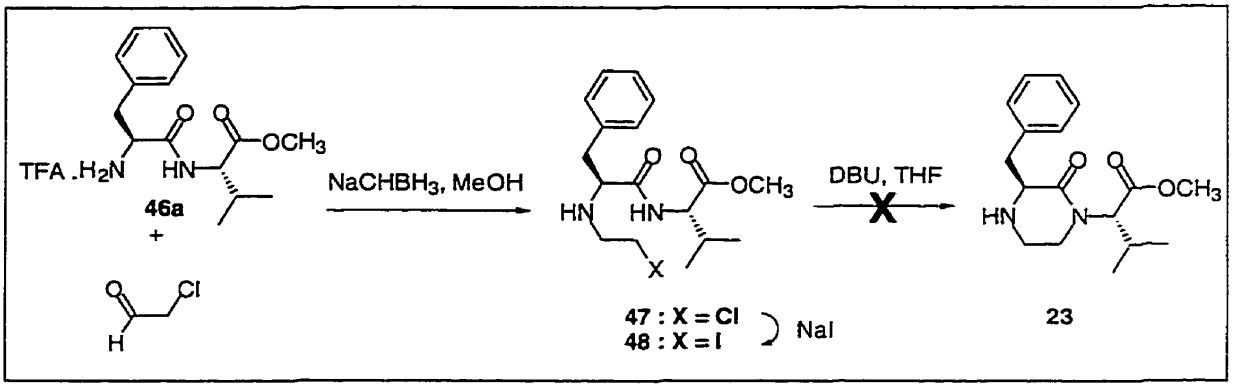


Scheme 20 : Unsuccessful use of bromoacetaldehyde dimethylacetal

It was then felt that perhaps bromoacetaldehyde could be generated from the acetal before reacting it with the dipeptide. Bromoacetaldehyde has been prepared by heating the acetal in presence of TFA followed by distillation of the product, but this literature procedure gave us a complicated mixture of products as observed by NMR.[35] We then gave up this route and tried using chloroacetaldehyde instead.

2.5.2 Reductive Amination Using Aqueous Chloroacetaldehyde

Chloroacetaldehyde is sold commercially not only in its protected form as its dimethylacetal, but also as a 50 % aqueous solution. On mixing the free amine dipeptide with the 50 % aqueous solution of chloroacetaldehyde in trimethyl orthoformate, we were unable to get the corresponding imine in a pure form. A substantial amount of the unreacted amine was recovered. This was not entirely unexpected as imine formation involves loss of a water molecule and the acetaldehyde was being added as an aqueous solution. The presence of this water obviously shifts the equilibrium back to the starting materials.



Scheme 21 : Reductive amination using chloroacetaldehyde

However, when the amine dipeptide TFA salt was reacted with the aqueous solution of chloroacetaldehyde and $NaCNBH_3$ in methanol, the desired product **47** was obtained in high yields (> 90 %) and purity as evidenced by the NMR of the crude product. Mass Spectrum also confirmed the structure of this compound and there was no evidence of the dialkylated product. Subsequent reaction to close the 2-oxopiperazine ring with DBU gave a complicated mixture of products as observed by TLC. In order to increase the leaving group ability, the chloro derivative was changed to the iodo derivative **48** by reaction with sodium iodide in refluxing methyl ethyl ketone. Unfortunately, on reacting of this iodo compound with DBU, none of the desired product **23** was obtained.

The Mitsunobu reaction described earlier in **Section 2.4** had created of a core system that was nearly identical to this core structure. The only difference was that the nitrogen atom was functionalized as a sulfonamide earlier and the cyclization was achieved easily. We felt that when the nitrogen atom was a secondary amine, the

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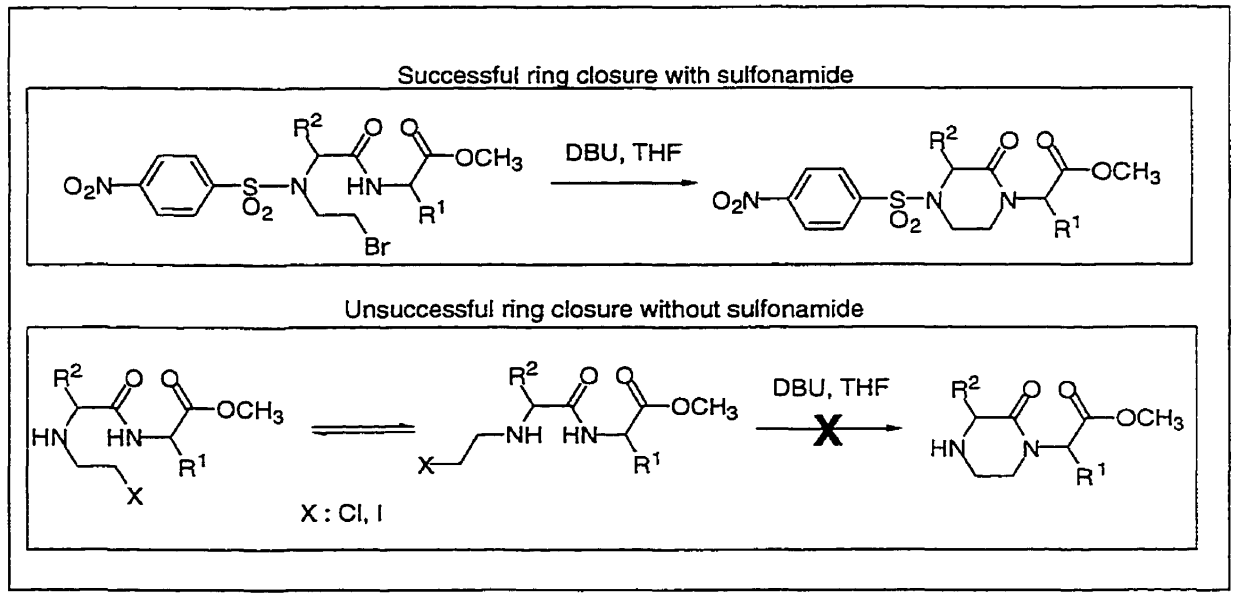
[35] Kayasuga-Mikado, K.; Hashimoto, T.; Negishi, T.; Negishi, K.; Hayatsu, H. *Chem. Pharm. Bull.* **1980**, *28*, 932.

chloroethyl group was free to rotate and thus could not be forced to only adopt a conformation where attack from the amide was feasible (**Scheme 22**). We felt that this was the reason behind the lack of cyclization when the secondary amine was used. It was clear that without proper substitution of the nitrogen, cyclization to product the 2-oxopiperazine-ring structure was difficult. We therefore decided to install a group (e.g. a sulfonamide) on the secondary amine that would force the chloroethyl group to adopt a conformation that led to facile cyclization.

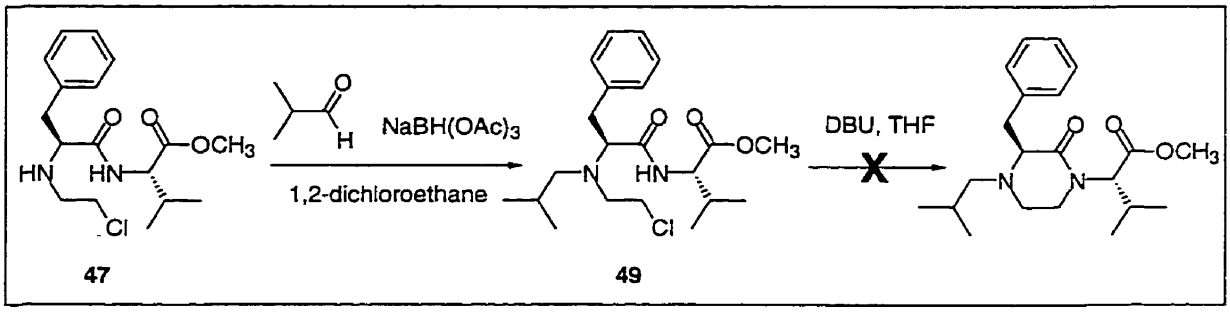


Scheme 22 : Probable explaination for unsuccessful ring closure

Therefore, the amine **47** was reacted with 4-nitrobenzenesulfonyl chloride and triethylamine in methylene chloride but none of the desired corresponding sulfonamide product could be isolated. The amine was next reacted with benzoyl chloride and 4-methylmorpholine in methylene chloride, but surprisingly none of the desired product was obtained again. In both cases, decomposition of the starting material was observed. Perhaps, the steric hindrance created by the secondary amine or the sensitive nature of the chloroethyl group was responsible for the failure of this reaction. Moreover, there is also

a possibility of aziridine-ring formation and subsequent side reactions. An attempt was also made to tritylate amine **47** by reaction with trityl chloride, but this too failed.

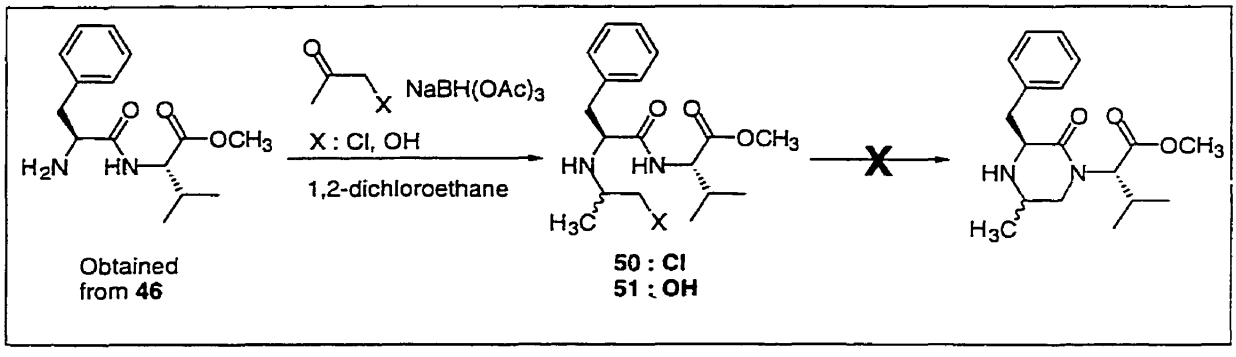
We next decided to reductively alkylate the secondary amine **47** by reaction with an aldehyde and sodium cyanoborohydride in acidic medium. Thus, the secondary amine **47** was reacted with isobutyraldehyde and sodium cyanoborohydride in methanol in presence of a few drops of acetic acid. The tertiary amine **49** so obtained was then reacted with DBU for the cyclization step but this unfortunately also did not proceed as desired. Changing the base from DBU to K_2CO_3 in DMF even at high temperatures resulted in no reaction at all, and all starting material was recovered unchanged.



Scheme 23 : Unsuccessful ring closure with i-butyl substituted nitrogen atom

So far, the focus of these reductive amination reactions had been reactions of the dipeptide with aldehydes. In order to expand the scope of the reaction and also create a further site for creation of diversity, it was felt that α-halo ketones, which by their greater bulk could prevent intramolecular aziridine ring formation, should be the next substrates for this reaction. However, when TFA.PheValOMe (**46a**) was reacted with 2-chloroacetone in MeOH with $NaCNBH_3$ and acetic acid, a complex mixture of products was obtained. The two step synthesis (i.e. initial formation of the imine, followed by its reduction) was then attempted. But none of the desired product could be isolated. Finally

following the procedures developed by Abdel-Magid *et al.*, the dipeptide PheValOMe (i.e. **46a** without the attached TFA salt) was reacted with 2-chloroacetone, $NaBH(OAc)_3$ and acetic acid in 1,2-dichloroethane to yield the corresponding product **50** in excellent yield.[36] The presence of the two diastereomers (due to the methyl group) was clearly visible in the ^1H and ^{13}C NMR spectra as nearly all peaks were doubled. It was treated with DBU in THF but no reaction occurred and most of the starting material was recovered. It was then reacted with sodium hydride to deprotonate the amide NH for cyclization. However, none of the product was observed again and all starting material was recovered unreacted.



Scheme 24 : Unsuccessful ring closure with i-propyl substituted nitrogen atom

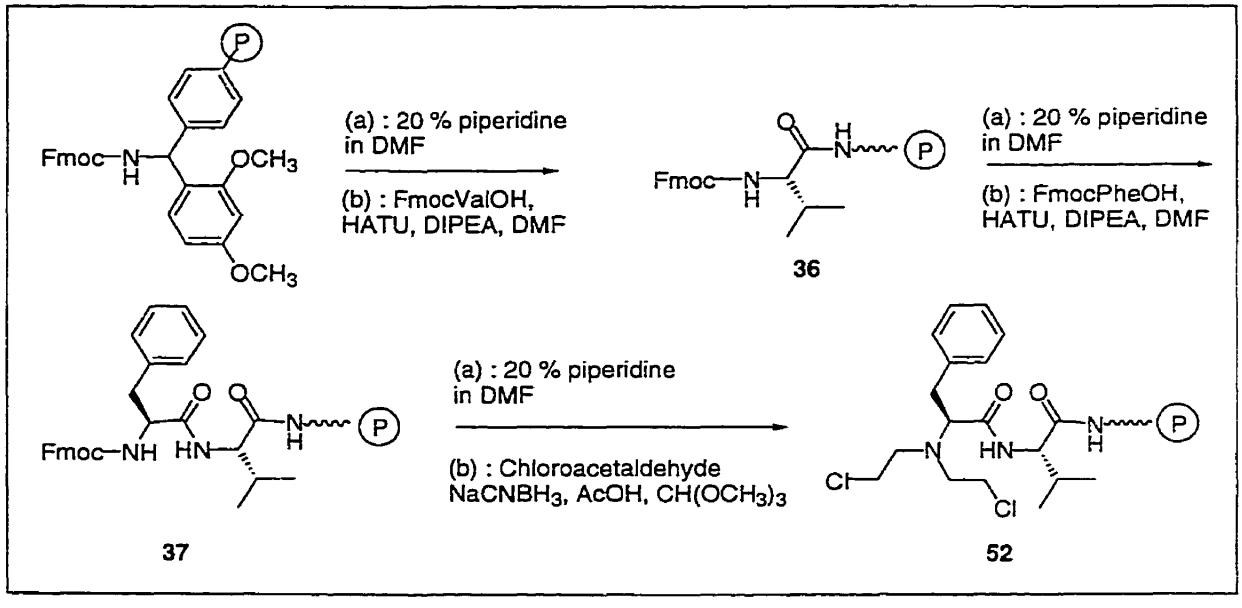
The dipeptide PheValOMe (**46a**) was next reacted with acetol and triacetoxyborohydride in presence of acetic acid in 1,2-dichloroetane and the alkylated alcohol **51** was produced. Unfortunately, this alcohol could not be converted to the tosylate, which could have then been cyclized. Dehydration with trifluoroacetic acid was also not successful. These setbacks resulted in our giving up this route.

Although this approach had failed in solution phase studies, we had been carrying out this route on solid support at the same time. A single step can be carried out

[36] Adbel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.

repeatedly on solid support and we were hoping that by repeating the cyclization step with DBU or another base, the cyclized product would eventually be formed.

2.5.3 Reductive Amination Approach on Solid Support



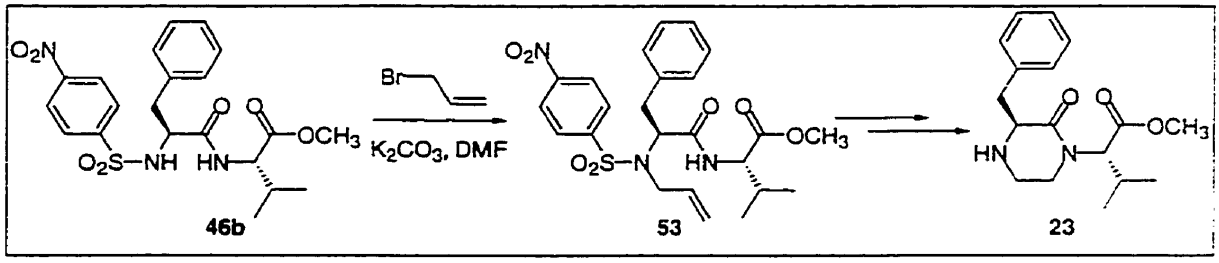
Scheme 25 : Reductive amination using chloroacetaldehyde on solid support

The reductive amination of dipeptides with haloacetaldehyde was attempted on solid support. Rink amide MBHA resin was transformed to the dipeptide resin as before and then reacted with excess chloroacetaldehyde and sodium cyanoborohydride in trimethyl orthoformate for 30 min. The mass spectrum of the compound obtained after cleavage from the resin corresponded to the dialkylated product and there was absolutely no evidence of the desired monoalkylated product. This was not entirely unexpected as the aldehyde was being used in excess. In order to prevent this dialkylation, this reaction was repeated with only 1.1 eq. of chloroacetaldehyde, but this time no reaction was observed. This reaction was then attempted by first synthesizing the imine which could

then be reduced to the desired secondary amine. With this in mind, excess chloroacetaldehyde was reacted with the dipeptide resin in trimethyl orthoformate as the dehydrating solvent. Unfortunately despite various attempts, the imine could never be obtained in pure form. There was a substantial amount of the unreacted dipeptide as evidenced by the mass spectrum of the product. This route was therefore given up entirely.

2.6 Sulfonamide N-Allyl Dipeptide Route to 2-Oxopiperazines

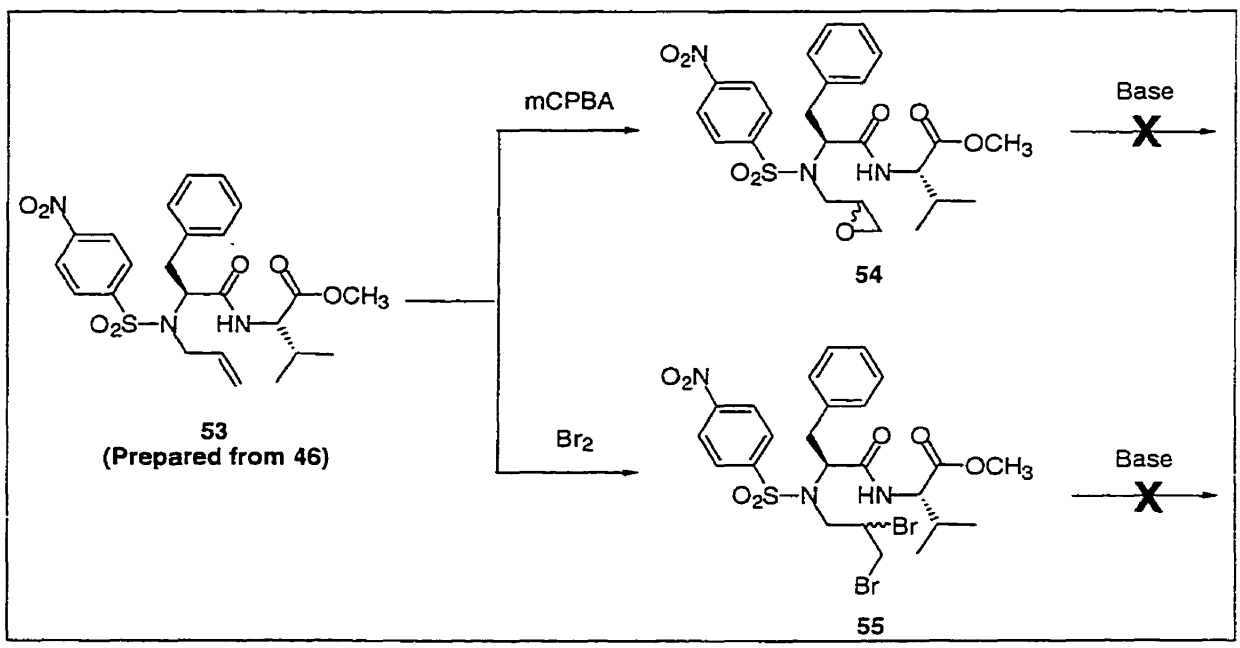
2.6.1 Synthesis of Sulfonamide N-Allyl Dipeptide



Scheme 26 : Sulfonamide N-allyl dipeptide route to 2-oxopiperazines

Sulfonamide dipeptides were successfully allylated and prenylated in excellent yields by Dr. Mohamed during the course his graduate studies (**Scheme 26**).[57] On subsequent ozonolysis and reductive treatment with methyl sulfide, the cyclized sulfonamide 2-oxopiperazine-ring was generated. The easy allylation of the sulfonamide dipeptide in solution phase seemed to suggest that it could be easily carried out under solid phase conditions. The alkene functionality could be a source of either an epoxide or a *vic* dibromide that could be potentially good substrates for the cyclization. With this in mind, 4-NBSPheValOMe **46b** was treated with allyl bromide and K_2CO_3 in DMF to yield the allylated sulfonamide dipeptide **53**. Its reaction with mCPBA in methylene chloride

proceeded in 35 % unoptimized yield to give the epoxide **54** as a mixture of diastereomers that were not separated. Reacting this mixture of the epoxides with P4-Phosphazene base in presence of LiCl in THF failed to yield the cyclized product. The allylated sulfonamide dipeptide **53** was next treated with Br_2 and DBU in THF with the hope that the bromonium ion formed would be attacked by the amide anion. However the reaction failed to proceed as hoped and instead yielded the *vic* dibromide **55** only. In order to achieve intramolecular attack by the amide to induce cyclization, this dibromide was next reacted with excess DBU in THF, but none of the desired product was obtained and nearly all the starting material was recovered unchanged. We therefore gave up this route.



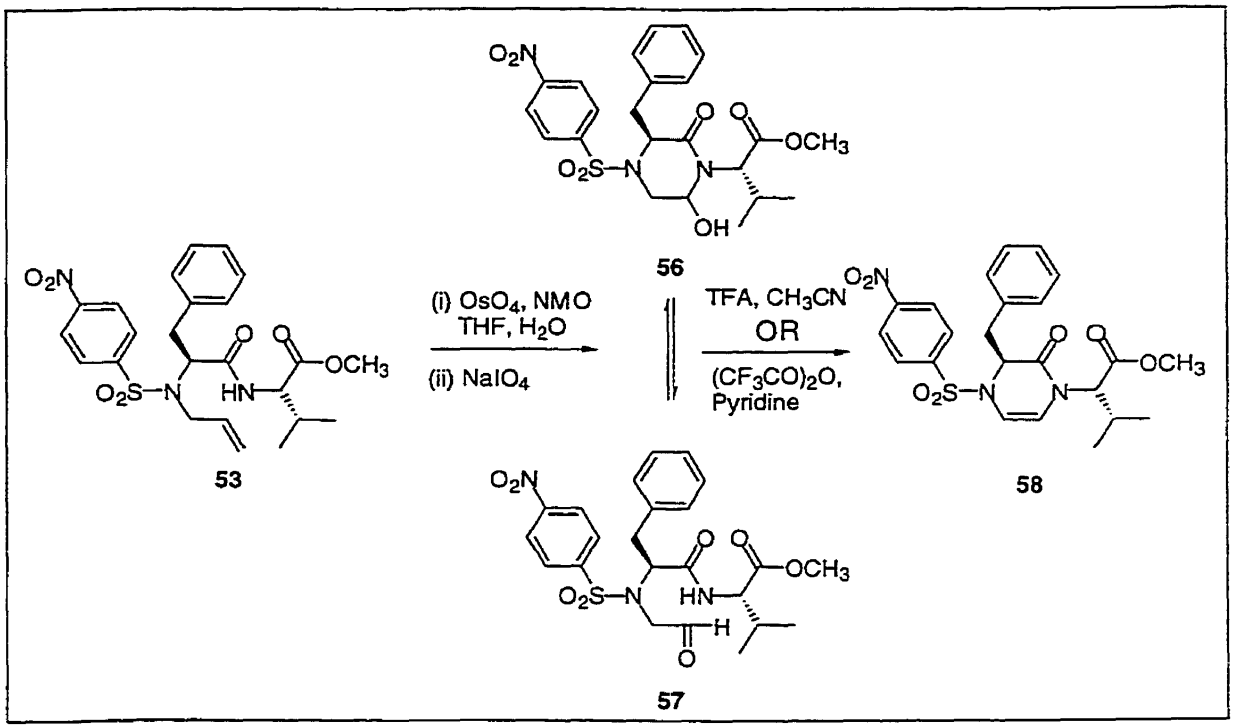
Scheme 27 : Unsuccessful ring closure reactions

2.6.2 Oxidation and Cyclization of N-allyl Dipeptide Derivatives

[37] Mohamed, N. *Ph.D. Thesis, Department of Chemistry, McGill University*, **1998**, Section 2.2.

We next decided to oxidatively cleave the allyl functionalized 4-nitrobenzenesulfonamide dipeptides and cyclize the aldehyde so generated to the 2-oxopiperazine derivative.

This approach (**Scheme 28**) began with the reaction of 4-NBSPheValOMe (**46b**) with allyl bromide and K_2CO_3 in DMF to generate the N-allyl product **53** in over 95 % yield without resorting to chromatographic purification. This allyl derivative was reacted with alkaline $KMnO_4$ solution in acetone but a complicated mixture of products was obtained. This was probably due to over oxidation as $KMnO_4$ is a powerful oxidizing agent. Hence we decided to use the more predictable OsO_4/NMO reagents for this transformation. The allyl derivative was therefore reacted with NMO and a catalytic amount (2 %) of OsO_4 in aqueous THF and the resulting diol was oxidized by subsequent addition of sodium periodate. TLC showed presence of two spots that were later separated and determined to be the cyclized oxopiperazyl amidol **56** and the uncyclized aldehyde **57**. These two compounds eluted very close to each other and required careful chromatographic separation. These two compounds are in equilibrium with each other and hence a method by which the equilibrium could be shifter towards the generation of only one compound was required to avoid tedious chromatography.

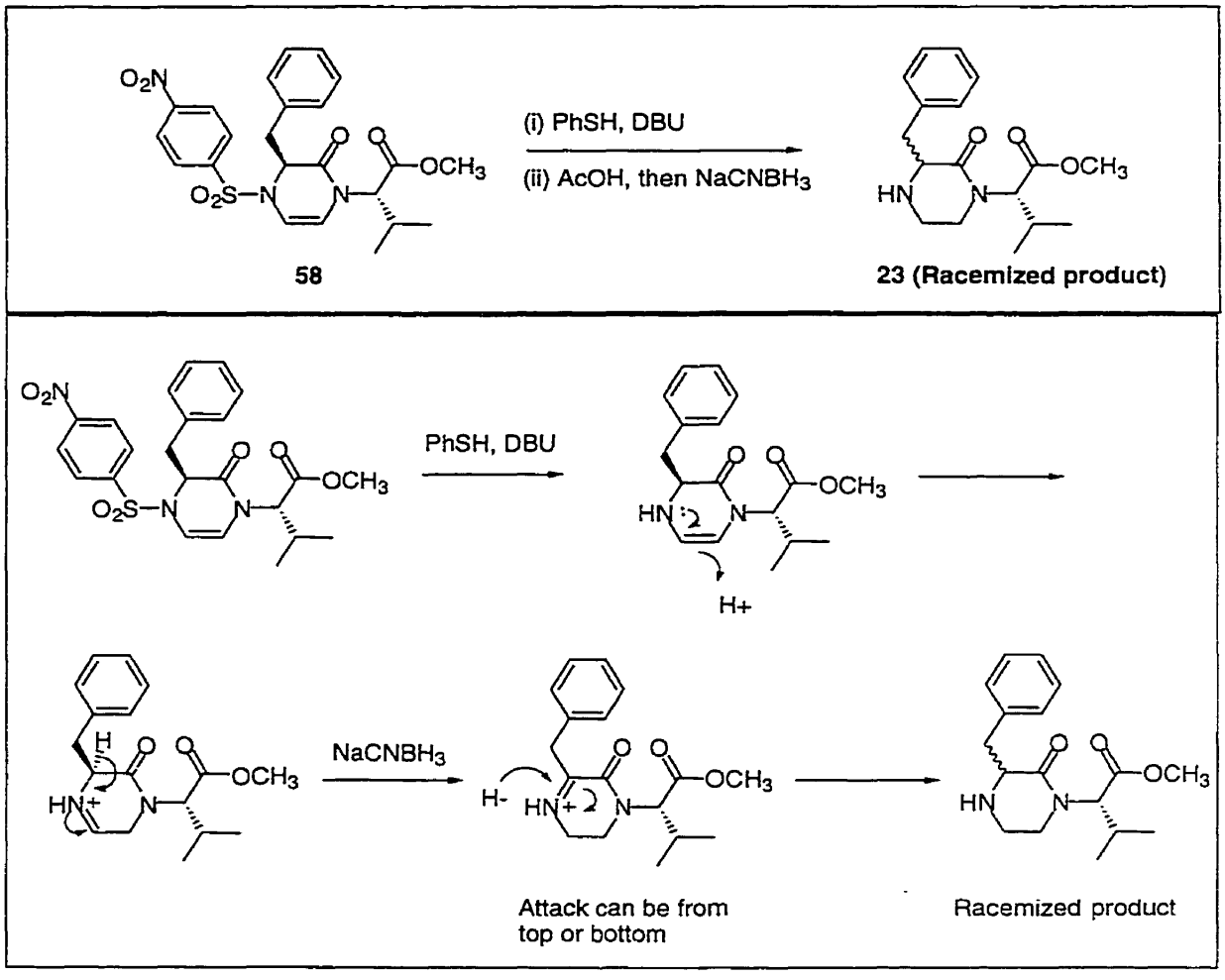


Scheme 28 : Cyclization via oxidative cleavage of sulfonamide N-allyl dipeptide ester

Initially, the mixture of **56** and **57** was treated with excess DBU in THF with the hope of getting the amide anion which would then give the cyclized amidol product **56** exclusively. However, this did not proceed as desired and a mixture was obtained. This equilibrium mixture was next reacted with excess of TFA in acetonitrile and refluxed for 24 hours to yield the dehydrated product **58** as shown in **Scheme 28**.

This compound **58** was seen as the unsaturated version of the desired sulfonamide 2-oxopiperazine product. This dehydrated product, however, could not be reduced to the sulfonamide oxopiperazyl derivative by reaction with Et_3SiH, $NaCNBH_3$ or $NaBH_4$ or $NaBH(OAc)_3$ in presence of acetic acid or TFA. Therefore, it was decided to remove the sulfonamide protection and then reduce the resulting enamine or tautomeric imine.

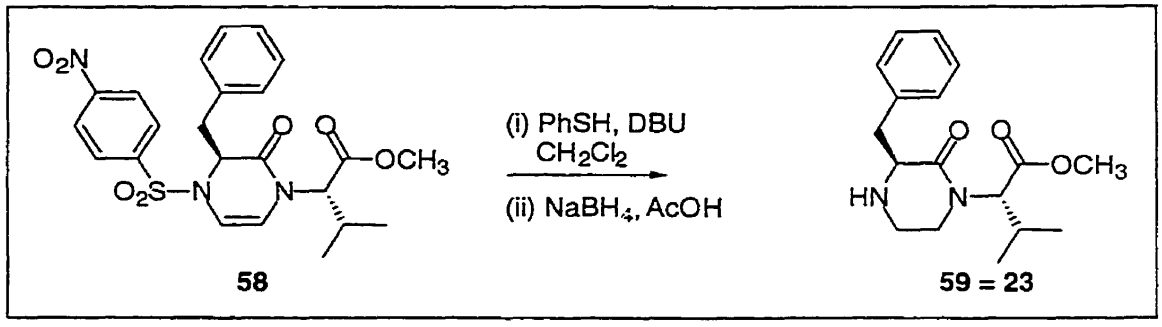
Accordingly, this alkene **58** was reacted with thiophenol/K_2CO_3 in DMF to deprotect the sulfonamide and the reaction quenched with acetic acid and $NaCNBH_3$ to give the desired 2-oxopiperazine derivative **23** after chromatography in a modest 20 % yield. Changing the solvent to CH_2Cl_2 and the base to DBU resulted in 50 % yield of the oxopiperazine product. In both these reactions, the reducing agent ($NaCNBH_3$) was added after the addition of acetic acid. It was also noticed that phenylalanine had racemized giving rise to diastereomers. This was clearly evident by the presence of duplicate peaks of nearly identical intensity in the ^1H and the ^{13}C NMR spectra of the products. In all the previous reactions, only negligible amounts of racemization had occurred as the NMR spectra of the compounds showed only a single set of peaks.



Scheme 29 : Racemization during deprotection and its proposed mechanism

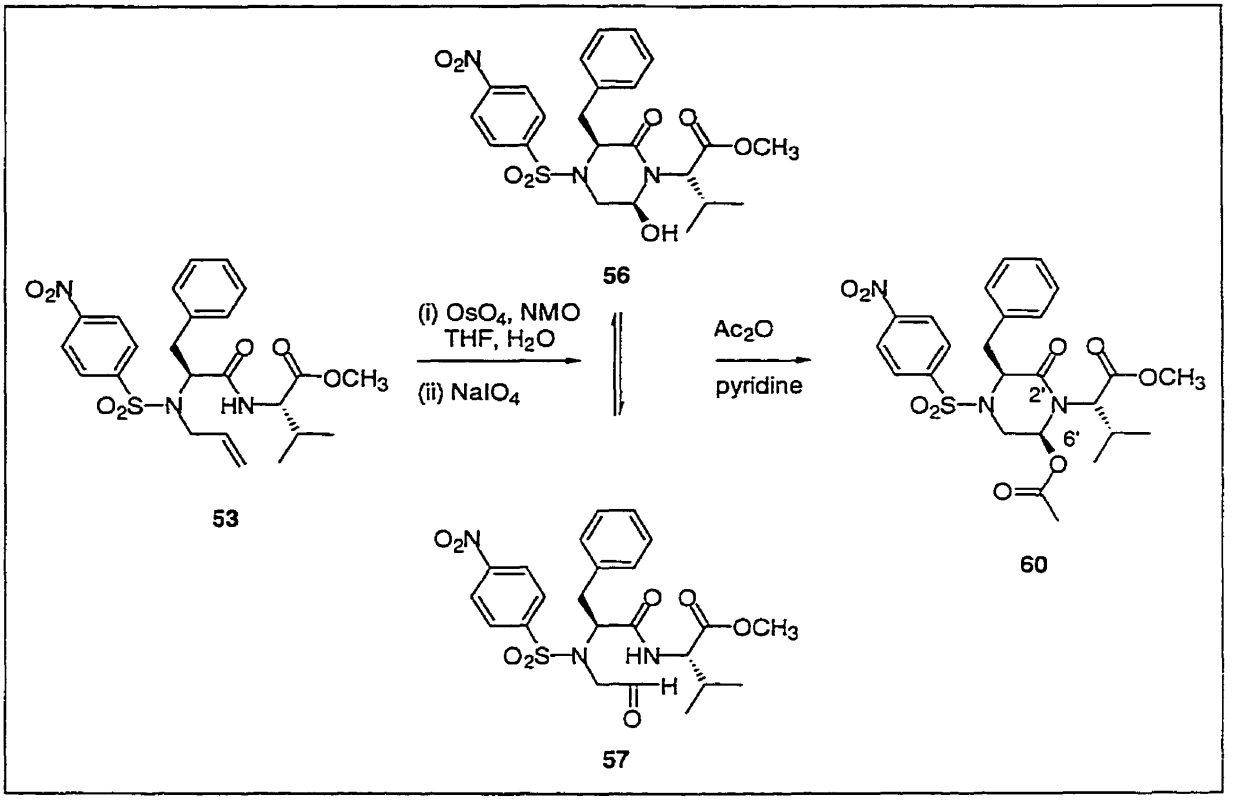
In order to prevent this racemization and also improve the yield of this process, a stronger reducing agent was needed. We decided to use $NaBH_4$ instead. Thus the above procedure was carried out in methylene chloride with DBU and thiophenol. This was followed by the addition of sodium borohydride after the sulfonamide had been deprotected as seen by TLC and then acetic acid was added. This order of addition seemed to be better suited as the reducing agent was present in the reaction medium when the enamine is being formed. Our hope was that this should give the enamine no time to racemize and it should instead get reduced immediately. This indeed turned out to be the

case (**Scheme 30**) and no racemization was observed. After chromatography, the desired 2-oxopiperaine derivative **59** was isolated in 60 % yield for this step using this procedure.



Scheme 30 : Deprotection and reduction procedure

In an alternate approach, the equilibrium mixture of the oxopiperazyl amidol **56** and the uncyclized aldehyde **57** was reacted with Ac_2O in pyridine, and the oxopiperazine-6-O-acetate product **60** was isolated in over 80 % yield (**Scheme 31**). This product was isolated as a single diastereoisomer as evidenced by the ^{1}H and ^{13}C NMR spectra. Moreover, the single proton at C-6' position (X of ABX system) showed coupling constants of 3.9 Hz each with the two protons on C-5' position. This showed that the C-6' proton occupied the equatorial position in the pseudo-chair conformation adopted by the 6-membered 2-oxopiperazine ring. All attempts using acetic acid and $NaBH_4$ or $NaCNBH_3$ to reductively cleave the acetate group and generate the desired 2-oxopiperazine product failed entirely. The starting acetate compound was recovered unchanged. Since the acetate group was not successfully removed, we decided to use the trifluoroacetate group instead (**Scheme 28**). The mixture of the oxopiperazyl amidol and the uncyclized aldehyde was therefore reacted with trifluoroacetic anhydride in pyridine at - 78 ^{0}C. This led to the isolation of the alkene **58** directly. This alkene had previously been successfully transformed to the desired 2-oxopiperazine derivative as described earlier.



Scheme 31 : Formation of acetate derivative

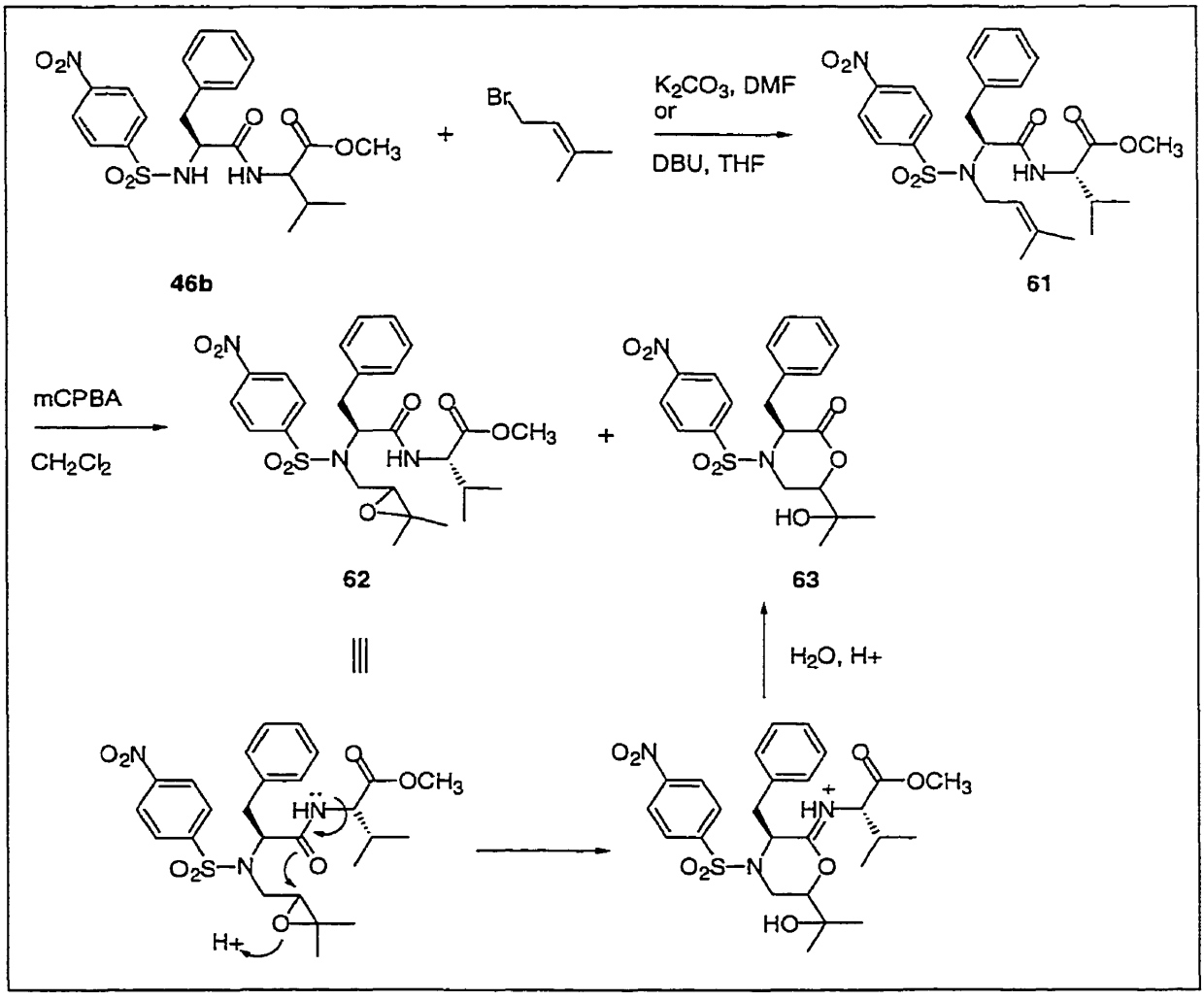
This route seemed to be ideal for carrying out the synthesis of 2-oxopiperazine derivatives on solid support. Unfortunately, when the reaction scheme was shown to the research director at Astra laboratories, objections to the use of OsO_4 were raised. OsO_4 is a known teratogen and is highly toxic. It's use, even in catalytic amounts, was unacceptable under regulations of the Astra laboratories where the solid phase studies were to be performed. Hence a new synthetic scheme was required which avoided the use of toxic OsO_4 entirely.

2.6.3 Epoxidation, Oxidative-cleavage and Cyclization of N-allyl dipeptide derivatives

At this point, we decided to use the epoxide **54** synthesized earlier to perform the crucial cyclization step towards the synthesis of the 2-oxopiperazine derivatives. In earlier reactions, we had failed to get the amide anion to attack the epoxide. On the other hand, Dr. Mohamed had earlier shown that the amide nitrogen spontaneously attacks a carbonyl carbon six atoms away. So if this alkene could be oxidatively cleaved to the corresponding aldehyde, cyclization to the desired 2-oxopiperazyl compound could be achieved. However, Dr. Mohamed had used ozonolysis for oxidation that was again unpractical for the purpose of automated synthesis on solid support. We therefore decided to use periodic acid for this crucial cyclization step. Reaction of epoxides with periodic acid is known to generate the corresponding diol that is immediately oxidatively cleaved to the aldehyde. This route thus bypasses the use of OsO_4. The previously synthesized sulfonamide N-allyl dipeptide **53** was epoxidized by reaction with mCPBA in methylene chloride in 35 % yield. In order to improve the yield, the reaction was then performed in slightly basic buffered solutions (initially with NaH_2PO_4, then with $NaHCO_3$) but the yield could only be improved to 42 %. mCPBA is sold commercially in 50 – 80 % purity with m-chlorobenzoic acid and water as the major impurities. These can be removed by washing a mCPBA solution with basic buffered (NaOH, NaH_2PO_4) solution. mCPBA was then purified and its subsequent use only marginally increased the yield of the epoxide **54** to 50 %.

We felt that perhaps the yield of this transformation could be improved by using the dimethylallyl derivative. The presence of two methyl groups on the alkene should make it more electron rich thereby increasing the efficiency of epoxidation. Thus N-(4-nitrobenzenesulfonyl), N'-(dimethylallyl)-L-phenylalanyl-L-valine methyl ester was

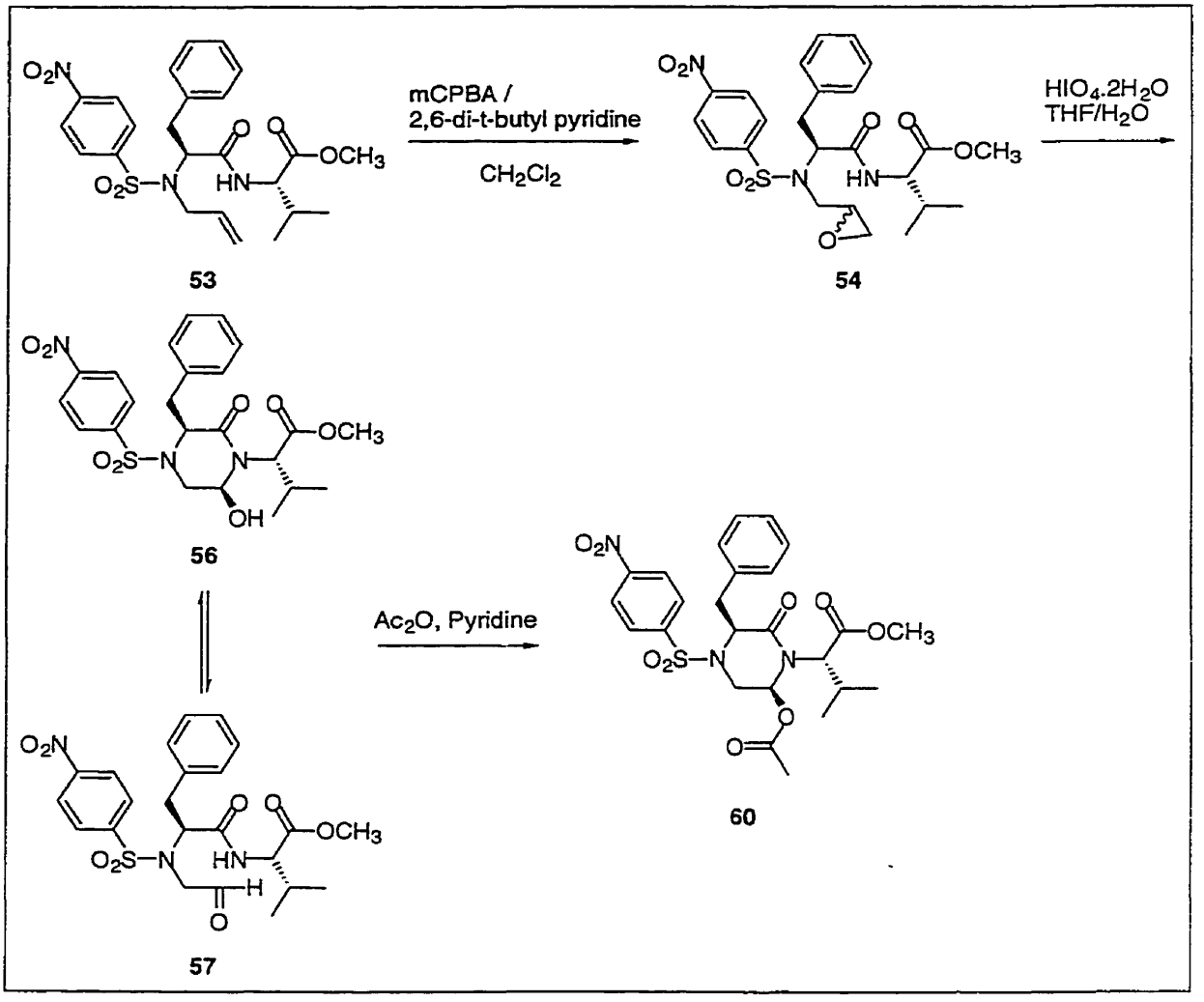
prepared by reaction of N-(4-nitrobenzenesulfonyl)phenylalanylvaline methyl ester with prenylbromide and DBU in THF in 90 % yield (**Scheme 32**). Epoxidation with mCPBA gave the product **62** in only 18 % yield. Unexpectedly the major product was **63** in 37 % yield, which we believe gets formed by the attack of the amide on the electrophilic epoxide and subsequent hydrolytic cleavage of the iminium species. The use of prenyl derivative was therefore given up and we decided to go back to the allyl derivative for achieving the required cyclization.



Scheme 32 : Side product formation with epoxidation of dimethylallyl derivative

The yield of the epoxidation step with the allyl derivative **54**, (**Scheme 27**) had been unacceptably low (< 50 %) and in an effort to increase the yield, the procedure by Svensson *et al.* was next attempted.[38] In this procedure, 2,6-di*tert*butyl piperidine was used as a base to neutralize the m-chlorobenzoic acid that is formed as a result of epoxidation. 4-fold excess each of prewashed mCPBA and 2,6-di*tert*butyl pyridine were used to achieve over 70 % yield for this step that required chromatographic purification to isolate the epoxides **54**. These diasteromeric epoxides were then reacted with periodic acid. As before, both the cyclic amidol **56** and the aldehyde **57** were produced as observed by TLC. Instead of separating them, the mixture was reacted with acetic anhydride in pyridine and the 2-oxopiperazine-O-acetyl product **60** was obtained as shown in **Scheme 33**.

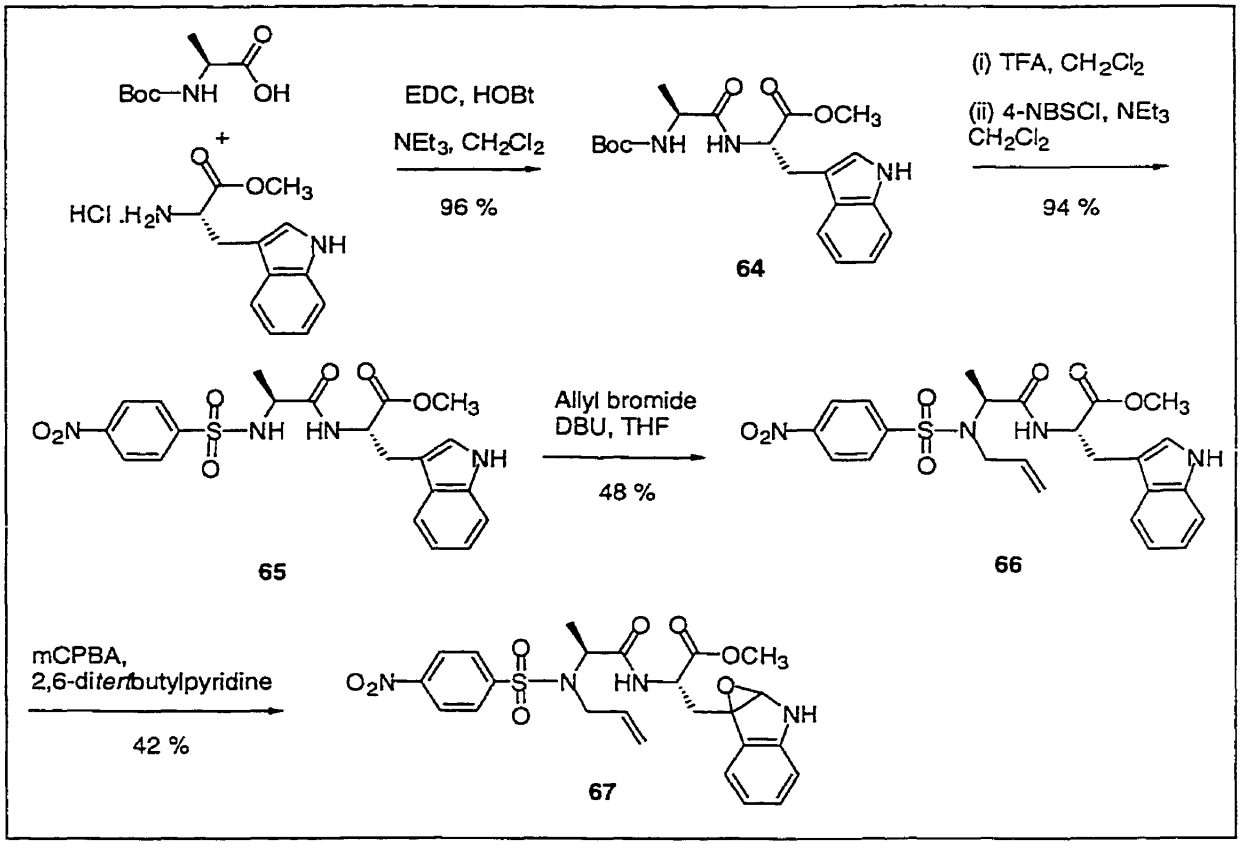
[38] Svensson, A.; Lindstrom, U. M.; Somfai, P. *Syn. Commun.* **1996**, *26*, 2875



Scheme 33 : Successful epoxidation and oxidative cleavage leading to cyclization

This route was also carried out on another dipeptide (Ala-Trp). As shown in **Scheme 34**, N-(*tert*Boc)AlaOH was coupled to TrpOMe.HCl using EDC and HOBt to give dipeptide **64**. The *tert*Boc group was removed with 50 % trifluoroacetic acid in methylene chloride and then this dipeptide was transformed to the 4-nitrobenzene sulfonamide **65** as before. This was then reacted with allyl bromide and DBU in THF to give the allylated product **66**. This was subjected to the epoxidation procedure as before using mCPBA and 2,6-di*tert*butylpyridine. However instead of the expected epoxide

product, we observed that the epoxidation had occurred at the indole ring to give **67** in 41 % yield.[39] Evidently the electron rich indole ring was the more reactive site. Although we did not follow this route and further, we decided to avoid using tryptophan in subsequent studies.



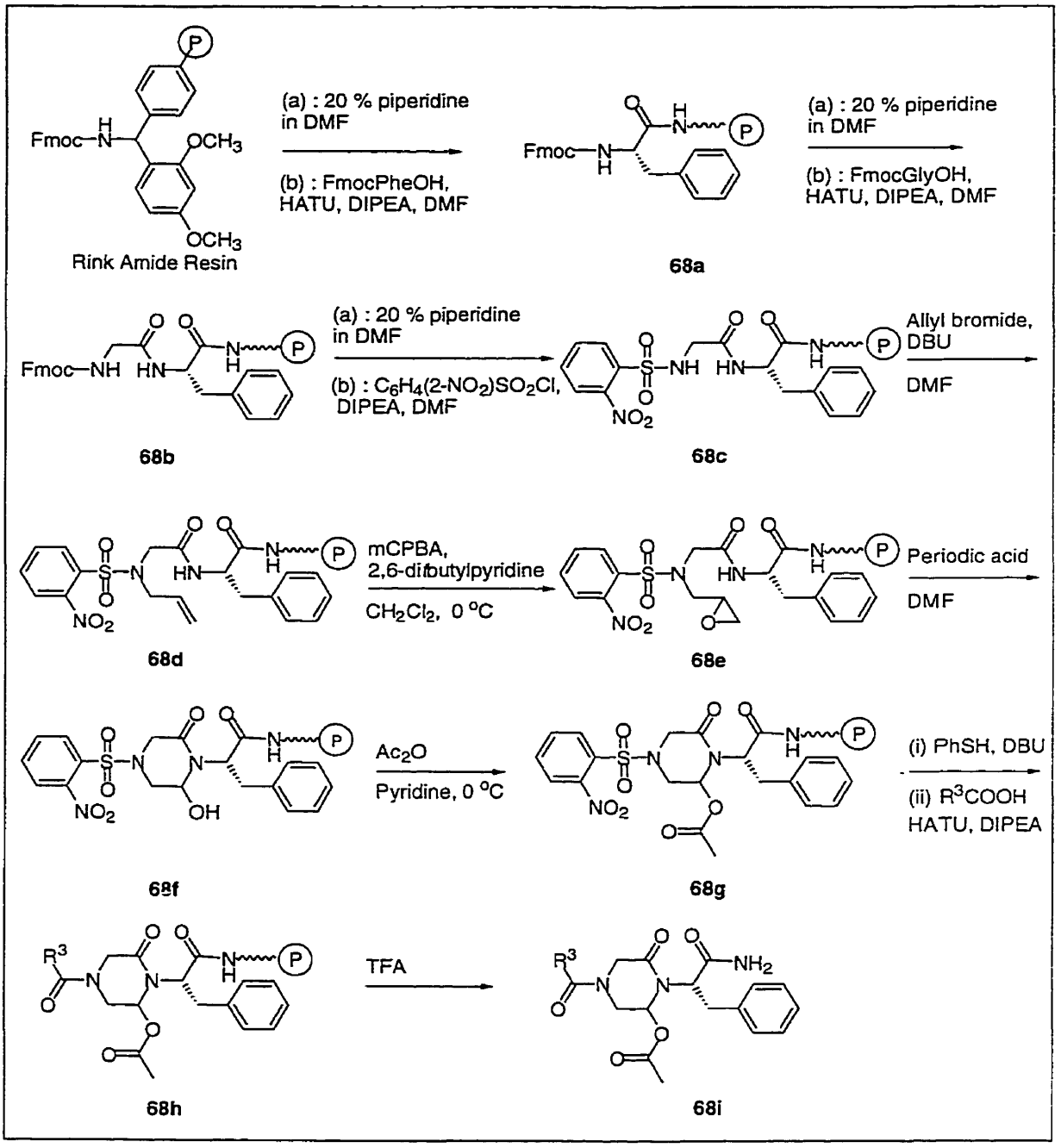
Scheme 34 : Unexpected epoxidation of the indole ring in AlaTrp dipeptide derivative

Inspite of this minor setback, this route seemed to be the ideal route for carrying out the synthesis of the ethylene-bridged dipeptides on solid support. We were thus ready to launch a final assault on the goal of synthesizing the 2-oxopiperazines on solid support.

[39] None of the desired product (with epoxidation occurring on the allyl group) was isolated.

2.6.4 Synthesis of 2-Oxopiperazines on Solid support

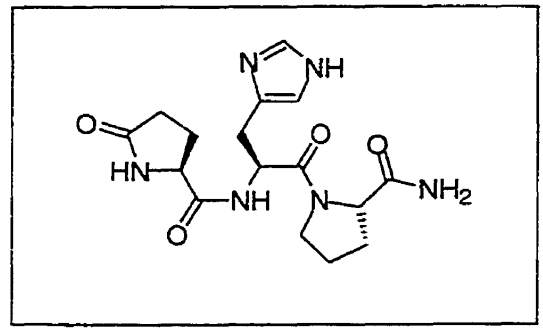
The final attempt at the synthesis of 2-oxopiperazines on solid support began with the attachment of N-Fmoc protected phenylalanine to Rink amide MBHA linker (**Scheme 35**). Fmoc deprotection was achieved by washing with 20 % piperidine in DMF. The free amine was next coupled to N-(Fmoc)glycine using HATU as the coupling reagent. Subsequent deprotection of the Fmoc group generated the free amine which was then converted to the sulfonamide **68c** by reaction with 2-nitrobenzenesulfonyl chloride. This 2-nitrobenzenesulfonamide was reacted with allyl bromide and DBU to generate the N-allylated sulfonamide dipeptide **68d**. This allylation step had to be carried out twice for complete conversion. The epoxidation of this allyl derivative was carried out by addition of mCPBA (10 eq.) and 2,6-di*tert*butyl pyridine (10 eq.) in methylene chloride at 0 °C. Cleavage of the product from the resin at this stage revealed the presence of a small amount of product. The major fractions in the LCMS trace were due to decomposition products obtained during the process of cleavage of the product from the resin, which uses trifluoroacetic acid. The epoxidized resin was next treated with periodic acid (3 eq.) and water in DMF. Cleavage of the product from a small amount of resin again revealed the presence of the desired cyclized product **68f**. However, the LCMS trace showed only a very small amount of product. There were other unidentified fragments in the spectrum. The last two transformations have not yet been optimized due to lack of time. Our efforts are continuing in this direction right now.



Scheme 35 : Successful synthesis of 2-oxpiperazine derivatives on solid support

2.7 Synthesis of Thyrotropin Releasing Hormone (TRH) Analogs

2.7.1 Introduction to TRH



Thyrotropin Releasing Hormone (TRH)

TRH (Glp-His-ProNH_2) was the first hypothalamic hormone to be isolated and characterized.[40] This tripeptide displays dual functions, acting both as a hormone and as a neuropeptide. TRH was initially classified as a hormone that releases prolactin and thyrotropin from the pituitary, but now it has also been shown to function as a neurotransmitter. This characterization is based primarily on its analeptic properties and its ability to reverse the sedation and hypothermia induced by pentobarbital, ethanol, and diazepam. TRH augments many of the neurotransmitter systems implicated in memory storage and retrieval independently of its hormonal activity, and is effective in ameliorating many forms of memory disruption. TRH - related alterations are associated with various disease states including Alzheimer's disease, depression, schizophrenia, epilepsy and metabolic disorders.[41]

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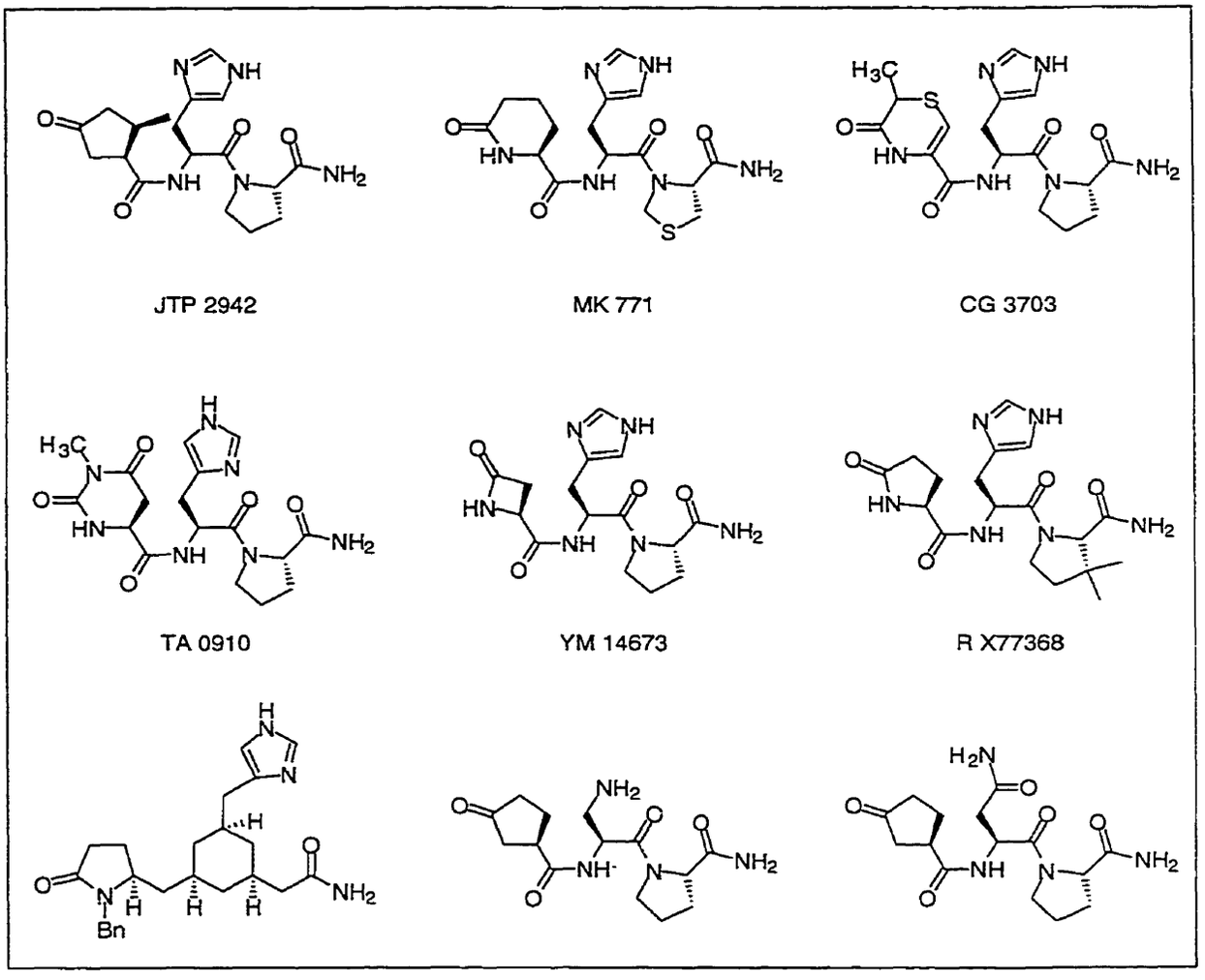
[40] (a) Bler, J.; Enzmann, F.; Folkers, K.; Bowers, C. Y.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 705. (b) Burgus, R.; Dunn, T. F.; Desiderio, D.; Vale, W.; Guillemin, R. C. *R. Acad. Sci. (Paris)* **1969**, *269*, 1870.

[41] Mazurov, A. A.; Andronati, S. A.; Korotenko, T. I.; Sokolenko, N. I.; Dyadenko, A. I.; Shapiro, Y. E.; Gorbatyuk, V. Y.; Voronina, T. A. *Bioorg. Med. Chem. Lett.* **1997**, *5*, 2029.

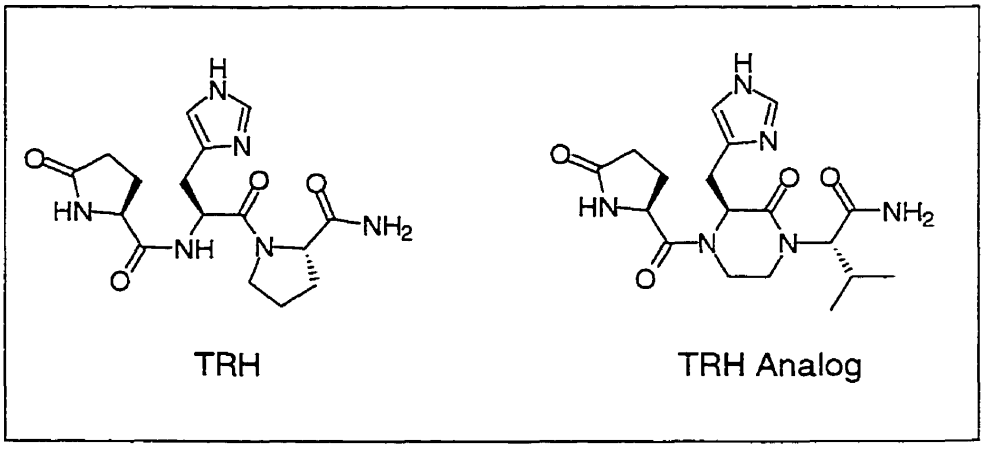


Some Representative TRH Analogs

The biological activities of TRH are brought about by the interaction of TRH with its receptor (TRH-R). This receptor is a member of a large family of transmembrane proteins (TM) that couple to G-proteins. To understand TRH signaling at the molecular level, it is important to determine the structure of the complex between TRH and its receptor. The transmembrane nature of these G-protein coupled receptors makes it nearly impossible to determine the three-dimensional structure of the TRH receptor. Moreover all attempts to crystallize this receptor with a bound ligand have been unsuccessful.

Consequently the only attempts to delineate the biologically active conformation of TRH have been by synthesizing various conformationally restricted analogs of TRH and then studying their activities.

Through these studies, it is known that there are at least three primary sites of interaction between TRH and its receptor. These include (a) the C-terminal amide group of proline with Arg-306 in transmembrane region – 7 (TM-7); (b) the imidazole ring of histidine with Tyr-282 in TM-6; (c) the carboxamide functionality on pyroglutamic acid with Tyr-106 and Asn-110 in TM-3. TRH is rapidly metabolized by the body, and hence an analog that retains these interactions while imparting rigidity to the peptide backbone would be an ideal TRH analog.[42] Accordingly we chose to synthesize a TRH analog based on the oxopiperazine ring system as shown below.



Proposed TRH Analog

[42] Various analogs of TRH having some or all of these functional groups have been synthesized. See : (a) Olson, G. L.; Cheung, H. -C.; Chiang, E.; Madison, V. S.; Sepinwall, J.; Vincent, G. P.; Winkour, A. Gary, K. A. *J. Med. Chem.* **1995**, *38*, 2866. (b) Bos, M.; Olson, G. L.; Vincent, G. P. *Helvt. Chim. Act.* **1994**, *77*, 463. (c) Ikeda, K.; Iwasaki, Y.; Kinoshita, M. *Neuroscience Lett.* **1998**, *250*, 9. (d) Toide, K.; Shinoda, M.; Takase, M.; Iwata, K.; Yoshida, H. *Eur. J. Pharmacol.* **1993**, *233*, 21.

In this TRH analog, the three major sites of interaction with the TRH-receptor are retained. The N-terminus pyroglutamic acid is kept intact, histidine is made a part of the oxopiperazine ring, and valine has replaced proline. The synthesis of this analog thus involves the generation of the oxopiperazine ring with His and Val, installation of Glp on the free N-terminus of this oxopiperazine and converting the C-terminus of valine to amide functionality.

2.7.2 Retrosynthesis and Histidine Protection

The central amino acid of TRH is histidine (His) which has an imidazole ring, the protection of which assumes critical importance. The protecting group must be stable to the conditions and reagents used earlier for the synthesis of the oxopiperazine ring. Thus it must be stable to piperidine, triethylamine, carbonate, thiophenolate and various peptide-coupling reagents. It must also prevent racemization, which is a major issue with histidine containing peptides. Tosyl, Boc, BOM, trityl, benzyl, and dinitrophenyl groups have all been used for protection of His depending on the particular conditions involved and most of these protected compounds are commercially available.[43] In addition, the amine and the carboxyl termini must also be suitably protected.

2.7.3 Initial Unsuccessful Attempts

2.7.3.1 Use of N-(*tert*Boc)His(Tos)OH

Tosyl protection of the imidazole seemed ideal for the synthesis as N-(*tert*Boc)His(Tos)OH is commercially available. Initial attempts at coupling N-

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[43] (a) Brown, T.; Jones, J. H.; Richards, J. D. *J. Chem. Soc. Perkin Trans. I* **1982**, 1553. (b) Sieber, P.; Riniker, B. *Tetrahedron Lett.* **1987**, *28*, 6031.

(*tert*Boc)His(Tos)OH with ValOMe using EDC and HOBt gave low yields (only 35 %) of the dipeptide **69** (**Scheme 36**). The cause of this unexpected low yield was later understood when a literature report where HOBt was used for removing tosyl protection from the imidazole ring came to our attention.[44] Thus instead of activating the acid, HOBt removed the tosyl group from the imidazole ring of histidine. When this coupling was carried out in absence of HOBt (i.e. with EDC alone), the yields went up to 90 % but the product had racemized. This was clearly evident by the ^{1}H and ^{13}C NMR spectrum with doubling of most of the peaks. Since this was completely undesirable, other peptide coupling reagents were tried. The reaction was attempted using BOP as the coupling reagent that does not require addition of HOBt. However there was very little improvement in the yield of the isolated product **69** (54 %). BOP has a hydroxybenzotriazole group that again reacts with the tosyl group on the imidazole ring. An attempt to circumvent this reactivity of the hydroxybenzotriazole group was made by using coupling reagents which neither required HOBt as an additive, nor had HOBt group in them. Pentafluorophenol activation of N-(*tert*Boc)His(Tos)OH, followed by coupling with ValOMe unfortunately also gave low yield of the dipeptide. Pentafluorophenol also attacked the tosyl group (thereby deprotecting histidine) apart from activating the acid for coupling. A small study was then done to determine the stability of the tosyl protection of the imidazole group towards a few reagents. Excess of thiophenol, pentafluorophenol, and HOBt completely removed the tosyl protection whereas secondary amines such as piperidine did not.

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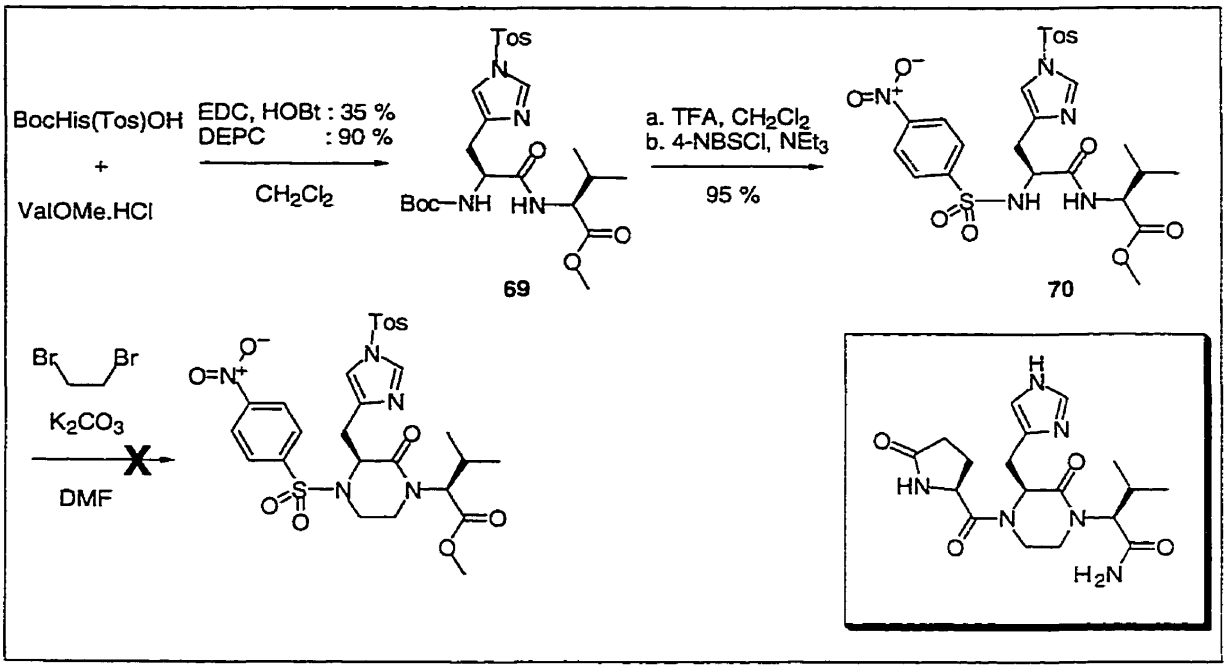
[44] Fuji, T.; Sakakibara, S. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 3146.

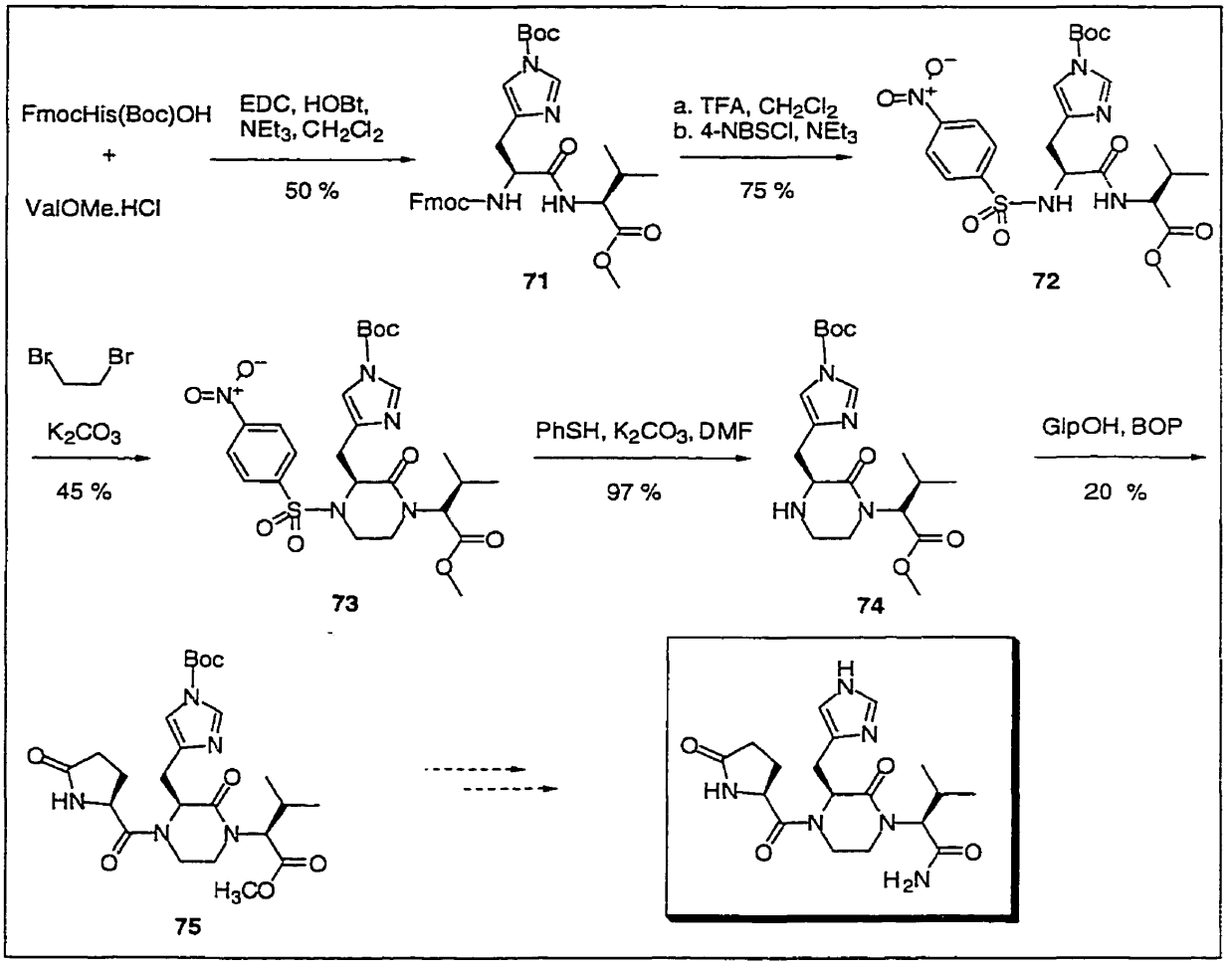


Scheme 36 : Use of BocHis(Tos)OH

Lastly, diethylphosphoryl cyanide (DEPC) was tried as the coupling reagent for this reaction.[45] Use of DEPC resulted in the generation of the dipeptide N-(*tert*Boc)His(Tos)ValOMe (**69**) in excellent yields (90 %) with the tosyl group intact. Furthermore, there was no indication of any racemized dipeptide. Removal of the *tert*Boc protection from the N-terminus of the dipeptide **69** furnished the TFA salt, with the tosyl group intact. Its transformation to the 4-nitrobenzenesulfonamide dipeptide **70** proceeded as usual by reaction with 4-nitrobenzenesulfonyl chloride in presence of triethylamine. Reaction of this sulfonamide dipeptide with K_2CO_3 and 1,2-dibromoethane in DMF at 60 °C gave a very low yield of the cyclized product. One of the side products in this reaction was the deprotected cyclized product in which the tosyl protection had come off. This may be again due the labily of the tosyl group on an imidazole group under basic

conditions. Hence this route using tosyl protection on the imidazole ring of His was abandoned.

2.7.3.2 Use of FmocHis(Boc)OH



Scheme 37 : Use of FmocHis(Boc)OH

A Boc group can also protect the imidazole group on histidine and N-(Fmoc)His(Boc)OH is commercially available as its cyclohexylammonium salt. Washing an ether solution with dilute sulfuric acid solution first neutralized the salt and the free

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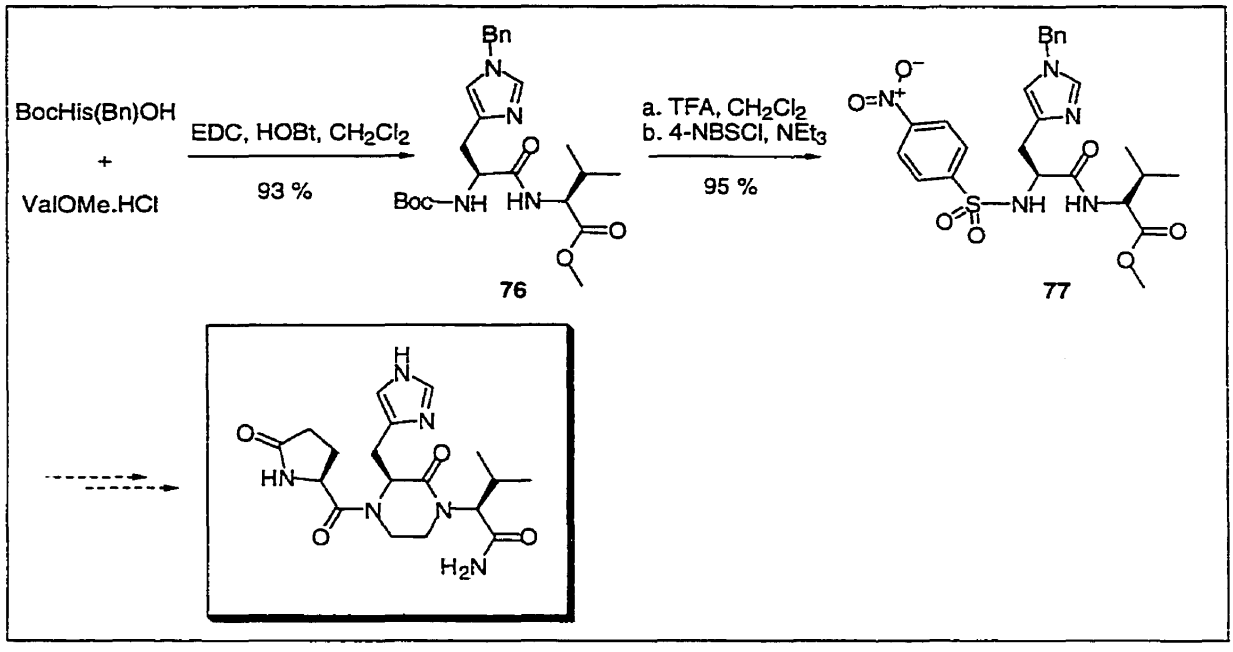
[45] Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, *18*, 1595.

acid was then coupled with valine methyl ester using BOP as the coupling reagent. Unlike the tosyl group, the Boc group is stable to nearly all nucleophiles. Fmoc deprotection of **71** proceeded with diethylamine or piperidine, and the free amine so obtained was reacted with 4-nitrobezenesulfonyl chloride in presence of triethylamine to furnish the sulfonamide **72** in 75 % yield for the two steps. On reacting this with 1,2 dibromoethane and K_2CO_3 in DMF at 60 °C, the desired cyclized product **73** was obtained. The 4-nitrobenzenesulfonamide group was then deprotected by reaction with thiophenol and K_2CO_3 in DMF to give the oxopiperazine derivative **74**. It was then coupled to pyroglutamic acid using BOP and triethylamine in methylene chloride. We had been carrying out a parallel synthesis of the desired TRH analog using trityl protection for the imidazole ring of histidine, which is described later. In that synthesis, the deprotection of the trityl group using trifluoroacetic acid led to decomposition of the starting material. Since the final deprotection step of the Boc protected imidazole ring also required the same set of conditions, we decided to discontinue this route.

2.7.3.3 Use of N-(*tert*Boc)His(Bn)OH



Scheme 38 : Use of BocHis(Bn)OH

The imidazole group on histidine can also be protected by a benzyl group and N-(*tert*Boc)His(Bn)OH is commercially available. One of its advantage is that removal of Boc group is easily performed in solution phase and we were hoping that the final deprotection of the benzyl group could be carried out easily by hydrogenation in neutral or mildly acidic conditions. Thus BocHis(Bn)OH was coupled to ValOMe with BOP as the coupling reagent in presence of triethylamine in methylene chloride as usual. TFA and methylene chloride were then added to this dipeptide **76** and solvent evaporation after 4 hours gave the TFA salt of the amine. This salt was an oil unlike all the previous TFA dipeptide salts that invariably came out a nice white powders. On examining the NMR, we realized that two molecules of TFA were bound to the dipeptide - one to the free amine and the other to the imidazole ring. The mass of the product obtained also correlated to two bound TFA molecules. This was then reacted with 4-nitrobenzenesulfonyl chloride and 3 equivalents of triethylamine **77** (2 eq. gave only 67

% yield!) in methylene chloride to obtain the sulfonamide dipeptide with the imidazole ring protected with a benzyl group. Reaction with 1,2-dibromoethane and K_2CO_3 in DMF was then performed, but surprisingly none of the desired product could be isolated. Starting material was obtained exclusively. Mitsunobu reaction (using PPh_3 and DEAD) was then attempted with bromoethanol and we were rather disappointed not to obtain the desired product. Hence this approach was given up.

2.7.4 Partial Synthesis of Novel TRH Analog

The synthesis was next attempted using trityl protection for the imidazole group of histidine. Coupling of N-(Fmoc)His(Trt)OH with valine methyl ester hydrochloride in presence of BOP (or HATU) and triethylamine gave the protected dipeptide **78**. This coupling was carried out in CH_2Cl_2 at 1 M concentration in nearly quantitative yield. Either 20 % piperidine or diethylamine in methanol removed the Fmoc group on the N terminus of histidine. The free amine so obtained was reacted without purification with 4-nitrobenzenesulfonyl chloride in presence of triethylamine in CH_2Cl_2 to give the corresponding sulfonamide **80**, which was purified by chromatography. The deprotection of Fmoc was also done using tetrabutylammonium fluoride (TBAF), but this reaction could not be pushed to completion and poor yields were consequently obtained.[46]

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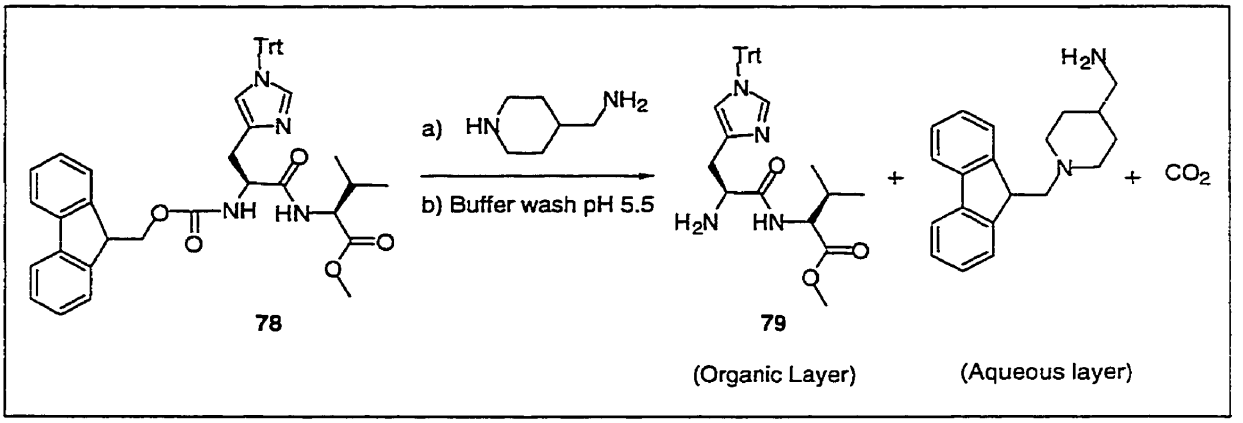
[46] (a) Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617. (b) Ueki, M.; Nishigaki, N.; Aoki, H.; Tsurusaki, T.; Katoh, T. *Chem. Lett.* **1993**, 721.



Scheme 39 : Deprotection of Fmoc using 4-aminomethyl piperidine

Subsequently, as shown in **Scheme 39** the procedure developed by Carpino *et al.* was used to isolate the free primary amine from the dibenzofulvene byproduct.[47] In this procedure, instead of piperidine, 4-aminomethyl piperidine was used to deprotect the Fmoc group and the 4-AMP-dibenzofulvene adduct was removed by washing the chloroform solution of the product with an aqueous phosphate buffer (pH 6.5). The deprotected amine **79** was thus left in the organic phase in nearly pure form and there was no need to perform chromatography. Its reaction with 4-nitrobenzenesulfonyl chloride and triethylamine in chloroform gave the 4-nitrobenzenesulfonamide dipeptide **80**. On heating this sulfonamide at 60 °C in DMF with excess 1,2-dibromoethane and K_2CO_3, oxopiperazine derivative **81** was obtained. The sulfonamide group was removed by $PhSH/K_2CO_3$ in DMF and the oxopiperazine **82** so obtained was ready for coupling with pyroglutamic acid. Since the oxopiperazine was a secondary amine, activation of the pyroglutamic acid as its acid fluoride was attempted, but the fluoride anion attacked the lactam ring exclusively instead of the terminal acid group in pyroglutamic acid. Thus the

[47] (a) Carpino, L. A.; Cohen, B. J.; Stephens, K. E.; Sadat-Aalaee, S. Y.; Tien, J. -H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732. (b) Beyermann, M.; Bienert, M.; Niedrich, H.; Carpino, L.A.; Sadat-Aalaee, D.

acid fluoride methodology developed earlier could not be applied. Fortunately, use of BOP (or HATU) as the coupling agent gave the desired coupled product **83** in good yields. There was absolutely no indication of ring-opening of the lactam group on pyroglutamic acid.

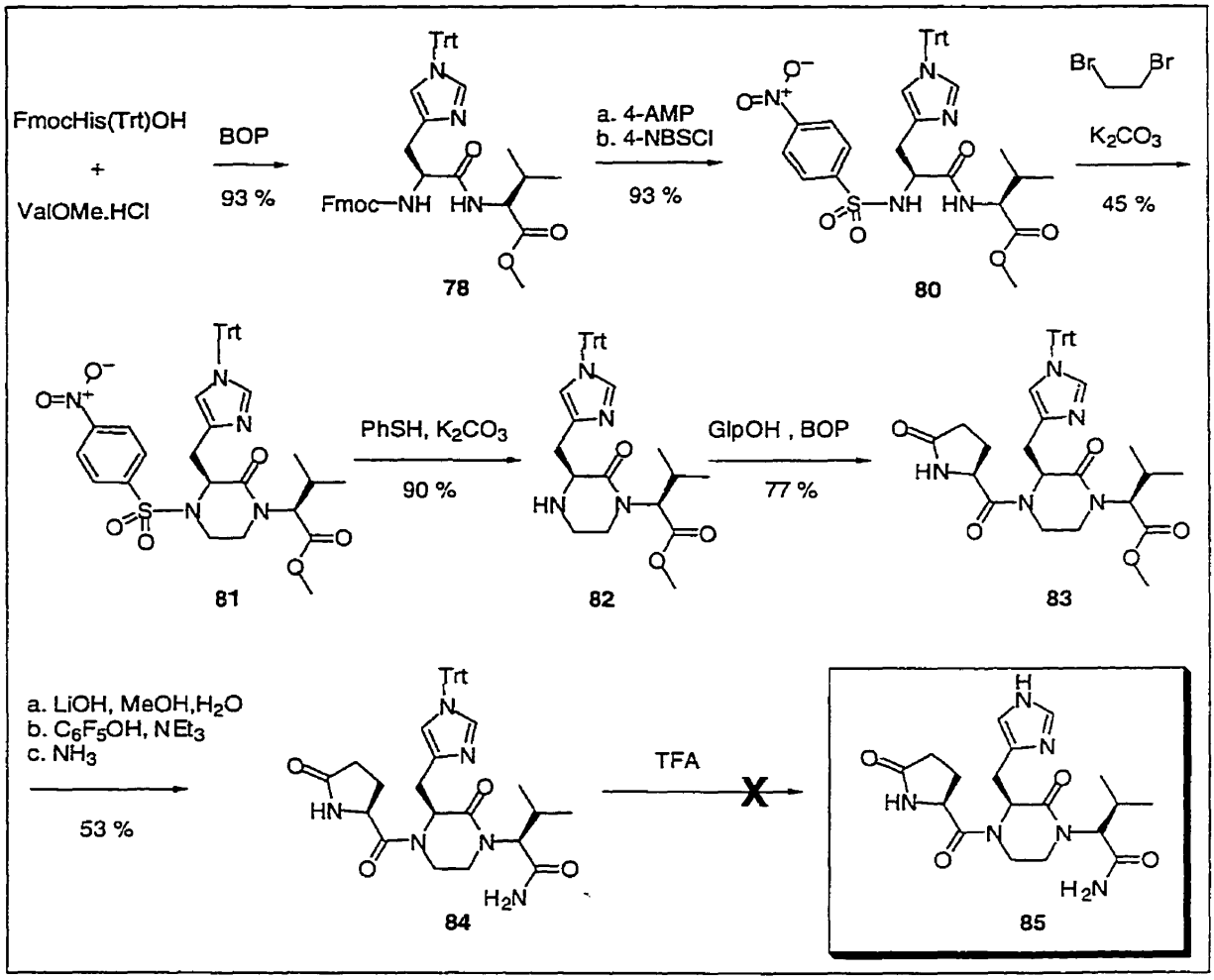
The remaining two transformation (i.e. conversion of the C-terminal ester to the amide, and deprotection of the N-imidazole trityl) proved to be more difficult. Methyl esters are usually converted to their corresponding amides by bubbling ammonia gas at high temperatures and pressure or in the presence of a catalyst like cyanide ion.[48] Treatment of the ester **83** with NH_3 even in presence of NaCN as catalyst, failed to transform the ester to the amide. The methodology developed by Weinreb *et al.* involving the use of $AlMe_3$ and NH_3 to generate NH_2^- ion in situ was also unsuccessful as the amide ion attacked the lactam ring of pyroglutamate.[49] Since the lactam group of the pyroglutamate residue was causing this problem, we decided to convert its precursor **82** to the corresponding C-terminal amide. Thus ester **82** was reacted with $AlMe_3/NH_3$ but no reaction occurred and all starting material was recovered unreacted. Therefore other methods were looked into for achieving this seemingly trivial transformation.

The methyl ester **83** was hydrolyzed with LiOH in aqueous methanol and the corresponding acid reacted with C_6F_5OH/DCC/DMAP to give the activated pentafluorophenol ester. Its treatment with 2 M ethanolic NH_3 solution gave the amide **84** in 53 % yield for the 3 steps. Similar route to convert the terminal ester to the amide was used by Olson *et al.* in the synthesis of their TRH analog.[42(a)]

J. Org. Soc. **1990**, *55*, 721. (c) Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673.

[48] (a) Hogberg, T.; Strom, P.; Edner, M.; Ramsby, S. *J. Org. Chem.* **1987**, *52*, 2033. (b) Chen, S. -T.; Jang, M. -K.; Wang, K. -T. *Synthesis* **1993**, 858.

[49] Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *48*, 4171.



Scheme 40 : Partial Success in the synthesis of novel TRH analog using FmocHis(Trt)OH

The trityl deprotection was next performed with dilute trifluoroacetic acid in methylene chloride. However none of the desired product could be obtained. Treatment with TFA led to an apparent decomposition of the tripeptide as only a fragment containing His and Val was isolated.[50] It is possible that the desired product went into the aqueous layer during workup, but this could not be verified as the aqueous layer was

[50] Hashimoto *et al.* have reported the cleavage of the pyroglutamate-peptide bond in dilute aqueous solutions. (a) Hoshimoto, T.; Ohki, K.; Sakura, N. *Chem. Pharm. Bull.* **1995**, *43*, 2068. (b) Hashimoto, T.; Saito, S.; Ohki, K.; Sakura, N. *Chem. Pharm. Bull.* **1996**, *44*, 877. (c) Ohki, K.; Sakura, N.; Hashimoto, T. *Chem. Pharm. Bull.* **1997**, *45*, 194.

never checked for the presence of the desired product. The use of milder acids for cleaving the trityl group was then studied. Unfortunately, none of the desired product was observed when trichloroacetic acid and dichloroacetic acid were used.

This transformation was then attempted on model compounds using even milder acidic conditions. Both N-(Fmoc)His(Trt)ValOMe and N-(4-NBS)His(Trt)ValOMe gave the corresponding desired free imidazole products when treated with 5 % acetic acid in methanol at reflux. Both these products were purified using chromatography. Therefore, 5 % acetic acid in methanol at reflux seemed like the ideal deprotecting reagent for trityl group from the imidazole ring. Indeed, treatment of the tripeptide amide **84** in 5 % acetic acid in methanol at reflux for 6 hours gave the desired TRH analog **85**. Aqueous work-up was performed to isolate the product but the organic layer contained none of the product. An examination of the aqueous layer showed that it contained the desired product mixed with inorganic salts. Evidently the presence of four amide bonds, coupled with the absence of any hydrophobic group, makes this compound extremely water-soluble and it therefore ended up in the aqueous layer during the work up. Gel filtration chromatography was attempted with Sephadex but the salt-free compound could not be isolated. The product crude **85** was at that stage only characterized by a ^{1}H NMR in D_2O.

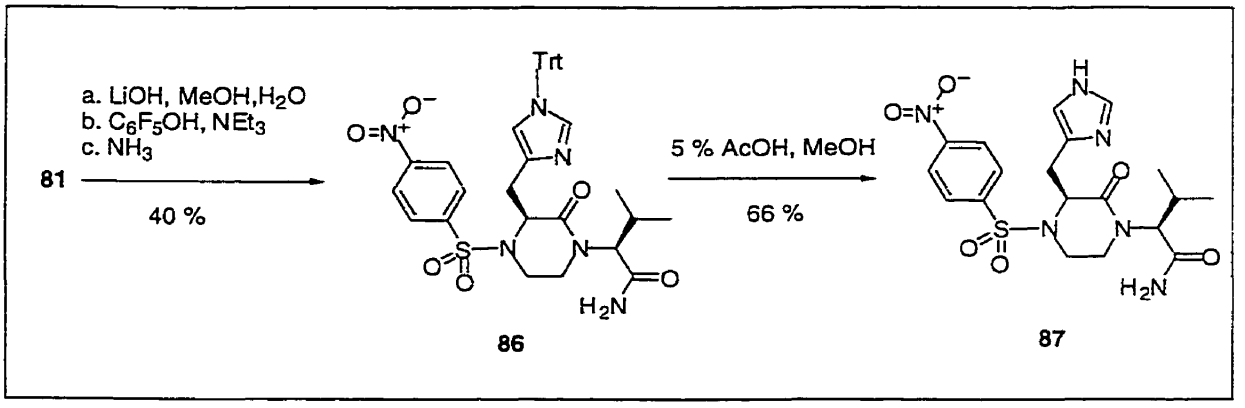
2.8.5 Synthesis of sulfonamide derived TRH analog

At this stage we decided to synthesize a TRH analog where the N-terminus was a 4-nitrobenzenesulfonyl group instead of pyroglutamate (**Scheme 41**). It was easily obtained from **81** by first hydrolyzing the ester followed by activating the acid by a pentafluorophenol ester and finally reacting it with ammonia to give **86**. The trityl protection on the imidazole was removed in presence of 5 % AcOH in refluxing

methanol. In view of the problems faced earlier, we decided not to do an aqueous work up but instead purified the product **87** by chromatography directly.

2.8.5 Synthesis of 2-Oxopiperazyl TRH analog

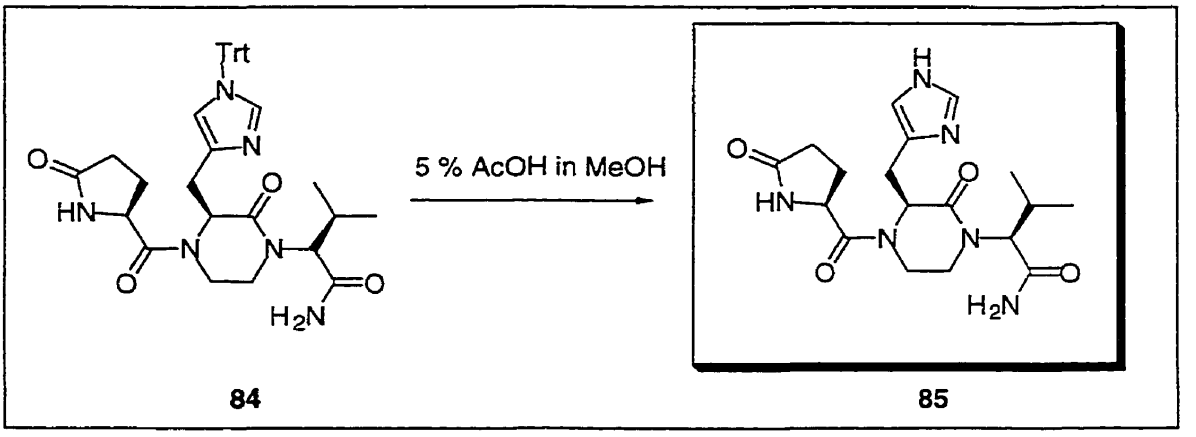
With this success, we decided to continue our quest towards the synthesis of the



Scheme 41 : Synthesis of sulfonamide derived TRH analog

desired TRH analog. The synthesis was repeated to generate compound **84** again which was reacted with 5 % acetic acid solution in methanol at reflux as before for 4.5 hours (**Scheme 42**). Initially, column chromatography was attempted, but no product could be isolated. Evidently the compound was too polar and therefore could not be isolated in this manner. The expected solubility pattern of the peptide product and the hydrocarbon byproduct are very different, as was evident from previous unsuccessful attempts, and this property was then utilized to isolate the pure desired tripeptide derivative **85**. The peptide product is polar and is expected to go into a methanol/water layer whereas the hydrocarbon product will preferentially dissolve in a non-polar hexanes layer. Thus the crude product was then partitioned into a hexanes/aqueous methanol system. The peptide went into the aqueous methanol layer and the trityl acetate into the hexanes layer.

Separation of the two layers and evaporation of the methanol layer under vacuum gave the desired product **85** whose characterization (^{1}H, ^{13}C NMR, HRMS) confirmed the structure.



Scheme 42 : Synthesis of 2-oxopiperazyl TRH analog

2.8 Conclusions and Future Prospects

The synthesis of 2-oxopiperazines derivatives using amino acids as precursors has been achieved in solution phase. However, on solid phase a reliable method for their synthesis remains to be established. The solid phase studies described in **Section 2.6.4** have demonstrated that the desired cyclized 2-oxopiperazine derivative does indeed get generated, albeit in small amounts. This route could not be optimized before the submission of this thesis due to lack of time. Our efforts are continuing in this direction right now in an effort to synthesize a library of such peptidomimetics.

There is also a need for the detailed computational study of these 2-oxopiperazine derivatives. Although we did perform a few rudimentary calculations on the various conformations, this issue needs to be looked into by an expert in the area of computational chemistry. The TRH analogs could also be studied to get an idea about their preferred conformations.

TRH analogs incorporating the 2-oxopiperazine ring using other amino acids (glutamic acid for N-terminus, phenylalanine for the middle residue, and alanine or leucine for the C-terminus) can be synthesized and their activity studied. This should give more information about the structure-activity relationship for these 2-oxpiperazine ring base TRH analogs.

Finally, the 2-oxopiperazine ring can be incorporated into various peptide fragments that show biological activity and the effect of this conformational restriction on the biological activity can be studied.

CHAPTER 3

Contributions to Knowledge

Contributions to Knowledge

1. Four synthetic routes towards the synthesis of 2-oxopiperazines from amino acids were established.

2. Synthesis of these 2-oxopiperazines using these methods was studied on solid support.

3. Two TRH analogs, incorporating the 2-oxopiperazine ring, were synthesized.

CHAPTER 4

Experimental Section

General Methods

All reactions were carried out using glassware that had been heated overnight in an oven at about 120 °C and then cooled in a dessicator containing drierite. All air and moisture sensitive experiments were carried out under nitrogen with freshly distilled solvents. Methylene chloride (CH_2Cl_2) was distilled over P_2O_5; diethyl ether and THF from sodium benzophenone ketyl; triethylamine and acetonitrile over CaH_2, and pyridine was distilled over KOH prior to use. Chemicals and resins were purchased from Aldrich Chemical Company Inc., Fluka Chemicals Inc., Chem-Impex International or Novabiochem and were used without further purification.

All melting points are uncorrected and were determined on a GallenKamp block. Thin Layer Chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Column chromatography was performed using the method described by Still *et al.* on Merck silica gel 40 - 63 um particle size.[51] Visualization was done by exposure to UV light followed by dipping into a solution of ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10 % aqueous sulfuric acid (100 ml) or into a ninhydrin solution (0.3 %) prepared with acetic acid (3 %) in n-butanol (97 %) followed by heating. For solid phase studies Kaiser test was done was taking a few dried resin beads and treating them to 2 ml each of the following solutions and heating for 5 minutes at 100 °C : (i) 5 g ninhydrin in 100 ml ethanol; (ii) 80 g liquefied phenol in 20 ml ethanol; (iii) 2 ml of a 0.001 M aqueous solution of potassium cyanide to 98 ml pyridine. A blue coloration of the resin beads indicate presence of amine end group on the resin beads. For MS determination, a few beads were taken in 50 % TFA in CH_2Cl_2

for 30 minutes and then filtered. The filtrate was collected and TFA and CH_2Cl_2 removed in vacuum. Methanol was added and the solution was analyzed.

^1H NMR spectra were recorded on JEOL CFP 270 and Varian UNITY 500 spectrometers at 270 and 500 MHz respectively. Peak assignments were made with 2D COrrelation SpectrosopY (COSY). ^{13}C NMR were recorded on Gemini 200, JEOL CFP 270 and Varian UNITY 500 spectrometers at 50.0 MHz, 67.5 MHz, 125.0 MHz respectively. Peak assignments were made with ^1H-detected heteronuclear Multiple Quantum Coherence (HMQC) and Distortionless Enhancement by Polarization Transfer (DEPT) experiments. Chemical shifts are reported on the δ scale in parts per million (ppm). Spin multiplicities are given with the following abbreviations : s (singlet), d (doublet), t (triplet), q (quartet), br (broad) and m (multiplet). Mass Spectra (MS) were obtained on a KRATOS MS 25RFA spectrometer in the direct-inlet mode.

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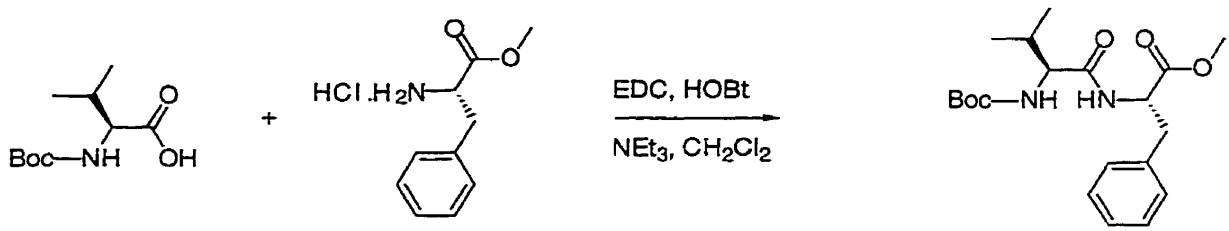
[51] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

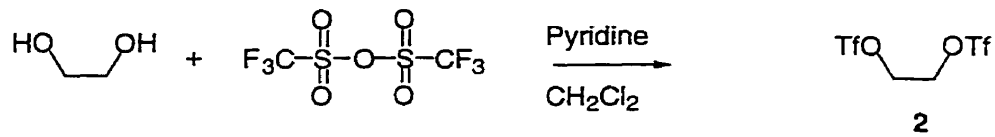
N-(*tert*Butoxycarbonyl)-L-valyl-L-phenylalanine methyl ester : (1)



1

N-(*tert*Butoxycarbonyl)valine (1.017 g 4.681 mmol), phenylalanine methyl ester hydrochloride (1.041 g, 4.83 mmol), EDC (1.425 g, 4.79 mmol), HOBt (0.659 g, 4.877 mmol) and triethylamine (0.65 ml, 4.67 mmol) were dissolved in dry CH_2Cl_2 at 0 °C and the solution stirred at 0 °C for 2 hours and then at room temperature for 36 hours. The solution was then washed with 10 % citric acid, 10 % $NaHCO_3$ and then with water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product as a white solid (1.281 g, 73 %). M.P 109 - 111 °C; R_f 0.42 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.84 (d, J = 6.4 Hz, 3 H, $CH(CH_3)_2$), 0.90 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 1.42 (s, 9 H, $C(CH_3)_3$), 2.00 - 2.15 (m, 1 H, $CH(CH_3)_2$), 3.05 - 3.10 (m, 2 H, $CHCH_2C_6H_5$), 3.69 (s, 3 H, $COOCH_3$), 3.88 (dd, J = 8.3 Hz, J = 6.2 Hz, 1 H, $CHCOOCH_3$), 4.85 (dd, J = 13.8 Hz, J = 5.9 Hz, 1 H, $CHCH_2C_6H_5$), 5.00 (d, J = 7.9 Hz, 1 H, CONH), 6.31 (d, J = COONH), 7.10 - 7.30 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.72 and 19.22 ($CH(CH_3)_2$), 28.36 ($(CH_3)_3C$), 30.92 ($CH(CH_3)_2$), 38.06 ($CHCH_2C_6H_5$), 52.35 (CH), 53.16 (CH), 59.97 ($COOCH_3$), 79.94 ($C(CH_3)_2$, 127.24 (C_6H_5), 128.68 (C_6H_5), 129.29 (C_6H_5), 135.74 (C_6H_5), 155.74 (COONH), 171.24 (CONH), 171.73 ($COOCH_3$); HRMS (EI) Expected for $C_{20}H_{30}N_2O_5$ 378.21546 Found 378.21520.

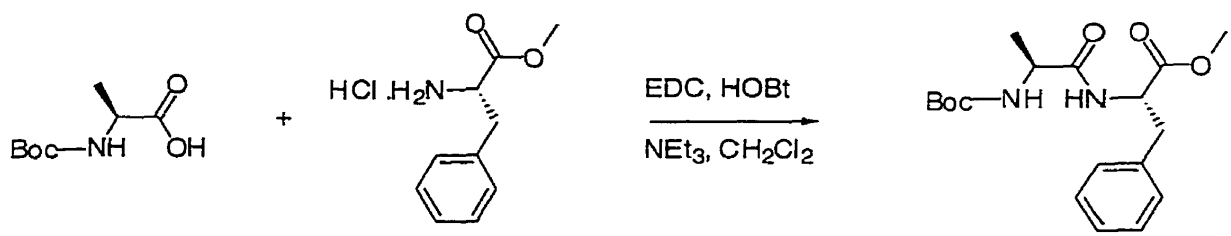
Ethylene glycol bistriflate : (2)



2

Triflic anhydride (5.96 ml, 35.45 mmol) was taken in 36 ml dry methylene chloride at 0 °C in a flask. In another flask, ethylene glycol (1.22 ml, 21.87 mmol) and pyridine (2.87 ml, 35.48 mmol) were taken in 5 ml dry methylene chloride and this solution was added to the solution of triflic anhydride dropwise over a period of 1 hour using a syringe pump. After the addition was over, the solution was filtered to remove the pyridine hydrochloride. The methylene chloride solution was washed with water and then dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (CH_2Cl_2) to give the product (2.697 g, 46 %). R_f 0.70 in CH_2Cl_2; ^1H NMR (270 MHz, $CDCl_3$) δ 4.736 (s, 4 H, CH_2CH_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) 71.98 (CH_2CH_2), 118.53 (q, J = 319 Hz, CF_3); MS (CI, NH_3) 344 (16.4 %, $(M + NH_4)^+$), 327 (0.9 %, $(M + H)^+$).

N-(*tert*Butoxycarbonyl)-L-alanyl-L-phenylalanine methyl ester : (3)

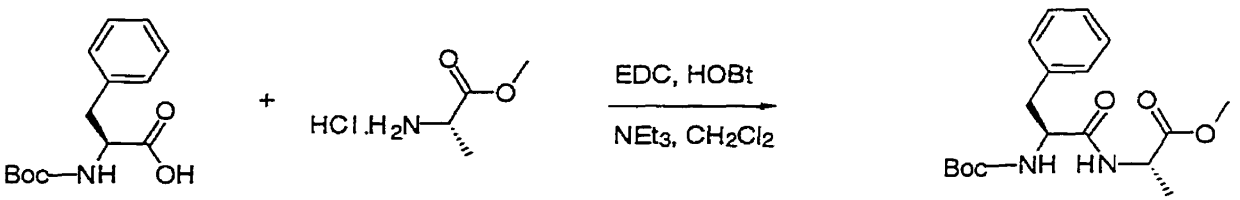


3

N-(*tert*Butoxycarbonyl)alanine (0.316 g 1.672 mmol), phenylalanine methyl ester hydrochloride (0.327 g, 1.514 mmol), EDC (0.322 g, 1.681 mmol) and triethylamine (0.23 ml, 1.65 mmol) were dissolved in 5 ml dry CH_2Cl_2 at 0 °C and the solution stirred at

0 °C for 2 hours and then at room temperature for 24 hours. The solution was then washed with 10 % citric acid, 10 % $NaHCO_3$ and then with water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product as a white solid (0.183 g, 35 %).M.P. 78 - 82 °C; R_f 0.44 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 1.24 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 1.38 (s, 9 H, $C(CH_3)_3$), 2.98 (A of ABX, J = 13.6 Hz, J = 6.4 Hz, 1 H, $CHCH_2C_6H_5$), 3.06 (B of ABX, J = 13.6 Hz, J = 5.9 Hz, 1 H, $CHCH_2C_6H_5$), 3.60 (s, 3 H, $COOCH_3$), 4.10 - 4.20 (m, 1 H, $CHCH_3$), 4.70 - 4.80 (m, 1 H, $CHCH_2C_6H_5$), 5.34 (d, J = 5.7 Hz, 1 H, CONH), 6.88 (d, J = 6.2 Hz, 1 H, COONH), 7.00 - 7.30 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.40 ($CHCH_3$), 28.35 ($C(CH_3)_3$), 37.94 ($CHCH_2C_6H_5$), 50.13 ($CHCH_3$), 52.30 ($COOCH_3$), 53.29 ($CHCH_2C_6H_5$), 80.04 ($C(CH_3)_3$), 127.09 (C_6H_5), 128.55 (C_6H_5), 129.31 (C_6H_5), 135.93 (C_6H_5), 155.39 (COONH), 171.83 (CONH), 172.55 ($COOCH_3$); MS (CI, NH_3) 351 (38.6 %, $(M + H)^+$), 251 (70.3 %, $(M - t\text{Boc})^+$).

N-(*tert*Butoxycarbonyl)-L-phenylalanyl-L-alanine methyl ester : (4)

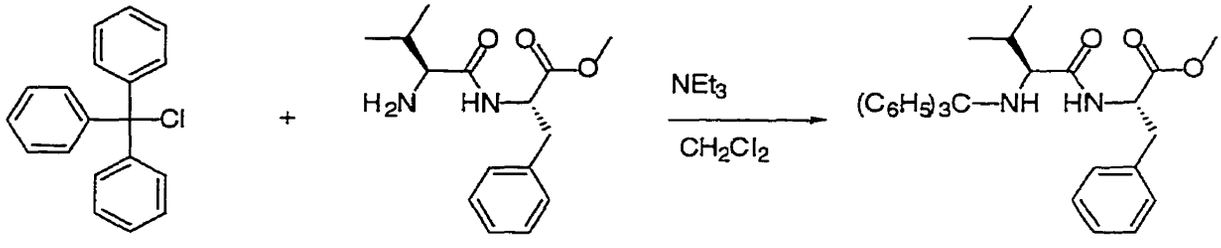


4

N-(*tert*Butoxycarbonyl)phenylalanine (1.214 g 4.576 mmol), alanine methyl ester hydrochloride (0.701 g, 5.021 mmol), EDC (0.969 g, 5.052 mmol) and triethylamine (1.40 ml, 10.04 mmol) were dissolved in 20 ml dry CH_2Cl_2 at 0 °C and the solution stirred at 0 °C for 2 hours and then at room temperature for 24 hours. The solution was then washed with 10 % citric acid, 10 % $NaHCO_3$ and then with water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product as a

white solid (0.627 g, 39 %). M.P. 100 - 103 °C; R_f 0.28 in 2 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) 1.32 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 1.38 (s, 9 H, $C(CH_3)_3$), 3.00 - 3.10 (m, 2 H, $CHCH_2C_6H_5$), 3.68 (s, 3 H, $COOCH_3$), 4.30 - 4.60 (m, 2 H, $CHCOOCH_3$ and $CHCH_2C_6H_5$), 5.06 (d, J = 6.7 Hz, 1 H, CONH), 6.54 (d, J = 6.9 Hz, COONH), 7.10 - 7.30 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.38 ($CHCH_3$), 28.31 ($C(CH_3)_3$), 38.44 ($CHCH_2C_6H_5$), 48.15 ($CHCH_3$), 52.42 ($COOCH_3$), 55.72 ($CHCH_2C_6H_5$), 80.26 ($C(CH_3)_3$), 126.97 (C_6H_5), 128.67 (C_6H_5), 129.43 (C_6H_5), 136.61 (C_6H_5), 155.41 (COONH), 170.88 (CONH), 172.92 ($COOCH_3$); MS (EI) 351 (55.2 %, $(M + H)^+$), 251 (79.0 %, $(M - t\text{Boc})^+$).

N-(Trityl)-L-valyl-L-phenylalanine methyl ester : (5)

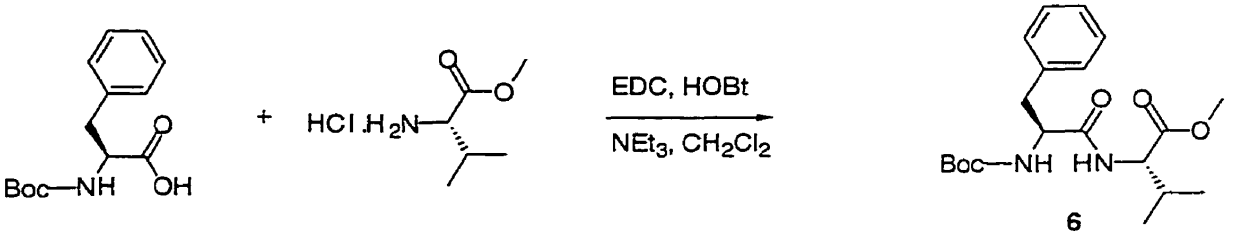


5

A solution of ValPheOMe (0.100 g, 0.255 mmol) was taken with trityl chloride (0.076 g, 0.273 mmol) in 2 ml dry methylene chloride and triethylamine (0.075 ml, 0.538 mmol) was added. The solution was stirred overnight and then washed with 10 % $NaHCO_3$ solution, water and then dried over $MgSO_4$. It was then filtered and the solvent removed under vacuum to give the product (0.135 g, quantitative); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.74 and 0.84 (two d, J = 7.0 Hz each, 3 H each, $CH(CH_3)_2$), 1.30 - 1.40 (m, 1 H, $CH(CH_3)_2$), 2.43 (d, J = 6.0 Hz, 1 H, NH), 2.93 (A of ABX, J = 14.0 Hz, J = 7.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.08 - 3.16 (m, 2 H, $CHCH_2C_6H_5$ and $CHCOOCH_3$), 3.70 (s, 3 H, $COOCH_3$), 4.44 (X of ABX, J = 12.0 Hz, J = 7.0 Hz, 1 H, $CHCH_2C_6H_5$), 7.00 -

7.40 (m, 15 H, $C(C_6\mathbf{H}_5)_3$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 16.85 and 20.20 ($CH(\mathbf{C}H_3)_2$), 33.24 ($\mathbf{C}H(CH_3)_2$), 38.45 ($CH\mathbf{C}H_2C_6H_5$), 52.83 ($COO\mathbf{C}H_3$), 60.99 (**C**H), 62.77 (**C**H), 71.76 ($\mathbf{C}(C_6H_5)_3$), 126.00 - 129.00 ($C(\mathbf{C}_6H_5)_3$), 136.06 ($\mathbf{C}_6H_5$), 145.61 ($\mathbf{C}_6H_5$), 171.23 (**C**ONH), 172.57 ($\mathbf{C}OOCH_3$).

N-(*tert*Butoxycarbonyl)-L-phenylalanyl-L-valine methyl ester : (6)



6

N-(*tet*Butoxycarbonyl)phenylalanine (2.078 g 7.832 mmol), valine methyl ester hydrochloride (1.419 g, 8.470 mmol), EDC (1.608 g, 8.388 mmol), HOBt (1.172 g, 8.676 mmol) and triethylamine (2.40 ml, 17.23 mmol) were dissolved in 30 ml dry CH_2Cl_2 at 0 °C and the solution stirred at 0 °C for 2 hours and then at room temperature for 24 hours. The solution was then washed with 10 % citric acid, 10 % $NaHCO_3$ and then with water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product as a white solid (2.726 g, 92 %); M. P. 117 - 120 °C; R_f 0.61 in 1 : 2 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 and 0.84 (two d, 6.9 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 1.38 (s, 9 H, $C(C\mathbf{H}_3)_3$), 2.00 - 2.10 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 3.00 - 3.10 (m, 2 H, $CHC\mathbf{H}_2C_6H_5$), 4.30 - 4.40 (m, 1 H, $C\mathbf{H}COOCH_3$), 4.40 - 4.50 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 5.09 (d, J = 7.9 Hz, 1 H, CONH), 6.45 (d, J = 8.4 Hz, COONH), 7.10 - 7.30 (m, 5 H, $C_6\mathbf{H}_5$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.83 and 18.88 ($CH(\mathbf{C}H_3)_2$), 28.30 ($C(\mathbf{C}H_3)_3$), 31.31 ($\mathbf{C}H(CH_3)_2$), 38.10 ($CH\mathbf{C}H_2C_6H_5$), 52.10 ($COO\mathbf{C}H_3$), 55.89 ($\mathbf{C}HCOOCH_3$), 57.30 ($\mathbf{C}HCH_2C_6H_5$), 80.24 ($\mathbf{C}(CH_3)_3$), 126.92 ($\mathbf{C}_6H_5$), 128.67 ($\mathbf{C}_6H_5$), 129.38 ($\mathbf{C}_6H_5$), 136.72 ($\mathbf{C}_6H_5$), 155.48 (**C**OONH), 171.27

(CONH), 171.84 ($COOCH_3$); MS (CI, NH_3) 379 (20 %, $(M + H)^+$), 279 (75.4 %, (M - *t*Boc)$^+$).

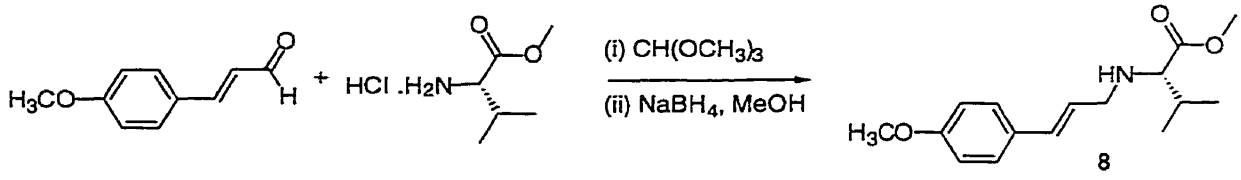
N-(4-Methoxycinnamyl)-L-valine methyl ester : (8)

Method A

Valine methyl ester hydrochloride (4.148 g, 24.744 mmol) was taken in 30 ml dry CH_2Cl_2 and 4-methoxycinnamaldehyde (3.995 g, 24.634 mmol) was added. The solution was heated and the CH_2Cl_2 distilled off slowly with the constant addition of fresh dry CH_2Cl_2. After 6 hours, the solution was cooled and $MgSO_4$ added. The suspension was stirred overnight, filtered and evaporated to yield the crude imine as an oil. The crude mixture was dried and then to its solution in 10 ml dry MeOH, $CeCl_3.7H_20$ (9.238 g, 24.796 mmol) and $NaCNBH_3$ (0.833 g, 13.265 mmol) were added in succession and the solution stirred at room temperature overnight. The solution was evaporated, CH_2Cl_2 and 10 % $NaHCO_3$ were added. The product was extracted in the organic layer, dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude which was chromatographed (3:1 Hexanes : Ethyl Acetate) to yield the product (3.755 g, 36.5%) as an oil. R_f 0.5 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.91 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 0.93 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 1.69 (br, 1 H, NH), 1.88 (septet, J = 6.6 Hz, 1 H, $CH(CH_3)_2$), 3.03 (d, J = 5.9 Hz, 1 H, NCHCO), 3.19 (A of ABX, J = 1.4 Hz, J = 6.4 Hz, J = 13.6 Hz, 1 H, $NHCH=CH_2$), 3.35 (B of ABX, J = 1.4 Hz J = 6.5 Hz, J = 13.6 Hz, 1 H, $NHCH=CH_2$), 3.65 (s, 3 H, $COOCH_3$), 3.77 (s, 3 H, OCH_3), 6.05 (X of ABX, J = 15.8 Hz, J = 6.4 Hz, 1 H, $NHCH=CH_2$), 6.41 (d, J = 15.8 Hz, 1 H, C_6H_4CH), 6.8 (d, J = 8.6 Hz, 2 H, C_6H_4), 7.25 (d, J = 8.6 Hz, 2 H, C_6H_4)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.57 and 18.99 ($CH(CH_3)_2$), 31.51 ($CH(CH_3)_2$), 50.69 (NCH_2), 51.25 ($CHCOOCH_3$), 55.07 ($C_6H_4OCH_3$), 66.28 ($CHCOOCH_3$), 113.74 ($C_6H_4CH=CH$), 125.78 (C_6H_4), 127.25 ($C_6H_4CH=CH_2$), 129.70 (C_6H_4), 130.91

(C_6H_4), 158.86 (C_6H_4), 175.62 (CO); MS (EI) m/z 277 (17.7 %, (M^+)), 218 (24.8 %, $(M - COOCH_3)^+$).

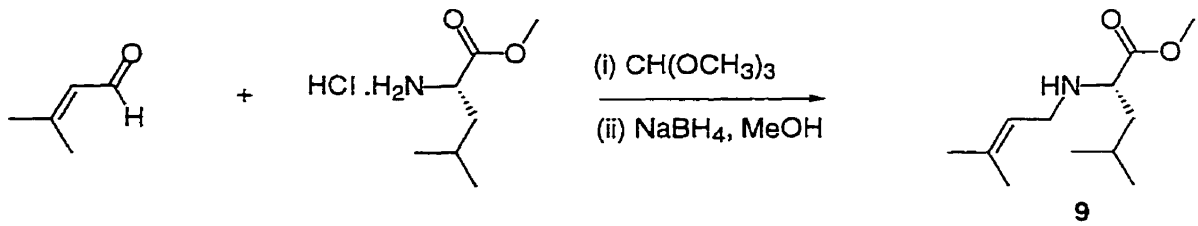
Method B



Valine methyl ester hydrochloride (0.371 g, 2.214 mmol) was taken in 5 ml dry trimethyl orthoformate and triethylamine (0.3 ml, 2.216 mmol) was added followed by 4-methoxycinnamaldehyde (0.360 g, 2.223 mmol). The solution was stirred for 18 hours at room temperature. The solution was then taken up in ethyl acetate and washed 3 times with water. The organic layers were collected, dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the imine as a pale yellow oil (0.564 g, 93 %). ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (d, J = 6.8 Hz, 3 H, $CH(CH_3)_2$), 0.87 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 2.24 (septet, J = 6.8 Hz, 1 H, $CH(CH_3)_2$), 3.46 (d, J = 7.1 Hz, 1 H, $CHCOOCH_3$), 3.67 (s, 3 H, $C_6H_4OCH_3$), 3.72 (s, 3 H, $COOCH_3$), 6.81 (d, J = 8.8 Hz, 2 H, C_6H_4), 6.84 (d, J = 6.8 Hz, 1 H, $CH=CHC_6H_4(OCH_3)$), 7.34 (d, J = 8.8 Hz, 2 H, C_6H_4), 7.86 (d, J = 7.8 Hz, 1H, N=CH=CH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.17 and 20.96 ($CH(CH_3)_2$), 33.33 ($CH(CH_3)_2$), 53.06 ($COOCH_3$), 56.40 ($C_6H_4OCH_3$), 81.28 ($NCHCOOCH_3$), 114.73 (C_6H_4), 125.29 (C_6H_4), 128.58 (C_6H_4), 129.06 ($CH=CHC_6H_4$), 142.85 ($CH=CHC_6H_4$), 160.55 (C_6H_4), 164.80 (N=CH), 172.23 ($COOCH_3$). The imine (0.457 g, 1.658 mmol) was dissolved in 8 ml freshly distilled methanol at 0 °C and $NaBH_4$ (0.030 g, 0.801 mmol) was added to it and stirred for 15 min. Methanol was evaporated in vacuum and ether was added. The ether layer was washed with 10 % $NaHCO_3$ and then with water. The organic layers were dried over

$MgSO_4$ and filtered. Solvent evaporation under vacuum gave a the product as a pale yellow oil (0.454 g, 99 %)

N-(Dimethyallyl)-L-leucine methyl ester : (9)



9

Leucine methyl ester hydrochloride (0.450 g, 2.477 mmol) was taken in 8 ml dry trimethyl orthoformate at room temperature and triethylamine (0.35 ml, 2.511 mmol) was added to it followed by 3-methyl-2-butenal (0.25 ml, 2.591 mmol) and stirred for 36 hours. Ether was added to the solution and it was washed 3 times with water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the imine as a pale yellow oil (0.487 g, 93 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.73 and 0.78 (two d, J = 6.7 Hz each, 3 H each, $CHCH_2CH(C\mathbf{H}_3)_2$), 1.38 (septet, J = 6.7 Hz, 1 H, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.58 - 1.63 (m, 2 H, $CHC\mathbf{H}_2CH(CH_3)$), 1.74 and 1.79 (two s, 3 H each, $(C\mathbf{H}_3)_2C=CH$), 3.57 (s, 3 H, $CHCOOC\mathbf{H}_3$), 3.72 - 3.77 (m, 1 H, $NCHCOOCH_3$), 5.93 (d, J = 9.4 Hz, 1 H, $(CH_3)_2C=C\mathbf{H}CH=N$), 8.03 (d, J = 9.4 Hz, 1 H, $(CH_3)_2C=CHC\mathbf{H}=N$); ^{13}C NMR (65 MHz, $CDCl_3$) δ 18.67 ($(C\mathbf{H}_3)_2C=CH$, Z - CH_3) 21.38 ($(C\mathbf{H}_3)_2C=CH$, E - CH_3), 23.03 and 24.25 ($(\mathbf{C}H_3)_2CH$), 26.57 ($(CH_3)_2\mathbf{C}H$), 42.31 ($\mathbf{C}H_2CH(CH_3)_2$), 51.94 ($COO\mathbf{C}H_3$), 71.75 ($\mathbf{C}HCOOCH_3$), 125.23 ($(CH_3)_2\mathbf{C}=CH$), 148.36 ($(\mathbf{C}H_3)_2C=CH$), 161.17 ($N=\mathbf{C}H$), 173.04 ($\mathbf{C}OOCH_3$). The imine (0.449 g, 2.124 mmol) was taken in 10 ml dry methanol at 0 °C and $NaBH_4$ (0.040 g, 1.057 mmol) was added to it. The solution was stirred at 0 °C for 15 min. and then solvent was evaporated in vacuum. Ether was added and washed 3 times with 10 % $NaHCO_3$ and once with water.

The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the product as a yellow oil (0.367 g, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 0.83 (J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 1.34 - 1.40 (m, 2 H, $CHCH_2(CH_3)_2$), 1.53 (s, 3 H, $CH=C(CH_3)_2$ Z - CH_3), 1.53 (s, 3 H, $CH=C(CH_3)_2$, E - CH_3), 1.57 - 1.67 (br m, 2 H, **NH** and $CH(CH_3)_2$), 3.22 (A of ABX, J = 12.5 Hz, J = 7.0 Hz, 1 H, $NHCH_2CH$), 3.09 (B of ABX, J = 12.5 Hz, J = 7.0 Hz, 1 H, $NHCH_2CH$), 3.19 (s, 3 H, $COOCH_3$), 5.10 - 5.20 (m, 1 H, $CH_2CH=C(CH_3)_2$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.75 ($(CH_3)_2C=CH$, Z - CH_3), 22.34 and 22.64 ($(CH_3)_2CH$), 24.91 ($(CH_3)_2C=CH$, E - CH_3), 25.70 ($CH(CH_3)_2$), 42.90 ($NHCH_2$), 45.57 ($CHCH_2CH(CH_3)_2$), 51.48 ($COOCH_3$), 59.30 ($CHCOOCH_3$), 122.51 ($(CH_3)_2C=CH$), 134.83 ($(CH_3)_2C=CH$), 176.61 ($COOCH_3$); MS (CI, NH_3) 214 (100 %, $(M + H)^+$).

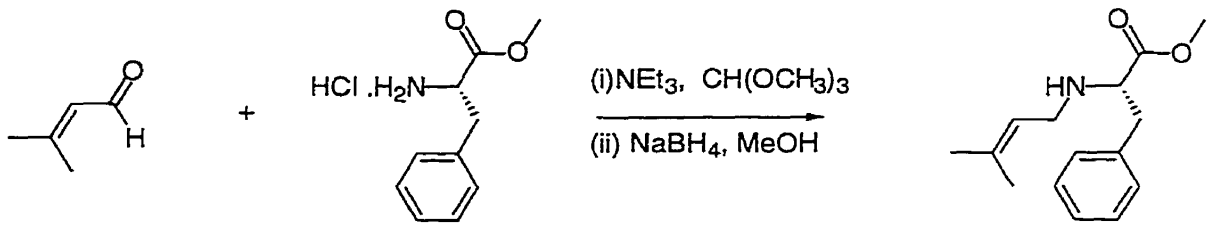
N-(Dimethylallyl)phenylalanine methyl ester : (10)

Method A

Phenylalanine methyl ester hydrochloride (2.01 g, 9.304 mmol) was added to Na_2CO_3 solution and the free amine was extracted with diethyl ether (1.463 g, 88 %). The free amine was dissolved in 10 ml dry trimethyl orthoformate and 3-methyl-2-butenal (0.8 ml, 8.29 mmol) was added to the solution which was then stirred at room temperature overnight. Ether was added to the solution and it was washed 3 times with water (30 ml each time). The ether layer was dried over $MgSO_4$ and filtered. Solvent evaporation led to the crude imine as a pale yellow oil 1.84 g (93 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.65 and 1.76 (two s, 3 H each, $CH=C(CH_3)_2$), 2.95 (A of ABX, J = 13.3 Hz, J = 8.9 Hz, 1 H, $CHCH_2C_6H_5$), 3.23 (B of ABX, J = 13.5 Hz, J = 5.2 Hz, 1 H, $CHCH_2C_6H_5$), 3.65 (s, 3 H, $COOCH_3$), 3.92 (X of ABX, J = 8.9 Hz, J = 5.2 Hz, 1 H, $CHCH_2C_6H_5$), 5.94 (d, J = 9.4 Hz, 1 H, $CH=C(CH_3)_2$), 7.05 - 7.25 (m, 5 H, C_6H_5), 7.66 (d, J = 9.4 Hz, 1 H, N=CH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.57 ($CH=C(CH_3)_2$, Z - CH_3), 26.66 ($CH=C(CH_3)_2$), E - CH_3), 40.06 ($CHCH_2C_6H_5$), 52.19 ($COOCH_3$), 75.19 (N=CH),

125.00 ($(CH_3)_2C$=**C**H), 126.55 (**C**$_6H_5$), 128.28 (**C**$_6H_5$), 129.77 (**C**$_6H_5$), 137.42 (**C**$_6H_5$), 148.77 ($(CH_3)_2$**C**=CH), 162.01 (N=**C**H), 172.40 (**C**OOCH$_3$); MS (EI) m/z 248 (100 %, $(M + H)^+$), 188 (22.8 %, $(M - COOCH_3)^+$), 156 (71.7 %, $(M - CH_2C_6H_5)^+$).

Method B



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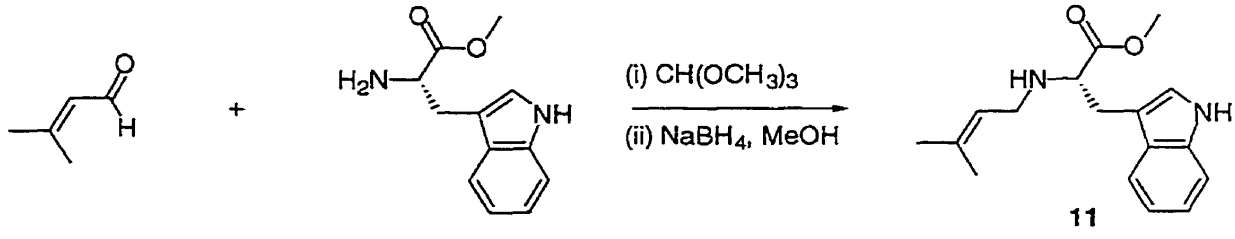
Phenylalanine methyl ester hydrochloride (0.502 g, 2.328 mmol) was taken in 6 ml dry trimethyl orthoformate and dry triethylamine (0.32 ml, 2.295 mmol) was added followed by 3-methyl-2-butenal (0.22 ml, 2.281 mmol). The solution was stirred for 24 hours at room temperature. The solution was then taken up in ethyl acetate and washed 3 times with H_2O. The organic layers were collected, dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the imine as a pale yellow oil (0.510 g, 90 %). This imine was taken in 10 ml dry methanol at 0 °C and $NaBH_4$ (0.041 g, 2 eq.) was added and the solution stirred for 30 mins. The solvent was evaporated under vacuum and ethyl ether added. The ether solution was washed with 10 % $NaHCO_3$, water and then dried over $MgSO_4$. Filtration and subsequent solvent evaporation under vacuum gave the product (0.468 g, 91 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.55 and 1.67 (two s, 3 H each, (CH_3)$_2$C=CH), 2.90 - 3.00 (m, 2 H, CHC**H**$_2C_6H_5$), 3.00 - 3.20 (m, 2 H, NC**H**$_2$), 3.45 - 3.50 (m, 1 H, C**H**COOCH$_3$), 3.61 (s, 3 H, COOC**H**$_3$), 5.10 - 5.20 (m, 1H, (CH_3)$_2$C=C**H**), 7.10 - 7.30 (m, 5 H, C$_6$**H**$_5$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.84 and 25.79 (CH=C(**C**H$_3$)$_2$), 39.84 (CH**C**H$_2$C$_6$H$_5$), 45.58 (N**C**H$_2$), 51.57 (COO**C**H$_3$), 62.37 (**C**HCOOCH$_3$), 122.37 ((CH_3)$_2$**C**=CH), 126.73 (**C**$_6H_5$), 128.46 (**C**$_6H_5$), 129.18 (**C**$_6H_5$),

135.05 ($(CH_3)_2$=**C**H), 137.35 ($\mathbf{C}_6H_5$), 175.17 ($\mathbf{C}OOCH_3$); MS (FAB, NBA) 248 (100 %, $(M + H)^+$).

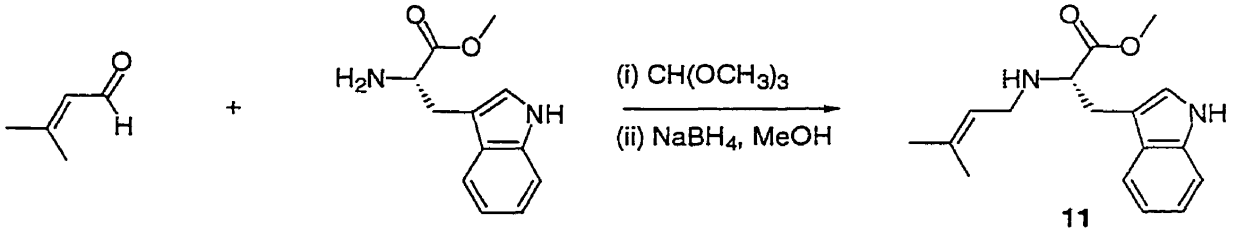
N-(Dimethylallyl)tryptophan methyl ester : (11)



Tryptophan methyl ester hydrochloride (2.039 g, 8.00 mmol) was added to Na_2CO_3 solution and the free amine was extracted quantitatively with diethyl ether. The free amine was dissolved in 10 ml dry trimethyl orthoformate and 3-methyl-2-butenal (0.8 ml, 8.29 mmol) was added to the solution which was then stirred at room temperature overnight. Ether was added to the solution and it was washed 3 times with water (30 ml each time). The ether layer was dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude imine as a pale yellow solid (2.04 g, 90 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.65 and 1.80 (two s, 3 H each, CH=C(C**H**$_3$)$_2$), 3.14 (A of ABX, J = 14.2 Hz, J = 8.5 Hz, 1 H, CHC**H**$_2$), 3.49 (B of ABX, J = 9.5 Hz, J = 5.5 Hz, 1 H, CHC**H**$_2$), 3.73 (s, 3 H, COOC**H**$_3$), 4.13 (X of ABX, J = 8.5 Hz, J = 5.0 Hz, 1 H, C**H**COOCH$_3$), 6.02 (d, 1 H, J = 9.5 Hz, C**H**=C(CH$_3$)$_2$), 6.95 (d, J = 2 Hz, 1 H, C=C**H**NH), 7.10 - 7.70 (m, 4 H, C_6**H**$_4$), 7.76 (d, J = 9.5 Hz, 1 H, N=C**H**), 8.4 (br s, 1H, N**H**); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.35 ((**C**H$_3$)$_2$C=CH), Z - CH_3), 26.45 ((**C**H$_3$)$_2$=CH), E - CH_3), 29.78 (CH**C**H$_2$), 52.03 (COO**C**H$_3$), 73.63 (**C**HCH$_2$), 110.99 ($\mathbf{C}_8H_5NH$), 111.01 ($\mathbf{C}_8H_5NH$), 118.63 ($\mathbf{C}_8H_5NH$), 119.13 ($\mathbf{C}_8H_5NH$), 121.70 ($\mathbf{C}_8H_5NH$), 123.41 ($\mathbf{C}_8H_5NH$), 124.79 ((CH$_3$)$_2$C=**C**H), 127.11 ($\mathbf{C}_8H_5NH$) 136.05 ($\mathbf{C}_8H_5NH$), 148.65 ((CH$_3$)$_2$**C**=CH), 161.71 (N=**C**H), 172.80 (**C**OOCH$_3$). The imine (0.110 g, 0.387 mmol) was taken in 3 ml dry methanol at 0 °C and $NaBH_4$ (0.010 g, 0.264) was added and the solution stirred for 10

minutes. Solvent was evaporated and ether added which was then washed with 10 % $NaHCO_3$, water and then dried over $MgSO_4$. Filtration and then solvent evaporation under vacuum gave the product as a pale yellow oil (0.110 g, 99 %). ^1H NMR (500 MHz, $CDCl_3$) δ 1.57 and 1.68 (two s, 3 H each, $(CH_3)_2C=CH$), 3.10 - 3.30 (m, 4 H, NCH_2 and $CHCH_2$-Indole), 3.65 (s, 3 H, $COOCH_3$), 3.90 - 3.94 (m, 1 H, $CHCOOCH_3$), 5.14 - 5.16 (m, 1 H, $(CH_3)_2C=CH$), 6.96 (d, J = 2.0 Hz, 1 H, $NHCH=C$), 7.12 (t, J = 8.0 Hz, 1 H, C_8H_5NH), 7.18 (t, J = 8.0 Hz, 1 H, C_8H_5NH), 7.30 (d, J = 8.0 Hz, 1 H, C_8H_5NH), 7.61 (d, J = 8.0 Hz, 1 H, C_8H_5NH), 8.65 (br s, 1 H, C_8H_5NH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.87 and 25.80 ($(CH_3)_2C=CH$), 29.49 ($CHCH_2$-Indole), 45.72 (NCH_2), 51.81 ($COOCH_3$), 61.51 ($CHCOOCH_3$), 110.91 (C_8H_5NH), 111.37 (C_8H_5NH), 118.75 (C_8H_5NH), 119.36 (C_8H_5NH), 121.99 (C_8H_5NH), 122.32 ($(CH_3)_2C=CH$), 123.17 (C_8H_5NH), 127.50 (C_8H_5NH), 135.22 ($(CH_3)_2C=CH$), 136.38 (C_8H_5NH), 175.64 ($COOCH_3$); MS (CI) 287 ($(M + H)^+$, 100 %), 156 (M - CH_2-Indole, 26.4 %).

N-(Carbobenzyloxy)-L-phenylalanyl-(N-4-methoxycinnamyl)-L-valine methyl ester : (12)



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Method A

N-(4-Methoxycinnamyl)valine methyl ester (0.193 g, 0.695 mmol) was taken in 0.6 ml dry THF at 0 °C and N-(Cbz)phenylalanine (0.204 g, 0.682 mmol) was added followed by HOBt (0.099 g, 0.731 mmol) and DCC (0.149 g, 0.731 mmol). The solution was stirred at 0 °C for 4 hours and then at room temperature for 3 days. Ice cold CH_2Cl_2 (10 ml) was added and the white residue (DCU) was filtered off. The filtrate was washed

with 5 % $KHSO_4$, 10 % $NaHCO_3$, H_2O, dried over $MgSO_4$ and filtered. The solvent was evaporated to yield crude product which was chromatographed (6:1 Hexanes : Ethyl acetate) to yield 0.017 g (5 %) of product as a pale yellow oil. R_f 0.15 in 4 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.76 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.95 (d, J = 6.4 Hz, 3 H, $CH(CH_3)_2$), 2.23 (septet, J = 6.4 Hz, 1 H, $CH(CH_3)_2$), 2.89 (A of ABX, J = 7.2 Hz, J = 13.4 Hz, 1 H, $CH_2C_6H_5$)), 3.07 (B of ABX, J = 7.2 Hz, J = 13.3 Hz, 1 H, $CH_2C_6H_5$), 3.56 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3)), 3.86 (A of ABX, J = 4.0 Hz, J = 17.4 Hz, 1 H), 4.40 (B of ABX, J = 6.8 Hz, J = 17.4 Hz, 1 H, $CH=CHCH_2$), 4.81 (d, J = 10.6 Hz, 1 H, $CHCOOCH_3$), 4.93 (m, 1 H, $CHCH_2C_6H_5$), 5.03 (s, 2 H,$COOCH_2C_6H_5$), 5.65 (m, 1 H, $CH=CHCH_2$), 6.37 (d, J = 16.1 Hz, 1 H, $CH=CHCH_2$), 6.82 (d, J = 8.7 Hz, 2 H C_6H_4), 7.30 - 7.16 (m, 12 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.52 and 20.02 ($CH(CH_3)_2$), 27.67 ($CH(CH_3)_2$), 39.41 ($CHCH_2C_6H_5$), 47.06 ($NCH_2CH=CH$), 51.90 (COONHCH), 52.54 ($COOCH_3$), 55.36 ($C_6H_4OCH_3$), 61.68 ($CHCOOCH_3$), 66.92 ($COOCH_2C_6H_5$), 114.02 ($C_6H_4CH=CH$), 122.94 (C_6H_4), 126.83 (C_6H_5), 127.88 (C_6H_5), 128.49 (C_6H_4), 129.67 (C_6H_5), 132.05 (C_6H_5), 136.35 (C_6H_5), 155.84 (NHCOO), 159.52 (C_6H_4), 171.08 (CON), 173.38($COOCH_3$); MS (FAB, NBA) m/z 559 ($M+H^+$).

Method B

N-(4-Methoxycinnamyl)valine methyl ester (0.323 g, 1.164 mmol) was taken in 0.6 ml dry DMF at 0 °C and N-(Cbz)phenylalanine (0.318 g, 1.0617 mmol) was added followed by PyBrOP (0.495 g, 1.061 mmol), DMAP (0.082 g, 0.0667 mmol) and DIEA (0.4 ml, 2.276 mmol). The solution was stirred at 0 °C for 15 min. and then at room temperature for 2 days. The solution was diluted with CH_2Cl_2 and washed with 5% $KHSO_4$, 10% $NaHCO_3$, H_2O, dried over $MgSO_4$ and filtered. The solvent was evaporated to yield crude product which was chromatographed (6:1 Hexanes : Ethyl Acetate) to yield 0.223 g (16 %) of product as a pale yellow oil.

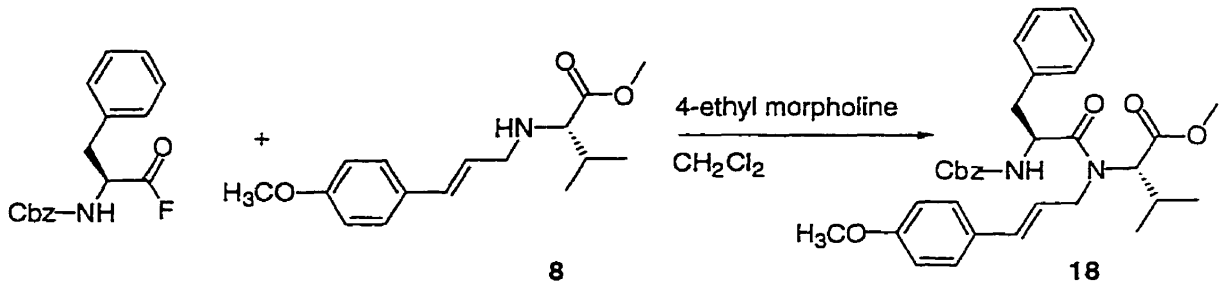
Method C

N-(4-Methoxycinnamyl)valine methyl ester (0.244 g, 0.880 mmol) was taken in 1 ml dry CH_2Cl_2 at 0 °C and N-(Cbz)phenylalanine (0.241 g, 0.806 mmol) was added followed by HOBt (0.145 g, 1.074 mmol) and DIC (0.125 ml, 0.798 mmol). The solution was stirred at 0 °C for 4 hours and then at room temperature for 2 days. The solution was diluted with CH_2Cl_2 and washed with 5 % $KHSO_4$, 10 % $NaHCO_3$, H_2O, dried over $MgSO_4$ and filtered. The solvent was evaporated to yield crude product which was chromatographed (6:1 Hexanes : Ethyl acetate) to yield 0.257 g (57 %) of product as a pale yellow oil.

Method D

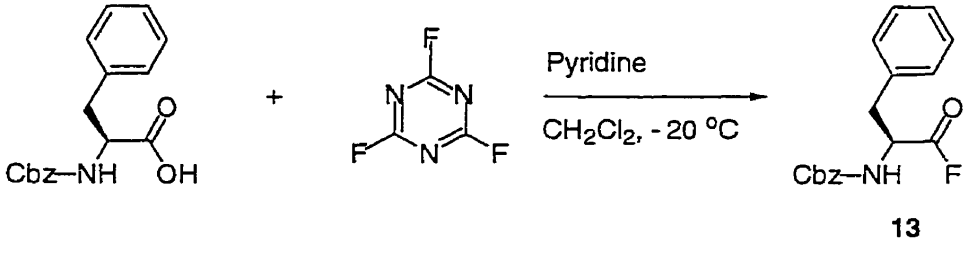
N-(4-Methoxycinnamyl)valine methyl ester (0.255 g, 0.920 mmol) was dissolved in 1 ml dry DMF and N-(Cbz)phenylalanine (0.256 g, 0.856 mmol) was added to it followed by HATU (0.315 g, 0.829 mmol) and DIPEA (0.29 ml, 1.665 mmol) and the solution was stirred at room temperature for 2 days and at 50 ^{0}C for a day. The solution was taken in 20 ml CH_2Cl_2 and washed successively with 10 % $NaHCO_3$, 5 % $KHSO_4$ and H_2O (3 times each),dried over $MgSO_4$ and filtered. Solvent evaporation gave crude product which was chromatographed (6 : 1 Hexanes : Ethyl acetate) to yield 0.195 g (38 %) product as a pale yellow oil.

Method E



N-(4-Methoxycinnamyl)valine methyl ester (0.065 g, 0.235 mmol) was dissolved in 0.4 ml dry CH_2Cl_2 and 0.180 g N-(Cbz)phenylalanine fluoride (0.180 g, 0.597 mmol) was added to it at room temperature followed by 4-ethylmorpholine (0.030 ml, 0.236 mmol) at room temperature. The solution was stirred at room temperature for 24 hours and then washed 3 times each with 10 % citric acid, 10 % $NaHCO_3$, and water. The organic extracts were dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude product which was purified by column chromatography (2 : 1 Hexanes : Ethyl acetate) to yield the product (0.100 g, 77%).

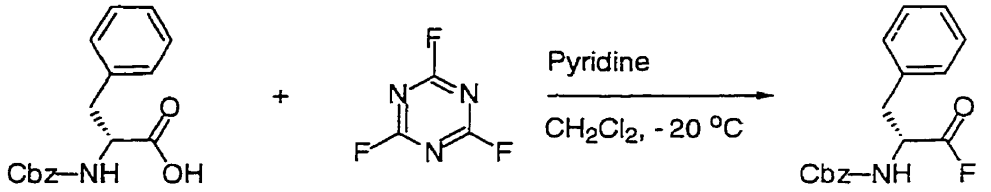
N-(Carbobenzyloxy)-L-phenylalanine fluoride : (13)



N-(Cbz)phenylalanine (0.179 g, 0.599 mmol) was dissolved in 2.5 ml of dry CH_2Cl_2 at -20 °C in a 10 ml flask under N_2. Pyridine (0.045 ml, 0.556 mmol) was added to the flask followed by cyanuric fluoride (0.1 ml, 1.165 mmol) and the flask was allowed to come to room temperature (2 hours). Ice was added to the flask and the organic layer extracted. The organic layer was washed 3 times with water, dried over $MgSO_4$ and

filtered. Solvent was evaporated under vacuum to yield the product as a colorless oil (0.183 g, quantitative) ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.10 - 3.20 (m, 2 H, $CHCH_2C_6H_5$), 4.80 - 4.90 (m, 1 H, $CHCH_2C_6H_5$), 5.10 (br s, 2 H, $NHCOOCH_2C_6H_5$), 7.10 - 7.40 (m, 10 H, two C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 36.7 ($CHCH_2C_6H_5$), 53.5 (d, J = 21.6 Hz, (CHC(O)F), 67.3 ($NHCOOCH_2C_6H_5$), 127.88 (C_6H_5), 128.26 (C_6H_5), 128.47 (C_6H_5), 128.66 (C_6H_5), 129.20 (C_6H_5), 133.9 (C_6H_5), 135.55 (C_6H_5), 155.3 (NHCOO), 161.6 (d, J = 346.3 Hz, (CHC(O)F).

N-(Carbobenzyloxy)-D-phenylalanine fluoride : (14)



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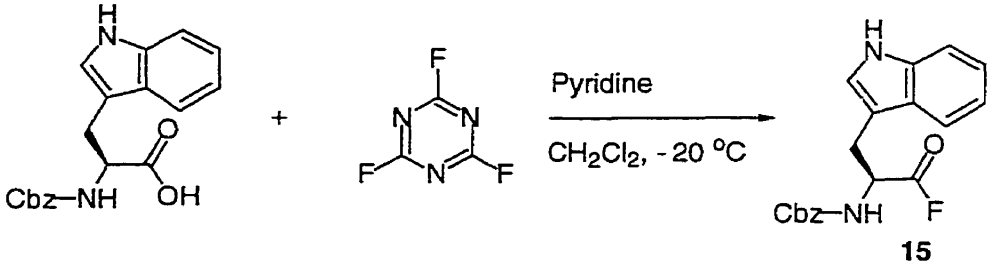
N-(Cbz)-D-phenylalanine (0.199 g, 0.665 mmol) was dissolved in 3.0 ml of dry CH_2Cl_2 at -20 °C in a 10 ml flask under N_2. Pyridine (0.060 ml, 0.741 mmol) was added to the flask followed by cyanuric fluoride (0.11 ml, 1.282 mmol) and the flask was allowed to come to room temperature (2 hours). Ice was added to the flask and the organic layer extracted. The organic layer was washed 3 times with water, dried over $MgSO_4$ and filtered. Solvent was evaporated under vacuum to yield the product as a white solid (0.199 g, quantitative). M.P. 82 - 83 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.15 - 3.20 (m, 2 H, $CHCH_2C_6H_5$), 4.75 - 4.90 (m, 1 H, $CHCH_2C_6H_5$), 5.10 (br s, 2 H, $NHCOOCH_2C_6H_5$), 7.10 - 7.40 (m, 10 H, two C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 36.95 ($CHCH_2C_6H_5$), 53.82 (d, J = 60.2 Hz, (CHC(O)F), 67.57 ($NHCOOCH_2C_6H_5$), 128.89 (C_6H_5), 127.27

(C_6H_5), 128.48 (C_6H_5), 128.67 (C_6H_5), 129.16 (C_6H_5), 129.25 (C_6H_5), 134.21 (C_6H_5), 135.83 (C_6H_5), 155.57 (NHCOO), 161.88 (d, J = 367.9 Hz, (CHC(O)F).

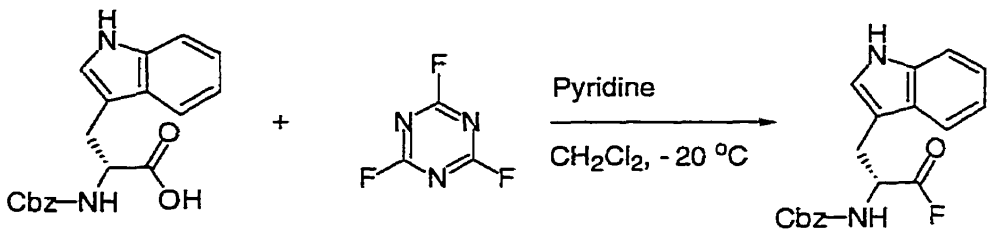
N-(Carbobenzyloxy)-L-tryptophan fluoride : (15)



15

N-(Cbz)tryptophan (0.501 g, 1.480 mmol) was dissolved in 5 ml of dry CH_2Cl_2 and 0.5 ml dry DMF at -20 °C in a 10 ml flask under N_2. Pyridine (0.120 ml, 1.483 mmol) was added to the flask followed by cyanuric fluoride (0.25 ml, 2.914 mmol) and the flask was allowed to come to room temperature (2 hours). Ice was added to the flask and the organic layer extracted. The organic layer was washed 3 times with water, dried over $MgSO_4$ and filtered. The solvent was evaporated under vacuum to yield the product as a colorless oil (0.500 g, 99 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.36 (d, J = 5.2 Hz, 2 H, CHC**H**$_2$), 4.80 - 4.90 (m, 1 H, C**H**CH$_2$), 5.10 (s, 2 H, NHCOOC**H**$_2$C$_6$H$_5$), 5.35 (d, J = 7.9 Hz, 1 H, N**H**CO), 6.98 (br s, 1 H, C$_8$**H**$_5$NH)), 7.10 - 7.40 (m, 9 H, C$_6$**H**$_5$ and C$_8$**H**$_5$NH), 8.60 (br s, 1 H, C$_8$H$_5$N**H**); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 26.89 (CH**C**H$_2$), 53.92 (d, J = 59.05 Hz, **C**HCOF), 67.47 (NHCOO**C**H$_2$), 108.41 (**C**$_8$H$_5$NH), 111.63, 118.34 (**C**$_8$H$_5$NH), 120.17 (**C**$_8$H$_5$NH), 122.65 (**C**$_8$H$_5$NH), 123.45 (**C**$_8$H$_5$NH), 127.27 (**C**$_8$H$_5$NH), 128.25 (**C**$_6$H$_5$), 128.44 (**C**$_6$H$_5$), 128.67 (**C**$_6$H$_5$), 135.97 (**C**$_8$H$_5$NH), 136.35 (**C**$_6$H$_5$), 155.86 (NHCOO), 162.37 (d, J = 370.35 Hz, COF).

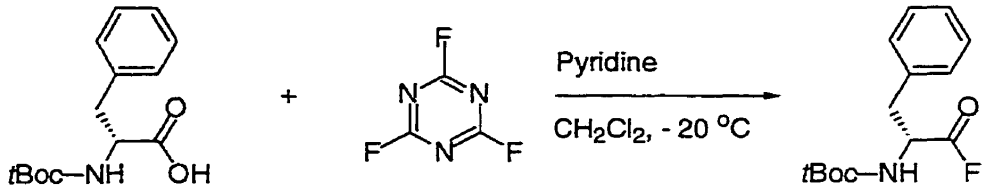
N-(Carbobenzyloxy)-D-tryptophan fluoride : (16)



16

N-(Cbz)-D-tryptophan (0.198 g, 0.587 mmol) was dissolved in 2.5 ml of dry CH_2Cl_2 and 0.3 ml dry DMF at -20 °C in a 10 ml flask under N_2. Pyridine (0.052 ml, 0.643 mmol) was added to the flask followed by cyanuric fluoride (0.100 ml, 1.165 mmol) and the flask was allowed to come to room temperature (2 hours). Ice was added to the flask and the organic layer extracted. The organic layer was washed 3 times with water, dried over $MgSO_4$ and filtered. Solvent was evaporated under vacuum to yield the product as a colorless oil (0.213 g, 99 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.36 (d, J = 5.7 Hz, 2 H, CHC**H**$_2$), 4.80 - 4.95 (m, 1 H, C**H**CH$_2$), 5.08 - 5.12 (m, 2 H, NHCOOC**H**$_2$C$_6$H$_5$), 5.33 (d, J = 8.1 Hz, 1 H, N**H**CO), 6.98 (d, J = 2.2 Hz, 1 H, C$_8$**H**$_5$NH)), 7.10 - 7.40 (m, 9 H, C$_6$**H**$_5$ and C$_8$**H**$_5$NH), 8.32 (br s, 1 H, C$_8$H$_5$N**H**); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 26.88 (CH**C**H$_2$), 53.92 (d, J = 58.5 Hz, **C**HCOF), 67.47 (NHCOO**C**H$_2$), 108.44 (**C**$_8$H$_5$NH), 111.61 (**C**$_8$H$_5$NH), 118.35 (**C**$_8$H$_5$NH), 120.19 (**C**$_8$H$_5$NH), 122.67 (**C**$_8$H$_5$NH), 123.40 (**C**$_8$H$_5$NH), 127.27 (**C**$_6$H$_5$), 128.25 (**C**$_8$H$_5$NH), 128.44 (**C**$_6$H$_5$), 128.67 (**C**$_6$H$_5$), 135.97 (**C**$_8$H$_5$NH), 136.34 (**C**$_6$H$_5$), 155.84 (NHCOO), 162.37 (d, J = 367.4 Hz, **C**OF).

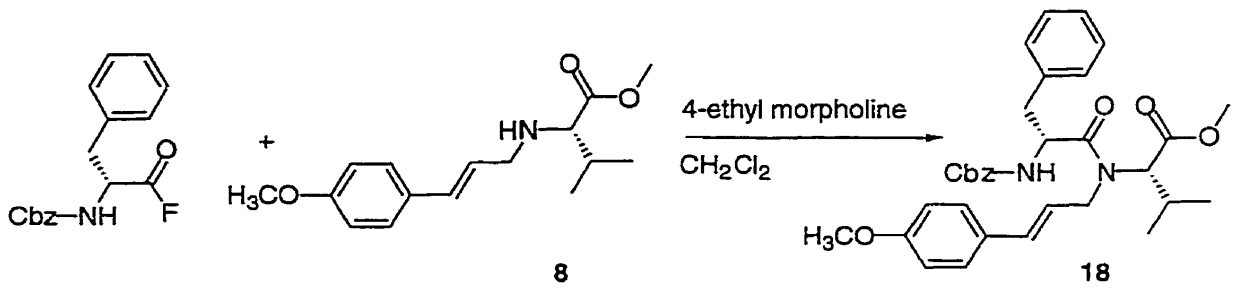
N-(*tert*Butoxycarbonyl)-L-phenylalanine fluoride : (17)



17

N-(*tert*Butoxycarbonyl)phenylalanine (0.249 g, 0.938 mmol) was dissolved in 5 ml of dry CH_2Cl_2 at -20 °C in a 10 ml flask under N_2. Pyridine (0.09 ml, 1.112 mmol) was added to the flask followed by cyanuric fluoride (0.16 ml, 1.864 mmol) and the flask was allowed to come to room temperature over a period of 2 hours. Ice was added to the flask and the organic layer extracted. The organic layer was washed 3 times with water, dried over $MgSO_4$ and filtered. Solvent was evaporated under vacuum to yield the product as a white solid (0.230 g, 92 %). M.P. 66 - 67 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 9 H, C(CH_3)$_3$), 3.10 - 3.20 (m, 2 H, CH$CH_2C_6H_5$), 4.70 - 4.80 (m, 1 H, C$HCH_2C_6H_5$), 4.80 - 4.90 (d, J = 6.3 Hz, 1 H, N$HCOOC(CH_3)_3$), 7.10 - 7.40 (m, 5 H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.69 C(CH_3)$_3$), 55.66 (d, J = 58.7 Hz, NHCHCOF)), 59 94 ($CHCH_2C_6H_5$), 81.73 (NHCOOC(CH_3)$_3$), 127.98 (C_6H_5), 129.30 (C_6H_5), 129.48 (C_6H_5), 134.76 (C_6H_5), 154.93 (NHCOO), 162.11 (d, J = 364.6 Hz, C(O)F).

N-(Carbobenzyloxy)-D-phenylalanyl-(N-4-methoxycinnamyl)valine methyl ester : (18)

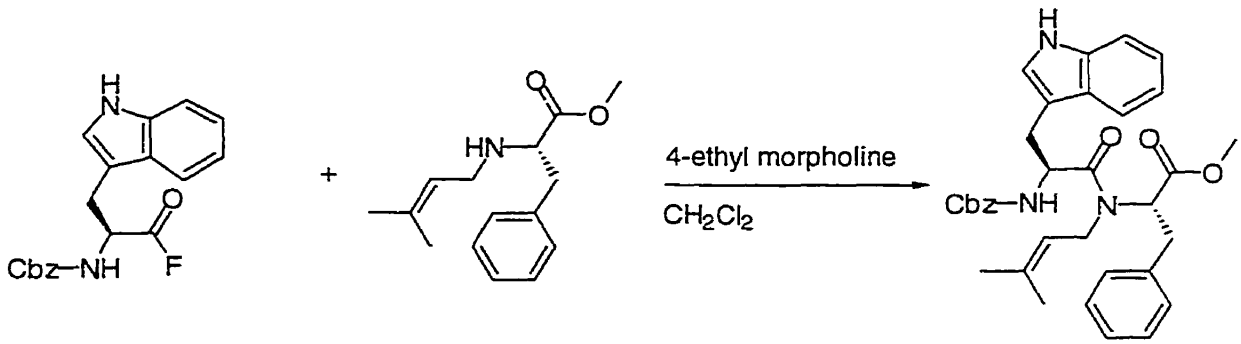


8 18

N-(4-Methoxycinnamyl)valine methyl ester (0.065 g, 0.236 mmol) was dissolved in 0.3 ml dry CH_2Cl_2 and N-(Cbz)-D-phenylalanine fluoride (0.190 g, 0.630 mmol) was added to it at room temperature followed by 4-ethylmorpholine (0.035 ml, 0.275 mmol). The solution was stirred at room temperature for 36 hours and then washed 3 times each with 10 % citric acid, 10 % $NaHCO_3$, and water. The organic extracts were dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude product which was purified by column chromatography (Hexanes : Ethyl acetate - 2: 1) to yield the product (0.079 g, 60%). R_f 0.18 in 4 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.69 and 0.93 (two d, J = 6.5 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 2.25 - 2.35 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.76 (A of ABX, J = 9.0 Hz, J = 13.5 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 2.91 (B of ABX, J = 5.5 Hz, J = 13.5 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.46 (s, 3 H, $OC\mathbf{H}_3$), 3.73 (s, 3 H, $CHCOOC\mathbf{H}_3$),4.10 - 4.15 (m, 1 H, $NC\mathbf{H}_2CH=CH$), 4.18 (d, J = 5.0 Hz, 1 H, $\mathbf{CH}COOCH_3$), 4.30 - 4.35 (m, 1 H, $NC\mathbf{H}_2CH=CH$), 4.65 - 4.70 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 4.94 - 4.96 (m, 2 H, $NHCOOC\mathbf{H}_2$), 6.00 (dt, J = 16.4 Hz, J = 6.0 Hz, 1 H, $NCH_2C\mathbf{H}=CH$), 6.50 (d, J = 16.8 Hz, 1 H, $NCH_2CH=C\mathbf{H}$), 6.88 (d, J = 9.0 Hz, 2 H, $C_6\mathbf{H}_4$), 7.10 - 7.30 (m, 12 H, $C_6\mathbf{H}_5$), 7.87 (d, J = 4.2 Hz, 1 H, $N\mathbf{H}COO$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.96 and 20.29 ($CH(\mathbf{C}H_3)_2$), 27.50 ($\mathbf{C}H(CH_3)_2$), 39.98 ($\mathbf{C}H_2C_6H_5$), 47.92 ($N\mathbf{C}H_2$), 52.28 ($COO\mathbf{C}H_3$), 55.35 ($COO\mathbf{C}H_2C_6H_5$), 62.45 (**C**H), 66.83 (**C**H), 114.04 ($NCH_2\mathbf{C}H$), 122.20 ($\mathbf{C}_6H_4$), 126.96 ($\mathbf{C}_6H_4$), 127 – 130 ($\mathbf{C}_6H_5$ and $\mathbf{C}_6H_4$), 132.40 ($\mathbf{C}H_2\mathbf{C}_6H_4$)), 136.45 ($\mathbf{C}_6H_5$), 155.57 ($\mathbf{C}_6H_4$), 159.52 ($NH\mathbf{C}OOCH_2$),

170.94 (**C**ON), 172.92 (**C**OOCH$_3$); MS (FAB, NBA) m/z 559 (9.1 %, (M + H)$^+$); 424 (1.6 %, (M – C_6H_5OCO)$^+$).

N-(Carbobenzyloxy)-L-tryptophanyl-N-(dimethylallyl)-L-phenylalanine methyl ester : (19)

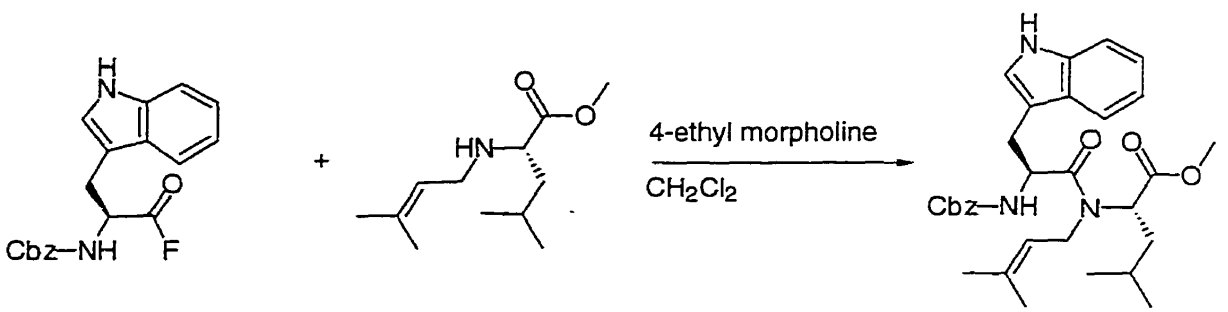


15 **10** **19**

N-(Dimethylallyl)phenylalanine methyl ester (1.634 g, 6.615 mmol) was taken in 7 ml dry methylene chloride with 0.5 ml DMF and N-(Cbz)tryptophan fluoride (7.238 g, 21.270 mmol) was added followed by 4-ethylmorpholine (0.9 ml, 7.071 mmol). The solution was stirred at room temperature for 12 hours and then diluted with methylene chloride. The solution was washed with 1 N HCl, 10 % $NaHCO_3$, water and then dried over $MgSO_4$. It was then filtered and solvent evaporation gave the crude which was chromatographed (2 : 1 Hexanes : Ethyl acetate) to give the product (2.646 g, 71 %). (R_f (0.22 in 1 : 1 Hexanes : Ethyl acetate); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30- 1.50 (m, 6 H, CH=C(C**H**$_3$)$_2$), 2.80 – 3.25 (m, 4 H, NC**H**$_2$-Indole, NC**H**$_2$CH), 3.55 (s, 3 H, COOC**H**$_3$), 3.60 – 3.80 (m, 2 H, CHC**H**$_2$C$_6$H$_5$), 4.30 – 4.40 (m, 1 H, C**H**CH$_2$-Indole), 4.50 – 4.60 (m, 1 H, C**H**CH$_2$C$_6$H$_5$), 4.60 – 4.70 (m, 1 H, NCH$_2$C**H**=C(CH$_3$)$_2$), 4.90 – 5.00 (m, 2 H, NHCOOC**H**$_2$), 6.90 – 7.50 (m, 16 H, two C$_6$**H**$_5$, C$_8$**H**$_5$N**H**), 10.50 – 10.60 (m, 1 H, CON**H**); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.72 and 25.88

($NCH_2CH=C(CH_3)_2$), 29.43 ($CHCH_2C_6H_5$), 35.10 (CH_2-Indole), 46.80 (NCH_2), 51.49 ($NHCOOCH_2$), 52.26 ($COOCH_3$), 60.97 ($CHCH_2$), 66.81 ($CHCH_2$), 111.23 (C_8H_5NH), 118.63 (C_8H_5NH), 119.75 (C_8H_5NH), 122.08 (C_8H_5NH), 123.28 (C_8H_5NH), 126.63 (C_6H_5), 127.97 (C_6H_5), 128.44 (C_8H_5NH), 128.56 (C_6H_5), 129.28 (C_6H_5), 136.15 (C_8H_5NH), 136.53 (C_6H_5), 138.06 (C_6H_5), 155.75 (COONH), 171.00 (CONH), 172.08 ($COOCH_3$); MS (FAB. NBA) m/z 568 (13.4 %, $(M + H)^+$), 500 (5.9 %, $(M - prenyl)^+$), 439 (8.2 %, (M - CH_2-Indole).

N-(Carbobenzyloxy)-L-tryptophanyl-N-(dimethylallyl)-L-leucine methyl ester : (20)

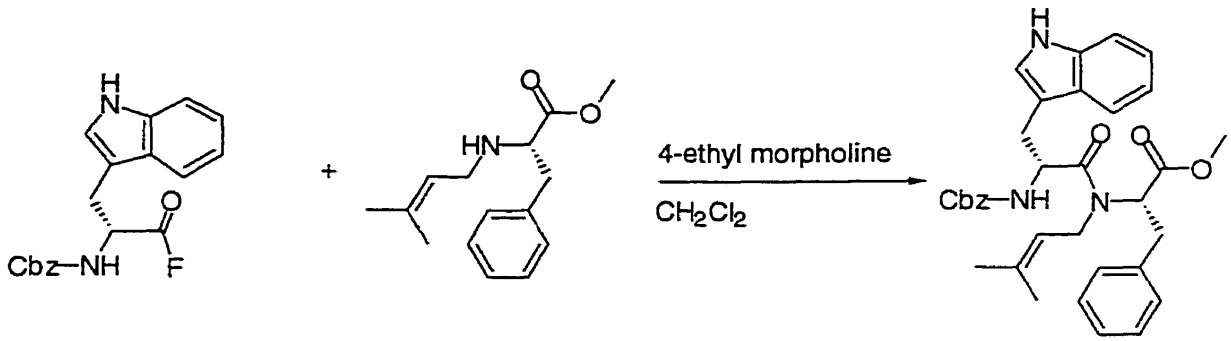


15 9 20

N-(Dimethylallyl)leucine methyl ester (0.339 g, 1.590 mmol) was dissolved in 1.5 ml dry CH_2Cl_2 and 1.461 g N-(Cbz)phenylalanine fluoride (4.291 mmol) was added to it at room temperature followed by 4-ethylmorpholine (0.25 ml, 1.965 mmol). The solution was stirred at room temperature for 36 hours and then washed 3 times each with 10 % citric acid, 10 % $NaHCO_3$, and water. The organic extracts were dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude product which was purified by column chromatography (Hexanes : Ethyl acetate - 2 : 1) to yield the product (0.563 g, 66.4 %). R_f 0.18 in 2:1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, DMSO-d_6) δ 0.77 and 0.78 (two d, J = 4.0 Hz each , 3 H each, $CH(CH_3)_2$), 1.30 - 1.60 (m, 2 H, $CH_2CH(CH_3)_2$), 1.43

and 1.47 (two s, 3 H each, ((CH_3)$_2$)C=CH), 1.50 - 1.60 (m, 1 H, C**H**(CH_3)$_2$), 2.92 (A of ABX, J = 13.5 Hz, J = 7.5 Hz, 1 H, CHC**H**$_2$-Indole), 3.05 (B of ABX, J = 14.0 Hz, J = 6.5 Hz, 1 H, CHC**H**$_2$-Indole), 3.51 (s, 3 H, COOC**H**$_3$), 3.70 (A of ABX, J = 17.0 Hz, J = 3.5 Hz, 1 H, NC**H**$_2$CH=C), 4.08 (B of ABX, J = 17.5 Hz, J = 6.5 Hz, 1 H, NC**H**$_2$CH=C), 4.62 (X of ABX, J = 15.0 Hz, J = 8.0 Hz, 1 H, C**H**CH$_2$-Indole), 4.75 - 4.80 (m, 2 H, C**H**COOCH$_3$ and NCH$_2$C**H**=C), 4.90 - 5.10 (m, 3 H, N**H**COOC**H**$_2$C$_6$H$_5$), 6.95 (t, J = 7.5 Hz, 1 H, C$_8$**H**$_5$NH), 7.00 - 7.10 (m, 2 H, C$_8$**H**$_5$NH), 7.20 - 7.40 (m, 5 H, C$_6$**H**$_5$), 7.50 (d, J = 7.5 Hz, 2 H, C$_8$**H**$_5$NH), 7.75 (d, J = 8.5 Hz, 2 H, C$_8$**H**$_5$NH), 10.86 (s, 1 H, C$_8$H$_5$N**H**); ^{13}C NMR (67.5 MHz, $CDCl_3$) 17.92 ((CH_3)$_2$C=CH), 21.97 and 22.99 (CH$_2$CH(**C**H$_3$)$_2$), 24.85 ((CH_3)$_2$**C**=CH), 25.62 (**C**H$_2$CH(CH$_3$)$_2$), 29.41 (CH$_2$**C**H(CH$_3$)$_2$), 37.91 (CH**C**H$_2$-Indole), 44.46 (N**C**H$_2$CH=C), 51.78 (**C**H$_2$C$_6$H$_5$), 52.09 (COO**C**H$_3$), 55.78 (**C**HCOOCH$_3$), 66.77 (**C**HCH$_2$-Indole), 110.22 (**C**$_8$H$_5$NH), 111.25 (**C**$_8$H$_5$NH), 118.78 (**C**$_8$H$_5$NH), 119.63 (**C**$_8$H$_5$NH), 120.62 (**C**$_8$H$_5$NH), 122.06 ((CH_3)$_2$**C**=CH), 123.35 (**C**$_8$H$_5$NH), 127.77 (**C**$_6$H$_5$), 127.98 (**C**$_8$H$_5$NH), 128.10 (**C**$_6$H$_5$), 128.55 (**C**$_6$H$_5$), 135.25 ((CH_3)$_2$C=**C**H), 136.54 (**C**$_8$H$_5$NH), 155.78 (NH**C**OOCH$_2$), 172.16 (N**C**O), 172.99 (**C**OOCH$_3$); MS (FAB, NBA) 534 (64.9 %, (M + H)$^+$), 466 (37.2 %, (M - prenyl)).

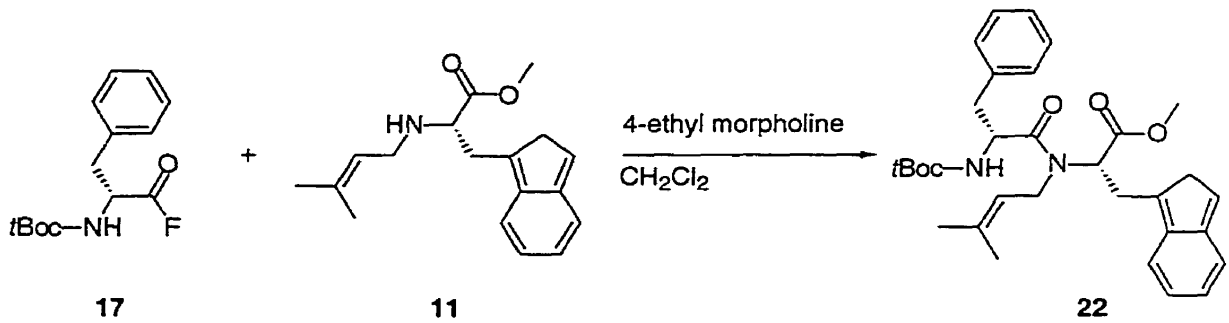
N-(Carbobenzyloxy)-D-tryptophanyl-N-(dimethylallyl)-L-phenylalanine methyl ester : (21)



16 **10** **21**

N-(Dimethylallyl)phenylalanine methyl ester (0.046 g, 0.186 mmol) was dissolved in 0.3 ml dry CH_2Cl_2 and N-(Cbz)-D-tryptophan fluoride (0.203 g, 0.596 mmol) was added to it at room temperature followed by 4-ethylmorpholine (0.026 ml, 0.204 mmol). The solution was stirred at room temperature for 36 hours and then washed 3 times each with 10 % citric acid, 10 % $NaHCO_3$ and water. The organic extracts were dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude product which was purified by column chromatography (Hexanes : Ethyl acetate - 1: 1) to yield the product (0.029 g, 28 %) R_f 0.38 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 and 1.38 (two s, 3 H each, C=C($\mathbf{CH_3}$)$_2$), 3.00 - 3.40 (m, 4 H, CH$\mathbf{CH_2}$-Indole and CH$\mathbf{CH_2}C_6H_5$), 3.45 - 3.55 (m, 1 H, N$\mathbf{CH_2}$), 3.67 (s, 3 H, COO$\mathbf{CH_3}$), 3.70 - 3.80 (m, 1 H, N$\mathbf{CH_2}$), 4.25 (dd, J = 9.0 Hz, J = 5.5 Hz, 1 H, $\mathbf{CH}$COOCH_3), 4.42 (dd, J = 10.0 Hz, J = 5.5 Hz, 1 H, $\mathbf{CH}$$CH_2$-Indole), 5.00 - 5.20 (m, 2 H, NHCOO$\mathbf{CH_2}C_6H_5$), 5.59 (d, J = 8.5 Hz, 1 H, N$\mathbf{H}$COO), 7.00 - 7.40 (m, 10 H, $C_6\mathbf{H_5}$ and $C_8\mathbf{H_5}$NH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.71 and 25.61 (($\mathbf{CH_3}$)$_2$C=CH), 29.42 (CH$\mathbf{CH_2}$), 35.05 (CHCH_2), 46.77 (N$\mathbf{CH_2}$), 51.48 (O$\mathbf{CH_2}C_6H_5$), 52.26 (COO$\mathbf{CH_3}$), 60.31 (**CH**), 66.79 (**CH**), 110.32 ($\mathbf{C_8H_5}$NH), 111.21 ($\mathbf{C_8H_5}$NH), 118.63 ($\mathbf{C_8H_5}$NH), 119.77 ($\mathbf{C_6H_5}$), 122.21 ($\mathbf{C_8H_5}$NH), 123.23 ($\mathbf{C_8H_5}$NH), 126.73 ($\mathbf{C_8H_5}$NH), 128.4 ($\mathbf{C_6H_5}$), 129.2 ($\mathbf{C_6H_5}$), 136.20 ($\mathbf{C_8H_5}$NH), 136.54 ($\mathbf{C_8H_5}$NH), 137.88 ($\mathbf{C_6H_5}$), 155.75 (**NCO**), 170.93 (NH**COO**), 172.57 (**COO**CH_3); MS (FAB, NBA) 568 (13.2 %, $(M+H)^+$), 439 (6.1 %, $(M-CH_2\text{-Indole})^+$).

N-(*tert*Butoxycarbonyl)-L-phenylalanyl-N-(dimethylallyl)-L-tryptophan methyl ester : (22)

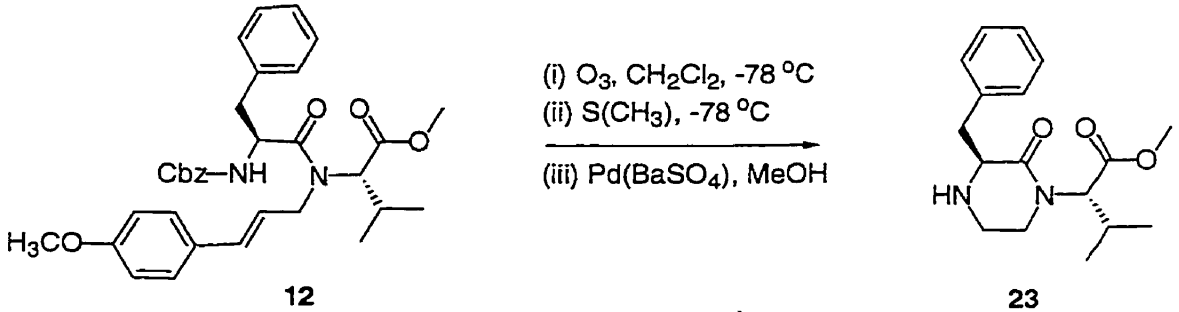


17 11 22

N-(Dimethylallyl)tryptophan methyl ester (0.098 g, 0.342 mmol) was dissolved in 0.3 ml dry CH_2Cl_2 and N-(Cbz)phenylalanine fluoride (0.217 g, 0.812 mmol) was added to it at room temperature followed by 4-ethylmorpholine (0.050 ml, 0.392 mmol). The solution was stirred at room temperature for 12 hours and then washed 3 times each with 10 % citric acid, 10 % $NaHCO_3$ and water. The organic extracts were dried over $MgSO_4$ and filtered. Solvent evaporation in vacuum gave the crude product which was purified by column chromatography (Hexanes : Ethyl acetate - 2 : 1) to yield the product (0.113 g, 62 %) R_f 0.32 in 2 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9 H, $C(\mathbf{CH_3})_3$), 1.42 and 1.47 (two s, 3 H each, $(\mathbf{CH_3})_2C$=CH), 2.89 (A of ABX, J = 13.0 Hz, J = 6.0 Hz, 1 H, $CHC\mathbf{H_2}C_6H_5$), 3.07 (B of ABX, J = 13.0 Hz, J = 8.0 Hz, 1 H, $CHC\mathbf{H_2}C_6H_5$), 3.17 (A of ABX, J = 15.0 Hz, J = 8.5 Hz, 1 H, $CHC\mathbf{H_2}$-Indole), 3.53 (B of ABX, J = 15.0 Hz, J = 6.0 Hz, 1 H, $CHC\mathbf{H_2}$-Indole), 3.60 - 3.70 (m, 2 H, $NC\mathbf{H_2}CH$=C), 3.67 (s, 3 H, $COOC\mathbf{H_3}$), 4.52 (t, J = 7.5 Hz, 1 H, $C\mathbf{H}CH_2$-Indole), 4.56 - 4.64 (m, 1 H, $NCH_2C\mathbf{H}$=C), 4.70 - 4.80 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 5.21 (d, J = 9.0 Hz, 1 H, N**H**COO), 6.71 (s, 1 H, N**H**C=C), 7.10 - 7.60 (m, 10 H, $C_6\mathbf{H_5}$ and $C_8\mathbf{H_5}$NH) 8.10 (br s, 1 H, $C_8H_5N\mathbf{H}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.22 and 26.36 ($(CH_3)_2C$=CH), 27.04 ($CHCH_2C_6H_5$), 29.78 ($C(\mathbf{CH_3})_3$), 41.07 ($CHCH_2$-Indole), 47.58 (NCH_2), 53.06 ($\mathbf{C}HCH_2C_6H_5$), 60.38 ($\mathbf{C}HCH_2$-Indole), 80.47 ($\mathbf{C}(CH_3)_3$), 111.68

(C_8H_5NH), 118.89 (C_8H_5NH), 119.82 (C_8H_5NH), 120.20 (C_8H_5NH), 122.34 ($(CH_3)_2C=CH$), 123.50 (C_8H_5NH), 126.99 (C_6H_5), 127.56 (C_6H_5), 128.70 (C_8H_5NH), 129.83 (C_6H_5), 130.00 (C_8H_5NH), 136.02 (C_8H_5NH), 136.37 (C_6H_5), 137.07 ($(CH_3)_2C=CH$), 154.91 (NHCOO), 170.96 (CON), 171.55 ($COOCH_3$); MS (FAB, NBA) 534 (34.6 %, $(M+H)^+$); 434 (26.5 %, $(M-t\text{BocNH})^+$).

Methyl (2S, 3'S)-2-[3'-benzyl-2'-oxopiperazin-1'-yl] -3-methyl butanoate : (23)

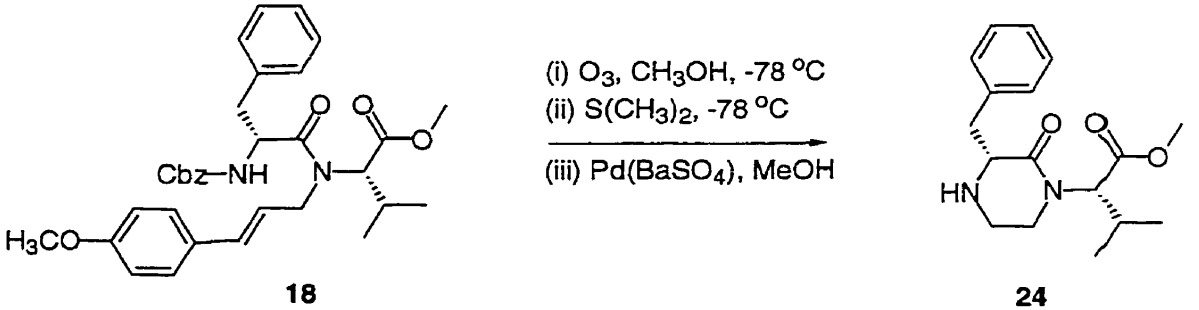


12 23

A solution of dipeptide **12** (0.175 g, 0.313 mmol) was taken in 30 ml dry CH_2Cl_2 at - 78 °C and O_3 was bubbled through the solution for 1.5 hours. Methyl sulfide (0.09 ml, 1.225 mmol) was then added to the solution at -78 °C and the solution allowed to come to room temperature and then stirred overnight. The solution was diluted with CH_2Cl_2 and washed with water, dried over $MgSO_4$, and filtered. Solvent evaporation gave the crude mixture of cyclized products that were purified by chromatography. A dry hydrogenation flask, flushed with N_2, was charged with 0.017 g Pd(C) (10%) and 5 ml ethyl acetate was added to it followed by a 5 ml solution of the mixture of cyclized products (0.095 g) and 1 ml glacial acetic acid. The solution was hydrogenated for 7 hr at 40 psi. The solution was filtered through a glass cinter to remove the catalyst and the filtrate was collected. The crude product was taken in $CHCl_3$ and washed with 10 % $NaHCO_3$ (3 times , 30 ml each time). The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum

gave the crude product which was chromatographed (1: 1 Hexanes : Ethyl acetate) to yield the product as a pale yellow oil (0.645 g, 95 %). R_f 0.22 in 3 : 1 : 0.5 Hexanes : Ethyl acetate : Methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 0.96 (d, J = 6.4 Hz, 3 H, $CH(CH_3)_2$), 1.93 (br, 1 H, NH), 2.10 - 2.20 (m, 1 H, $CH(CH_3)_2$), 2.85 - 2.90 (m, 1 H, $CHCH_2C_6H_5$), 2.90 - 3.00 (m, 1 H, $NHCH_2CH_2N$), 3.00 - 3.10 (m, 1 H, $NHCH_2CH_2N$), 3.20 - 3.30 (m, 1 H, $NHCH_2CH_2N$), 3.35 (dd, J = 3.5 Hz, J = 13.5 Hz, 1 H, $CHCH_2C_6H_5$), 3.40 - 3.46 (m, 1 H, $NHCH_2CH_2N$), 3.69 (s, 3 H, $COOCH_3$), 3.70 - 3.72 (m, 1 H, $CHCH_2C_6H_5$), 4.92 (d, J = 10.8 Hz, 1 H, $CHCH(CH_3)_2$), 7.20 - 7.40 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 19.14 and 19.73 ($CHCH_3)_2$), 26.90 $CH(CH_3)_2$, 38.59 ($CH_2C_6H_5$), 42.21 and 44.50 ($NHCH_2CH_2N$), 51.89 ($COOCH_3$), 61.02 ($CHCH(CH_3)_2$), 61.11 ($CHCH_2C_6H_5$), 126.75 (C_6H_5), 128.68 (C_6H_5), 129.57 (C_6H_5), 138.19 (C_6H_5), 169.91 (NCO), 171.62 ($COOCH_3$); HRMS (FAB, NBA) Expected for $(C_{17}H_{24}N_2O_3 + H)^+$ 305.18652, Found 305.18652.

Methyl(2S, 3'R)-2-[3'benzyl-2'oxopiperazin-1'yl]-3-methyl butanoate : (24)

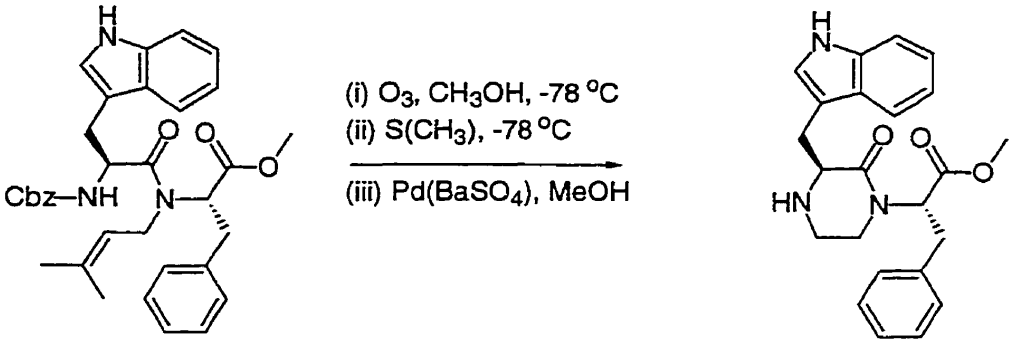


18 **24**

A solution of **18** (0.080 g, 0.143 mmol) was taken in 20 ml dry methanol at -78 ^{0}C and O_3 was bubbled for 2 hours. Dimethyl sulfide (0.90 ml) was then added and the solution allowed to come to room temperature and stirred for 12 hours. The solution was

washed with water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude that was purified by chromatography. A dry hydrogenation flask flushed with N_2 was charged with 0.040 g $Pd(BaSO_4)$ and 5 ml methanol was added to it followed by a 5 ml solution of the mixture of cyclized products (0.040 g). The solution was hydrogenated for 7 hr at 40 psi. The solution was filtered through a glass cinter to remove the catalyst and the filtrate was collected Evaporation of the solvent gave the crude product which was chromatographed (5 : 2.5 : 0.5 , Hexanes : Ethyl acetate : Methanol) to yield the product as a pale yellow oil (0.019 g, 71 %). R_f 0.40 in 5 : 2.5 : 0.5 Hexanes : Ethyl acetate : Methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (d, J = 6.5 Hz, 3 H, $CH(C\mathbf{H}_3)_2$), 1.01 (d, J = 6.5 Hz, 3 H, $\mathbf{CH}(CH_3)_2$), 1.62 (br, 1 H, N**H**), 2.20 - 2.25 (m, 1 H, $\mathbf{CH}(CH_3)_2$), 2.90 - 2.95 (m, 1 H, $CHC\mathbf{H}_2C_6H_5$), 2.90 - 2.95 (m, 1 H, $NHC\mathbf{H}_2CH_2N$), 3.07 - 3.11 (m, 1 H, $NHC\mathbf{H}_2CH_2N$), 3.15 - 3.20 (m, 1 H, $NHCH_2C\mathbf{H}_2N$), 3.27 - 3.33 (m, 1 H, $NHCH_2C\mathbf{H}_2N$), 3.43 (dd, J = 3.50 Hz, J = 10 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.40 - 3.46 (m, 1 H, $NHCH_2C\mathbf{H}_2N$), 3.71 (s, 3 H, $COOC\mathbf{H}_3$), 3.70 - 3.72 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 4.95 (d, J = 10.5 Hz, 1 H, $C\mathbf{H}CH(CH_3)_2$), 7.20 - 7.40 (m, 5 H, $C_6\mathbf{H}_5$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.37 and 19.65 ($CHC\mathbf{H}_3)_2$), 26.44 ($\mathbf{C}H(CH_3)_2$), 38.18 ($\mathbf{C}H_2C_6H_5$), 41.98 ($NH\mathbf{C}H_2\mathbf{C}H_2N$), 44.18 ($NHCH_2\mathbf{C}H_2N$), 51.75 ($COO\mathbf{C}H_3$), 60.43 ($\mathbf{C}H$-$CH(CH_3)_2$) 60.86 ($\mathbf{C}HCH_2C_6H_5$), 126.51 ($\mathbf{C}_6H_5$)), 128.49 ($\mathbf{C}_6H_5$)), 129.25 ($\mathbf{C}_6H_5$)), 138.08 ($\mathbf{C}_6H_5$)), 169.62 (**N**CO), 170.78 ($\mathbf{C}OOCH_3$); MS (CI, NH_3) m/z 305 (92.0 %, M+ H)$^+$); 277 (100.0 %, (M – $CH_2C_6H_5$)$^+$).

Methyl (2S, 3'S)-2-[3'-methindolyl-2'-oxopiperazin-1'-yl]-3-phenyl propanoate : (25)

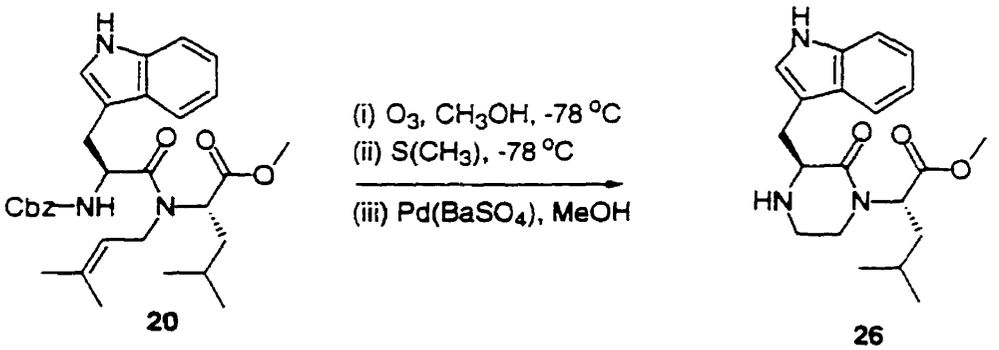


19 **25**

A solution of **19** (1.595 g, 2.811 mmol) was taken in methanol (40 ml) at - 78 °C and O_3 was passed for 2 hours. Dimethyl sulfide (2.0 ml) was then added and the solution was allowed to come to room temperature and stirred overnight. The solvent was removed under and ethyl acetate added which was then washed with water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude mixture that was purified by chromatography. A dry hydrogenation flask flushed with N_2 was charged with 0.10 g Pd(C) (10%) and 0.10 g $Pd(OH)_2$ and 5 ml ethanol was added to it followed by a 5 ml solution of the mixture of cyclized products (0.30 g). The solution was hydrogenated for 7 hr at 40 psi. The solution was filtered through a glass cinter to remove the catalyst and the filtrate was collected. Evaporation of the solvent gave the crude product which was chromatographed (1: 1 : 0.5, Hexanes : Ethyl acetate : MeOH) to yield the product as a pale yellow oil (0.173 g, 75 %). R_f 0.25 in 2 : 1 : 0.5 Hexanes : Ethyl acetate : Methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 - 2.70 (br, 1 H, N**H**), 2.80 - 2.90 (m, 1 H, NHC**H**$_2$CH$_2$), 2.90 - 3.10 (m, 1 H, NHC**H**$_2$CH$_2$), 3.10 - 3.15 (m, 1 H, CHC**H**$_2$C$_6$H$_5$), 3.15 - 3.25 (m, 1 H, CHC**H**$_2$-Indole), 3.40 - 3.50 (m, 2 H, NHCH$_2$C**H**$_2$N, CHC**H**$_2$C$_6$H$_5$), 3.60 - 3.70 (m, 2 H, CHC**H**$_2$-Indole, NHCH$_2$C**H**$_2$N), 3.75 (s, 3 H, COOC**H**$_3$), 5.10 - 5.20 (m, 1 H, C**H**CH$_2$-Indole), 7.10 - 7.30 (m, 7 H, C$_6$H$_5$, C$_8$**H**$_5$NH) 7.56 (t, J = 8.0 Hz, 1 H, C$_8$**H**$_5$NH), 7.87 (dd, J = 8 Hz, 1 H, C$_8$**H**$_5$NH), 8.46 (s, 1 H, C$_8$H$_5$N**H**), 8.72 (d, J = 8.5 Hz, 1 H, C$_8$**H**$_5$NH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 34.47 (CHCH$_2$C$_6$H$_5$), 42.28 (CHCH$_2$-Indole), 42.96 (NHCH$_2$CH$_2$N), 46.64 (NHCH$_2$CH$_2$N),

52.37 ($CHCOOCH_3$), 56.33 ($CHCH_2C_6H_5$), 58.68 ($CHCH_2$-Indole), 122.41 ($C_8\mathbf{H}_5NH$), 123.06 ($C_8\mathbf{H}_5NH$), 126.76 ($C_8\mathbf{H}_5NH$), 126.83 (C6H5), 128.50 (C6H5), 128.89 ($C_8\mathbf{H}_5NH$), 128.98 (C_6H_5), 130.95 ($C_8\mathbf{H}_5NH$), 134.94 ($C_8\mathbf{H}_5NH$), 137.15 (C_6H_5), 139.78 ($C_8\mathbf{H}_5NH$), 159.75 ($C_8\mathbf{H}_5NH$), 169.75 (NCO), 170.79 ($COOCH_3$); MS (FAB, NBA) 424 (48.6 %, $(M + CH_3OH + H)^+$), 261 (100 %, $M - CH_2$-Indole)$^+$).

Methyl (2S, 3'S)-2-[3'-methindolyl-2'-oxopiperazin-1'-yl]-4-methyl pentanoate : (26)

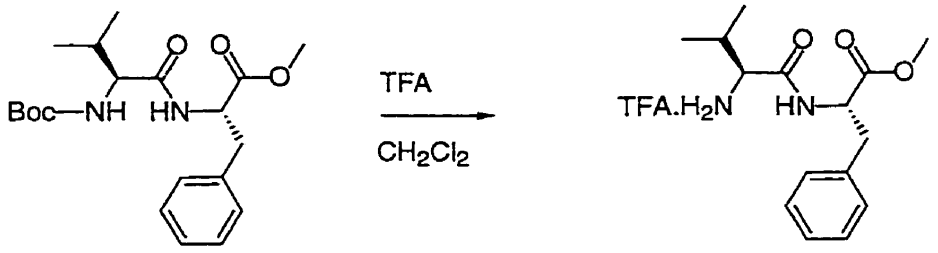


20 **26**

A solution of the dipeptide **20** (0.536 g, 1.005 mmol) was taken in 30 ml methanol at - 78 °C and O_3 was bubbled for 1.5 hours. Dimethyl sulfide was then added and the solution allowed to come to room temperature and stirred for 12 hours. Solvent was evaporated under vacuum and ether added which was then washed with water. It was dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude that was purified by chromatography. A dry hydrogenation flask flushed with N_2 was charged with 0.145 g $Pd(BaSO_4)$ (10%), 0.075 g $Pd(OH)_2$ and 5 ml ethyl acetate was added to it followed by a 5 ml solution of the mixture of cyclized products (0.170 g). The solution was hydrogenated for 7 hours at 40 psi. The solution was filtered through a glass cinter to remove the catalyst and the filtrate was collected. The solvent was evaporated and the crude product which was chromatographed (2 : 1 : 0.5 Hexanes : Ethyl acetate : Methanol) to yield the product as a

pale yellow oil (0.017 g, 14 %); R_f 0.35 in 1 : 1 : 0.5 Hexanes : Ethyl acetate : Methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 0.99 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 1.55 - 1.65 (br, 1 H, NH), 1.65 - 1.75 (m, 1 H, $CH(CH_3)_2$), 1.75 - 1.80 (m, 2 H, $CH_2CH(CH_3)_2$), 3.15 - 3.25 (m, 2 H, $NHCH_2CH_2N$), 3.30 - 3.35 (m, 1 H, $NHCH_2CH_2N$), 3.40 - 3.45 (m, 1H, $NHCH_2CH_2N$), 3.51 (dd, J = 8.0 Hz, J = 15.0 Hz, 1 H, $CHCH_2$-Indole), 3.72 (s, 3 H, $COOCH_3$), 3.81 (dd, J = 18.5 Hz, J = 2.5 Hz, 1 H, $CHCH_2$-Indole), 3.98 (dd, J =8.0 Hz, J = 2.0 Hz, 1 H, $CHCH_2$-Indole), 5.36 (dd, J = 10.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2(CH_3)_2$), 7.17 (t, J = 7.5 Hz, 1 H, C_8H_5NH), 7.57 (t, J = 8.0 Hz, 1 H, C_8H_5NH), 7.98 (t, J = 8.0 Hz, 1 H, C_8H_5NH), 8.45 (s, 1 H, C_8H_5NH), 8.73 (d, J = 8.0 Hz, 1 H, C_8H_5NH), 11.47 (s, 1 H, C_8H_5NH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 21.44 and 23.39 ($CH(CH_3)_2$), 24.88 ($CH(CH_3)_2$), 36.84 ($CHCH_2CH(CH_3)_2$), 42.79 ($CHCH_2$-Indole), 43.09 ($NHCH_2CH_2N$), 44.66 ($NHCH_2CH_2N$), 52.30 ($CHCOOCH_3$), 53.99 ($CHCOOCH_3$), 56.45 ($CHCH_2$-Indole), 121.72 (C_8H_5NH), 123.30 (C_8H_5NH), 131.07 (C_8H_5NH), 135.49 (C_8H_5NH), 139.91 (C_8H_5NH), 159.65 (C_8H_5NH), 169.93 (CON), 172.25 ($COOCH_3$); MS (FAB, NBA) 390 (75. %, M + MeOH + 1)$^+$); 227 (100.0 %, M – CH_2-Indole)$^+$).

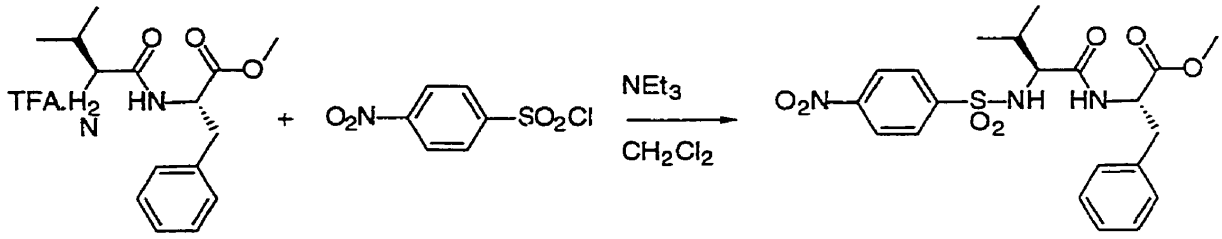
L-Valyl-L-phenylalanine methyl ester trifluoroacetate : (27)



1 **27**

A solution of N-(*tert*)BocValPheOMe (**1**) (0.102 g, 0.27 mmol) in 1 ml CH_2Cl_2 and 0.8 ml TFA was stirred at room temperature for 45 min. The solvents were evaporated in vacuum to yield a white solid. This was taken in toluene and the solvent evaporated in vacuum three times to yield a white solid (0.105 g, 99.4 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.88 and 0.93 (two d, J = 7.0 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 2.10 (septet, J = 7.0 Hz, 1 H, $C\mathbf{H}(CH_3)_2$), 2.95 (A of ABX, J = 8.5 Hz, J = 12.0 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.05 (B of ABX, J = 5.5 Hz, J = 14.0 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.58 (s, 3 H, $COOC\mathbf{H}_3$), 3.60 - 3.62 (br, 1 H, $C\mathbf{H}COOCH_3$), 4.54 (X of ABX, J = 14.0 Hz, J = 7.0 Hz, 1 H, $C\mathbf{H}CH_2C_6H_5$), 7.20 - 7.30 (m, 5 H, $C_6\mathbf{H}_5$), 8.00 - 8.10 (br, 3 H, $N\mathbf{H}_3^+$) ,8.85 (d, J = 7.5 Hz, 1 H, $N\mathbf{H}CO$); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 18.63 and 19.24 ($CH(\mathbf{C}H_3)_2$), 30.67 ($CH\mathbf{C}H_2C_6H_5$), 37.55 ($\mathbf{C}H(CH_3)_2$), 52.21 ($COOCH_3$), 54.01 ($CHCOOCH_3$), 59.00 ($\mathbf{C}HCH_2C_6H_5$), 116.75 (q, J =289.2 Hz, $\mathbf{C}F_3$), 127.66 ($\mathbf{C}_6H_5$), 129.01 ($\mathbf{C}_6H_5$), 130.12 ($\mathbf{C}_6H_5$), 135.35 ($\mathbf{C}_6H_5$), 161.88 (q, J = 41.2 Hz, $\mathbf{C}OOCF_3$), 169.88 (**C**ONH), 172.61 ($\mathbf{C}OOCH_3$).

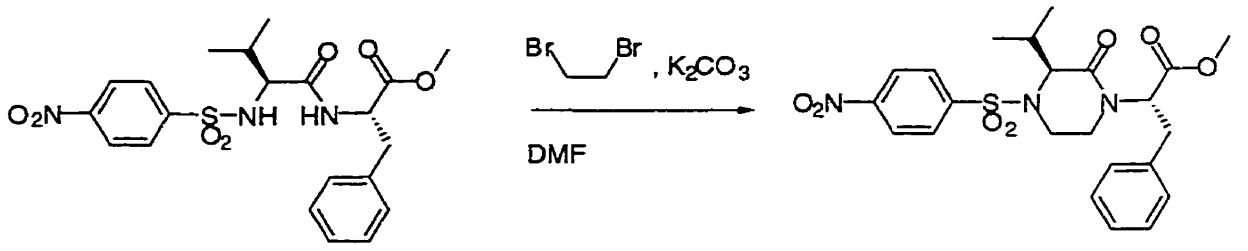
N-(4-Nitrobenzenesulfonyl)-L-valyl-L-phenylalanine methyl ester : (28)



27 28

A solution of the deprotected dipeptide **27** (0.100 g, 0.255 mmol) in CH_2Cl_2 at 0 °C was treated with 4-nitrobenzenesulfonyl chloride (0.063 g, 0.286 mmol) and NEt_3 (0.080 ml, 0.575 mmol) and the solution stirred for 6 hours. The solution was washed with 10 % $NaHCO_3$, 10 % citric acid and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.120 g, 93 %) as a pale yellow oil. R_f 0.17 in 2 : 1 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 0.77 and 0.88 (two d, J = 6.7 Hz each, 3 H each, $CH(CH_3)_2$), 1.94 - 2.04 (m, 1 H, $CH(CH_3)_2$), 2.92 (d, J = 5.9 Hz, 2 H, $CHCH_2C_6H_5$), 3.63 - 3.69 (m, 1 H, $CHCH(CH_3)_2$), 3.65 (s, 3 H, OCH_3), 4.62 (dd, J = 7.7 Hz, J = 5.9 Hz, 1 H, $CHCOOCH_3$), 6.01 (d, J = 8.9 Hz, 1 H, $NHSO_2$), 6.32 (d, J = 7.7, 1 H, NHCO), 6.92 - 6.96 and 7.22 - 7.28 (m, 5 H, C_6H_5), 7.95 (d, J = 8.9 Hz, 2 H, C_6H_4), 8.21 (d, J = 8.9 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 Hz, $CDCl_3$) δ 17.28 and 19.17 ($CH(CH_3)_2$), 31.89 ($CH(CH_3)_2$, 37.79 ($CHCH_2C_6H_5$), 52.23 ($COOCH_3$), 53.54 ($CHCH(CH_3)_2$), 62.20 ($CHCOOCH_3$)), 124.28 (C_6H_4), 127.42 (C_6H_5), 128.44 (C_6H_4), 128.77 (C_6H_5), 129.07 (C_6H_5), 135.42 (C_6H_5). 145.99 (C_6H_4), 150.02 (C_6H_4), 169.91 (CONH), 171.56 ($COOCH_3$); MS (EI) 463 (0.8 %, $(M)^+$), 404 (4.4 %, $M - COOCH_3)^+$).

Methyl (2S, 3'S)-2-[3'isopropyl-4'-(4-nitrobenzenesulfonyl)-2'-oxopiperazin-1'yl]-phenyl propanoate : (29)

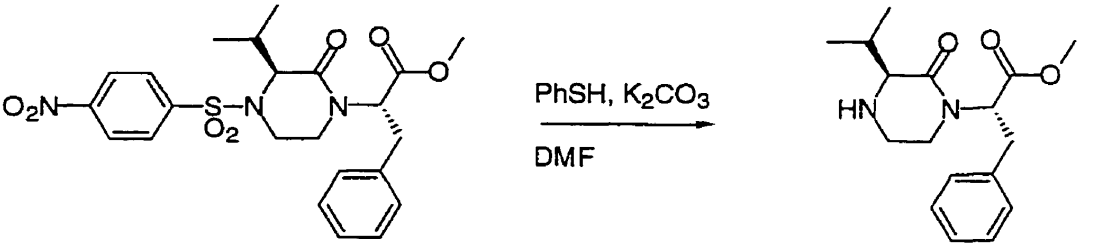


28 29

A solution of N-(4-nitrobenzenesulfonyl)valyl-phenylalanine methyl ester (0.108 g, 0.233 mmol) in DMF was treated with K_2CO_3 (0.344 g, 2.49 mmol) at 60 °C for 30 min. 1,2-dibromoethane (0.1 ml, 1.160 mmol) was added to the solution which was stirred at 60 °C for 8 hours and at room temperature for 24 hours. The solvent was evaporated under vacuum and then ether was added. The ether solution was washed with 10 % citric acid solution (3 times, 30 ml each), 10 % $NaHCO_3$ (3 times, 30 ml each) and water. The ether layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude product which was chromatographed (2 : 1 Hexanes : Ethyl acetate) to yield the product (0.028 g, 25 %) as a yellow oil. R_f 0.26 in 2 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.58 and 0.88 (two d, J = 6.5 Hz each, 3 H each, $CH(CH_3)_2$), 1.59 - 1.66 (m, 1 H, $CH(CH_3)_2$), 2.74 (A of ABX, J = 12.5 Hz, J = 14.5 Hz, 1 H, $CHCH_2C_6H_5$), 3.15 - 3.20 (m, 2 H, $NHCH_2CH_2N$), 3.34 (B of ABX, J = 5.0 Hz, J = 15.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.43 (dt, J = 5.5 Hz, J = 13.0 Hz, 1 H, $NHCH_2CH_2N$), 3.56 (s, 3 H, OCH_3), 3.56 - 3.60 (m, 1 H, $NHCH_2CH_2N$), 3.92 (d, J = 9.0 Hz, 1 H, $CHCH(CH_3)_2$), 5.42 (dd, J = 5.0 Hz, J = 12.0 Hz, 1 H, $CHCOOCH_3$), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.96 (d, J = 8.5 Hz, 2 H, C_6H_4), 8.32 (d, J = 8.5 Hz, 2 H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.80 and 19.62 ($CH(CH_3)_2$), 31.28 ($CH(CH_3)_2$), 34.50 ($CHCH_2C_6H_5$), 39.78 and 41.69 ($O_2SNCH_2CH_2N$), 52.12 ($COOCH_3$), 55.23 (CH), 65.00 (CH), 124.33 (C_6H_4), 127.04 (C_6H_4), 128.23 (C_6H_4), 128.49 (C_6H_5), 128.60 (C_6H_4), 128.63 (C_6H_5),

143.96 (C_6H_5), 161.84, (C_6H_4) 167.71 (CONH), 170.19 ($COOCH_3$); HRMS (EI) Expected for $C_{23}H_{27}N_3O_7S$ 489.1570, Found 489.1565.

Methyl (2S, 3'S) -2-[3'isopropyl-2'-oxopiperazin-1'yl]-phenyl propanoate : (30)

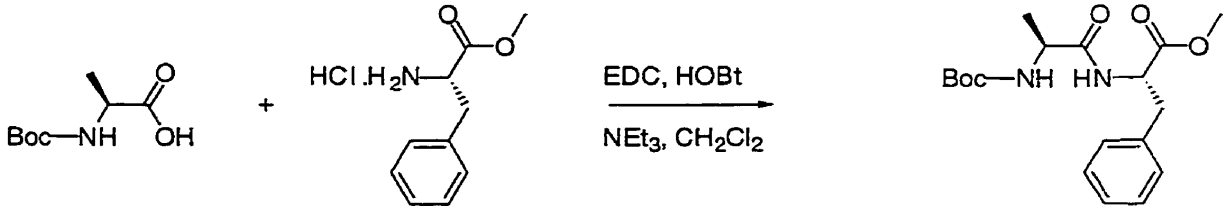


29 **30**

A solution of 0.023 g K_2CO_3 in 0.3 ml dry DMF was treated with benzenethiol (0.01 ml, 0.097 mmol) at room temperature. After 45 minutes, a solution of sulfonamide **29** (0.021 g, 0.0422 mmol) in 0.2 ml DMF was added to it and the solution stirred at room temperature for 5 hours. The solvent was removed under vacuum and ethyl acetate added. The solution was washed with 1 N HCl and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.013 g, 100 %) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.51 and 0.88 (two d, J = 7.0 Hz each, 3 H each, $CH(CH_3)_2$), 1.60 - 1.80 (br, 1 H, NH), 2.35 - 2.40 (m, 1 H, $CH(CH_3)_2$), 2.80 - 2.90 (m, 1 H, $CHCOOCH_3$), 2.92 - 2.98 (m, 1 H, NCH_2CH_2NH), 3.02 - 3.12 (m, 2 H, NCH_2CH_2NH and $CHCH_2C_6H_5$), 3.31 (dt, J = 10.5 Hz, J = 4.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.37 - 3.42 (m, 2 H, NCH_2CH_2NH), 3.75 (s, 3 H, $COOCH_3$), 5.15 (dd, J = 12.5 Hz, J = 4.0 Hz, 1 H, $CHCH_2C_6H_5$), 7.10 - 7.40 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 16.40 and 19.17 ($CH(CH_3)_2$), 30.23 ($CH(CH_3)_2$), 34.50 ($CHCH_2C_6H_5$), 42.44 ($HNCH_2CH_2N$), 46.71 ($HNCH_2CH_2N$), 51.90 ($COOCH_3$), 58.53 ($CHCH(CH_3)_2$), 64.55 ($CHCOOCH_3$), 126.70 (C_6H_5), 128.51 (C_6H_5), 128.85 (C_6H_5), 137.27 (C_6H_5), 170.32 (NHCO),

171.10 ($COOCH_3$); MS (EI) 304; HRMS (EI) Expected for $C_{17}H_{24}N_2O_3$ 304.1787, Found 304.1784.

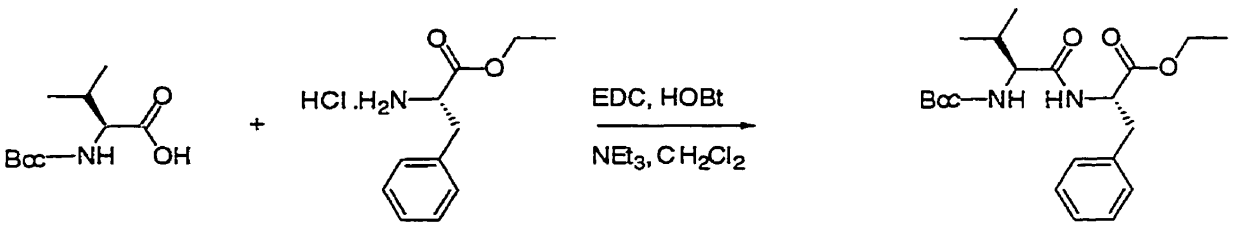
N-(*tert*-Butoxycarbonyl)-L-alanyl-L-phenylalanine methyl ester : (31 a)



31a

A solution of N-(*tert*)BocAlaOH (1.011 g, 5.342 mmol) and PheOMe.HCl (1.324 g, 6.138 mmol) in 10 ml dry CH_2Cl_2 at 0 °C was treated with EDC (1.105 g, 5.762 mmol) HOBt (0.812 g, 6.007 mmol) and triethylamine (0.8 ml, 5.739 mmol). The solution was stirred at room temperature for 12 hours and then diluted with more CH_2Cl_2. The solution was washed with 1N HCl, 10 % $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (1.752 g, 94 %) as a white powder. M. P. 78 - 82 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.25 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 1.38 (s, 9 H, $C(CH_3)_3$), 3.02 (A of ABX, J = 13.9 Hz, J = 6.2 Hz, 1 H, $CHCH_2C_6H_5$), 3.11 (B of ABX, J = 13.9 Hz, J = 5.9 Hz, 1 H, $CHCH_2C_6H_5$), 3.65 (s, 3 H, $CHCOOCH_3$), 4.10 - 4.20 (m, 1 H, $CHCH_3$), 4.79 (X of ABX, J = 13.6 Hz, 6.2 Hz, 1 H, $CHCH_2$) 5.15 (d, J = 7.4 Hz, 1 H, NHCO), 6.71 (d, J = 7.4 Hz, 1 H, NHCOO), 7.05 - 7.25 (m, 5 H, $CHCH_2C_6H_5$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.39 ($CHCH_3$), 28.34($C(CH_3)_3$), 37.95 ($CHCH_2C_6H_5$), 50.10 ($CHCH_2$), 52.30 ($CHCOOCH_3$), 53.27 ($CHCH_3$), 80.10 ($C(CH_3)_3$), 127.11 (C_6H_5), 128.57 (C_6H_5), 129.32 (C_6H_5), 135.91 (C_6H_5), 155.37 (NHCOO), 171.80 (CONH), 172.46 ($COOCH_3$); MS (FAB, NBA) m/z 351 (71.9 %, $(M + H)^+$), 251 (83.1 %, $M - t\text{Boc})^+$).

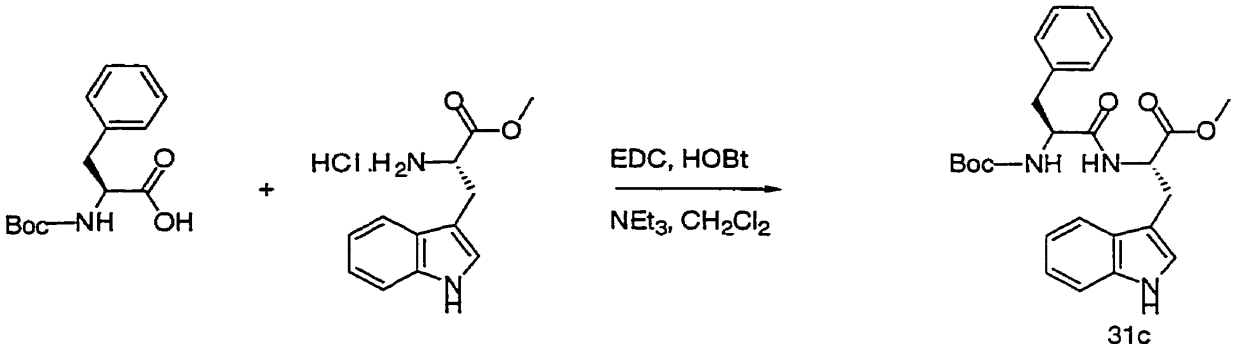
N-(*tert*-Butoxycarbonyl)-L-valyl-L-phenylalanine ethyl ester : (31b)



31b

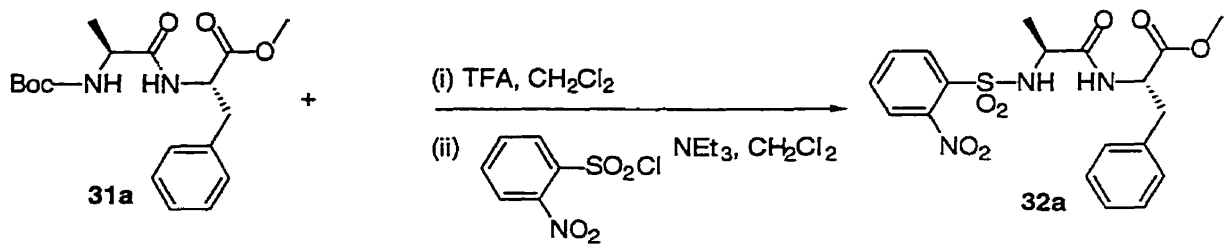
A solution of N-(*tert*)BocValOH (1.051 g, 4.836 mmol) in 8 ml dry CH_2Cl_2 at 0 °C was treated with PheOEt.HCl (1.005 g, 4.378 mmol), EDC (0.922 g, 4.809 mmol), HOBt (0.654 g, 4.843 mmol) and triethylamine (0.7 ml, 5.02 mmol) and the solution was stirred overnight at room temperature. It was diluted with CH_2Cl_2 and washed with 1 N HCl, 10 % $NaHCO_3$, water , dried over $MgSO_4$, and filtered. Solvent evaporation under vacuum gave the product as a white solid (1.675 g, 88.5 %). M. P. 109 - 111 °C; R_f 0.56 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.83 and 0.87 (two d, J = 6.8 each, 3 H each, $CH(CH_3)_2$), 1.15 (t, J = 7.0 Hz, 3 H, $COOCH_2CH_3$), 1.39 (s, 9 H, $C(CH_3)_3$), 2.04 (septet, J = 6.7 Hz, 1 H, $CH(CH_3)_2$), 3.04 (d, J = 5.9 Hz, 2 H, $CH_2C_6H_5$), 3.85 - 3.95 (m, 1 H, $CHCH(CH_3)_2$), 4.08 (q, J = 7.1 Hz, 2 H, $COOCH_2CH_3$), 4.75 - 4.85 (m, 1 H, $CHCOOC_2H_5$), 5.15 (d, J = 8.9 Hz, 1 H, CONH), 6.54 (d, J = 7.9 Hz, 1 H, NHCOO), 7.05 - 7.10 and 7.15 - 7.25 (m, 5 H, $CHCH_2C_6H_5$), ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.09 ($COOCH_2CH_3$), 17.80 and 19.21 ($CH(CH_3)_2$), 28.36 ($C(CH_3)_4$), 31.00 ($CH(CH_3)_2$), 38.09 ($CHCH_2C_6H_5$), 53.25 ($CHCH_2$), 59.89 ($CHCH(CH_3)_2$), 61.46 ($COOCH_2CH_3$), 79.72 ($C(CH_3)_3$), 127.08 (C_6H_5), 128.55 (C_6H_5), 129.35 (C_6H_5), 135.90 (C_6H_5), 155.76 (NHCOO), 171.35 ($COOCH_2CH_3$ and CONH)); MS (FAB,NBA), m/z 393 (71.1 %, $(M + H)^+$); 293 (90.1 %, M – *t*Boc)$^+$).

N-(*tert*-Butoxycarbonyl)-L-phenylalanyl-L-tryptophan methyl ester : (31c)



A solution of N-(*tert*)BocPheOH (1.024 g, 3.860 mmol) in 10 ml dry CH_2Cl_2 at 0 °C was treated with TrpOMe.HCl (1.090 g, 4.281 mmol), EDC (0.809 g, 4.220 mmol), HOBt (0.564 g, 4.171 mmol) and triethylamine (0.6 ml, 4.305 mmol) and the solution was stirred overnight at room temperature. It was diluted with CH_2Cl_2 and washed with 1 N HCl, 10 % $NaHCO_3$, water , dried over $MgSO_4$, and filtered. Solvent evaporation under vacuum gave the product as a white solid (1.737 g, 96.8 %) ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9 H, $(CH_3)_3C$), 2.96 - 3.40 (m, 2 H, $CHCH_2$-Indole), 3.20 - 3.30 (m, 2 H, $CHCH_2C_6H_5$), 3.63 (s, 3 H, $COOCH_3$), 4.30 - 4.40 (m, 1 H, $CHCH_2$-Indole), 4.80 - 4.90 (m, 2 H, $CHCH_2C_6H_5$ and CONH), 6.87 (s, 1 H C=CH-NH), 7.00 - 7.40 (m, 10 H, C_6H_5 and C_8H_6N), 8.04 (s, 1 H, C=CH-NH); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 27.78 ($CHCH_2$-Indole), 28.28 ($C(CH_3)_3$), 35.43 ($CHCH_2C_6H_5$), 52.38 ($COOCH_3$), 53.15 ($CHCH_2C_6H_5$), 55.74 ($CHCH_2$-Indole), 80.20 ($C(CH_3)_3$), 109.49 (C_8H_5NH), 111.53 (C_8H_5NH), 118.45 (C_8H_5NH), 119.59 (C_8H_5NH), 122.17 (C_8H_5NH), 123.24 (C_8H_5NH), 126.98 (C_6H_5), 127.57 (C_8H_5NH), 128.65 (C_6H_5), 129.46 (C_6H_5), 136.25 (C_8H_5NH), 136.70 (C_6H_5), 155.41(NHCOO), 171.17 (CONH), 171.96 ($COOCH_3$); MS (FAB, NBA) m/z 466 (49.4 %, $(M + H)^+$); 410 (28.7 %, $M - C(CH_3)_4)^+$); 366 (73.8 %, $M - C(CH_3)_3OOC)^+$).

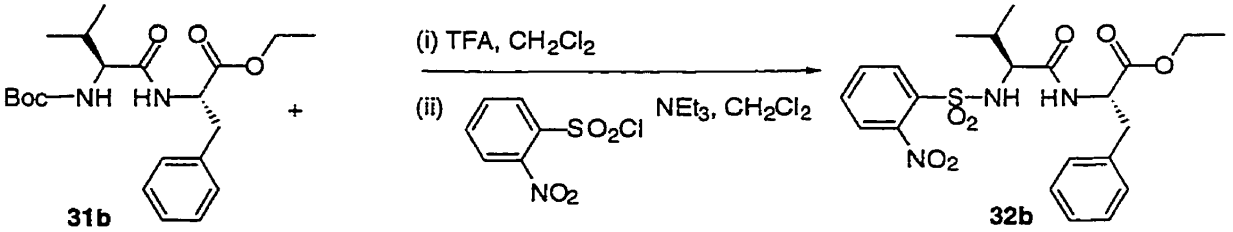
N-(2-Nitrobenzenesulfonyl)-L-alanyl-L-phenylalanine methyl ester : (32 a)



A solution of dipeptide **31a** in 4 ml CH_2Cl_2 was treated with 10 ml TFA at room temperature and the solution stirred for 4 hours. The solvents were evaporated with toluene three times under vacuum to yield the product (1.758 g, 97 %) as a white solid. ^{1}H NMR (270 MHz, DMSO d_6) δ 1.33 (d, J = 6.9 Hz, 3 H, CHC**H**$_3$), 2.94 (A of ABX, J = 14.0 Hz, J = 9.3 Hz, 1 H CHC**H**$_2$C$_6$H$_5$), 3.10 (B of ABX, J = 13.7 Hz, J = 5.3 Hz, 1 H, CHC**H**$_2$C$_6$H$_5$), 3.62 (s, 3 H, CHCOOC**H**$_3$), 3.77 - 3.85 (m, 1 H, C**H**CH$_3$), 4.53 (X of ABX, J = 13.4 Hz, J = 8.2 Hz, 1 H, C**H**CH$_2$C$_6$H$_5$), 7.20 - 7.30 (m, 5 H, CHCH$_2$C$_6$**H**$_5$), 8.00 - 8.10 (br s, 3 H, N**H**$_3$), 8.82 (d, J = 7.4 Hz, 1 H, N**H**CO); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 17.62 (CH**C**H$_3$), 36.80 (CH**C**H$_2$), 48.48 (**C**HCH$_3$), 52.62 (CHCOO**C**H$_3$), 54.46 (**C**HCH$_2$), 127.27 (**C**$_6$H$_5$), 128.91 (**C**$_6$H$_5$), 129.61 (**C**$_6$H$_5$), 137.46 (**C**$_6$H$_5$), 170.35 (NH**C**O), 171.96 (**C**OOCH$_3$); MS (FAB, NBA) m/z 251 (100.0 %, M$^+$). A solution of this trifluoroacetate salt (0.300 g, 0.893 mmol) in 5 ml dry CH_2Cl_2 was treated with 2-nitrobenzenesulfonyl chloride (0.232 g, 1.046 mmol) and triethylamine (0.30 ml, 2.152 mmol) at 0 °C and the solution stirred at room temperature overnight. The solution was diluted with CH_2Cl_2 and washed with 1 N HCl, 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product (0.365 g,93.8 %) as a white solid. R_f 0.20 in 1 : 2 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.26 (d, J = 7.2 Hz, 3 H, CHC**H**$_3$), 2.93 (A of ABX, J = 13.8 Hz, J = 6.7 Hz, 1 H, CHC**H**$_2$C$_6$H$_5$), 3.03 (B of ABX, J = 13.8 Hz, J = 5.7 Hz, 1 H CHC**H**$_2$C$_6$H$_5$), 3.64 (s, 3 H, COOC**H**$_3$), 4.00 - 4.10 (m, 1 H, C**H**), 4.60 - 4.70 (m, 1 H, C**H**), 6.12 (d, J = 7.7 Hz, 1 H, CON**H**), 6.81 (d, J = 7.9 Hz, 1 H, N**H**SO$_2$), 7.00 - 7.30 (m, 5 H, C$_6$**H**$_5$), 7.80 - 8.10 (m, 4 H, C$_6$**H**$_4$); ^{13}C

NMR (67.5 MHz, $CDCl_3$) δ 19.34 ($CHCH_3$), 37.83 ($CHCH_2$), 52.84 ($CHCH_3$), 53.35 ($COOCH_3$), 53.53 ($CHCH_2$), 125.67 (C_6H_4), 127.24 (C_6H_5), 128.67 (C_6H_4), 129.28 (C_6H_5), 130.90 (C_6H_5), 133.06 (C_6H_4), 133.58 (C_6H_4), 133.97 (C_6H_4), 135.81 (C_6H_5), 147.82 (C_6H_4), 170.71 (CONH), 171.54 ($COOCH_3$); MS (EI) m/z 436 (8.3 %, $(M + H)^+$), 376 (3.2 %, $(M - COOCH_3)^+$).

N-(2-Nitrobenzenesulfonyl)-L-valyl-L-phenylalanine ethyl ester : (32b)

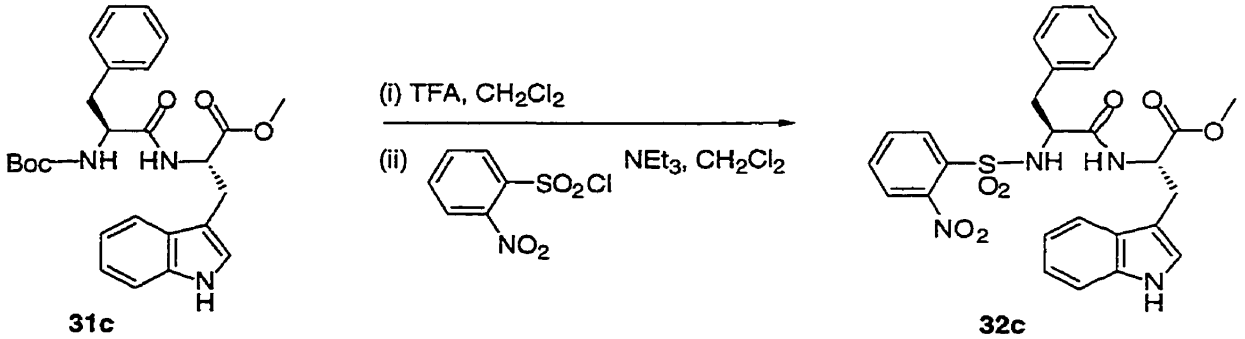


31b 32b

A solution of N-(*tert*)BocValPheOEt (1.650 g, 4.217 mmol) in 4 ml CH_2Cl_2 was treated with TFA (10 ml) at room temperature for 6 hours. Solvent evaporation with toluene three times gave the product as a white solid. ^1H NMR (500 MHz, DMSO-d_6) δ 0.90 and 0.94 (two d, J = 7.0 Hz each, 3 H each, $CH(CH_3)_2$), 1.08 (t, J = 7.0 Hz, 3 H, $COOCH_2CH_3$), 2.10 - 2.15 (m, 1 H, $CH(CH_3)_2$), 2.96 (A of ABX, J = 14.0 Hz, J = 8.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.02 (B of ABX, J = 14.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.62 (d, J = 5.0 Hz, $CHCH(CH_3)_2$), 4.02 (q, J = 7.0 Hz, $COOCH_2CH_3$), 4.51 (X of ABX, J = 15.0 Hz, J = 7.0 Hz, 1 H, $CHCH_2C_6H_5$), 7.20 - 7.30 (m, 5 H, C_6H_5), 8.0 - 8.1 (br s, 3 H, NH_3^+), 8.86 (d, J = 7.0 Hz, 1 H, NHCO); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 14.45 ($COOCH_2CH_3$), 17.72 and 18.83 ($CH(CH_3)_2$), 30.41 ($CH(CH_3)_2$), 37.06 ($CHCH_2C_6H_5$), 54.50 (CH), 57.60 (CH), 61.27 ($COOCH_2CH_3$), 127.28 (C_6H_5), 128.89 (C_6H_5), 129.63 (C_6H_5), 137.28 (C_6H_5), 171.38 ($COOCH_2CH_3$). A solution of this trifluoroacetate salt (0.501 g, 1.233 mmol) in 10 ml dry CH_2Cl_2 at room temperature was treated with 2-nitrobenzenesulfonyl chloride (0.300 g, 1.355 mmol) and triethylamine

(0.38 ml, 2.726 mmol). The solution was stirred overnight and then washed with 1 N HCl, 10 % $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product as white solid (0.547 g, 93 %) ^1H NMR (270 MHz, $CDCl_3$) δ 0.76 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.82 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 1.15 (t, J = 7.2 Hz, 3 H, $COOCH_2CH_3$), 2.05 - 2.15 (m, 1 H, $CH(CH_3)_2$), 2.86 - 2.92 (m, 2 H, $CHCH_2C_6H_5$), 3.81 (dd, J = 8.2 Hz, J = 4.9 Hz, 1 H, $NHCHCH(CH_3)_2$), 4.07 (q, J = 7.2 Hz, 2 H, $COOCH_2CH_3$), 4.60 - 4.70 (m, 1 H, CHCOO), 6.13 (d, J = 8.2 Hz, 1 H, $NHSO_2$), 6.60 (d, J = 7.9 Hz, 1 H, NHCO), 7.00 - 7.10 and 7.20 - 7.30 (m, 5 H, $CHCH_2C_6H_5$), 7.60 - 7.70 (m, 2 H, $C_6H_4NO_2$), 7.83 (dd, J = 7.7 Hz, J = 1.7 Hz, 1 H, $C_6H_4NO_2$), 7.99 (dd, J = 7.4 Hz, J = 2.0 Hz, 1 H, $C_6H_4NO_2$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.06 ($COOCH_2CH_3$), 17.06, and 19.81 ($CH(CH_3)_2$), 31.35 ($CH(CH_3)_2$), 38.04 ($CHCH_2C_6H_5$), 53.47 ($CHCH_2C_6H_5$), 61.68 ($CHCOOCH_3$), 63.05 ($COOCH_2CH_3$), 125.63 (C_6H_4), 127.21 (C_6H_5), 128.66 (C_6H_4), 129.23 (C_6H_5), 130.57 (C_6H_5), 132.95 (C_6H_4), 133.28 (C_6H_4), 133.85 (C_6H_4), 135.84 (C_6H_5), 147.76 (C_6H_4), 169.68 (CONH), 170.99 ($COOCH_2CH_3$); MS (CI, NH_3) m/z 478 (70.0 %, $(M + H)^+$), 404 (22.3 %, $M - COOC_2H_5)^+$).

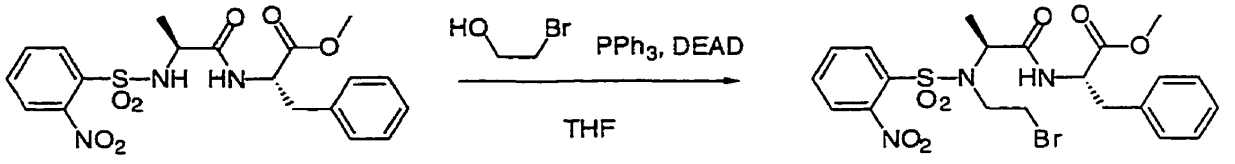
N-(2-Nitrobenzenesulfonyl)-L-phenylalanyl-L-tryptophan methyl ester : (32c)



31c 32c

A solution of N-(*tert*)BocPheTrpOEt **31c** (1.730 g, 3.720 mmol) in 4 ml CH_2Cl_2 was treated with TFA (10 ml) at room temperature for 6 hours. Solvent evaporation with toluene three times gave the product as a white solid (1.770g, 99.2 %) ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.90 (dd, J = 16.0 Hz, J = 8.0 Hz, 1 H, CHC**H**$_2$-Indole), 3.06 - 3.12 (m, 2 H, CHC**H**$_2$-Indole and CHC**H**$_2$C$_6$H$_5$), 3.17 (dd, J = 15.0 Hz, J = 6.0 Hz, 1 H, CHC**H**$_2$C$_6$H$_5$), 3.57 (s, 3 H, COOC**H**$_3$), 4.00 - 4.10 (m, 1 H, **C**HCH$_2$C$_6$H$_5$), 4.61 (dd, J = 14.0 Hz, J = 7.5 Hz, 1 H, **C**HCH$_2$-Indole), 7.00 - 7.40 (m, 10 H, C$_6$**H**$_5$ and C$_8$**H**$_6$N), 7.48 (d, J = 8.0 Hz, 1 H, C=C**H**-NH), 8.10 - 8.20 (br s, 3 H, N**H**$^{3+}$), 8.97 (d, J = 7.5 Hz, N**H**CO), 10.93 (d, J = 0.5 Hz, C=CH-N**H**). A solution of this trifluoroacetate salt (1.770 g, 3.692 mmol) in 10 ml dry CH_2Cl_2 at room temperature was treated with 2-nitrobenzenesulfonyl chloride (0.956 g, 4.313 mmol) and triethylamine (1.25 ml, 8.968 mmol). The solution was stirred overnight and then washed with 1 N HCl, 10 % $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product as a white solid (2.023 g, 99.4 %) ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.63 (A of ABX, J = 14.1 Hz, J = 9.6 Hz, 1 H, CHC**H**$_2$-Indole), 3.10 (B of ABX, J = 16.0 Hz, 4.2 Hz, 1 H, CHC**H**$_2$-Indole), 3.20 - 3.30 (m, 2 H, CHC**H**$_2$C$_6$H$_5$), 3.61 (s, 3 H, COOC**H**$_3$), 4.10 - 4.30 (m, 1 H, C**H**CH$_2$), 4.80 - 4.90 (m, 1 H, C**H**CH$_2$), 6.18 (d, J = 6.9 Hz, N**H**SO$_2$), 6.90 - 7.70 (m, aromatic H), 8.54 (br s, C=CH-N**H**); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 27.66 (CH**C**H$_2$-Indole), 38.22 (CH**C**H$_2$C$_6$H$_5$), 52.53 (COO**C**H$_3$), 53.27 (**C**HCH$_2$C$_6$H$_5$), 59.56 (**C**HCH$_2$-Indole), 109.41 (**C**$_8$H$_5$NH), 111.69 (**C**$_8$H$_5$NH), 118.81 (**C**$_8$H$_5$NH), 122.16 (**C**$_8$H$_5$NH), 123.61 (**C**$_8$H$_5$NH), 125.77 (**C**$_8$H$_5$NH), 127.12 (**C**$_6$H$_5$), 127.57 (**C**$_8$H$_5$NH), 128.51 (**C**$_6$H$_5$), 128.69 (**C**$_6$H$_5$), 129.16 (**C**$_6$H$_4$), 130.61 (**C**$_6$H$_4$), 133.11 (**C**$_6$H$_4$), 133.57 (**C**$_6$H$_4$), 135.44 (**C**$_6$H$_5$), 136.29 (**C**$_8$H$_5$NH), 146.97 (**C**$_6$H$_4$), 170.07 (**C**ONH), 171.86 (**C**OOCH$_3$).

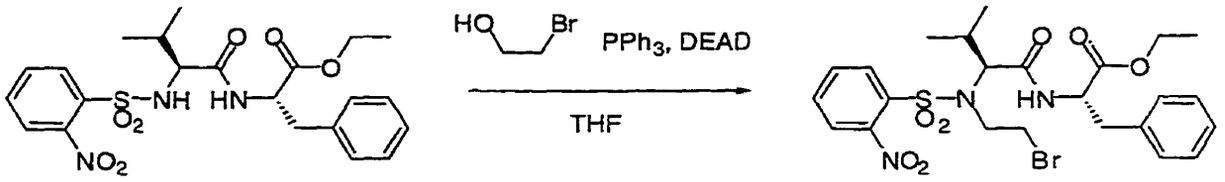
N-(2-Bromoethyl)-N-(4-nitrobenzenesufonyl)-L-alanyl-L-phenylalanine methyl ester : (33a)



32a **33a**

A solution of N-(2-nitrobenzenesulfonyl)AlaPheOMe (**32a**) (0.103 g, 0.237 mmol) in 2 ml dry THF at 0 °C was treated with PPh_3 (0.095 g, 0.361 mmol), bromoethanol (0.028 ml, 0.395 mmol) and then DEAD (0.06 ml, 0.381 mmol) was added dropwise and the solution was stirred at room temperature overnight. Solvent was evaporated under vacuum and ethyl acetate added, washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude which was chromatographed (1 : 2 Hexanes : Ethyl acetate) to give the product (0.087 g, 68 %) as an oil. R_f 0.23 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 1.29 (d, J = 7.3 Hz, 3 H, $CHCH_3$), 2.90 (A of ABX, J = 15.7 Hz, J = 8.9 Hz, 1 H, $CHCH_2C_6H_5$), 3.10 - 3.15 (m, 1 H, NCH_2CH_2Br), 3.18 (B of ABX, J = 14.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.35 - 3.55 (m, 2 H, NCH_2CH_2Br), 3.68 (s, 3 H, $COOCH_3$), 4.40 (q, J = 7.0 Hz, 1 H, $CHCH_3$), 4.70 - 4.80 (m, 1 H, $CHCH_2C_6H_5$), 6.79 (d, J = 8.0 Hz, 1 H, CONH), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.70 - 7.80 (m, 3 H, C_6H_4), 8.04 (d, J = 7.5 Hz, 1 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.62 ($CHCH_3$), 28.28 (NCH_2CH_2Br), 37.53 ($CHCH_2C_6H_5$), 45.95 (NCH_2CH_2Br), 52.26 ($CHCOOCH_3$), 53.49 ($CHCH_2C_6H_5$), 55.14 ($CHCH_3$), 124.70 (C_6H_4), 127.23 (C_6H_5), 128.73 (C_6H_4), 128.97 (C_6H_5), 131.24 (C_6H_5), 132.02 (C_6H_4), 132.05 (C_6H_4), 134.19 (C_6H_4), 135.49 (C_6H_5), 147.72 (C_6H_4), 169.64 (NHCO), 171.37 ($COOCH_3$); HRMS (FAB, NBA) Calculated for $[C_{21}H_{24}N_3O_7SBr + H]^+$ 542.05986, Found 542.05966

N-(2-Bromoethyl)-N-(4-nitrobenznesulfonyl)-L-valyl-L-phenylalanine ethyl ester : (33b)

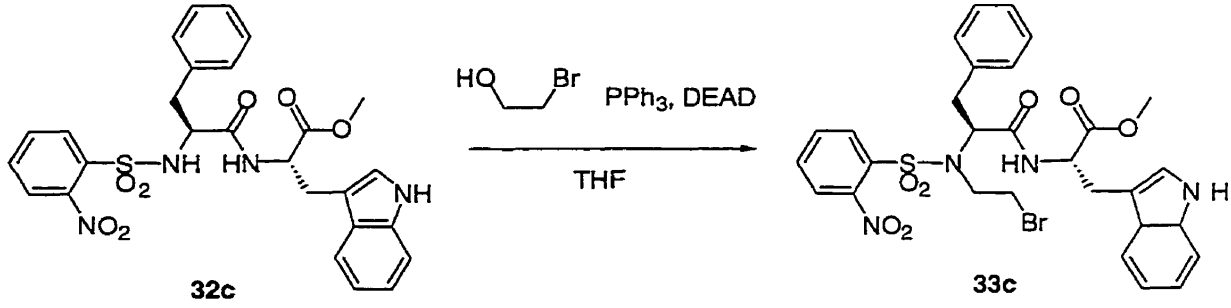


32b **33b**

A solution of sulfonamide **32b** (0.110 g, 0.232 mmol) in 2 ml dry THF at 0 °C was treated with triphenylphosphine (0.091 g, 0.342 mmol) and bromoethanol (0.029 ml, 0.409 mmol). DEAD (0.058 ml, 0.368 mmol) was added dropwise to the solution that was allowed to come to room temperature and then stirred for 24 hours. The solvent was then evaporated under vacuum and ethyl acetate added. This was washed with 10 % $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude product which was chromatographed (1 : 2 Hexanes : Ethyl acetate) to yield the product (0.128 g, 95 %) as an oil. R_f 0.06 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.41 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 0.86 (d, J = 6.4 Hz, 3 H, $CH(CH_3)_2$), 1.20 - 1.30 (m, 3 H, $COOCH_2CH_3$), 2.15 - 2.25 (m, 1 H, $CH(CH_3)_2$), 2.80 - 3.00 (m, 2 H, NCH_2CH_2Br), 3.05 - 3.20 (m, 2 H, $CHCH_2C_6H_5$), 3.30 - 3.40 (m, 1 H, NCH_2CH_2Br), 3.72 (d, J = 10.0 Hz, 1 H, $CH(CH_3)_2$), 3.75 - 3.90 (m, 1 H, NCH_2CH_2Br), 4.17 (q, J = 7.2 Hz, 2 H, $COOCH_2CH_3$), 4.70 - 4.80 (m, 1 H, $CHCH_2C_6H_5$), 6.67 (d, J = 7.9 Hz, 1 H, NHCO), 7.15 - 7.30 (m, 5 H, C_6H_5), 7.75 - 7.60 (m, 1 H, $C_6H_4NO_2$), 7.65 - 7.75 (m, 2 H, $C_6H_4NO_2$), 7.95 - 8.00 (m, 1 H, $C_6H_4NO_2$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.13 ($COOCH_2CH_3$), 18.04 and 19.72 ($CH(CH_3)_2$), 26.96 ($CH(CH_3)_2$), 28.67 (NCH_2CH_2Br), 37.95 ($CHCH_2C_6H_5$), 46.45 (NCH_2CH_2Br), 53.74 ($CHCH_2C_6H_5$), 61.63 ($COOCH_2CH_3$), 65.13 ($CHCH(CH_3)_2$), 124.24 (C_6H_4), 127.29 (C_6H_5), 128.85 (C_6H_5), 129.36 (C_6H_5), 130.79 (C_6H_4), 131.89 (C_6H_4), 132.96 (C_6H_4), 134.19 (C_6H_4), 135.81 (C_6H_5), 147.96 (C_6H_4), 169.35

(CONH), 171.13 ($COOCH_2$); MS (FAB, NBA) m/z 586 (14.9 %, $(M + H)^+$); 584 (10.5 %, $(M + H)^+$).

N-(2-Bromoethyl)-N-(2-nitrobenzenenesulfonyl)-L-phenylalanyl-L-tryptophan methyl ester : (33c)

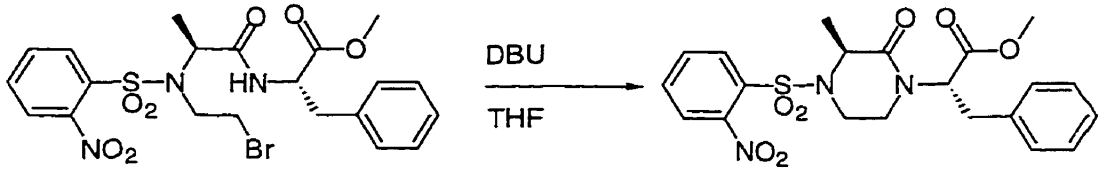


32c 33c

A solution of N-(2-nitrobenzenesulfonyl)PheTrpOMe (0.257 g, 0.468 mmol) in 4 ml dry THF at 0 °C was treated with triphenylphosphine (0.182 g, .695 mmol) and bromoethanol (0.054 ml, 0.762 mmol). DEAD (0.115 ml, 0.661 mmol) was added dropwise to the solution that was allowed to come to room temperature and then stirred for 24 hours. The solvent was then evaporated under vacuum and ethyl acetate added. This was washed with 10 % $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude product which was chromatographed (1 : 1 Hexanes : Ethyl acetate) to yield the product (0.212 g, 70 %) as an oil. R_f 0.27 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 2.80 - 2.90 (m, 2 H, NCH_2CH_2Br and $CHCH_2C_6H_5$), 3.05 - 3.10 (m, 1 H, NCH_2CH_2Br), 3.14 (A of ABX, J = 14.5 Hz, J = 8.5 Hz, 1 H, $CHCH_2$-Indole), 3.20 (B of ABX, J = 14.0 Hz, J = 8.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.32 (B of ABX, J = 15.0 Hz, J = 4.5 Hz, 1 H, $CHCH_2$-Indole), 3.60 - 3.65 (m, 1 H, NCH_2CH_2Br), 3.65 (s, 3 H, $COOCH_3$), 3.76 (X of ABX further coupled to NH, J = 15.1 Hz, J = 11.5 Hz, J = 5.5 Hz, 1 H, NCH_2CH_2Br), 4.51 (X of ABX, J = 7.5 Hz, J = 7.5 Hz, 1 H, $CHCH_2C_6H_5$), 4.68 - 4.74 (m, 1 H, $CHCH_2$-Indole), 6.75 (d, J

= 7.0 Hz, 1 H, NHCO), 7.00 - 7.20 (m, 13 H, C_6H_5, C_6H_4, C_8H_5NH), 8.28 (br s, C=CH-NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.08 ($CHCH_2C_6H_5$), 29.86 (CH_2Br), 36.23 ($CHCH_2$-Indole), 47.75 (NCH_2CH_2Br), 53.65 ($CHCH_2$-Indole), 53.97 ($COOCH_3$), 61.74 ($CHCH_2C_6H_5$), 109.76 (C_8H_5NH), 111.99 (C_8H_5NH), 118.82 (C_8H_5NH), 120.12 (C_8H_5NH), 122.72 (C_8H_5NH), 124.18 (C_8H_5NH), 124.82 (C_8H_5NH), 127.17 (C_6H_5), 128.70 (C_6H_5), 129.43 (C_6H_4), 131.22 (C_6H_4) 132.28 (C_6H_4), 132.56 (C_6H_4), 134.20 (C_6H_4), 135.93 (C_6H_5) , 136.53 (C_8H_5NH), 147.68 (C_6H_4), 168.97 (CONH), 171.63 ($COOCH_3$); MS (FAB, NBA) m/z 659 (6.8 %, $(M + H)^+$); 657 (6.5 %, $(M + H)^+$.

Methyl (2S)-2-[3'-methyl-4'-(4-nitrobenzenesulfonyl)-2'-oxopiperazin-1'-yl]-3-phenyl propionate : (34a)

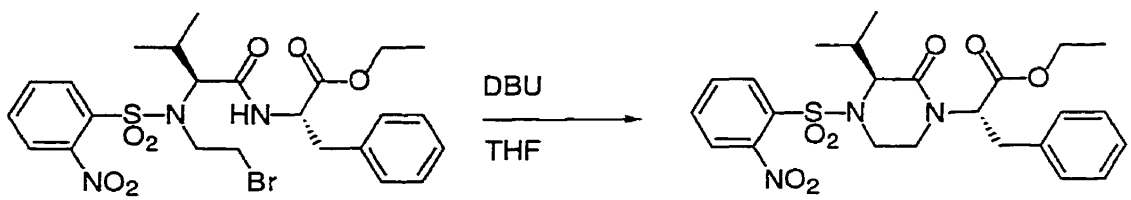


33a **34a**

A solution of **33a** (0.042 g, 0.077 mmol) in 2 ml dry THF at 0 °C and DBU (0.030 ml, 0.201 mmol) was added. The solution was stirred for 24 hours and the solvent evaporated under vacuum. Ethyl acetate was added and the solution was washed with dilute HCl and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.034 g, 97 %) as an oil. R_f 0.10 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 1.10 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 2.95 - 3.40 (m, 6 H, NCH_2CH_2N and $CHCH_2C_6H_5$), 3.68 (s, 3 H, $COOCH_3$), 4.40 (q, J = 7.1 Hz, 1 H, $CHCH_3$), 5.27 (dd, J = 11.6 Hz, J = 5.4 Hz, 1 H, $CHCH_2C_6H_5$), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.60 - 7.70 (m, 3 H, $SO_2C_6H_4NO_2$), 7.95 - 8.05 (m, 1 H, $SO_2C_6H_4NO_2$), ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.20 ($CHCH_3$), 34.21 ($CHCH_2C_6H_5$), 39.55 and 44.01 (NCH_2CH_2N), 52.52

($COOCH_3$), 54.53 ($CHCH_3$), 57.37 ($CHCH_2$), 124.56 (C_6H_4), 127.08 (C_6H_5), 128.70 (C_6H_4), 128.80 (C_6H_5), 130.92 (C_6H_5), 132.22 (C_6H_4), 133.50 (C_6H_4), 133.96 (C_6H_4), 136.16 (C_6H_5), 147.30 (C_6H_4) 168.10 (NHCO), 170.29 ($COOCH_3$); MS (FAB, NBA) m/z 462 (19.2 %, $(M + H)^+$), 402 (8.1 %, $M - COOCH_3$).

Ethyl (2S)-2-[3'-isopropyl-4'-(4-nitrobenzenesulfonyl)-2'-oxopiperazin-1'-yl]-phenyl propionate : (34b)

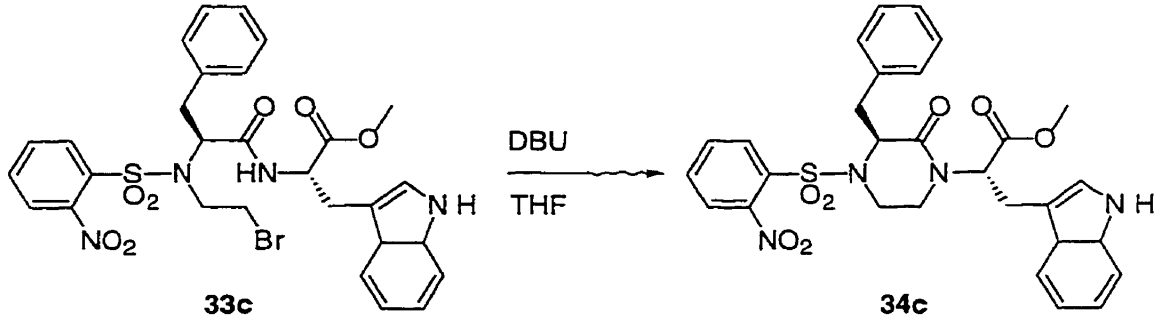


33b **34b**

A solution of **33b** (0.145 g, 0.248 mmol) in 7 ml dry THF at 0 °C was treated with DBU (0.088 ml, 0.581 mmol) and the solution was stirred for 24 hours. Solvent was evaporated and ethyl acetate added which was then washed with 1 N HCl and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.078 g, 62.5 %). R_f 0.31 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.69 and 0.71 (two d, J = 7.5 Hz each, 3 H each, $CH(CH_3)_2$), 1.19 (t, J = 7.0 Hz, 3 H, $COOCH_2CH_3$), 1.78 (m, 1 H, $CH(CH_3)_2$), 2.92 (dd, J = 15 Hz, J = 12 Hz, 1H, $CHCH_2C_6H_5$), 3.10 - 3.20 (m, 1 H, NCH_2CH_2N), 3.20 - 3.30 (m, 1 H, NCH_2CH_2N), 3.30 - 3.40 (m, 2 H, NCH_2CH_2N, $CHCH_2C_6H_5$), 3.75 - 3.85 (m, 1 H, NCH_2CH_2N), 4.00 - 4.15 (m, 3 H, $CHCOOCH_2CH_3$), 5.31 (dd, J = 11.5 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.60 - 7.70 (m, 3 H, C_6H_4), 7.95 (d, J = 7.0 Hz, 1 H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.91 ($COOCH_2CH_3$), 19.28 and 19.38 ($CH(CH_3)_2$), 31.41 ($CH(CH_3)_2$), 34.10 ($CHCH_2C_6H_5$), 40.57 and 42.36 (NCH_2CH_2N), 56.96 ($CHCH_2C_6H_5$), 61.28 ($COOCH_2CH_3$), 64.36 ($CHCH(CH_3)_2$), 124.16 (C_6H_4),

126.79 (C_6H_5), 128.47 (C_6H_5), 128.51 (C_6H_5), 130.62 (C_6H_4), 131.86 (C_6H_4), 132.74 (C_6H_4), 133.67 (C_6H_4), 136.17 (C_6H_5), 147.96 (C_6H_4), 166.97 (CONH), 169.68 ($COOCH_2CH_3$); MS (CI, NH_3) m/z 504 (41.0 %, $(M + H)^+$); 460 (21.2 %, $M - NO_2)^+$).

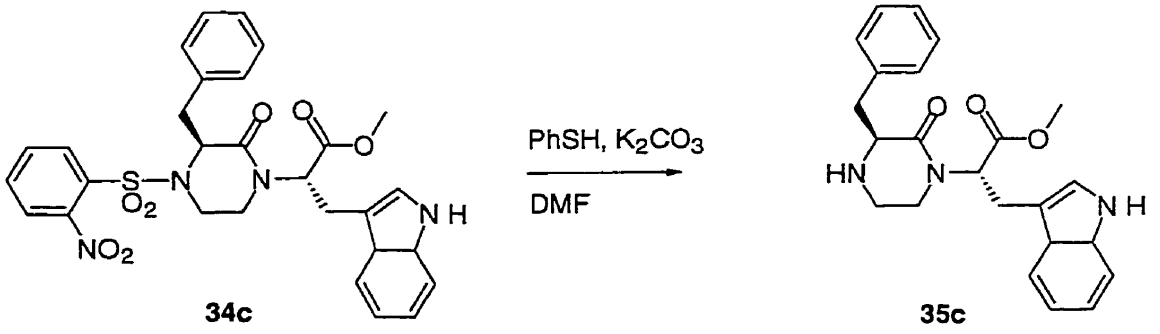
Methyl (2S)-2-[3'-benzyl-4'-(2-nitrobenzenesulfonyl)-2'oxopiperazin-1'-yl]-3-indole propanoate : (34c)



A solution of **33c** (0.180 g, 0.274 mmol) in 5 ml dry THF at 0 °C was treated with DBU (0.4 ml, 2.635 mmol) and the solution was stirred for 24 hours. Solvent was evaporated and ethyl acetate added which was then washed with 1 N HCl and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.092 g, 58 %). R_f 0.21 in 1 : 2 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 2.80 (dd, J = 14.0 Hz, J = 8.0 Hz, 1 H, $CHCH_2$-Indole), 2.95 - 3.15 (m, 3 H, $CHCH_2$-Indole and NCH_2CH_2N), 3.31 (A of ABX, J = 15.0 Hz, J = 11.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.46 (B of ABX, J = 15.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.72 (s, 3 H, $COOCH_3$), 3.70 - 3.80 (m, 2 H, NCH_2CH_2Br), 4.65 (dd, J = 8.0 Hz, J = 4.0 Hz, 1 H, $CHCH_2$-Indole further coupled to NH with J = 1.5 Hz), 5.05 (X of ABX, J = 11.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 6.90 – 7.60 (m, 13 H, C_6H_5, C_6H_4, C_8H_5NH), 8.32 (br s, 1 H, C_8H_5NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.68 ($CHCH_2C_6H_5$), 37.62 ($CHCH_2$-Indole), 39.68 and 45.27 (NCH_2CH_2N), 52.25 ($COOCH_3$), 58.44 ($CHCH_2$-Indole), 60.55 ($CHCH_2C_6H_5$), 110.05 (C_8H_5NH), 111.32 (C_8H_5NH), 117.94 (C_8H_5NH), 119.40

(C_8H_5NH), 122.00 (C_8H_5NH), 123.00 (C_8H_5NH), 124.22 (C_8H_5NH), 126.69 (C_6H_5), 127.99 (C_6H_5), 129.39 (C_6H_4), 130.30 (C_6H_4), 132.03 (C_6H_4), 133.14 (C_6H_4), 133.28 (C_6H_4), 135.98 (C_6H_5), 136.22 (C_6H_4), 166.60 (CON), 170.24 ($COOCH_3$), MS (ES, + ve) m/z 577.33 ($(M + H)^+$); 599.31 ($(M + Na)^+$).

Methyl (2S, 3'S)-2-[3'-benzyl-2'-oxopiperazin-1'-yl] 3-indole propanoate : (35c)



34c **35c**

A solution of K_2CO_3 (0.227 g, 1.645 mmol) in 0.3 ml dry DMF was treated with thiophenol (0.16 ml, 1.558 mmol) and the solution stirred for 30 minutes. The sulfonamide **34c** (0.090 g, 0.159 mmol) was added to this as a 0.3 ml solution in DMF and then stirred for 6 hours. Solvent was evaporated under high vacuum and ethyl acetate added which was washed with 1 N HCl and water and dried over $MgSO_4$. Filtration and solvent evaporation gave crude which was chromatographed (10 % MeOH in Ethyl acetate) to yield the product (0.008 g, 13 %) as a pale yellow oil. R_f 0.26 in Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 2.70 - 2.80 (m, 2 H, $CHCH_2$-Indole and $HNCH_2CH_2N$), 2.85 - 2.95 (m, 1 H, $HNCH_2CH_2N$), 3.00 (dt, J = 11.0 Hz, J = 3.0 Hz, 1 H, $HNCH_2CH_2N$), 3.20 - 3.30 (m, 1 H, $HNCH_2CH_2N$), 3.30 - 3.40 (m, 2 H, $CHCH_2$-Indole and $CHCH_2C_6H_5$), 3.46 (dd, J = 16.0 Hz, J = 4.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.76 (s, 3 H, $COOCH_3$), 5.30 (dd, J = 11.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 6.95 (d, J = 2.0 Hz, 1 H, C=CH-NH), 7.10 - 7.60 (m, 9 H, C_6H_5 and C_8H_5NH), 8.00 - 8.05 (br s, C=CH-NH); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 23.76 ($CHCH_2C_6H_5$), 37.91 ($CHCH_2$-Indole), 41.75 and 46.03 (NCH_2CH_2N), 52.16 ($COOCH_3$), 57.36 ($CHCH_2C_6H_5$), 60.51 ($CHCH_2$-Indole), 111.02 (C_8H_5NH), 118.27 (C_8H_5NH), 119.38 (C_8H_5NH), 121.98 (C_8H_5NH), 126.36 (C_6H_5), 128.42 (C_6H_5), 129.36 (C_6H_5), 138.13 (C_6H_5), 160.35 (CON), 161.84 ($COOCH_3$); MS (FAB, NBA) m/z 392; (ES, +ve) 392.36; HRMS (FAB, NBA) Calculated for $C_{23}H_{26}O_3N_3$ 392.19742, Found 392.19744.

Solid Phase Reactions : (Compounds 36 to 43)

Rink amide MBHA resin was taken in a solid phase synthesis vessel and washed twice with 20 % piperidine in DMF. It was then washed thrice each with DMF, CH_2Cl_2 and methanol. It was then dried and reacted with N-(Fmoc)ValOH (3 eq.), HATU (3 eq.) and DIPEA (6 eq.) in DMF for 6 hours. The resin was filtered and washed 3 times with DMF, CH_2Cl_2, methanol and then dried. A few beads were taken out and Kaiser test was performed. When the Kaiser test showed complete coupling had occurred to give N-(Fmoc)Val-MBHA resin **36** (79 % purity, MS Obs. 339 ($(M + H)^+$), the terminal N-(Fmoc) group was removed by washing the resin twice with 20 % piperidine in DMF. The resin was then washed 3 times each with DMF, CH_2Cl_2, methanol and dried. N-(Fmoc)PheOH was coupled to the free amine as before to give N-(Fmoc)PheVal-MBHA resin **37** (81 % purity, MS Obs. 486 ($(M + H)^+$). Kaiser test was performed as before to check for complete coupling. The terminal N-(Fmoc) group was then removed as before with 20 % piperidine in DMF. After washing and drying, the terminal amine group was reacted with 2-nitrobenzenesulfonyl chloride (3 eq.) and DIPEA (6 eq.) in DMF for 6 hours to generate N-(2-nitrobenzenesulfonyl)PheVal-MBHA resin **38** (89 % purity, MS Obs. 449 ($(M + H)^+$). Alternatively, the terminal amine on was reacted with 2,4-dinitrobenzenesulfonyl chloride (3 eq.) and DIPEA (6 eq.) in DMF for 4 hours to give the N-(2,4-dinitrobenzenesulfonyl)PheVal-MBHA resin **39** (63 % purity, MS Obs. 494 ($(M + H)^+$).

Mitsunobu reactions were performed by taking resin **38** or **39** with at least 5 eq. of phosphine (PPh_3 or $P(n\text{-}Bu)_3$), 10 eq. of bromoethanol in DMF, THF or CH_2Cl_2 and adding 5 eq. of DEAD or TMAD at 0 °C and then stirring at room temperature for a minimum of 12 hours. MS of the compound cleaved from the resin always showed peaks corresponding to the starting material.

N-(Fmoc)Phe-MBHA (**40**) and N-(Fmoc)ValPhe-MBHA (**41**) were synthesized in a manner analogous to those described above for **36** and **37**. N-(Fmoc)ValPhe-MBHA resin (**41**) was washed twice with 20 % piperidine in DMF to deprotect the terminal Fmoc group. ValPhe-MBHA resin so generated was reacted with chlorodiethylphosphate (3 eq.) and DIPEA (6 eq.) in DMF for 6 hours to give the diethylphosphoramidate (**42**). Similarly, reaction of ValPhe-MBHA resin with trifluoroacetic anhydride (3 eq.) and pyridine (6 eq.) in DMF at 0 °C for 6 hours gave trifluoroacetamideValPhe-MBHA resin (**43**).

Mitsunobu reactions were performed by taking resin **42** or **43** with at least 5 eq. of phosphine (PPh_3 or $P(n\text{-}Bu)_3$), 10 eq. of bromoethanol in DMF, THF or CH_2Cl_2 and adding 5 eq. of DEAD or TMAD at 0 °C and then stirring at room temperature for a minimum of 12 hours.

N-(2-nitrobenzenesulfonyl)-L-phenylalanine methyl ester : (44)

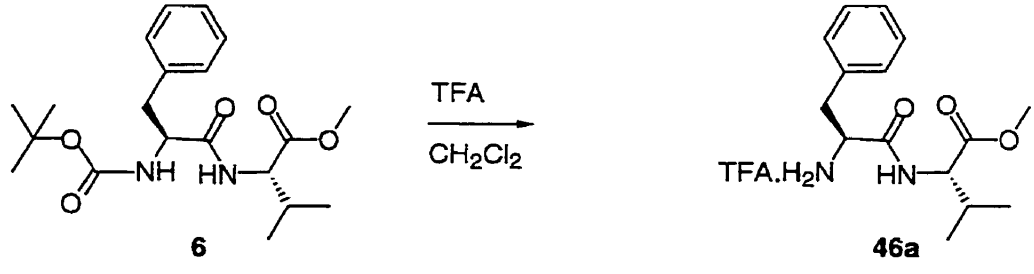
Phenylalanine methyl ester hydrochloride (0.501 g, 2.324 mmol) was taken with 2-nitrobenzenesulfonyl chloride (0.572 g, 2.582 mmol) in 10 ml CH_2Cl_2 at 0 °C and triethylamine (0.71 ml, 5.094 mmol) was added to it. The solution was stirred for 3 hours and the washed with 1 N HCl, 10 % $NaHCO_3$, water and dried over $MgSO_4$. It was then filtered and solvent evaporation gave the product (0.735 g, 86.8 %). ^1H NMR (400 MHz, $CDCl_3$) δ 3.00 - 3.20 (m, 2 H, $CHCH_2$), 3.50 (s, 3 H, $COOCH_3$), 4.40 - 4.50 (m, 1 H,

$CHCH_2$), 5.90 - 6.00 (br s, 1 H, NH), 7.00 - 7.30 (m, 5 H, C_6H_5), 7.60 - 8.00 (m, 4 H, C_6H_4); MS (ES, + ve) 365.19 $(M + H)^+$).

N-(Bromoethyl), N-(2-nitrobenzenesulfonyl)-L-phenylalanine methyl ester : (45)

Sulfonamide **44** (0.735 g, 2.018 mmol) was taken with triphenylphosphine (0.800 g, 3.051 mmol) and bromoethanol (0.25 ml, 3.391 mmol) in 5 ml THF at 0 °C and DEAD (0.50 ml, 3.175 mmol) was added dropwise and the solution maintained at 0 °C for an hour and then stirred at room temperature for 18 hours. The solvent was evaporated under vacuum and the crude product was chromatographed (2 : 1 Hexanes : Ethyl acetate) to give the product (0.500 g, 52.5 %). R_f 0.65 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (400 MHz, $CDCl_3$) δ 2.95 - 3.00 (m, 1 H, $CHCH_2C_6H_5$), 3.20 - 3.30 (m, 2 H, $CHCH_2C_6H_5$, NCH_2CH_2Br), 3.70 (s, 3 H, $COOCH_3$), 3.70 - 3.85 (m, 3 H, NCH_2CH_2Br), 4.85 - 4.95 (m, 1 H, $CHCH_2C_6H_5$), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.50 - 7.90 (m, 4 H, C_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$) 29.36 (CH_2Br), 36.52 ($CHCH_2$), 47.30 (NCH_2), 52.48 ($COOCH_3$), 61.09, ($CHCOOCH_3$) 124.23 (C_6H_4), 127.23 (C_6H_5), 128.73, (C_6H_5) 128.80 (C_6H_5), 130.88 (C_6H_4), 131.76 (C_6H_4), 131.98 (C_6H_4), 134.07 (C_6H_4), 135.45 (C_6H_5), 148.05 (C_6H_4), 170.60 ($COOCH_3$); MS (ES, + ve) m/z 473, 471 $(M + H)^+$).

L-Phenylalanyl-L-valine methyl ester trifluoroacetate : (46a)



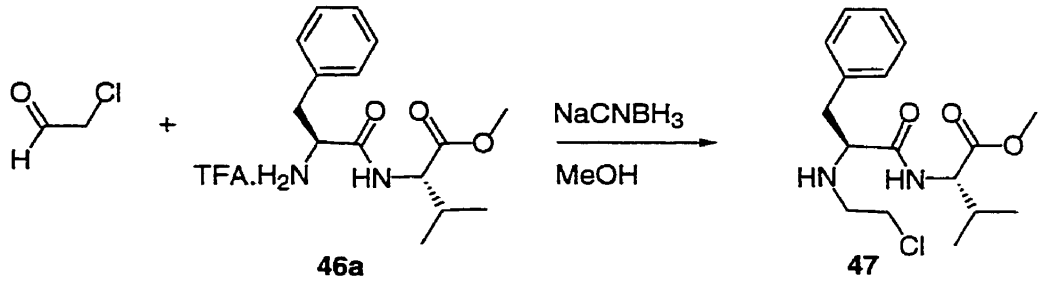
6 **46a**

A solution of N-(*tert*)BocPheValOMe (6.730 g, 17.782 mmol) was taken in 10 ml methylene chloride and 10 ml TFA was added. The solution was stirred for 5 hours and then solvent evaporated with toluene 3 times to give a white solid (7.16 g, quantitative). M.P. (165 - 168 °C); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 18.71and 19.34 ($CH(\mathbf{C}H_3)_2$), 30.57 ($\mathbf{C}H(CH_3)_2$), 37.48 ($\mathbf{C}H_2C_6H_5$), 52.24 ($COO\mathbf{C}H_3$), 53.61 ($\mathbf{C}HCOOCH_3$), 58.21 ($\mathbf{C}HCH_2C_6H_5$), 116.52 (q, J = 287.6 Hz, $CF_3\mathbf{C}OO$), 127.52 ($\mathbf{C}_6H_5$), 128.96 ($\mathbf{C}_6H_5$), 130.07 ($\mathbf{C}_6H_5$), 135.34 ($\mathbf{C}_6H_5$), 161.59 (q, J = 40.3 Hz, $\mathbf{C}F_3COO$), 168.88 (**C**ONH), 171.72 ($\mathbf{C}OOCH_3$).

N-(4-nitrobenzenesulfonyl)phenylalanyl-L-valine methyl ester : (46b)

A solution of **46a** (0.274 g, 0.697 mmol) in 4 ml dry CH2Cl2 was taken at 0 °C with 4-nitrobenzenesulfonyl chloride (0.218 g, 0.984 mmol) and triethylamine (0.21 ml, 2.10 mmol). The solution was stirred for 4 hours at room temperature and then washed with 1 N HCl, water, dried over MgSO4 and filtered. Solvent evaporation gave the product (0.310 g, 96 %). R_f 0.1 in 2 : 1 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 and 0.83 (two d, J = 3.2 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 2.00 - 2.10 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.92 (A of ABX, J = 8.6 Hz, J = 14.1 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.05 (B of ABX, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.71 (s, 3 H, $COOC\mathbf{H}_3$), 3.90 - 4.10 (m, 1 H, $C\mathbf{H}COOCH_3$), 4.40 - 4.50 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 6.00 - 6.10 (s, 1 H, $SO_2N\mathbf{H}$), 6.58 (d, J = 8.2 Hz, 1 H, CON**H**), 7.00 - 7.20 (m, 5 H, $C_6\mathbf{H}_5$), 7.76 (d, J = 8.9 Hz, 2 H, $C_6\mathbf{H}_4$), 8.12 (d, J = 8.9 Hz, 2 H, $C_6\mathbf{H}_4$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.76 and 18.80 ($CH(\mathbf{C}H_3)_2$), 31.35 ($\mathbf{C}H(CH_3)_2$), 38.93 ($CH\mathbf{C}H_2C_6H_5$), 52.93 ($COO\mathbf{C}H_3$), 57.59 ($\mathbf{C}HCOOCH_3$), 58.64 ($\mathbf{C}HCH_2$), 124.26 ($\mathbf{C}_6H_4$), 127.18 ($\mathbf{C}_6H_5$), 128.18 ($\mathbf{C}_6H_5$), 129.31 ($\mathbf{C}_6H_5$), 135.48 ($\mathbf{C}_6H_5$), 145.94 ($\mathbf{C}_6H_4$), 170.14 (**C**ONH), 171.71 ($\mathbf{C}OOCH_3$); MS (CI, NH_3) m/e 464 (100 %, $(M + H)^+$), 434 (35.5 %, $(M - COOCH_3)^+$).

N-(Chloroethyl)-L-phenylalanyl-L-valine methyl ester : (47)



46a **47**

Method A

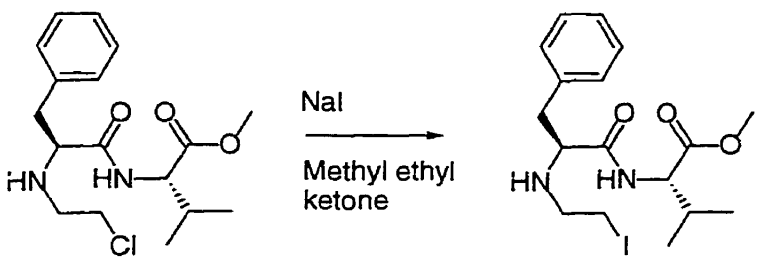
A solution of TFA.PheValOMe **46a** (0.212 g, 0.540 mmol) in 5 ml MeOH at 0 °C was mixed with chloroacetaldehyde (0.1 ml, 0.787 mmol) and $NaCNBH_3$ (0.026 g, 0.414 mmol) was added to it. The solution was stirred for 15 mins. and then solvent was evaporated and ethyl acetate added. The solution was washed with 10 % $NaHCO_3$ and water and the organic layer dried over $MgSO_4$. Solvent evaporation gave the product as a light yellow oil (0.181 g, 98 %) ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86 and 0.89 (two d, J = 6.9 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 2.10 - 2.20 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.60 - 2.90 (m, 3 H, $CHC\mathbf{H}_2C_6H_5$ and $NCH_2C\mathbf{H}_2Cl$), 3.22 (dd, J = 13.8 Hz, J = 3.7 Hz, 1 H, $NC\mathbf{H}_2$), 3.34 (dd, J = 9.7 Hz, J = 3.7 Hz, 1 H, $NC\mathbf{H}_2CH_2Cl$), 3.45 - 3.55 (m, 2 H, $C\mathbf{H}CH_2C_6H_5$ and $NC\mathbf{H}_2CH_2Cl$)), 3.69 (s, 3 H, $COOC\mathbf{H}_3$), 4.49 (dd, J = 9.3 Hz, J = 4.8 Hz, $C\mathbf{H}COOCH_3$), 7.15 - 7.30 (m, 5 H, $C_6\mathbf{H}_5$), 7.76 (d, J = 9.4 Hz, 1 H, N**H**CO); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.73 and 19.17 $CH(C\mathbf{H}_3)_2$), 31.02 ($C\mathbf{H}(CH_3)_2$), 39.64 ($CHC\mathbf{H}_2C_6H_5$), 44.44 ($NCH_2C\mathbf{H}_2Cl$), 49.64 ($N\mathbf{C}H_2CH_2Cl$), 52.14 ($COOCH_3$), 56.69 ($\mathbf{C}HCOOCH_3$), 63.69 ($CHCH_2C_6H_5$), 127.14 ($\mathbf{C}_6H_5$), 128.91 ($\mathbf{C}_6H_5$), 129.12 ($\mathbf{C}_6H_5$), 137.08 ($\mathbf{C}_6H_5$), 172.42 (NHCO), 173.60 ($\mathbf{C}OOCH_3$); MS (CI, NH_3) m/z 341 (31.5 %, $(M + H)^+$); 305 (74.1 %, $(M - Cl)^+$).

Method B

A solution of TFA.PheValOMe **46a** (0.326 g, 0.829 mmol) in ethyl acetate was washed with saturated Na_2CO_3 solution and then water. The organic layer was collected,

dried over $MgSO_4$, and filtered to yield the free amine in quantitative yield. A solution of the free amine in 5 ml 1,2-dichloroethane was treated with chloroacetaldehyde (0.105 ml, 1.653 mmol), sodium triacetoxyborohydride (0.257 g, 01.213 mmol) and acetic acid (0.050 ml) and the solution stirred for 6 hours at room temperature. The solution was diluted with dichloromethane and then washed with 1 N NaOH and water. The organic layer was dried over $MgSO_4$, filtered and solvent evaporation under vacuum gave the product as pale yellow oil (0.234 g, 83 %).

N-(Iodoethyl)-L-phenylalanyl-L-valine methyl ester : (48)

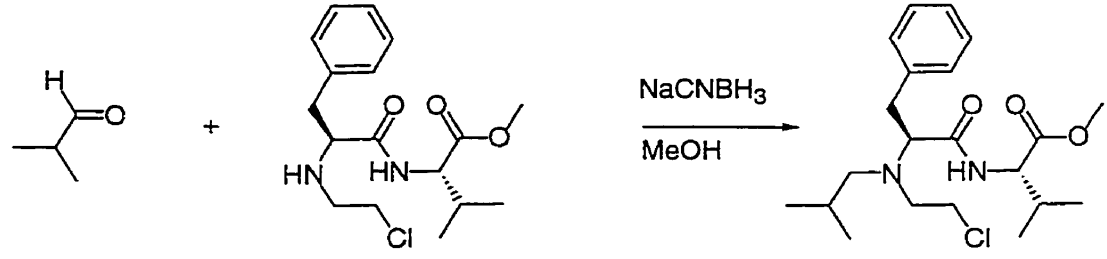


47 48

A solution of **47** (0.031 g, 0.078 mmol) in 3 ml methyl ethyl ketone with sodium iodide (0.034 g, 0.229 mmol) and the solution refluxed for 8 hours. After cooling the solvent was evaporated under vacuum and ethyl acetate added. The solution was then washed with water, dried over $MgSO_4$, and filtered. The solvent was evaporated under vacuum to give the product (0.041 g, quantitative) ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (d, J = 7.2 Hz, 3 H, $CH(CH_3)_2$), 0.90 (d, J = 7.4 Hz, 3 H, $CH(CH_3)_2$), 2.20 (septet, J = 6.9 Hz, 1 H, $CH(CH_3)_2$), 2.65 - 2.90 (m, 3 H, $NHCH_2CH_2I$), 3.10 - 3.20 (m, 2 H, $CHCH_2C_6H_5$), 3.20 - 3.40 (m, 2 H, NCH_2), 3.60 - 3.65 (m, 1 H, $CHCOOCH_3$), 3.70 (s, 3 H, $COOCH_3$), 4.50 (dd, J = 9.3 Hz, J = 4.8 Hz, 1 H, $CHCH_2C_6H_5$), 7.15 - 7.35 (m, 5 H, C_6H_5); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 4.28 (CH_2I), 18.35 ($CH(CH_3)_2$), 18.99 ($CH(CH_3)_2$), 20.57 ($CH(CH_3)_2$), 20.82 ($CH(CH_3)_2$), 26.64 ($CH(CH_3)_2$), 31.23

($\mathbf{C}H(CH_3)_2$), 32.53 ($\mathbf{C}H_2C_6H_5$), 51.96 ($COO\mathbf{C}H_3$), 53.97 ($N\mathbf{C}H_2CH_2I$), 57.52 (**C**H), 59.57 ($N\mathbf{C}H_2CH(CH_3)_2$)), 66.87 (**C**H), 126.13 ($\mathbf{C}_6H_5$), 128.45 ($\mathbf{C}_6H_5$), 129.11 ($\mathbf{C}_6H_5$), 140.08 ($\mathbf{C}_6H_5$), 172.07 (**C**ONH), 172.36 ($\mathbf{C}OOCH_3$); MS (FAB, NBA) m/z 433 ((M + H)$^+$).

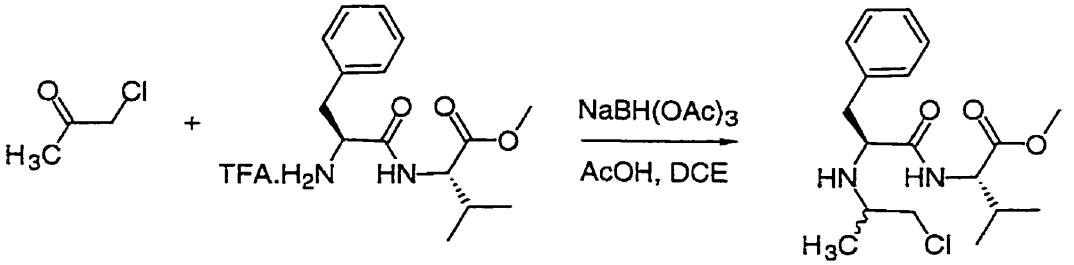
N-Chloroethyl, N-isobutyl-L-phenylalanyl-L-valine methyl ester : (49)



A solution of amine **47** (0.200 g, 0.585 mmol) in 2.50 ml MeOH was treated with isobutyraldehyde (0.080 ml, 0.881 mmol), $NaCNBH_3$ (0.022 g, 1.050 mmol) and acetic acid (3 drops) at room temperature and the solution stirred for 6 hours. Solvent was evaporated under vacuum and ethyl acetate added. The solution was then washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and then filtered. Solvent evaporation under vacuum gave the product (0.196 g, 84.6 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (d, J = 6.5 Hz, 3 H, $NCH_2CH(C\mathbf{H}_3)_2$), 0.90 - 0.96 (m, 9 H, $NCH_2(C\mathbf{H}_3)_2$ and $CH(C\mathbf{H}_3)_2$), 1.65 - 1.75 (m, 1 H, $NC\mathbf{H}_2CH(CH_3)_2$), 2.10 - 2.20 (m, 2 H, $C\mathbf{H}(CH_3)_2$ and $NC\mathbf{H}_2CH(CH_3)_2$), 2.35 (dd, J = 14.0 Hz, J = 6.5 Hz, 1 H, $NC\mathbf{H}_2CH(CH_3)_2$), 2.80 - 2.90 (m, 3 H, $NC\mathbf{H}_2CH_2Cl$ and $CHC\mathbf{H}_2C_6H_5$), 3.36 (dd, J = 14.0 Hz, J = 6.0 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.50 - 3.53 (m, 2 H, $NCH_2C\mathbf{H}_2Cl$), 3.62 - 3.66 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 3.68 (s, 3 H, $COOC\mathbf{H}_3$), 4.46 (dd, J = 9.0 Hz, J = 5.5 Hz, 1 H, $C\mathbf{H}COOCH_3$), 7.20 - 7.60 (m, 5 H, $C_6\mathbf{H}_5$), 7.51 (d, J = 9. Hz, N**H**CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.23 ($NCH_2CH(\mathbf{C}H_3)_2$), 18.78, 20.46, 20.63 ($NCH_2CH(\mathbf{C}H_3)_2$ and $CH(\mathbf{C}H_3)_2$), 26.58 ($NCH_2\mathbf{C}H(CH_3)_2$), 31.01

($CHCH(CH_3)_2$), 32.37 ($CHCH_2C_6H_5$), 42.36 ($NCH_2CH_2Cl_2$), 51.79 ($COOCH_3$), 52.82 (NCH_2CH_2Cl), 57.49 ($CHCOOCH_3$), 59.72 (NCH_2CH_2Cl), 66.89 ($CHCH_2C_6H_5$), 125.98 (C_6H_5), 128.30 (C_6H_5), 128.98 (C_6H_5), 140.03 (C_6H_5), 171.92 (NHCO), 172.48 ($COOCH_3$); MS (FAB, NBA) m/z 397(85.8 %, $(M + H)^+$).

N-(Chloroisopropyl)-L-phenylalanyl-L-valine methyl ester : (50)

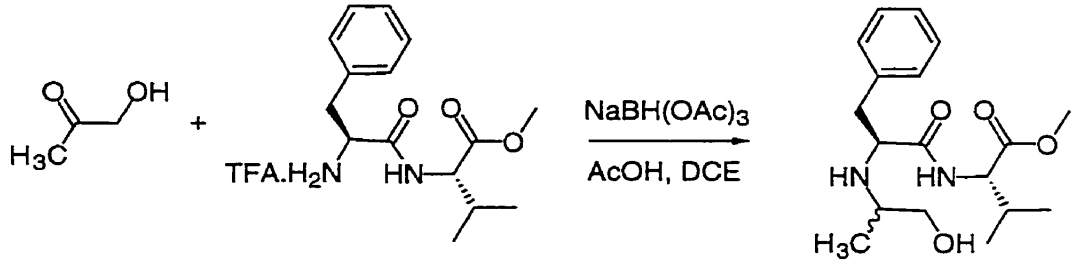


46a **50**

46a (0.222 g, 0.565 mmol) was taken in ethyl acetate and washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the free amine (0.152 g, 97 %). The free amine was taken in 3 ml 1,2-dichloroethane with chloroacetone (0.044 ml, 0.552 mmol) and sodium triacetoxyborohydride (0.162 g, 0.7667 mmol) was added followed by acetic acid (0.031 ml, 0.542 mmol) and the solution stirred for 4 hours. The solution was washed with 1 N NaOH, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product (0.195 g, quantitative). ^1H NMR (270 MHz, $CDCl_3$) 0.70 - 0.90 (m, 9 H, $CH(CH_3)_2$, $CHCH_3$), 2.10 - 2.20 (m, 1 H, $CH(CH_3)_2$), 2.58 (A of ABX, J = 13.6 Hz, J = 10.1 Hz, 1 H, $CHCH_2C_6H_5$), 2.65 - 2.80 (m, 1 H, $CHCH_3$), 3.10 - 3.40 (m, 3 H, CH_2Cl, $CHCOOCH_3$), 3.44 (B of ABX, J = 10.9 Hz, J = 4.9 Hz, 1 H, $CHCH_2C_6H_5$), 3.63 (s, 3 H, $COOCH_3$), 4.45 (X of ABX, J = 9.3 Hz, J = 4.9 Hz, 1 H, $CHCH_2C_6H_5$), 7.10 - 7.30 (m, 5 H, C_6H_5), 7.88 (d, J = 9.6 Hz, 1 H, CONH); ^{13}C NMR (17.01 and 17.52 ($CH(CH_3)_2$), 18.86 ($CHCH_3$), 30.71 ($CH(CH_3)_2$), 39.58 ($CHCH_2C_6H_5$), 50.23 (CH_2Cl), 51.77 (NCH), 53.02 ($COOCH_3$), 56.49 ($CHCOOCH_3$),

61.70 (**C**HCH$_2$C$_6$H$_5$), 126.82 (**C**$_6$H$_5$), 128.57 (**C**$_6$H$_5$), 128.83 (**C**$_6$H$_5$), 137.00 (**C**$_6$H$_5$), 172.02 (**C**ONH), 173.73 (**C**OOCH$_3$); MS (CI, NH$_3$) m/z 355 (1.2 %, (M + H)$^+$).

N-(hydroxyisopropyl)-L-phenylalanyl-L-valine methyl ester : (51)



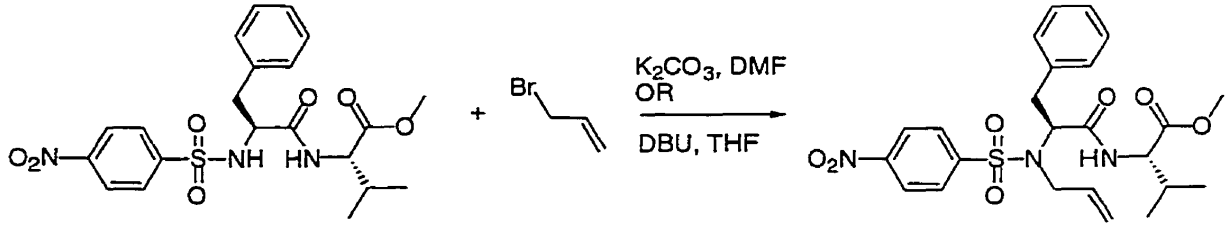
46a **51**

46a (0.310 g, 0.788 mmol) was taken in ethyl acetate and washed with 10 % NaHCO$_3$, water, dried over MgSO$_4$ and filtered. Solvent evaporation gave the free amine (0.250 g). The free amine was taken in 4 ml 1,2-dichloroethane with acetol (0.060 ml, 0.876 mmol) and sodium triacetoxyborohydride (0.235 g, 1.110 mmol) was added followed by acetic acid (3 drops) and the solution stirred for 4 hours. The solution was washed with 1 N NaOH, water, dried over MgSO$_4$ and filtered. Solvent evaporation gave the product (0.235 g, 89 %). ^{1}H NMR (270 MHz, CDCl$_3$) δ 0.65 - 0.66 (m, 3 H, CHC**H**$_3$), 0.80 - 0.90 (m, 6 H, CH(C**H**$_3$)$_2$), 2.00 - 2.20 (m, 1 H, C**H**(CH$_3$)$_2$), 2.40 - 2.60 (m, 1 H, C**H**CH$_3$), 3.10 - 3.50 (m, 4 H, CHC**H**$_2$C$_6$H$_5$, C**H**$_2$OH), 3.50 - 3.55 (m, 4 H, C**H**COOC**H**$_3$), 4.49 (dd, J = 9.6 Hz, J = 4.9 Hz, 1 H, C**H**CH$_2$C$_6$H$_5$), 7.10 - 7.30 (m, 5 H, C$_6$**H**$_5$), 8.26 (d, J = 9.6 Hz, 1 H, N**H**); ^{13}C NMR (67.5 MHz, CDCl$_3$) δ 16.26 (CH**C**H$_3$), 17.41 and 18.91 (CH(**C**H$_3$)$_2$), 30.59 (**C**H(CH$_3$)$_2$), 39.59 (CH**C**H$_2$C$_6$H$_5$), 52.05 (COO**C**H$_3$), 54.70 (**C**H), 56.35 (**C**H), 62.16 (**C**H), 66.43 (**C**H2OH), 126.70 (**C**$_6$H$_5$), 128.37 (**C**$_6$H$_5$), 128.75 (**C**$_6$H$_5$), 137.25 (**C**$_6$H$_5$), 173.54 (**C**ONH), 174.46 (**C**OOCH$_3$); MS (FAB, NBA) m/z 337 (89 %, (M + H)$^+$), 245 (19.6 %, (M - Bn)$^+$).

N, N'-(Dichloroethyl)-L-phenylalanine-L-valine-MBHA resin : (52)

Resin **37** was washed twice with 20 % piperidine in DMF and then thrice each with DMF, CH_2Cl_2, methanol and then dried. It was then reacted with chloroacetaldehyde (3 eq.) and sodium cyanoborohydride (1 eq.) in trimethyl orthoformate for 12 hours to give the dialkylated product (MS Obs. 390, 388 $(M + H)^+$).

N-(allyl), N'-(4-Nitrobenzenesulfonyl)-L-phenylalanyl-L-valine methyl ester : (53)



46b **53**

Method A

A solution of N-(4-Nitrobenzenesulfonyl)-L-phenylalanyl-L-valine methyl ester (**46b**) (0.142 g, 0.306 mmol) in 2 ml dry DMF was taken with K_2CO_3 (0.087 g, 0.633 mmol) and stirred for 20 minutes during which time the solution changed its color from light yellow to dark brown. Allyl bromide (0.130 ml, 1.502 mmol) was then added and the solution stirred for 6 hours. DMF was removed under vacuum and ethyl acetate added. The solution was then washed with 1 N HCl, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product as a bright yellow oil (0.145 g, 94.2 %). R_f 0.25 in 2 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.94 and 0.97 (two d, J = 7.0 Hz each, 3 H each, $CH(CH_3)_2$), 2.15 – 2.25 (m, 1 H, $CH(CH_3)_2$), 2.87 (A of ABX J = 15.0 Hz, J = 9.5 Hz, 1 H, $CHCH_2C_6H_5$), 3.24 (B of ABX J = 15.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.74 (s, 3 H, $COOCH_3$), 4.03 (A of ABX J = 16.0 Hz, J = 6.0 Hz, 1 H, $NCH_2CH=CH_2$), 4.10 (B of ABX J = 16.0 Hz, J = 7.0 Hz, 1 H, $NCH_2CH=CH_2$), 4.42 (X of ABX J = 8.0 Hz, J = 5.0 Hz, 1 H, $CHCOOCH_3$), 4.67 (X of ABX, J = 9.5 Hz, J =

6.0 Hz, 1 H, C**H**$CH_2C_6H_5$), 5.18 (d, J = 10.0 Hz, 1 H, CH=C**H**$_2$), 5.32 (d, J = 18.0 Hz, 1 H, CH=C**H**$_2$), 5.70 – 5.80 (m, 1 H, C**H**=CH_2), 6.91 (d, J = 8.0 Hz, 1 H, N**H**CO), 7.00 – 7.15 (m, 5 H, C_6**H**$_5$), 7.58 (d, J = 9.0 Hz, 2 H, C_6**H**$_4$), 8.05 (d, J = 9.0 Hz, 2 H, C_6**H**$_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.46 and 18.92 (CH(C**H**$_3$)$_2$), 30.69 (**C**H$(CH_3)_2$), 34.47 (CH**C**$H_2C_6H_5$), 47.70 (N**C**H_2CH=CH_2), 52.06 (COO**C**H_3), 57.50 (**C**HCOOCH_3), 62.02 **C**H$CH_2C_6H_5$), 119.72 (NCH_2CH=**C**H_2), 123.87 (**C**$_6H_4$), 126.66 (**C**$_6H_4$), 127.98 (**C**$_6H_5$), 128.51 (**C**$_6H_5$), 128.95 (**C**$_6H_5$), 133.04 (NCH_2**C**H=CH_2), 136.68 (**C**$_6H_5$), 145.39 (**C**$_6H_4$), 149.54 (**C**$_6H_4$), 168.97 (NH**C**O), 171.66 (**C**OOCH$_3$); MS (FAB, NBA) m/z 504 (7.6 %, $(M + H)^+$).

Method B

A solution of N-(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valine methyl ester (**46b**) (0.398 g, 0.859 mmol) was taken in 5 ml dry THF and allyl bromide (0.37 ml, 4.275 mmol) was added followed by DBU (0.39 ml, 2.574 mmol) and the solution was stirred for 2 hours. Solvent was removed under vacuum and ethyl acetate was added. The solution was washed with 1 N HCl, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (5 : 2 Hexanes : Ethyl acetate) to give the product (0.345 g, 80 %) as an oil.

N-(2,3 epoxypropyl), N-(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valyl methyl ester : (54)

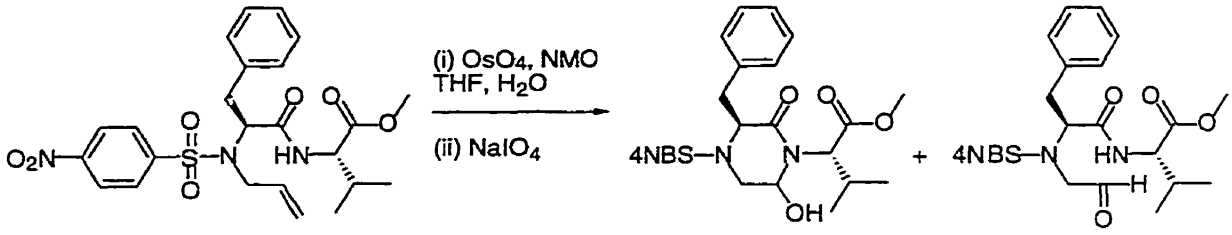
A solution of N-(allyl), N-(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valine methyl ester **53** (0.180 g, 0.357 mmol) in 1.5 ml dry CH_2Cl_2 was taken with mCPBA (prewashed with NaH_2PO_4/NaOH buffer ; 0.132 g, 0.766 mmol) and 2,6-di*tert*butyl pyridine (0.16 ml, 0.712 mmol) was added at 0 °C and the solution stirred overnight. The solution was diluted with CH_2Cl_2 and washed with sodium thiosulphate solution, 0.5 N NaOH solution and water, dried over $MgSO_4$ and filtered. The organic layer was evaporated to give the product

(0.123 g, 65 %). ^{1}H and ^{13}C NMR spectra showed doubling of peaks due the presence of two diastereomers. MS (FAB, NBA) m/z 520 (10.3 %, $(M + H)^+$), 460 $(M - COOCH_3)^+$).

N-(2,3 dibromopropyl), N-(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valyl methyl ester : (55)

Allyl derivative **53** (0.067 g, 0.144 mmol) was taken in 1.5 ml dry THF at 0 °C and bromine (0.010 ml, 0.194 mmol) was added followed by DBU (0.070 ml, 0.462 mmol) and the solution stirred for 4 hours at room temperature. The solvent was removed under vacuum and the crude product chromatographed (2 : 1 Hexanes : Ethyl acetate) to give the product (0.052 g, 54 %). R_f 0.32 in 2 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.93 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 2.10 - 2.20 (m, 1 H, $CH(CH_3)_2$), 3.00 - 3.30 (m, 2 H, CH_2Br), 3.40 - 3.50 (m, 2 H, $CHCH_2C_6H_5$), 3.50 - 3.60 (m, 1 H, NCH_2), 3.70 (s, 3 H, $COOCH_3$), 4.10 - 4.20 (m, 1 H, NCH_2), 4.40 - 4.70 (m, 2 H, $CHCOOCH_3$ and $CHCH_2$), 5.00 - 5.10 (m, 1 H, CHBr), 6.72 (d, J = 8.7 Hz, 1 H, CONH), 7.00 - 7.20 (m, 5 H, C_6H_5), 7.70 - 7.80 (m, 2 H, C_6H_4), 8.10 - 8.20 (m, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.62 and 18.78 ($CH(CH_3)_2$), 31.19 ($CHCH_3)_2$), 35.10 ($CHCH_2$), 36.00 (CH_2Br), 50.82 (NCH_2), 52.30 ($CHCOOCH_3$), 57.64 ($COOCH_3$), 62.49 ($CHCH_2$), 70.26 (CHBr), 124.21 (C_6H_4), 127.28 (C_6H_5), 128.21 (C_6H_5), 128.76 (C_6H_5), 128.91 (C_6H_4), 135.51 (C_6H_5), 144.75 (C_6H_4), 150.04 (C_6H_4), 171.17 (CONH), 171.64 ($COOCH_3$).

Cyclized amidol : 56 and Uncyclized aldehyde : 57



53 56 57

Method A : Reaction of 53 with $NMO/OsO_4/NaIO_4$

A solution of alkene **53** (0.157 g, 0.312 mmol) in 0.3 ml THF was treated with NMO (0.040 g, 0.341 mmol) and OsO_4 (0.090 ml of a 0.072 M solution in benzene, 0.006 mmol) at room temperature and the solution stirred for 6 hours. When TLC showed no more starting material (approx. 8 hours), $NaIO_4$ (0.278 g, 1.296 mmol) was added and the solution diluted with water and stirred overnight at room temperature. The solution was taken in ethyl acetate and washed with water and dried over $MgSO_4$. Filtration and solvent evaporation under vacuum gave the crude product that was chromatographed to yield two products **56** and **57**.

56 : 33.4 %

^1H NMR (500 MHz, $CDCl_3$) δ 0.08 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.96 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 2.30 – 2.40 (m, 1 H, $CH(CH_3)_2$), 2.96 (A of ABX, J = 10.5 Hz, J = 6.5 Hz, 1 H, $CHCH_2C_6H_5$), 3.20 (A of ABX, J = 14.0 Hz, J = 6.5 Hz, 1 H, NCH_2CH), 3.34 (B of ABX, J = 13.0 Hz, J = 4.0 Hz, 1 H, NCH_2CH), 3.57 (B of ABX, J = 15.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.69 (s, 3 H, $COOCH_3$), 4.36 (d, J = 9.5 Hz, 1 H. $CHCOOCH_3$), 4.38 – 4.42 (m, 1 H, $CHCH_2C_6H_5$), 5.06 – 5.12 (m, 1 H, NCH_2CH), 7.16 – 7.28 (m, 5 H, C_6H_5), 7.71 (d, J = 9.0 Hz, 2 H, C_6H_4), 8.24 (d, J = 9.0 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 19.78 ($CH(CH_3)_2$), 20.60 ($CH(CH_3)_2$), 28.72 ($CH(CH_3)_2$), 37.71 ($CHCH_2C_6H_5$), 47.81 (NCH_2), 52.49 ($COOCH_3$), 60.67 (CH), 62.51 (CH), 77.14 $NCH_2CH(OH)N$), 127.58 (C_6H_5), 128.89 (C_6H_5), 129.02 (C_6H_5),

130.08 ($\mathbf{C}_6H_4$), 135.91 ($\mathbf{C}_6H_5$), 142.57 ($\mathbf{C}_6H_4$), 150.35 ($\mathbf{C}_6H_4$), 167.37 (CON), 172.41 ($\mathbf{C}OOCH_3$); MS (FAB, NBA) m/z 506 (2.7 %, $(M + H)^+$); 488 (9.5 %, $(M-H_2O)^+$).

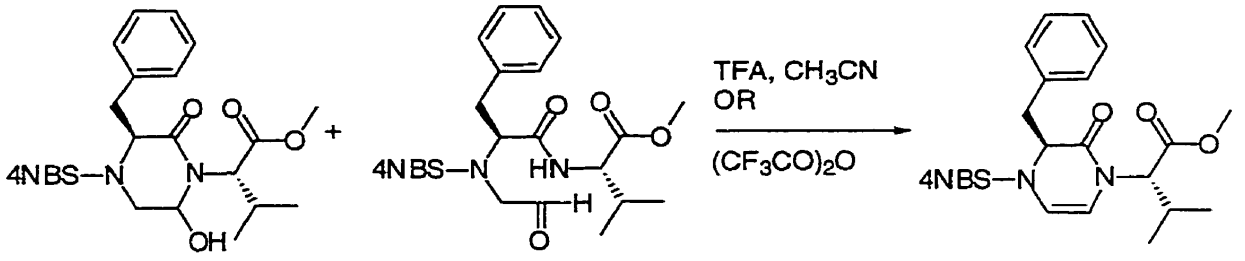
57 : 19.8 %

^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85 and 0.88 (two d, J = 6.9 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 2.05 – 2.15 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.70 (A of ABX, J = 14.5 Hz, J =8.4 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.00 (B of ABX, J =14.5 Hz, J = 6.8 Hz, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.65 (s, 3 H, $COOC\mathbf{H}_3$), 4.00 – 4.30 (m, 2 H, $NC\mathbf{H}_2$), 4.30 – 4.40 (m, 1 H, $C\mathbf{H}COCH_3$), 4.50 (X of ABX, J = 8.4 Hz, J =6.6 Hz, 1 H, $C\mathbf{H}CH_2C_6H_5$), 6.53 (d, J = 7.9 Hz, 1 H, CONH), 6.80 – 6.30 (m, 5 H, $C_6\mathbf{H}_5$), 7.70 (d, J = 8.7 Hz, 2 H, $C_6\mathbf{H}_4$), 8.09 (d, J = 8.9 Hz, 2 H, $C_6\mathbf{H}_4$), 9.45 (s, 1 H, CHO); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.68 and 18.86 ($CH(\mathbf{C}H_3)_2$), 30.98 ($\mathbf{C}H(CH_3)_2$), 35.52 ($CH\mathbf{C}H_2$), 52.54 ($\mathbf{C}OOCH_3$), 53.19 ($N\mathbf{C}H_2$), 57.44 (CH), 61.82 (CH), 124.20 ($\mathbf{C}_6H_4$) , 127.18 ($\mathbf{C}_6H_5$), 128.40 ($\mathbf{C}_6H_4$), 128.75 ($\mathbf{C}_6H_4$), 128.99 ($\mathbf{C}_6H_5$), 129.99 ($\mathbf{C}_6H_5$), 135.52 ($\mathbf{C}_6H_5$), 144.42 ($\mathbf{C}_6H_4$), 150.06 ($\mathbf{C}_6H_4$), 168.77 ($\mathbf{C}ONH$), 171.60 ($\mathbf{C}OOCH_3$), 196.59 (CHO); MS (FAB, NBA) m/z 506 (3.5 %, $(M + H)^+$).

Method B : Reaction of 54 with $HIO_4.2H_2O$

The mixture of epoxides **54** (0.316 g, 0.609 mmol) was taken in 1.5 ml mixture of THF/H_2O (1 : 1) at room temperature and periodic acid (0.529 g, 2.321 mmol) was added and the solution stirred for 8 hours. Solvent was evaporated and ethyl acetate added which was then washed with water, dried over $MgSO_4$ and filtered. The solvent was evaporated to yield the crude, which was then used directly in the next step.

Methyl (2S, 3'S)-2-[3'-benzyl-5'-ene-4'-(4-nitrobenzenesulfonyl)-2'-oxopiperazin-1'-yl]-3-methyl butanoate : (58)



56 57 58

Method A : Using TFA in actonitrile

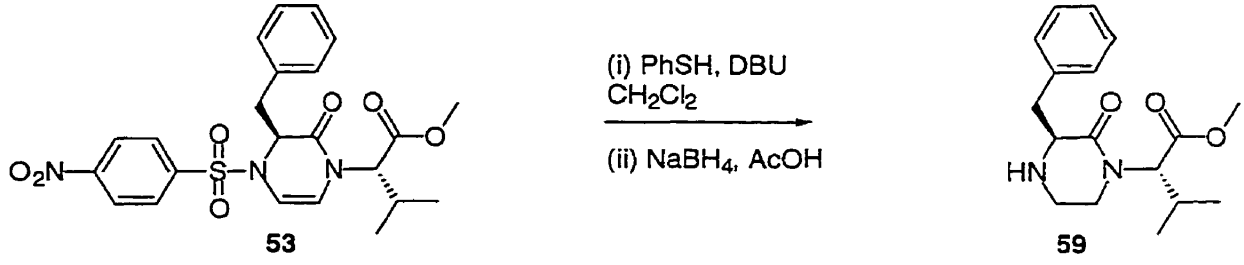
A solution of the crude cyclized amidol **56** and uncyclized aldehyde **57** (0.038 g, 0.076 mmol) in 2 ml dry acetonitrile was mixed with TFA (0.040 ml) and the solution refluxed for 48 hours. Solvent was evaporated under vacuum and ethyl acetate added. The solution washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. The solvent was evaporated under vacuum to yield the product was a light yellow oil (0.034 g, 93 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.73 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 0.86 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 2.00 – 2.50 (m, 1 H, $CH(CH_3)_2$), 2.81 (A of ABX, J = 14.0 Hz, J = 10.0 Hz, 1 H, $CHCH_2C_6H_5$), 2.90 (B of ABX, J = 14.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2C_6H_5$), 3.44 (s, 3 H, $COOCH_3$), 4.45 (d, J = 10.0 Hz, 1 H, $CHCOOCH_3$), 4.60 – 4.65 (m, 1 H, $CHCH_2C_6H_5$), 6.00 – 6.10 (m, 2 H, NCH=CHN), 7.10 – 7.30 (m, 5 H, C_6H_5), 7.80 (d, J = 9.0 Hz, 2 H, C_6H_4), 8.20 (d, J = 9.0 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.73 ($CH(CH_3)_2$), 19.35 ($CH(CH_3)_2$), 28.58 ($CH(CH_3)_2$), 36.18 ($CHCH_2$), 51.93 ($COOCH_3$), 60.09 (CH), 60.26 (CH), 106.17 and 117.84 (CH=CH), 124.25 (C_6H_4), 127.38 (C_6H_5), 128.07 (C_6H_5), 128.59 (C_6H_5), 129.53 (C_6H_4), 134.68 (C_6H_4), 143.25 (C_6H_5), 150.17 (C_6H_4), 163.29 (CON), 170.05 ($COOCH_3$); MS (FAB, NBA) m/z 488 (13.1 %, $(M + H)^+$);

Method B : Using trifluoroacetic anhydride in pyridine

A mixture of the amidol **56** and the uncyclized aldehyde **57** (0.039 g, 0.076 mmol) was taken in 1 ml dry pyridine at 0 °C and trifluoroacetic anhydride (0.027 ml, 0.191

mmol) was added. The solution was stirred for 3 hours and then ethyl acetate added. The solution was washed with 1 N HCl, 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude which was chromatographed to give the product (0.015 g, 40 %).

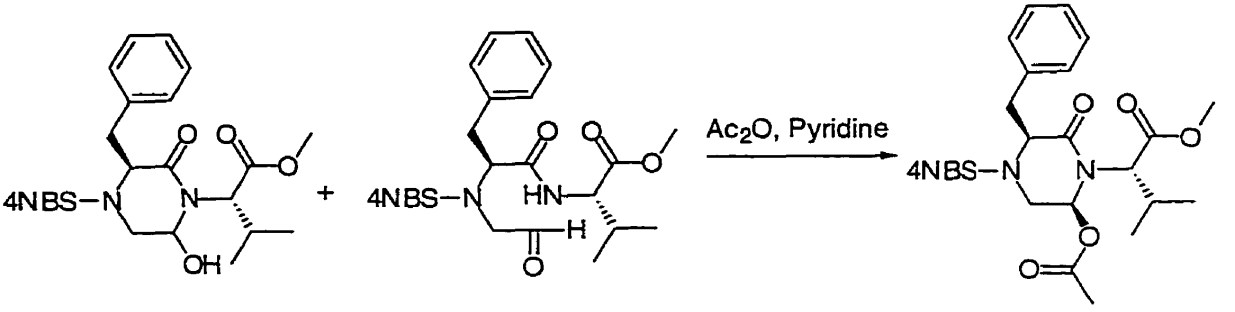
Methyl (2S, 3'S)-2-[3'-benzyl-2'-oxopiperazin-1'-yl]-3-methyl butanoate : (59)



53 59

A solution of alkene **58** (0.060 g, 0.124 mmol) in CH_2Cl_2/THF and DBU (0.050 ml, 0.330 mmol) and benzenethiol (0.032 ml, 0.312 mmol) were added. Excess $NaBH_4$ and acetic acid (2 drops) were then added. The solution was stirred for 10 minutes and then washed with 10 % $NaHCO_3$, water and dried over $MgSO_4$. It was then filtered and solvent evaporation gave the crude which was chromatographed (10 % Methanol in Ethyl acetate) to give the product (0.022 g, 59 %). ^1H and ^{13}C NMR were identical to those obtained for **23**.

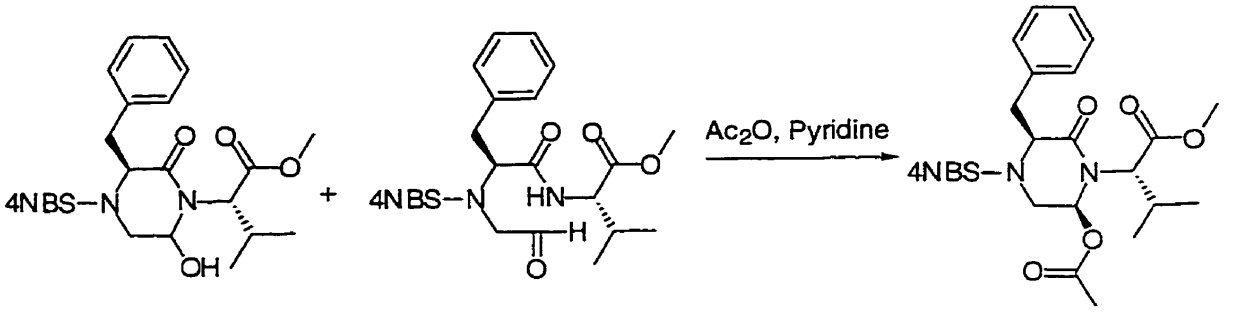
Methyl (2S, 3'S)-2-[6'-O-acetyl-3'-benzyl-4'-(4-nitrobenzenesulfonyl)-2'-oxopiperazin-1'-yl]-3-methyl butanoate : (60)



56 **57** **60**

Method A : Using products obtained from Scheme 28

A mixture of cyclized amidol **56** and the uncyclized aldehyde **57** (0.037 g, 0.072 mmol) in 0.2 ml pyridine was reacted with acetic anhydride (0.040 ml, 0.424 mmol) at 0 °C and the solution stirred for 6 hours. Ethyl acetate was added and the solution washed 4 times with 1 N HCl, 10 % $NaHCO_3$ water, dried over $MgSO_4$ and then filtered. Solvent evaporation gave the crude which was chromatographed (3 : 1 Hexanes : Ethyl acetate) to give the product (0.032 g, 81.2 %). R_f 0.21 in 1 : 2 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 0.94 (d, J =6.5 Hz, 3 H, $CH(CH_3)_2$), 2.00 – 2.10 (m, 4 H, $CH(CH_3)_2$ and CH_3COO), 3.40 – 3.50 (m, 3 H, $CHCH_2C_6H_5$ and NCH_2), 3.60 (s, 3 H, $COOCH_3$), 3.70 – 3.76 (m, 1 H, NCH_2), 4.00 – 4.50 (m, 2 H, $CHCOOCH_3$ and $CHCH_2C_6H_5$), 6.41 (X of ABX, J = 3.9 Hz each, 1 H, NCH_2CHN), 7.20 – 7.30 (m, 5 H, C_6H_5), 7.80 (d, J = 8.5 Hz, 2 H, C_6H_4), 8.28 (d, J = 8.5 Hz, 2 H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.35 ($CH(CH_3)_2$), 19.82 ($CH(CH_3)_2$), 20.69 ($CH(CH_3)_2$), 28.65 (CH_3CO), 38.92 ($CHCH_2C_6H_5$), 46.10 (NCH_2), 51.94 ($COOCH_3$), 61.13 (CH), 62.12 (CH), 76.27 ($CHOCOCH_3$), 124.20 (C_6H_4), 127.13 (C_6H_5), 128.50 (C_6H_5), 128.77 (C_6H_4), 129.80 (C_6H_4), 135.72 (C_6H_4), 142.04 (C_6H_5), 150.15 (C_6H_4), 167.89 ($OCOCH_3$), 169.17 (CON), 170.82 ($COOCH_3$); MS (FAB, NBA) m/z 548 (4.0 %, $(M + H)^+$); 488 (26.7 %, $(M - COOCH_3)^+$).

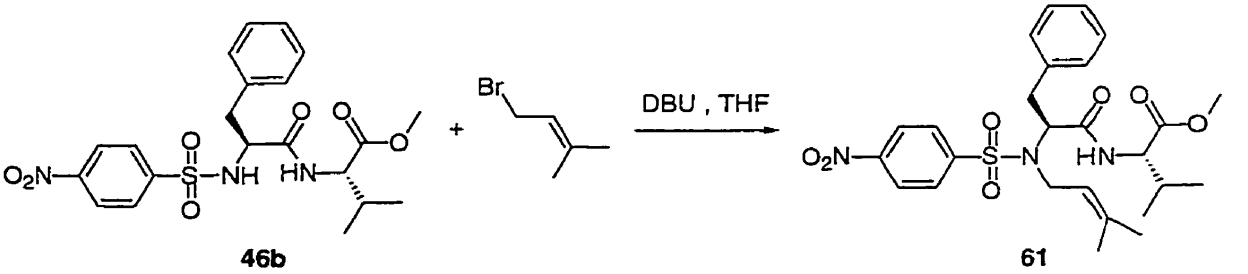


56 57 60

Method B : Using products obtained from Scheme 33

The crude mixture of **56** and **57** obtained (after reaction of epoxide with HIO_4) was taken in 1.5 ml pyridine at 0 °C and 0.5 ml acetic anhydride was added and the solution was stirred at room temperature for 6 hours. Ethyl acetate was added and the solution was washed with 1 N HCl (5 times) and $NaHCO_3$ (3 times) and water, dried over $MgSO_4$ and filtered. The solvent was removed under vacuum and the crude was chromatographed (5 : 2 Hexanes : ethyl acetate) to yield the acetate product **60** (0.128 g, 39 % for 3 steps). ^1H and ^{13}C NMR spectra were identical to the ones obtained earlier.

N-(Dimethylallyl), N'(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valine methyl ester : (61)

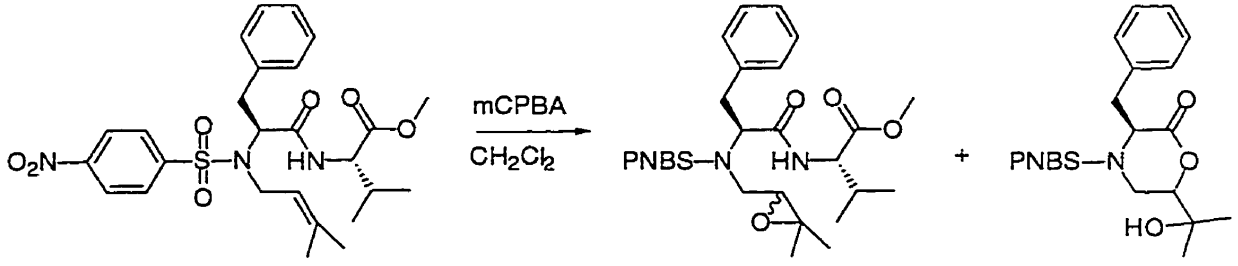


46b 61

Sulfonamide **46b** (0.504 g, 1.087 mmol) was taken in 5 ml dry THF and prenyl bromide (0.64 ml, 5.552 mmol) was added to it followed by DBU (0.48 ml, 3.168 mmol) and the solution stirred for 4 hours. Solvent was evaporated under vacuum and ethyl

acetate added. It was washed with 1 N HCl, water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.528 g, 91.4 %) ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 and 0.93 (two d, J = 6.9 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 1.57 and 1.65 (two s, 3 H each, $CH=C(C\mathbf{H}_3)_2$), 2.10 - 2.20 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.70 - 2.80 (m,1 H, $CHC\mathbf{H}_2C_6H_5$), 3.10 - 3.20 (m, 1 H, $CHC\mathbf{H}_2C_6H_5$), 3.68 (s, 3 H, $COOC\mathbf{H}_3$), 3.90 - 4.10 (m, 2 H, $NC\mathbf{H}_2$), 4.30 - 4.40 (m, 1 H, $C\mathbf{H}COOCH_3$), 4.60 - 4.70 (m, 1 H, $C\mathbf{H}CH_2C_6H_5$), 4.90 - 5.00 (m, 1 H, $NCH_2C\mathbf{H}$), 6.90 - 7.10 (m, 5 H, $C_6\mathbf{H}_5$), 7.40 -7.50 and 7.90 - 8.00 (m, 4 H, $C_6\mathbf{H}_4$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.26 ($CH(\mathbf{C}H_3)_2$), 19.50 ($CH=C(\mathbf{C}H_3)_2$), 20.63 ($CH(\mathbf{C}H_3)_2$), 27.28 ($\mathbf{C}H(CH_3)_2$), 32.38 ($CH=\mathbf{C}(CH_3)_2$), 35.95 ($\mathbf{C}H_2C_6H_5$), 44.54 ($N\mathbf{C}H_2$), 53.40 ($COO\mathbf{C}H_3$), 58.83 (**C**H), 63.17 (**C**H), 119.99 ($C=\mathbf{C}(CH_3)_2$), 124.23 ($\mathbf{C}_6H_4$), 127.08 ($\mathbf{C}_6H_5$), 128.33 ($\mathbf{C}_6H_4$), 128.93 ($\mathbf{C}_6H_5$), 129.43 ($\mathbf{C}_6H_5$), 129.59 ($\mathbf{C}_6H_4$), 137.32 ($\mathbf{C}_6H_5$), 146.17 ($C=\mathbf{C}(CH_3)_2$), 149.63 ($\mathbf{C}_6H_4$), 169.17 (**C**ONH), 171.61 ($COOCH_3$); MS (CI, NH_3) m/z 532 (5.4 %, $(M + H)^+$), 464 (19.3 %, $(M - prenyl)^+$).

N-(2,3-epoxy,3-methylbutyl), N-(4-nitrobenzenesulfonyl)-L-phenylalanyl-L-valyl methyl ester : (62)



61 **62** **63**

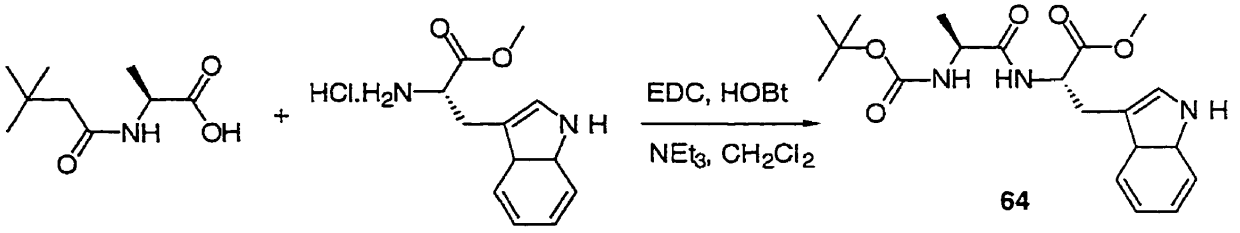
A solution of **61** (0.117 g, 0.219 mmol) was taken in 4 ml CH2Cl2 at 0 °C with mCPBA (0.221 g) and Na_2HPO_4 and the solution stirred for 5 hours. It was washed with thiosulfate solution, 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent

evaporation under vacuum gave the product (0.027 g, 23 %). ^{1}H and ^{13}C NMR complicated due the presence of diastereomers. MS (CI, NH_3) 548 (3.0 %, $(M + H)^+$), 532 (7.6 %, $(M - O)^+$).

Side Product : (63)

Obtained from the above reaction in 25 % yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.95 and 0.99 (two s, 3 H each, $C(CH_3)_2$), 2.50 - 2.70 (m, 2 H, NCH_2), 3.25 - 3.35 (m, 1 H, $CHCH_2C_6H_5$), 3.55 - 3.65 (m, 1 H, $CHCH_2C_6H_5$), 3.70 - 3.75 (m, 1 H, NCH_2CH), 4.40 - 4.45 (m, 1 H, $CHCH_2C_6H_5$), 7.25 - 7.40 (m, 5 H, C_6H_5), 7.90 - 8.00 and 8.30 - 8.40 (m, 4 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 25.03 and 25.82 $C(CH_3)_2$), 41.43 ($CHCH_2C_6H_5$), 43.00 (NCH_2), 59.12 ($CHCH_2C_6H_5$), 69.99 (COH), 80.11 (CH), 124.82 (C_6H_4), 127.91 (C_6H_5), 128.83 (C_6H_5), 128.97 (C_6H_5), 130.41 (C_6H_4), 134.78 (C_6H_5), 140.99 (C_6H_4), 150.67 (C_6H_4), 167.52 (COO); MS m/z 435 (21.8 %, $(M + H)^+$), 417 (13.8 %, $(M - OH)^+$).

N-(*tert*Butoxycarbonyl)-L-alanyl-tryptophan methyl ester : (64)

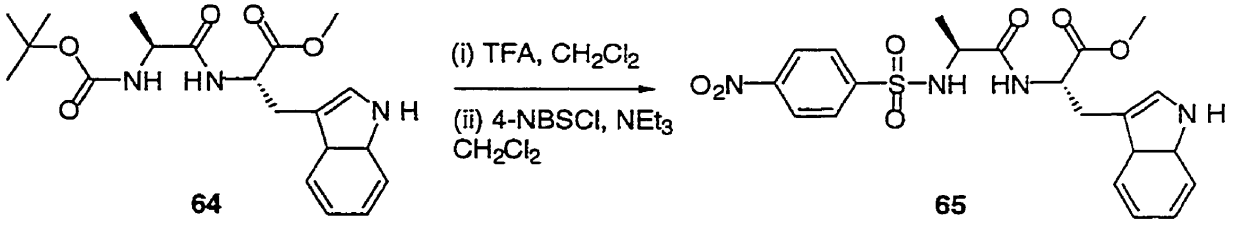


64

A solution of N-(*tert*)Butoxycarbonyl-L-alanine (1.011 g, 5.345 mmol), tryptophan methyl ester hydrochloride (1.501 g, 5.894 mmol), EDC (1.170 g, 6.105 rmmol) and HOBt (0.810 g, 5.994 mmol) were dissolved in 20 ml dry CH_2Cl_2 at 0 °C under nitrogen and triethylamine (1.63 ml, 11.695 mmol) was added. The solution was allowed to come to room temperature and stirred for 8 hours. It was then diluted with CH_2Cl_2 and washed

with 1 N HCl, 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. The organic solvent was evaporated in vacuum to yield the product (2.006 g, 96.4 %). R_f 0.12 in 1 : 1 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 1.28 (d, J = 5.5 Hz, 3 H, $CHCH_3$), 1.41 (s, 9 H, $C(CH_3)_3$), 3.30 (d, J = 5.0 Hz, 2 H, $CHCH_2$-Indole), 3.64 (s, 3 H, $COOCH_3$), 4.15 – 4.20 (br m, 1 H, $CHCH_3$), 4.85 – 4.95 (m, 1 H, $CHCH_2$-Indole), 5.00 – 5.10 (br s, 1 H, COONH), 6.60 – 6.70 (br s, 1 H, CONH), 7.00 (s, 1 H, C=CHNH), 7.10 (t, J = 7.5 Hz, 1 H, C_8H_5NH), 7.16 (t, J = 7.5 Hz, 1 H, C_8H_5NH), 7.32 (d, J = 8.0 Hz, 1 H, C_8H_5NH), 7.51 (d, J = 8.0 Hz, 1 H, C_8H_5NH), 8.40 – 8.50 (br s, 1 H, C_8H_5NH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.34 ($CHCH_3$), 27.53 ($CHCH_2$-Indole), 28.21 ($C(CH_3)_3$), 50.12 (CH), 52.30 ($COOCH_3$), 52.89 (CH), 80.00 ($C(CH_3)_3$), 109.58 (C_8H_5NH), 111.30 (C_8H_5NH), 118.43 (C_8H_5NH), 119.48 (C_8H_5NH), 122.08 (C_8H_5NH), 123.02 (C_8H_5NH), 127.53 (C_8H_5NH), 136.08 (C_8H_5NH), 155.30 (C_8H_5NH), 155.30 (COONH), 172.04 (CONH), 172.36 ($COOCH_3$); MS (EI) m/z 389 (4.28 %, $(M)^+$).

4-(Nitrobenzenesulfonyl)-L-alanyl-L-tryptophan methyl ester : (65)

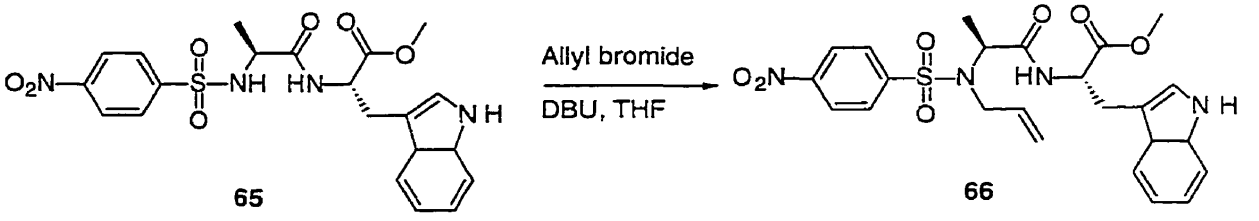


64 65

A solution of N-(*tert*Butoxycarbonyl)-L-alanyl-L-tryptophan methyl ester (2.000 g, 5.135 mmol) in 10 ml dry CH_2Cl_2 was treated with 10 ml dry TFA at room temperature and stirred for 2 hours. Solvent evaporation with toluene three times under vacuum gave the product (2.020 g, 97.5 %) as a white solid. A solution of this TFA salt (2.000 g, 4.956 mmol) in 20 ml dry CH_2Cl_2 was reacted with 4-nitrobenzenesulfonyl chloride (1.115 g, 5.031 mmol) and 4-ethylmorpholine (1.45 ml, 11.393 mmol) at 0 °C and the solution

stirred for 6 hours. The solution was diluted with CH_2Cl_2 and washed with 1 N HCl, 10 % $NaHCO_3$, water and dried over $MgSO_4$. The organic layer was filtered and evaporated under vacuum to yield the crude which was chromatographed (1 : 1 Hexanes : Ethyl acetate) to yield the product (2.214 g, 94 .1 %) as a white foam. ^1H NMR (270 MHz, $CDCl_3$) δ 1.24 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 3.20 – 3.30 (m, 2 H, $CHCH_2$-Indole), 3.69 (s, 3 H, $COOCH_3$), 3.80 – 4.00 (m, 1 H, CH), 4.70 – 4.80 (m, 1 H, CH), 5.53 (d, J = 8.2 Hz, 1 H, CONH), 6.38 (d, J = 7.9 Hz, 1 H, COONH), 6.96 (d, J = 2.5 Hz, 1 H, C_8H_5NH), 7.10 – 7.50 (m, 4 H, C_8H_5NH), 7.86 (d, J = 8.9 Hz, 2 H, C_6H_4), 8.17 (d, J = 8.9 Hz, 2 H, C_6H_4), 8.22 – 8.26 (br s, 1 H, C_8H_5NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.20 ($CHCH_3$), 28.78 ($CHCH_2$-Indole), 53.10 (CH), 53.72 ($COOCH_3$), 54.37 (CH), 110.02 (C_8H_5NH), 112.06 (C_8H_5NH), 118.67 (C_8H_5NH), 120.29 (C_8H_5NH), 122.92 (C_8H_5NH), 123.36 (C_8H_5NH), 124.69 (C_6H_4), 128.00 (C_6H_4) 128.45 (C_8H_5NH), 136.31 (C_8H_5NH), 145.85 (C_6H_4), 150.12 (C_6H_4), 170.14 (CONH), 171.45 ($COOCH_3$).

N-(4-Nitrobenzenesulfonyl)-N-(allyl)-L-alanyl-L-tryptophan methyl ester : (66)

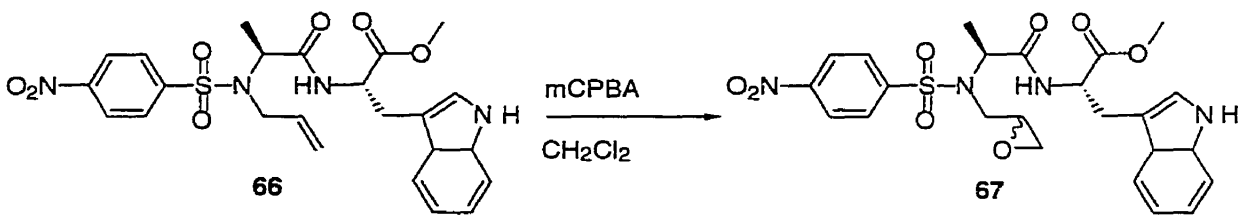


65 66

A solution of N-(4-Nitrobenzenesulfonyl)-L-alanyl-L-tryptophan methyl ester (2.210 g, 4.657 mmol) in 20 ml dry THF was reacted with DBU (2.10 ml, 13.860 mmol) and allyl bromide (2.80 ml, 32.356 mmol) at room temperature and the solution stirred for 5 hours. THF was removed under vacuum and ethyl acetate added. The solution was washed with 1 N HCl and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave

the crude which was chromatographed (2 : 1 Hexanes : Ethyl acetate) to give the product (1.152 g, 48 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.12 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 3.23 (A of ABX, J = 14.7 Hz, J = 7.7 Hz, 1 H, $CHCH_2$-Indole), 3.40 (B of ABX, J = 14.7 Hz, J = 4.7 Hz, 1 H, $CHCH_2$-Indole), 3.51 (A of ABX, J = 16.0 Hz, J = 7.0 Hz, 1 H, $NCH_2CH=CH_2$), 3.62 (B of ABX, J = 16.0 Hz, J = 5.7 Hz, 1 H, $NCH_2CH=CH_2$), 3.73 (s, 3 H, $COOCH_3$), 4.43 (q, J = 7.2 Hz, 1 H, $CHCH_3$), 4.70 – 4.78 (m, 1 H, $CHCH_2$-Indole), 4.80 (dd, J = 10.0 Hz, J = 1.1 Hz, 1 H, $NCH_2CH=CH_2$), 4.98 (dd, J = 16.9 Hz, 1 H, $NCH_2=CH_2$), 5.15 – 5.30 (m, 1 H, $NCH_2CH=CH_2$), 6.65 (d, J = 6.9 Hz, 1 H, CONH), 7.10 – 7.40 (m, C_8H_5NH), 7.58 (d, J = 7.9 Hz, 1 H, C_8H_5NH), 7.88 (d, J = 8.9 Hz, 2 H, C_6H_4), 8.25 (d, J = 8.9 Hz, 2 H, C_6H_4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.32 ($CHCH_3$), 27.51 ($CHCH_2$-Indole), 47.62 (NCH_2), 52.59 ($COOCH_3$), 52.86 (CH), 55.68 (CH), 111.49 (C_8H_5NH), 118.61 (C_8H_5NH), 119.27 ($NCH_2CH=CH_2$), 120.26 (C_8H_5NH), 122.61 (C_8H_5NH0, 123.41 (C_8H_5NH), 124.47 (C_6H_4), 127.45 (C_8H_5NH), 128.39 (C_6H_4), 133.11 ($NCH_2CH=CH_2$), 136.54 (C_8H_5NH), 145.70 (C_6H_4), 150.10 (C_8H_5NH), 160.46 (C_6H_4), 169.40 (CONH), 171.87 ($COOCH_3$).

N-(2,3-epoxypropyl), N'-(4-nitrobenzenesulfonyl)-L-alanyl-L-tryptophan methyl ester : (67)



A solution of N-(Allyl), N'-(4-nitrobenzenesulfonyl)-L-alanyl-L-tryptophan methyl ester (1.152 g, 2.239 mmol) in CH_2Cl_2 at 0 °C was treated with mCPBA (1.465 g, 8.488 mmol) and 2,6-di*tert*butylpyridine (2.0 ml, 8.906 mmol) and the solution stirred for 8 hours. The solution was washed with 0.5 N NaOH, saturated $NaHCO_3$, and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude which was

chromatographed (1: 1 Hexanes : Ethyl acetate) to yield the product. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20 – 1.25 (m, 3 H, $CHCH_3$), 3.60 – 3.70 (m, 2 H, $CHCH_2$-Indole), 3.74 (s, 3 H, $COOCH_3$), 3.80 – 3.90 (m, 2 H, NCH_2), 4.51 (q, J = 7.0 Hz, 1 H, $CHCH_3$), 4.80 – 4.90 (m, 2 H, $CHCH_2$-Indole and $NCH_2CH=CH_2$), 5.09 (d, J = 17.5 Hz, 1 H, $NCH_2CH=CH_2$), 5.50 – 5.60 (m, 1 H, $NCH_2CH=CH_2$), 7.16 (t, J = 7.5 Hz, 1 H, C_8H_5ONH), 7.30 (d, J = 7.5 Hz, 1 H, CONH), 7.56 (t, J = 8.0 Hz, 1 H, C_8H_5ONH), 7.86 (dd, J = 8.0 Hz, J = 1.0 Hz, 1 H, C_8H_5ONH), 7.97 (d, J = 9.0 Hz, 1 H, C_8H_5ONH), 8.29 (d, J = 8.5 Hz, 1 H, C_8H_5ONH), 8.47 (d, J = 1 H, 1 H, C_8H_5ONH), 8.71 (d, J = 9.0 Hz, 1 H, C_8H_5ONH), 11.26 (s, 1 H, C_8H_5ONH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.22 ($CHCH_3$), 41.19 ($CHCH_2$), 47.85 (NCH_2), 48.41 (CH(O)C), 52.83 ($COOCH_3$), 55.60 (CH), 60.25 (CH), 119.40 ($NCH_2CH=CH_2$), 121.06 (C_8H_5NHO), 121.70 (C_8H_5NHO), 123.14 (C_8H_5NHO), 124.33 (C_6H_4), 128.34 (C_6H_4), 130.61 (C_8H_5NHO), 133.08 ($NCH_2CH=CH_2$), 135.71 (C_8H_5NHO), 139.81 (C_8H_5NHO), 145.50 (C_8H_5NHO), 149.99 (C_6H_4), 160.12 (C_6H_4), 169.73 (CONH), 170.96 ($COOCH_3$).

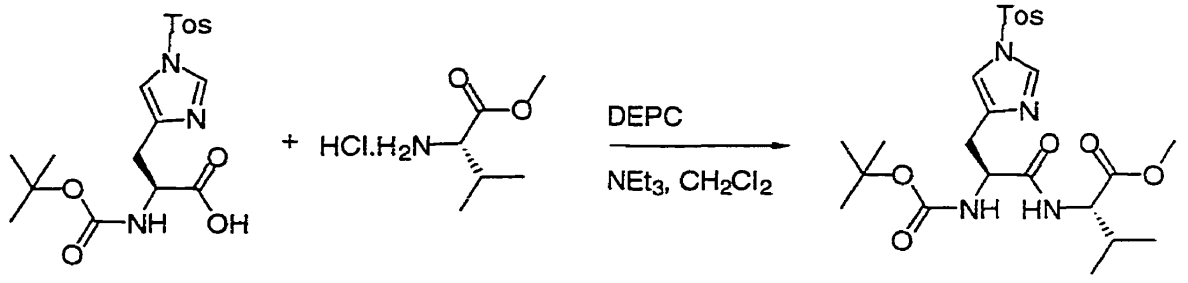
Solid phase compounds : (68 a to 68 f)

Rink amide MBHA resin was washed twice with 20 % piperidine in DMF and then thrice each with DMF, CH_2Cl_2, and methanol. It was then dried and reacted with N-(Fmoc)PheOH (3 eq.), HATU (3 eq.) and DIPEA (6 eq.) for 6 hours. The resin was filtered and washed thrice with DMF, CH_2Cl_2 and methanol to give N-(Fmoc)phenylalanine-MBHA resin (**68a**). The terminal Fmoc group was removed by washing twice with 20 % piperidine in DMF and the resin was again washed thrice each with DMF, CH2Cl2 and methanol. It was then dried and reacted with N-(Fmoc)GlyOH (3 eq.) using HATU (3 eq.) and DIPEA (6 eq.) in DMF for 6 hours to give N-(Fmoc)GlyPhe-MBHA resin (**68b**). The terminal Fmoc protection was removed as before with 20 % piperidine in DMF. The resin was then washed thrice each with DMF, CH_2Cl_2

and methanol. It was then dried and reacted with 2-nitrobenzenesulfonyl chloride (3 eq.) and DIPEA (6 eq.) to generate the sulfonamide **68c**. This was reacted with allyl bromide (4 eq.) and DBU (8 eq.) in DMF to give N-(allyl), N'-(2-nitrobenzenesulfonyl)GlyPhe-MBHA resin (**68c**). This allyl derivative was reacted with mCPBA (10 eq.) and 2,6-di*tert*butyl pyridine (10 eq.) in CH_2Cl_2 at 0 °C for 2 hours and then at room temperature for 12 hours gave the epoxide **68d**. It was reacted with periodic acid (4 eq.) in aqueous DMF for 12 hours to give a small amount of **68e**.

N-(*tert*-Butoxycarbonyl)-L-histidyl(N-tosyl)-L-valine methyl ester : (69)

Method A



69

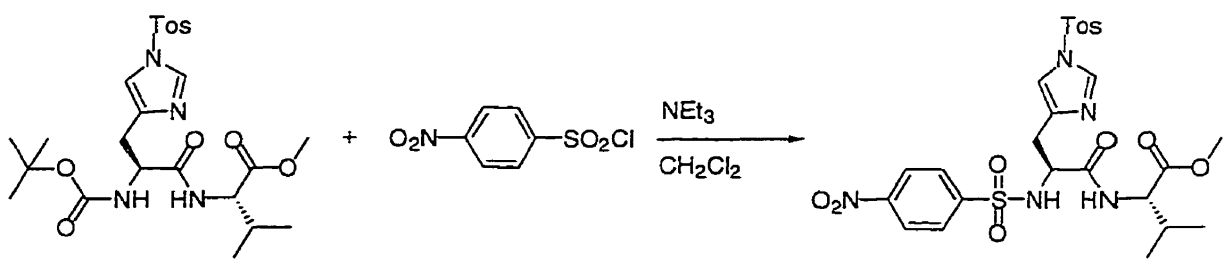
A solution of N-(*tert*)BocHis(Tos)OH (0.200 g, 0.488 mmol) in 2.5 ml dry CH_2Cl_2 at 0 °C was treated with EDC (0.160 g, 0.537 mmol) valine methyl ester (0.091 g, 0.537 mmol), HOBt (0.073 g, 0.537 mmol) and triethylamine (0.075 ml, 0.538 mmol) and the solution was stirred for 12 hours. The CH_2Cl_2 solution was washed with 1 N HCl (3 times 30 ml), 10 % $NaHCO_3$ (3 times 30 ml), and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (1 : 1 Hexanes : Ethyl acetate) to yield the product as a transparent oil (0.050 g, 20 %). R_f 0.32 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65 – 0.75 (m, 6 H, $CH(CH_3)_2$), 1.41 (s, 9 H, $C(CH_3)_3$), 1.90 – 2.00 (m, 1 H, $CH(CH_3)_2$), 2.41 (s, 3 H, $C_6H_4CH_3$), 2.86 (A of ABX, J = 15.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2$), 3.06 (B of ABX, J = 15.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2$), 3.66 (s, 3 H, $COOCH_3$), 4.35 – 4.40 (m, 1 H, $CHCOOCH_3$), 4.40 – 4.45 (m, 1 H, $CHCH_2C_6H_5$), 6.07 (d, J = 7.5 Hz, 1 H, NHCOO), 7.07 (s, 1 H, $C_3H_3N_2$-Tos), 7.10 (br s, CONH), 7.33 (d, J = 8.5 Hz, 2 H, C_6H_4), 7.78 (d, J = 8.5 Hz, 2 H, C_6H_4), 7.91 (s, 1 H, $C_3H_3N_2$-Tos); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.58 and 18.62 ($CH(CH_3)_2$), 21.43 ($CH(CH_3)_2$), 28.25 ($C(CH_3)_3$), 30.08 ($CHCH_2$-Imd), 31.11 ($C_6H_4CH_3$), 51.66 ($COOCH_3$), 54.38 (CH), 57.35 (CH), 80.00 ($C(CH_3)_3$), 114.98, ($C_3H_3N_2$), 127.36 (C_6H_4), 130.34

(C_6H_4), 135.34 ($C_3H_3N_2$), 136.18 (C_6H_4), 140.82 ($C_3H_3N_2$), 146.14 (C_6H_4), 155.44 (NHCOO), 171.01 (CONH), 171.65 ($COOCH_3$); HRMS (EI) Expected for $C_{24}H_{34}N_4O_7S$ 522.2148, Found 522.2144.

Method B

A solution of BocHis(Tos)OH (0.152 g, 0.371 mmol) was taken with ValOMe.HCl in 2 ml dry CH_2Cl_2 at 0 °C under N_2. DEPC (0.060 ml, 0.395 mmol) and triethylamine (0.115 ml, 0.825 mmol) were added and the solution stirred for 12 hours. Solution was diluted with CH_2Cl_2 and washed with 10 % HCl, 10 % $NaHCO_3$, water and then filtered. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation gave the product (0.164 g, 89.4 %).

N-(4-Nitrobenzenesulfonyl)-L-histidyl(tosyl)-L-valine methyl ester : (70)

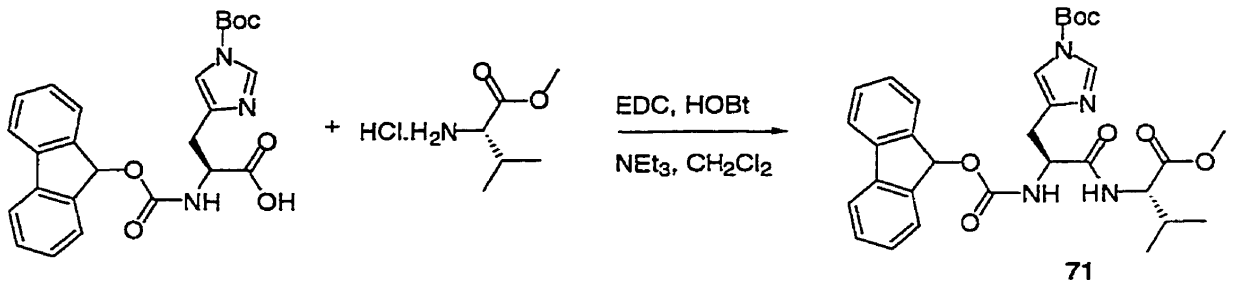


69 70

A solution of N-(*tert*)BocHis(Tos)ValOMe (0.050 g, 0.096 mmol) in 0.5 ml CH_2Cl_2 and 2 ml TFA was stirred at room temperature for 45 min. The solvents were evaporated in vacuum to yield a white solid. This was taken in toluene and the solvent evaporated in vacuum three times to yield a white solid (99.4 %) M.P (73 - 75 °C). ^1H

NMR (270 MHz, DMSO-d_6) δ 0.89 (d, J = 6.6 Hz, 6 H, $CH(CH_3)_2$), 2.05 (septet, J = 6.4 Hz, 1 H, $CH(CH_3)_2$), 2.31 (s, 3 H, $C_6H_4CH_3$), 2.80 – 3.10 (m, 2 H, $CHCH_2$), 3.65 (s, 3 H, $COOCH_3$), 4.10 – 4.30 (m, 2 H, $CHCH_2$ and $CHCOOCH_3$), 7.51 (s, 1 H, $C_3H_2N_2$), 7.52 (d, J = 7.9 Hz, 2 H, C_6H_4), 7.97 (d, J = 8.4 Hz, 2 H, C_6H_4), 8.10 –8.20 (m, 3 H, NH_3^+), 8.40 (d, J =1.4 Hz, 1 H, $C_3H_2N_2$), 8.75 (d, J = 8.15 Hz, 1 H, CONH); MS (FAB, NBA) 537 ($(M + H)^+$). A solution of this deprotected dipeptide salt (0.052 g, 0.132 mmol) in CH_2Cl_2 at 0 °C was treated with 4-nitrobenzenesulfonyl chloride (0.040 g, 0.167 mmol) and NEt_3 (0.040 ml, 0.286 mmol) in CH_2Cl_2 and the solution stirred for 6 hours. The solution was washed with 1 N HCl (3 times 30 ml), water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product (0.064 g, 100 %) as a yellow solid. ^1H NMR (270 MHz, $CDCl_3$) δ 0.77 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.87 (d, J = 6.7 Hz, 3 H, $CH(CH_3)_2$), 1.90 - 2.10 (m, 1 H, $CH(CH_3)_2$), 2.90 - 3.00 (m, 2 H, $CHCH_2C_6H_5$), 3.64 - 3.70 (m, 1 H, $CHCOOCH_3$), 3.66 (s, 3 H, $COOCH_3$), 4.60 - 4.70 (m, 1 H, $CHCH_2C_6H_5$), 6.03 (d, J = 9.1 Hz, 1 H, CONH), 6.35 (d, J = 7.6 Hz, 1 H, SO_2NH), 6.90 - 7.30 (m, 5 H, C_6H_5), 7.95 (d, J = 8.6 Hz, 2 H, C_6H_4), 8.21 (d, J = 8.8 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.25 and 19.17 ($CH(CH_3)_2$), 31.89 ($CH(CH_3)_2$), 37.77 ($CHCH_2$-Imd), 52.55 ($COOCH_3$), 53.53 ($CHCOCH_3$), 62.16 ($CHCH_2$-Imd), 115.79 ($C_3H_2N_2$), 124.28 (C_6H_4), 125.22 (C_6H_4), 128.38 (C_6H_4), 129.09 (C_6H_4), 136.91 ($C_3H_2N_2$), 141.50 ($C_3H_2N_2$), 143.06 (C_6H_4) 146.66 (C_6H_4), 150.06 (C_6H_4), 151.57 (C_6H_4), 170.05 (CONH), 171.48 ($COOCH_3$).

N-(Fluorenmethyloxy)-L-histidyl(N-*tert*-butoxycarbonyl)-L-valine methyl ester : (71)

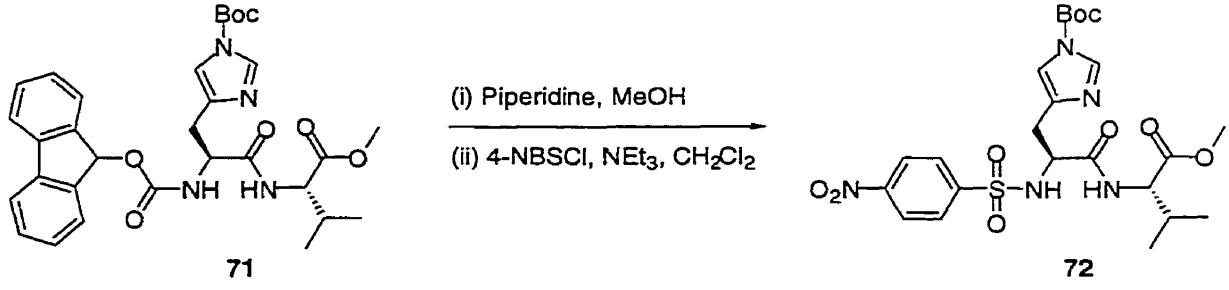


71

A solution of N-(Fmoc)His(Boc)OH.CHA (2.477 g, 4.295 mmol) in ethyl acetate was washed with dilute H_2SO_4 (3 times 30 ml). The ethyl acetate layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave FmocHis(Boc)OH in quantitative yield. The free acid was taken in 10 ml dry CH_2Cl_2 and ValOMe.HCl (0.727 g), EDC (0.825 g), HOBt (0.591 g) and triethylamine (0.6 ml, 4.305 mmol) were added. The solution was stirred at 0 °C for 3 hours and then at room temperature for 12 hours. The solution was diluted with 20 ml CH_2Cl_2 and washed with 10 % $NaHCO_3$, 1N HCl, water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude which was chromatographed (1: 2 Hexanes : Ethyl Acetate) to yield the product (1.442 g, 50 %). R_f 0.33 in 1 : 3 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 - 0.82 (m, 6 H, $CH(CH_3)_2$), 1.57 (s, 9 H, $C(CH_3)_3$), 2.08 - 2.12 (m, 1 H, $CH(CH_3)_2$), 3.02 (A of ABX, J = 6.0 Hz, J = 15.0 Hz, 1 H, $NHCHCH_2$), 3.14 (B of ABX, J = 5.0 Hz, J = 15.0 Hz, 1 H, $NHCHCH_2$), 3.66 (s, 3 H, $COOCH_3$), 4.23 (t, J = 7.0 Hz, 1 H, NHCOOCH), 4.35 (d, 2 H, J = 7.0 Hz, $NHCOOCHCH_2$), 4.47 (dd, J = 5.0 Hz, J = 9.0 Hz, 1 H, $CHCOOCH_3$), 4.58 - 4.62 (m, 1 H, $NHCHCH_2$), 6.66 (d, J = 7.5 Hz, $NHCHCH_2$), 7.20 (s, 1 H, C=CH-N), 7.28 (t, J = 7.5 Hz, 2 H, $C_{12}H_8$), 7.37 (t, J = 7.5 Hz, 2 H, $C_{12}H_8$), 7.49 (d, J = 7.5 Hz, 1 H, NHCO), 7.56 - 7.60 (m, 2 H, $C_{12}H_8$), 7.73 (d, J = 7.0 Hz, 2 H, $C_{12}H_8$), 8.05 (s, 1 H, N=CH-N); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.68 and 18.88 ($CH(CH_3)_2$, 27.87

($C(CH_3)_3$), 30.46 ($CHCH_2$-Imd), 31.06 ($CH(CH_3)_2$), 47.15 (CH), 52.05 ($COOCH_3$), 54.75 ($CHCH_2$-Imd), 57.41 ($CHCOOCH_3$), 67.38 (CH_2), 85.74 ($C(CH_3)_3$), 114.97 (C=CHN), 120.00 ($C_{12}H_8$), 125.27 ($C_{12}H_8$), 127.13 ($C_{12}H_8$), 127.73 ($C_{12}H_8$), 136.77 (C-N=CH), 139.16 (C-N=CH), 141.31 ($C_{12}H_8$), 143.91 ($C_{12}H_8$), 146.85 ($C_{12}H_8$), 156.25 ($C_{12}H_8$), 171.12 (CONH), 171.95 ($COOCH_3$); MS (FAB, NBA) m/z 591 (11.3 %, $(M+H)^+$).

N-(4-Nitrobenzenesulfonyl)-histidyl(N-*tert*-butoxycarbonyl)-L-valine methyl ester : (72)

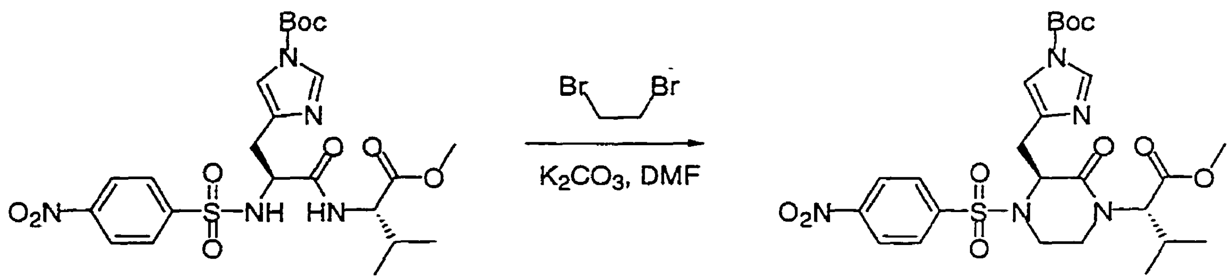


71 72

A solution of N-(Fmoc)His(Boc)ValOMe (1.441 g, 1.929 mmol) in 10 ml dry CH_2Cl_2 was treated with 1 ml diethylamine at room temperature. The solution was stirred for 5 hours and then solvent evaporated. The free amine was taken in 15 ml dry CH_2Cl_2 at 0 °C and treated with 4-nitrobenzenesulfonyl chloride (0.474 g, 2.137 mmol) and triethylamine (0.3 ml, 2.152 mmol). The solution was stirred for 3 hours and then washed with 1N HCl, 10 % $NaHCO_3$, and water, dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the crude which was chromatographed (1: 2 Hexanes : Ethyl Acetate) to yield the product (0.742 g, 75 %). R_f 0.42 in Hexanes : Ethyl acetate 1 : 2; ^1H NMR (500 MHz, $CDCl_3$) δ 0.65 and 0.75 (two d, J = 7.0 Hz, each, 3 H each, $CH(CH_3)_2$), 1.59 (s, 9 H, $C(CH_3)_3$), 1.95 - 2.05 (m, 1 H, $CH(CH_3)_2$), 2.76 (A of ABX, J =

15.0 Hz, J = 5.0 Hz, 1 H, CHC**H**$_2$-Imd), 3.06 (B of ABX, J = 15.0 Hz, J = 5.0 Hz, 1 H, CHC**H**$_2$-Imd), 3.67 (s, 3 H, COOC**H**$_3$), 4.20 - 4.25 (m, 1 H, CHC**H**$_2$-Imd), 4.32 (dd, J = 8.5 Hz, J = 5.0 Hz, 1 H, C**H**COOCH$_3$), 7.12 (s, 1 H, C=C**H**), 8.02 (s, 1 H, C**H**=N), 8.05 - 8.10 (m, 2 H, C_6H_4), 8.30 - 8.35 (m, 2 H, C$_6$**H**$_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.21 and 18.42 (CH(CH_3)$_2$), 27.62 (C(CH_3)$_3$), 26.91 (CHCH$_2$-Imd), 30.69 (CH(CH_3)$_2$), 51.99 (COOCH$_3$), 56.26 (**C**HCH$_2$-Imd), 57.21 (CHCOOCH$_3$), 86.35 (**C**(CH$_3$)$_3$), 115.16 ($C_3H_2N_2$), 124.17 (**C**$_6$H$_4$), 128.39 (**C**$_6$H$_4$), 136.48 ($C_3H_2N_2$), 137.42 ($C_3H_2N_2$), 145.39 (C_6H_4), 146.18 (C_6H_4), 149.94 (Imd-**C**OO)), 169.22 (CONH), 171.28 (COOCH$_3$): MS (FAB, NBA) m/z 554 (64.5 %, $(M + H)^+$); 454 (66.6 %, $(M - t\text{Boc})^+$).

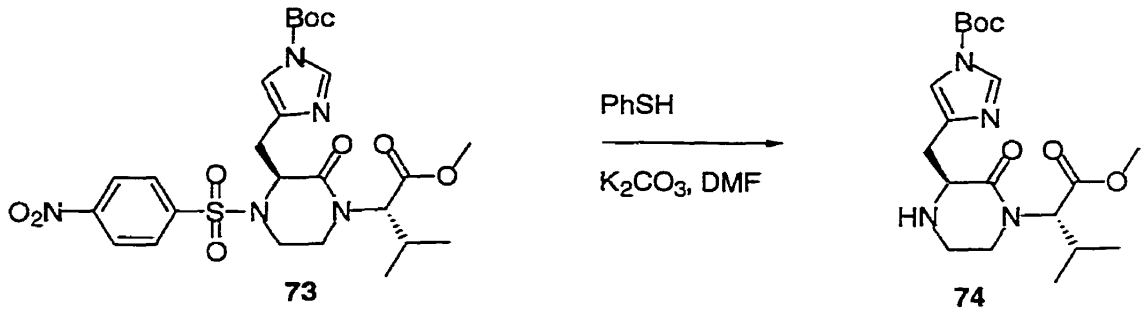
Methyl (2S, 3'S) [3'-(2-methimidazolyl(N-*tert*butoxycarbonyl)-4'(4-nitrobenzenesulfonyl)-2'oxopiperazin-1'yl]-3-methyl butanoate : (73)



A solution of sulfonamide **72** (0.740 g, 1.337 mmol) in 50 ml dry DMF was treated with K_2CO_3 (1.871 g, 13.537 mmol) at 60 °C for 30 min. and then 1,2-dibromoethane (1.15 ml, 13.344 mmol) was added. The solution was stirred at 60 °C for 24 hours and then DMF was evaporated under high vacuum and ethyl acetate added which was washed with 1 N HCl and 10 % $NaHCO_3$, water, dried over $MgSO_4$ and

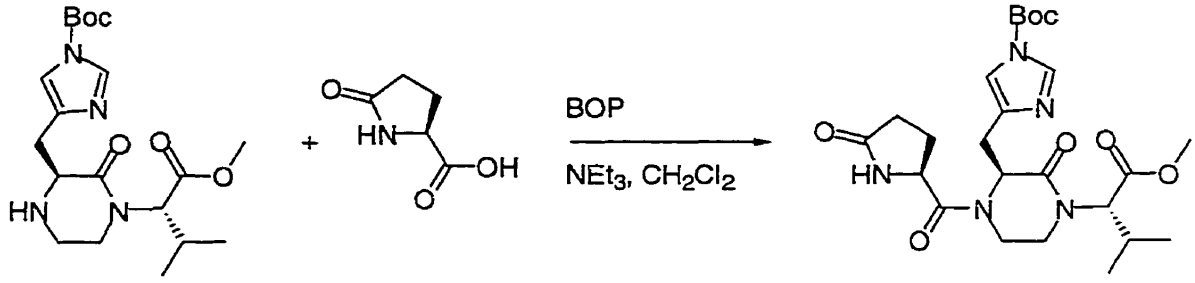
filtered. The solvent was evaporated under vacuum to yield the crude which was chromatographed (1 : 2 Hexanes : Ethyl acetate) to yield the product (0.310 g, 45 %) as a white solid (M.P. 145 - 147 °C). R_f 0.35 in 1 : 2 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.72 and 0.93 (two d, J = 7.0 Hz, each, 3 H each, $CH(CH_3)_2$), 1.58 (s, 9 H, $C(CH_3)_3$), 2.00 - 2.10 (m, 1 H, $CH(CH_3)_2$), 3.10 - 3.20 (m, 2 H, $CHCH_2$-Imd) and $HNCH_2CH_2N$), 3.23 (B of ABX, J = 15.0 Hz, J = 7.0 Hz, 1 H, $CHCH_2$-Imd), 3.30 - 3.40 (m, 1 H, $HNCH_2CH_2N$) , 3.45 - 3.55 (m, 1 H, NCH_2CH_2N), 3.58 (s, 3 H, $COOCH_3$), 3.70 - 3.80 (m, 1 H, $HNCH_2CH_2N$), 4.70 (X of ABX, J = 6.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2$-Imd), 4.80 (d, J = 11.0 Hz, 1 H, $CHCOOCH_3$), 7.10 (s, 1 H, C=CH), 7.85 (s, 1 H, CH=N), 7.98 (d, J = 9 Hz, 2 H, C_6H_4), 8.29 (d, J = 9.0 Hz, 2 H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.43 and 19.34 ($CH(CH_3)_2$), 26.77 ($CH(CH_3)_2$) 27.65 ($C(CH_3)_3$), 31.55 ($CHCH_2$-Imd), 40.72 and 41.80 (NCH_2CH_2N), 51.73 ($COOCH_3$), 58.32 ($CHCH_2$-Imd), 60.73 ($CHCOOCH_3$), 85.75 ($C(CH_3)_3$), 115.29 ($C_3H_2N_2$), 124.19 (C_6H_4), 128.34 (C_6H_4), 136.25 (C_6H_4), 137.80 ($C_3H_2N_2$), 137.80 ($C_3H_2N_2$), 144.96 (C_6H_4), 146.49 (C_6H_4), 149.97 (Imd-COO), 166.33 (CON), 170.40 ($COOCH_3$); MS (EI) 580 (12.6 %, $(M + H)^+$); 480 (15.6 %, $(M - t\text{Boc})^+$).

Methyl (2S, 3'S) [3'-(2-methimidazolyl(N-*tert*butoxycarbonyl)-2' oxopiperazin-1'yl]-3-methyl butanoate : (74)



K_2CO_3 (0.191 g, 1.381 mmol) was taken in 0.2 ml dry DMF at room temperature and thiophenol (0.1 ml, 0.974 mmol) was added to it. After 30 min. of stirring, sulfonamide **73** (0.089 g, 0.154 mmol) was added as a 0.5 ml solution in DMF. The solution was stirred for 24 hours and then solvent was removed under high vacuum and ethyl acetate added. The solution was washed with 10 % $NaHCO_3$ and water. The solution was dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (15 % MeOH in EtOAc) to yield the product (0.046 g, 97 %). R_f 0.45 in 15 : 85 Methanol : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.68 and 0.92 (two d, J = 7.0 Hz each, 3 H each, $CH(C\mathbf{H}_3)_2$), 1.60 (s, 9 H, $C(C\mathbf{H}_3)_3$), 2.05 – 2.15 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 2.65 – 2.75 (br s, 1 H, N**H**), 2.90 – 3.25 (5 H, $CHC\mathbf{H}_2$-Imd, $NC\mathbf{H}_2C\mathbf{H}_2N$), 3.40 – 3.50 (m, 1 H, $NC\mathbf{H}_2CH_2N$), 3.67 (s, 3 H, $COOC\mathbf{H}_3$), 3.80 – 3.85 (m, 1 H, $C\mathbf{H}CH_2$-Imd), 4.83 (d, J = 10 Hz, 1 H, $C\mathbf{H}COOCH_3$), 7.15 (s, 1 H, $C_3\mathbf{H}_2N_2$), 7.96 (s, 1 H, $C_3\mathbf{H}_2N_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.32 and 19.34 ($CH(\mathbf{C}H_3)_2$), 26.56 ($\mathbf{C}H(CH_3)_2$), 27.66 ($C(\mathbf{C}H_3)_3$), 30.05 ($CH\mathbf{C}H_2$-Imd), 41.96 and 44.22 ($N\mathbf{C}H_2\mathbf{C}H_2N$), 51.63 ($COO\mathbf{C}H_3$), 59.42 ($\mathbf{C}HCH_2$-Imd), 60.59 ($\mathbf{C}HCOOCH_3$), 85.28 ($\mathbf{C}(CH_3)_3$), 114.73 ($\mathbf{C}_3H_2N_2$), 136.38 ($\mathbf{C}_3H_2N_2$), 139.94 ($\mathbf{C}_3H_2N_2$), 146.75 (Imd-**C**OO), 169.56 (**C**ON), 171.28 (**C**OOCH$_3$); MS (FAB, NBA) m/z 395 (51.2 %, $(M + H)^+$); 295 (23.7 %, $(M - t\text{Boc})^+$).

Gln-His(Boc)ValOMe CYCLIZED : (75)

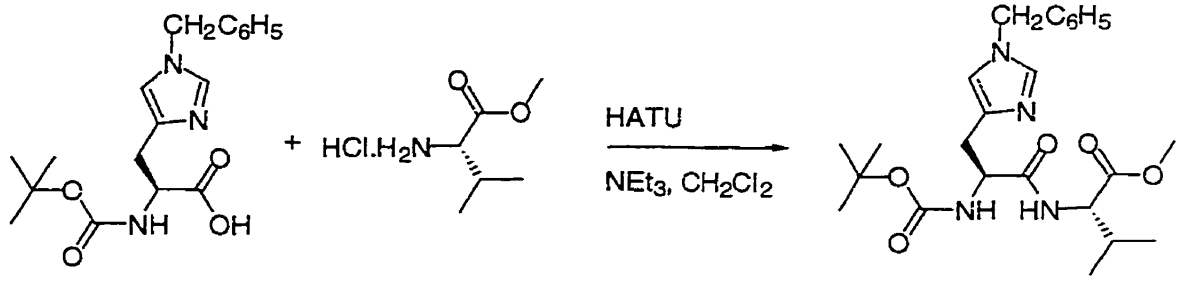


74 **75**

A solution of amine **74** (0.043 g, 0.109 mmol), GlnOH (0.161 g, 0.125 mmol) and BOP (0.06 g, 0.126 mmol) in 0.2 ml dry CH_2Cl_2 at room temperature under N_2 was treated with triethylamine (0.020 ml, 0.143 mmol) and the solution stirred for 2 days. The solution was diluted with CH_2Cl_2 and washed with 1 N HCl, 10 % $NaHCO_3$, and water; dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (15 % MeOH in Ethyl acetate) to give the product (0.009 g, 20 %). R_f 0.17 in 15 : 85 Methanol : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.73 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.99 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 1.61 (s, 9 H, $C(CH_3)_3$), 2.10 – 2.20 (m, 2 H, ($CH(CH_3)_2$), $CHCH_2CH_2CO$), 2.30 – 2.40 (m, 3 H, $CHCH_2CH_2CO$), 3.20 – 3.35 (m, 2 H, $CHCH_2$-Imd), 3.40 – 3.50 (m, 2 H, NCH_2CH_2N), 3.55 – 3.65 (m, 2 H, NCH_2CH_2N), 3.71 (s, 3 H, $COOCH_3$), 4.61 (dd, J = 5.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2CH_2CO$), 4.96 (d, J = 10 Hz, 1 H, $CHCOOCH_3$), 5.20 – 5.30 (m, 1 H, $CHCH_2$-Imd), 6.67 (br s, 1 H, NHCO), 7.28 (br s, 1 H, $C_3H_2N_2$), 8.30 (br s, 1 H, $C_3H_2N_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.52 and 19.29 ($CH(CH_3)_2$), 24.34 and 26.79 ($CHCH_2CH_2CO$), 26.79 ($CHCH_2$), 29.23 ($CH(CH_3)_2$), 41.32 and 42.40 (NCH_2CH_2N), 51.94 ($COOCH_3$), 53.25 ($CHCH_2$), 56.69 ($CHCH_2$-Imd), 60.49 ($CHCOOCH_3$), 115.91 ($C_3H_2N_2$), 135.68 ($C_3H_2N_2$), 135.71 ($C_3H_2N_2$), 145.43 (NCOO), 166.97 (CON), 170.13

(CON), 170.61 (CON), 177.56 ($COOCH_3$); HRMS (FAB, NBA) Expected for $[C_{24}H_{35}N_5O_7 + H]^+$ 506.26147; Found 506.26162.

N-(*tert*Butoxycarbonyl)-L-histidyl(N-benzyl)-L-alanine methyl



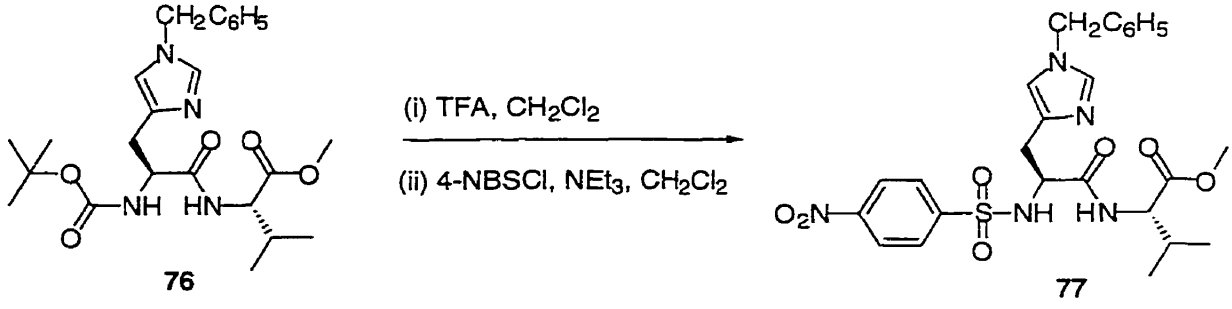
76

ester : (76)

A solution of N-(*tert*)BocHis(N-benzyl)OH (3.018 g, 8.739 mmol), alanine methyl ester hydrochloride (1.335 g, 9.567 mmol) and HATU (3.631 g, 9.550 mmol) in 9 ml dry CH_2Cl_2 at 0 °C was treated with triethylamine (2.60 ml, 18.654 mmol). The solution was stirred for 12 hours at room temperature and then washed with 1 N HCl, 10 % $NaHCO_3$ and water. The organic layer was fried over $MgSO_4$ and solvent evaporation under vacuum gave the product as a white foam (3.756 g, 99 %). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.14 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 1.39 (s, 9 H, $C(CH_3)_3$), 2.86 (A of ABX, J = 14.7 Hz, J = 5.9 Hz, 1 H, $CHCH_2$-Imd), 3.06 (B of ABX, J = 14.2 Hz, J = 3.2 Hz, 1 H, $CHCH_2$-Imd), 3.63 (s, 3 H, $COOCH_3$), 4.30 - 4.50 (m, 2 H, $CHCH_3$, $CHCH_2$-Imd), 4.97 (s, 2 H, $CH_2C_6H_5$), 6.31 (d, J = 3.9 Hz, NHCOO), 6.67 (s, 1 H, C=CH-$NCH_2C_6H_5$), 7.05 - 7.10 and 7.25 - 7.30 (m, 5 H, C_6H_5), 7.42 (s, 2 H, NHCO and CN=CHN$CH_2C_6H_5$); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.23 ($CHCH_3$), 28.37 ($C(CH_3)_3$), 30.27 (Imd-$CH_2C_6H_5$),

38.64 (CH**C**H_2), 48.02 (**C**HCOOCH$_3$), 50.95 (**C**$H_2C_6H_5$), 52.30 (COO**C**H_3), 79.82 (**C**$(CH_3)_3$), 117.29 (**C**$_3H_2N_2$), 127.50 (**C**$_6H_4$), 128.38 (**C**$_6H_4$), 129.03 (**C**$_6H_4$), 135.95 (**C**$_3H_2N_2$), 136.67 (**C**$_3H_2N_2$), 138.54 (**C**$_6H_4$), 155.50 (NH**C**OO), 171.33 (**C**ONH), 172.50 (**C**OOCH$_3$); MS (FAB, NBA) m/z 431 (81.0 %, $(M+H)^+$), 331 (73.2 %, $(M - t\text{Boc})^+$).

N-(4-Nitrobenzenesulfonyl)-L-histidyl(N-benzyl)-L-alanine methyl ester : (77)

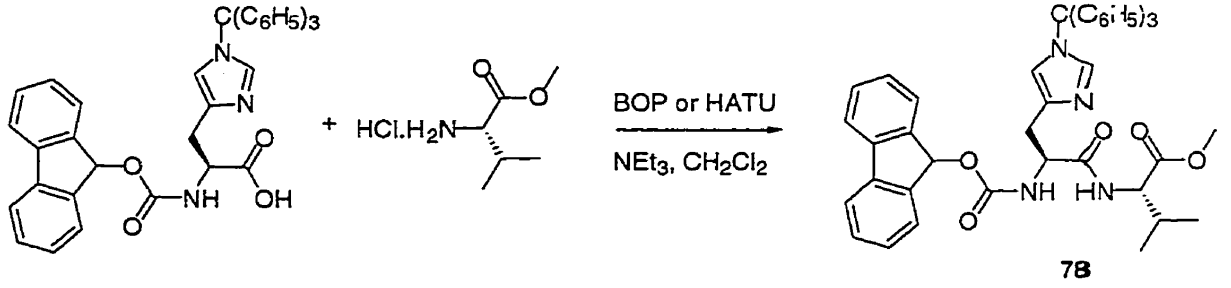


76 77

A solution of dipeptide **76** (3.750 g, 8.711 mmol) in 10 ml dry CH_2Cl_2 was treated with 10 ml TFA and the solution stirred for 1 hour. Solvent evaporation with toluene three times under vacuum gave the product as a transparent oil.^{1}H NMR (500 MHz. $CDCl_3$) δ 1.25 (d, J = 7.0 Hz, CHC**H**$_3$), 3.05 - 3.15 (m, 2 H, CHC**H**$_2$-Imd), 3.59 (s, 3 H, COOC**H**$_3$), 4.05 - 4.15 (br m, 1 H, C**H**CH$_2$-Imd), 4.25 - 4.35 (m, 1 H, C**H**CH$_3$), 5.37 (s, 2 H, C**H**$_2C_6H_5$), 7.10 - 7.25 (m, 6 H, C=C**H**-N(C$_6$**H**$_5$), 7.50 (s, 1 H, C**H**=NCH$_2C_6H_5$), 8.30 - 8.40 (br s, 3 H, N**H**$_3^+$), 8.85 (d, J = 6.5 Hz, 1 H, N**H**CO). A solution of this TFA salt of the dipeptide and 4-nitrobenzenesulfonyl chloride (1.965 g, 8.867 mmol) in 50 ml dry CH_2Cl_2 at 0 °C under N_2 was treated with triethylamine (3.70 ml, 26.546 mmol) and the solution stirred at 0 °C for 1 hour and then at room temperature for 4 hours. The solution

was washed with 1 N HCl, 10 % $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and filtered. Solvent evaporation under vacuum gave the product as a white solid (3.553 g, 85.5 %). Recrystallized from Hexanes - Ethyl acetate (M.P. 182 - 184 °C); R_f 0.12 in 1 : 4 Hexanes : Ethyl acetate; ^1H NMR (270 MHz, $CDCl_3$) δ 1.12 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 2.60 (A of ABX, J = 14.8 Hz, J = 5.9 Hz, 1 H, $CHCH_2$-Imd), 3.03 (B of ABX, J = 14.8 Hz, J = 4.4 Hz, 1 H, $CHCH_2$-Imd), 3.68 (s, 3 H, $COOCH_3$), 4.00 – 4.05 (m, 1 H, $CHCOOCH_3$), 4.30 – 4.40 (m, 1 H, $CHCH_2$), 4.98 (br s, 2 H, Imd-$CH_2C_6H_5$), 6.63 (s, 1 H, NH), 7.10 - 7.15 and 7.30 - 7.35 (m, 5 H, C_6H_5), 7.41 (d, J = 1.2 Hz, 1 H, NH), 8.07 (d, J = 8.9 Hz, 2 H, C_6H_4), 8.30 (d, J = 8.9 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.05 ($CHCH_3$), 29.49 ($CHCH_2$), 48.30 ($CH_2C_6H_5$), 51.13 (CH), 52.51 ($COOCH_3$), 56.76 (CH), 117.48 ($C_3H_2N_2$), 124.35 (C_6H_5), 127.50 (C_6H_4), 128.66 (C_6H_5), 128.80 (C_6H_4), 129.17 (C_6H_5), 135.15 ($C_3H_2N_2$), 136.17 ($C_3H_3N_2$), 137.44 (C_6H_5), 145.77 (C_6H_4), 150.17 (C_6H_4), 169.43 (CONH), 172.59 ($COOCH_3$); MS (FAB, NBA) m/z 516 (12.4 %, $(M + H)^+$).

N-(Fluorenmethyloxy)-L-histidyl(N-trityl)-L-valine methyl ester : (78)



78

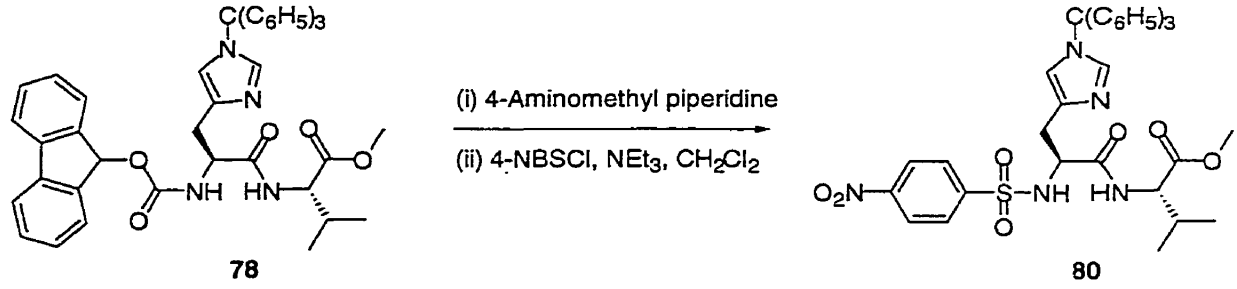
Method A

A solution of N-(Fmoc)His(Trt)OH (0.502 g, 0.810 mmol) in 3 ml dry CH_2Cl_2 was treated with valine methyl ester hydrochloride (0.166 g, 0.990 mmol), EDC (0.178 g, 0.930 mmol) and triethylamine (0.30 ml, 2.15 mmol). The solution was stirred for 16 hours and then taken up in ethyl acetate. The solution was washed with 10 % $NaHCO_3$ (3 times 30 ml) and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (Hexanes : Ethyl acetate 1 : 1) to yield the product (0.410 g, 70 %) as a white foam. R_f = 0.15 in 1 : 1 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 - 0.90 (m, 6 H, $CH(CH_3)_2$), 2.10 - 2.20 (m, 1 H, $CH(CH_3)_2$), 3.00 (A of ABX, J = 15.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2$-Imd), 3.11 (B of ABX, J = 15.0 Hz, J = 5.0 Hz, 1 H, $CHCH_2$-Imd), 3.66 (s, 3 H, $CHCOOCH_3$), 4.22 - 4.26 (m, 1 H, $NHCOOCHCH_2$), 4.30 - 4.40 (m, 2 H, $NHCOOCHCH_2$), 4.49 (dd, J = 8 Hz, J = 4.5 Hz, 1 H, $CHCOOCH_3$), 4.58 (X of ABX, J = 11.5 Hz, J = 7.0 Hz, 1 H, $CHCH_2$-Imd), 6.69 (s, 1 H, $C=CHN(C_6H_5)_3$), 6.89 (d, J = 6.5 Hz, $NHCOOCHCH_2$), 7.06 - 7.14 and 7.22 - 7.40 (m, 19 H, $(C_6H_5)_3$ and $C_{12}H_8$), 7.62 (d, J = 6.5 Hz, 2 H, $C_{12}H_8$), 7.75 (d, J = 7.0 Hz, 2 H, $C_{12}H_8$), 7.83 (d, J = 7.5 Hz, NHCO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.67 and 18.89 ($CH(CH_3)_2$), 30.30 ($CHCH_2$-Imd), 30.92 ($CH(CH_3)_2$), 46.95 (Fmoc-CH), 51.82 ($COOCH_3$), 54.71 (CH) 57.29 (CH), 67.06 ($NHCOOCHCH_2$), 75.18 ($C(C_6H_5)_3$) 119.25 ($C_{19}H_{15}$), 119.76 ($C_3H_2N_2$), 125.14 ($C_{12}H_{10}$), 125.08 ($C_{12}H_{10}$), 126.89 ($C_{12}H_{10}$), 126.90 ($C_{19}H_{15}$), 127.47 ($C_{19}H_{15}$), 127.88 ($C_{19}H_{15}$), 129.58 ($C_3H_2N_2$), 138.21 ($C_3H_2N_2$), 141.06 ($C_{12}H_{10}$), 142.15 ($C_{12}H_{10}$), 143.74 ($C_{12}H_{10}$), 143.81 (NHCOO) 171.22 (CONH), 171.84 ($COOCH_3$); HRMS (FAB, NBA) Expected for $[C_{46}H_{44}N_4O_5 + H]^+$ 733.33900, Found 733.33928.

Method B

A solution of N-(Fmoc)His(Trt)OH (1.510 g, 2.434 mmol) in 2.5 ml dry CH_2Cl_2 was treated with valine methyl ester hydrochloride (0.467 g, 2.783 mmol), BOP (1.181 g, 2.670 mmol) and triethylamine (0.40 ml, 2.70 mmol). The solution was stirred for 12 hours and then taken up in ethyl acetate. The solution was washed with 10 % $NaHCO_3$ (3 times 30 ml) and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (Hexanes : Ethyl acetate 1 : 1) to yield the product (1.348 g, 99 %) as a white foam.

N-(4-Nitrobenzenesulfonyl)-L-histidyl(N-trityl)-L-valine methyl ester : (80)



78 80

Method A

A solution was the dipeptide **78** (0.400 g, 0.546 mmol) in 2.5 ml dry DMF was treated with 2.5 ml morpholine and 1 ml dry piperidine. The solution was stirred at RT for 3 hours and then solvent evaporation gave the crude product that was used without further purification. R_f = 0.05 in 1 : 2 Hexanes : Ethyl acetate. A solution of the free amine in 4 ml dry CH_2Cl_2 at 0 °C was treated with 4-nitrobenzenesulfonyl chloride (0.197 g, 0.889 mmol) and triethylamine (0.135 ml, 0.967 mmol) and the solution was stirred for 12 hours at room temperature. The solution was taken in ethyl acetate and washed with

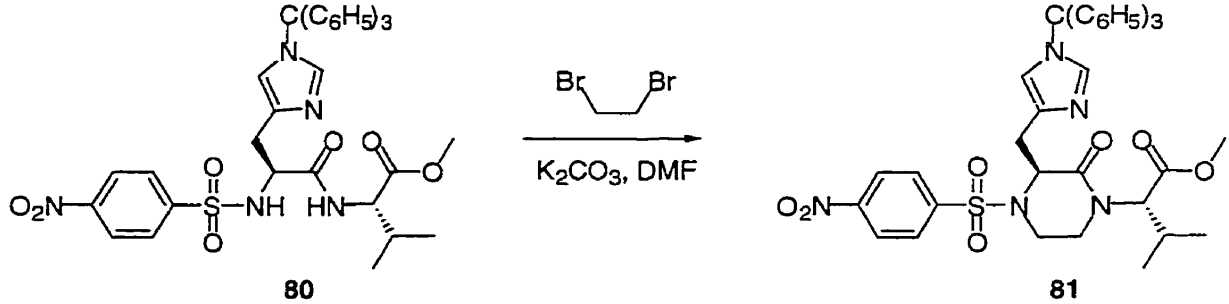
10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (1: 1 Hexanes : Ethyl acetate) to yield the product (0.195 g, 52 %) as a white solid. M.P. 192 - 194 °C; R_f = 0.32 in 1 : 2 Hexanes : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.72 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.76 (d, J = 6.5 Hz, 3 H, $CH(CH_3)_2$), 2.00 – 2.10 (m, 1 H, $CH(CH_3)_2$), 2.67 (A of ABX , J = 15.0 Hz, J = 6.0 Hz, 1 H, $CHCH_2$), 3.04 (B of ABX, J = 15.0 Hz, J = 4.0 Hz, 1 H, $CHCH_2$), 3.69 (s, 3 H, $COOCH_3$), 4.08 – 4.10 (m, 1 H, $CHCH_2$), 4.36 (dd, J = 9.0 Hz, J = 5.0 Hz, 1 H, $CHCOOCH_3$), 6.60 (s, 1 H, $C=CHNC(C_6H_5)_3$), 7.00 - 7.36 (m, 16 H, $C\text{-}CH=N(C_6H_5)_3$), 7.59 (d, J = 9.0 Hz, 1 H, CONH), 8.09 (d, J = 8.5 Hz, 2 H, $C_6H_4\text{-}NO_2$), 8.30 (d, J = 8.5 Hz, 2 H, $C_6H_4\text{-}NO_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.50 and 18.73 ($CH(CH_3)$), 29.81 ($CH(CH_3)$), 30.86 ($CHCH_2$), 51.92 ($COOCH_3$), 56.73 ($CHCOOCH_3$), 57.28 ($CHCH_2$), 73.45 ($C(C_6H_5)_3$), 119.68 ($C_3H_2N_2$), 124.10 (C_6H_4), 127.96 ($C_{19}H_{15}$), 128.08 ($C_{19}H_{15}$), 128.46 (C_6H_4), 129.48 ($C_{19}H_{15}$), 135.55 ($C_3H_2N_2$), 138.18 ($C_{19}H_{15}$), 141.80 ($C_3H_2N_2$), 145.63 (C_6H_4), 149.87 (C_6H_4), 169.71 (CONH), 171.30 ($COOCH_3$); MS (FAB, NBA) m/z 696 ($(M + H)^+$); HRMS (FAB, NBA) Expected for $[C_{37}H_{37}N_5O_7S + H]^+$ 696.24920; Found 696.24902.

Method B

A solution of dipeptide **78** (0.450 g, 0.614 mmol) was taken in 5 ml $CHCl_3$ and 5 ml 4-aminomethyl piperidine was added. The solution was stirred for 2 hours, diluted with CHCl3 and washed twice with saturated NaCl solution. It was then washed 5 times with phosphate buffer (prepared by adding 90 g $NaH_2PO_4.H_2O$ and 32.7 g Na_2HPO_4 in 500 ml distilled water : pH 5.5), water, dried over $MgSO_4$ and filtered. Partial solvent evaporation under vacuum gave the free amine dipeptide **79** as a solution in $CHCl_3$. It

was taken with 4-nitrobenzenesulfonyl chloride (0.163 g, 0.735 mmol) and triethylamine (0.10 ml, 0.717 mmol) and the solution stirred for 5 hours. It was washed with 1 N HCl, 10 % $NaHCO_3$, and water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the product **80** (0.400 g, 93.6 %).

Methyl (2S, 3'S) [3'-(2-methimidazolyl(N-trityl)-4'-(4-nitrobenzenesulfonyl)-2'oxopiperazin-1'yl]-3-methyl butanoate : (81)

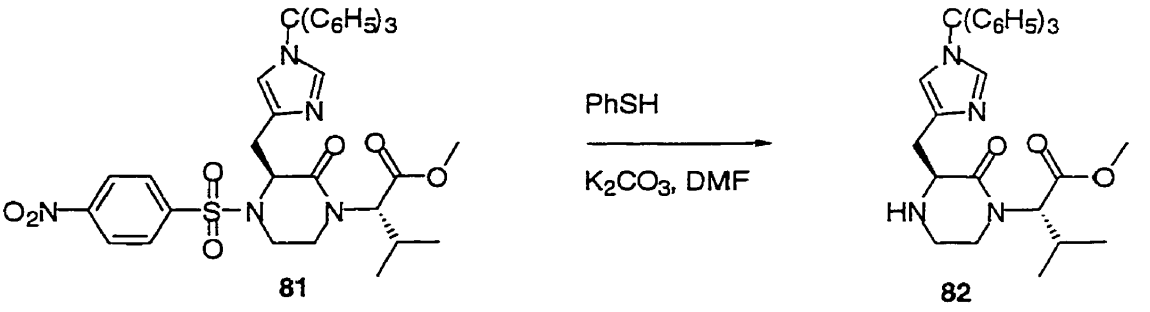


80 **81**

A solution of N-(4-Nitrobenzenesulfonyl)His(Trt)ValOMe **80** (0.093 g, 0.134 mmol) in 1 ml dry DMF was treated with K_2CO_3 (0.143 g ,1.032 mmol) and the solution was heated at 60 °C for 30 min. after which 1,2-dibromoethane (0.11 ml, 1.277 mmol) was added. The solution was stirred for 14 hours and then solvent was evaporated under high vacuum. Ethyl acetate was added and the solution was washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (1: 1 Hexanes : Ethyl acetate) to give the product (0.038 g, 40 %) as a white foam. R_f = 0.18 in 1 : 2 Hexanes : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.74 (d, J = 6.8 Hz, 3 H, $CH(CH_3)_2$), 0.90 (d, J = 6.3 Hz, 3 H, $CH(CH_3)_2$), 1.90 - 2.10 (m, 1 H, $CH(CH_3)_2$), 3.05 - 3.10 (m, 1 H, NCH_2CH_2N), 3.18 (d, J = 6.5 Hz, 2 H, $CHCH_2$-Imd)

3.25 – 3.30 (m, 1 H, NCH_2CH_2N), 3.45 - 3.50 (m, 1 H, NCH_2CH_2N) 3.53 (s, 3 H, $COOCH_3$), 3.60 - 3.65 (m, 1 H, NCH_2CH_2N), 4.69 (t, J = 6.5 Hz, 1 H, $CHCH_2$-Imd) 4.75 (d, J = 10.5 Hz, 1 H, $COOCH_3$), 6.67 (s, 1 H, $C=CHN(C_6H_5)_3$), 7.10 - 7.15 and 7.30 – 7.40 (m, 16 H, $C(C_6H_5)_3$ and CN=CHN), 7.95 (d, J = 9.0 Hz, 2 H, C_6H_4), 8.26 (d, J = 9.0 Hz, 2 H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.70 and 19.36 ($CH(CH_3)$), 27.12 ($CH(CH_3)$), 32.03 ($CHCH_2$), 40.42 and 41.02 ($SO_2NCH_2CH_2N$), 51.58 ($COOCH_3$), 58.81 ($CHCOOCH_3$), 60.43 ($CHCH_2$), 75.08 ($C(C_6H_5)_3$), 120.11 ($C_3H_2N_2$), 124.31 (C_6H_4), 127.84 ($C_{19}H_{15}$), 127.88 ($C_{19}H_{15}$), 128.28 (C_6H_4), 129.59 ($C_{19}H_{15}$), 135.49 ($C_3H_2N_2$), 138.13 ($C_{19}H_{15}$), 142.22 ($C_3H_2N_2$), 145.11 (C_6H_4), 149.95 (C_6H_4), 166.88 (CONH), 170.61 ($COOCH_3$); HRMS (FAB, NBA) Expected for $[C_{39}H_{39}N_5O_7S + H]^+$ 722.26485; Found 722.26452.

Methyl (2S, 3'S) [3'-(2-methylimidazolyl(N-trityl)-2'oxopiperazin-1'yl]-3-methyl butanoate : (82)

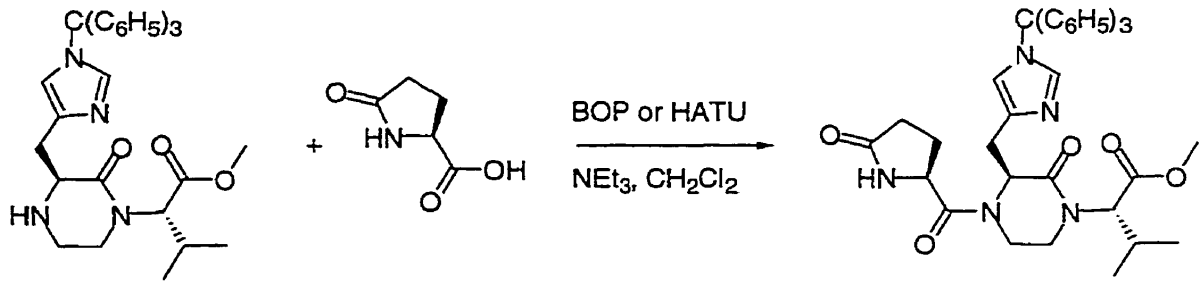


81 82

A solution of K_2CO_3 (0.166 g, 1.201 mmol) in 0.2 ml dry DMF at room temperature was treated with benzenethiol (0.085 ml, 0.828 mmol). The solution was

stirred at room temperature for 30 min. and **81** (0.144 g, 0.200 mmol) was added to it as a 0.5 ml solution in DMF. The solution was stirred at room temperature for 12 hours. The solvent was evaporated under vacuum and ethyl acetate was added which was then washed with 10 % $NaHCO_3$, water, dried over $MgSO_4$ and filtered. Solvent evaporation gave the crude which was chromatographed (10 : 1 Ethyl Acetate : Methanol) to yield the product (88. 3 %). R_f = 0.05 in Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 and 0.95 (two d, J = 7.0 Hz each, 3 H each, $CH(CH_3)_2$), 2.10 – 2.20 (m, 1 H, $CH(CH_3)_2$), 2.80 - 2.85 (br, 1 H, NH), 2.89 (A of ABX, J = 15.0 Hz, J = 9.0 Hz, 1 H, $CHCH_2$-Imd), 2.92 – 3.00 (m, 1 H, $HNCH_2CH_2N$), 3.14 - 3.20 (m, 1 H, $HNCH_2CH_2N$) 3.21 (B of ABX, J = 15.0 Hz, J = 3.0 Hz, 1 H, $CHCH_2$-Imd) 3.26 - 3.32 (m, 1 H, $HNCH_2CH_2N$), 3.42 - 3.46 (m, 1 H, $HNCH_2CH_2N$), 3.68 (s, 3 H, $COOCH_3$), 3.78 (X of ABX, J = 9.0 Hz, J = 3.0 Hz, 1 H, $CHCH_2$-Imd), 4.92 (d, J = 11.0 Hz, 1 H, $CHCOOCH_3$), 6.66 (s, 1 H, $CCHN(C_6H_5)_3$), 7.10 - 7.40 (m, 16 H, $C(C_6H_5)_3$ and $CN=CHN(C_6H_5)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.74 and 19.36 ($CH(CH_3)_2$), 26.63 ($CH(CH_3)_2$), 30.35 ($CHCH_2$), 41.95 ($NHCH_2CH_2N$), 44.26 ($NHCH_2CH_2N$), 51.60 ($CHCOOCH_3$), 59.75 ($CHCOOCH_3$), 60.58 ($CHCH_2$), 75.02 ($C(C_6H_5)_3$), 119.25 ($C_3H_2N_2$), 127.83 ($C_{19}H_{15}$), 129.57 ($C_{19}H_{15}$), 138.10 ($C_3H_2N_2$), 138.42 ($C_{19}H_{15}$), 142.27 ($C_3H_2N_2$), 170.07 (CONH), 171.42 ($COOCH_3$); MS (ES, + ve) Expected for (($C_{33}H_{36}N_4O_3$ + H)$^+$) 537; Found 537.

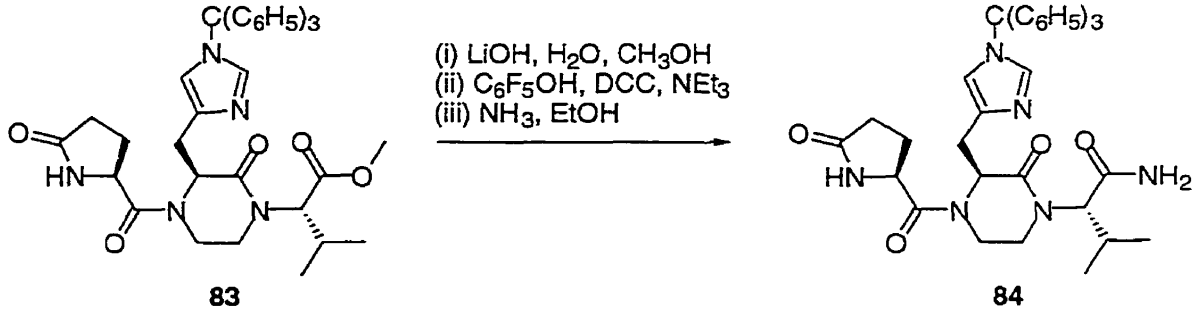
Pyroglutamic acid His(Trt)Val OMe CYCLIZED : (83)



82 **83**

A solution of **82** (0.096 g, 0.178 mmol) in 0.2 ml dry CH_2Cl_2 was treated with pyroglutamic acid (0.028 g, 0.214 mmol), BOP (0.100 g, 0.226 mmol), and triethylamine (0.03 ml, 0.215 mmol). The solution was stirred at room temperature for 48 hours. The solution was diluted with 20 ml CH_2Cl_2 and washed with 10 % $NaHCO_3$, 1 N HCl, water, dried over $MgSO_4$ and solvent evaporation under vacuum gave the crude which was chromatographed (1: 4 Hexanes : Ethyl acetate) to yield the product (0.091 g, 79 %) as a white solid. M.P. 186 - 188 °C; R_f = 0.07 in 10 : 1 Ethyl acetate : Methanol; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.67 and 0.91 (two d, J = 6.6 Hz each, 3 H each, $CH(CH_3)_2$), 2.00 - 2.30 (m, 5 H, $CH(CH_3)_2$ and $CHCH_2CH_2CO$), 3.00 - 3.15 (m, 2 H, $CHCH_2$-Imd), 3.15 - 3.25 and 3.30 - 3.50 (m, 4 H, NCH_2CH_2N), 3.65 (s, 3 H, $COOCH_3$), 4.36 - 4.46 (m, 1 H, $CHCH_2CH_2CONH$), 4.90 (d, J = 10.5 Hz, 1 H, $CHCOOCH_3$), 5.07 (dd, J = 5.9, J = 11.8 Hz, 1 H, $CHCH_2$-Imd), 6.58 - 6.62 (m, 1 H, NH), 6.65 (s, 1 H, $CN=CHN(C_6H_5)_3$), 7.02 - 7.06 and 7.20 - 7.30 (m, 16 H, $C(C_6H_5)_3$ and $CN=CHN(C_6H_5)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.53 and 19.32 ($CH(CH_3)_2$), 24.26 ($CHCH_2CH_2CON$), 26.80 ($CH(CH_3)_2$), 28.97 ($CHCH_2$-Imd), 29.29 ($CHCH_2CH_2CONH$), 40.64 and 42.46 (NCH_2CH_2N), 51.79 ($COOCH_3$), 53.35 ($CHCH_2CH_2CONH$), 56.56 ($CHCH_2$-Imd), 60.32 ($CHCOOCH_3$), 75.08 ($C(C_6H_5)_3$), 119.71 ($C_3H_2N_2$), 127.86 ($C_{19}H_{15}$), 129.53 ($C_{19}H_{15}$), 135.87 ($C_3H_2N_2$), 138.41 ($C_{19}H_{15}$), 142.16 ($C_3H_2N_2$), 167.13 (NCO), 169.13 (NCO), 170.72 ($COOCH_3$),

177.62 (NHCO); HRMS (FAB, NBA) Expected for [$C_{38}H_{41}N_5O_5$ + H] 648.31859, Found 648.31876.

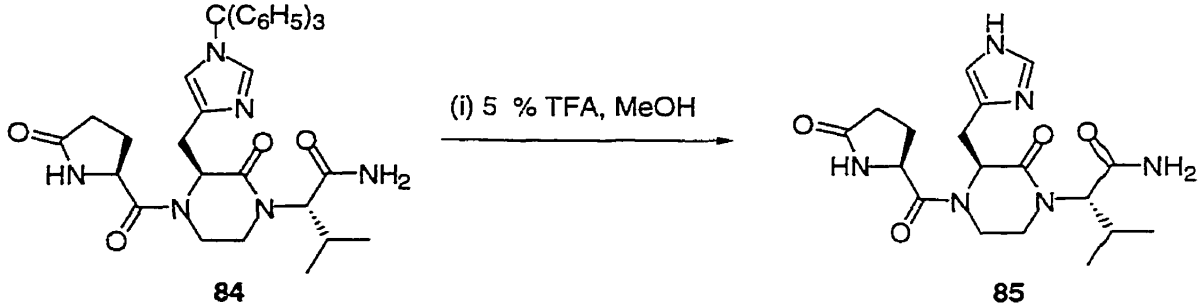


83 84

PygHis(Trt)Val NH_2 Cyclized : (84)

A solution of ester **83** (12 mg, 0.018 mmol) was taken in 0.2 ml of 1 : 1 solution of methanol and water at room temperature and treated with 2.4 mg LiOH. The solution was stirred for 6 hours and then dilute HCl and ethyl acetate were added. The organic layer was collected, dried over $MgSO_4$, filtered, and the solvent evaporated off. The solid so obtained was then treated with 5 mg C_6F_5OH, 5 mg DCC, 1 mg DMAP and 3 µl NEt_3 in 0.3 ml dry CH_2Cl_2 at 0 °C. The solution was stirred at 0 °C for 15 min. and then at room temperature overnight. After TLC showed that the reaction was complete, the solution was diluted with CH_2Cl_2 and filtered to remove DCU. The solvent was evaporated from the filtrate and then it was treated with 1.5 ml of 2 M NH_3 solution in ethanol at room temperature for 8 hours. 1 N HCl was then added to the solution, which was then extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and then filtered. Solvent evaporation gave the crude which was chromatographed (5 % Methanol in Ethyl acetate) to yield the product as a yellow oil (6.2 mg, 52.2 %). R_f 0.31 in 1 : 10

Methanol : Ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.62 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.70 (d, J = 6.0 Hz, 3 H, $CH(CH_3)_2$), 2.00 - 2.40 (m, 5 H, $NHCOCH_2CH_2$ and $CH(CH_3)_2$), 2.80 – 2.95 (m, 2 H, $CHCH_2$-Imd), 3.05 – 3.20 (m, 2 H, NCH_2CH_2N), 3.30 - 3.40 (m, 2 H, NCH_2CH_2N), 4.24 – 4.34 (m, 1 H, CHNHCO), 4.58 (d, 1 H, $CHCH(CH_3)_2$), 4.98 – 5.10 (m, 1 H, $CHCH_2$-Imd), 5.60 and 6.20 (two br s, 1 H each, $CONH_2$), 6.56 (br s, 1 H, NHCO), 6.56 (s, 1 H, C=CH-$NC(C_6H_5)_3$), 7.00 - 7.40 (m, 15 H, $(C_6H_5)_3$), 7.35 (s, 1 H, C-N=CHNC$(C_6H_5)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ, 18.15 and 19.31 ($CH(CH_3)_2$), 25.25 ($CH(CH_3)_2$), 29.16 and 29.67 (CH_2CH_2CO), 31.54 ($CHCH_2$-Imd), 40.42 and 41.56 (NCH_2CH_2N), 51.90 ($CHCH_2CH_2$), 56.48 ($CHCH_2$), 61.42 ($CHCOOCH_3$), 75.18 ($C(C_6H_5)_3$), 119.79 ($C_3H_2N_2$), 127.95 ($C_{19}H_{15}$), 129.53 ($C_{19}H_{15}$), 135.73 ($C_3H_2N_2$), 138.83 ($C_{19}H_{15}$), 141.95 ($C_3H_2N_2$), 168.04 (CO), 169.32 (CO), 170.91 (CO), 177.79 (CO); HRMS (FAB, NBA) Expected for $[C_{37}H_{40}N_6O_4 + H]^+$ 633.31893, Found 633.31883.



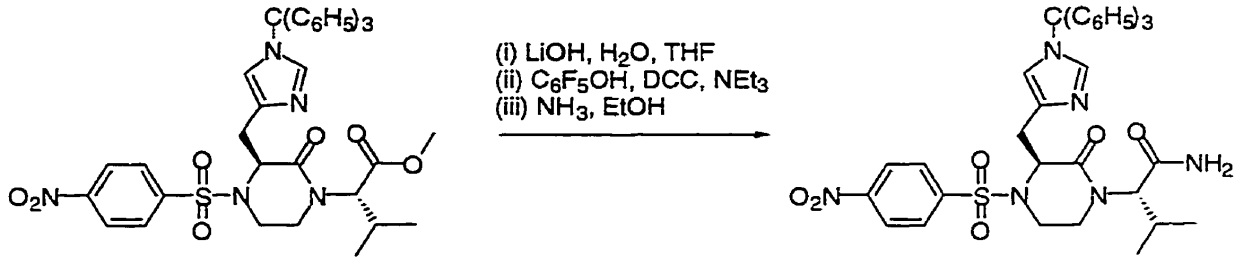
84 85

PygHisValNH$_2$ (Cyclized) : 85

A solution of **84** (9 mg) in 2 ml MeOH was treated with 0.1 ml acetic acid and refluxed for 6 hours. The solvent was evaporated to remove excess CH_3COOH and then

methanol and hexanes were added in equal amounts by volume. A few drops of water were added to create two layers. The two layers were thoroughly mixed and then the top hexanes layer was pipetted out. The methanol/water mixture was evaporated under vacuum to give the product **85** (4.5 mg, 82 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 - 1.00 (m, 6 H, $CH(CH_3)_2$), 1.80 - 2.00 (m, 1 H, $CHCH_2CH_2O$), 2.05 - 2.10 (m, 1 H, $CH(CH_3)_2$), 2.20 -2.40 (m, 3 H, $CHCH_2CH_2O$), 3.20 - 3.60 (m, 5 H, CH_2-Imd, NCH_2CH_2N), 2.80 - 2.85 (m,1 H, NCH_2CH_2N), 4.60 - 4.65 (m, 1 H, $CHCH_2CH_2CO$), 4.90 - 4.95 (m, 1 H, $CHCH(CH_3)_2$), 5.15 - 5.20 (m, 1 H, $CHCH_2$-Imd), 6.95 (br s, 1 H, $C_3H_3N_2$), 8.10 (br s, 1 H, $C_3H_3N_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.73 and 19.29 ($CH(CH_3)_2$), 21.83 ($CH(CH_3)_2$), 24.39 ($CHCH_2CH_2CO$) and 27.17 ($CHCH_2$-Imd), 29.23 ($CHCH_2CH_2CO$), 40.90 and 42.43 (NCH_2CH_2N), 53.68 ($CHCH_2CH_2$), 56.58 ($CHCH_2$-Imd), 60.87 ($CHCOOCH_3$), 118.06 ($C_3H_2N_2$), 129.90 ($C_3H_2N_2$), 134.31 ($C_3H_2N_2$), 167.14 (CO), 170.22 (CO), 176.34 (CO), 179.36 (CO); HRMS (FAB, NBA) m/z Expected for $[C_{18}H_{26}N_6O_4 + H]^{+\cdot}$ 391.20938, Found 391.20947.

Methyl (2S, 3'S)-2-[3'-methimidazoyl(N-trityl)-4'-(4-nitrobenzene sulfonyl)-2'-oxopiperazin-1'-yl]-3-methyl butylamide : (86)

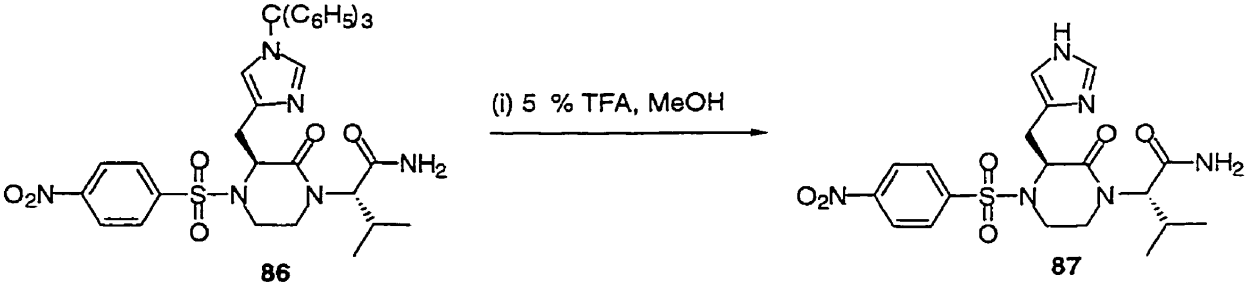


81 **86**

A solution of **81** (0.109 g, 0.151 mmol) was taken in 2 ml of 1 : 1 solution of methanol and water at room temperature and treated with 40 mg LiOH. The solution was stirred for 6 hours and then HCl and ethyl acetate were added. The organic layer was collected and solvent evaporated off. It was then treated with 44 mg C_6F_5OH, 38 mg DCC, 13 mg DMAP and 30 µl NEt_3 in 3 ml dry CH_2Cl_2 at 0 °C. The solution was stirred at 0 °C for 15 min. and then at room temperature overnight. After TLC showed that the reaction was complete, the solution was diluted with CH_2Cl_2 and filtered to remove DCU. The solvent was evaporated from the filtrate and then it was treated with 3 ml of 2 M NH_3 solution in ethanol at room temperature for 8 hours. 1 N HCl was then added to the solution that was then extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and then filtered. Solvent evaporation gave the crude which was chromatographed (Hexanes : Ethyl acetate 1: 3 to 10 % MeOH in Ethyl acetate)to give the product (0.043 g, 40.3 %). R_f 0.67 in 1 : 3 Methanol : Ethyl acetate; ^1H NMR (500 MHz, $CDCl_3$) δ 0.67 and 0.89 (two d, J = 6.6 Hz each, 3 H each, $CH(CH_3)_2$), 2.08 - 2.14 (m, 1 H, $CH(CH_3)_2$), 2.98 - 3.02 (m, 2 H, $CHCH_2$-Imd and $SO_2NCH_2CH_2N$), 3.10 - 3.20 (m, 2 H, $CHCH_2$-Imd and $SO_2NCH_2CH_2N$), 3.34 - 3.44 (m, 1 H, $SO_2NCH_2CH_2N$), 3.62 - 3.68 (m, 1 H, $SO_2NCH_2CH_2N$), 4.64 - 4.70 (m, 1 H, $CHCH_2$-Imd), 4.74 (d, J = 10.7 Hz, 1 H, $CHCONH_2$), 5.36 - 5.40 and 6.30 - 6.34 (two br s, 1 H each, $CONH_2$), 6.62 (s, 1 H, $C=CH-NC(C_6H_5)_3$), 7.05 - 7.15 and 7.30 - 7.40 (m, 16 H, $C-N=CHNC(C_6H_5)_3$), 7.93 (d, J

= 8.8 Hz, 2 H, $C_6\mathbf{H}_4$), 8.23 (d, J = 8.8 Hz, 2 H, $C_6\mathbf{H}_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.06 and 19.32 ($CH(\mathbf{C}H_3)_2$), 25.36 ($\mathbf{C}H(CH_3)_2$), 31.76 ($CH\mathbf{C}H_2$), 33.75 and 39.80 ($NCH_2\mathbf{C}H_2N$), 57.46 (**C**H), 58.61 (**C**H), 75.16 ($\mathbf{C}(C_6H_5)_3$), 120.15 ($\mathbf{C}_3H_2N_2$), 124.43 ($\mathbf{C}_6H_4$), 127.90 ($\mathbf{C}_6H_5$), 127.93 ($\mathbf{C}_6H_5$), 128.02 ($\mathbf{C}_6H_5$), 128.10 ($\mathbf{C}_6H_4$), 129.41 ($\mathbf{C}_3H_2N_2$), 129.52 ($\mathbf{C}_6H_5$), 129.55 ($\mathbf{C}_6H_5$), 138.25 ($\mathbf{C}_3H_2N_2$), 142.11 ($\mathbf{C}_6H_5$), 145.08 ($\mathbf{C}_6H_4$), 150.13 ($\mathbf{C}_6H_4$), 167.36 (**C**ON), 170.29 ($\mathbf{C}OOCH_3$); MS (ES, +ve) Expected for 707 Found 707.

Methyl (2S, 3'S)-2-[3'-methimidazoyl-4'-(4-nitrobenzenesulfonyl) -2'-oxopiperazin-1'-yl]-3-methyl butylamide : (87)



86 87

A solution of **86** (0.041 g, 0.059 mmol) in 3 ml methanol was treated with 0.15 ml acetic acid and the solution refluxed for 12 hours at the end of which time TLC showed no more starting material. The solvent was evaporated under vacuum and the crude product was chromatographed (10 % MeOH in Ethyl acetate) to give the product (0.018 g, 66.2 %) as a white solid. R_f 0.15 in 1 : 9 Methanol : Ethyl acetate; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.69 (d, J = 6.8 Hz, 3 H, $CH(C\mathbf{H}_3)_2$), 0.94 (d, J = 6.3 Hz, 3 H, $CH(C\mathbf{H}_3)_2$), 2.10 - 2.20 (m, 1 H, $C\mathbf{H}(CH_3)_2$), 3.04 (dt, J = 12.9 Hz, J = 3.7 Hz, 1 H, $SO_2NC\mathbf{H}_2CH_2N$), 3.24 - 3.44 (m, 4 H, $CHC\mathbf{H}_2$-Imd $SO_2NC\mathbf{H}_2CH_2N$, $SO_2NCH_2C\mathbf{H}_2N$), 3.60 - 3.70 (m, 1 H,

$SO_2NCH_2CH_2N$), 4.37 (d, J = 7.0 Hz, 1 H, C**H**$CONH_2$), 4.59 (dd, J = 5.6 Hz, J = 5.6 Hz, 1 H, C**H**CH_2-Imd), 5.28 - 5.34 and 6.04 - 6.12 (two br s, 1 H each, $CHCONH_2$), 6.81 (s, 1 H, C=C**H**-$NC(C_6H_5)_3$), 7.53 (d, J = 1.0 Hz, 1 H, C-N=C**H**$NC(C_6H_5)_3$), 7.94 (d, J = 9.0 Hz, 2 H, C_6H_4), 8.32 (d, J = 8.8 Hz, 2 H, C_6H_4); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 18.31 and 19.56 ($CH(CH_3)_2$), 25.53 ($CH(CH_3)_2$), 29.76 ($CHCH_2$), 40.77 and 41.89 (NCH_2CH_2N), 58.87 (CH), 62.73 (CH), 118.00 ($C_3N_2H_2$), 124.64 (C_6H_4), 128.51 (C_6H_4), 129.00 ($C_3N_2H_2$), 134.95 ($C_3N_2H_2$), 144.58 (C_6H_4), 150.50 (C_6H_4), 167.51 (NCO), 170.46 ($COOCH_3$); HRMS (FAB, NBA) m/z Expected for $[C_{19}H_{25}N_6O_6S + H]^+$ 465.15563, Found 465.15498.