Development of Chemoselective and Stereoselective Strategies for Peptide Synthesis and Related Organic Transformations

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Doctor of Philosophy in Chemistry

Submitted by

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Dedicated

to

My Parents & My Brother

Indian Institute of Technology Guwahati

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STATEMENT

I do hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology Guwahati, India under the guidance of Dr. Bhubaneswar Mandal.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

30th June 2014

Thalluri Kishore

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CERTIFICATE

This is to certify that **Thalluri Kishore** has been working under my supervision since July 2009 as a regular registered Ph. D. student. I am forwarding his thesis entitled "**Development of Chemoselective and Stereoselective Strategies for Peptide Synthesis and Related Organic Transformations**" for being submitted for the Ph. D. (Science) degree of this institute. I certify that he has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in his thesis and this work has not been submitted elsewhere for a degree.

30th June 2014

Dr. Bhubaneswar Mandal

Supervisor

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Synopsis

Chapter 1: Introduction

Peptides are macromolecules that contain amino acid monomers linked together by peptide (amide) bonds. Polypeptides of specific sequence of more than ~50 amino acid residues with well defined 3 dimensional structures and biological functions are usually known as proteins. Proteins are present in every living cells and takes part in a variety of biochemical activities. They appear as enzymes, hormones, antibiotics, toxins and receptors. Therefore scientists are interested to produce peptides in an easier way. This interest has developed a major research field known as peptide chemistry.

There are mainly two approaches for peptide synthesis, the first one is the *solution phase peptide synthesis* and the second one is the *solid phase peptide synthesis*. Solution phase peptide synthesis involves mixing of amino acid residues without using any solid resin. Depending on the requirement, protecting groups are used either at carboxyl end or at amine end. Solid phase peptide synthesis (SPPS) is a milestone in the area of peptide synthesis pioneered by Prof. Robert Bruce Merrifield. General principle of SPPS involves: (a) attachment of the first amino acid to resin, (b) *N*-deprotection with the appropriate reagent, (c) coupling of the next amino acid, and (d) cleavage from the resin.

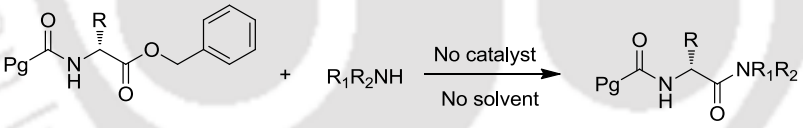
Usually existing methods involve costly coupling reagents. Moreover, those coupling reagents are used in 3-5 fold molar excess especially in SPPS. Thus solid by products, which are very difficult to separate from the products often, get generated. For example, in case of condensation using DCC (dicyclohexylcarbodiimide), the by product, *N, N'*-dicylcohexylurea (DCU), is generated, which is harmful to the environment and is very difficult to remove from the product. In case of an activated ester, it gets hydrolyzed easily because of its high reactivity. Racemization is also an important issue in peptide chemistry. To address the above mentioned drawbacks, we wanted to develop new strategies for peptide synthesis.

Chapter 2: Inactive ester mediated synthesis of amides and peptides

Active ester based methods for peptide synthesis such as via para-nitrophenyl esters or penta-fluorophenolic esters are used often. However, inactive ester based peptide synthesis is not reported in literature yet. We wanted to develop such a method for peptide synthesis. For that, we started our journey to find a greener method for the conversion of inactive esters to amides.

Solvent free and catalyst free amidation of inactive benzyl esters

Although there are many protocols available for amidation of esters, popular methods employ additional catalyst or reagent. We have developed a method for amide synthesis from inactive esters, e.g. benzyl ester of *N*-protected amino acids, without using any catalyst and extra solvent at 40 °C as shown in Scheme 1. Further, we explored the scope of the reaction with various types of *N*-protected amino acids with various amines with high yields (up to 85%, 18 examples). The common *N*-protecting groups such as Boc and Cbz were found to be compatible under the present reaction conditions.



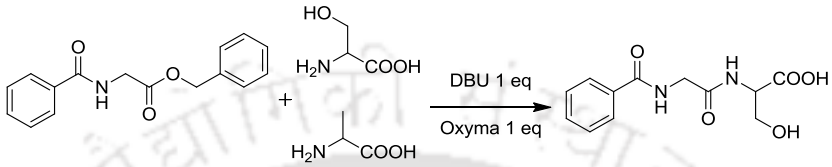
Scheme 1: *Synthesis of amide of N-protected amino acids from their benzyl ester*

Benzyl ester mediated native chemical ligation at serine and threonine site

After successful synthesis of amides from correspoding benzyl esters in a reagent economic way, we went for designing a native chemical ligation (NCL) strategy for the convergent synthesis of peptides using the same method. Ligation chemistry is an extremely valuable tool for the assembly of peptide fragments to provide polypeptide and protein for biological studies.

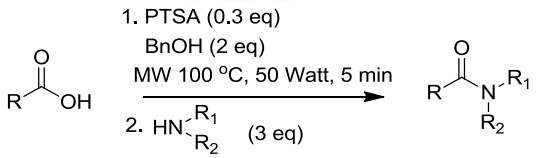
We prepared esters by the reaction of *N*-protected amino acids with alcohol under microwave irradiation in presence of catalytic amount of *p*-toluene sulphonic acid (*p*-TsOH, 0.3 equiv). Serine and alanine were dissolved in water in presence of 1 equiv of DBU, and it was added to the ester which was pre-dissolved in acetonitrile in presence of oxyma. It was

observed that serine attacked chemoselectively to ligate to form the corresponding dipeptide. There was no dipeptide formed by ligation with alanine (*Scheme 2*), as noted by LC-MS studies. We have extended this method to many peptides as well.



Scheme 2: *NCL with benzyl ester of benzoyl glycine at serine.*

After successful completion of the preparation of amides from inactive esters and using it for a chemoselective ligation, we prepared amides directly from *N*-protected amino acids where the *C*-terminus protection and activation could be achieved in one step (*Scheme 3*). We have protected carboxylic acid group with benzyl alcohol as benzyl ester using catalytic amount of *para*-toluenesulfonic acid (*p*-TsOH), further the ester was reacted with amine to form amide in the same pot. We can recover and reuse the catalyst. Here, benzyl alcohol works as solvent as well as carboxylic acid group activator. Unreacted starting materials also could be recovered. Therefore, finally, we get a method where we don't use any extra reagent and don't generate any waste; still a conversion from acid to amide is achieved with high atom economy. We explored the scope of the reaction with various types of carboxylic acids and *N*-protected amino acids with various amines with high yields (up to 75%, 22 examples). We also have designed a continuous flow operation process based on this method and demonstrated that such process can reduce the overall E-Factor from 38% to 4%.



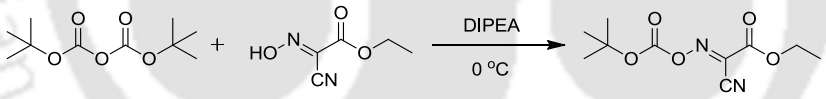
Scheme 3: *Synthesis of amide from N-protected amino acid*

In summary, we have discussed a novel catalyst free and solvent free method for thesynthesis of amides from inactive bezyl esters in this chapter. The extention of the method for development of a novel chemoselective ligation strategy at serine and threonine

sites is also described. Finally, a reagent economic way for amide synthesis directly from carboxylic acids where protection and activation could be achieved in one step with high atom economy is also discussed.

Chapter 3: Ethyl 2-(*tert*-butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma) as a Novel Coupling Reagent for Racemization-Free Esterification, Amidation, and Peptide Synthesis

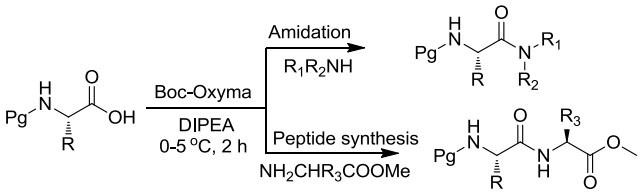
In the previous chapter, we described the amide and peptide synthesis from *N*-protected amino acids. Bulky amino acids took long time to complete the reaction and generated lesser yield in that method. Some of the bulky amino acids did not react at all. In our continued effort to develop methods for synthesis of peptides with all kinds of amino acids, we developed a new coupling reagent, tertiary butyl carbonate ester of ethyl 2-hydroxyimino-2-cyanoacetate (Boc-Oxyma). We prepared Boc-Oxyma by the reaction of Boc anhydride (1.2 equiv) and Oxyma (1 equiv) in presence of DIPEA (1 equiv) at 0 °C in 1 hour (*Scheme 4*). Then we have used the reagent for various reactions, which are described in this chapter.



Scheme 4: *Preparation of Boc-Oxyma*

Boc-Oxyma as new coupling reagent for racemization free amidation and peptide synthesis

Amides are versatile functional groups in the realm of organic synthesis and biomolecules, as they constitute a significant part of the chemical space of biologically active compounds. The newly developed coupling reagent, Boc-Oxyma, was used for the synthesis of amides and peptides. We added Boc-Oxyma to the carboxalic acid/ *N*-protected amino acid which was dissloved in ethyl acetate in DIPEA and the reaction mixture was stirred for 1 h at 0 °C for pre-activation followed by the addition of amine or methylester of amino acids to form amides and peptides respectively (*Scheme 5*, 24 examples, yields up to 95%).



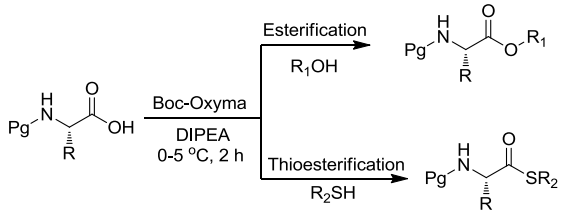
Scheme 5: *Synthesis of amides and peptides using Boc-Oxyma*

The efficiency of the new coupling reagent was also verified for solid phase peptide synthesis. We have successfully prepared the octapeptide H-Gly-Asn-Phe-Gly-Ala-Ile-Leu-Gly-NH_2, which contains the hexapeptide sequence NFGAIL, known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes, using Boc-Oxyma as coupling reagent by stepwise coupling of the constituent amino acids on rink amide MBHA resin using Fmoc chemistry.

The efficiency of the coupling reagentas racemization suppressant was checked by synthesizing a tripeptide, Z-Gly-Phe-Val-OMe by solution phase peptide synthesis via the a segment coupling of Z-Gly-Phe-OH and H-Val-OMe and compared the results to those reported by others with a variety of well known coupling reagents such as HATU, HDMA, HBTU and HDMD. Unlike the other coupling reagents, no racemization was observed while using Boc-Oxyma, monitored by RP-HPLC and NMR techniques.

Epimerization free synthesis of esters and thioesters using Boc-Oxyma

Esters are very important components in the organic, medicinal, and pharmaceutical chemistry. We have developed a method for racemization free esterification and thioesterification that uses nearly equimolar amounts of acids and the corresponding nucleophiles, such as alcohols and thiols using Boc-Oxyma as a coupling reagent with the simple and mild reaction conditions (*Scheme 6*).



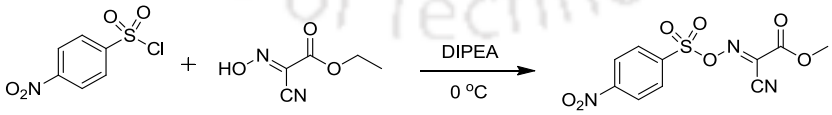
Scheme 6: *Synthesis of esters and thioesters from acids using Boc-Oxyma*

We performed esterification of various acids with different alcohols to explore the scope of the coupling reagent (23 examples). Excellent yield (up to 95%), racemization suppression, and functional group tolerance was noted. A mechanistic investigation was also performed.

Development of a novel and efficient coupling reagent, Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-Cyanoacetate (Boc-Oxyma), for simple, mild, and racemization free esterification, thioesterification, amidation, and peptide synthesis that uses equimolar amounts of acids with equimolar amounts of alcohols, thiols, and amines, respectively, is reported in this chapter. This method can be used for both solid phase and solution phase peptide synthesis.

Chapter 4: Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY) Mediated Racemization free Synthesis of Hydroxamic Acids and Ureas from Carboxylic Acids

In continuation to the development of stereoselective amide and peptide synthesis, we further wanted to develop a method for a racemization free synthesis of peptide hydroxamates and ureas using a new coupling reagent. Peptide hydroxamates and ureas constitute valuable tools in chemical biology as well as interesting leads in medicinal chemistry. We developed a new coupling reagent, Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY). We prepared the 4-NBsOXY by the reaction of 4-nitro benzene sulfonyl chloride and Oxyma in presence of DIPEA as shown in Scheme 7. Then we have used the reagent for hydroxamates and urea synthesis.



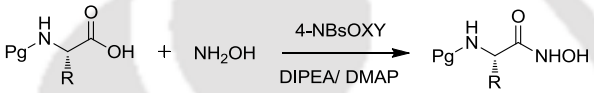
Scheme 7: *Preparation of 4-NBsOXY*

Racemization free synthesis of hydroxamic acids using 4-NBsOXY as a coupling reagent

Hydroxamic acids exhibit wide range of biological activities such as antihypertensive drugs and anti bacterial agent. We described a method for synthesizing racemization free

hydroxamic acids at ambient conditions without using any acid chlorides or strong bases by the new coupling reagent, 4-NBsOXY.

Hydroxamic acids were prepared by pre-activation of the *N*-protected amino acid or carboxylic acids with the coupling reagent 4-NBsOXY in presence of DIPEA and catalytic amount of DMAP (0.1 mol %) at room temperature for 30 min in anhydrous THF followed by the addition of hydroxylamine hydrochloride in DMF with DIPEA (1.5 equiv). It required 2 h for complete conversion at room temperature as monitored by TLC (17 examples, yields upto 82%) (*Scheme 8*).

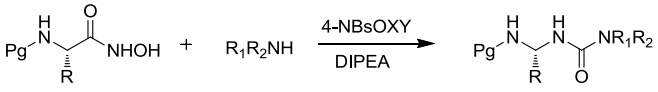


Scheme 8: *Synthesis of hydroxamic acids using 4-NBsOXY*

We have successfully applied this methodology to a wide variety of carboxylic acids that included aromatic, aliphatic,long chain carboxylic acids, and *N*-protected amino acids with excellent yield. The reactions were compatible with common *N*-protecting groups, e.g. Bn, Cbz, Fmoc, and Boc. We have investigated the racemization probability of the current protocol by comparison of the HPLC profile of DL-Boc-phenylalanine hydroxamic acid and L-Boc-phenylalanine hydroxamic acid. From the HPLC data we concluded that there was no detectable racemization for the preparation of hydroxamic acids using 4-NBsOXY. Mechanistic investigation was also carried out.

4-NBsOXY mediated racemization free synthesis of ureas via Lossenrearragement

The urea linkages have interesting applications in medicinal chemistry and in structural chemistry. We developed a method for the preparation of ureas via Lossen rearrangement of hydroxamic acids into corresponding isocyanate by the reaction of 4-NBsOXY (1 equiv) in presence of DIPEA. Stirring was continued at 0 °C for 90 min followed by the addition of amine to the reaction mixture at room temperature as shown in Scheme 9 (21 examples).

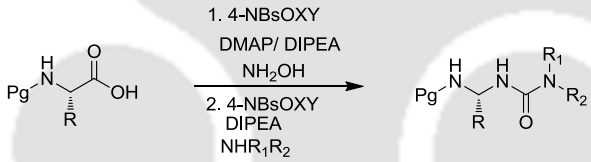


Scheme 9: *Synthesis of peptide ureas using 4-NBsOXY*

The reaction worked well with aliphatic, aromatic, long chain *N*-protected amino acids containing hydroxamic acids with good yield (up to 92%). This methodology was compatible with common amino protecting groups, such as Boc, Fmoc, Cbz, and benzyl as well as various side chain alcohol protecting groups of amino acids, such as, ^{t}Bu and Bzl.

4-NBsOXY mediated single pot synthesis of ureas from carboxylic acids

Finally, we achieved a one-pot synthesis protocol of ureas from carboxylic acids using 4-NBsOXY and obtained up to 71% yield as shown in Scheme 10. One pot protocol requires 2 equiv of 4-NBsOXY; one equiv for activation of the carboxylic acid into Oxyma ester which converts into hydroxamic acid followed by the addition of hydroxylamine hydrochloride, further one equiv of 4-NBsOXY was necessary for conversion of hydroxamic acid into urea via isocyanate (Lossen rearrangement). The scope of the one pot protocol was investigated with a variety of *N*-protected amino acids.



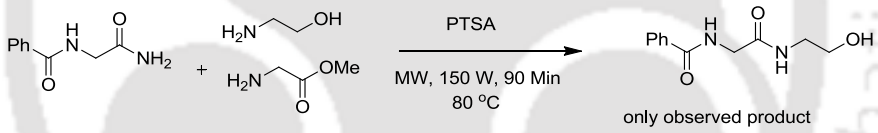
Scheme 10: *Synthesis of peptide ureas direct from acids*

We have developed an efficient method for the racemization free synthesis of ureas and hydroxamic acids under milder reaction conditions utilizing Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY) as a recyclable reagent. The reaction is compatible with common *N*-protecting groups, such as, Boc, Fmoc, Cbz, benzyl and various side chain alcohol protecting groups of amino acids, such as, ^{t}Bu and OBzl. Steroselectivity and mechanism of the reaction were also studied in detail.

Chapter 5: Chemoselective and Stereoselective Synthesis of Peptide Alcohols from Amides via Transamidation

After successful completion of the stereoselective synthesis of amides, peptides, hydroxamates and peptide ureas, we wanted to demonstrate the synthesis of another important class of organic compounds, peptide alcohols. Peptides containing a *C*-terminal

alcohol function (*C*-terminal peptide alcohols) exhibit a range of biological activities, such as the antibiotic properties of the peptaibols and the potent and unique biological activity of the metabolically stable somatostatin analogue octreotide (Sandostatin), which is used clinically for the diagnosis and treatment of a variety of neuroendocrine tumors and gastrointestinal disorders. *C*-terminal peptide alcohols cannot be synthesized by usual solid-phase peptide synthesis (SPPS). Therefore, other SPPS methods that involve reductive cleavage of a peptide ester linkage, use of redox-sensitive resins, solid-phase azirine/oxazolone method, and aminolysis of a peptide ester linkage with amino alcohol were necessary to develop. However, specially modified resins are essential for all those methods.We developed a new procedure for chemoselective and stereoselective peptide alcohol synthesis from amide via transamidation using readily available and inexpensive PTSA (*para*-toluenesulfonic acid) under microwave irradiation as shown in Scheme 11.(18 examples, yields upto 90%)



Scheme 11: *Synthesis of peptide alcohols*

We extended this methodology to the synthesis of peptide alcohol from *C*-terminal amide, which is the most readily available *C*-terminal functionality in usual SPPS. We prepared three peptide alcohols by this strategy, gramicidine A, one of its derivatives, and an anthranilic acid containing α/β-hybrid peptide using this method**.**

Thus, we have developed an efficient method for the preparation of peptide alcohols under simple and milder reaction conditions in presence of commercially available and cost effective PTSA from amides under microwave irradiation that has been extended to the synthesis of biologically important *C*-terminal peptide alcohol synthesis.

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Thalluri Kishore

Abbreviations

Boc	*tert*-butyloxycarbonyl
Boc-Oxyma	Ethyl 2-(tert-butoxycarbonyloxyimino)-2-cyanoacetate
BOP	Benzotriazol-1-yloxy tris(dimethylamino) phosphoniumhexafluorophosphate
Bzl	Benzyl
Cbz	Benzyloxycarbonyl
CDI	*N*,*N*-carbonyldiimidazole
$CHCl_3$	Chloroform
CH_3CN	Acetonitrile
DCM	Dichloromethane
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide
DABCO	1,4-diazabicyclo[2.2.2]octane
DIPEA	Diisopropylethyl amine
DMAP	*N*,*N*-dimethylpyridin-4-amine
DMF	*N, N* dimethyl formamide
ESI MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
Et_3N	Triethylamine
Fmoc	9-Fluorenylmethoxycarbonyl
FT-IR	Fourier transformation infra red spectroscopy
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene)-*N*-methylmethanaminiumhexafluorophosphate*N*-oxide
HBTU	*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino) methylene]*N*-methylmethanaminiumhexafluorophosphate*N*-oxide

HDMA	1-((dimethylamino)-(morpholino)methylene)-1*H* [1,2,3]triazolo[4,5-*b*]pyridiniumhexafluorophosphate3-oxide
HDMB	1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazoliumhexafluorophosphate3-oxide
HOBt	1-hydroxy benzotriazole
HPLC	High pressure liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
mM	millimolar
MW	Molecular weight
mL	milli liter
4-NBsOXY	Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)
NMI	*N*-methyl imidazole
NMP	*N*-methyl pyrolidine
NMR	Nuclear magnetic resonance
OtBu	*tert*-butyl ester
Oxyma	Ethyl 2-cyano-2-(hydroxyimino)acetate
PTSA	Para-toulenesulphonic acid
PyBOP	Benzotriazole-1-yl-oxy-tris-pyrolidine-phosphonium hexafluorophosphate
RP	Reverse phase
SPPS	Solid phase peptide synthesis
tBu	*tert*-butyl
TCT	Tri chloro triazene
TFA	Trifluoroacetic acid
TFE	Trifluoroethanol
TFFH	Tetramethylfluoroformamidiniumhexafluorophosphate
THF	Tetra hydrofuran

Amino Acids:

Ala	A	Alanine
Arg	R	Arganine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine

Chapter 1: Introduction

1.1. Introduction

Novel methodologies for the synthesis of amides, esters, peptides, peptidyl hydroxamic acid, peptidyl-ureas, and peptidyl-alcohols are demonstrated in this thesis. These functionalities are present in most of the pharmaceuticals and natural products. They appear as enzymes, hormones, antibiotics, toxins, and receptors, which constitute a major portion of muscle, hair, and skin. Before going into the discussion about the newly developed protocols for the synthesis of the mentioned classes of compounds, their importance, existing methods for their synthesis, and drawbacks associated with the existing methods are discussed.

1.2. Amino acids

Mostly, compounds associated with peptides have been considered as target molecules in this thesis. Part of a protein is called a peptide. Peptides are synthesized by condensation of amino acids. The linkage between the α-amino acids in proteins and peptides is called a peptide bond, which is nothing but an amide bond. The central carbon atom of an α-amino acid (*Figure 1.2.1.)* is called α-carbon atom, which is linked to a carboxylic acid group, an amino group, a hydrogen atom, and a distinct side chain (R). R can be a simple hydrogen atom or alkyl chains bearing acidic, basic, hydrophobic, hydrophilic, redox-active, bulky or compact moieties. These R groups decide the chemical reactivity of a particular amino acid. Amino acids such as Serine, Threonine, Cysteine, Tyrosine, Asparagine, and Glutamine come under a category of polar amino acids. Charged amino

acids are Aspartic acid, Glutamic acid, Lysine, Arginine, and Histidine. Glycine, Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, and Methionine are hydrophobic and non-polar amino acids. There are 20 standard amino acids that are usually found in proteins. All the standard amino acids are L-amino acids other than glycine.

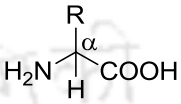


Figure 1.2.1.*General structure of an amino acid*

1.3. Importance of amides, esters, peptides, peptide hydroxamic acids, peptidylureas and peptide alcohols

1.3.1. Importance of amides and esters

Amide bonds are essential for making the peptide bonds in proteins such as enzymes, and are the prevalent subunits in pharmaceutical molecules and natural products. For example, carnosine,[2] a dipeptide (*Figure 1.3.1.1*), synthesized from the amino acids histidine and beta-alanine, was discovered by a Russian chemist V. Gulevich. Carnosine acts as anantioxidant and antiglycating agent. The natural dipeptide L-Carnosine was found to inhibit diabetic nephropathy by protecting the podocytes and mesangial cells. Zinc carnosine (ZnC) is a health food product claimed to possess health-promoting and gastrointestinal supportive activity. Similarly, Atorvastatin,[3] (*Figure 1.3.1.1*) a calcium salt under the trade name Lipitor, is a member of the drug class known as statins and used for lowering blood cholesterol. It prevents strokes through anti-inflammatory

activity. It is a best-selling drug in pharmaceutical history. Likewise, valsartan[4] (Angiotan or Diovan, *Figure 1.3.1.1*) is an angiotensin II receptor. Valsartan expand the blood vessels and reduces blood pressure and congestive heart failure (CHF). Valsartan also used in the treatment and prevention of Alzheimer's disease. In 2005, Valsartan was prescribed more than 12 million times in the United States.

Ester bond is among the most common chemical function present in synthetic or natural products. Phorbol ester is a tetracyclic diterpenoid ester. It is a potent tumor-promoting agent and it can be used as an effective biopesticide and insecticide.[5a] Similarly, caffeic acid phenethyl ester (CAPE) is the ester of caffeic acid and phenethyl alcohol. It has antimitogenic, anticarcinogenic, and anti-inflammatory properties[5b] (*Figure 1.3.1.1).*

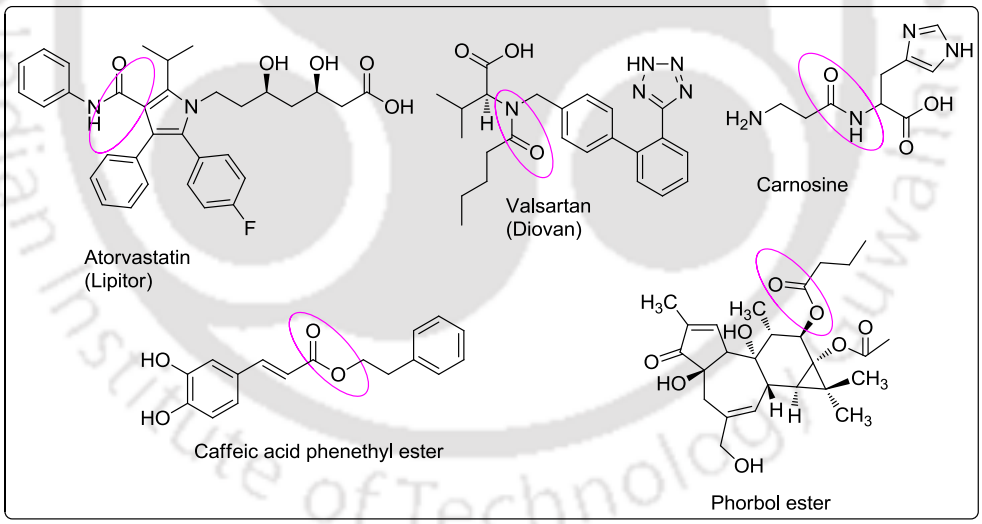


Figure 1.3.1.1. *Biologically active amides and ester*

1.3.2. Importance of peptides

Peptides play important role in biomedicine and biotechnology. Oxytocin[6] (*Figure 1.3.2.1*) is a mammalian neurohypophysical hormone. It acts primarily as a neuromodulator in the brain. Oxytocin activates the flow of milk from breast (milk letdown) by stimulating the contraction of muscle cells located near the milk-containing glands within seconds after an infant begins to suckle. Oxytocin was first synthesized by Vincent du Vigneaud in 1953, and he received the Nobel Prize for Chemistry in 1955 for this work. Oxytocin plays an important role in the neuroanatomy of intimacy, specifically in sexual reproduction, in particular during and after childbirth. A tri-peptide Pro-Leu-Gly-NH_2[7] activates the brain. L-prolyl-L-leucyl-glycinamide (Pro-Leu-Gly-NH_2, *Figure 1.3.2.1*) possesses some anti-parkinsonian activity and that, if used with levodopa, can reduce some of the drug-induced dyskinesias. The tri-peptide Pro-Leu-Gly-NH_2 is used as a potential therapeutic in treatment of Parkinson's disease and depression. The peptide, KLVFF (*Figure 1.3.2.1*), can bind full-length *Aβ*-peptide and prevent its assembly into amyloid fibrils.[8]

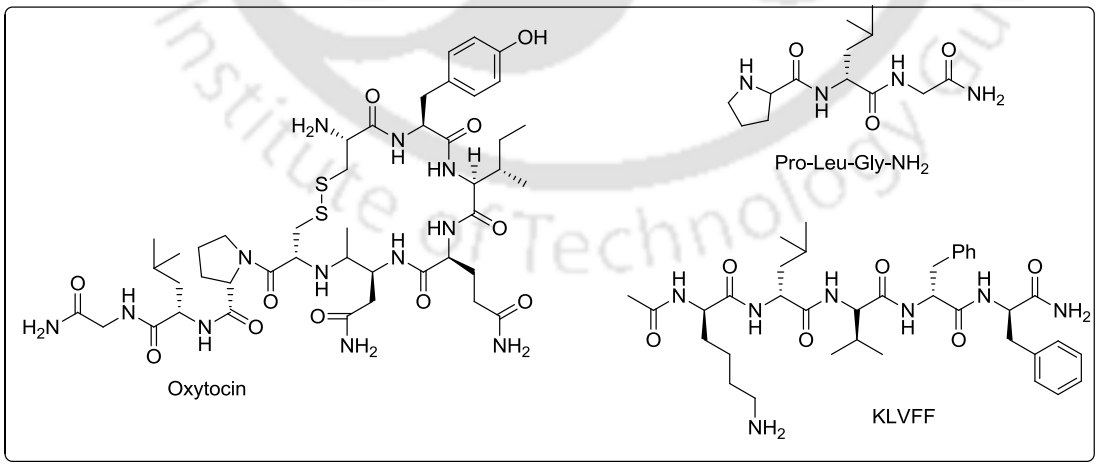


Figure 1.3.2.1. *Biological active peptides*

1.3.3. Importance of peptide hydroxamic acid

Peptide hydroxamates are valuable tools for chemical biology as well as interesting leads for medicinal chemistry. Peptide hydroxamic acids exhibit wide range of biological activities such as anti-hypertensive and anti-bacterial drugs. Vorinostat (*Figure 1.3.3.1,* also known as suberanilo-hydroxamic acid or suberoyl + anilide + hydroxamic acid abbreviated as SAHA) [9] is a prototype of the newly developed second-generation hybrid polar compounds. It is an anticancer agent against both hematologic and solid tumors. It inhibits the activity of histone deacetylases (HDACs), including all known human class I and class II HDACs. Trocade (*Figure 1.3.3.1,* also known as Ro 32-3555 or Cipemast) [10] is also a class of hydroxamic acids. It was developed by Roche. It is a selective matrix mettaloproteinase-1 inhibitor, and it has been used as an anti-arthritis agent. Deferoxamine[11] (*Figure 1.3.3.1,* also known as desferrioxamine B, desferoxamine B, DFO-B, DFOA, DFB or desferal) is a hydroxamic acid siderophore, and is used medically to remove excess iron from the body. GM6001 is a class of peptide hydroxamic acids which are reversible metallopeptidase inhibitors.

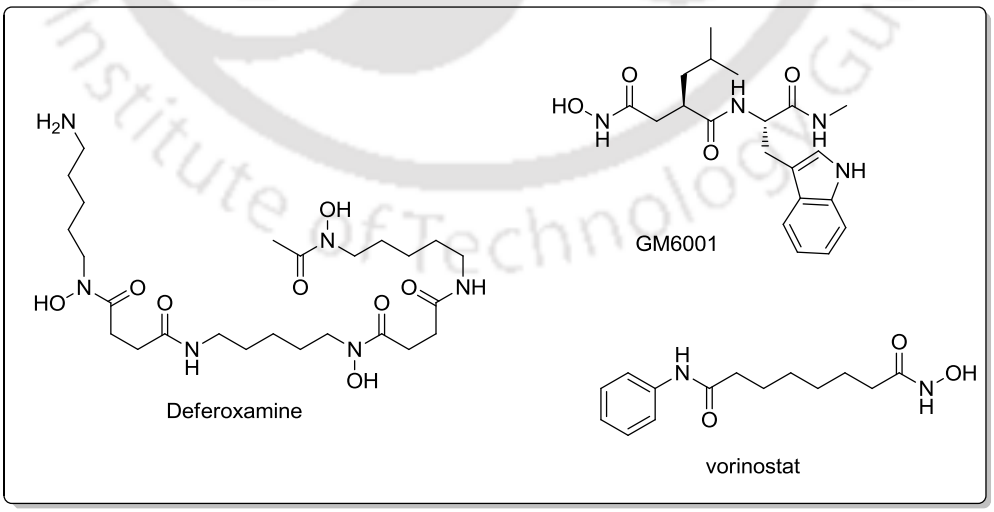


Figure 1.3.3.1. *Biological active peptide hydroxamic acids*

1.3.4. Importance of peptidyl ureas

The urea linkages have interesting applications in medicinal chemistry and in structural chemistry.[12] Unsymmetrically substituted ureas are potential inhibitors of HIV-1 protease,[13] microbial alkaline protease,[14] and several other classes of enzymes.[15] For example, A77003[16] was administered to asymptomatic HIV-1-infected patients in a phase I trial as an inhibitor of the human immunodeficiency virus type 1 (HIV-1) protease. Similarly, α-MAPI[17] is a microbial alkaline protease inhibitor (*Figure 1.3.4.1*) which reached clinical trials.

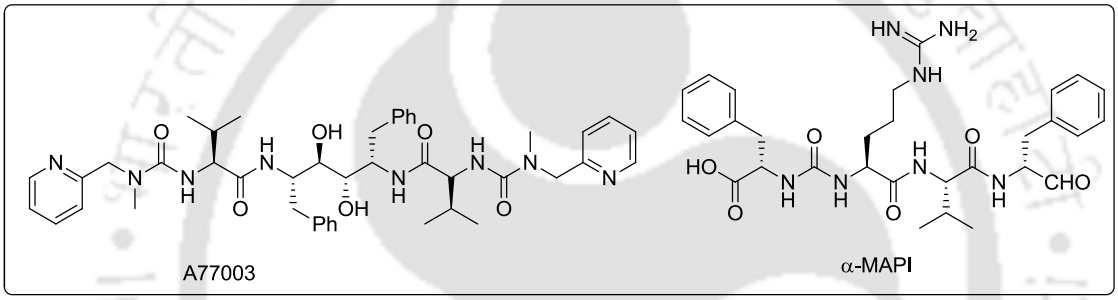


Figure 1.3.4.1. *Biologically active peptidyl ureas*

1.3.5. Importance of peptide alcohols

Peptide alcohols or Peptaibols are non-ribosomal peptides. Hundreds of peptaibols antibiotics have been described in the past half-century. Octreotide[18] (*Figure 1.3.5.1*) is a cyclic deca-peptide alcohol. It was first synthesized by the chemist Wilfried Bauer in 1979. It acts as a potent inhibitor of growth hormone glucagon and insulin. The usage of the salt form of this drug was approved by the food and drug administration. Similarly, gramicidin[19] is an antibiotic linear polypeptide chain with alternating L- and D-amino acids. Gramicidin is active against gram-positive bacteria as well as against selective gram-negative bacteria. Culicinin D (*Figure 1.3.5.1*), a member of the linear peptaibol

family of peptides isolated from the cultures of the fungus, *Culicinomycesclavisporus*, is an anticancer agent.[20] Similarly, SPF-5506-A_4 (*Figure 1.3.5.1*) is a new peptide alcohol,[21] which act as an inhibitor of amyloid-β peptide formation in primary guinea pig cerebral cortex neuron cell culture dose-dependently with an IC_{50} of 0.1 g/ml. Cytotoxicity was not observed at concentrations of <3 g/ml.

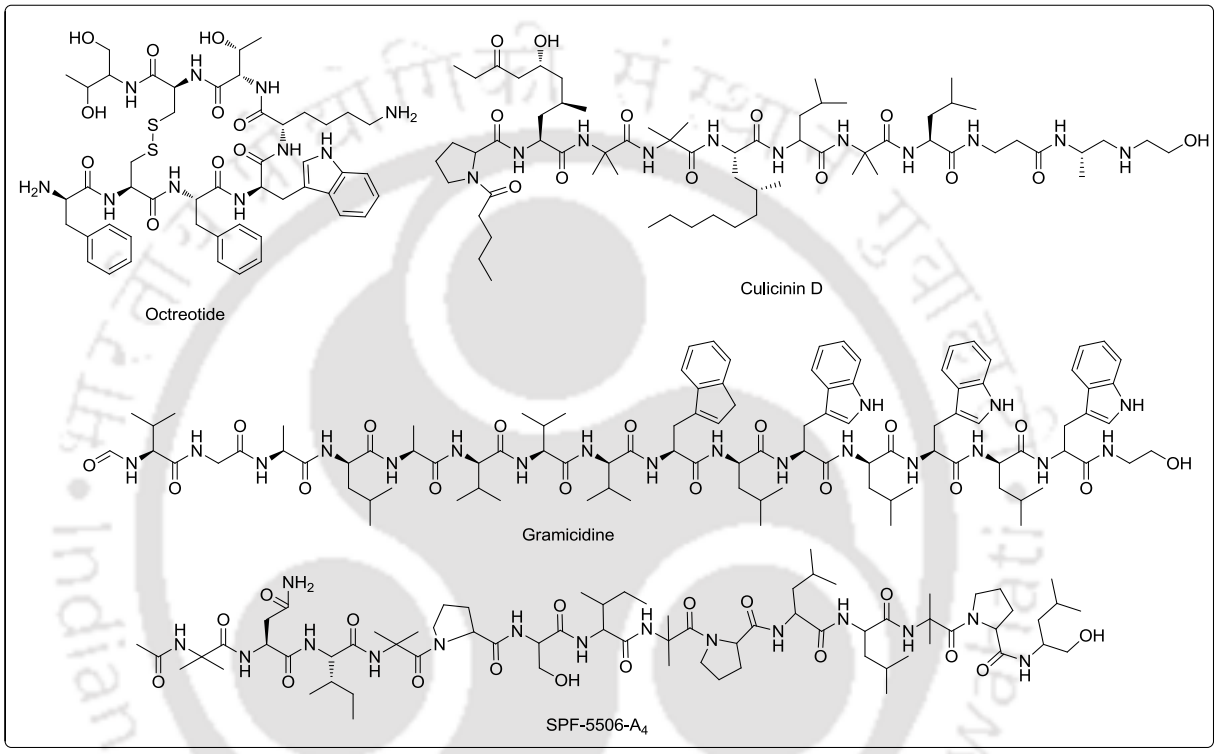


Figure 1.3.5.1. *Biologically active peptide alcohols*

1.4. Existing methods for the synthesis of amides, esters and peptides

Amide or ester bond formation between a carboxylic acid and an amine or alcohol, respectively, is formally a condensation reaction. Amide bonds are typically synthesized by mixing an amine with a carboxylic acid; an acid-base reaction occurs first to form a stable salt. The direct condensation of the salt can be achieved at high temperature (160–180 °C),[24] which is usually quite incompatible with the presence of other common

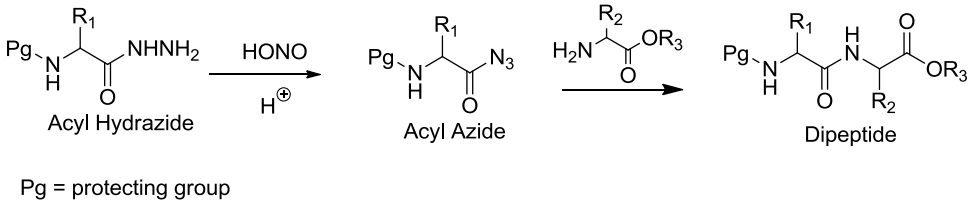
peptide protecting groups, such as Boc, Fmoc, and Cbz. Therefore, activation of the acid is necessary to reduce the energy barrier, and this can be achieved by transformation of the carboxylic acid function to more reactive functional groups, such as anhydrides, acid halides, and active esters, in presence of mixed anhydrides and acid chlorides.

Peptides are synthesized by coupling of carboxylic acid group of one amino acid to the amino group of another amino acid using appropriate protection and deprotection. There are mainly two approaches for peptide synthesis, i.e. *solution phase peptide synthesis* without any solid support and the *solid phase peptide synthesis* (SPPS) using polymeric resin as a support.

1.4.1. Solution phase synthesis

Until 1963, when Prof. Robert Bruce Merrifield[22] started solid phase peptide synthesis, solution phase peptide synthesis was the only available method employed by chemists. Research is still going on to make the solution phase synthesis better. Solution phase peptide synthesis involves mixing of amino acid residues without using any solid resin. Depending on the requirement, protecting groups are used either at carboxyl end or amine end.

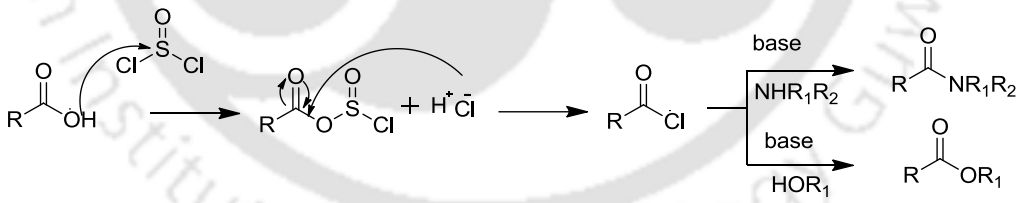
Curtius developed the first chemical synthesis of a dipeptide in 1881.[23] Curtius method involves preparation of acyl azide from acyl hydrazide by the reaction of sodium nitrite with aqueous acetic acid or hydrochloric acid as shown in Figure 1.4.1.1. Acyl azide reacts with the next amino acid in basic medium to form dipeptide.



Scheme 1.4.1.1. *Curtius approach of peptide synthesis*

1.4.1.1. Synthesis of amides, esters, and peptides via acid halides

The most obvious method for activating the carboxyl group of an amino acid for peptide bond formation under milder reaction condition is via simple acid halides. Fisher and co-workers in 1903 have first introduced an acid chloride method in peptide chemistry.[25] Activation of acid function into acid chloride to form amide/ester with amine/alcohol can be achieved by thionyl chloride,[26] oxalyl chloride,[27] phosphorus trichloride,[28] and phosphorus pentachloride.[29] The mechanism of acid chloride formation from acid using thionyl chloride is illustrated in Scheme 1.4.1.1.1. However, because of its high reactivity, it undergoes various side reactions including the loss of configuration.

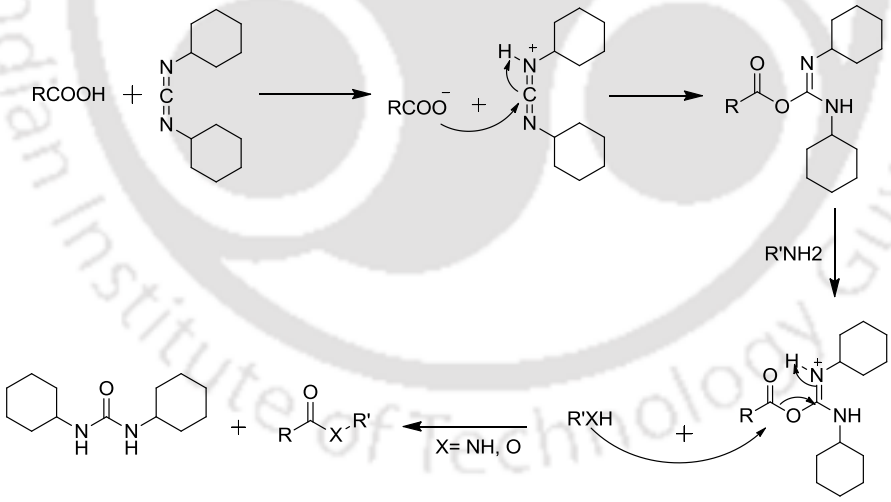


Scheme 1.4.1.1.1*. Mechanism for acyl chloride formation using thionyl chloride*

1.4.1.2. Synthesis of amides, esters, and peptides via anhydrides

Carboxylic acid anhydrides are highly reactive species that readily react with a vast range of nucleophiles, such as alcohols, thiols, and amines to form esters, thioesters, and

amides, respectively. The advantage of this method is that it works at 0 °C to room temperature. The process of coupling individual amino acids can be accomplished through employment of the carbodiimide,[30] 1-ethoxycarbonyl-1,2-dihydroquinoline (EDDQ),[31] cyclic anhydrides (*N*-carboxy anhydrides or Leuch's anhydrides),[32] urethane-protected *N*-carboxy anhydrides (UNCAs).[33] The carbodiimide method involves coupling *N*- and *C*- protected amino acids by dicyclohexylcarbodiimide (DCC) as the coupling reagent. Essentially, this coupling reagent promotes dehydration between the free carboxyl group of an *N*-protected amino acid and the free amino group of the *C*-protected amino acid, resulting in the formation of an amide bond with precipitation of the byproduct, *N*,*N'*-dicylcohexylurea (DCU). This method, however, is hampered by the side reactions which can result in racemization, the formation of (4H)-oxazolones and *N*-acylureas.

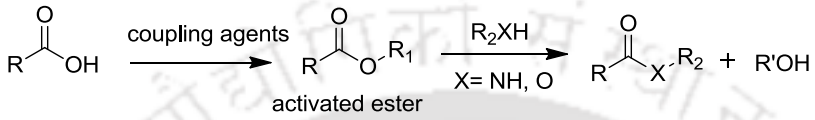


Scheme 1.4.1.2.1. *Preparation of amide and ester using DCC*

1.4.1.3. Synthesis of amides, esters, and peptides via active esters

Schwyzer and co-workers started the preparation of amides, esters, and peptides using activated ester at the *C*-terminus of the amino acid such as para-nitrophenyl ester,[34]

HOBt ester,[35] HOAt ester,[36] pentafluorophenolic ester,[37] *N*-hydroxysuccinimide (HOSu),[38] *N*-hydroxy-5-norbornene-endo-2,3-dicarboxyimide(HONB) esters,[39] and 2, 4, 5-trichlorophenolic ester[40] (*Scheme 1.4.1.3.1*). The presence of these activated esters helps in amidation in shorter time and under milder conditions with good yields. Activated esters such as aromatic ester are commonly used for aminolysis.



Scheme 1.4.1.3.1. *Synthesis of amide through activated ester*

On the other hand, inactive esters such as methyl, ethyl, and benzyl esters are less commonly used. Moreover, these esters require harsh conditions such as the use of high temperatures and addition of catalysts, which increases electrophilic nature of the carbonyl; to name a few, Lewis acids such as $TiCl_4$,[41] sodium methoxide,[42] sodium hydride,[43] sodium cyanide,[44] Grignard reagents,[45] and boron based reagents[46] are used. Han *et al.* introduced the use of group IV metal alkoxides in combination with additives such as HOBt and HOAt.[47]

1.4.1.4. Synthesis of amides and esters via acid halides using microwave activation

Microwave (MW) activation has become a successful alternative to the conventional high temperature reaction condition. It has been used to perform direct condensation of amines and carboxylic acids without prior activation. The use of direct microwave heating helps to reduce the reaction time, side reactions, and increase yields.[48] Microwave irradiation may be used with or without catalyst for condensation reaction.[49]

Different kinds of catalysts such as K-10 montmorillonite[50] imidazole,[51] zeolite-HY[52] and polyphosphoric acid[53] have been used for amide synthesis under microwave reaction conditions. The direct MW-assisted amide formation is compatible with optically active compounds. Both optically active amines and carboxylic acids were found to react smoothly without racemization to give the desired optically active amides which makes this method potentially a powerful tool for asymmetric peptide synthesis.

1.4.1.5. Synthesis of amides, esters, and peptides using coupling reagents

In the early 1970s, Castro and Coste[54] have introduced CloP and BroP as peptide coupling reagents with noticeable racemization. After discovery of HOBt and HOAt as a additives to suppress the racemization in peptide synthesis, in 1975, Coste and coworkers have developed a HOBt and HOAt based phosphonium salt type coupling reagents for peptide synthesis with good yield. At first, they introduced a BOP[55] (benzotriazol-1-yloxy)tris(dimethylamino)-phosphoniumhexafluorophosphate) as a coupling reagent, which contains the racemization suppressant HOBt. Although BOP is an excellent coupling reagent with respect to yield and racemization suppression, the byproduct hexamethylphosphoramide (HMPA), a toxic compound, is generated when condensation is performed using BOP. In order to prevent the generation of the undesirable toxic by-product HMPA, PyCloP, PyBroP,[56] and PyBOP[57] was developed by replacing the dimethylamine moiety with pyrrolidine. HOAt based phosphonium salt type coupling reagents, such as (7-azabenzotriazol-1-yloxy) tris-(dimethylamino)phosphoniumhexafluorophosphate (AOP) and (7-azabenzotriazol-1-yloxy)tris-(pyrrolidino) phosphoniumhexafluorophosphate (PyAOP), have also been

prepared and found to be more efficient than HOBt based phosphonium salt type coupling reagents.

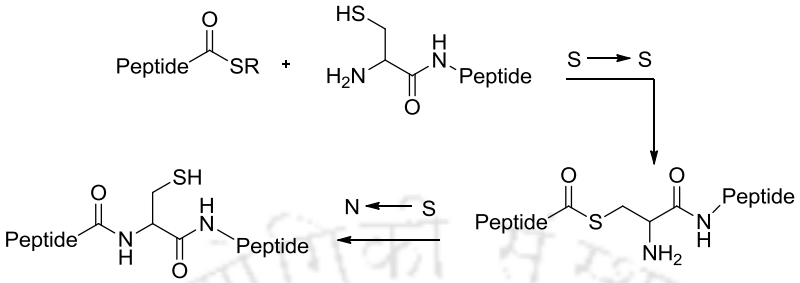
El-Faham and Albericio reported a phosphonium salt of Oxyma, O-[(cyano-(ethoxycarbonyl)methylidene)-amino]yloxytripyrrolidinophosphonium hexafluorophosphate (PyOxm),[58] as an efficient racemization suppressing coupling reagent for the assembly of hindered peptides. Oxyma performs better than classical benzotriazole. Similarly, the recently introduced uronium salt, COMU,[59] renders a higher percentage of cyclic peptides than other known phosphonium salts in cyclization models.

Aminium salts or uranium salts based coupling reagents bear a positive carbon atom instead of the phosphonium residue. The amminium or uronium based reagents react with carboxylic acids to form HOAt/HOBt derived active esters, which then react with amines to form amide. Many coupling reagents based on the HOBt/HOAt system and uronium/aminium salts have been developed, such as HATU,[60] HBTU,[61] TDMB,[62] HPyOPfp,[63] HAPyU,[64] *etc*.

1.4.1.6. Synthesis of peptides via Native Chemical Ligation

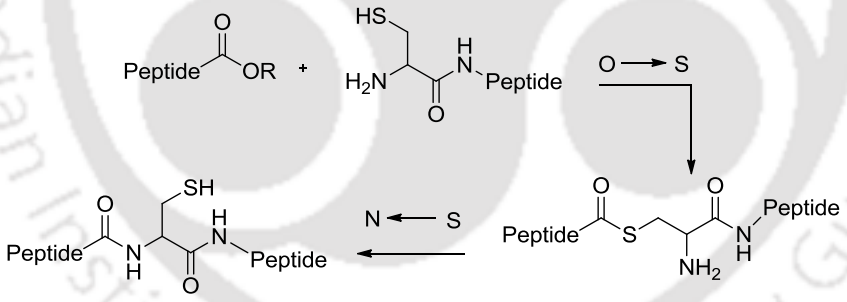
Native Chemical Ligation chemistry is an extremely valuable tool for the assembly of large peptide fragments to form polypeptide and protein for biological studies. Native chemical ligation (NCL) was introduced by Kent and co-workers in 1994.[65] NCL is a major breakthrough in the field of peptide chemistry. Native chemical ligation is a reversible trans-thioesterification and trans-esterification reaction followed by intramolecular amide formation. Kent and co-workers have developed a NCL method from thioester of peptide with cysteine as shown in Scheme 1.4.1.6.1. In this method, reversible thiol-thioester exchange takes place initially. In second step, intramolecular

amide-formation by thioester-amide exchange takes place; this step is irreversible under the reaction conditions. Consequently, only the desired amide-containing product is formed even in the presence of internal cysteine residues in either peptide segment.



Scheme 1.4.1.6.1. *NCL with thioester*

Danishefsky[66] and co-workers have described an oxo-ester mediated Native Chemical Ligation with *para*-nitrophenyl ester under mild reaction condition. In this method, an intermolecular *O-S* acyl transfer takes place followed by intramolecular *S-N* acyl transfer as shown in scheme 1.4.1.6.2.



Scheme 1.4.1.6.2. *NCL with oxo-oester*

1.4.2. Solid phase peptide synthesis

Solid phase peptide synthesis (SPPS) is a milestone in the area of peptide synthesis pioneered by Prof. Robert Bruce Merrifield.[22] He developed a method to synthesize peptides and proteins using solid resin famous as Merrifield resin.

General principle of SPPS involves:

a) Attachment of the first amino acid to a resin

b) Deprotection of *N*-protection with the appropriate reagent

c) Coupling of the next amino acid

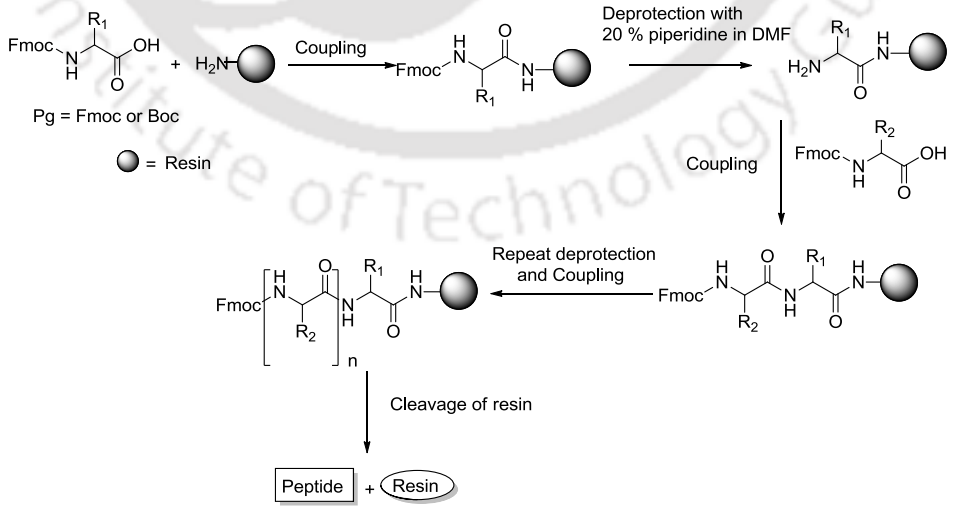
d) Chain elongation by repeating step b and c

e) Cleavage from the resin and deprotection

Two important strategies of this method are Fmoc based SPPS and Boc based SPPS.

1.4.2.1. Fmoc based SPPS

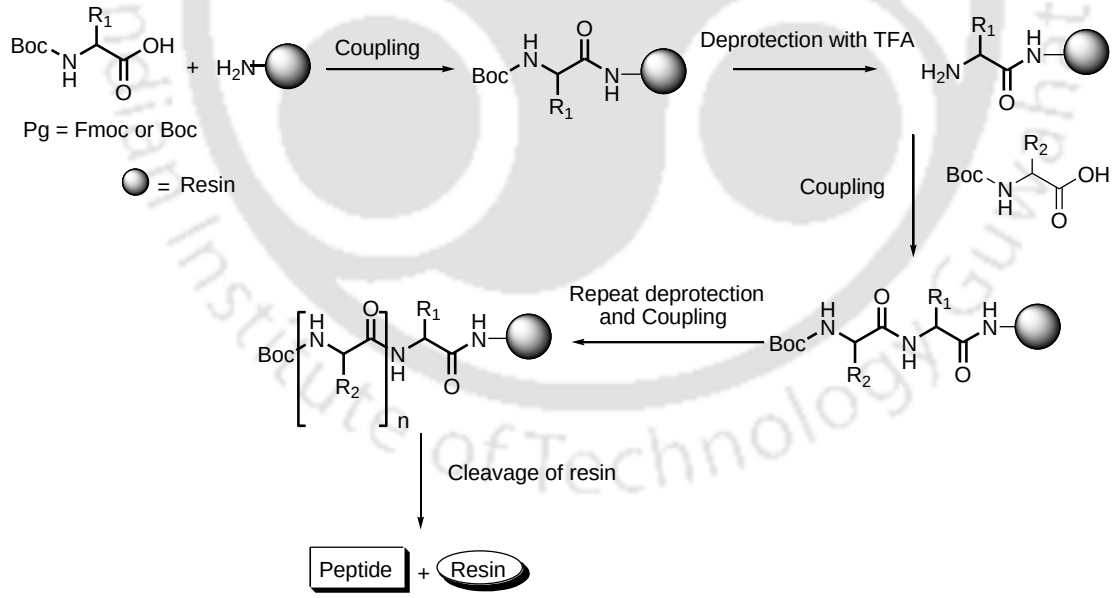
In Fmoc based SPPS,[67] Fmoc protected amino acid is attached to resin via an acid labile linker followed by deprotection of Fmoc, which is usually carried out by piperidine. The second Fmoc protected amino acid is coupled utilizing a pre-activated species or in situ activation by coupling reagents, such as HBTU, BOP, BOP-Cl, *O*-(7-azabenzotrizol-1-yl)-1,1,3,3-tetra-methyluronium hexafluorophosphate (HATU), *etc*. After the desired peptide is synthesized, the resin bound peptide is deprotected and detached from the solid support as shown in scheme 1.4.2.1.1.



Scheme 1.4.2.1.1. *Fmoc based SPPS*

1.4.2.2. Boc based SPPS

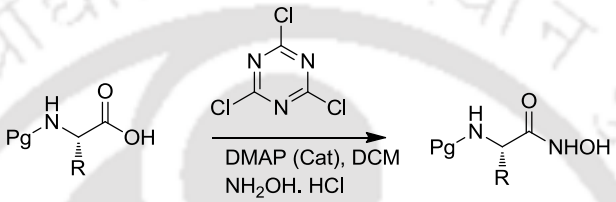
The general scheme which outlines the strategy of Boc based SPPS[68] is shown in Scheme 1.4.2.2.1. Boc protected amino acid is attached to resin via HF cleavable linker. Deprotection of Boc is done by treatment of the amino acid with TFA. The second Boc protected amino acid is coupled utilizing a pre-activated species or in situ activation. After the desired peptide is synthesized, the resin bound peptide is deprotected and detached from the solid support via HF cleavage (*Scheme 1.4.2.2.1*). For many years, DCC was the primary choice as coupling reagent for this strategy. The major problems encountered were the precipitation of dicyclohexyl urea during the activation and acylation processes and side reactions associated with its usage. Later, many new activating agents, such as PyBroP, PyBOP, and BOP were developed for pursuing Boc chemistry.



Scheme 1.4.2.2.1. *Boc based SPPS*

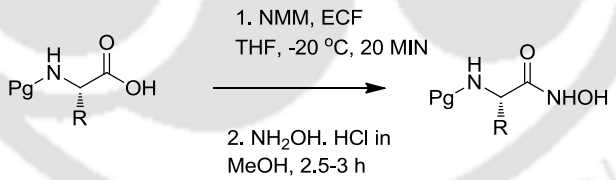
1.5. Existing methods for the synthesis of peptide hydroxamic acids

Usually hydroxamic acids were prepared by the reaction of carboxylic acid or *N*-protected amino acids with hydroxylamine hydrochloride using various catalysts or reagents. Giampaolo *et al*. have synthesized the hydroxamic acids using cyanuric chloride (TCT) directly from *N*-protected amino acids with hydroxylamine hydrochloride in presence of DMAP as shown in Scheme 1.5.1.[69]



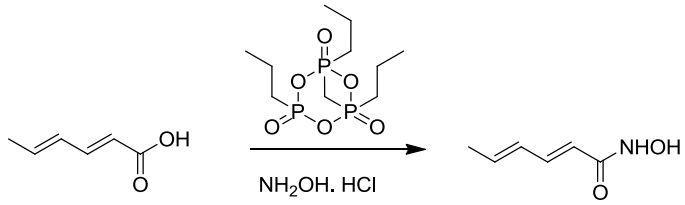
Scheme 1.5.1. *Synthesis of hydroxamic acids by TCT*

Suresh babu *et al.*[70] have prepared the peptide hydroxamic acids using alkyl chloroformates from *N*-protected amino acids in presence of NMM with hydroxyl amine hydrochloride as shown in Scheme 1.5.2.



Scheme 1.5.2. *Synthesis of hydroxamic acids*

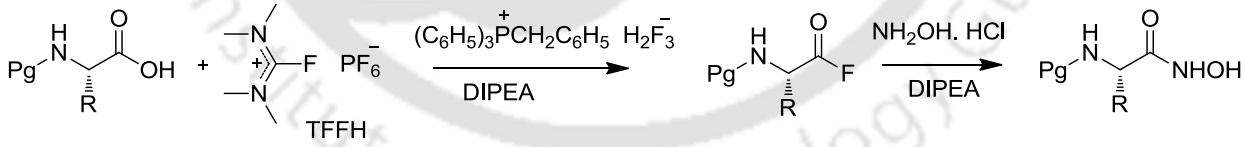
Giovanni Appendino *et al.* have reported (*Scheme 1.5.3*) a method for the synthesis of hydroxamic acids from α,*β*-unsaturated carboxylic acids and hydroxylamine hydrochloride using cyclic phosphonic anhydride (PPAA).[71]



Scheme 1.5.3. *Preparatioon of hydroxamic acids using cyclic phosphonic anhydride*

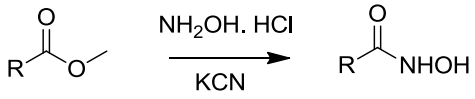
Handling of acid chlorides is difficult, also they produce HCl during the reaction. Therefore usage of acid chlorides is restricted in peptide chemistry. TCT is prepared from the dangerous precursors such as HCN and the byproduct from this reaction is extremely hygroscopic. Therefore, to avoid such difficulties, hydroxamic acids were prepared using peptide coupling reagents from esters and resin-bound esters.

Abdul-Ghani *et al.* has synthesized (*Scheme 1.5.4*) the peptide hydroxamic acids using TFFH as a coupling reagent in presence of the fluoride additive PTF with good yield. First they activated the carboxylic acids or *N*-protected amino acids with TFFH/PTF for 5 min in presence of DIPEA followed by addition of hydroxylamine hydrochloride at 0 °C.[72]



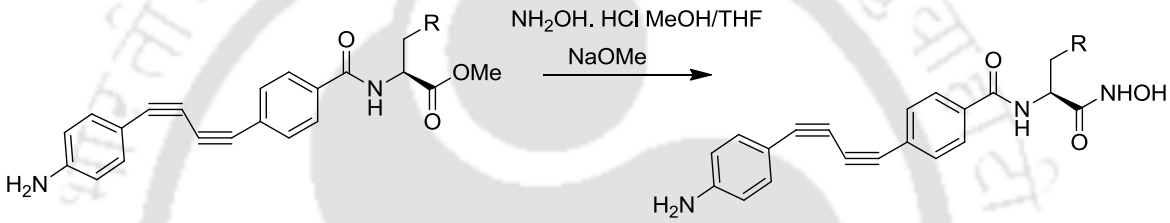
Scheme 1.5.4. *Preparation of hydroxamic acids using TFFH*

Chih *et al.*[73] has developed a method for the solution phase and solid phase synthesis of hydroxamic acids directly from the inactive ester (methyl ester) with hydroxylamine hydrochloride in presence of KCN as shown in Scheme 1.5.5.



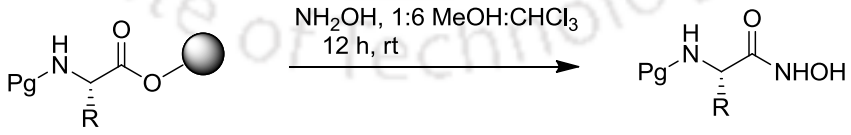
Scheme 1.5.5. *Preparation of hydroxamic acids from esters*

Pei Zhou *et al.*[74] have also prepared hydroxamic acids directly from the inactive ester (methyl ester) with hydroxylamine hydrochloride in presence of sodium methoxide as shown in Scheme 1.5.6.



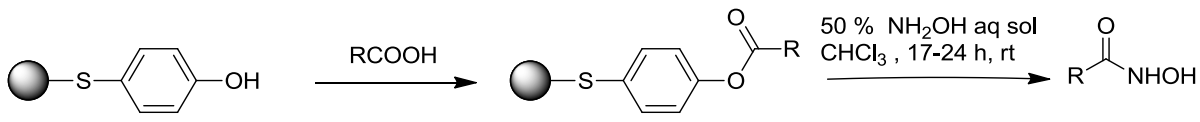
Scheme 1.5.6. *Preparation of hydroxamic acids using sodium methoxide*

Lubell *et al.*[75] has prepared enantiopure hydroxamic acids from the nucleophilic displacement of carboxylates linked to oxime resin using hydroxylamine in a MeOH: $CHCl_3$ solution at room temperature (*Scheme 1.5.7*).



Scheme 1.5.7. *Synthesis of hydroxamic acids from oxime resin*

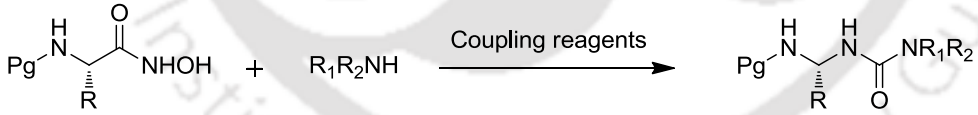
Ping Pang *et al.* has reported the synthesis of a library of discrete hydroxamic acids using the hydroxythiophenol (Marshall) resin from carboxylic acids with good yield (*Scheme 1.5.8*).[76]



Scheme 1.5.8. *Synthesis of hydroxamic acids using Marshall resin*

1.6. Existing methods for the synthesis of ureas

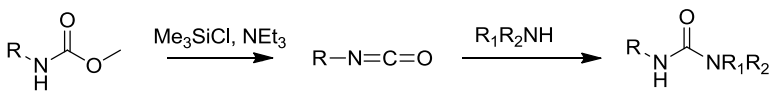
Over the years numerous synthetic methods have been developed for the synthesis of ureas. Urea and its derivatives were synthesized via azide, isocynate or carbamate intermediates. Various coupling reagents have been used in last two decades such as CDI, *N, N*-disuccinimido carbonate, EDC/DMAP, and 1,1-carbonylbisbenzotriazole (*Scheme 1.6.1*).[77]



Coupling reagents = CDI, EDC/DMAP, *N, N*-disuccinimido carbonate and 1,1-carbonylbisbenzotriazole

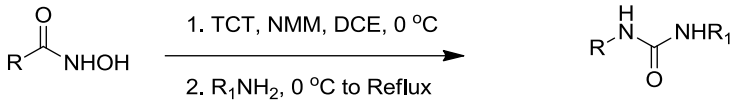
Scheme 1.6.1. *Preparation of ureas using coupling reagents*

Stein *et al*. has reported a mild efficient method for urea synthesis from carbamates via isocyanates in presence of Me_3SiCl (*Scheme 1.6.2*).[78]



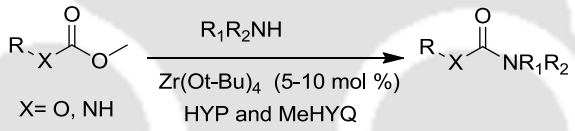
Scheme 1.6.2. *Preparation of ureas using trimethylsilylchloride*

Papot and co-workers developed (*Scheme 1.6.3*) an efficient method for urea synthesis *via* Lossen rearrangement using 2,4,6-trichloro-1,3,5-triazine (TCT) as a promoter from hydroxamic acids.[79]



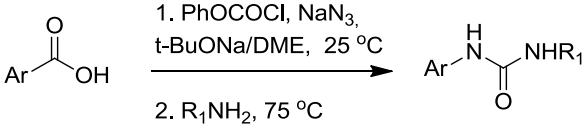
Scheme 1.6.3. *Preparation of ureas using TCT*

Han and co-workers demonstrated a zirconium (IV)-catalyzed protocol for the synthesis of ureas from dialkyl carbonates and carbamates employing 2-hydroxypyridine (HYP) and 4-methyl-2-hydroxyquinoline (MeHYQ) as catalytic additives as shown in Scheme 1.6.4.[80]



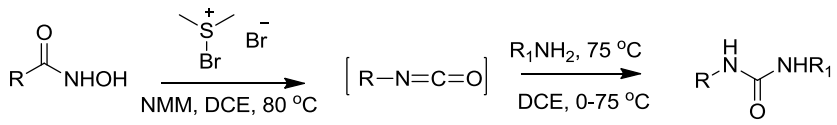
Scheme 1.6.4. *Preparation of ureas using zirconium (IV) catalyst*

Leogane and co-workers[81] developed Curtius rearrangement for the synthesis of ureas from carboxylic acids in presence of di-tert-butyl dicarbonate and sodium azide *via* isocynate formation followed by the addition of amine. This method was only applicable for aromatic carboxylic acids as shown in Scheme 1.6.5.



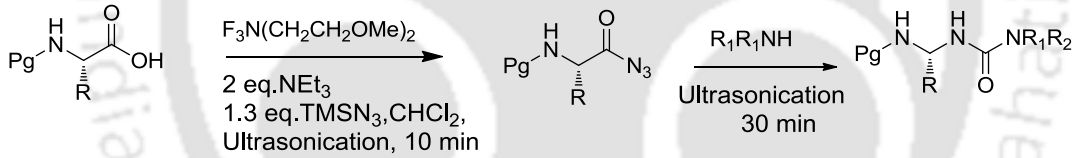
Scheme 1.6.5. *Direct preparation of ureas from acids*

Yadav and co-workers[82] described (*Scheme 1.5.6*) a bromodimethyl sulfonium bromide (BDMS)-mediated Lossen rearrangement for synthesis of unsymmetrical ureas from hydroxamic acids.



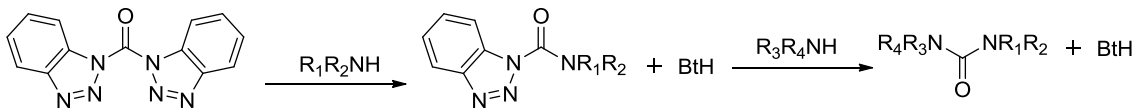
Scheme 1.6.6. *Preparation of ureas using BDMS*

Suresh babu *et al.*[83] has developed a method for the preparation of urea-linked peptidomimetics and neoglycopeptides from acids in presence of Deoxo-Fluor and $TMSN_3$ under Curtius rearrangement conditions. The reaction was carried out under ultrasonication. (*Scheme 1.5.7*)



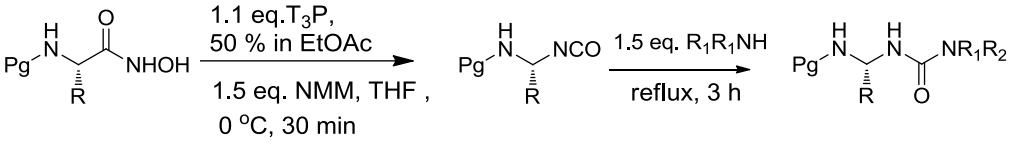
Scheme 1.6.7. *Preparation of ureas using Deoxo-Fluor and $TMSN_3$*

Katritzky[84] has reported a safe and versatile method for the synthesis of unsymmetrical tetra substituted ureas from 1,1-carbonylbisbenzotriazole with amines as shown in Scheme 1.5.8.



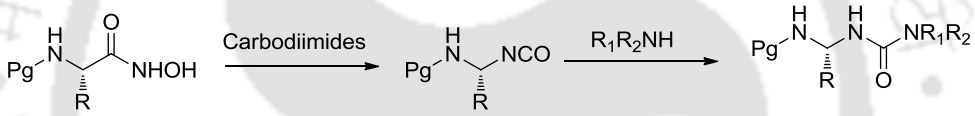
Scheme 1.6.8. *Preparation of ureas using 1,1-carbonylbisbenzotriazole*

Suresh babu and co-workers[85] described a 1-Propanephosphonic acid cyclic anhydride (T_3P) mediated Lossen rearrangement of hydroxamic acids to corresponding ureas via isocynate intermediate. (*Scheme 1.5.9*)



Scheme 1.6.9. *Preparation of ureas using 1-Propanephosphonic acid cyclic anhydride*

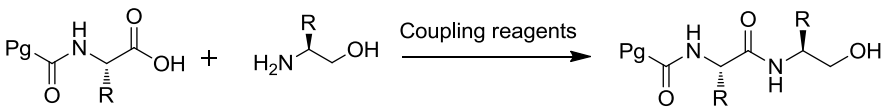
Koshland and co-workers[85b] has reported the method for the preparation of ureas in presence of carbodiimide via isocynate intermediate as shown in Scheme 1.5.10.



Scheme 1.6.10. *Preparation of ureas using carbodiimide*

1.7. Existing methods for the synthesis of peptide alcohols

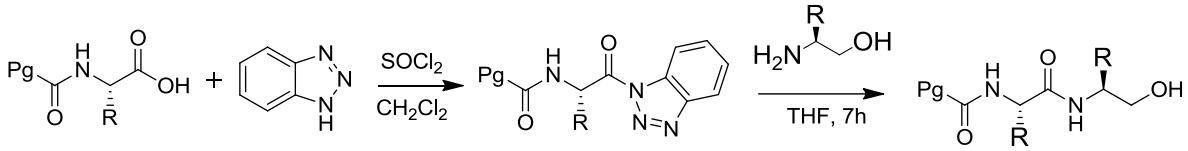
Peptaibols or *C*-terminal peptide alcohols have been synthesized from *C*-terminal carboxylic acids using various coupling reagents [86] as shown in Scheme 1.7.1.



Coupling reagents = DCC/HOBT, EDC/HOBT,DEPBT.

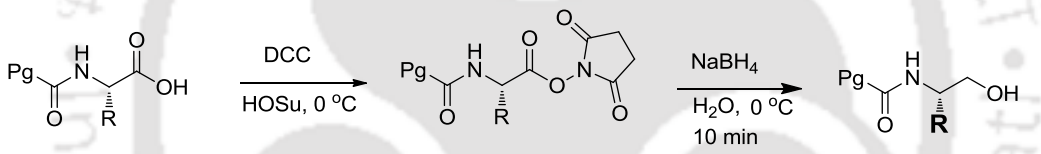
Scheme 1.7.1. *Synthesis of peptide alcohols using coupling reagents*

Katritzky *et al.* have developed a method for the preparation of peptide alcohols using benzotrizole in presence of $SOCl_2$ as shown in Scheme 1.7.2.[87]



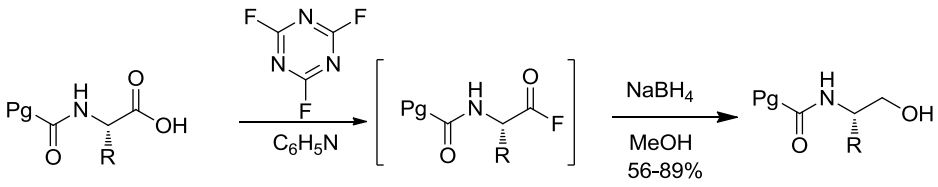
Scheme 1.7.2. *Synthesis of peptide alcohols using thionyl chloride*

Gopi and co-workers (*Scheme 1.7.3*) described the preparation of peptide alcohols from *N*-protected amino acids *via* *N*-hydroxysuccinimide active ester using DCC/HOSu followed by reduction of activated ester with $NaBH_4$.[88]



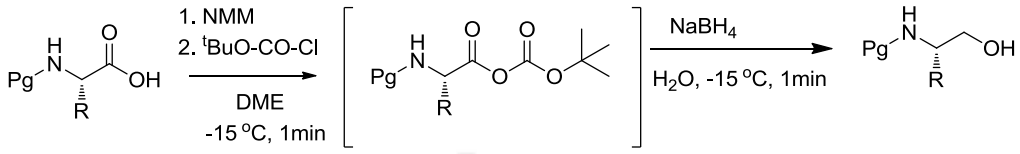
Scheme 1.7.3. *Synthesis of peptide alcohols using N-hydroxysuccinimide active ester*

Noula and co-workers (*Scheme 1.7.4*) have reported the synthesis of peptide alcohols using acid fluorides in presence of strong base pyridine followed by the reduction with $NaBH_4$ in water at 0 °C.[89]



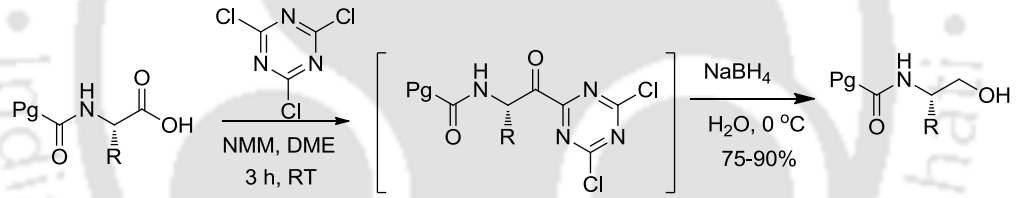
Scheme 1.7.4. *Synthesis of peptide alcohols using acid fluorides*

Martinez *et al.* have synthesized chiral peptide alcohols using anhydrides in presence of NMM, followed by the reduction with $NaBH_4$ in water at 0 °C [90] (*Scheme 1.7.5*).



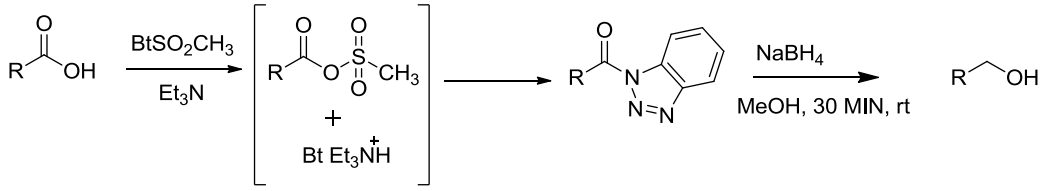
Scheme 1.7.5. *Synthesis of peptide alcohols using anhydrides*

Tadde and co-workers (*Scheme 1.7.6*) have developed a method for peptide alcohols using trichlorotriazine (cyanuric chloride) in presence of NMM, followed by the reduction with $NaBH_4$ in water at 0 °C.[91]



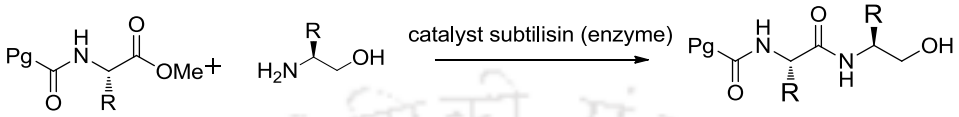
Scheme 1.7.6. *Synthesis of peptide alcohols using trichlorotriazine*

Kaur and co-workers has reported chemoselective reduction of carboxylic acids into corresponding alcohols via sodium borohydride in presence of $BtSO_2CH_3$. (*Scheme 1.7.7*).[92]



Scheme 1.7.7. *Synthesis of peptide alcohols using $NaBH_4$ and $BtSO_2CH_3$*

Stepanov and co-workers[93] has described method for the preparation of peptidyl amino alcohols from esters in presence of subtilisin catalyst by enzymatic synthesis. (*Scheme 1.7.8*)



Scheme 1.7.8. *Synthesis of peptide alcohols by enzymatic synthesis.*

1.8. Drawbacks of the existing methods

The activation of carboxylic acids for the formation of amides or esters is an important process usually carried out using acid halides, anhydrides, azides, active esters, and peptide coupling reagents. This is followed by the reaction with the amine moiety of another amino acid to produce the desired peptide. Nonetheless, acid chlorides and anhydrides are more reactive, and because of their reactivity, many side reactions are possible which are difficult to control. Acid labile *N*-protecting groups of amino acids get cleaved in presence of acid chloride. Therefore, the practical application of the acid chloride method is restricted. In case of acyl azides, many side reactions are possible where acyl azide rearranges to acyl nitrene with the loss of nitrogen gas. Acyl nitrene undergoes Curtius rearrangement (also known as Wolff rearrangement), which leads to the formation of isocyante. This isocyanate reacts with free amino group of amino acids to form urea. On the other hand, activated esters help in amidation in shorter time and

under milder conditions. However, because of its high reactivity, it gets hydrolyzed easily and generates byproducts. The effective formation of peptides, peptide hydroxamic acids, ureas, and peptide alcohols not only depends on the reaction rate and yield but also on the suppression of the undesired racemization. Usually existing methods involve costly coupling reagents. Moreover, those coupling reagents are used in excess sometimes. Thus solid byproducts, which are very difficult to separate from the products often, get generated. For example, in case of DCC method, the byproduct, *N*, *N'*-dicylcohexyl urea (DCU), is harmful to the environment and is very difficult to remove from the product. Furthermore, these coupling reagents are often very difficult to prepare and involves multi-step synthesis that require harsh reagents. Therefore, invention of environmentally friendly methods and cost effective coupling reagents for peptide synthesis and related condensation reactions with highest level of efficacy and racemization suppression still remains a challenge. Therefore, we fixed some specific objectives to develop newer strategies for peptide synthesis and other organic transformations to get rid of some of the mentioned problems associated with the existing methods.

1.9. Objectives of the thesis

1 We wanted to develop a greener method for amide synthesis from inactive esters instead of active esters under milder reaction conditions as the first target.

2 We also wanted to develop a native chemical ligation at serine/ threonine sites from inactive esters using the method obtained from the first objective (Most of the reported native chemical ligation methods are from activated esters at cysteine site. Cysteine residue in proteins is about 1.4%. Because of its rare presence, scientists are extensively interested in ligation at serine and threonine sites).

3 Next, we wanted to develop a protocol for amide synthesis directly from carboxylic acids where protection and activation can be achieved in one step to increase atom economy, decrease cost and waste.

4 We wanted to develop an eco-friendly coupling reagent for racemization free esterification, amidation, and peptide synthesis.

5 We wanted to develop a new coupling reagent for the stereoselective synthesis of peptide hydroxamic acids and ureas. The methods for the stereoselective synthesis of such compounds are not described often.

6 We also wanted to develop a new method for the preparation of peptide alcohols. Most of the reported protocols for such biologically important compounds involve the use of specially modified resins, which are more expensive.

Chapter 2: Inactive Ester Mediated Synthesis of Amides and Peptides

2.1. Solvent free and catalyst free amidation of inactive benzyl esters

Amide bond is among the most common chemical function present in synthetic or natural molecules and is important in peptide chemistry. The amide bond plays a major role in the elaboration and composition of biological systems (Chapter 1, section 1.3.1).[94] An in-depth analysis of the comprehensive medicinal chemistry database revealed that the carboxamide group appears in more than 25% of known drugs.[95]

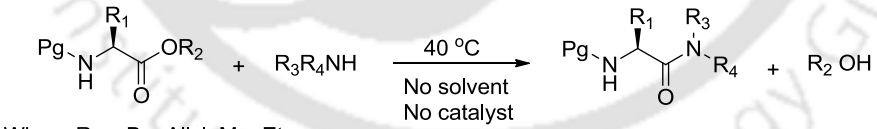
There are plethora of methods and strategies for amide and peptide synthesis. Relevant examples of these methods are mentioned in Chapter I. Although there are many protocols available for amidation from esters, all those methods employ additional catalyst and harsh conditions, such as highly basic or acidic media, high temperature and/or longer reaction time. Herein, we describe the catalyst free and solvent free amide synthesis from benzyl ester of various *N*-protected amino acids at 40 °C.

At first, we have prepared the benzyl amide of benzoyl glycine by the reaction of benzyl ester of *N*-benzoyl glycine with 10 equiv. of benzyl amine under microwave at 160 °C for 30 min, and it worked well. This result inspired us to explore the possibility of preparation of amides from benzyl ester of various *N*-protected amino acids with various amines. Next, we went for finding out the best reaction condition. For optimization of the reaction condition,

we performed the same reaction at different temperature, for example, at 160 °C, 120 °C, 100 °C, and 40 °C. No decrement in the yield of the product with decrement of the temperature was noted. After several trial and error, we found that 3 equiv. of amine was good enough for complete conversion of the starting material to corresponding amide at 40 °C.

We prepared various amides under the optimized condition with various benzyl esters of *N*-protected amino acids and simple amines (*Table 2.1.1).* Especially in case of ethanol amine which facilitates *O* to *N* acyl shift with benzyl ester of benzoyl glycine, it took nearly 20 min for completion. In case of alanine, phenylalanine, and valine derivatives, longer time was necessary. Further we were interested to explore the feasibility of the current methodology for secondary amines, e.g. in case of piperidine, it took 6 h to complete the reaction (*entry 10*). The reaction did not work with diisopropyl amine even after 24 h (*entry 18*).

Table 2.1.1. *Synthesis of amides from benzyl ester of N-protected amino acids*



Where R_2 = Bn, Allyl, Me, Et
Pg = Bz, Cbz, Boc

Entry	Ester	Amine	Time	Isolated yield (%)[a]
1.	Bz-Gly-OBn	$NH_2CH_2CH_2OH$	20 min	73%
2.	Bz-Gly-OBn	$nBuNH_2$	20 min	82%
3.	Bz-Gly-OBn	$BnNH_2$	2.5 h	70%
4.	Bz-Gly-OBn	NH_2CH_2Py	2.5 h	76%
5.	Bz-Gly-OBn	NH_2Cyclohexyl	5 h	85%
6.	Cbz-Gly-OBn	NH_2CH_2Py	1.5 h	80%
7.	Cbz-Gly-OBn	$BnNH_2$	2.5 h	78%
8.	Cbz-Gly-OBn	NH_2Ph	4 h[a]	72%
9.	Cbz-Gly-OBn	NH_2Cyclohexyl	5 h	80%
10.	Cbz-Gly-OBn	Piperidine	6 h	70%
11.	Bz-Ala-OBn	$NH_2CH_2CH_2OH$	1 h	83%
12.	Bz-Ala-OBn	$BnNH_2$	10 h	77%
13.	Bz-Ala-OBn	NH_2CH_2Py	10 h	74%
14.	Cbz-Val-OBn	$nBuNH_2$	24 h	51%
15.	Cbz-Val-OBn	$BnNH_2$	36 h	40%
16.	Boc-Phe-OBn	$nBuNH_2$	10 h	72%
17.	Boc-Phe-OBn	NH_2CH_2Py	23 h	85%
18.	Bz-Gly-OBn	iPr_2NH	36 h[b]	NA

[a]*All the products were characterized using NMR, IR and HRMS. Moreover, the structure of the products of entry no. 7 and entry no. 13 were also confirmed from the X-ray crystallographic data.*

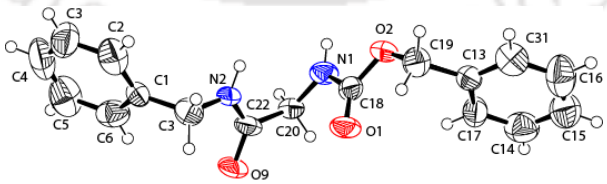


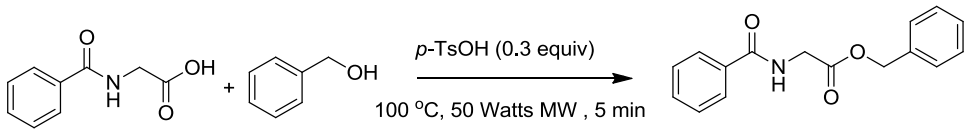
Figure 2.1.1. *ORTEP diagram of Cbzglycinebenzylamide with 50% ellipsoid.*

Thus, the conversion of inactive benzyl ester to amides at 40 °C was found to be possible without addition of any solvent or catalyst. The yields of the reactions were also found to be very good to excellent. This is an example of a reagent economic green reaction.

2.2. Inactive benzyl ester mediated chemoselective ligation at serine

After successful synthesis of amides from inactive benzyl ester in a reagent economic way, we went for designing a native chemical ligation (NCL) type ligation protocol for the convergent synthesis of peptides using the same method. Ligation chemistry is an extremely valuable tool for the assembly of peptide fragments to synthesize polypeptide and protein for biological studies (Chapter 1, section 1.4.1.6). NCL involves a chemo selective ligation reaction between a *C*-terminal thioester and an *N*-terminal Cys to yield a native peptide bond. Although being an extremely powerful method, cysteine based NCL is associated with few drawbacks. For example, the presence of the cysteine residue in proteins is about 1.4%.[96] Moreover, peptide based pharmaceuticals rarely contain an internal cysteine. Now scientists are extensively searching for alternative of cysteine for "native chemical ligation" methods over the past decade.[97] In this direction, people are more interested in the development of ligations at serine and threonine sites.[98] Up to now, active esters, such as thio-ester, *p*-nitro phenyl, and phenyl esters mediated NCL with cysteine and salicylaldehyde ester mediated ligation with serine is demonstrated.[99] In our endeavor to develop a novel chemoselective ligation protocol, aliphatic amino acid esters would ligate to serine.

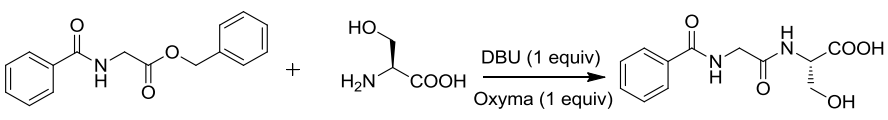
To achieve the mentioned target, initially we prepared the esters by the reaction of *N*-protected amino acids with alcohol under microwave irradiation at 100 ºC, 50 watts for 5 min in presence of catalytic amount of *p*-toluene sulphonic acid (*p*-TsOH, 0.3 equiv, *Scheme 2.2.1)*.



Scheme 2.2.1. *Preparation of benzyl ester of N-protected amino acids*

Next, the benzyl ester of the *N*-protected amino acid was pre-dissolved in acetonitrile and subjected to treatment with unprotected free amino acid serine in presence of phosphate buffer solution of various pH such as 6.6, 7.0, and 7.4 to establish the peptide bond. This reaction was monitored with TLC for four days with 40% of yield. It was found that the benzyl esters are quite stable. Then we went on checking the possibility of constructing the peptide bond with DBU instead of phosphate buffer solution. It took 36 h for the complete disappearance of the ester. The most important advantage of this method is the base itself catalysis the coupling reaction.

For optimization of the reaction condition, we started the reaction under microwave irradiation with benzyl ester of benzoyl glycine, which was pre-dissolved in various solvents in presence of DBU. Serine was dissolved in water in presence of various bases and was added to the ester solution. We found DBU and acetonitrile at 80 °C was the best combination (*Table 2.2.1*). There was no increment of the yield with the addition of additives to the reaction. On the other hand, the addition of Oxyma and HOBt helps to suppress the racemization. Because of the explosive character of HOBt, we selected Oxyma as an additive (*Scheme 2.2.2*).



Scheme 2.2.2. *Preparation of dipeptide using bezyl ester mediated ligation*

Table 2.2.1. *Optimization of the reaction conditions*

Entry	Base	Additive	Solvent	Temp (^{o} C) 150 watt	Yield (%)[a]
1	DBU (1.0)		CH_3CN	80	75
2	DBU (1.0)		toulene	80	32
3	DBU (1.0)		H_2O	80	20
4	DBU (1.0)		DMF	80	62
5	DBU (1.0)		THF	80	53
6	DBN (1.0)		CH_3CN	80	63
7	DABCO (1.0)		CH_3CN	80	28
8	NMM (1.0)		CH_3CN	80	15
9	DIPEA (1.0)		CH_3CN	80	10
10	Et_3N (1.0)		CH_3CN	80	12
11	DBU (1.0)	HOBt (1.0)	CH_3CN	80	72
12	DBU (1.0)	1,2,3-Triazole (1.0)	CH_3CN	80	27
13	DBU (1.0)	Oxyma (1.0)	CH_3CN	80	75
14	DBU(1.0)	Imidazole(1.0)	CH_3CN	80	40
15	DBU(1.0)	benzotriazole	CH_3CN	80	31
16	DBU (1.0)	catechol	CH_3CN	80	47
17	DBU(1.0)		CH_3CN	80	75
18	DBU (0.5)		CH_3CN	80	58
19	DBU (0.2)		CH_3CN	80	48
20	DBU (0.1)		CH_3CN	80	34
21	—		CH_3CN	80	-

[a]*Time required to complete the reaction was 5 h. Temperature was fixed in a CEM microwave synthesizer and the upper limit of the microwave energy was fixed at 150 Watt.*

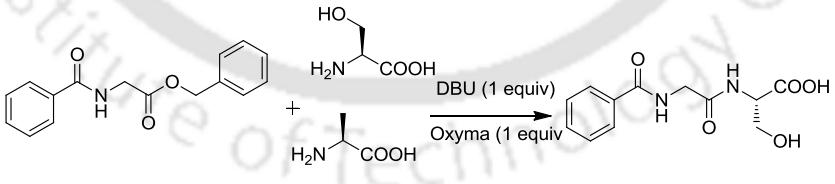
Table 2.2.2 *Standardization of temperature* [a]

Entry	Temp	Time (h)	Yield
1	80	5 h	75%
2	60	10 h	54%
3	50	14 h	34%
4	rt	42 h	70%[b]

[a]*Reaction condition: Bz-Gly-OBz (1 equiv), L-serine (2 equiv), DBU (1 equiv), Oxyma (1 equiv);*

[b]*Reaction was carried out at room temperature.*

To explore the chemoselectivity of the method, we performed a competition reaction to attach serine and alanine to the benzyl ester of benzoyl glycine under the optimized reaction conditions. In this case, serine ligated chemoselectively to the ester to form the corresponding dipeptide. There was no dipeptide formed by ligation with alanine (*Scheme 2.2.3.)*. It was confirmed by LC-MS study as shown in Figure 2.2.3.



Scheme 2.2.3. *NCL with benzyl ester of benzoyl glycine at serine*

A time dependent LC-MS experiment clearly demonstrated slow disappearance of the peak (*Rt 5 min*) corresponding to the parent benzyl ester and simultaneous increment of the intensity of the peak (*Rt 3.18 minutes*) corresponding to the desired dipeptide, Bz-Gly-Ser-OH (*Figure 2.2.1*). Absence of a peak corresponding to the other possible dipeptide, Bz-

Gly-Ala-OH, demonstrates the mentioned selectivity. However, partial hydrolysis generating the peak (*Rt 3.45 minutes*) corresponding to Bz-Gly-OH was also noted.

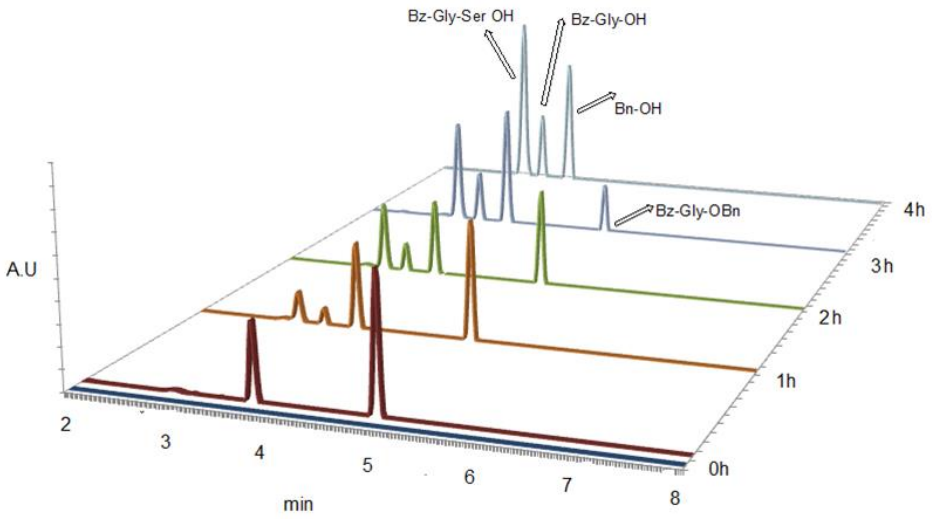
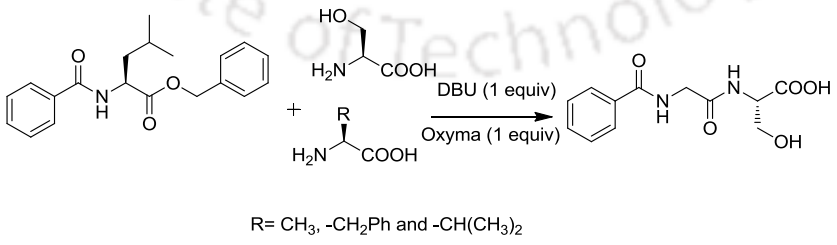


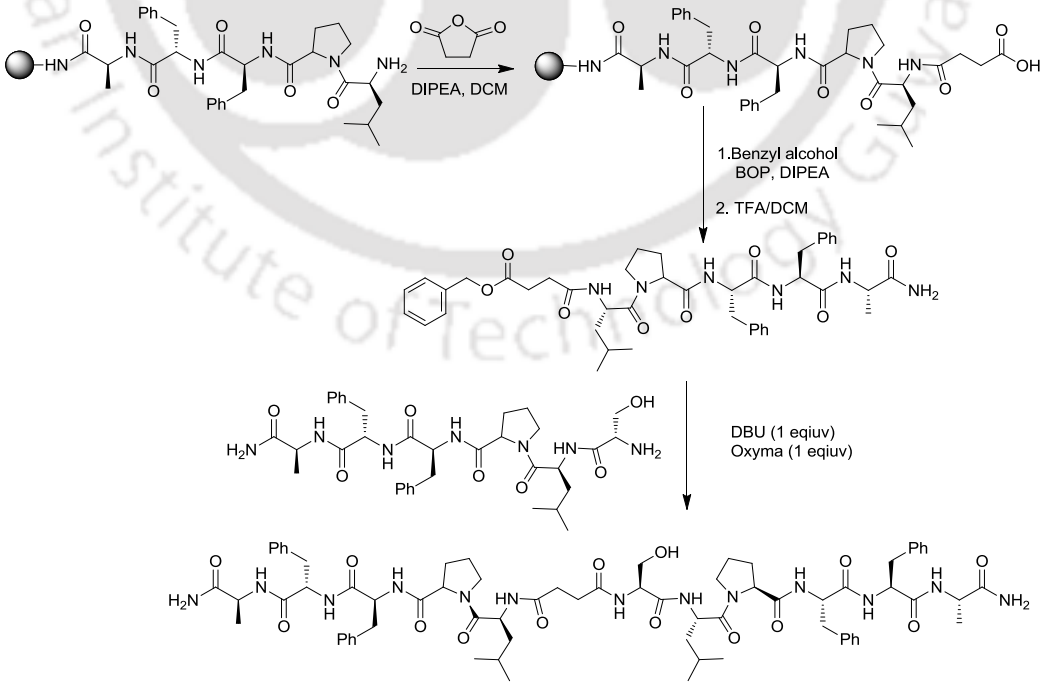
Figure 2.2.1. *LC-MS study of ligation of serine with benzyl ester of benzoyl glycine*

Under the optimized reaction condition, further we have checked the chemoselectivity of serine compared with amino acids like alanine, phenylalanine and leucine using benzyl ester of benzoyl leucine. In all the cases, we got the product corresponding to only serine with good yield (*Scheme 2.2.4*)



Scheme 2.2.4. *NCL with benzyl ester of benzoyl leucine*

The scope of this method was extended for the preparation of the poly peptides. At first, we prepared SLPFFA by stepwise condensation of amino acids on the Rink amide resin. The benzyl ester derivative of LPFFA was also prepared on the solid support by reaction of the succinic anhydride in DCM followed by the addition of benzyl alcohol in presence of the coupling reagent, BOP. After the complete synthesis of the desired peptide sequence, the peptide was cleaved from the resin using TFA/DCM/H_2O. The crude peptide was precipitated using ether followed by centrifugation to get solid desired peptide. Then, the obtained peptide was used for NCL with another peptide of the sequence SLPFFA, which was prepared following similar strategy, to get the desired retro-in verso type peptide under the optimized reaction condition in solution as depicted in the Scheme 2.2.4. All three polypeptides were purified by RP-HPLC and characterized by ESI-MS. The spectra of the final peptide are demonstrated in Figure 2.2.2 as a representative example.



Scheme 2.2.5. *Synthesis of a retro-in verso type peptide by the novel chemoselective ligation method*

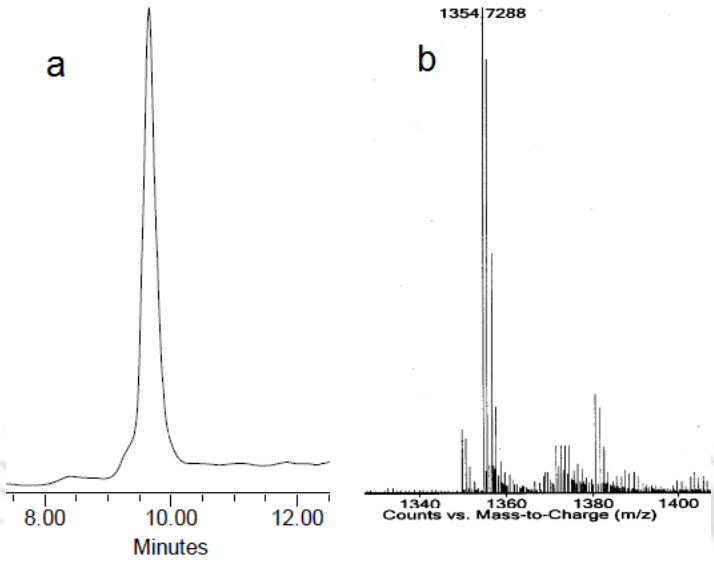
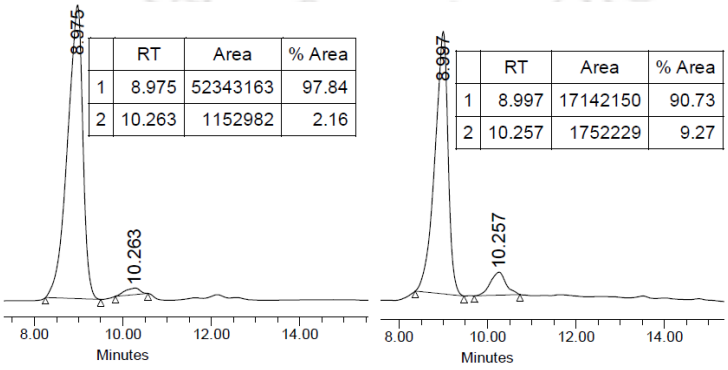


Figure 2.2.2. *HPLC profile and HRMS of the newly synthesized retro-inverso type polypeptide*

The extent of racemization involved in this protocol was checked using HPLC. From the HPLC profile (Symmetry C8, 5 mm 3.0× 15.0 mm analytical column, linear gradient of 0 to 100% CH_3CN in H_2O with 0.1% formic acid, run for 20 min) of Bz-Lue-Ser-OH, 9.1% racemization was observed, where as 2.1% racemization was observed in presence of Oxyma. In case of Bz-Phe-Ser-OH, 2.6% racemization was observed as shown in Figure 2.2.2.



	RT	Area	% Area
1	8.975	52343163	97.84
2	10.263	1152982	2.16

	RT	Area	% Area
1	8.997	17142150	90.73
2	10.257	1752229	9.27

Figure 2.2.2. *HPLC profile of Bz-Leu-Ser-OH, a) with Oxyma, b) without Oxyma*

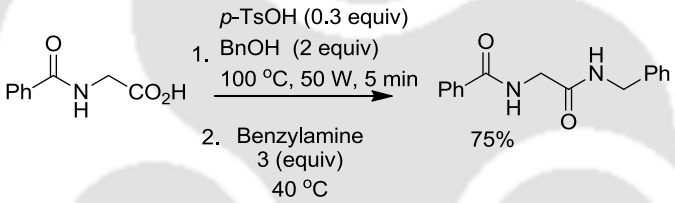
Thus, we have demonstrated the proof of principle of inactive benzyl ester mediated novel chemoselective ligation on serine site. The method will be further explored by one of my junior lab mate.

2.3. An environment friendly method development for amide synthesis

After successful completion of the preparation of amides and peptides from inactive esters, we wanted to develop a new method for amide synthesis direct from acids via benzyl ester of *N*-protected amino acid. The American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable recently recognized amide synthesis as one of the most utilized but problematic syntheses in pharmaceutical industry and indicated as a high priority research area. Direct amide bond formation from free acid and amine requires high temperature and harsh reaction conditions.[100] Although there are many protocols available for amidation from carboxylic acids, still there is a demand for reagent economized and waste minimized amide synthesis protocols.

Our idea of reagent economized and waste minimized amide synthesis involves, the preparation of benzyl ester of benzoyl glycine from benzoylglycine and benzyl alcohol in presence of PTSA using a microwave reactor at 100 ºC, 50 watts for 5 min. Isolated yield of the ester was 77%. Next, the whole amount of isolated ester was stirred at 40 °C for 2.5 h in presence of 3 equiv of benzyl amine, which resulted in amide with 70 % of yield. Therefore, final yield after two steps is about 53% (from acid to amide via ester). To improve the final yield we wanted to use the concept of pot economy. We observed that when benzoyl glycine was treated with *p*-TsOH and benzyl alcohol under microwave at 300 Watts for 1 min

followed by addition of 3 equiv of benzyl amine in the same pot, incubation at 40 °C for 3 h resulted in higher yield of the corresponding amide (isolated yield 75% after two steps, *Scheme 2.3.1*). Percentage yield of the isolated amide, recovered unreacted amine, and benzyl alcohol obtained in this way is reported in the respective tables. We can recover and reuse the catalyst. Here, benzyl alcohol works as solvent as well as carboxyl group activator. Unreacted starting materials also could be recovered. Therefore, finally we get a method where we don't use any extra reagent and don't generate any waste; still a conversion from acid to amide is achieved with high atom economy.



Scheme 2.3.1. *Model reaction with optimized conditions*

We explored the scope of the reaction with various types of carboxylic acids and *N*-protected amino acids with various amines under the optimized reaction condition with high yields (up to 75%). We observed that the reaction was very slow in case of bulky amino acids like alanine and valine. Reaction was not complete even after 36 h in the case of valine. We extended our work to aromatic and aliphatic amines, which generally exist in solid state (*Table 2.3.1*). However, rising the reaction temperature to 90 °C was necessary in the cases of aniline derivatives and 70 °C was sufficient in the case of TRIS to obtain good yield.

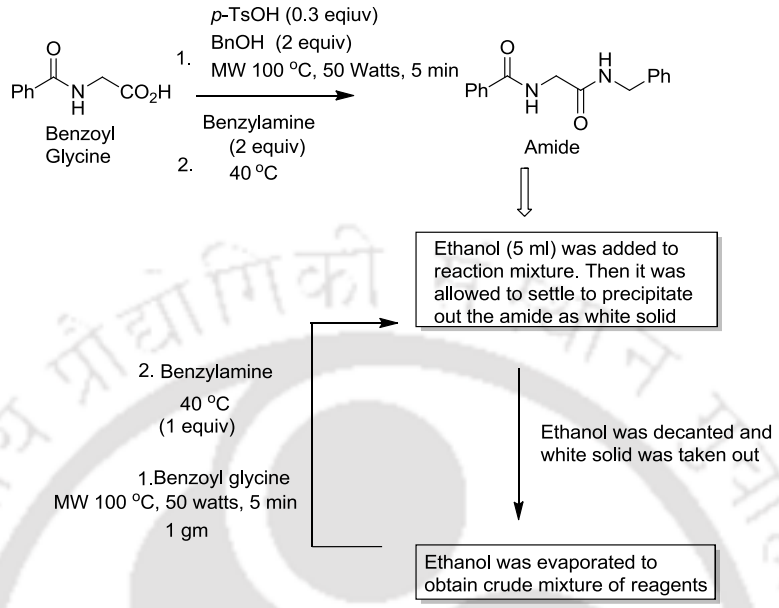
Table 2.3.1. *Preparation of amides direct from acids*

Entry	Acid	Amine	Time	Yield (%)
1	Bz-Gly-OH	n-Butyl	20 min	67
2	Bz-Gly-OH	Ethanol	20 min	60
3	Bz-Gly-OH	Benzyl	3 h	75
4	Bz-Gly- OH	Cyclohexyl	5 h	64
5	Cbz-Gly-OH	Benzyl	2.5 h	68
6	Cbz-Gly-OH	Cyclohexyl	5 h	66
7	Bz-Ala-OH	Benzyl	10 h	66
8	Cbz-Val-OH	n-Butyl	48 h	30
9	Cbz-Val-OH	Benzyl	36 h	36
10	Bz-Gly-OH	Tris	16 h[a]	68
11	Bz-Gly-OH	p-toluidine	24 h[b]	65
12	Bz-Gly-OH	2,3 dimethyl aniline	24 h[b]	67
13	Bz-Gly-OH	*p*-chloro aniline	24 h[b]	67
14	Bz-Ala-OH	Tris	21 h[b]	68
15	$PhCH_2COOH$	Tris	19 h[b]	67
16	$PhCH_2COOH$	Benzyl	5 h	84
17	$PhCH_2COOH$	Cyclohexyl	5 h	48
18	$PhCH_2COOH$	n-Butyl	3 h	93
19	$PhCH_2COOH$	Aniline	7 h[c]	26
20	PhCOOH	Benzyl	8 h	31
21	$C_{11}H_{23}COOH$	n-Butyl	12 h	87
22	$C_{11}H_{23}COOH$	Benzyl	24 h	89

[a]*Reaction was carried out at 70 °C on oil bath.* [b]*Reaction was carried out at 90 °C on oil bath.*
[C]*Reaction was carried out at 100 °C on oil bath.*

Further, we thought whether a continuous flow operation process is possible to design to make the process more efficient. To achieve the continuous flow operation protocol, first we

washed the crude reaction mixture with ethanol (green solvent) after completion of the reaction, which facilitates amide to precipitate out and separate easily from the reaction mixture. The ethanol wash after filtration was later evaporated to obtain PTSA, benzyl alcohol, and the unreacted starting materials back as a mixture. More benzoyl glycine was added to it and again subjected to microwave irradiation at 300 watts for 1 min followed by addition of the benzyl amine. Stirring was continued at 40 °C as before to obtain the amide product (*Scheme 2.3.2*). This process was continued up to three cycles. After three cycles the amide could not be precipitated after the addition of ethanol. Therefore, instead of ethanol wash, we went for acidic wash to remove benzyl amine. Organic layer was evaporated and the crude reaction mixture was washed again with ethanol to obtain the white solid amide product and continued as before up to six cycles. The yield in each cyclic operation is demonstrated in Table 2.3.2. After considering the recovered 500 mg of the starting carboxylic acid can be reused, final yield after six cycles becomes 70%. The E-factor of this continuous operation was found to be 3.87 (E-factor was calculated considering up to 6 cycles), which is much less than the E-factor of the batch process where product precipitation protocol was not used (30.8). However, the E-factor of the batch process, considering the recovery of the reaction mixture by product-precipitation protocol, comes down to 0.3.



Scheme 2.3.2. *Reuse of reagents*

Table 2.3.2. *Reuse of reagents*

Run	Yield
1	80%
2	72%
3	70%
4	60%[a]
5	54%[a]
6	62%[b]

[a]*Yield calculated after acidic workup followed by precipitation of amide, b) after recovery of all the starting materials.*

We have demonstrated a reagent economized and waste minimized amide synthesis where chemical waste generation, evident from the E-factor calculation, is drastically reduced.

2.4. Conclusion

In summary, we have synthesized the amides from inactive esters under solvent free and catalyst free conditions. We have extended the method for developing a inactive ester mediated native chemoselective ligation strategy at serine sites. Found a reagent economic and waste minimized way for amide synthesis directly from carboxylic acids.

2.5. Experimental Section

2.5.1. Materials and methods

All the reagents were purchased from commercial sources and were used without any further treatment. All amino acids except glycine were of the L-configuration unless otherwise stated. Melting points are uncorrected and were determined with a Buchi-540 apparatus. Thin layer chromatograms were run on glass plates precoated with silica gel G for TLC, using solvent systems EtOAc/Hexane. Purification of the reaction products was carried out by column chromatography using silica gel (60-120 mesh) using eluent EtOAc/Hexane.All evaporations were performed under reduced pressure using *Buchi* evaporator. ^{1}H NMR and ^{13}C NMR (400 MHz, 600 MHz for ^{1}H and 100 MHz, 150 MHz for ^{13}C) were recorded using *DRX*-400 Varian and Bruker spectrometers using $CDCl_3$ and MeOH-D_4 as solvents. Chemical shifts are reported in parts per million (*ppm*), internal reference (0.05% to 1 % tetramethylsilane). Abbreviations to denote the multiplicity of a particular signal are s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). High resolution mass spectra were recorded on a Micromass *Q-TOF* ESI MS instrument (model HAB273). The deviation

from the observed m/z value to the theoretical value is less than 5 ppm in each case. IR was recorded on a Perkin Elmer spectrum one FT-IR spectrometer. X-ray data were collected on a Bruker *SMART APEX* equipped with a CCD area detector using Mo. Specific rotation was measured on Rudolph Research Analytical instrument Autopol 1 at 589 nm, 27.1 °C, temperature correction off. Biotageintiator and CEM microwave reactor were used for microwave irradiation. HPLC analysis was carried out with a chiral column, (5 µm, 2.1× 150 mm) and Symmetry C_{18} (5 µm, 3.5 × 150 mm) column coupled to a UV-Visible detector and HPLC grade solvents were used for HPLC analysis.

2.5.2. General procedure for the preparation of amides from benzyl ester of *N*-protected amino acids:

To the benzyl ester of *N*-protected amino acid (0.5 mmol), amine (1.5 mmol) was added, and the reaction mixture stirred at 40 °C. Progress of the reaction was monitored by TLC. After complete conversion, reaction mixture was directly loaded on to the silica gel (60-120 mesh) column for purification.

2.5.3. General procedure for the preparation of dipeptide from benzyl esters by NCL:

Benzyl ester of benzoyl glycine (1 equiv) was taken in sealed pressure tube (5 mL) and dissolved in acetonitrile, serine, and alanine (or other amino acids) were dissolved in water in presence of DBU (1 equiv) was added and irradiated using Biotage intiator microwave reactor at 50 Watts, 80 °C for 5 h. Reaction was monitored by TLC. Product was purified by column chromatography. After the completion of the reaction in order to extract product from the mixture of water and acetonitrile, acetonitrile was evaporated by rotavapour. After

evaporation of the reaction mixture, the reaction mixture was acidified with concentrated HCl upto pH 1-2 followed by the addition of brine solution and product was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated by rotary evaporator. The crude reaction mixture was purified by column chromatography using mixture of ethyl acetate and hexane maintaining 1% of acetic acid throughout.

2.5.4. General procedure for the preparation of SLPFFA using SPPS:

The peptides SLPFFA was synthesized on Rink amide resin (loading 1.1 mmol/g) by stepwise coupling of Fmoc protected amino acids. Each coupling was performed with 2 equivalents of Fmoc amino acid, 2.5 equivalents of BOP, and 5 equivalents of DIPEA (for first coupling three equivalents were used). After each coupling resin was washed with DMF and DCM for 5 min each (1 min x 5) and Kaiser test was performed. Fmoc group was removed using 20% piperidine in DMF mixture for 21 min (7 min x 3). After final Fmoc removal, peptide was cleaved from the resin using a mixture of TFA/DCM/H_2O (80:19:1) 3 mL for 3 h. Crude peptide was precipitated using cold diethyl ether, centrifuged and washed 3 times with cold ether. Peptide was purified on semi preparative HPLC and lyophilized to obtain the final product.

2.5.5. General procedure for the preparation of benzyl ester of the succinyl derivative of LPFFA-NH_2 using SPPS:

To the *N*-terminal free LPFFA-Resin, succinic anhydride (2 equiv) in DCM was added to the resin to get respective succinic acid derivative of LPFFA-NH_2. The reaction was monitored by HPLC and ESI-MS of a test cleavage. To that succinic acid derivative, benzyl

alcohol (2 equiv) was added in presence of the coupling reagent BOP (2 equiv) and DIPEA (4 equiv). After the complete synthesis of the desired peptide sequence, the peptide was cleaved from the resin using TFA/DCM. The crude peptide was precipitated using ether followed by centrifugation to get desired peptide as white solid. The final polypeptide was purified by RP-HPLC and characterized by ESI-MS.

2.5.6. General procedure for the preparation of poly peptide using NCL:

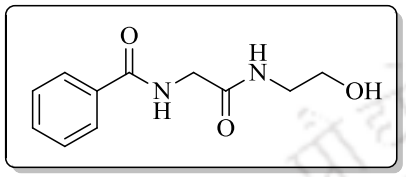
The peptides, benzyl ester of the succinyl derivative of LPFFA and SLPFFA were mixed in presence of DBU with Oxyma under (Biotage intiator) microwave reactor at 50 Watts, 80 °C for 5 h. The reaction was monitored by HPLC and ESI-MS. The final polypeptide was purified by RP-HPLC and characterized by ESI-MS.

2.5.7. General procedure for the preparation of amides direct from acids:

Benzoyl glycine (1 equiv) was taken in a sealed pressure tube (5 mL) and dissolved in benzyl alcohol (2 equiv), *para*-toluene sulphonic acid (0.3 equiv) was added and irradiated using Biotageintiator microwave reactor at 50 Watts, 100 °C for 5 min. After getting the starting materials converted in to the respective esters, benzyl amine (2 equiv) was added and stirring was continued at 40 °C. Reaction monitored by TLC. Product purified by column chromatography.

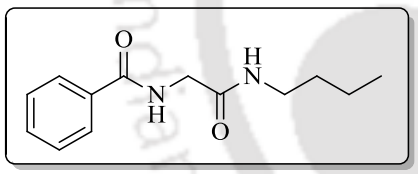
2.6. Characterization data

1) *N*-2-(2-hydroxyethylamino)-2-oxoethylbenzamide (product of entry 1 in Table 2.2.1)



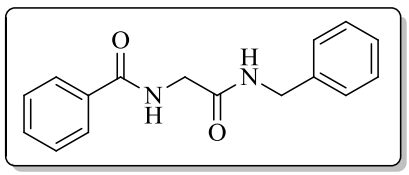
White solid, Yield 73%; Mp. 128 ºC; ^{1}H NMR (400 MHz, MeOH-D_4) δ 7.79-7.35 (m, 5H), 3.94 (s, 2H), 3.52-3.46 (m, 2H), 3.25-3.16 (m, 2H); ^{13}C NMR (100 MHz, MeOH-D_4)δ 170.8, 169.3, 133.8, 131.7, 128.3, 127.3, 60.3, 42.9, 41.7, 21.3; FT-IR (KBr) 1638, 3437 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{11}H_{14}N_2NaO_3$ is 245.0902 found 245.0902.

2) *N*-2-butylamino2-oxoethyl-benzamide (product of entry 2 in Table 2.2.1)



White solid, Yield 82%; Mp. 126 ºC;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.38 (m, 5H, Ph), 6.78 (br, 1H), 4.12-4.10 (d, *J* = 4.8 Hz, 2H, $NHCH_2CO$), 3.27-3.22 (m, 2H), 1.51-1.43 (m, 2H), 1.36-1.28 (m, 2H), 0.89-0.85 (t, *J* = 7.4 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 168.2, 133.6, 132.1, 128.8, 127.4, 41.1, 39.7, 31.6, 20.2, 13.8; FT-IR (KBr) 1539, 1635, 1652, 3298, 3320 cm^{-1};HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{13}H_{19}N_2O_2$ is 235.1447 found 235.1458.

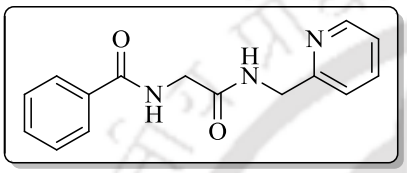
3) *N*-2-benzyl amino-2-oxoethyl benzamide (product of entry 3 in Table 2.2.1)



White solid, Yield 70%; Mp.158 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J* = 8.4 Hz, 1H), 7.50-7.24 (m, 10H,), 6.98 (br, 1H), 4.45-4.43 (d, *J* = 6 Hz, 2H), 4.16-4.15 (d, *J* = 4.8 Hz, 2H);^{13}C NMR (100MHz, $CDCl_3$) δ 169.2, 168.0, 138.0, 132.0,

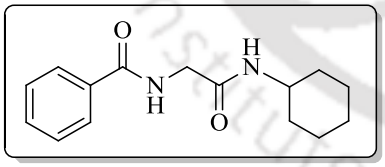
128.8, 128.7, 127.9, 127.6, 127.3, 44.0, 43.8; FT-IR (KBr) 1639, 1667, 3293 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{17}N_2O_2$ is 269.1290 found 269.1290.

4) *N*-pyridin-2-yl-methyl carbamoyl methyl benzamide (product of entry 4 in Table 2.2.1)



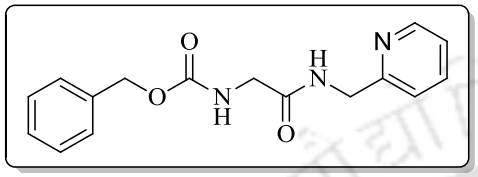
White solid, Yield 83%; Mp. 129 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50-8.49 (d, *J* = 4.4 Hz, 1H), 7.84-7.82 (d, *J* = 7.6 Hz, 1H), 7.68-7.18 (m, 8H), 4.59-4.57 (d, *J* = 5.2 Hz, 2H), 4.23-4.21 (d, *J* = 4.8 Hz, 2H), 3.06 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 167.9, 156.1, 148.5, 137.8, 133.8, 131.9, 128.7, 127.4, 122.9, 122.5, 44.3, 43.7;FT-IR (KBr) 1546, 1654, 1668, 3283 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{15}H_{16}N_3O_2$ is 270.1243 found 270.1256.

5) *N*-2-cyclohexylamino-2-oxoethyl benzamide (product of entry 5 in Table 2.2.1)



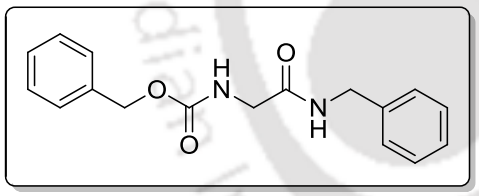
White solid, Yield 85%; Mp. 165 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.81 (d, *J* = 7.2 Hz, 1H), 7.53-7.37 (m, 5H), 6.77-6.75 (d, *J* = 7.6 Hz, 1H), 4.11-4.10 (d, *J* = 4.8 Hz, 2H), 3.74-3.72 (t, *J* = 4 Hz, 1H), 1.87-1.11 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 168.0, 133.7, 131.9, 128.7, 127.3, 48.6, 44.1, 32.9, 25.6, 24.9; FT-IR (KBr) 1542, 1644, 1665, 2934 cm^{-1}, 3303; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{15}H_{21}N_2O_2$ is 261.1603 found 261.1611.

6) Pyridin-2yl-methyl carbamoylmethylcarbamic acid benzyl ester (entry 6 in Table 2.2.1)



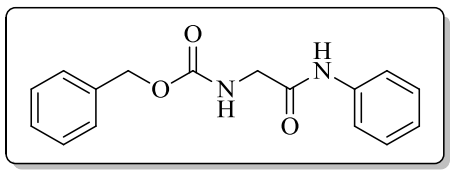
White solid, Yield 80%; Mp.133 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51-8.50 (d, *J* = 3.6 Hz, 1H), 7.74-7.71 (t, *J* = 7.2 Hz, 1H), 7.41-7.26 (m, 7H), 5.67 (br, 1H), 5.1 (s, 2H), 4.58-4.57 (d, *J* = 3.6 Hz, 2H), 3.95-3.94 (d, *J* = 4.4 Hz, 2H), 2.64 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 156.8, 156.4, 148.9, 137.1, 128.5, 128.2, 128.1, 122.5, 122.2, 67.1, 44.5, 44.4; FT-IR (KBr) 1652, 1726, 3281 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{18}N_3O_3$ is 300.1348 found 300.1345.

7) Benzylcarbamoyl-methyl carbamic acid benzyl ester (entry 7 in Table 2.2.1)



White solid, Yield 78%; Mp. 120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.23 (m, 10H), 5.09 (s, 2H), 4.44-4.42 (d, *J* = 5.6 Hz, 2H), 3.89-3.87 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 156.8, 137.9, 136.2, 128.7, 128.6, 128.3, 128.1, 127.7, 127.6, 67.2, 44.6, 43.5; FT-IR (KBr) 1663, 1715, 3322 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{17}H_{19}N_2O_3$ is 299.1396 found 299.1406

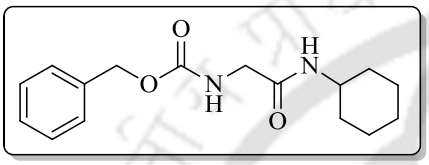
8) Phenylcarbamoylmethyl-carbamic acid-benzyl ester (entry 8 in Table 2.2.1)



White solid, Yield 72%; Mp.147 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.10 (m, 10H), 5.57 (br, 1H), 5.13 (s, 2H), 3.99-3.98 (d, *J* = 4 Hz, 2H); ^{13}C NMR

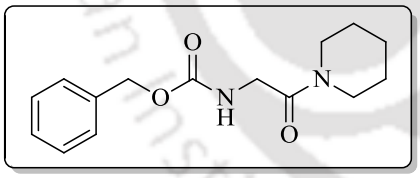
(100 MHz, $CDCl_3$) δ 170.0, 156.5, 136.3, 135.3, 129.3, 128.8, 128.7, 128.5, 128.3, 128.2, 67.3, 43.0; FT-IR (KBr) 1675, 1696, 2929, 3340 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{16}H_{16}N_2NaO_3$ is 307.1059 found 307.1062.

9) Cyclohexylcarbamoylmethylcarbamic acid benzyl ester (entry 9 in Table 2.2.1)



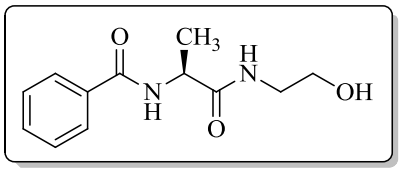
Brown solid, Yield 80%; Mp.110 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 5H), 6.1 (br, 1H), 5.6 (br, 1H), 5.0 (s, 2H), 3.79-3.78 (d, *J* = 4 Hz, 2H), 3.74-3.70 (m, 1H), 1.84-1.09 (m 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 156.8, 136.3, 128.7, 128.4, 128.2, 67.3, 48.5, 44.8, 33.0, 25.6, 24.9; FT-IR (KBr) 1655, 1718, 2924, 3347 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{23}N_2O_3$ is 291.1709 found 291.1710.

10) 2-Oxo-piperidin-1-yl-ethyl-carbamic acid benzyl ester (entry 10 in Table 2.2.1)



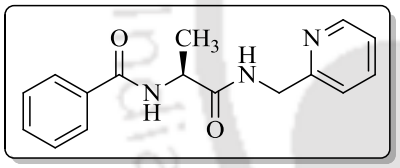
White solid, Yield 70%, Mp.114 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.29 (m, 5H), 5.84 (br, 1H), 5.09 (s, 2H), 3.98-3.97 (d, *J* = 4 Hz, 2H), 3.54-3.52 (t, *J* = 5.2 Hz, 2H), 3.29-3.27 (t, *J* = 5.2 Hz, 2H), 1.70 (s, 2H,), 1.63-1.61 (d, *J* = 4.8 Hz, 2H), 1.54-1.52 (d, *J* = 5.6 Hz, 2H);^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 156.3, 136.6, 128.5, 128.1, 128.0, 66.8, 45.4, 43.1, 42.6, 34.0, 26.2, 25.7, 25.4, 25.0, 24.3; FT-IR (KBr) 1641, 1707, 2936, 3293 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{15}H_{20}N_2NaO_3$ is 299.1372 found 299.1366.

11) *N*-1-(2-hydroxy-ethyl carbamoyl) - ethyl benzamide (entry 11 in Table 2.2.1)



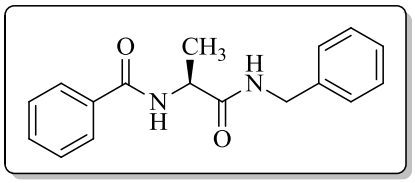
White solid, Yield 83%, Mp.127 ºC; ^{1}H NMR (400 MHz, MeOH-D_4) 7.78-7.34 (m, 5H), 4.49-4.43 (m, 1H), 3.51-3.49 (d, *J* = 5.2 Hz, 2H), 3.23-3.16 (m, 2H), 1.84 (s, 1H), 1.37-1.35 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, MeOH-D_4) δ 175.6, 170.1, 135.3, 133.0, 129.6, 128.7, 61.6, 51.3, 43.1, 18.4; FT-IR (KBr) 1622, 3300, 3425 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{17}N_2O_3$ is 237.1239 found 237.1250. $[\alpha]^{27}_D$ = - 20.00 (c = 0.2, $CHCl_3$)

12) *N*-1 pyridin-2-yl-methylcarbomyl ethyl benzamide (entry 12 in Table 2.2.1)



White solid, Yield 74%; Mp.154 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53-8.52 (d, *J* = 4 Hz, 1H), 7.84-7.24 (m, 8H), 4.87-4.80 (m, 1H), 4.66-4.54 (dd, *J* = 4.8 Hz, 2H), 1.53-1.51 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 167.3, 156.3, 148.6, 137.6, 131.8, 128.6, 127.3, 122.7, 122.3, 49.4, 44.4, 29.8, 18.9; FT-IR (KBr) 1644, 1667, 3300 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{18}N_3O_2$ is 284.1399 found 284.1403. $[\alpha]^{27}_D$ = + 10.00 (c = 0.2, $CHCl_3$)

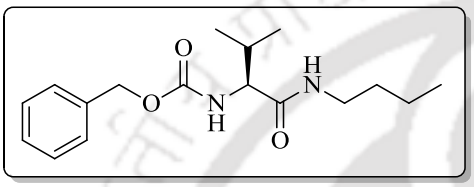
13) *N*-1-benzylcarbamoyl ethyl benzamide (entry 13 in Table 2.2.1)



White solid, Yield 77%; Mp.169 ºC;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.24 (10H), 7.13-7.14 (d, *J* = 4 Hz, 1H), 4.83-4.76 (m, 1H), 4.503-4.379 (dd, *J* = 6 Hz, 2H,), 1.49-1.47 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 167.4, 138.1, 138.8, 131.9, 128.8, 128.7, 127.8, 127.6, 127.3, 49.4, 43.7, 18.9; FT-IR (KBr) 1632, 1657,

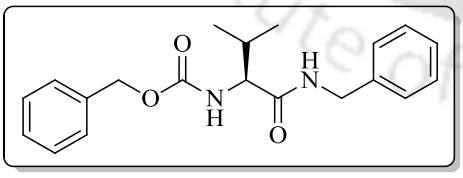
3305 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{17}H_{19}N_2O_2$ is 283.1447 found 283.1434. $[\alpha]^{27}{}_D$ = - 0.003 (c = 0.2, $CHCl_3$)

14) 1-Butyl carbomyl-2-methyl-propyl carbamic acid benzyl ester (entry 14 in Table 2.2.1)



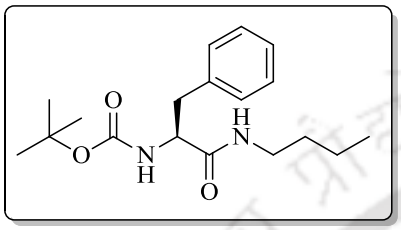
White solid, Yield 40%; Mp. 139 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 5H), 5.95 (br, 1H), 5.41-5.39 (d, *J* = 8 Hz, 1H), 5.1 (s, 2H), 3.92-3.82 (t, *J* = 8.4 Hz, 1H), 3.27-3.18 (m, 2H), 2.11-2.10 (m, 1H), 1.47-1.45(m, 2H), 1.35-1.29 (m, 2H), 0.96-0.83(m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 156.7, 136.4, 128.6, 128.2, 128.0, 67.0, 60.8, 39.3, 31.6, 31.2, 20.1, 19.3, 18.2, 13.8; FT-IR (KBr) 1641, 1690, 3293 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{17}H_{27}N_2O_3$is 307.2022 found 307.2032. $[\alpha]^{27}{}_D$ = - 15.00 (c = 0.2, $CHCl_3$)

15) 1-Benzylcarbamoyl -2-methyl-propyl-carbamic acid bezyl ester (entry 15 in Table 2.2.1)



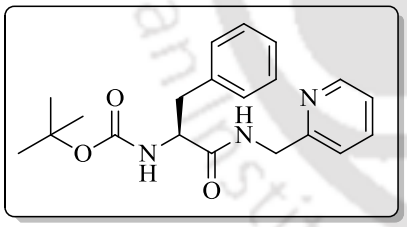
White solid, Yield 40%; Mp.169 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.24 (m, 10H), 6.29 (br, 1H), 5.37-5.35 (d, *J* = 7.6 Hz, 1H), 5.04 (s, 2H), 4.47-4.35 (dd, *J*= 5.2 Hz, 2H), 3.98-3.95 (t, *J* = 6.8 Hz, 1H), 2.14-2.12 (m, 2H), 0.95-0.93 (d, *J* = 6.4 Hz, 3H), 0.91-0.89 (d, *J* = 6.8 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 156.7, 138.0, 136.3, 128.9, 128.7, 128.3, 128.1, 127.9, 127.7, 67.2, 60.8, 43.7, 31.2, 19.4, 18.1; FT-IR (KBr) 1650, 1690, 3291 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{20}H_{25}N_2O_3$ is 341.1865 found 341.1872. $[\alpha]^{27}{}_D$ = + 40.00 (c = 0.2, $CHCl_3$)

16) 1-Butylcarbamoyl -2-phenyl-ethyl-carbamic acid tert butyl ester (entry 16 in Table 2.2.1)



White solid, Yield 72%; Mp. 130 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.19 (m, 5H), 5.73, (br, 1H), 5.12 (br, 1H), 4.27-4.25 (d, *J* = 6.4 Hz, 2H), 3.14-2.97 (m, 4H), 1.41 (s, 9H), 0.87-0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 155.6, 137.1, 129.4, 128.7, 126.9, 80.1, 56.1, 39.2, 39.0, 31.5, 28.4, 20.0, 13.8; FT-IR (KBr) 1654, 1680, 3343 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{18}H_{29}N_2O_3$ is 321.2178 found 321.2180. $[\alpha]^{27}{}_D$ = - 20.00 (c = 0.2, $CHCl_3$)

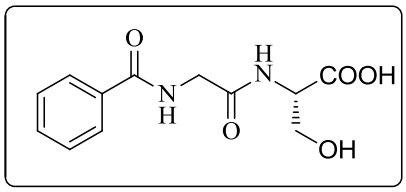
17) 2-phenyl-1-pyridin-2-ylmethylcarbamoyl ethyl carbamic acid tert butyl ester (entry 17 in Table 2.2.1)



White solid, Yield 85%; Mp. 120 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44-8.43 (d, *J* = 4 Hz, 1H), 7.65-7.62 (t, *J* = 6.4 Hz, 1H), 7.22-7.14 (m, 8H), 5.16 (br, 1H), 4.49-4.48 (d, *J* = 4 Hz, 2H), 4.45 (br, 1H), 3.07-3.05 (d, *J* = 6.4 Hz, 2H), 1.36 (s, 9H);^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 156.4, 155.5, 148.9, 137.0, 136.8, 129.4, 128.7, 126.9, 122.5, 122.1, 80.2, 56.0, 44.5, 38.8, 28.4; FT-IR (KBr) 1650, 1661, 1684, 3332 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{20}H_{26}N_3O_3$ is 356.1974 found 356.1962. $[\alpha]^{27}$ D = - 5.00 (c = 0.2, $CHCl_3$).

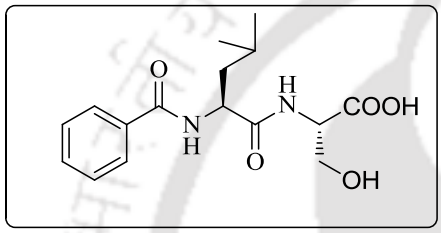
18) 2-(2-benzamidoacetamido)-3-hydroxypropanoic acid (A)

White solid, Yield, 75%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.83-7.82 (d, *J* = 6.4 Hz, 2H), 7.52-7.39 (m. 3H), 4.52 (m. 1H), 4.14-4.13 (d, *J* = 6.4 Hz, 2H,), 3.88-3.920 (2H, m);^{13}C



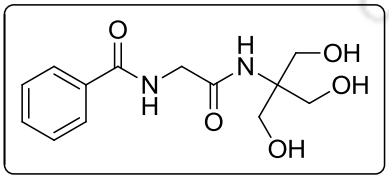
NMR (100 MHz, CD_3OD) δ 172.8, 167.6, 167.6, 133.5, 131.3, 128.1, 127.1, 61.8, 54.7, 43.0; HRMS (-Ve), $[M-H]^-$, calculated for $C_{12}H_{13}N_2O_5$ is 265.0824, found 265.1544. $[\alpha]^{27}$ D = - 27.00 (c = 0.2, MeOH).

19) 2-(2-benzamido-4-methylpentanamido)-3-hydroxypropanoic acid (B)



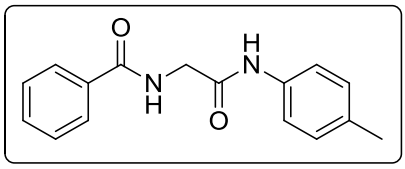
White solid, Yield 68%;^{1}H NMR (400 MHz, CD_3OD) δ 7.98-7.97 (d, *J* = 6.4 Hz, 1H), 7.78-7.74 (m, 5H), 7.43-7.28 (m, 5H), 4.85 (m, 1H),4.57 (m. 1H), 3.99-3.96 (d, *J* = 6.4 Hz, 1H), 3.81-3.79 (d, *J* = 6.4 Hz, 1H), 1.67 (m, 1H), 1.27 (m, 1H), 0.85-0.84 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.5, 173.0, 168.7, 133.3, 132.1, 128.7, 127.5, 62.6, 60.6, 54.8, 41.3, 24.9, 23.0, 22.8. HRMS (-Ve), $[M-H]^-$, calculated for $C_{12}H_{13}N_2O_5$ is 321.1450, found 321.2904. $[\alpha]^{27}{}_D$ = - 42.00 (c = 0.2, $CHCl_3$).

20) *N*-[(2-Hydroxy-1,1-bis-hydroxymethyl-ethylcarbamoyl)-methyl]-benzamide (entry 10 in Table 2.3.1)



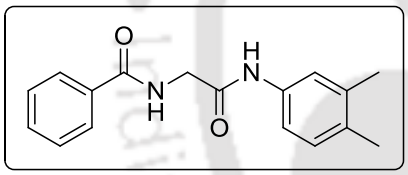
Semi solid, Yield 84%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87-7.44 (m, 5H), 4.06 (s, 2H), 3.74 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 170.6, 133.1, 129.6, 128.5, 126.9, 63.5, 62.2, 44.6; FT-IR (KBr) 1544, 1641, 2918, 3432 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{13}H_{18}N_2O_5$ is 305.1113 found 305.1117.

21) *N*-(p-Tolylcarbamoyl-methyl)-benzamide (entry 11 in Table 2.3.1)



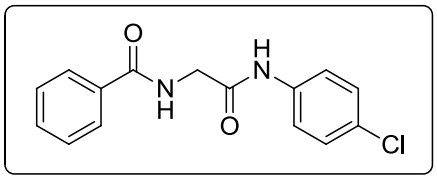
White solid, Yield 65%; Mp. 230 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (br, 1H), 7.83-7.06 (m, 10H), 5.68 (br, 1H), 4.32-4.31 (d, *J* = 5.6 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 167.4, 135.4, 133.4, 131.5, 130.8, 129.1, 128.3, 127.2, 119.8; FT-IR (KBr) 1638, 1676, 2925, 3269 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{16}N_2O_2$ is 269.1290 found 269.1285.

22) *N*-[(3,4-Dimethyl-phenylcarbamoyl)-methyl]-benzamide (entry 12 in Table 2.3.1)



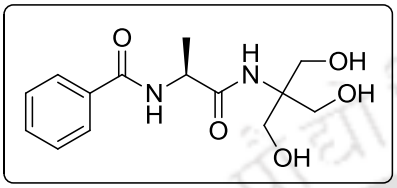
White solid, Yield 67%; Mp. 218 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (br, 1H), 7.87-7.03 (m, 10H), 4.35-4.34 (d, 2H, *J* = 5.2 Hz), 2.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 167.4, 136.9, 135.7, 131.6, 129.7, 128.4, 127.2, 121.2, 117.4, 44.3, 19.8, 19.1; FT-IR (KBr) 1638, 1693, 2921, 3283 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{17}H_{18}N_2NaO_2$ is 305.1266 found 305.1251.

23) *N*-[(4-Chloro-phenylcarbamoyl)-methyl]-benzamide (entry 13 in Table 2.3.1)



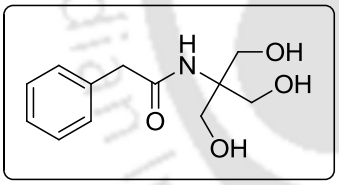
White solid, Yield 67%; Mp. 232 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93-7.33(m, 10H), 4.25 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 167.8, 136.4, 133.2, 131.8, 129.1, 128.6, 128.4, 127.0, 121.1, 43.5; FT-IR (KBr) 1638, 1680, 3192, 3306 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{15}H_{13}ClN_2NaO_2$ is 311.0563 found 311.0565.

24) *N*-[1-(2-Hydroxy-1,1-bis-hydroxymethyl-ethylcarbamoyl)-ethyl]-benzamide (entry 14 in Table 2.3.1)



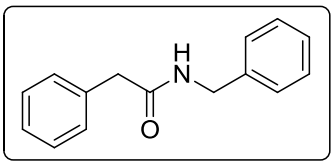
Semi solid, Yield 68%; ^{1}H NMR (400 MHz, CD_3OD) δ7.76-7.7.35 (m, 5H), 4.51-4.44 (q, *J* = 7.2 Hz, 1H), 3.62 (s, 6H), 1.37-1.36 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 170.5, 135.1, 133.1, 129.7, 128.6, 63.5, 62.3, 51.8, 18.1; FT-IR (KBr) 1640, 1671, 2922, 3436 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{20}N_2O_5$ is 297.1450 found 297.1455.

25) *N*-(2-Hydroxy-1,1-bis-hydroxymethyl-ethyl)-2-phenyl-acetamide (entry 15 in Table 2.31)



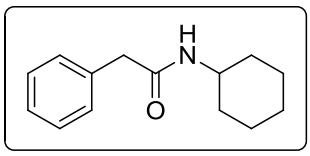
White solid, Yield 67%; Mp. 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19-7.13 (m, 5H), 3.59 (s, 6H), 3.46 (s, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 136.7, 130.3, 129.7, 128.0, 63.6, 62.5, 44.3; FT-IR (KBr) 1542, 1644, 2934, 3283 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{17}NO_4$ is 240.1236 found 240.1233.

26) *N*-Benzyl-2-phenyl-acetamide (entry 16 in Table 2.3.1)



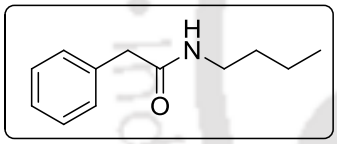
White solid, Yield 68%; Mp. 118 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.16 (m, 10H), 5.76 (br, 1H), 4.41-4.39 (d, 2H, *J*= 6 Hz), 3.62 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 138.3, 135.0, 129.4, 129.0, 128.7, 127.5, 127.4, 127.3, 43.7, 43.6; FT-IR (KBr) 1552, 1638, 2925, 3289 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{15}NO$ is 226.1232 found 226.1231.

27) *N*-Cyclohexyl-2-phenyl-acetamide (entry 17 in Table 2.3.1)



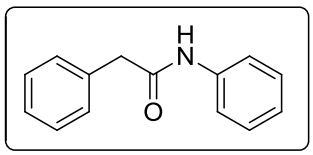
White solid, Yield 48%; Mp. 133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.23 (m, 5H), 5.23 (br,1H), 3.76-3.74 (t, *J* = 5.2 Hz, 1H), 3.54 (s, 2H), 1.70 (s, 2H), 1.83-1.54 (m, 4H), 1.41-1.25 (m, 4H), 1.13-0.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 135.3, 129.4, 129.0, 127.3, 48.3, 44.0, 32.9, 25.5, 24.8; FT-IR (KBr); 1556, 1635, 2929, 3273 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{19}NO$ is 218.1545 found 218.1540.

28) *N*-Butyl-2-phenyl-acetamide (entry 18 in Table 2.3.1)



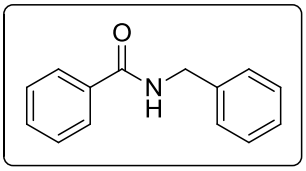
White solid, Yield 93%; Mp. 38 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.24 (m, 5H), 5.40 (br, 1H), 3.56 (s, 2H), 3.22-317 (m, 2H), 1.41-1.35 (m, 2H), 1.27-1.21 (m, 2H), 0.88-0.85 (t, 3H, *J*= 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 135.4, 128.9, 128.3, 128.0, 126.6, 43.1, 39.1, 31.2, 19.8, 13.5; FT-IR (KBr) 1559, 1630, 2957, 3253 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{17}NO$ is 192.1388 found 192.1387.

29) *N*-Diphenyl-acetamide (entry 19 in Table 2.3.1)



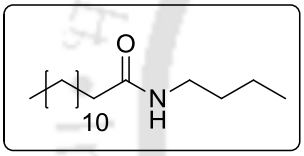
White solid, Yield 26%; Mp. 116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.42-7.06 (m, 10H), 3.73 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 169.8, 137.9, 134.7, 129.5, 129.1, 128.9, 127.5, 124.5, 120.2, 44.6; FT-IR (KBr) 1601, 1657, 3256, 3285 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{13}NO$ is 212.1075 found 212.1071.

30) *N*-Benzyl-benzamide (entry 20 in Table 2.3.1)



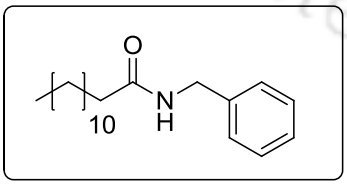
White solid, Yield 31%; Mp. 162, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.77 (m, 2H), 7.43-7.25 (m, 8H), 6.5 (br, 1H) 4.64-4.63 (d, 2H, *J*= 5.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 138.4, 134.4, 131.6, 128.8, 128.6, 127.9, 127.5, 127.1, 44.1; FT-IR (KBr) 1551, 1637, 2924, 3290 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{13}NO$ is 212.1075 found 212.1073.

31) *N*-butyldodecanamide (entry 21 in Table 2.3.1)



White solid, Yield 87%; Mp. 55 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.52 (br, 1H), 3.23-3.18 (q, *J*= 6.8 Hz, 2H), 2.14-2.10(t, *J*= 7.2 Hz, 2H), 1.60-1.56 (m, 2H), 1.48-1.41(m, 2H), 1.22(s, 18H), 0.90-0.82 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 39.2, 36.7, 31.9, 31.7, 29.6, 29.5, 29.4, 29.4, 25.9, 22.7, 20.1, 14.1, 13.7; FT-IR (KBr) 1552, 1638, 2919, 3296 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{33}NO$ is 256.2640 found 256.2639.

32) *N*-benzyldodecanamide (entry 22 in Table 2.3.1)



White solid, Yield 89%; Mp. 82 °C, ^{1}H NMR (400 MHz, CDCl3) δ 7.30-7.19 (m, 5H), 5.69 (br, 1H), 4.37-4.36 (d, , *J*= 5.6 Hz, 2H), 2.15-2.11 (t, *J*= 7.6 Hz, 2H), 1.59-1.54 (m, 2H), 1.18(s,16H), 0.82-0.79 (t, *J*= 6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3), δ 173.5, 138.6, 128.5, 128.3, 127.6, 43.3, 36.6, 31.9, 29.6, 29.5, 29.4, 29.3, 25.8, 22.7, 14.1; FT-IR (KBr) 1555, 1632, 2916, 3292 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{19}H_{31}NO$ is 290.2484 found 290.2482.

2.7. Selected spectra

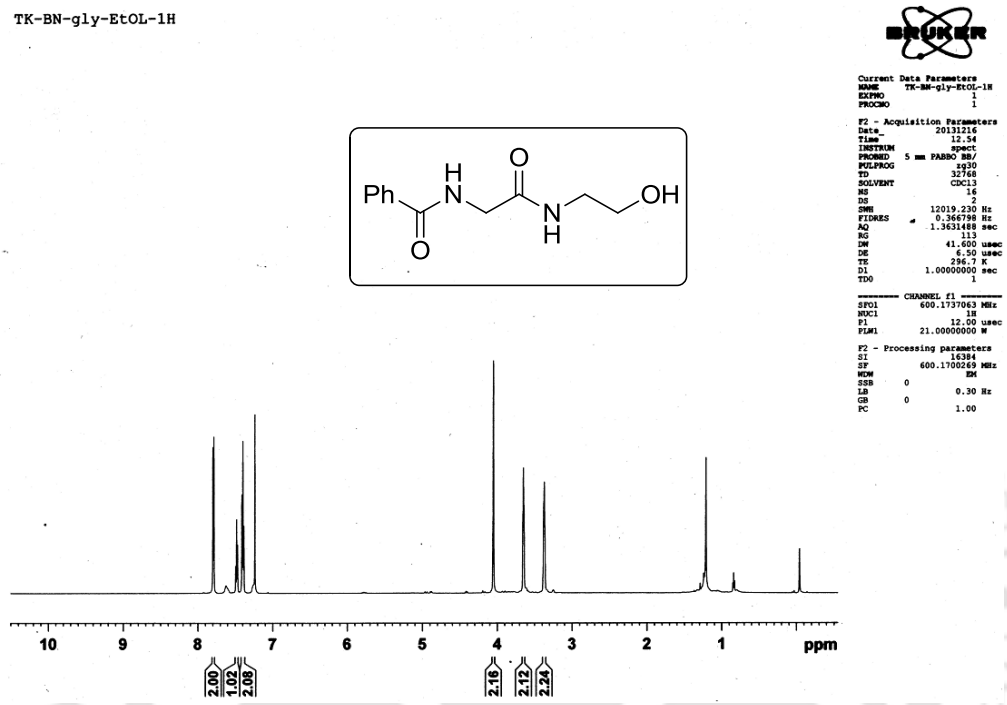


Figure S1. ^{1}H NMR spectra of the product of entry 1 in Table 2.2.1

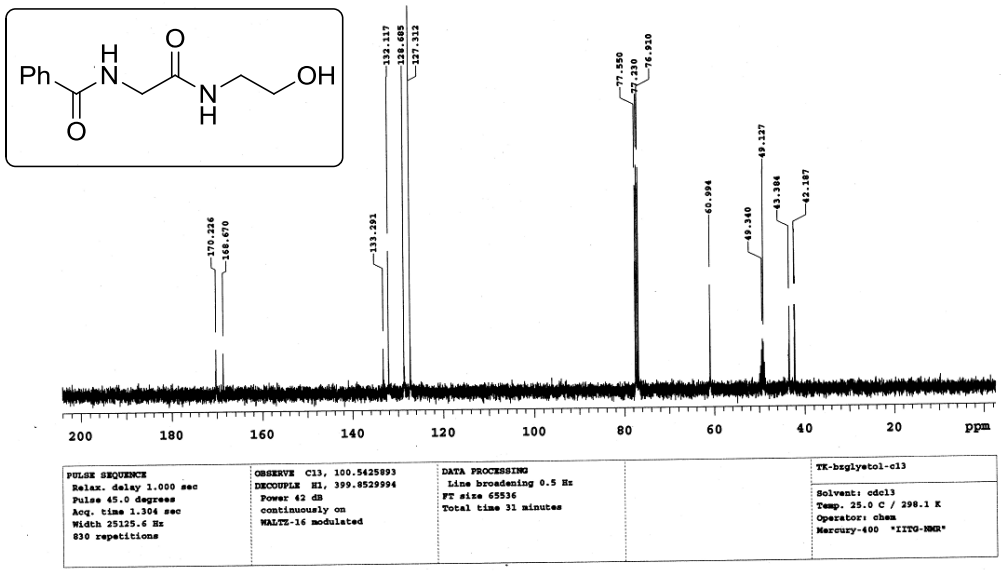


Figure S2. ^{13}C NMR spectra of the product of entry 1 in Table 2.2.1

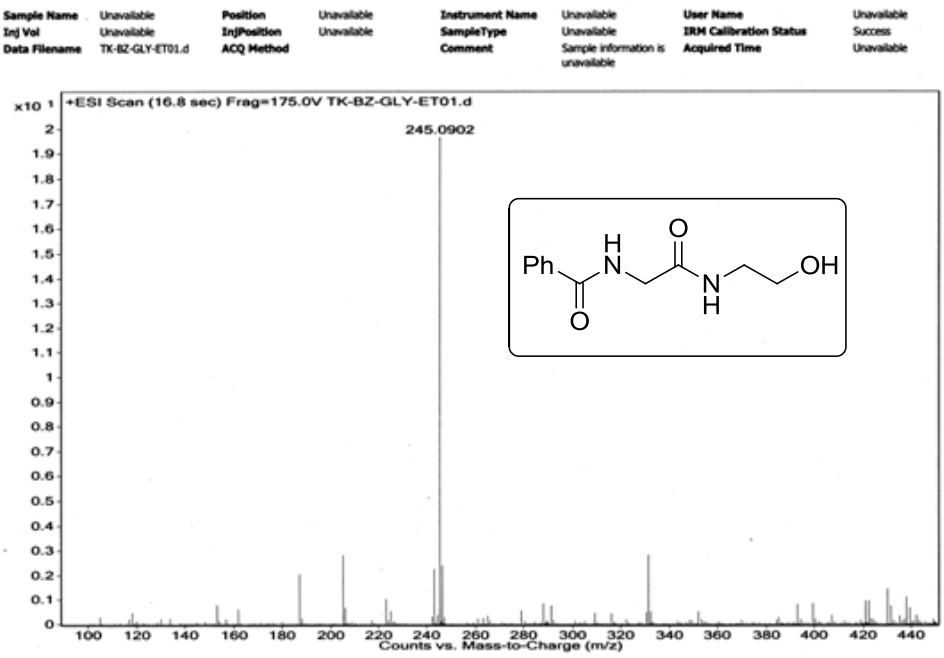


Figure S3. HRMS spectra of the product of entry 1 in Table 2.2.1

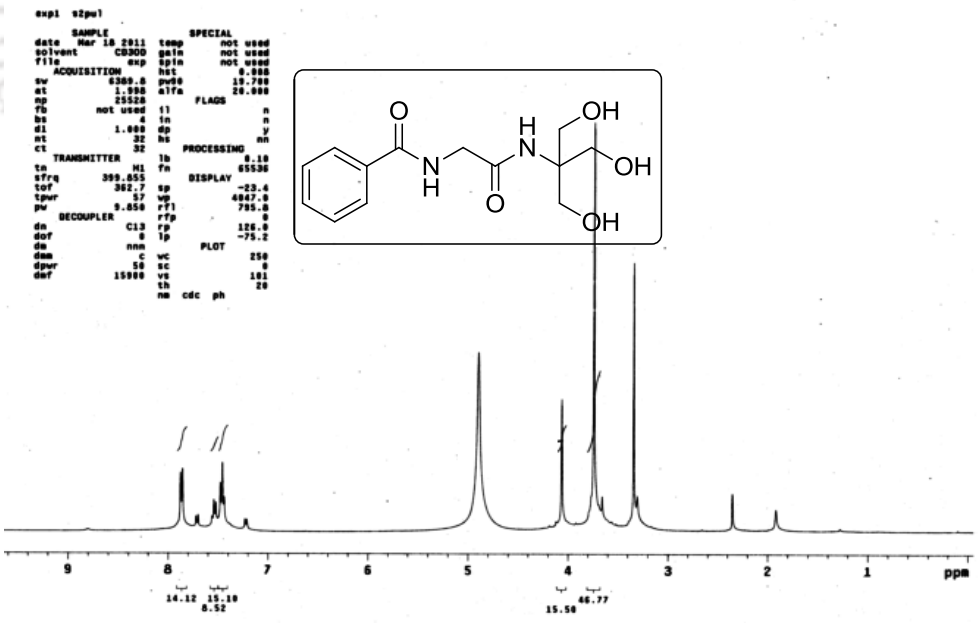


Figure S4. ^{1}H NMR spectra of the product of entry **10** in Table 2.3.1

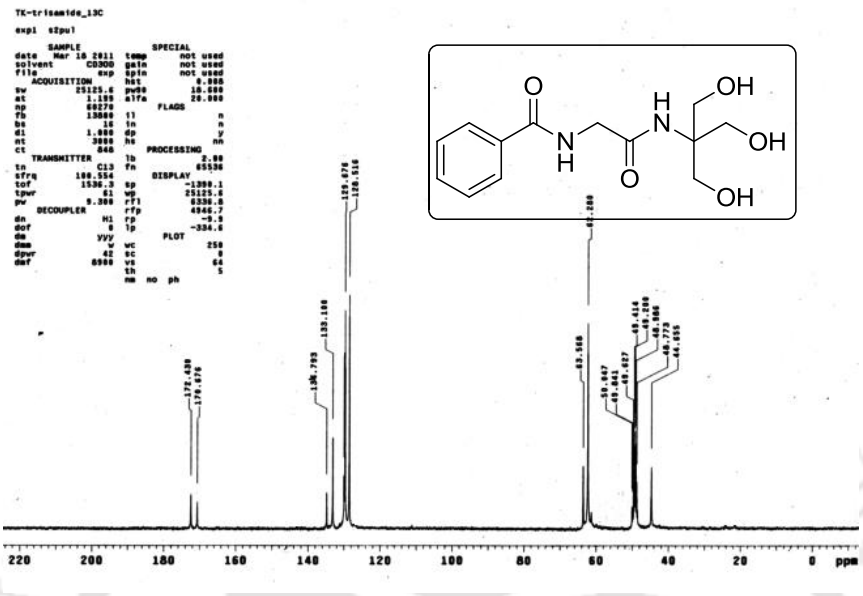
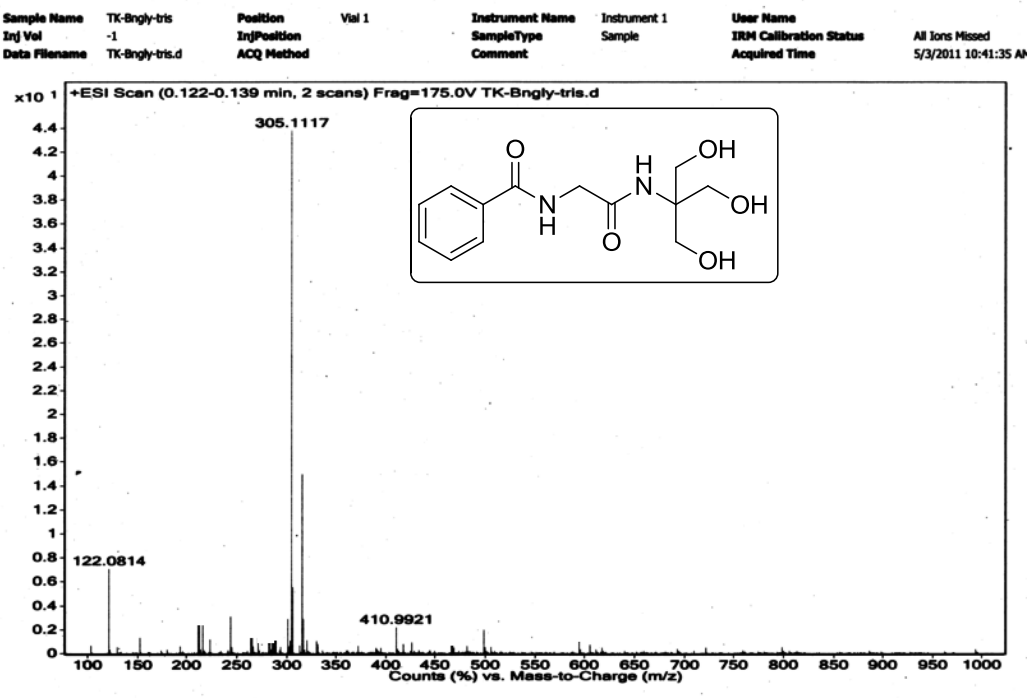


Figure S5. ^{13}C NMR spectra of the product of entry 10 in Table



2.3.1

Figure S6. HRMS spectra of the product of entry **10** in Table 2.3.1

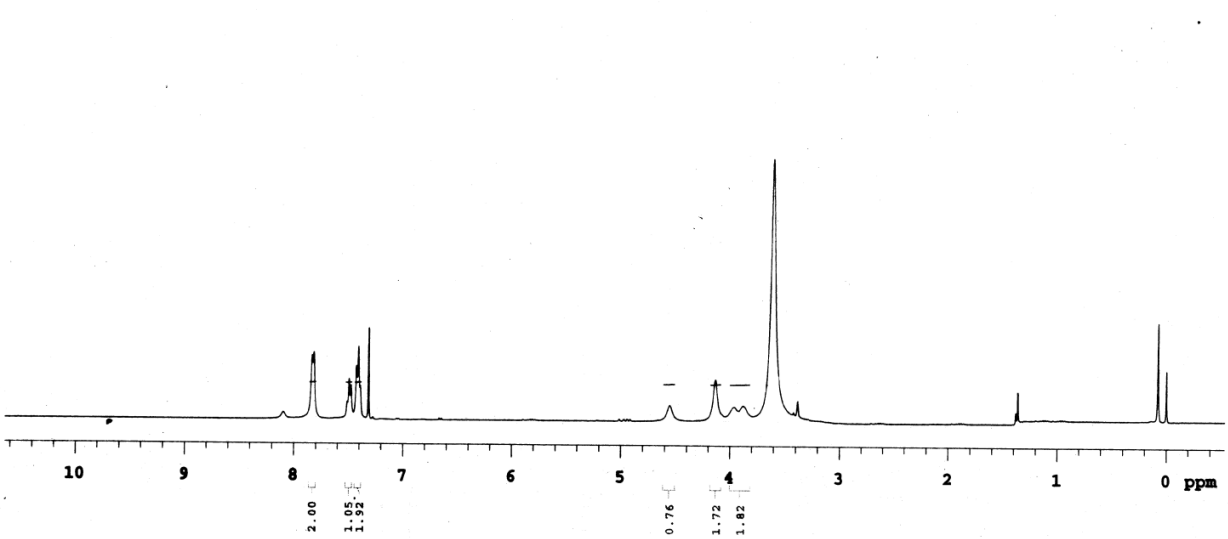


Figure S7. [1]H NMR spectra of the product of Bz-Gly-Ser-OH

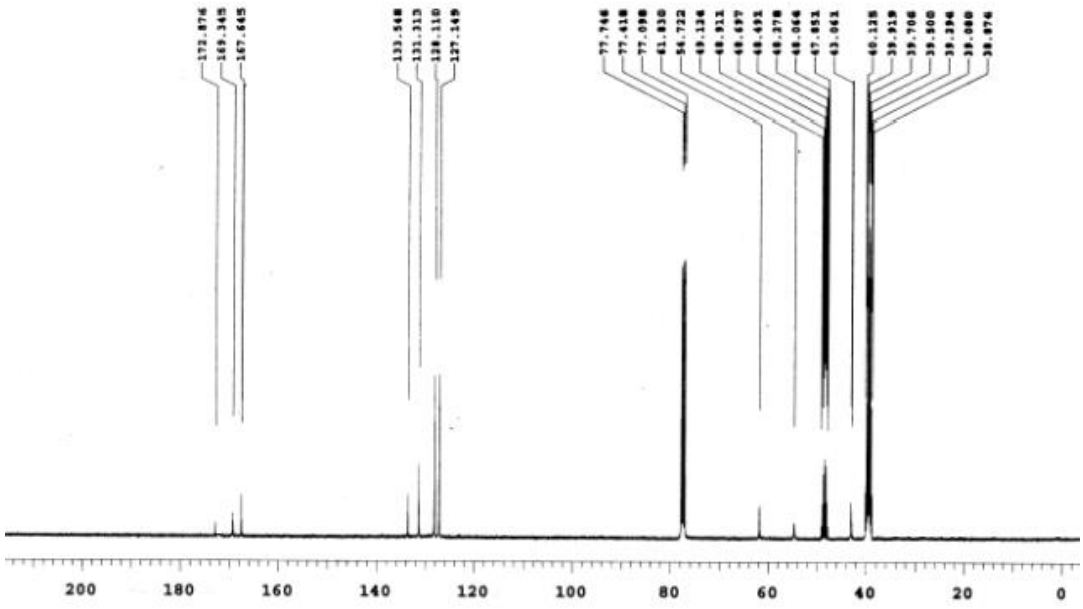


Figure S8.[1]H NMR spectra of the product of Bz-Gly-Ser-OH

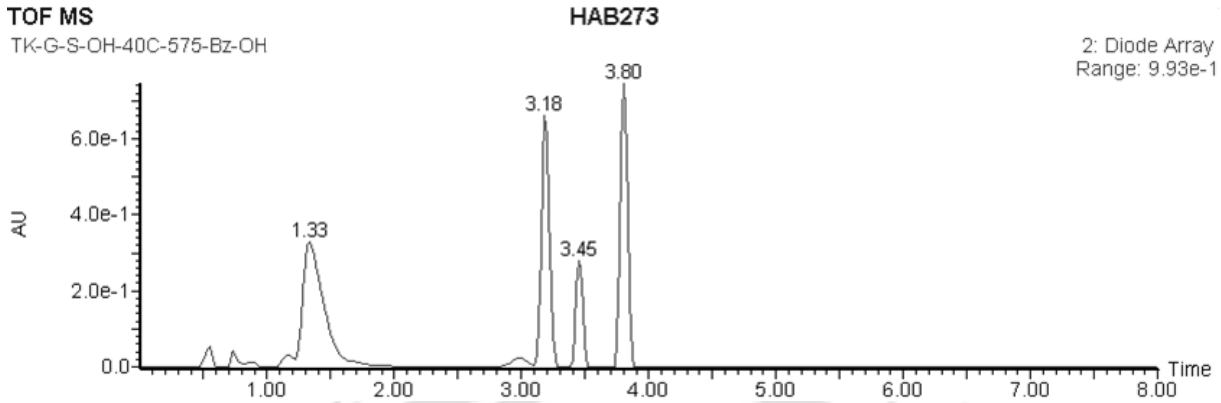


Figure S9.LC-MS profile of Bz-Gly-Ser-OH

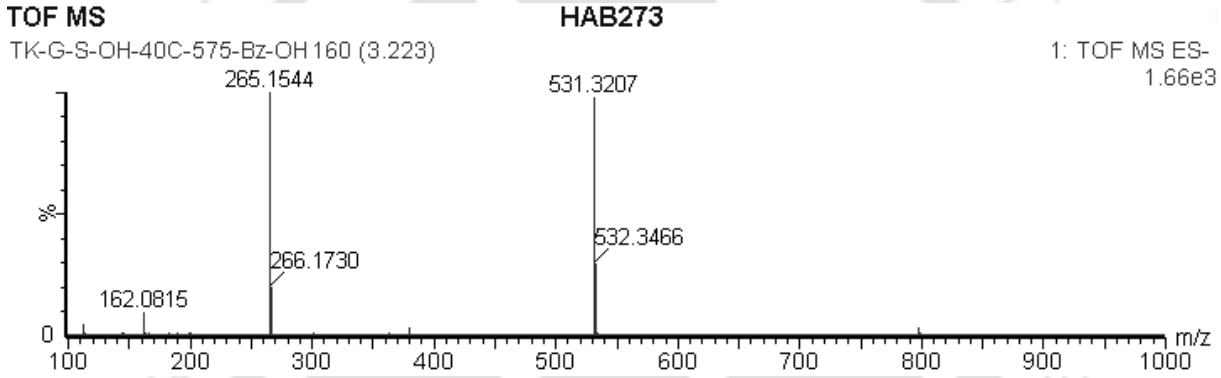


Figure S10. Mass of Bz-Gly-Ser-OH

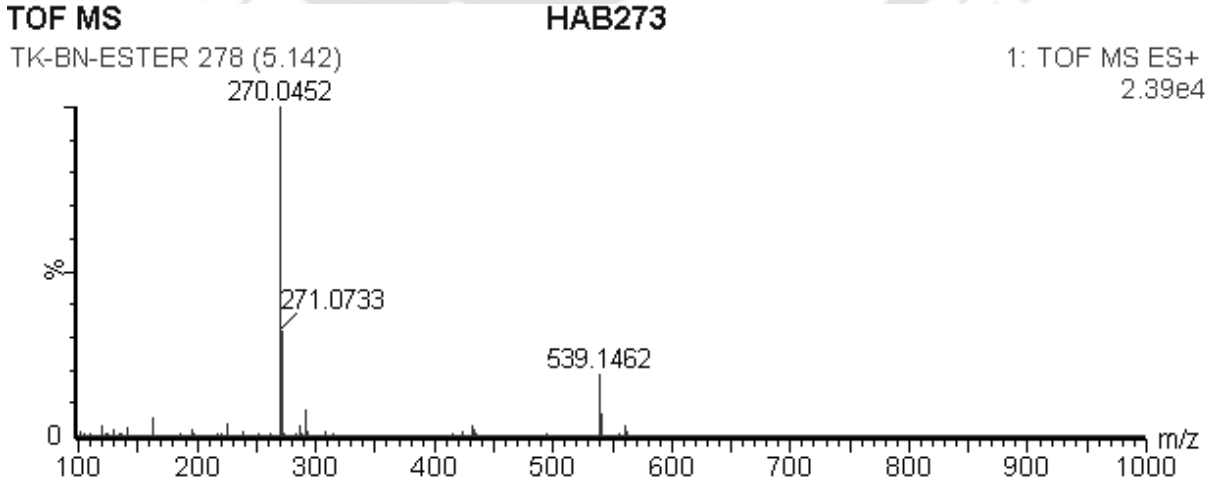


Figure S11. Mass of Bz-Gly-OBz

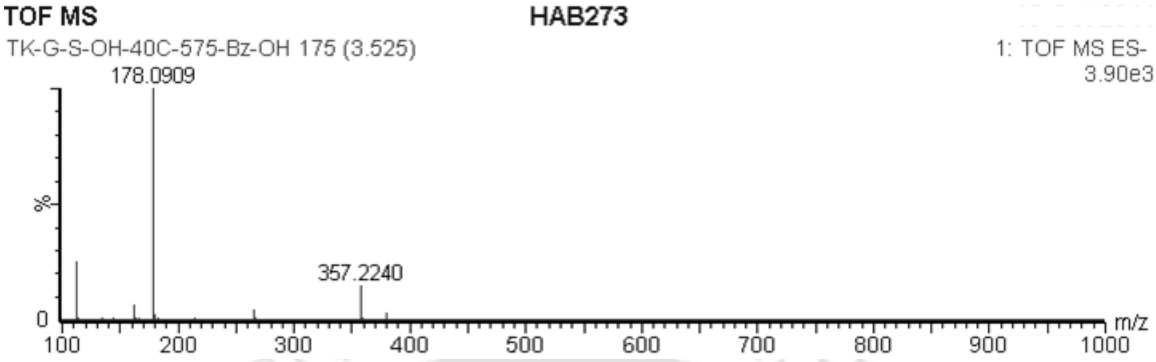
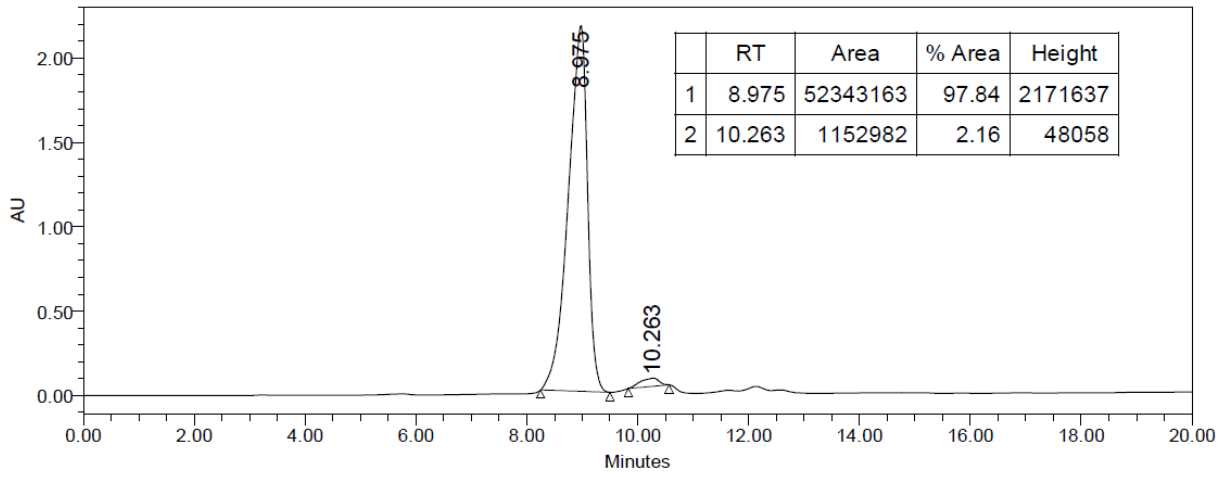
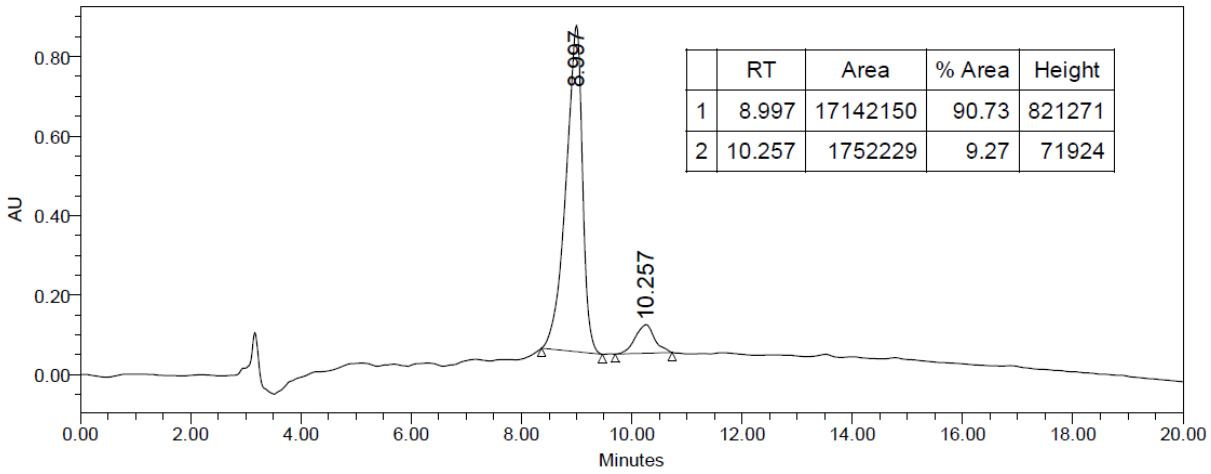


Figure S12. Mass of Bz-Gly-OH



	RT	Area	% Area	Height
1	8.975	52343163	97.84	2171637
2	10.263	1152982	2.16	48058

Figure S13. HPLC profileof compound Bz-Leu-Ser-OH (with Oxyma)



	RT	Area	% Area	Height
1	8.997	17142150	90.73	821271
2	10.257	1752229	9.27	71924

Figure S12. HPLC profileof compoundBz-Leu-Ser-OH(with out Oxyma)

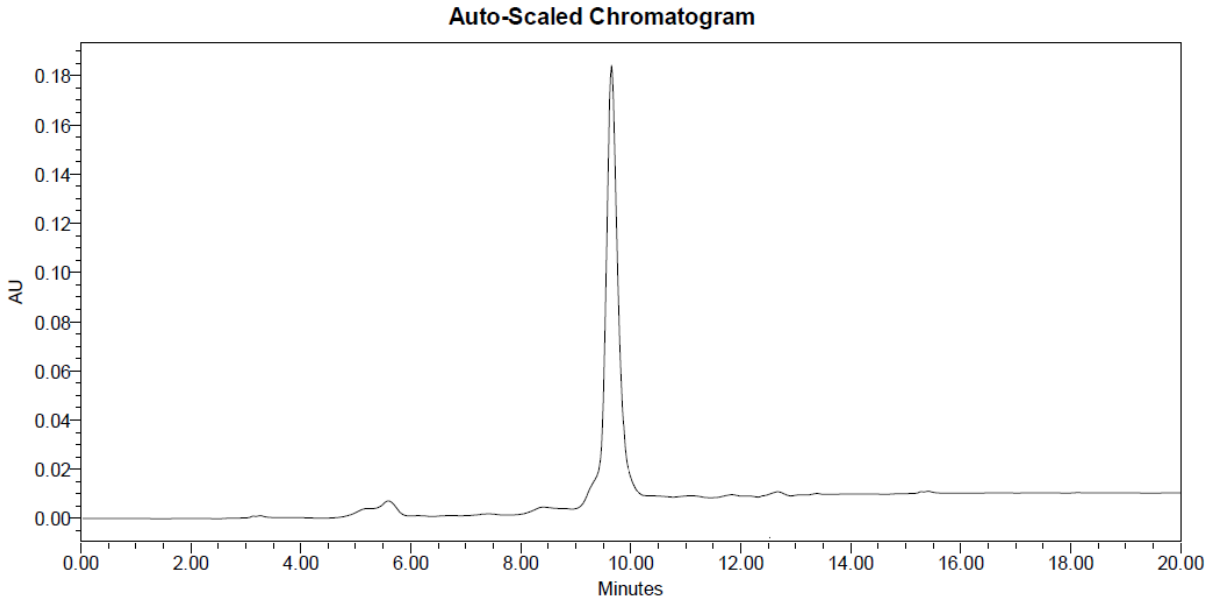


Figure S12. HPLC profileof polypeptide

Sample Name	TK-NCL-LIGATION	**Position**	-1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	-10	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	TK-NCL-LIGATION.d	**ACQ Method**		**Comment**		**Acquired Time**	4/23/2014 11:22:57 AM

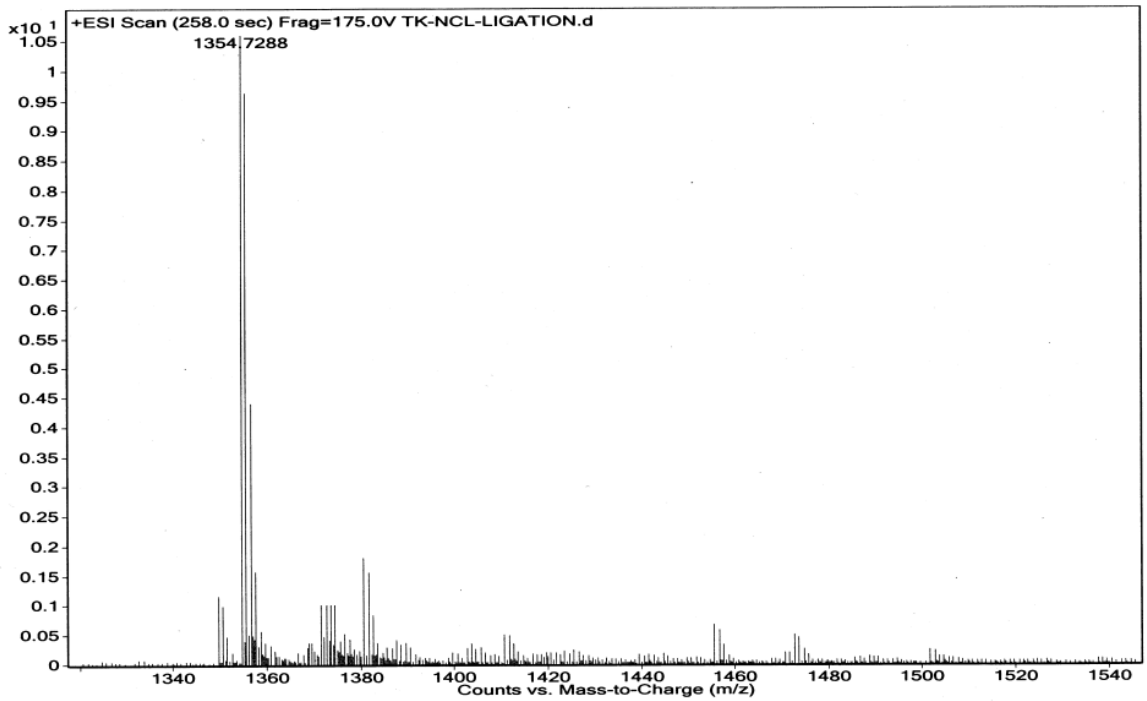
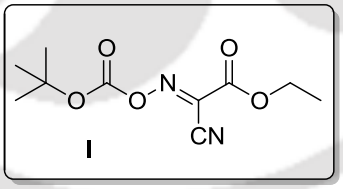


Figure S13. HRMS of polypeptide

Chapter 3: Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2 Cyanoacetate (Boc-Oxyma) as a New Coupling Reagent for Racemization-Free Esterification, Amidation, and Peptide Synthesis

In the previous chapter, we described the synthesis of amides and peptides with *N*-protected amino acids. Few bulky amino acids, such as leucine, valine, and phenylalanine took longer time to complete the reaction, and yield was also less. To address this problem, we were interested to develop a new coupling reagent. In this chapter, we have described the development of the new coupling reagent, Ethyl 2-(tert-butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma, *Figure 3.1*), which was successfully applied for the synthesis of amides, peptides, esters, and thioesters in good yield with broad substrate scope.



I

Figure 3.1. *Ethyl 2-(tert-butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma, I)*

3.1. Boc-Oxyma as a new coupling reagent for racemization free amidation and peptide synthesis

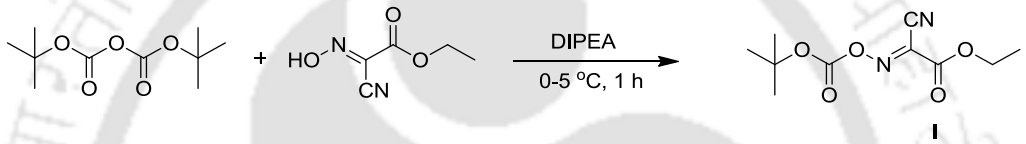
Amides are the versatile functional groups in the realm of organic synthesis[101] as they constitute a significant part of the chemical space of biologically active compounds[102]

(Chapter 1, section 1.3.1). Amide bonds are present in proteins such as enzymes, and they are the prevalent subunits in pharmaceutical molecules and natural products.[103]

For the last two decades, a great effort has been devoted towards the development of efficient methods for the synthesis amide and peptides from carboxylic acids and *N*-protected amino acids using various coupling reagents (we have discussed few methodologies in Chapter 1). To date, numerous coupling reagents have been developed for peptide synthesis. Among them, the most effective coupling agents are carbodiimides, phosphonium, and uroniumsalts.[104-110] Most of them constitute benzotriazole or aza-benzotriazole ring system as an important fragment of their structure. After two decades of domination of benzotriazole-based coupling reagents, Albericio *et al.* have introduced a new class of peptide coupling reagents based on ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma)[111-112] as benzotriazoles are explosive. Oxyma is superior to its counterparts as racemization suppressant and environmentally benign reagent.[113] Moreover, a major disadvantage of existing popular coupling reagents (carbodiimide, HOBt, and Oxyma based coupling reagents)[113] developed to date is that they generate substantial amount of undesired by-products. Furthermore, these coupling reagents are often very difficult to prepare and involves multi-step synthesis that require harsh reagents. Therefore, invention of an environmentally friendly and cost effective coupling reagent with highest level of efficacy, but lowest level of epimerization, still remains a challenge. We report the development of a new Oxyma based coupling reagent, Ethyl 2-(tert-Butoxycarbonyloxyimino)-2-Cyanoacetate (Boc-Oxyma, **I**, *Figure 3.1*) for racemization free synthesis of amides and peptides.

3.1.1. Reaction optimization and substrate scope for amides and peptides synthesis using I

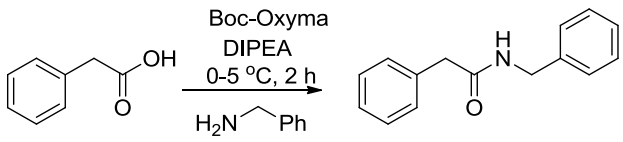
Initially, we prepared the newly designed coupling reagent Boc-Oxyma by the reaction of Boc anhydride (1.2 equiv) with ethyl-2-hydroxyimino-2-cyanoacetate (Oxyma, 1 equiv) in presence of DIPEA or triethyl amine (1 equiv) at 0-5 ºC for 1 h (*Scheme 3.1.1.1*) with 95% yield.



Scheme 3.1.1.1. *Prepation of the coupling reagent, Boc-Oxyma*

First, the reaction conditions were optimized (*Table 3.1.1.1*) for amidation and peptide synthesis using newly synthesized coupling reagent Boc-Oxyma. For optimization, we added the coupling reagent, Boc-Oxyma, to a stirred solution of phenylacetic acid and DIPEA in various solvents, such as DCM, $CHCl_3$, CH_3CN, THF, EtOAc, and DMF for 1 h at 0 ºC for pre-activation followed by the addition of benzyl amine. Stirring was continued for 2 h. The solvent ethyl acetate afforded higher yield (92%) of the desired product.

Table 3.1.1.1. *Solvent screening*

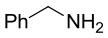
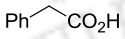
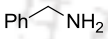
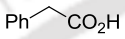
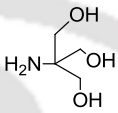
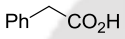
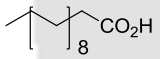
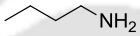
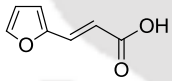
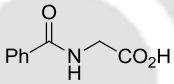
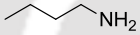
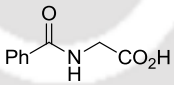
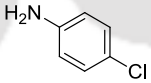
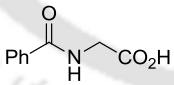
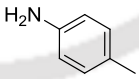
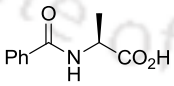
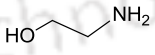
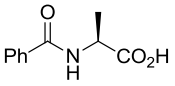
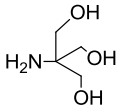
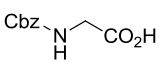
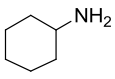
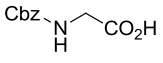
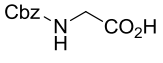


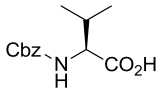
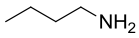
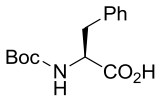
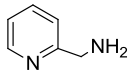
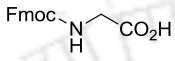
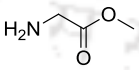
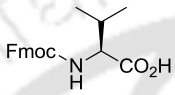
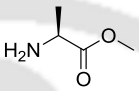
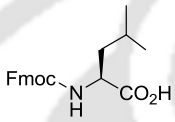
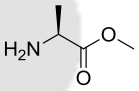
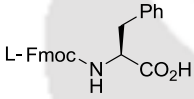
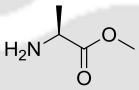
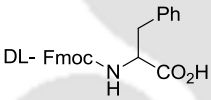
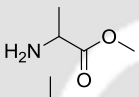
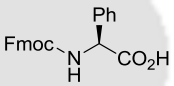
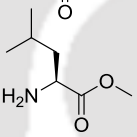
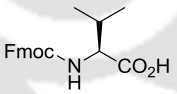
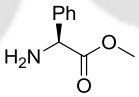
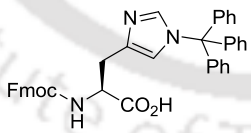
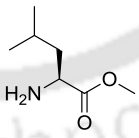
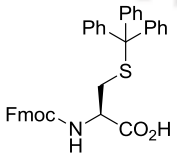
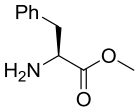
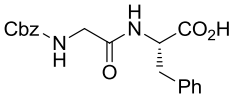
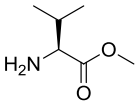
Entry	Solvent	Yield[a]
1	Dichloromethane	50 %
2	Chloroform	55 %
3	Acetonitrile	40 %
4	Tetrahydrofuran	35 %
5	Ethyl acetate	92 %
6	Dimethylformamide	70 %

[a]*Isolated yield after column chromatography.*

The scope of the amidation and peptide synthesis was investigated by analysing the reactivity of various aromatic carboxylic acids, long chain aliphatic carboxylic acids, and *N*-protected amino acids containing various side chains with variety of amines and methyl ester of amino acids under the above optimized reaction condition. The yields of the reactions were also good to excellent (up to 94%, *Table 3.1.1.2)*. The current coupling protocol is compatible with all common amino protecting groups, e.g. Cbz (*Table 3.1.1.2, entries 12-15 and 24*), Boc (*entry 16*) and Fmoc *(entries 17-23*). The reaction works well for hindered (*entry 6*) and non-hindered (*entry 13)* secondary amines. Several of *N*-hydroxyalkyl amides were also prepared chemoselectively by direct coupling of the carboxylic acid and amino alcohol under mild conditions (*entries 3, 10, and 11*). The amidation of aniline derivatives also works smoothly with the use of **I** at mild temperatures (0 to 5 °C) and with better yields (*entries 4, 8, 9, and 14)*. The use of Boc-Oxyma was also successfully applied to solution phase peptide coupling with good yields even with bulky amino acids, such as leucine, valine, and phenylalanine *(entries 17-24).*

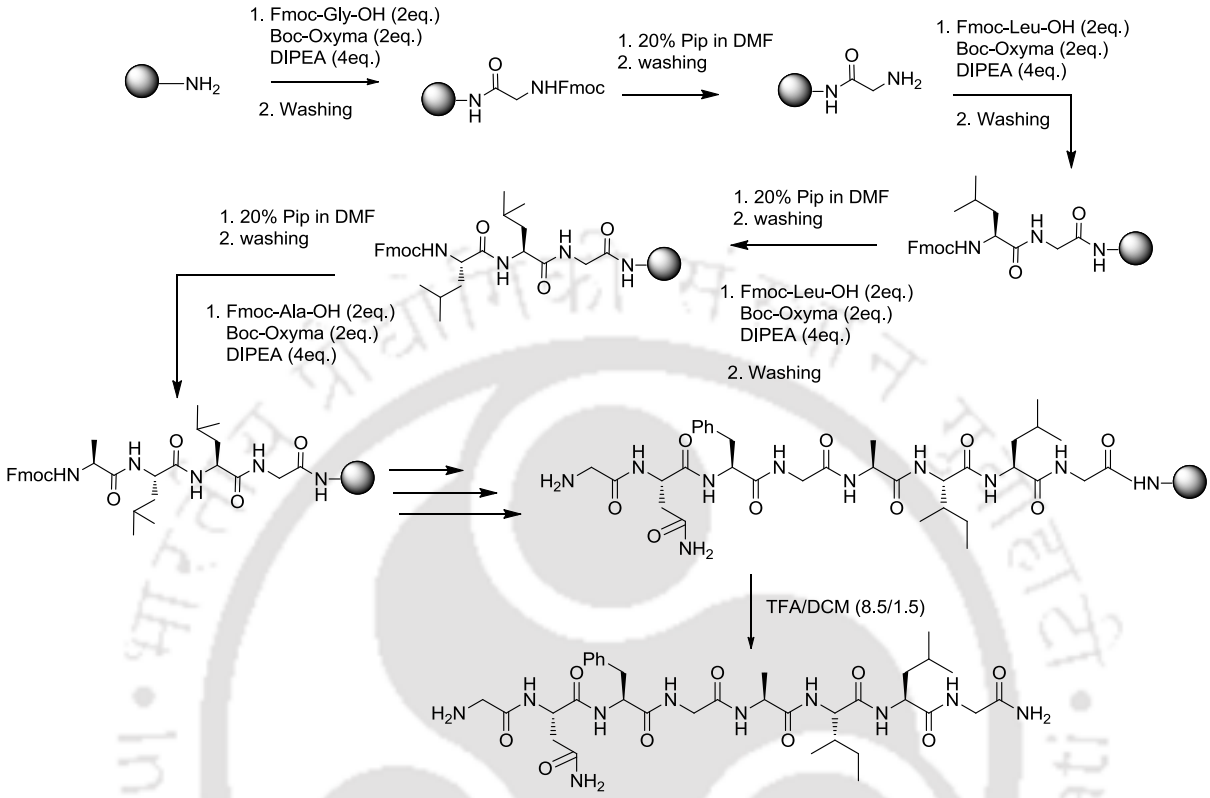
Table 3.1.1.2. *Scope of the amide and peptide synthesis using Boc-Oxyma*

Entry	Acid	Amine	Product ID	Product Yield %[a]
1			1a	91
2			2a	92
3			3a	90
4			4a	85
5			5a	95
6			6a	90
7			7a	93
8			8a	90
9			9a	89
10			10a	91
11			11a	91
12			12a	94
13			13a	91
14			14a	87

15			15a	93
16			16a	91
17			17a	91
18			18a	90
19			19a	88
20			20a	89
21			21a	91[b]
22			22a	90
23			23a	91
24			24a	85
25			25a	87
26			26a	90

[a]*Isolated yield after column chromatography.* [b] *All amino acids are L-amino acids, other than 21*

To extend the scope of the new coupling reagent **I**, we prepared the octapeptide H-Gly-Asn-Phe-Gly-Ala-Ile-Leu-Gly-NH_2 *(scheme 3.1.1.2)*, which contains the hexapeptide sequence, NFGAIL, known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes. We synthesized the octapeptide by stepwise coupling of the constituent amino acids using **I** as a coupling reagent on rink amide MBHA resin following Fmoc/^{t}Bu orthogonal protection strategy. Couplings of amino acids with **I** proceeded smoothly at each step in less than 2 h and were confirmed by Kaiser's test. The HPLC retention times and ESI-MS data for each fragment are reported in Table 3.1.1.3. The yield of the final peptide after cleavage from the resin with TFA followed by ether precipitaion and semi-preparative HPLC was around 40% with respect to the resin loading. The HPLC profile and ESI-MS spectrum of the purified peptide are provided below (*Figure 3.1.1.1*).



Scheme 3.1.1.2. *Synthesis of peptide sequence GNFGAILG-NH_2 on SPPS using Boc-Oxyma*

Table 3.1.1.3. *HPLC retention times and observed mass (ESI-MS) of the peptides after each coupling step on solid phase.*

Entry	Peptide	HPLC Retention time min	Expected mass m/z	Observed mass m/z
1	Fmoc-L-G-NH_2	12.618	$[M+H]^+$ 410.20	410.21
2	Fmoc-I-L-G-NH_2	13.083	$[M+H]^+$ 523.29	523.31
3	Fmoc-A-I-L-G-NH_2	13.216	$[M+H]^+$ 594.32	594.35
4	Fmoc-G-A-I-L-G-NH_2	13.047	[M+Na]+ 673.33	673.36
5	Fmoc-F-G-A-I-L-G-NH_2	13.430	[M+Na]+ 820.40	820.41
6	Fmoc-N-F-G-A-I-L-G-NH_2	13.448	$[M+Na+H]^{+2}$ 467.72	467.17
7	G-N-F-G-A-I-L-G-NH_2	10.992	$[M+H]^+$ 747.41	747.35

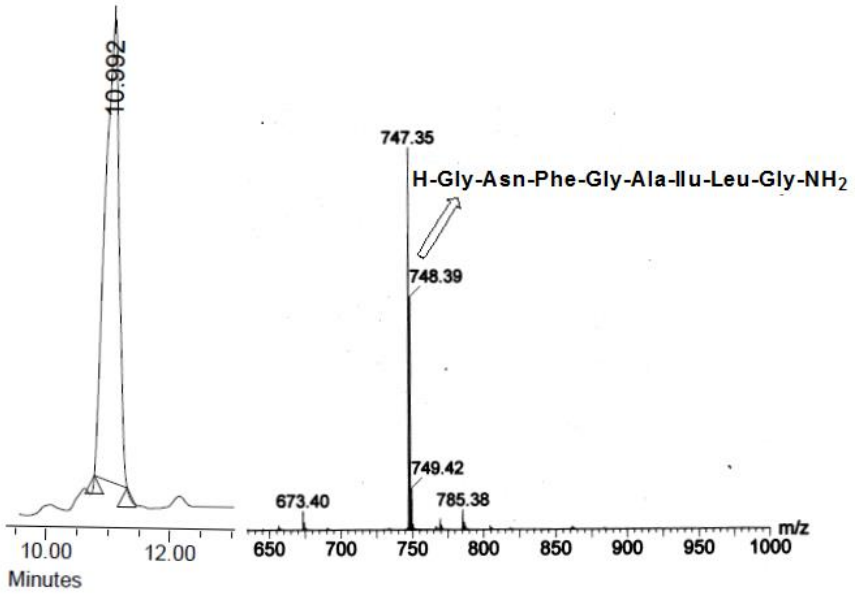


Figure 3.1.1.1. *HPLC profile and ESI-MS of the final peptide after purification.*

3.1.2. Racemization suppression efficiency of I for amides and peptides synthesis

We have checked the racemization probability of the current protocol during the amidation as well as peptide synthesis by comparison of the HPLC profiles, ^{1}H and ^{13}C NMR spectra of Fmoc-L-Phe-L-Ala-OMe (*entry 20, Table 3.1.1.2)* and that of the Fmoc-DL-Phe-L-Ala-OMe (*entry 21, Table 3.1.1.2, Figure 3.1.2.1).* One peak was observed at the retention time 11.6 min in the HPLC profile, which corresponds to a single stereoisomeric product of Fmoc-L-Phe-L-Ala-OMe. On the other hand, two peaks with retention times of 12.7 min and 14.2 min appeared in the HPLC profile corresponding to the two diastereomers present in the product, Fmoc-DL-Phe-L-Ala-OMe. The mass of the peaks in the HPLC profiles was verified using ESI-MS. The doublet centered at $\delta = 1.32$ ppm in the ^{1}H NMR and the peak at $\delta = 17.8$ ppm in the ^{13}C NMR correspond to the protons and the carbon of the methyl group of the alanine residue of the single isomer of the di-peptide product, on the other hand, the doublets centered at $\delta = 1.33$ and 1.22 ppm in the ^{1}H NMR and the peaks at $\delta = 18.3$ and 18.2 ppm in the ^{13}C NMR indicate the presence of two diastereomers for the same proton and carbon respectively. From the data of HPLC profiles, ^{1}H and ^{13}C NMR, it can be concluded that there was no detectable racemization in the amidation and peptide synthesis by using **I** as the coupling reagent.

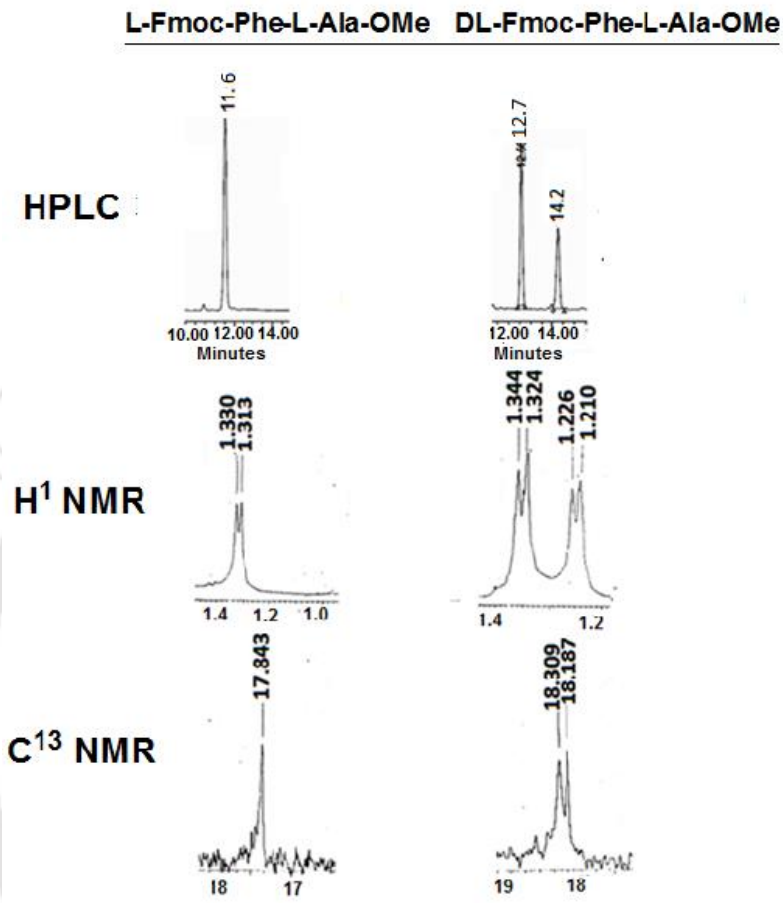


Figure 3.1.2.1. *HPLC profiles (18 min, linear gradient, 0 to 100% CH_3CN in water), ^1H NMR and ^{13}C NMR of the products of the reactions entered as 20 and 21 in Table 3.1.1.2.*

The racemization suppression efficiency of the new coupling reagent was checked by comparison of the reported data obtained for a tripeptide, Z-Gly-Phe-Val-OMe (*entry 26, Table 3.1.1.2*), synthesized using the well-known coupling reagents such as HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene)-*N*-methylmethanaminium hexafluorophosphate-*N*-oxide), HDMA (1-((dimethylamino)-(morpholino)methylene)-1*H* [1,2,3]triazolo[4,5-*b*]pyridiniumhexafluorophosphate3-oxide),

HBTU (*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino) methylene]*N*-methylmethanaminium hexafluorophosphate*N*-oxide), and HDMB (1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazoliumhexafluorophosphate3-oxide),[114] with that using Boc-Oxyma by solution phase peptide synthesis via a segment coupling of Z-Gly-Phe-OH and H-Val-OMe. Unlike the other coupling reagents, no racemization was observed while using **I** by RP-HPLC and NMR techniques *(Table 3.1.2.1).*

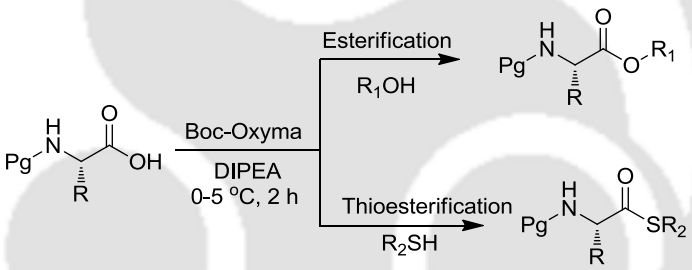
Table 3.1.2.1. *Comparison of % yield and % of racemization of a tripeptide using various coupling reagents with* ***I****.*

Entry	Peptide	Coupiling Reagent	yield (%)	Racemization (%)
1	Z-Gly-Phe-Val-OMe	HATU	90	1.56
2		HDMA	90	0.65
3		HBTU	89	5.90
4		HDMB	90	2.90
5		I	90	nd[a]

[a] *"nd" implies "no racemization could be detected by HPLC, ^{1}H and ^{13}C NMR".*

3.2. Synthesis of esters and thioesters using Boc-Oxyma

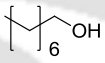
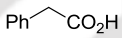
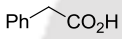
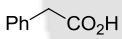
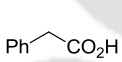
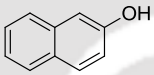
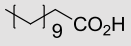
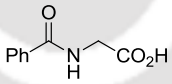
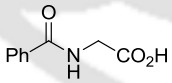
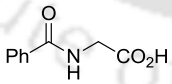
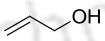
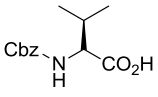
After successful synthesis of amides and peptides using **I**, we further extended its scope for the synthesis of esters and thioesters. It was observed that the esterification reaction of carboxylic acid with an equimolar amount of an alcohol was completed within 2 h in presence of equimolar amount of Boc-Oxyma in ethyl acetate solvent with good to excellent yields up to 95%.

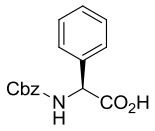
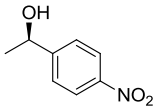
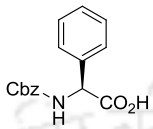
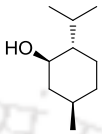
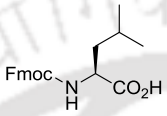
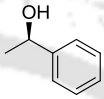
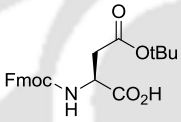
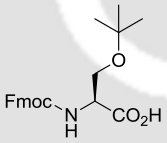
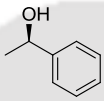
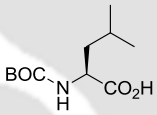
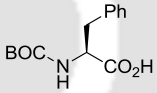
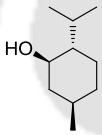
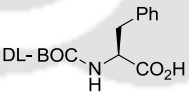
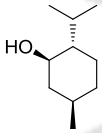
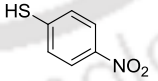
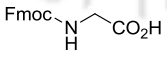
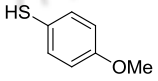
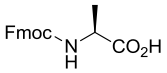
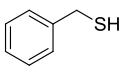


Scheme 3.2.1. *Synthesis of esters and thioesters from carboxylic acids using Boc-Oxyma*

We extended the scope of the method for esterification under the optimized reaction condition with various aromatic acids (*Table 3.2.1, entries 1-3*), aliphatic acids (*entries 4-7*), a long chain aliphatic acid (*entry 8*) and *N*-protected amino acids (*entries 9-20*). All the esterification reactions worked smoothly with good yields and without any side reactions. The current methodology is compatible with all the common *N*-protecting groups, such as Cbz, Fmoc, and Boc *(entries 12-20*). Various *N*-protected amino acids with bulky side chains, such as alanine, phenylalanine, phenylglycine, valine, and isoleucine were also tested and all of them resulted in excellent yields (*entries 12-20*).

Table 3.2.1. *Esterification using* ***I*** *as coupling reagent*

Entry	Acids	Alcohols	Product	
			ID	Yield %[a]
1	Ph CO_2H	MeOH	1b	92
2	Ph CO_2H	PhOH	2b	90
3	Ph CO_2H		3b	94
4			4b	93
5			5b	90
6			6b	87
7			7b	95
8			8b	93
9		MeOH	9b	93
10		EtOH	10b	95
11			11b	93
12			12b	92

13			13b	89
14			14b	91
15			15b	90
16		PhOH	16b	90
17			17b	92
18			18b	91
19			19b	92
20			20b	91[b]
21	Ph-CO2H		21b	87
22			22b	91
23			23b	92

[a]*Isolated yield after column chromatography* [b)]*DL-Boc-Phe-OH was used as substrate.*

The esterification of secondary alcohols requires a high temperature with strong bases, such as DBU with benzotriazole derived coupling reagents, e.g. TATU [1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate] and TBTU [2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate][115] and esterification with tertiary alcohols does not proceed even with high temperatures and strong bases with benzotriazole-derived coupling agents.[115] Esterification with *tert*-butyl alcohol have been achieved in presence of strong base MTBD [7-methyl-1,5,7-triazabicyclo-[4.4.0]dec-5-ene] using COMU as a coupling reagent with good yield.[15] On the other hand, we have prepared the esters with a variety of secondary alcohols (*Table 3.2.1, entry 5, 7, 8, 13, 14, 17 and 19)* and even with *tert*-butanol (*Table 3.2.1, entry 6*) in good to excellent yields using Boc-oxyma in presence of mild base DIPEA. Boc-Oxyma mediated esterification proceeds smoothly with a wide variety of alcohols. The scope of the thioesterification using Boc-Oxyma was tested by analyzing the reactivity of various carboxylic acids and with various thiols under these optimized reaction conditions (*Table 3.2.1, entries 20-23*). Thioesterification using **I** also proceeds with excellent yields.

3.2.1. Racemization suppression efficiency of I for esters and thioesters synthesis

The racemization associated with the use of Boc-Oxyma in the esterification was also investigated by comparison of the HPLC profiles, ^{1}H and ^{13}C NMR spectra of L-Boc-Phe-O-(*R*)-(-)-Menthyl (*entry 19b, Table 3.2.1)* and DL-Boc-Phe-O-(*R*)-(-)-Menthyl (*entry 20b, Table 3.2.1)*. One peak at the retention time 9 min in the HPLC, was observed for L-Boc-Phe-O-(*R*)-(-)-Menthyl which corresponds to a single stereoisomer, on the other hand two peaks with retention times of 9.0 min and 9.5 min appeared in the HPLC profile, corresponding to the two isomers of DL-Boc-Phe-O-(*R*)-(-)-Menthyl. The mass of the peaks

in the HPLC profiles was verified using ESI-MS. In ^{1}H NMR and ^{13}C NMR, the peaks at δ = 1.42 ppm and δ = 20.9 ppm respectively correspond to the tertiary butyl proton and carbon respectively of the Boc group of the ester product (*entry 19b, Table 3.2.1*) and confirm the presence of only one optical isomer. The peaks at δ = 1.42 and 1.40 ppm in ^{1}H NMR and at δ = 21.0 and 20.9 ppm in ^{13}C NMR of the product of the reaction with DL-Boc-Phe-OH (*entry 20b, Table 3.2.1*) indicates the presence of two diastereomers of the resulting ester (*Figure 3.2.1.1*). From the above comparative study, it can be concluded that the Boc-Oxyma mediated esterification conserves the chiral integrity of the substrate in the product.

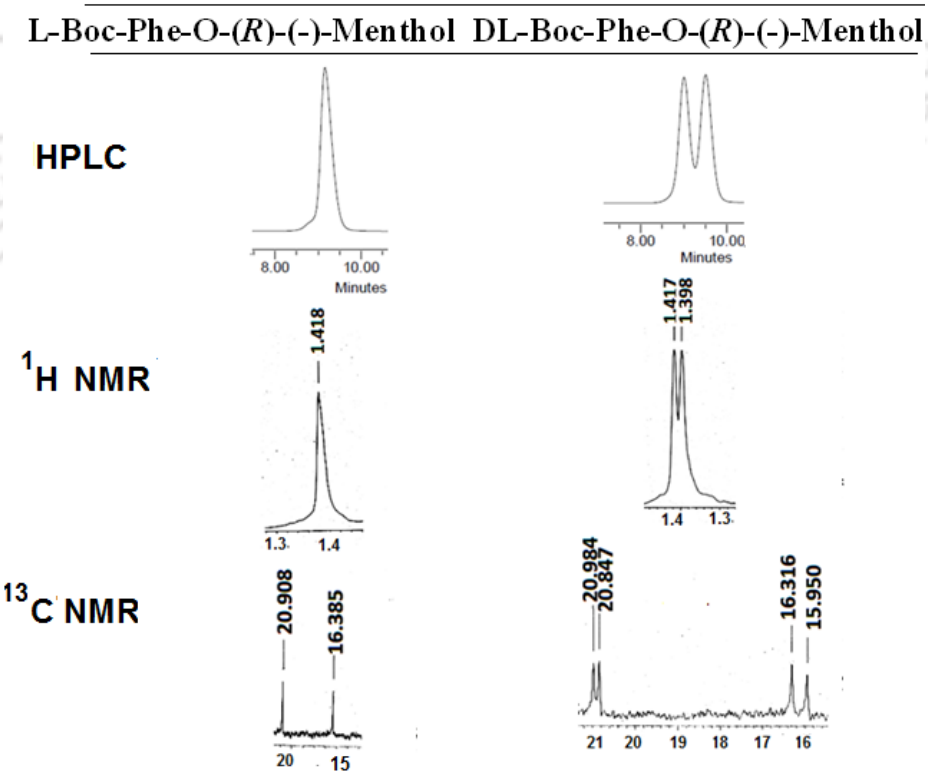


Figure 3.2.1.1. *Examination of racemization by comparison of the HPLC profiles, ^{1}HNMR and ^{13}C NMR of the product of entries 19 and 20 respectively*

3.3. Mechanistic study

A plausible mechanism of condensation reaction using current methodology is shown in Figure 3.3.1. The intermediate, Oxyma ester of the carboxylic acid, [A], was formed via the mixed anhydride intermediate, [X] (*Figure 3.3.1*), by the reaction of a carboxylic acid with **I**. The mixed anhydride [X] would be generated by the nucleophilic attack of the carboxylate anion at the carbonyl carbon of **I**. Subsequently, the active ester [A] is likely to be generated by further attack of the Oxyma anion at the carbonyl carbon of the mixed anhydride [X] followed by the release of CO_2 and tert-butanol. This plausible mechanism was confirmed by NMR, IR, and XRD. At first we took the phenylacetic acid in the presence of DIPEA in $CDCl_3$ in an NMR tube. Boc-Oxyma was added in the same NMR tube and ^{13}C NMR spectra were recorded over time. Initially, at five minutes, three carbonyl peaks were present, one corresponding to the phenylacetic acid at 175.0 ppm (a) and the other two at 155.9 and 147.5 ppm (b and c) corresponding to Boc-Oxyma. At 30 min, two new peaks were appeared at 169.8 (d) and 163.0 ppm (e) corresponding to the Oxyma ester of phenylacetic acid [A]. At 60 min, two new peaks (d, e) were observed, while the peaks corresponding to the starting materials (a, b, and c) completely disappeared, indicating complete conversion of starting carboxylic acid and the reagent **I** to the intermediate Oxyma ester [A]. However, with the addition of benzylamine (the nucleophile), peaks d and e shifted to new positions, f and g, respectively, as the intermediate [A] was converted to the amide product and the Oxyma was regenerated. When benzyl alcohol was added as the nucleophile, the benzyl ester carbonyl carbon, h, was observed. Finally, when benzoic acid was used instead of phenylacetic acid, the corresponding transient intermediate [A] could be isolated and its structure was resolved crystallographically.

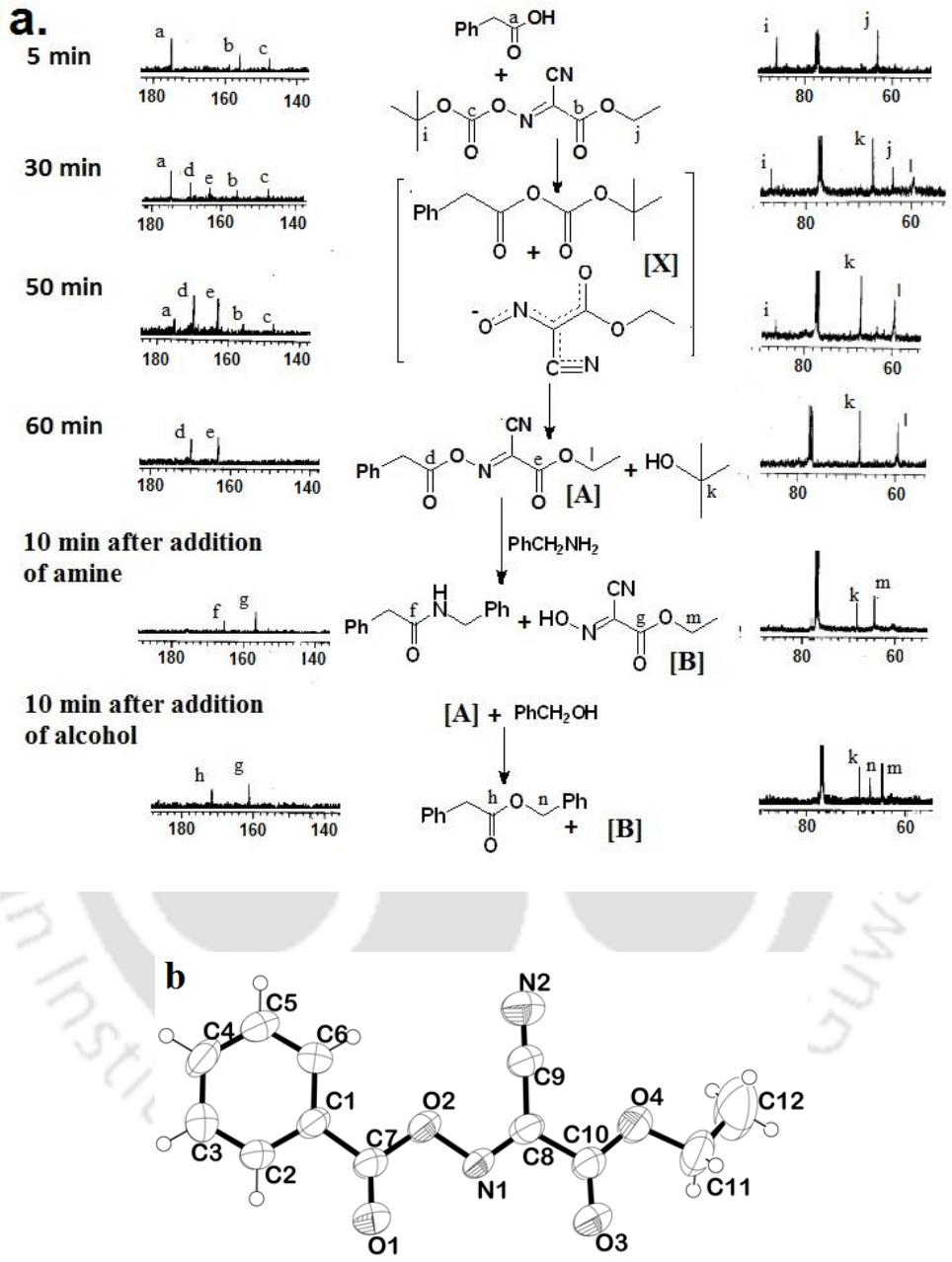


Figure 3.3.1. *Plausible mechanism of Boc-Oxyma mediated coupling reaction. (a) Time dependent ^{13}C NMR experiments. δ C_{ppm} a = 175.0, b = 155.9, c = 147.5, d = 169.8, e = 163.0, f = 165.9, g = 156.4, h = 171.5, i = 86.4, j = 63.4, k = 67.1, l = 59.5, m = 64.0, n = 66.6. b) ORTEP molecular diagram with ellipsoid at 50% probability of [A], when benzoic acid was used, CCDC # 903487.*

The evolution of tert-butanol was confirmed by both ^{1}H NMR and ^{13}C NMR. Before starting the reaction, we recorded the ^{13}C NMR of Boc-Oxyma, the tert-butyl group of which was observed at 86.4 ppm. During the reaction, we observed that, tert-butyl group of Boc-Oxyma gradually shifted from 86.4 ppm (i) to 67.1 ppm (*k, corresponding to the tert-butyl carbon of the tert-butanol that is generated during the reaction, right side panel in figure 3.3.2*). In the ^{1}H NMR, the peak corresponding to the tert-butyl proton of Boc-Oxyma gradually shifted from 1.60 ppm to 1.20 ppm, which corresponds to the tert-butanol protons during the reaction as shown in Figure 3.3.2.

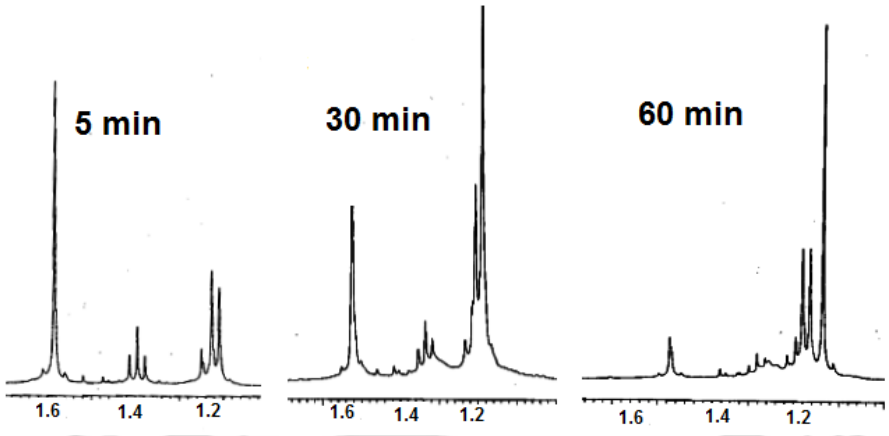


Figure 3.3.2. *^{1}H NMR spectra for evolution of tert-butanol.*

The mechanism of the reaction was also confirmed from IR data as shown in Figure 3.3.3. The CO stretching frequency of Boc-anhydride appeared at 1807 cm^{-1}. IR stretching frequency of the carbonyl (C=O) of phenylacetic acid was observed at 1703 cm^{-1} (a), for Boc-Oxyma at 1808 (p), 1766 (c) (it is in the form of carbonate, carbonate gives two stretching frequencies) and 1741 cm^{-1} (b). When the Oxyma ester of phenylacetic acid was formed by mixing of Boc-Oxyma and phenylacetic acid in presence of DIPEA, the stretching frequencies were shifted from 1808, 1766 (p, c) to 1715 (d) (there was no peak at

1808, because the anhydride was converted into ester) and from 1741 (b) to 1724 (e). Latter nucleophile benzylamine was added to the Oxyma ester of phenylacetic acid to form amide. After the formation of amide the stretching frequency of carbonyl (C=O) shifted from 1715 to 1646 (amide peak) and 1724 to 1720 (by-product Oxyma). Similarly for esterification (with benzyl alcohol) the stretching frequencies of carbonyl (C=O) shifted from 1715 to 1736 (ester peak) and 1724 to 1727 (by-product Oxyma) as shown in Figure 3.3.3.

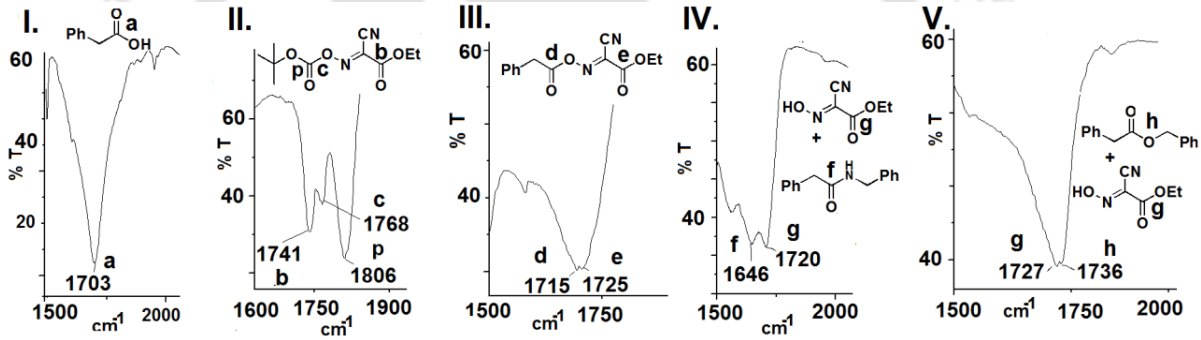


Figure 3.3.3. *IR spectra of (I) phenylacetic acid, (II) Boc-Oxyma, (III) the reaction mixture after 1h, Oxyma ester of phenylacetic acid, intermediate [A], (IV) 10 min after addition of benzylamine, (V) 10 min after addition of benzyl alcohol.*

3.4. Conclusion

We have developed a new coupling reagent, Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-Cyanoacetate (**I**) for simple, mild, and racemization free, amidation, peptide synthesis, esterification, and thioesterification that uses equimolar amounts of acids with equimolar amounts of amines, alcohols, and thiols respectively with equimolar amounts of **I**. The coupling reagent **I** was successfully used for both solid phase and solution phase peptide synthesis. Boc-Oxyma is much easier to prepare than other popular coupling reagents and the byproduct, Oxyma, can be easily recovered and reused to regenerate **I**. Thus, use of this reagent reduces chemical waste generation. Boc-Oxyma was successfully used for the esterification of secondary and tertiary alcohols with mild base DIPEA, whereas other coupling reagents such as, COMU, TBTU and TATU need stronger bases.

3.5. Experimental Section

3.5.1. Materials and methods

As described in chapter 2 section 2.4.1

3.5.2. Procedure for the preparation of Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-Cyanoacetate (Boc-Oxyma, I):

Di-*tert*-butyl dicarbonate (1.2 equiv) was added to a solution of Ethyl cyano(hydroxyimino)acetate (oxyma) (1 equiv) and DIPEA (1 equiv) in 1 ml $CHCl_3$ at 0-5 ºC and the reaction mixture was stirred for 1 h. After complete disappearance of Oxyma, the solvent $CHCl_3$ and byproduct tertiary butanol were evaporated with a rotary evaporator to obtain pure Boc-Oxyma.

3.5.3. General Procedure for the Synthesis of Esters, Amides and Thioesters:

Boc-Oxyma was added to a solution of acid (1 equiv) and DIPEA (1 equiv) in 1 mL of EtOAc. The reaction mixture was stirred for 1 h at 0-5 ºC followed by the addition of alcohol, amine or thiol (1 equiv). The reaction mixture was stirred at the same temperature for another 2 h. After completion of the reaction, the reaction mixture was diluted with 50 ml of ethyl acetate; the organic phase was washed with 5% citric acid (2×20 mL), saturated $NaHCO_3$ (2×20 mL) and dried over anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the product which was purified by column chromatography.

3.5.4. General Procedure for the Synthesis of di peptides:

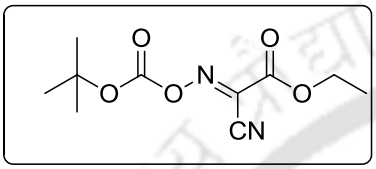
To a solution of *N*-protected amino acid (1 equiv) and DIPEA (1 equiv) in 1 mL of EtOAc, Boc-Oxyma (1 equiv) was added. The reaction mixture was stirred for 1 h at 0-5 ºC followed by the addition of methyl ester of the second amino acid (1 equiv) and DIPEA (1 equiv) in 1 mL of EtOAc. The reaction mixture was stirred at the same temperature for more 2 h. After completion of the reaction, the reaction mixture was diluted with 50 ml of ethylacetate, the organic phase was washed with 5% citric acid (2×20 mL), saturated $NaHCO_3$ (2×20 mL) and dried over Na_2SO_4 anhydrous. Finally, Na_2SO_4 was filtered and the solvent was evaporated. The product was purified by silica gel column chromatography.

3.5.5. Solid phase synthesis of GNFGAILG-NH_2 with Boc-Oxyma:

The octapeptide was manually assembled stepwise on Fmoc-Rink Amide MBHA resin using Fmoc/^{t}But protection strategy. The pre-activation time of Fmoc-amino acids with Boc-Oxyma (3 equiv) and DIPEA (5 equiv) was 1 h. The coupling time was 2 h. Fmoc deprotection was carried out with the use of piperidine/DMF (1:4, 3 × 7 min). The peptide was cleaved from the resin with the use of TFA/DCM (1:1) mixture for 2.5 h. Purification of the peptide was carried out by semi-preparative HPLC and subsequent lyophilization afforded the final peptide as a white powder.

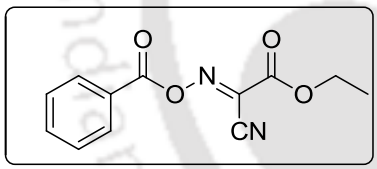
3.6. Characterization data

Boc-Oxyma (Coupling reagent)



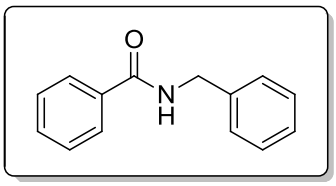
Red color liquid, Yield 96 %; ^{1}H NMR (400 MHz, CDCl3) δ 4.44-4.39 (q, *J* = 8 Hz, 2H), 1.54 (s, 9H) 1.38-1.34 (t, *J* = 8.4, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 156.7, 148.3, 129.9, 106.6, 87.4, 64.3, 27.3, 13.8; IR (KBr) 3444, 2925, 1764, 1742 cm^{-1}; HRMS (ESI) m/z calculated for $C_{10}H_{14}N_2NaO_5$ is 265.0800 found 265.0728.

Bz-Oxyma (intermediate [A])



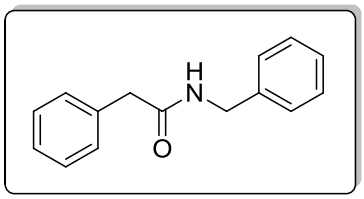
Yield: 96 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18-7.51 (m, 5H), 4.52-4.46 (q, *J* = 8 Hz, 2H), 1.44-1.40 (t, *J* = 8.4, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 157.0, 135.3, 131.7, 130.6, 129.2, 125.6, 107.0, 67.7, 13.9 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{12}H_{10}N_2NaO_4$ is 269.0538 found 269.0007.

1) *N*-Benzyl-benzamide (1a, Table 3.1.1.2)



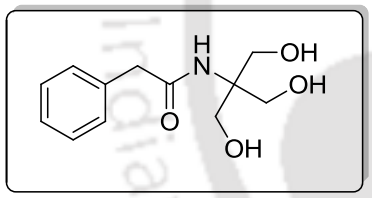
White solid, Yield 91 %; Mp. 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.77 (m, 2H), 7.43-7.25 (m, 8H), 4.64-4.63 (d, *J*= 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 138.4, 134.4, 131.6, 128.8, 128.6, 127.9, 127.5, 127.1, 44.1; FT-IR (KBr): 3290, 2924, 1637, 1551 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{14}NO$ is 212.1075 found 212.1073.

2) *N*-Benzyl-2-phenyl-acetamide (2a, Table 3.1.1.2)



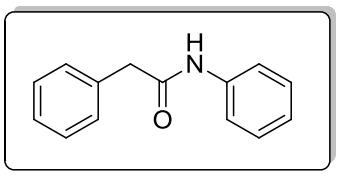
White solid, Yield 91 %; Mp 162 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.16 (m, 10H), 5.76 (br, 1H), 4.41-4.39 (d, *J*= 6 Hz, 2H), 3.62 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 138.3, 135.0, 129.4, 129.0, 128.7, 127.5, 127.4, 127.3, 43.7, 43.6; FT-IR (KBr): 3289, 2925, 1638, 1552 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{15}H_{16}NO$ is 226.1232 found 226.1231.

3) *N*-(2-Hydroxy-1,1-bis-hydroxymethyl-ethyl)-2-phenyl-acetamide (3a, table 3.1.1.2)



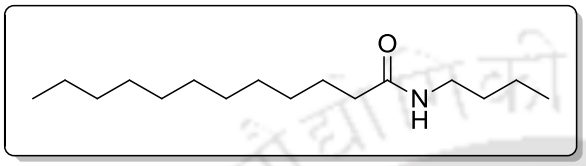
White solid, Yield 90 %; Mp. 125 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ 7.19-7.13 (m, 5H), 3.59 (s, 6H), 3.46 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 136.7, 130.3, 129.7, 128.0, 63.6, 62.5, 44.3; FT-IR (KBr): 3283, 2934, 1644, 1542 cm-1; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{18}NO_4$ is 240.1236 found 240.1233.

4) 2-*N*-Diphenyl-acetamide (4a, Table 3.1.1.2)



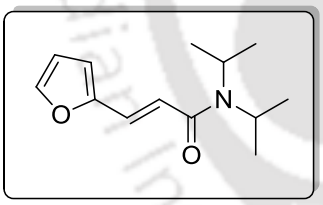
White solid; Yield 85%; Mp. 116 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.06 (m, 10H), 3.73 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 137.9, 134.7, 129.5, 129.1, 128.9, 127.5, 124.5, 120.2, 44.6; FT-IR (KBr) 3285, 3256, 1657, 1601 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{14}NO$ is 212.1075 found 212.1071.

5) *N*-butyldodecanamide (5a, Table 3.1.1.2)



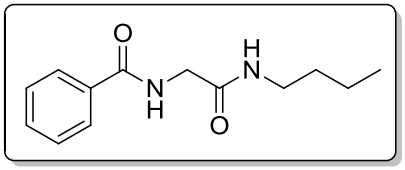
White solid, Yield: 95 %; Mp: 56 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52 (br, 1H), 3.23-3.18 (q, *J*= 6.8 Hz, 2H), 2.14-2.10 (t, *J*= 7.2 Hz, 2H), 1.60-1.56 (m, 2H), 1.48-1.41 (m, 2H), 1.22 (s, 18H), 0.90-0.82 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 39.2, 36.7, 31.9, 31.7, 29.6, 29.5, 29.4, 29.4, 25.9, 22.7, 20.1, 14.1, 13.7; FT-IR (KBr, ν/cm^{-1}): 3296, 2919, 1638, 1552; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{34}NO$ is 256.2640 found 256.2639.

6) 3-Furan-2-yl-*N*,*N*-diisopropyl-acrylamide (6a, Table 3.1.1.2)



White solid, Yield: 90 %; Mp: 125 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J*= 1.6 Hz, 1H),7.36-7.32 (d, *J*= 15.2 Hz, 1H), 6.74-6.71 (d, *J*= 14.8 Hz, 1H), 6.46-6.45 (d, *J*= 3.6 Hz, 1H), 6.40-6.39 (dd, *J*= 2 Hz, 1H), 4.10-4.05 (q, *J*= 7.4 Hz, 1H), 1.35-1.30 (d, *J*= 11.6 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 152.1, 143.6, 128.2, 118.2, 113.1, 112.2, 48.0, 46.0, 21.7, 20.8; FT-IR (KBr, ν/cm-1) 3458, 2970, 1646; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{13}H_{20}NO_2$ is 222.1494 found 222.1498.

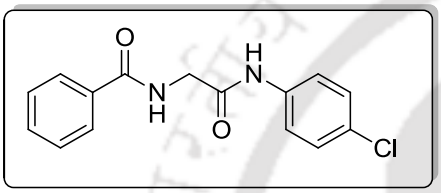
7) *N*-(2-(butylamino)-2-oxoethyl)benzamide (7a, Table 3.1.1.2)



White solid, Yield: 93 %; Mp: 128 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.38 (m, 5H), 6.78 (br, 1H), 4.12-4.10 (d, *J* = 4.8 Hz, 2H), 3.27-3.22 (m, 2H), 1.51-1.43

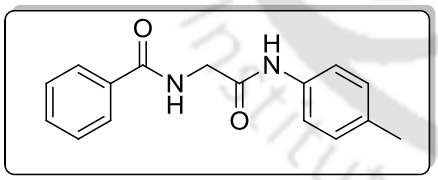
(m, 2H), 1.36-1.28 (m, 2H), 0.89-0.85 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 168.2, 133.6, 132.1, 128.8, 127.4, 41.1, 39.7, 31.6, 20.2, 13.8; FT-IR (KBr, ν/cm^{-1}): 3320, 3298, 1652, 1635; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{13}H_{19}N_2O_2$ is 235.1447 found: 235.1458.

8) *N*-[(4-Chloro-phenylcarbamoyl)-methyl]-benzamide (8a, Table 3.1.1.2)



White solid; Yield: 90 %; Mp: 232 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93-7.33 (m, 10H), 4.25 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, CD_3OD for solubility) δ 168.8, 167.8, 136.4, 133.2, 131.8, 129.1, 128.6, 128.4, 127.0, 121.1, 43.5; FT-IR (KBr, ν/cm^{-1}): 3306, 3192, 1680, 1638 ; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{15}H_{13}ClN_2NaO_2$ is 311.0563 found 311.0565.

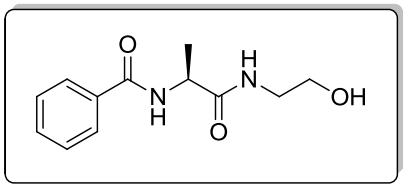
9) *N*-(p-Tolylcarbamoyl-methyl)-benzamide (9a, Table 3.1.1.2)



White solid, Yield: 89 %; Mp. 230 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (br, 1H), 7.83-7.06 (m, 10H), 5.68 (br, 1H), 4.32-4.31 (d, *J* = 5.6 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, CD_3OD for solubility) δ 167.8, 167.4, 135.4, 133.4, 131.5, 130.8, 129.1, 128.3, 127.2, 119.8; FT-IR (KBr) 3269, 2935, 1676, 1638 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{17}N_2O_2$ is 269.1290 found: 269.1285.

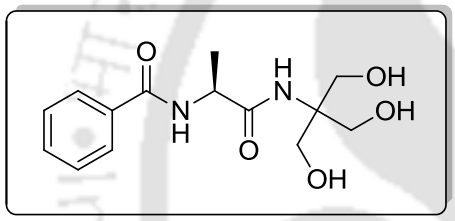
10) *N*-1-(2-hydroxy-ethylcarbamoyl)-ethyl benzamide (10a, Table 3.1.1.2)

White solid, Yield 91 %; Mp. 127 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.78-7.34 (m, 5H), 4.49-4.43 (m, 1H), 3.51-3.49 (d, *J* = 5.2 Hz, 2H), 3.23-3.16 (m, 2H), 1.84 (s, 1H), 1.37-1.35



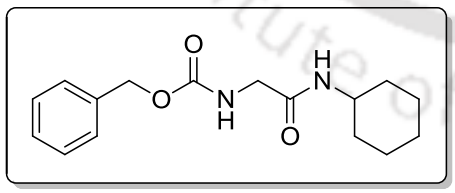
(d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD)δ 175.6, 170.1, 135.3, 133.0, 129.6, 128.7, 61.6, 51.3, 43.1, 18.4; FT-IR (KBr) 3425, 3300, 1622 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{17}N_2O_3$ is 237.1239 found: 237.1250. $[\alpha]^{27}_D$ = - 15.00 (c = 0.2, $CHCl_3$).

11) *N*-[1-(2-Hydroxy-1,1-bis-hydroxymethyl-ethylcarbamoyl)-ethyl]-benzamide (11a, Table 3.1.1.2)



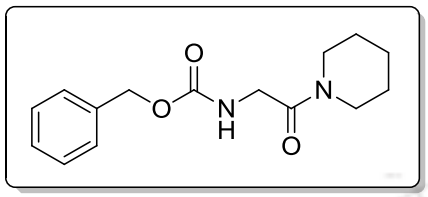
Semi solid, Yield: 91%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.76-7.73 (m, 5H), 4.51-4.44 (q, *J* = 7.2 Hz, 1H), 3.62 (s, 6H), 1.37-1.36 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.0, 170.5, 135.1, 133.1, 129.7, 128.6, 63.5, 62.3, 51.8, 18.1; FT-IR (KBr) 3436, 2922, 1671, 1640 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{14}H_{21}N_2O_5$ is 297.1450 found 297.1455.

12) Benzyl (2-(cyclohexylamino)-2-oxoethyl)carbamate (12a, Table 3.1.1.2)



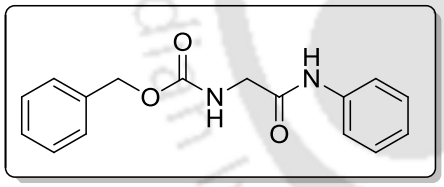
Brown solid, Yield 94 %; Mp. 110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 5H), 6.1 (br, 1H), 5.6 (br, 1H), 5.0 (s, 2H), 3.79-3.78 (d, *J* = 4 Hz, 2H), 3.74-3.70 (m, 1H), 1.84-1.09 (m, 10H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 156.8, 136.3, 128.7, 128.4, 128.2, 67.3, 48.5, 44.8, 33.0, 25.6, 24.9; FT-IR (KBr) 3347, 2924, 1718, 1655 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{16}H_{23}N_2O_3$ is 291.1709 found: 291.1710.

13) Benzyl (2-oxo-2-(piperidin-1-yl)ethyl)carbamate (13a, Table 3.1.1.2)



White solid, Yield 91 %; Mp. 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.29 (m, 5H), 5.84 (br, 1H), 5.09 (s, 2H), 3.98-3.97 (d, *J* = 4 Hz, 2H), 3.54-3.52 (t, *J* = 5.2 Hz, 2H), 3.29-3.27 (t, *J* = 5.2 Hz, 2H), 1.70 (s, 2H), 1.63-1.61 (d, *J* = 4.8 Hz, 2H), 1.54-1.52 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 156.3, 136.6, 128.5, 128.1, 128.0, 66.8, 45.4, 43.1, 42.6, 34.0, 26.2, 25.7, 25.4, 25.0, 24.3; FT-IR (KBr) 3293, 2936, 1707, 1641 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{15}H_{20}N_2NaO_3$ is 299.1372 found: 299.1366.

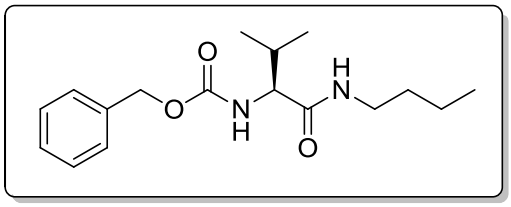
14) Benzyl (2-oxo-2-(phenylamino)ethyl)carbamate (14a, Table 3.1.1.2)



Light red solid, Yield: 87 %; Mp. 147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.10 (m, 10H), 5.57 (br, 1H), 5.13 (s, 2H), 3.99-3.98 (d, *J* = 4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 156.5, 136.3, 135.3, 129.3, 128.8, 128.7, 128.5, 128.3, 128.2, 67.3, 43.0; FT-IR (KBr) 3340, 2929, 1696, 1675 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{16}H_{16}N_2NaO_3$ is 307.1059 found 307.1062.

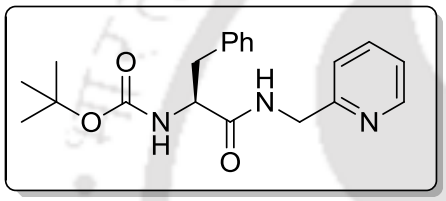
15) Benzyl (1-(butylamino)-3-methyl-1-oxobutan-2-yl)carbamate(15a, Table 3.1.1.2)

White solid, Yield: 93%; Mp. 139 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 5H), 5.95 (br, 1H), 5.41-5.39 (d, *J* = 8 Hz, 1H), 5.1 (s, 2H), 3.92-3.82 (t, *J* = 8.4 Hz, 1H), 3.27-3.18 (m, 2H), 2.11-2.10 (m, 1H) 1.47-1.45 (m, 2H), 1.35-1.29



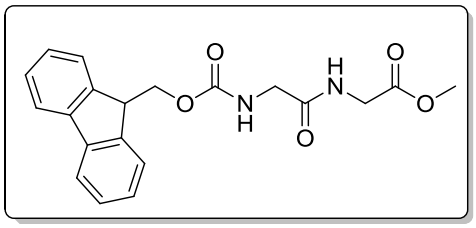
(m, 2H), 0.96-0.83 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 156.7, 136.4, 128.6, 128.2, 128.0, 67.0, 60.8, 39.3, 31.6, 31.2, 20.1, 19.3, 18.2, 13.8; FT-IR (KBr) 3293, 1690, 1641 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{17}H_{27}N_2O_3$ is 307.2022 found 307.2032. $[\alpha]^{27}_D$ = - 12.00 (c = 0.2, $CHCl_3$).

16) Tert-butyl (1-oxo-3-phenyl-1-((pyridin-2-ylmethyl)amino)propan-2-yl)carbamate (16a, Table 3.1.1.2)



White solid, Yield: 91 %; Mp. 120 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44-8.43 (d, *J* = 4 Hz, 1H), 7.65-7.62 (t, *J* = 6.4 Hz, 1H), 7.22-7.14 (m, 8H), 5.16 (br, 1H), 4.49-4.48 (d, *J* = 4 Hz, 2H), 4.45 (br, 1H), 3.07-3.05 (d, *J* = 6.4 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 156.4, 155.5, 148.9, 137.0, 136.8, 129.4, 128.7, 126.9, 122.5, 122.1, 80.2, 56.0, 44.5, 38.8, 28.4; FT-IR (KBr) 3332, 1684, 1661, 1650 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{20}H_{26}N_3O_3$ is 356.1974 found: 356.1962. $[\alpha]^{27}_D$ = - 4.00 (c = 0.2, $CHCl_3$).

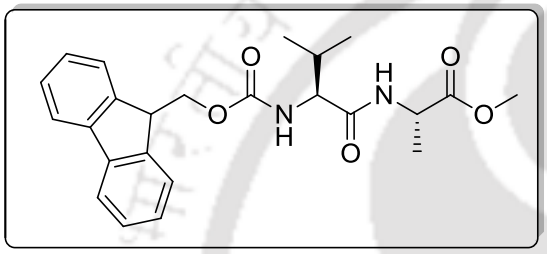
17) Methyl 2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)acetate (17a, Table 3.1.1.2)



White solid, Yield 91 %; Mp. 162 ºC; ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.76-7.74 (d, *J*= 7.6 Hz, 2H), 7.58-7.56 (d, *J* = 7.6 Hz, 2H), 7.40-7.37 (t, *J* = 7.2 Hz, 2H), 7.31-7.27 (t, *J* = 7.2 Hz, 2H), 6.80 (br, 1H), 5.73 (br, 1H), 4.43-4.41 (d, *J* = 6.8 Hz, 2H), 4.22-4.19 (t, *J* = 7.2 Hz, 1H), 4.04-4.03 (d, *J* = 6.8 Hz, 2H) 3.92-3.91 (d, *J* = 6.8 Hz, 2H), 3.73 (s, 3H); ^{13}C NMR (100 MHz,

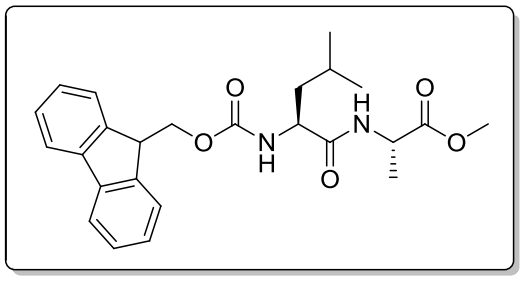
$CDCl_3$) δ 169.9, 169.7, 156.8, 143.7, 141.2, 128.6, 128.5, 128.3, 127.1, 67.1, 47.0, 44.2, 41.2, 33.8; FT-IR (KBr) 3316, 2931, 1734, 1692, 1654 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{20}H_{20}N_2NaO_5$ is 391.1270 found 391.1366.

18) 2-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-propionic acid methyl ester (18a, Table 3.1.1.2)



White solid, Yield 90 %; Mp. 205 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.6 Hz, 2H), 7.60-7.58 (d, *J* = 6.8 Hz, 2H), 7.41-7.37 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (t, *J* = 7.2 Hz, 2H), 6.54-6.52 (d, *J* = 6.8 Hz, 1H), 5.54-5.52 (d, *J* = 8 Hz, 1H), 4.60-4.58 (d, *J* = 7.2 Hz, 2H), 4.44-4.35 (m, 1H), 4.23-4.21(d, *J* = 6.4 Hz, 1H) 4.04-4.0 (t, *J* = 7.4 Hz, 1H), 3.73 (s, 3H), 2.11-2.08 (m, 1H), 1.41-1.39 (d, *J* = 6.8 Hz, 3H) 0.98-0.96 (d, *J* = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 171.2, 156.6, 144.0, 141.4, 127.8, 127.2, 125.2, 120.1, 67.2, 60.4, 52.6, 48.2, 47.3, 31.9, 29.8, 19.2, 18.2; FT-IR (KBr) 3296, 2953, 1745, 1692, 1650 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{24}H_{28}N_2NaO_5$ is 447.1896 found 447.1908. $[\alpha]^{27}_D$ = - 17.10 (c = 1, $CHCl_3$).

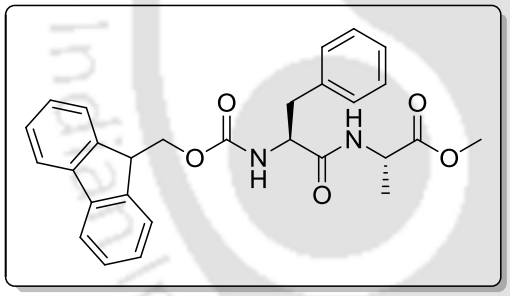
19) Methyl 2-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-methylpentanamido)propanoate (19a, Table 3.1.1.2)



White solid, Yield 88 %; Mp. 162 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.6 Hz, 2H), 7.59-7.57 (d, *J* = 6.8 Hz, 2H), 7.41-7.37 (t, *J* = 7.2 Hz, 2H), 7.32-7.28 (t, *J* = 7.2 Hz, 2H),

6.62-6.60 (d, *J* = 6.8 Hz, 1H), 5.36-5.34 (d, *J* = 8 Hz, 1H), 4.58-4.52 (m, 1H), 4.41-4.35 (m, 3H), 4.22-4.19 (t, *J* = 6.4 Hz, 1H), 3.73 (s, 3H), 1.81-1.74 (m, 1H), 1.65-1.63 (m, 2H), 1.40-1.38 (d, *J* = 6.8 Hz, 3H) 1.01-0.98 (d, *J* = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 163.1, 156.6, 143.8, 141.1, 129.3, 128.6, 127.5, 126.9, 124.9, 66.6, 57.4, 52.2, 46.9, 38.2, 38.3, 28.1, 18.0; FT-IR (KBr) 3307, 2925, 1754, 1694, 1654 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{25}H_{31}N_2O_5$ is 439.2233 found 439.2047. $[\alpha]^{27}_D$ = - 32.00 (c = 1, $CHCl_3$).

20) 2-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-phenyl-propionylamino]-propionic acid methyl ester (20a, Table 3.1.1.2)

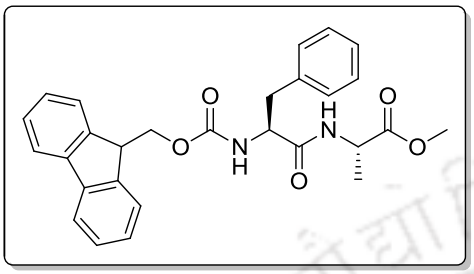


White solid, Yield 89 %; Mp. 182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.73 (d, *J* = 7.6 Hz, 2H), 7.53-7.50 (t, *J* = 6.8 Hz, 2H), 7.40-7.36 (t, *J* = 7.2 Hz, 2H), 7.30-7.18 (m, *J* = 7.2 Hz, 7H), 6.63 (br, 1H), 5.37 (br, 1H), 4.51-4.39 (m, 3H), 4.32-4.29 (m, 1H), 4.18-4.14 (t, *J* = 6.8 Hz, 1H) 3.09-3.03 (dd, *J* = 6 Hz, 2H), 3.68 (s, 3H), 1.33-1.31 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.9, 156.0, 143.7, 141.1, 136.5, 129.4, 128.4, 127.6, 127.0, 126.8, 125.1, 119.8, 67.0, 56.0, 52.3, 48.2, 46.9, 38.4, 17.8; FT-IR (KBr) 3307, 2925, 1754, 1694, 1654 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{28}H_{28}N_2NaO_5$ is 473.2076 found 473.2402. $[\alpha]^{27}_D$ = - 11.00 (c = 0.8, $CHCl_3$).

21) 2-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-phenyl-propionylamino]-propionic acid methyl ester (21a, Table 3.1.1.2)

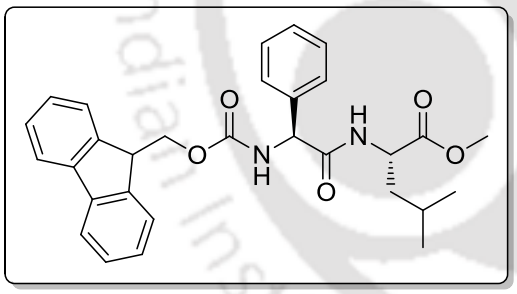
White solid, Yield 91 %; Mp. 182 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J* = 7.6

Hz, 4H), 7.55-7.12 (m, 20H), 6.41 (br, 1H), 6.29 (br, 1H), 5.52-5.50 (d, *J* = 6.8 Hz, 1H),



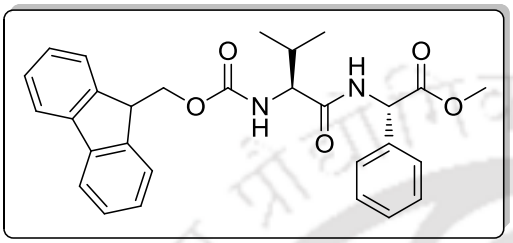
5.45-5.43 (d, *J* = 6.8 Hz, 1H 4.53-4.37 (m, 6H), 4.35-4.31 (m, 2H), 4.19-4.16(t, *J* = 6.8 Hz, 2H) 3.10-2.97 (m, 4H), 3.68 (s, 6H), 1.33-1.32 (d, *J*= 7.2 Hz, 3H), 1.22-1.20 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 173.1, 171.1, 170.8, 156.2,144.0, 141.5, 136.8, 129.6, 128.8, 128.3, 127.9, 127.6, 127.3, 127.2, 125.3, 120.2, 67.4, 56.3, 52.6, 48.3, 48.2, 47.3, 39.2, 38.9, 18.3, 18.1.

22) 2-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-2-phenyl-acetylamino]-4-methyl-pentanoic acid methyl ester (22a, Table 3.1.1.2)



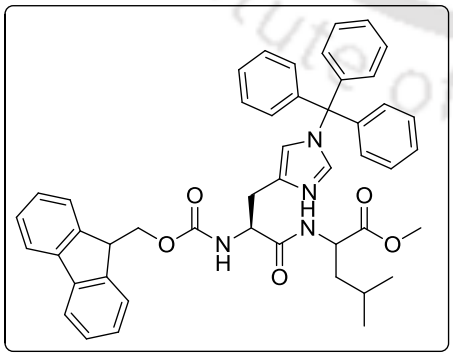
White solid, Yield 90 %; Mp. 194 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.73 (d, *J* = 7.6 Hz, 2H), 7.56-7.22 (m, 11H), 6.21-6.20 (d, *J* = 4.8 Hz, 1H), 5.29(br, 1H), 4.60-4.55 (m, 1H), 4.36-4.34 (d, *J* = 7.2 Hz, 1H) 4.19-4.16 (t, *J* = 6.8 Hz, 2H), 3.61 (s, 3H), 1.63-1.60 (m, 2H), 1.54-1.47 (m, 1H), 0.92-0.91 (d, *J* = 4.4 Hz, 3H) 0.90-0.89 (d, *J* = 4.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 170.0, 155.9, 143.7, 143.7, 141.2, 137.6, 128.8, 128.2, 127.6, 127.1, 127.0, 125.1, 119.9, 67.2, 58.1, 52.0, 51.1, 47.0, 41.0, 24.7, 22.6, 21.8; FT-IR (KBr) 3433, 1742, 1684, 1655; 1654 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{30}H_{33}N_2O_5$ is 501.2389 found 501.2418. $[\alpha]^{27}_D$ = + 46.00 (c = 1, $CHCl_3$).

23) [2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-phenyl-acetic acid methyl ester (23a, Table 3.1.1.2)



White solid, Yield 91%; Mp. 200 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.74 (d, *J* = 7.6 Hz, 2H), 7.56-7.55 (d, *J* = 7.6 Hz, 2H), (7.40-7.23 (m, 9H), 6.90-6.88 (d, *J* = 6.8 Hz, 1H), 5.54-5.52 (d, *J* = 6.4 Hz, 1H), 5.45-5.43 (d, *J* = 9.6 Hz, 1H), 4.40-4.28 (m, 1H), 4.19-4.18 (d, *J* = 6.8, 1H) 4.10-4.07 (t, *J* = 7.8 Hz, 2H), 3.7 (s, 3H), 2.16-2.13 (m, 1H), 1.01-0.99 (d, *J* = 6.8 Hz, 3H), 0.96-0.95 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 169.1, 156.6, 144.0, 143.9, 141.4, 135.9, 129.1, 128.7, 127.8, 127.4, 127.2, 125.2, 120.0, 67.2, 60.2, 56.7, 52.9, 47.2, 31.6, 19.2, 18.1; FT-IR (KBr) 3445, 1739, 1681, 1650 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{29}H_{31}N_2O_5$ 487.2233 found 487.2373.

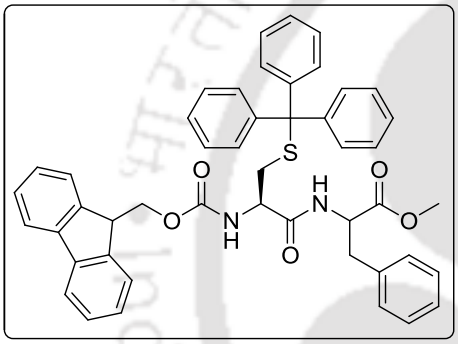
24) Methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-trityl-1H-imidazol-4-yl)propanamido)-4-methylpentanoate (24a, Table 3.1.1.2)



White solid, Yield 85%; Mp. 202 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86-7.85 (d, *J*= 7.6 Hz, 2H), 7.76-7.74 (d, *J*= 7.2 Hz, 2H), 7.61-7.59 (d, *J*= 7.2 Hz, 2H), 7.39-7.09 (m, 19H), 6.90-6.88 (S, 1H), 6.61-6.60 (d, *J*= 6.4 Hz, 1H), 4.56-4.54 (m, 2H), 4.33-4.32 (d, *J*= 6.8, 2H) 4.12-4.11 (t, *J*= 7.6 Hz, 1H), 3.65 (s, 3H), 3.04-3.03 (d, *J* = 6.8 Hz, 2H), 1.60-1.54 (m, 2H), 1.27-1.23 (t, *J* = 6.8 Hz, 1H), 0.91-0.88 (d, *J*= 6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 171.5, 156.4, 144.0, 144.0, 142.4,

142.3, 141.3, 138.6, 138.5, 137.0, 129.8, 128.3, 128.2, 127.8, 127.2, 125.4, 125.3, 120.1, 120.0, 119.6, 75.6, 67.3, 60.5, 54.9, 52.2, 5.1, 47.2, 41.3, 24.9, 22.9, 22.0; FT-IR (KBr) 3435, 1746, 1692, 1643 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{47}H_{47}N_4O_5$ 747.3546 found 747.3558. $[\alpha]^{27}_D$ = + 3.00 (c = 1, $CHCl_3$).

25) Methyl 2-((R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tritylthio)propanamido)-3-phenylpropanoate (25a, Table 3.1.1.2)

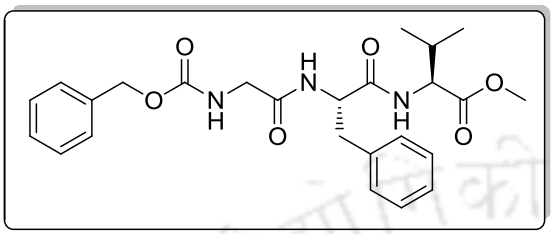


White solid, Yield 87%; Mp. 192 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.72 (d, *J*= 7.6 Hz, 2H), 7.55-7.54 (d, *J*= 7.6 Hz, 2H), 7.40-7.00 (m, 24H), 6.38-6.36 (d, *J*= 7.2 Hz, 1H), 4.99-4.97 (m, 1H), 4.76-4.74 (d, *J*= 6.8, 2H), 4.35-4.26 (m, 1H), 4.18-4.15 (t, *J*= 7.2 Hz, 1H), 3.65 (s, 3H), 3.06-2.98 (m, 2H), 2.64-2.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 169.9, 156.0, 144.4, 143.8, 141.3, 153.7, 129.7, 129.6, 129.4, 128.6, 128.2, 127.2, 127.0, 125.2, 125.1, 120.1, 67.4, 67.1, 54.0, 53.3, 52.4, 47.1, 37.8, 34.0 ; FT-IR (KBr) 3452, 1766, 1649, 1636 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{47}H_{43}N_2O_5S$ 747.2893 found 747.3549. $[\alpha]^{27}_D$ = + 28.60 (c = 1, $CHCl_3$).

26) 2-[2-(2-Benzyloxycarbonylamino-acetylamino)-3-phenyl-propionylamino]-3-methyl-butyric acid methyl ester (26a, Table 3.1.1.2)

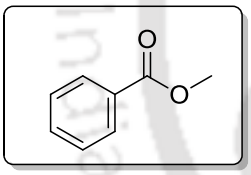
White solid, Yield 90 %; Mp. 101 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.13 (m, 10H), 6.99 (br, 1H), 6.2 (br, 1H), 5.8 (br, 1H), 5.08 (s, 2H), 4.58-4.54 (m, 1H), 4.43-4.38 (m, 1H),

3.85-3.84 (d, *J*= 4.4 Hz, 2H), 3.73 (s, 3H), 304-3.00(m, 2H), 2.14 (m, 1H), 0.94-0.93 (d, *J*= 7.2 Hz, 3H), 0.91-0.90 (d, *J*= 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ171.8, 171.2, 169.4, 156.6, 136.5, 136.3, 129.3, 128.4, 128.3, 128.0, 127.9, 126.7, 66.9, 57.2, 54.3, 52.0, 44.2, 38.4, 31.1, 18.8, 18.7; FT-IR (KBr): 3406, 1728, 1677, 1651, 1646 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{25}H_{32}N_3O_6$ is 470.2291 found: 470.2294.



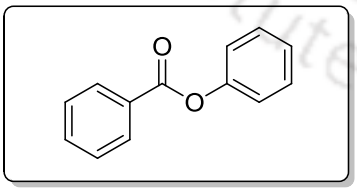
Esters

25) Benzoic acid methyl ester (1b, Table 3.2.1)



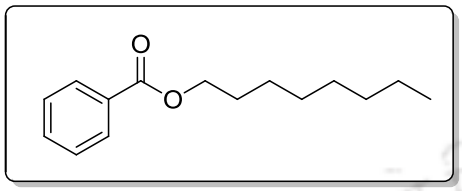
White solid, Yield 92 %; Mp. 112 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-7.38 (m, 5H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 133.0, 130.3, 129.7, 128.5, 52.2;FT-IR (KBr) 3451, 2961, 1730 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_8H_9O_2$ is 137.0603 found 137.0649.

26) Benzoic acid phenyl ester (2b, Table 3.2.1)



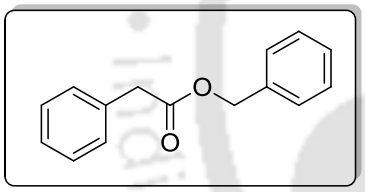
White solid, Yield 90 %; Mp. 70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21-8.19 (m, 2H), 7.64-7.48 (m, 3H), 7.50-7.15 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 151.2, 133.7, 130.3, 129.6, 129.5, 128.7, 126.0, 125.8; FT-IR (KBr) 3454, 2923, 1731 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{13}H_{10}NaO_2$ is 221.0578 found 221.0583.

27) Benzoic acid octyl ester (3b, Table 3.2.1)



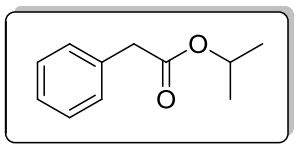
Colorless oil, Yield 94 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05-7.41 (m, 5H), 4.33-4.29 (t, *J* = 6.8 Hz, 2H), 1.80-1.73 (m, 2H), 1.46-1.41 (m, 2H), 1.37-1.28 (m, 8H), 0.89-0.87 (t, *J* = 4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$)δ 166.9, 132.9, 130.7, 129.7, 128.5, 65.3, 32.0, 29.4, 29.4, 28.9, 26.2, 22.8, 14.2; FT-IR (KBr) 3455, 2929, 1721 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{15}H_{23}O_2$ is 235.1698 found 235.1692.

28) Phenyl acetic acid benzyl ester (4b, Table 3.2.1)



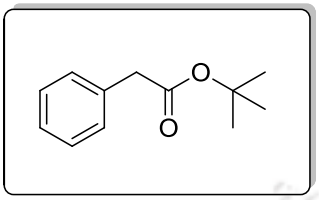
White solid, Yield 93 %; Mp. 52 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.25 (m, 10H), 5.11 (s, 2H), 3.64 (s,2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 135.9, 133.9, 129.3, 128.5, 128.5, 128.2, 128.1, 127.1, 66.5, 41.3; FT-IR (KBr):3436, 2917, 1733 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{15}H_{14}NaO_2$ is 249.0891 found 249.0893.

29) Phenyl acetic acid isopropyl ester (5b, Table 3.2.1)



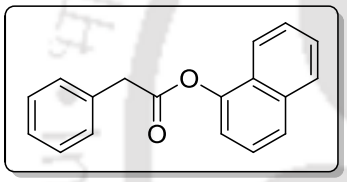
Colorless oil, Yield 90 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.23 (m, 5H), 5.04-4.97 (m, 1H), 3.57 (s, 2H), 1.22 (d, *J* = 0.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 134.4, 129.3, 128.6, 127.0, 68.2, 41.8, 21.8; FT-IR (KBr) 3440, 2981, 1732 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{11}H_{15}O_2$ is 179.1072 found 179.0885.

30) Phenyl acetic acid *tert*- butyl ester (6b, Table 3.2.1)



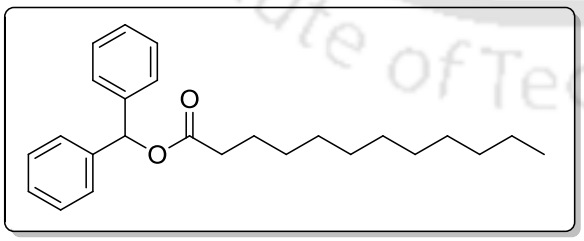
Colorless oil, Yield 87 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.22 (m, 5H), 3.51 (s, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 134.7, 129.2, 128.5, 126.8, 80.7, 42.6, 28.0; FT-IR (KBr): 3230, 2989, 1754 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{12}H_{16}NaO_2$ is 215.1048 found 215.0990.

31) Phenyl acetic acid naphthalen-1-yl ester (7b, Table 3.2.1)



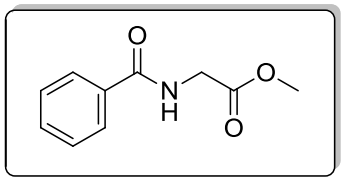
White solid, Yield 95 %; Mp. 52 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.80 (d, *J* = 8 Hz, 1H), 7.70-7.68 (d, *J* = 8.4, 1H), 7.61-7.59 (d, *J* = 8.4, 1H), *7*.48-7.20 (m, 9H), 3.99 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 146.5, 134.6, 133.5, 129.4, 128.8, 127.9, 127.4, 126.8, 126.4, 126.0, 125.3, 121.0, 118.0, 41.4; FT-IR (KBr) 3444, 2925, 1748 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{18}H_{14}NaO_2$ is 285.0891 found 285.0892.

32) Dodecanoic acid benzhydryl ester (8b, Table 3.2.1)



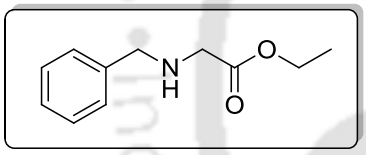
White solid, Yield 93 %; Mp. 55 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.22 (m, 10H), 5.79 (s, 1H), 2.31-2.27 (t, *J*= 7.6 Hz, 2H), 1.59-1.57 (m, 2H), 1.25 (s, 16H), 0.89-0.86 (t, *J*= 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.7, 158.8, 143.6, 128.4, 127.5, 126.6, 63.3, 34.1, 32.0, 29.7, 29.5, 29.4, 29.3, 29.1, 24.1, 22.7, 14.1; FT-IR (KBr) 3390, 2921, 1705 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{25}H_{34}NaO_2$ is 389.2456 found 389.2480.

33) Benzoylamino-acetic acid methyl ester (9b, Table 3.2.1)



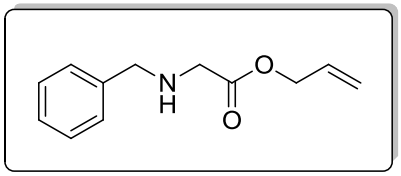
White solid, Yield 93 %; Mp. 82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.81 (d, *J* = 7.2 Hz, 2H), 7.54-7.50 (t, *J* = 7.2 Hz, 1H), 7.46-7.43 (t, *J* = 7.2 Hz, 2H), 6.75 (br, 1H), 4.26-4.25 (d, *J* = 4.8 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 167.7, 133.6, 131.7, 128.4, 127.1, 52.3, 41.7; FT-IR (KBr) 3433, 2925, 1736, 1627 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{10}H_{11}NNaO_3$ is 216.0637 found 216.0631.

34) Benzylamino acetic acid ethyl ester (10b, Table 3.2.1)



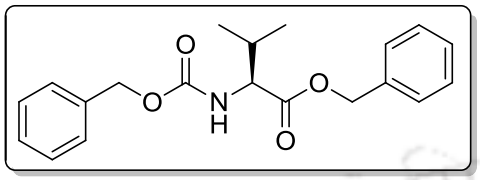
White solid, Yield 95 %; Mp. 61 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.80 (d, *J* = 8 Hz, 2H), 7.53-7.49 (t, *J* = 6.8 Hz, 1H), 7.45-7.42 (t, *J* = 7.2 Hz, 2H), 6.79 (br, 1H), 4.28-4.22 (m, 4H), 1.32-1.29 (t, *J* = 7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 167.8, 133.4, 131.5, 128.3, 127.0, 61.2, 41.6, 13.9; FT-IR (KBr) 3435, 3340, 1757, 1642 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{11}H_{14}NO_3$ is 208.0974 found 208.0982.

35) Benzylamino acetic acid allyl ester (11b, Table 3.2.1)



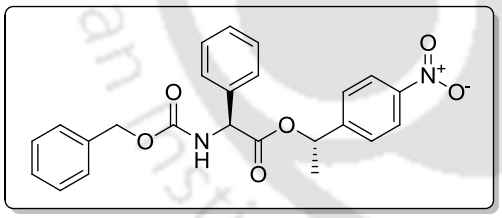
Yellow oil, Yield: 93 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.8-7.78 (d, *J* = 8.4 Hz, 2H), 7.52-7.48 (t, *J* = 8.2 Hz, 1H), 7.44-7.40 (t, *J* = 8 Hz, 2H), 6.68 (br, 1H), 5.96-5.86 (m, 1H), 5.36-5.31 (dd, *J* = 16 Hz, 1H), 5.27-5.25 (dd, *J* = 10.4 Hz, 1H), 4.68-4.67 (d, *J* = 6 Hz, 2H), 4.26-4.25 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 167.8, 133.6, 131.7, 131.5, 128.5, 127.2, 118.8, 60.0, 41.8; FT-IR (KBr) 3427, 2928, 1751, 1650 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{12}H_{13}NNaO_3$ is 242.0793 found 242.0791.

36) Benzyl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate (12b, Table 3.2.1)



Colorless oil, Yield 92 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 10H), 5.3-5.27 (d, *J* = 8.8 Hz, 1H), 5.19-5.11 (m, 2H), 5.09 (s, 2H), 4.36-4.32 (m, 1H), 2.19-2.14 (m, 1H), 0.94-0.92 (d, *J* =6.8 Hz, 3H), 0.84-0.82 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 156.2, 136.3, 135.3, 128.4, 128.3, 128.3, 128.2, 127.9, 66.8, 59.0, 31.1, 18.8, 17.3, FT-IR (KBr) 3342, 2963,1727, 1538 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{20}H_{24}NO_4$ is 342.1705 found 342.1714. $[\alpha]^{27}_D$ = - 2.70(c = 1, $CHCl_3$).

37) 1-(4-nitrophenyl)ethyl 2-(benzyloxycarbonylamino)-2-phenylacetate (13b, Table 3.2.1)

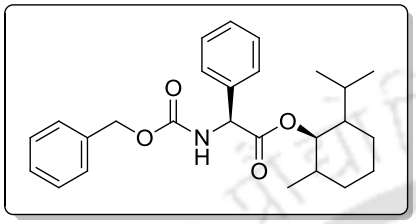


White solid, Yield 93 %; Mp. 155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20-8.18 (d, *J*= 8.4 Hz, 2H), 7.98-7.96 (d, *J*= 8.4 Hz, 1H), 7.54-7.00 (m, 12H), 5.92-5.91 (d, *J*= 6.4 Hz, 1H), 5.78-5.77 (q, *J*= 7.6 Hz, 1H), 5.06 (s, 2H), 1.41-1.39 (d, *J*= 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 155.5, 147.9, 147.6, 136.1, 129..0, 128.8, 128.5, 128.2, 128.1, 127.4, 127.1, 126.7, 126.2, 72.9, 67.1, 58.2, 21.7; FT-IR (KBr) 3404, 1726, 1716 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{24}H_{22}N_2NaO_6$ is 457.1376 found 457.1388.

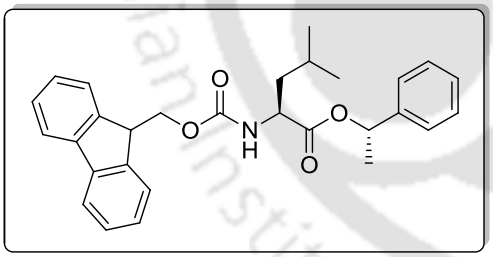
38) 2-isopropyl-6-methylcyclohexyl 2-(benzyloxycarbonylamino)-2-phenylacetate (14b, Table 3.2.1)

White solid, Yield 93 %; Mp. 60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.12 (m, 10H),

5.98-5.96 (d, *J*= 6.8 Hz, 1H), 5.36-5.34 (d, *J*= 7.2 Hz, 1H), 5.06 (s, 2H), 4.75-4.64 (m, 1H), 2.03-2.00 (m, 1H),1.95-1.86 (m, 1H), 1.73-1.57 (m, 1H), 1.37-1.28 (m, 1H), 1.05-1.95 (m, 4H), 0.89-0.87 (d, *J*= 6.8 Hz, 3H), 0.81-0.74 (dd, *J* = 6.4 Hz, 3H), 0.58-0.56 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 155.3, 137.0, 136.2, 128.6, 128.5, 128.3, 128.2, 128.2, 128.0, 127.2, 127.0, 76.0, 66.8, 58.0, 46.7, 40.6, 39.8, 34.0, 31.3, 26.1, 23.3, 21.9, 20.6; FT-IR (KBr) 3368, 1735, 1708 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{26}H_{33}NNaO_4$ is 446.2307 found 446.2356.

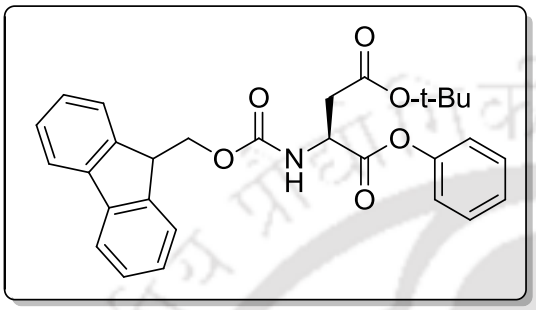


39) 1-phenylethyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-methylpentanoate (15b, Table 3.2.1)



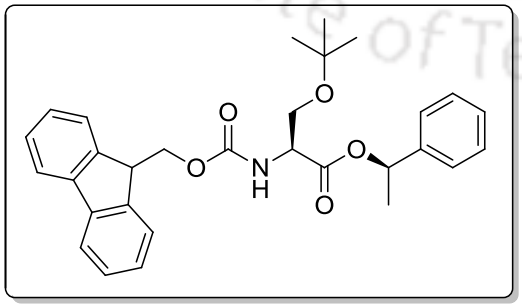
White solid, Yield 92 %; Mp. 150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J*= 7.6 Hz 2H), 7.58-7.25 (m, 11H), 5.92-5.90 (q, *J*= 2.8 Hz, 1H), 5.22-5.20 (d, *J*= 8.4 Hz, 1H) 4.91-4.86 (q, *J*= 6.4 Hz, 1H), 4.39-4.38 (d, *J*= 4 Hz, 2H), 4.21-4.18 (t, *J*= 6.8 Hz, 1H), 1.69- 1.52 (m, 2H), 1.49-1.48 (d, *J*= 6.4 Hz, 3H), 0.97-0.95 (d, *J*= 6 Hz, 3H), 0.92-0.87 (dd, *J*= 6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 156.0, 144.1, 143.9, 141.4, 128.7, 128.2, 127.8, 127.2, 126.2, 126.1, 125.5, 125.2, 73.6, 67.0, 52.8, 47.3, 42.0, 29.8, 25.3, 23.0, 22.3; FT-IR (KBr)3429, 1727, 1711cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{29}H_{31}NNaO_4$ is 480.2150 found 480.2764.

40) 4-tert-butyl 1-phenyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)succinate (16b, Table 3.2.1)



White solid, Yield 90 %; Mp 122-124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J*= 7.6 Hz, 2H), 7.59-7.58 (d, *J*= 7.6 Hz, 2H), 7.40-7.36 (t, *J*= 7.2 Hz, 2H), 7.30-7.19 (m, 3H), 7.10-7.08 (d, *J*= 7.6 Hz, 2H), 6.90-6.82 (m, 2H), 5.94-5.92 (d, *J* = 8.4 Hz, 1H), 4.45-4.43 (d, *J* = 7.2 Hz, 2H), 4.38-4.34 (t, *J*= 7.6 Hz, 1H), 4.27-4.23 (t, *J*= 6.8 Hz, 1H) 3.15-3.10 (dd, *J*= 4.4 Hz, 1H), 2.92-2.87 (dd, *J*= 4.4 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.7, 156.1, 150.5, 143.7, 141.2, 129.4, 128.7, 127.6, 127.0, 126.1, 125.0, 121.2, 82.0, 67.3, 50.7, 47.0, 37.8, 27.9; FT-IR (KBr) 3361, 2935, 1750, 1720, 1690 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{30}H_{33}NNaO_5$ is 510.1893 found 510.2304. $[\alpha]^{27}_{D}$ = + 4.40 (c = 0.5, $CHCl_3$).

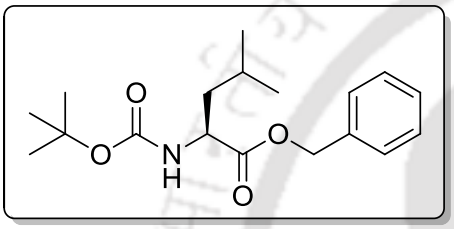
41) 3-tert-Butoxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid 1-phenyl-ethyl ester (17b, Table 3.2.1)



White solid, Yield:92 %; Mp. 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.76 (d, *J*= 7.6 Hz 2H), 7.64-7.61 (t, *J*= 6.4 Hz, 2H), 7.42-7.25 (m, 9H), 5.99-5.97 (d, *J*= 6.4 Hz, 1H) 4.92-4.87 (q, *J*= 6.4 Hz, 1H), 4.54-4.53 (d, *J*= 2.4 Hz, 2H), 4.42-4.36 (m, 1H), 4.27-4.26 (t, *J*= 5.2 Hz, 1H), 3.91-3.89 (dd, *J*= 2 Hz, 1H), 3.85-3.82 (dd, *J*= 2.4 Hz, 1H), 1.51- 1.49 (d, *J*= 6.4 Hz, 3H), 1.81 (s, 9H); ^{13}C NMR

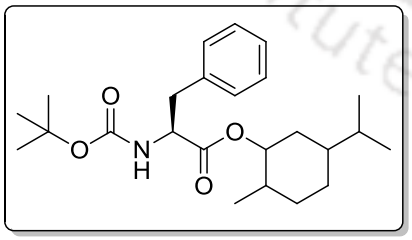
(100 MHz, $CDCl_3$) δ 170.0, 156.2, 144.0, 141.3, 141.0, 128.5, 128.4, 128.0, 127.7, 127.3, 127.1, 126.2, 126.0, 125.4, 73.5, 70.2, 67.2, 62.3, 54.8, 47.1, 27.3, 22.0; FT-IR (KBr) 3443, 2975, 1727, 1700 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{30}H_{33}NNaO_5$ is 510.2256 found 510.2284.

42) 2-*tert*-butoxycarbonylamino-4-methyl-penatnoic acid benzyl ester (18b, Table 3.2.1)



White semi-solid, Yield 91 %; ^1H NMR (400 MHz, $CDCl_3$)δ 7.36-7.32 (m, 5H), 5.18-5.09 (m, 2H), 4.89-4.87 (d, *J* = 8 Hz, 1H), 4.33-4.32 (m, 1H), 1.40 (s, 12H), 0.90-0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 155.4, 135.5, 128.5, 128.2, 128.1, 79.7, 66.8, 52.2, 41.5, 28.3, 24.7, 22.8, 21.8; FT-IR (KBr) 3442, 1712, 1638 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{18}H_{27}NNaO_4$ is 344.1838 found 344.1838. $[\alpha]^{27}_D$ = - 12.40 (c = 1, $CHCl_3$).

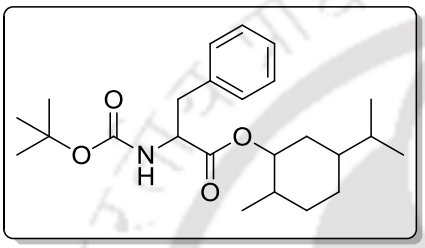
43) L-2-*tert*-Butoxycarbonylamino-3-phenyl-propionic acid 5-isopropyl-2-methyl-cyclohexyl ester (19b, Table 3.2.1)



White solid, Yield 92 %; Mp. 162 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29-7.14 (m, 5H), 5.01-4.99 (d, *J* = 7.2 Hz, 1H), 4.73-4.68 (m,1H), 4.54-4.52 (d, *J* = 6.4 Hz, 1H), 3.12-3.09 (dd, *J* = 6 Hz, 1H), 3.05-3.00 (dd, *J* = 6 Hz, 1H), 1.89-1.86 (m, 1H), 1.77-1.76 (m, 1H), 1.67-1.64 (m, 1H), 1.41 (s, 9H), 1.34-1.31 (m, 1H), 1.04-1.0 (m, 4H), 0.92-0.85 (dd, *J* = 6.4 Hz, 6H), 0.72-0.71 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 155.2, 136.3, 129.6, 128.5, 127.0, 79.8, 75.6, 54.6, 47.0, 40.8, 38.3, 34.3, 31.5, 28.4, 26.1, 23.4, 22.1, 20.9, 16.3; FT-IR (KBr) 3394, 2945, 1721,

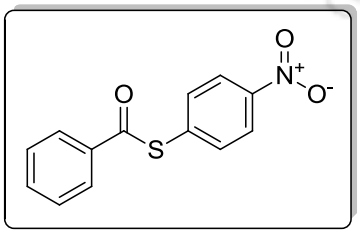
1687 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{27}H_{37}NNaO_4$ is 426.2620 found 426.2797. $[\alpha]^{27}_D$ = + 27.20 (c = 1, $CHCl_3$).

44) DL-2-*tert*-Butoxycarbonylamino-3-phenyl-propionic acid 5-isopropyl-2-methyl-cyclohexyl ester (20b, Table 3.2.1)



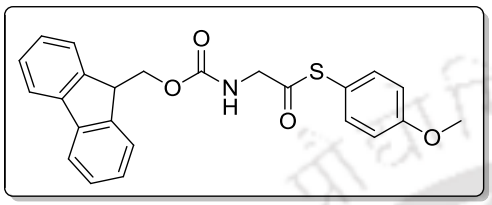
White solid, Yield: 91 %; Mp. 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.15 (m, 10H), 5.01-5.00 (d, *J* = 7.2 Hz, 1H), 4.95-4.93 (d, *J* = 8 Hz, 1H) 4.72-4.67 (t, *J* = 10.4 Hz, 2H), 4.54-4.52 (d, *J* = 6.4 Hz, 2H), 3.13-3.04 (dd, *J* = 5.2, Hz, 2H), 3.02-2.97 (dd, *J* = 6 Hz, 2H), 2.03-1.86 (m, 2H), 1.77-1.72 (m, 2H), 1.67-1.64 (m, 2H), 1.41 (s, 9H), 1.39 (s, 9H), 1.32-1.29 (m, 2H), 1.03-1.93 (m, 8H), 0.90-0.85 (dd, *J* = 6.4 Hz, 12H), 0.72-0.70 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 171.5, 155.1, 136.2, 129.6, 129.4, 128.5, 128.4, 126.9, 79.7, 75.7, 75.5, 54.7, 54.6, 47.0, 46.8, 40.7, 38.2, 34.2, 31.4, 29.7, 28.3, 26.0, 25.8, 23.3, 23.0, 22.0, 20.9, 20.8, 16.3, 15.9 ESI-MS, m/z $[M+Na]^+$ calculated for $C_{27}H_{37}NNaO_4$ is 426.26, found 426.27.

45) Thiobenzoic acid S-(4-nitro-phenyl) ester (21b, Table 3.2.1)



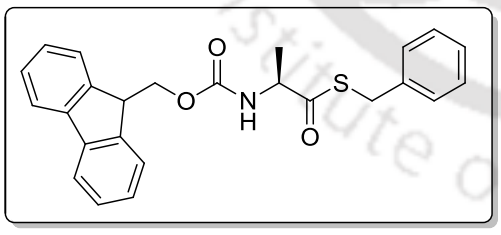
Yellow solid, Yield 87 %; Mp. 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25-8.22 (dd, *J*= 2 Hz, 2H), 7.96-7.94 (dd, *J*= 1.2 Hz, 2H), 7.66-7.64 (dd, *J*= 2 Hz, 2H), 7.59-7.44 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 137.4, 134.3, 133.3, 128.7, 126.3, 124.9, 124.2, 123.6; FT-IR (KBr) 3390, 2927, 1750, 1289 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{13}H_9NNaO_3S$ is 280.0201 found 280.0200.

46) (9H-Fluoren-9-ylmethoxycarbonylamino)-thioacetic acid S-(4-methoxy-phenyl) ester (22b, Table 3.2.1)



White solid, Yield 91 %; Mp. 168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77-7.75 (d, *J*= 7.6 Hz 2H), 7.61-7.60 (d, *J*= 6.4 Hz, 2H), 7.41-7.25 (m,7H), 6.95-6.93(d, *J*= 6.4 Hz, 1H), 5.45 (s, 1H), 4.46-4.44 (d, *J*= 2.4 Hz, 2H), 4.26-4.23 (t, *J*= 6.8 Hz, 1H), 4.21-4.20 (d, *J*= 5.2 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1, 161.0, 156.3, 143.8, 141.4, 136.4, 132.8, 127.9, 127.2, 125.2, 120.1, 116.9, 115.2, 67.5, 55.5, 47.3, 29.8; FT-IR (KBr) 3349, 1724, 1683, 1249 cm^{-}1; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{24}H_{21}NNaO_4S$ is 442.1089 found 442.1081.

47) (9H-Fluoren-9-ylmethoxycarbonylamino)-thiopropionic acid *S*-benzyl ester (23b, Table 3.2.1)



White solid, Yield 92 %; Mp. 172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.73 (d, *J*= 7.6 Hz, 2H), 7.60-7.25 (m, 11H), 5.28-5.27 (d, *J*= 2.4 Hz, 1H), 4.51-4.46 (q, *J*= 6.8 Hz, 1H),), 4.38-4.36 (d, *J*= 6.8 Hz, 2H) 4.22-4.19 (t, *J*= 7.2 Hz, 1H), 4.10 (s, 2H), 1.41-1.39 (d, *J*= 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.9, 155.7, 143.9, 143.8, 141.4, 137.0, 128.9, 128.7, 127.8, 127.4, 127.1, 125.1, 67.1, 56.7, 47.2, 33.3, 18.8; FT-IR (KBr) 3309, 1735, 1696, 1273 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{25}H_{23}NNaO_3S$ is 440.1296 found 440.1374.

3.7. Selected spectra

3.7.1. ^{1}H NMR, ^{13}C NMR and HRMS of Compounds:

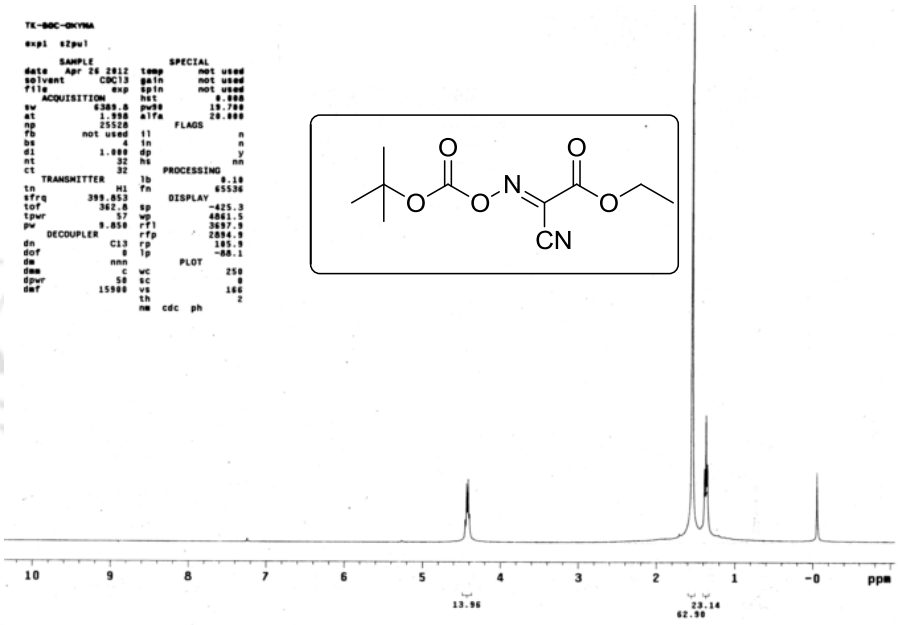


Figure S1. ^{1}H NMR spectra of Boc-Oxyma

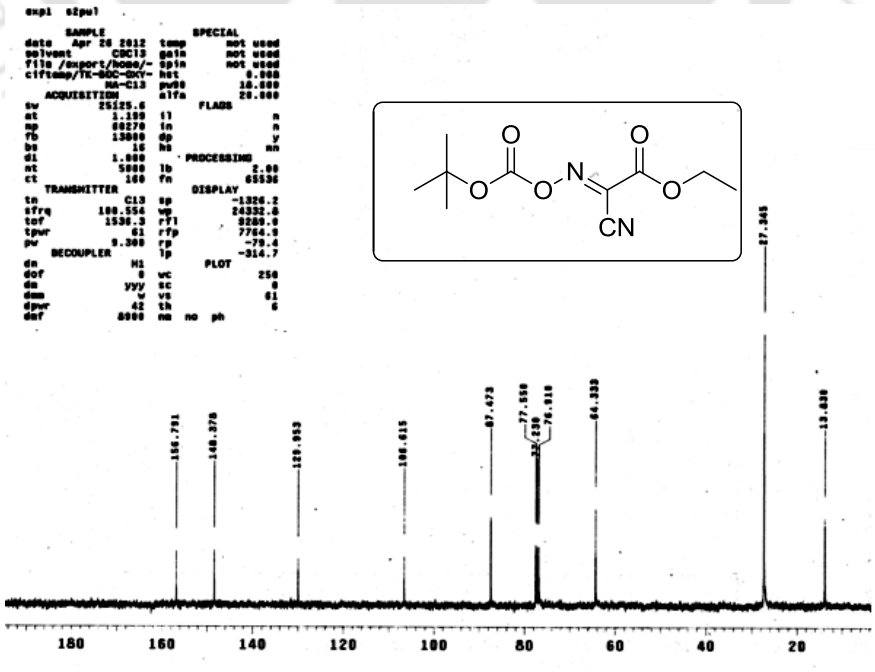


Figure S2. ^{13}C NMR spectra of Boc-Oxyma

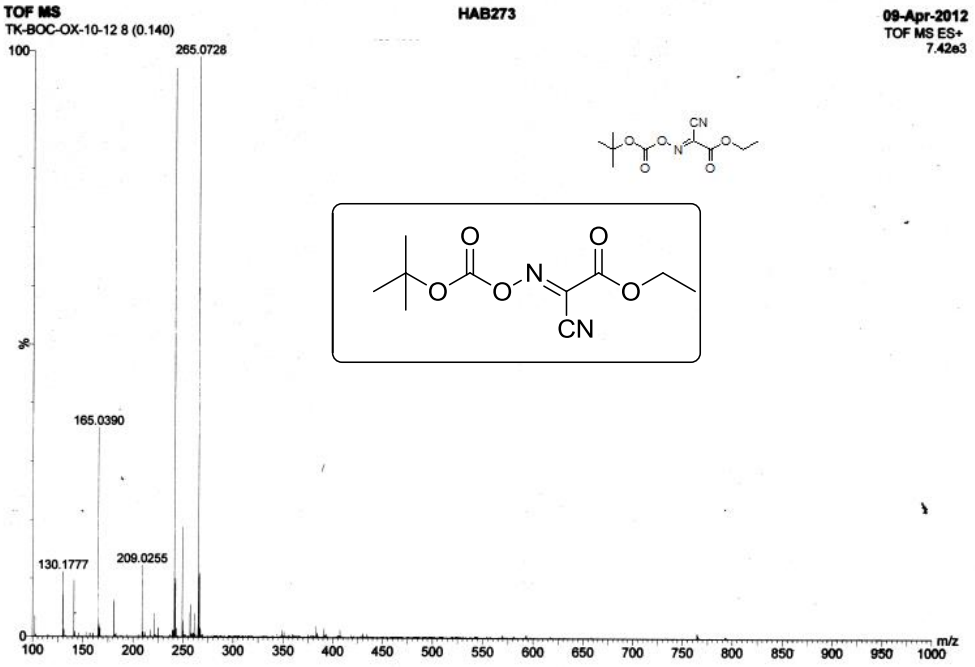


Figure S3. HRMS of intermediate Boc-Oxyma

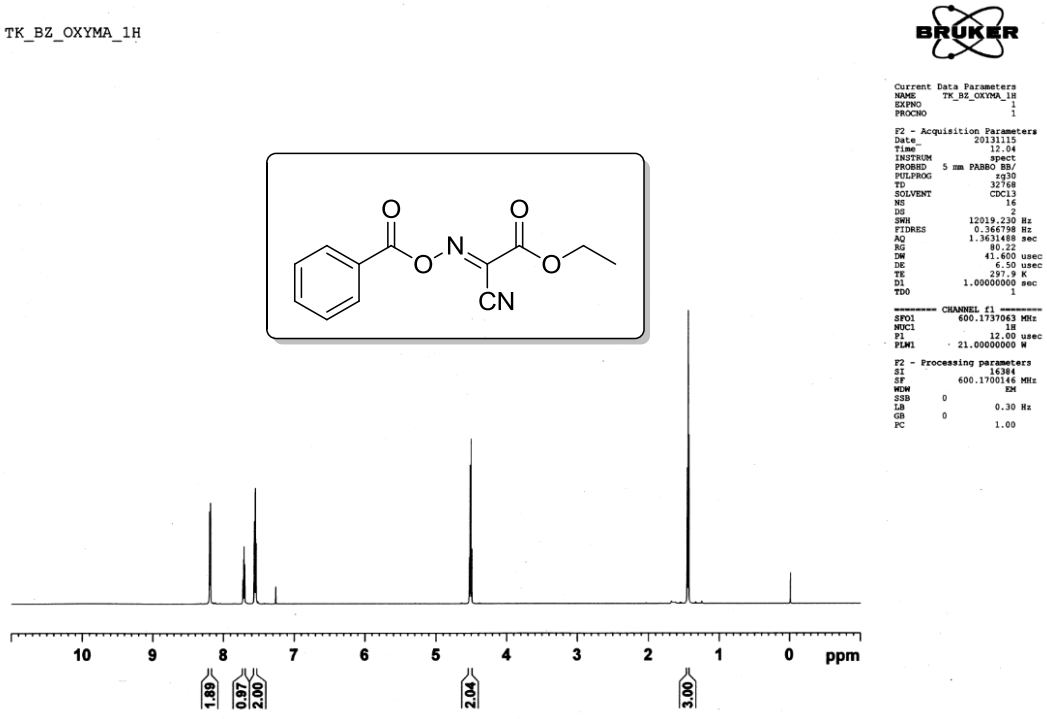


Figure S4. ^{1}H NMR spectra of inetrmediate **[A]**

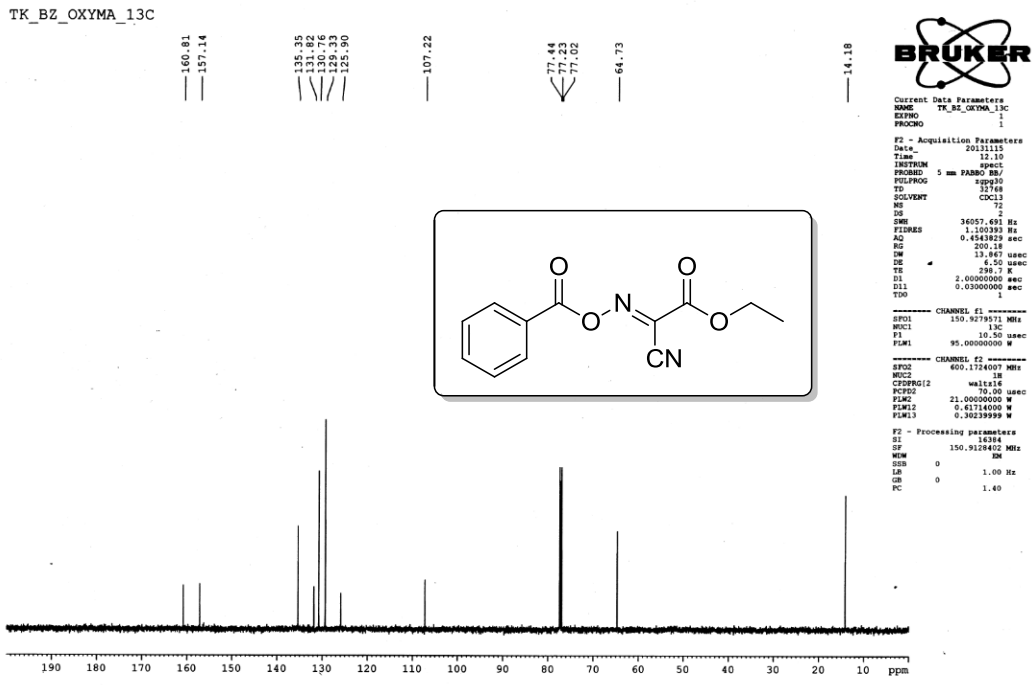


Figure S5. ^{13}C NMR spectra of inetrmediate **[A]**

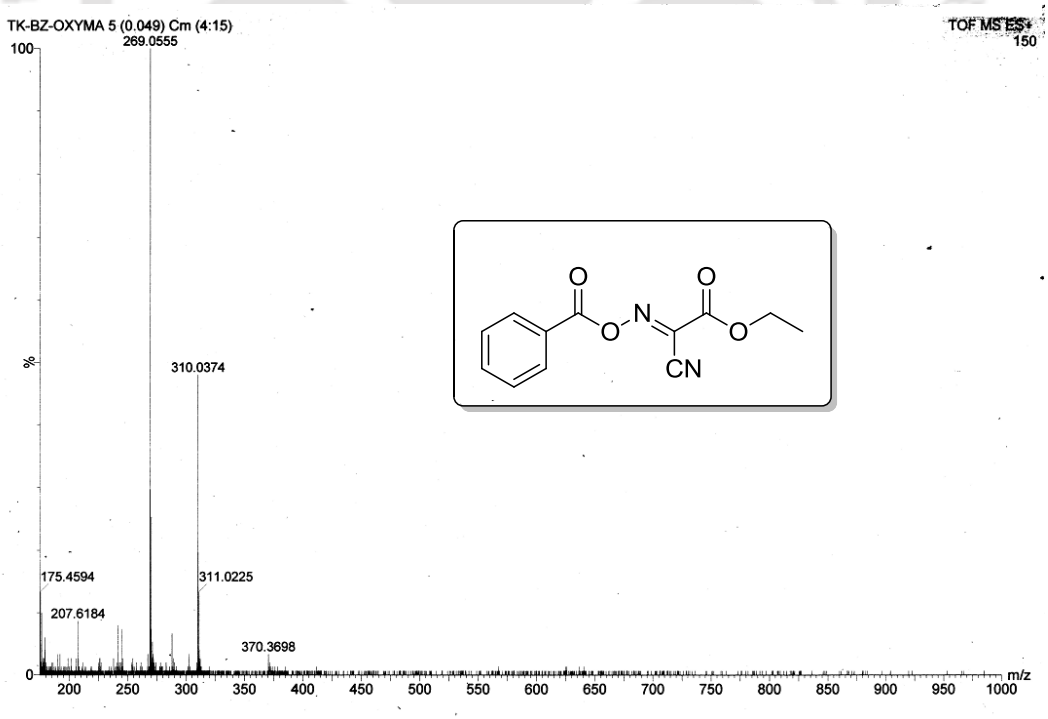


Figure S6. HRMS of inetrmediate **[A]**

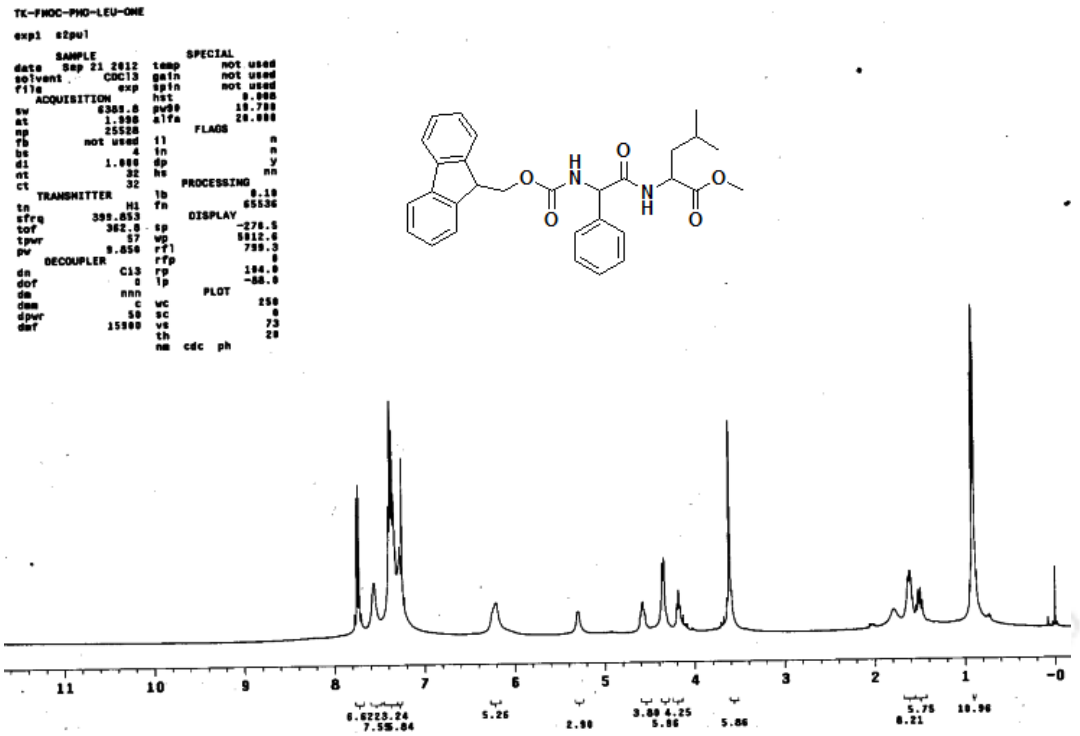


Figure S7. [1]H NMR spectra of compound 22a in table 3.1.1.2

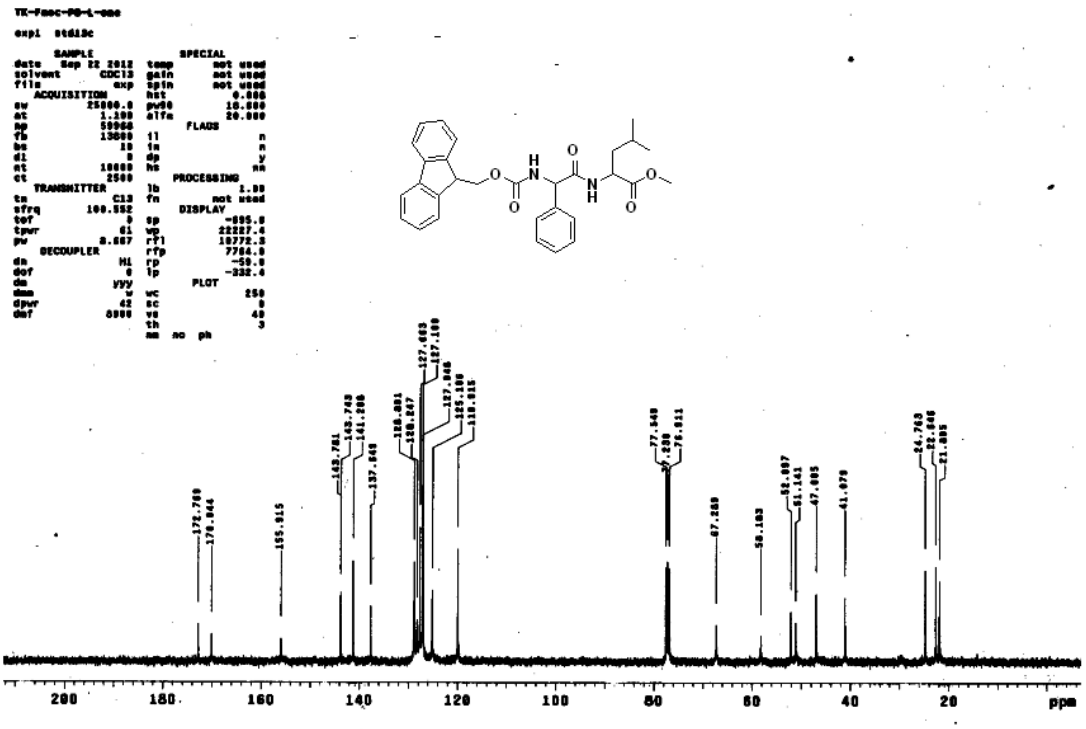


Figure S8. [13]C NMR spectra of compound 22a in table 3.1.1.2

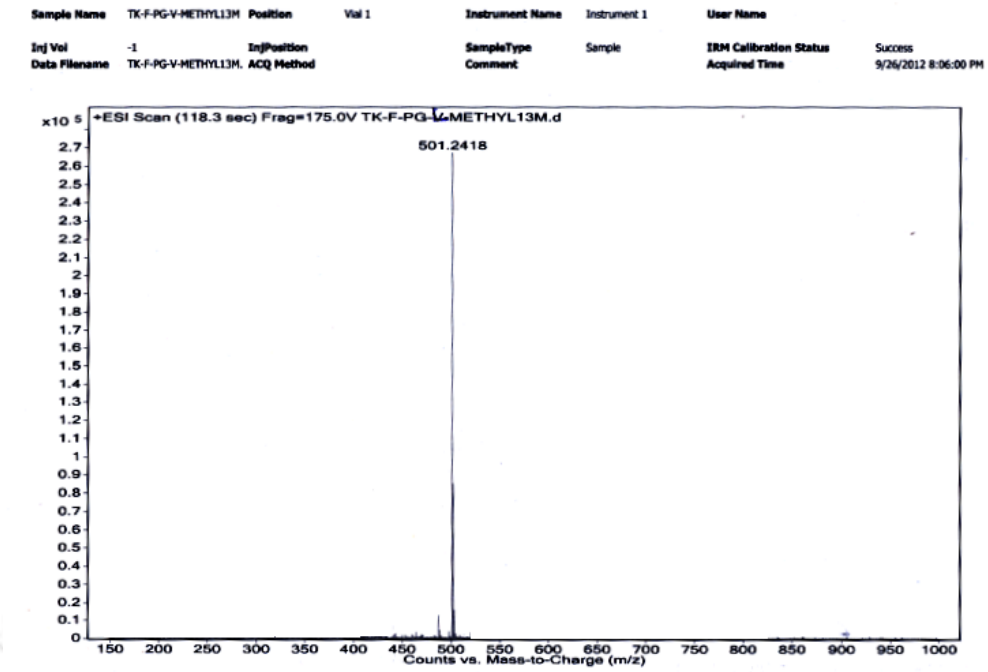


Figure S9. HRMS of compound 22a in table 3.1.1.2

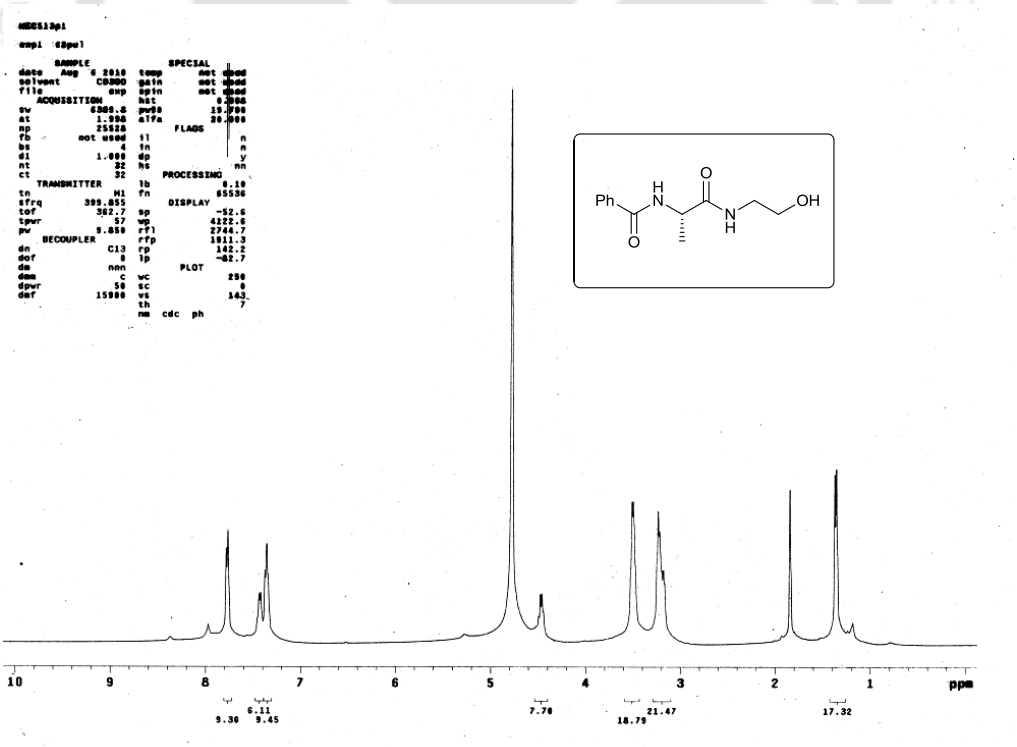


Figure S10. ^{1}H NMR spectra of compound 10a in table 3.1.1.2

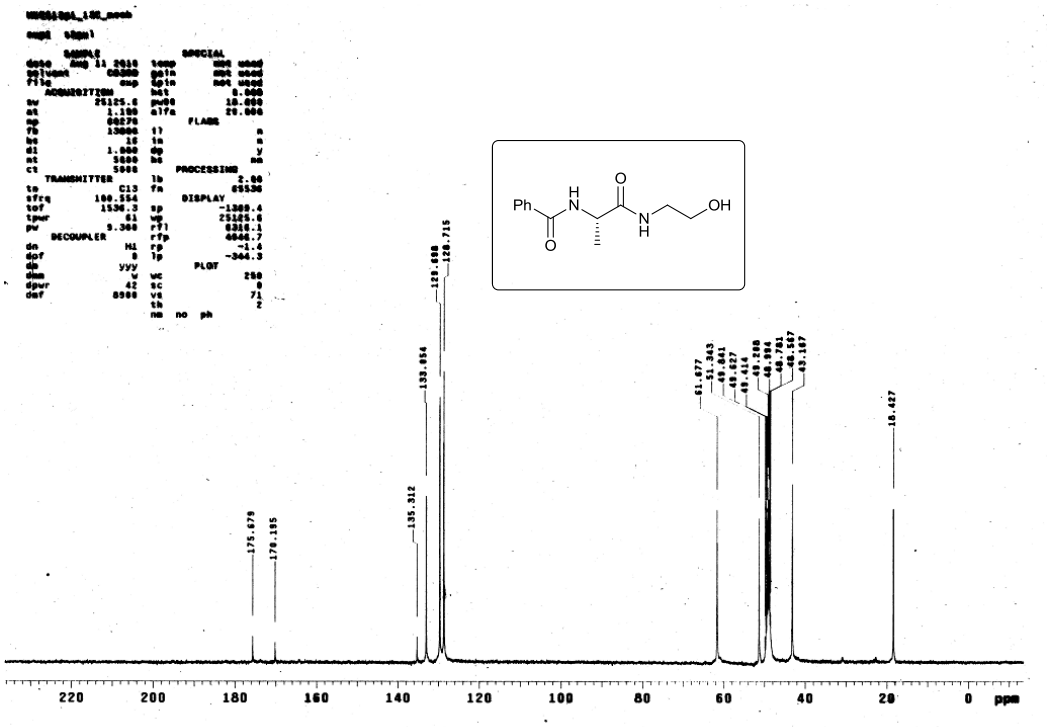


Figure S11. ^{13}C NMR spectra of compound 10a in table 3.1.1.2

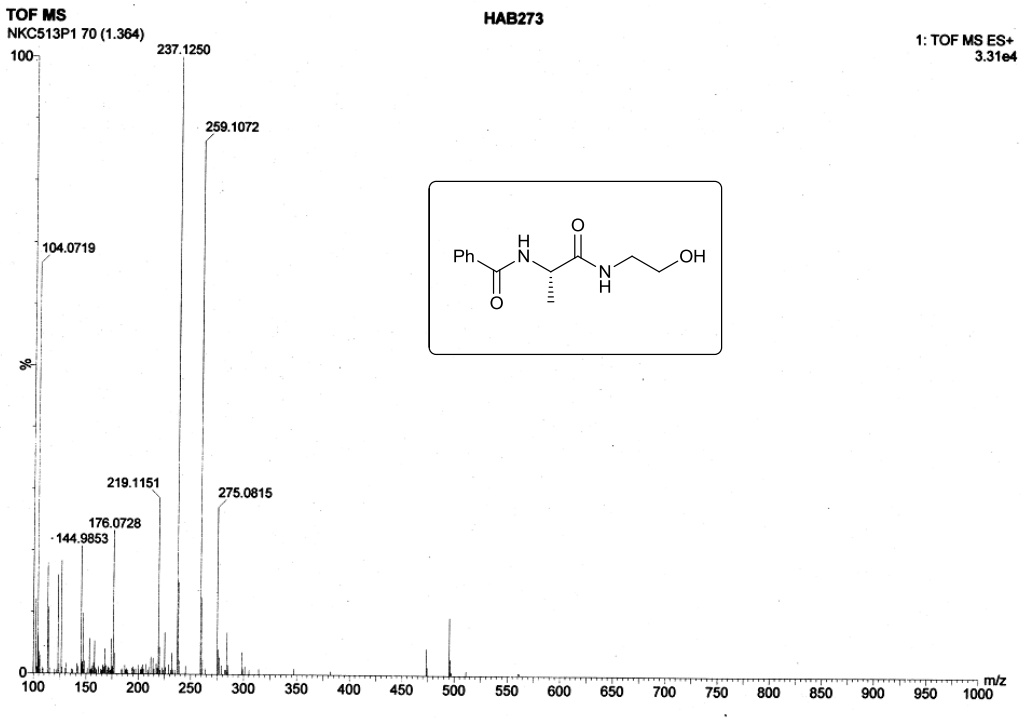


Figure S12. HRMS spectra of compound 10a in table 3.1.1.2

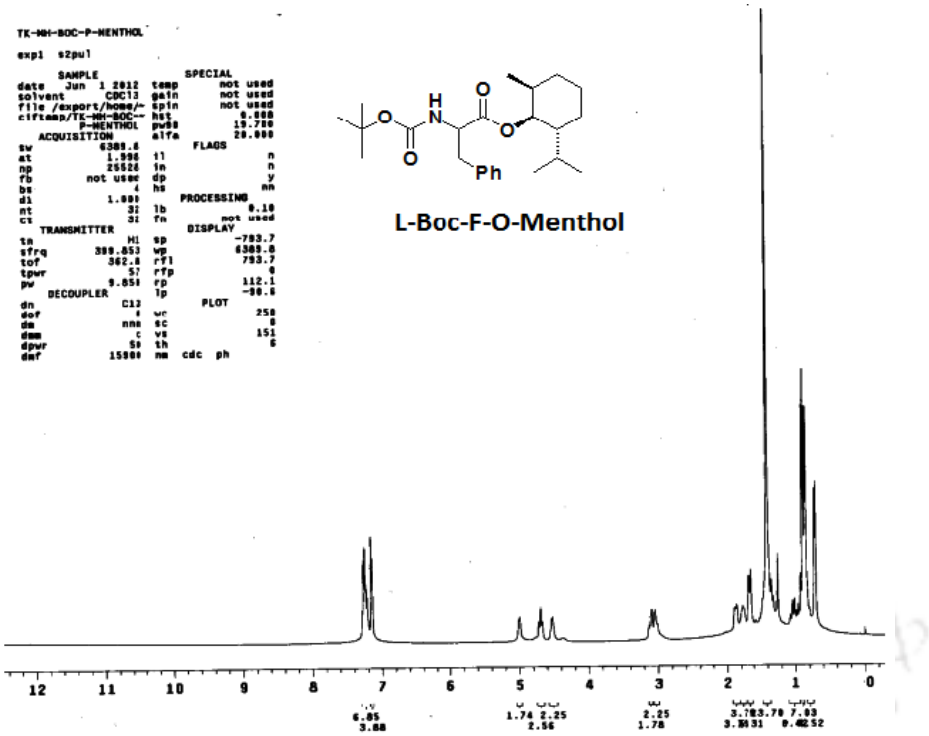


Figure S13. ^{1}H NMR spectra of compound 19b in table 3.2.1

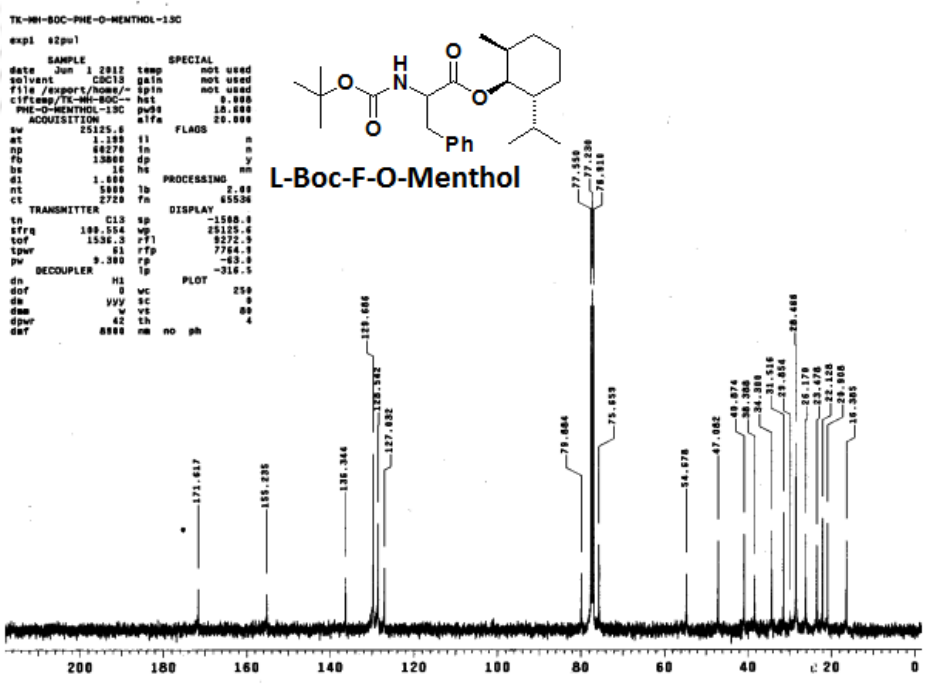


Figure S14. ^{13}C NMR spectra of compound 19b in table 3.2.1

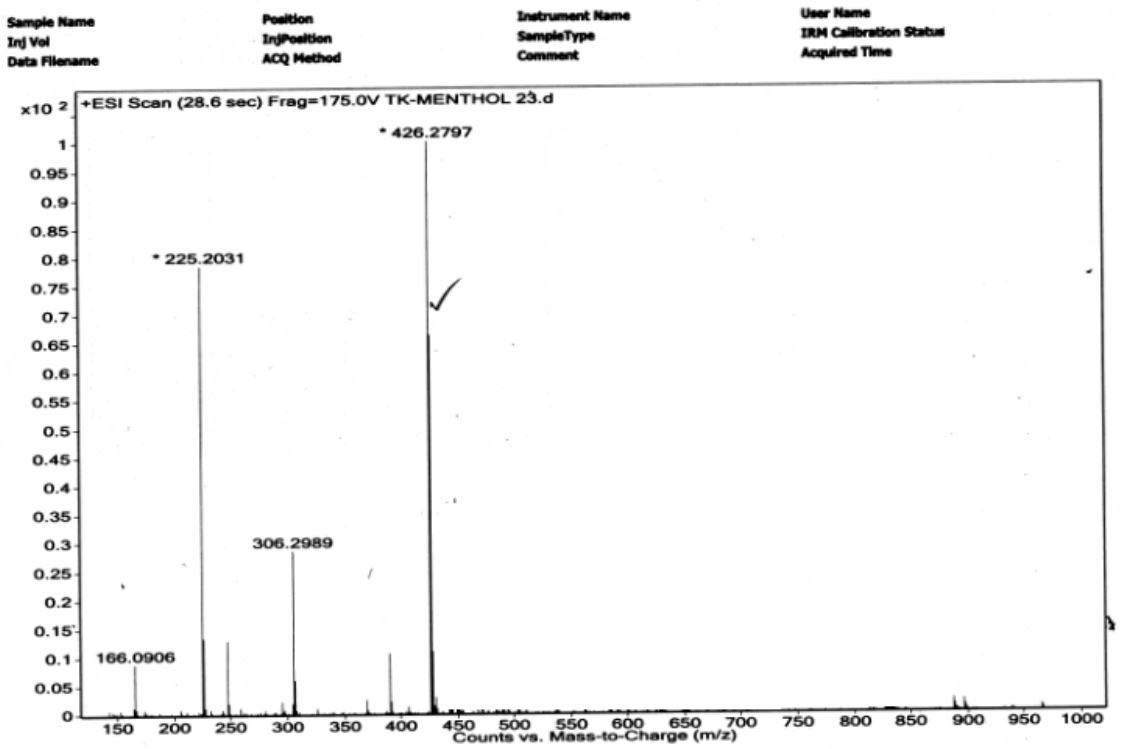


Figure S15. HRMS spectra of compound 19b in table 3.2.1

3.7.2. Data for racemization test:

(A) For amidation

1) Reverse phase HPLC, run time 20 min (linear gradient 0 to 100 % CH_3CN in water)

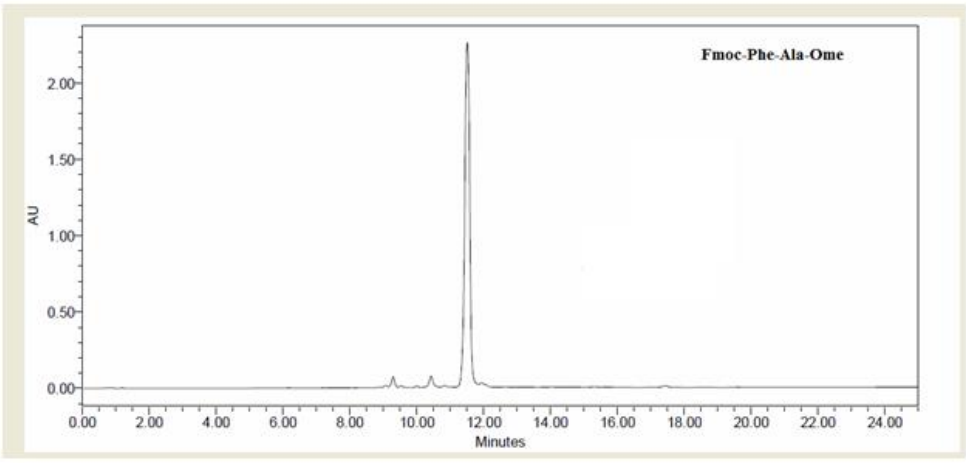


Figure S16. HPLC profile of L-Fmoc-Phe-L-Ala-OMe

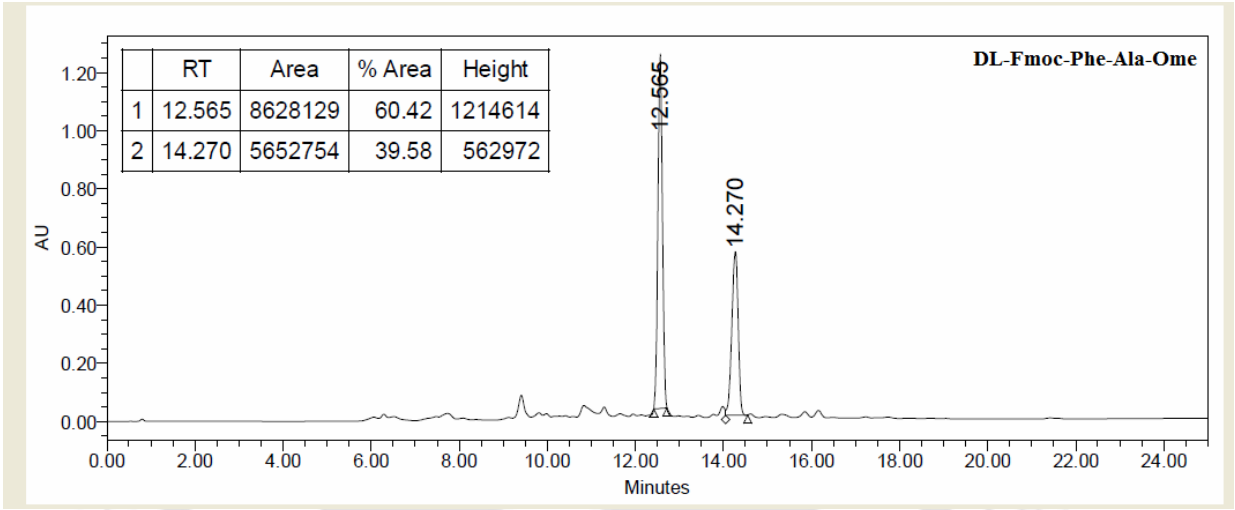


Figure S17. HPLC profile of DL-Fmoc-Phe-L-Ala-OMe

B) For esterification

1) Reverse phase HPLC, isocratic gradient, 20 min (70 % CH_3CN in water)

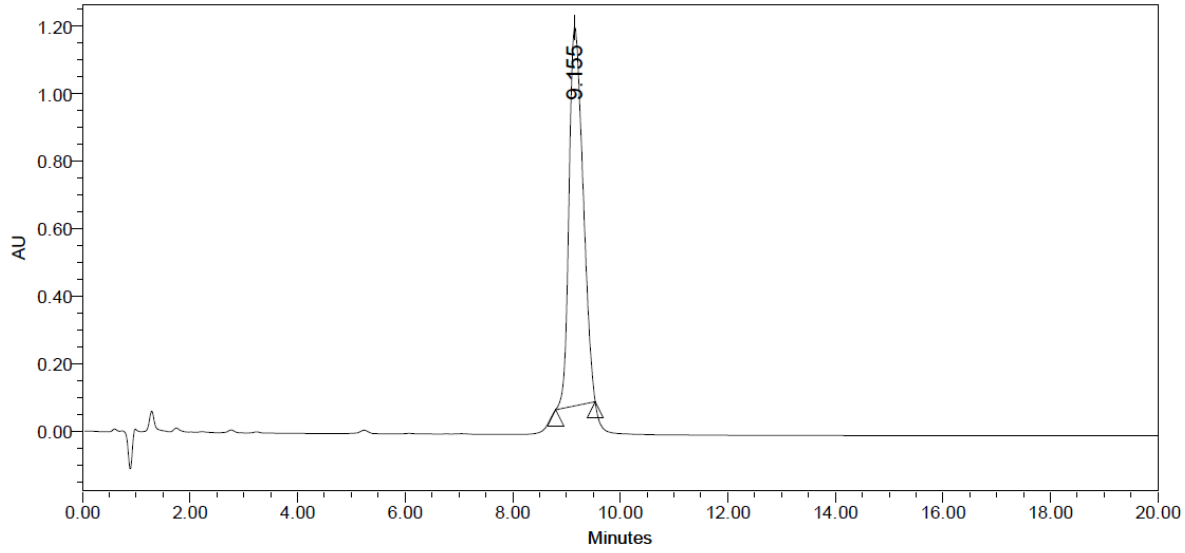
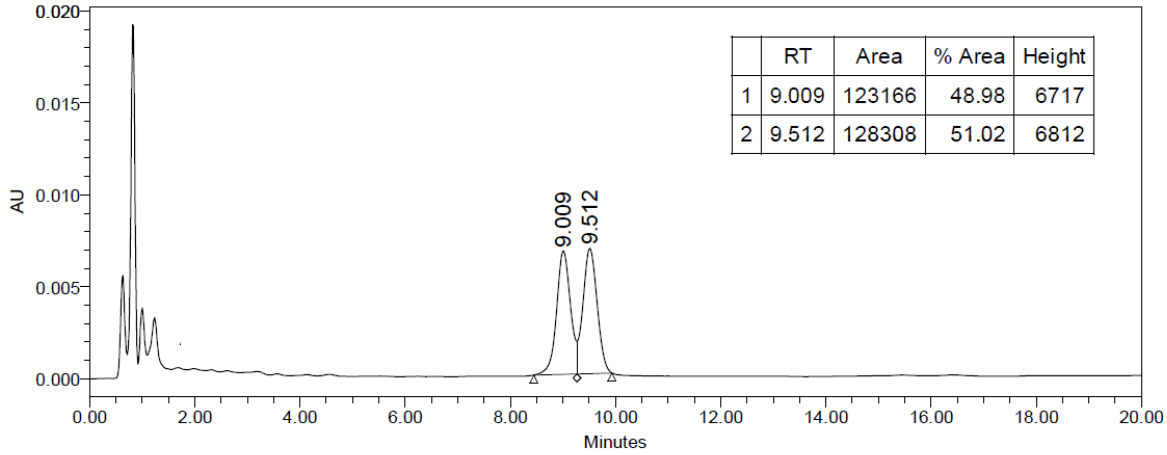


Figure S18. HPLC profile of L-Boc-Phe-(1S, 2S, 5R) menthol



	RT	Area	% Area	Height
1	9.009	123166	48.98	6717
2	9.512	128308	51.02	6812

Figure S19. HPLC profile of DL-Boc-Phe-(1S, 2S, 5R) menthol

3.7.3. HPLC and HRMS of NFGAILG-NH_2

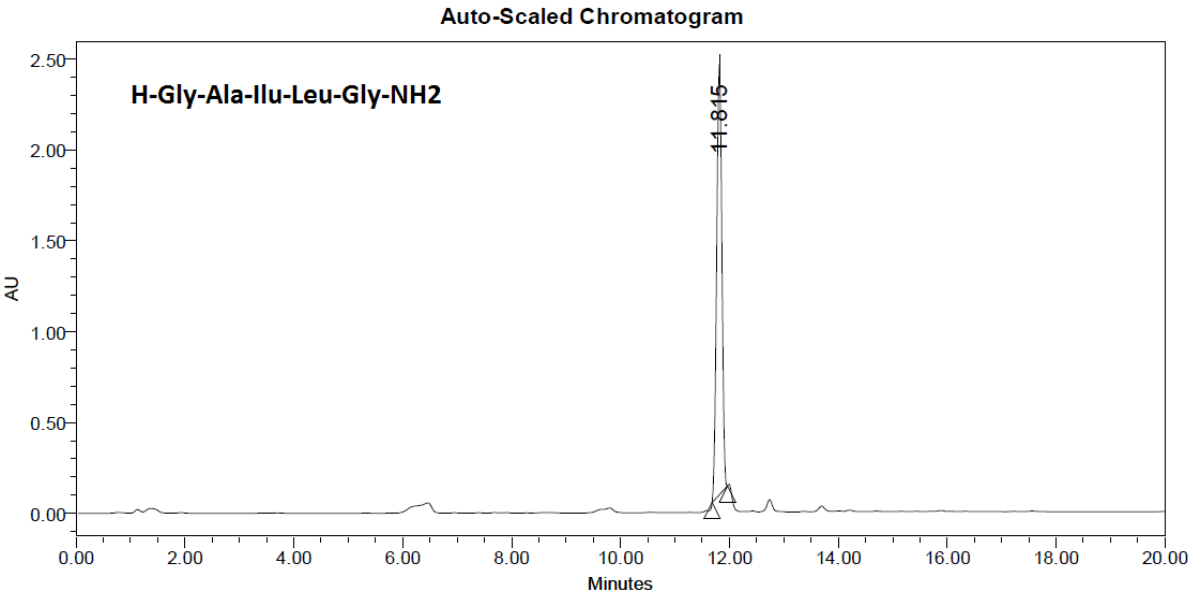


Figure S20. HPLC profile of fragment GAILG-NH_2

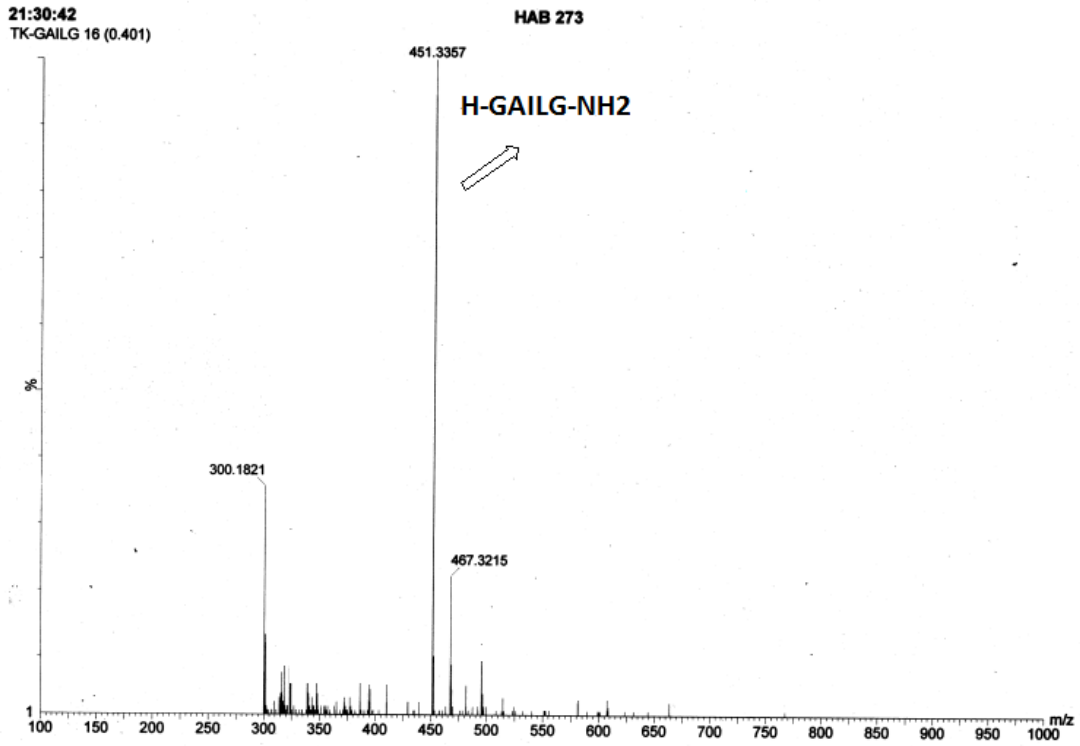


Figure S21. HPLC profile of GAILG-NH_2

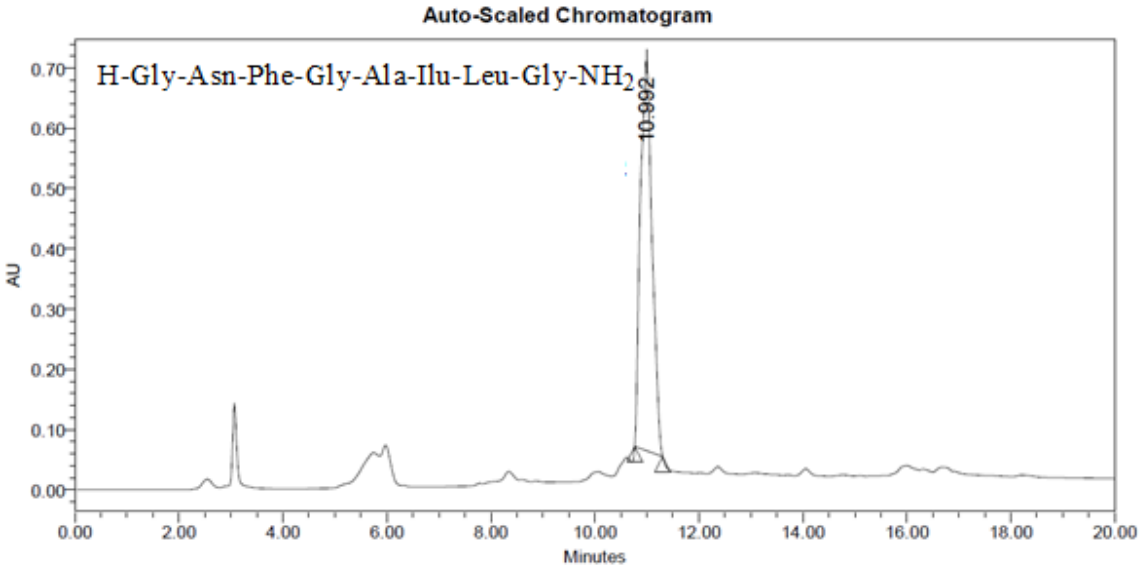


Figure S22. HPLC profile of GNFGAILG-NH_2

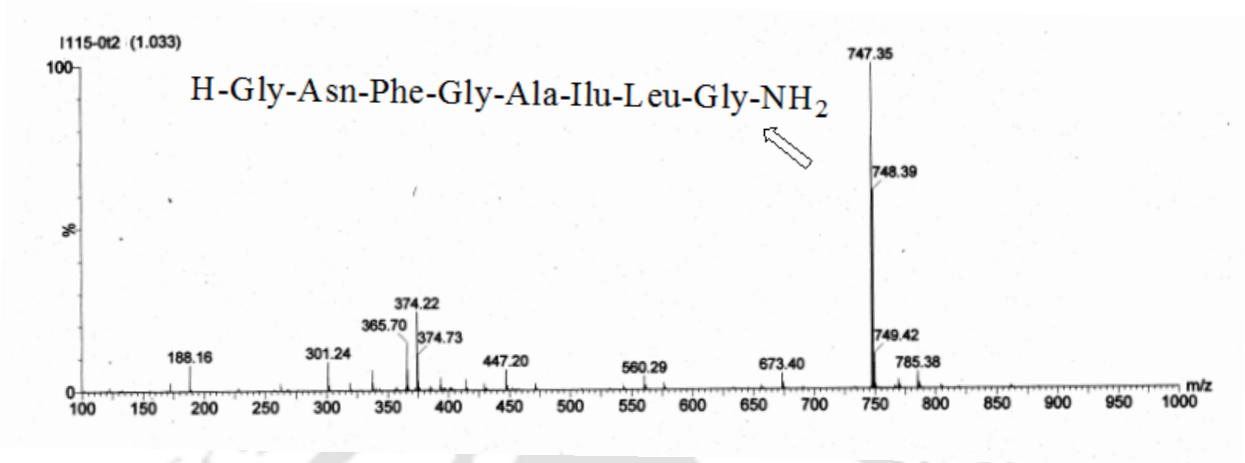
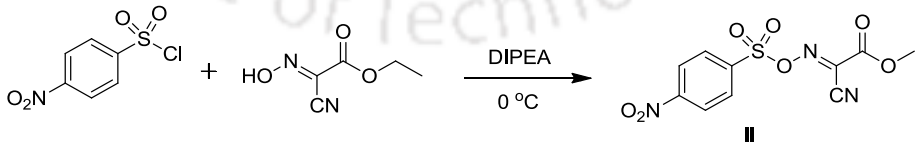


Figure S23. HPLC profile of GNFGAILG-NH_2

Chapter 4: Ethyl 2-cyano-2-(4-nitrophenyl sulfonyloxyimino) acetate (4-NBsOXY) Mediated Racemization free Synthesis of Hydroxamic Acids and Ureas from Carboxylic Acids

In continuation to the development of racemization free amide and peptide synthesis methodologies, we were further interested to develop a method for racemization free synthesis of peptide hydroxamates and ureas using another novel coupling reagent. Peptide hydroxamates and ureas constitute valuable tools in chemical biology as well as interesting leads in medicinal chemistry.[116] In this chapter, we described the development of a new coupling reagent and racemization free synthesis of hydroxamates and ureas using the same coupling reagent. At first we have synthesized the new coupling reagent, Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino) acetate (4-NBsOXY) by the reaction of 4-nitro benzene sulfonyl chloride with Oxyma in presence of DIPEA with 95% yield (*Scheme 4.1*).



Scheme 4.1. *Preparation of 4-NBsOXY, II*

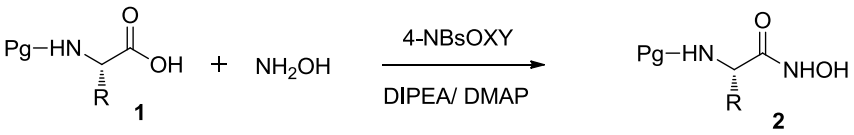
Then, we explored the applicability of this reagent for racemization free synthesis of peptide hydroxamic acids and ureas directly from carboxylic acids/*N*-protected amino acids.

4.1. Racemization free synthesis of hydroxamic acids using 4-NBsOXY as a coupling reagent

4.1.1. Development of a new method for hydroxamic acid synthesis using 4-NBsOXY

The major disadvantages of the existing methods (Chapter 1, section 1.5) were the generation of undesired by-products, usage of toxic reagents, harsh reaction conditions, and chemical waste as well as racemization. To address some of these drawbacks, we wanted to develop a new method for the preparation of hydroxamic acids using the new coupling reagent, Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino) acetate (4-NBsOXY, II, *Scheme 4.1*).

Initially, we performed a reaction, in which the new coupling reagent 4-NBsOXY (which was prepared as shown in *Scheme 4.1*) was added to a solution of benzoic acid and DIPEA in THF and the reaction mixture was stirred for 30 min at room temperature for pre-activation followed by the addition of hydroxylamine hydrochloride in DMF with DIPEA. The product, benzhydroxamic acid was obtained in 61% yield within 90 min. To increase the yield of product, we optimized the reaction condition with different bases such as DIPEA, NMM, Et_3N, and DABCO (1,4-Diazabicyclo[2.2.2]octane) along with catalytic amount of DMAP (0.1 mol%) using the same reaction conditions (*Table 4.1.1.1*). We got 82% of the product with DIPEA/DMAP. It required 2 h for complete conversion at room temperature as monitored by TLC.



Scheme 4.1.1.1. *Synthesis of hydroxamic acids using 4-NBsOXY*

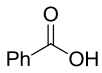
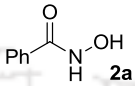
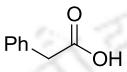
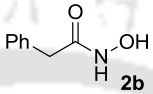
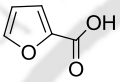
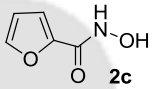
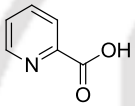
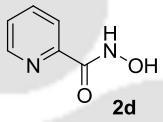
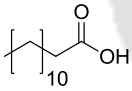
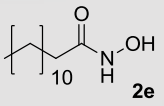
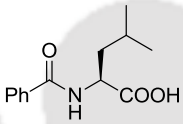
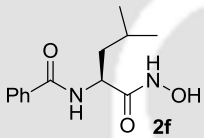
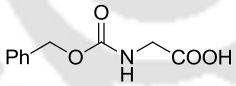
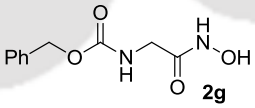
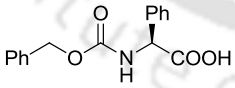
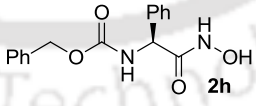
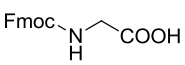
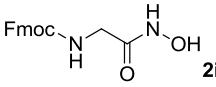
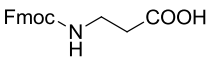
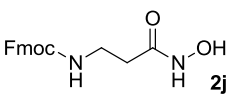
Table 4.1.1.1. *Optimization of the reaction conditions*[a]

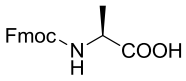
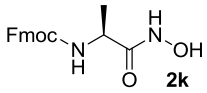
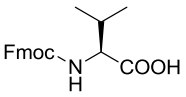
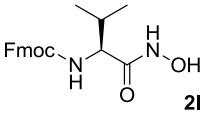
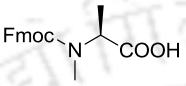
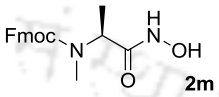
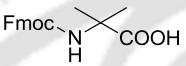
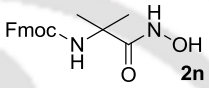
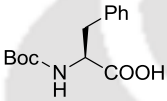
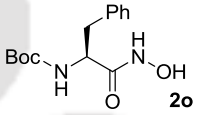
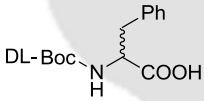
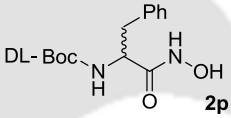
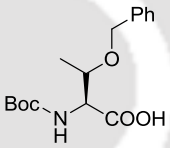
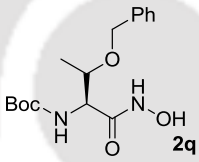
Entry	Base (1 mmol)	DMAP	Yield[b]
1	DIPEA	0.1 mmol %	82
2	DIPEA	-	61
3	NMM	0.1 mmol %	73
4	NMM	-	52
5	Et_3N	0.1 mmol %	50
6	DABCO	0.1 mmol %	30

[a]*Reaction conditions: substrate 1a (122 mg, 1 mmol), 4-NBsOXY (327 mg, 1 mmol), DMAP (12.2 mg, 0.1mmol), DIPEA (322.5 mg, 2.5 mmol), Hydroxylamine hydrochloride (103.5 mg, 1.5 mmol) and THF (2 ml), at room temperature, time of the reaction was fixed to 2.5 h.* [b]*Isolated yield.*

This methodology was applied to a wide variety of carboxylic acids that included aromatic, aliphatic, long chain carboxylic acids, and *N*-protected amino acids (*Table 4.1.1.2.*) using the optimized condition. Good yields were obtained in all the cases. The reactions were compatible with all common *N*-protecting groups, e.g. Bn (**2f**), Cbz (**2g** and **2h**), Fmoc (**2i**, **2j**, **2k**, **2l**, **2m,** and **2n**) and Boc (**2o**, **2p,** and **2q**).

Table 4.1.1.2. *Wide scope of the synthesis of hydroxamic acid using II*

Entry	Acid	Structure	Yield[a]
a		 **2a**	82
b		 **2b**	79
c		 **2c**	76
d		 **2d**	74
e		 **2e**	81
f		 **2f**	81
g		 **2g**	78
h		 **2h**	78
i		 **2i**	80
j		 **2j**	81

k		 **2k**	80
l		 **2l**	75
m		 **2m**	77
n		 **2n**	75
o		 **2o**	80
p		 **2p**	81
q		 **2q**	80

[a]*Isolated yield. Reaction condition was similar to that mentioned in Table 1.*

4.1.2. Investigation on racemization

We investigated the racemization probability of the this method by comparison of the HPLC profiles of L-Boc-phenylalanine hydroxamic acid (**2o**) and DL-Boc-phenylalanine hydroxamic acid (**2p**) (chiral column, 5 µm, 2.1× 150 mm, isocratic gradient of 10 % isopropanol in hexane, 20 min). We observed single peak at the retention time 3.24 min in the HPLC for L-Boc-phenylalanine hydroxamic acid (**2o**), which corresponds to a single

enantiomeric product. On the other hand, two peaks with retention times of 3.06 and 3.81 min appeared in the HPLC profile corresponding to the two enantiomers of DL-Boc-phenylalanine hydroxamic acid (**2p**) as shown in Figure 4.1.2.1. The mass of the peaks in the HPLC profiles was verified using ESI-MS. Thus, it was concluded that there was no detectable racemization for the preparation of hydroxamic acids using reagent **II**.

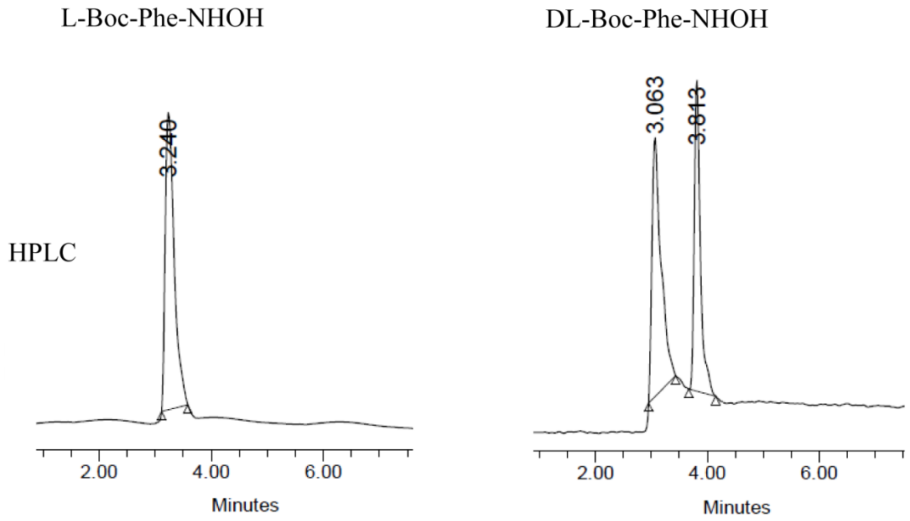


Figure 4.1.2.1. *HPLC profile diagrams of products* ***2o*** *&* ***2p***

4.1.3. Probable mechanism of the reaction

A possible reaction mechanism is shown in Figure 4.1.3.1 and Scheme 4.1.3.1. In the first step, the intermediate **IV** (could be isolated and characterized by ^{1}H, ^{13}C NMR, and HRMS) was formed *via* transient intermediate **III**. In the second step, hydroxylamine hydrochloride reacts with **IV** to produce the desired hydroxamic acid. DMAP may act as an acyl transfer catalyst as known for Steglich esterification.[117]

To confirm the proposed mechanism, we performed the reaction in NMR tube using benzoic acid, hydroxylamine hydrochloride, and 4-NBsOXY in presence of DIPEA/DMAP. At first we have taken the benzoic acid 4-NBsOXY and DIPEA/DMAP in an NMR tube and recorded ^{13}C NMR in $CDCl_3$. At the initial stage, the peak for the carbonyl carbon (**a**) of benzoic acid was noted at δ 172.8 ppm as well as the carbonyl carbon (**b)** and the aromatic carbon at para position (**c)** of 4-NBsOXY was noted at 155.5 ppm and 151.9 ppm, respectively. At 30 min, these peaks were replaced by four new peaks, two peaks at 160.4 and 156.7 ppm (**d** and **e**) correspond to the intermediate **IV** and the other two peaks at 151.7 and 148.0 ppm (**f** and **g**) correspond to the by-product 4-nitrobenzene sulfonic acid. After 30 min we have added hydroxylamine hydrochloride in DIPEA/DMAP to the above reaction mixture in the same NMR tube. ^{13}CNMR was recorded after 90 min of the addition of hydroxylamine hydrochloride. The peaks **d** and **e** were replaced by the peaks at 164.7 ppm (**h**) and 158.7 ppm **(i)**, which correspond to the hydroxamic acid product and the by-product, Oxyma, respectively. These observations clearly support the proposed mechanism as depicted in Figure 4.2.3.1. A plausible pathway explaining the involvement of DMAP is also proposed based on the existing literature (*Scheme 4.1.3.1*).

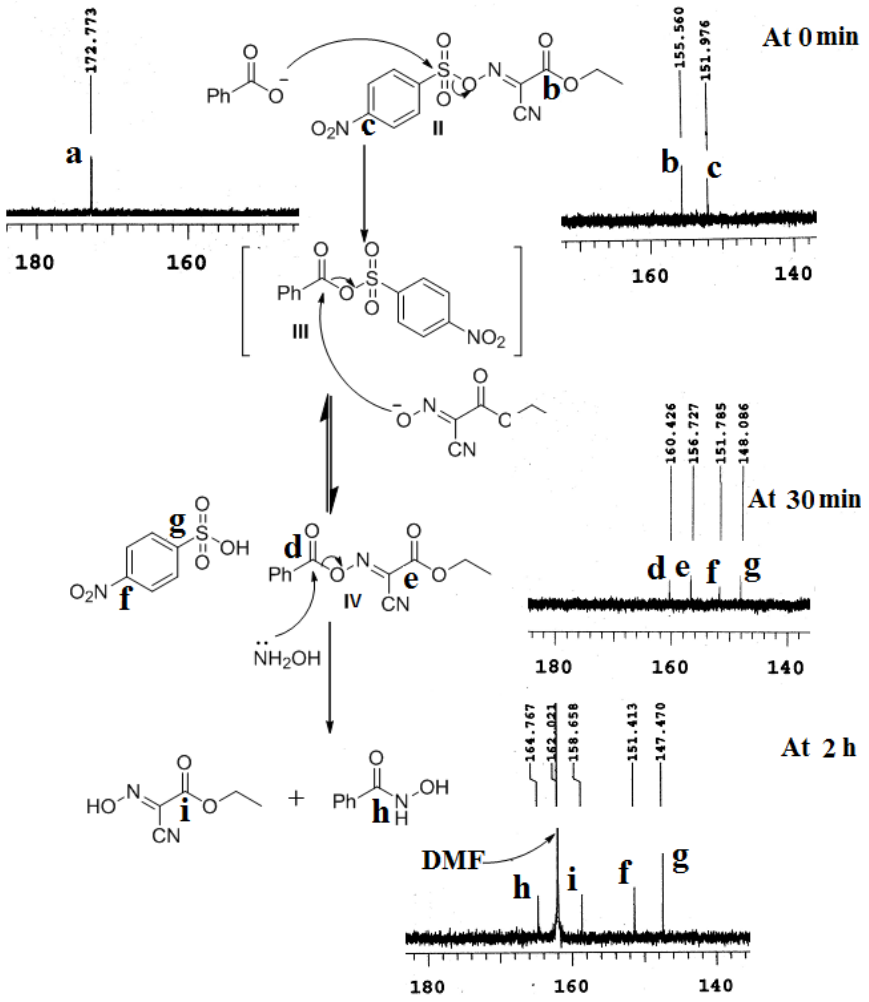
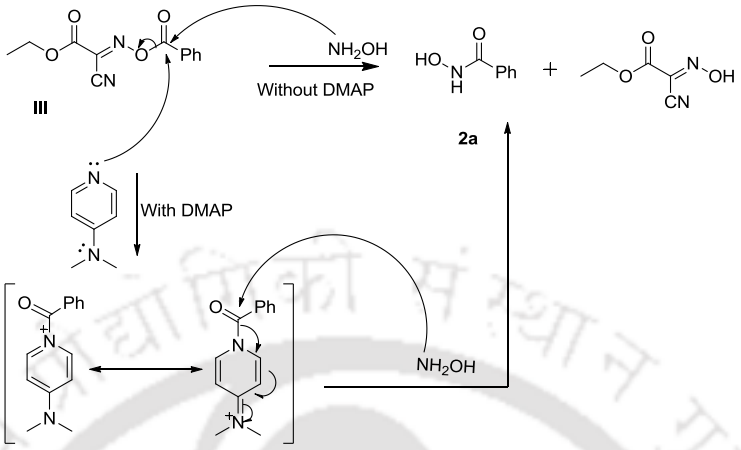


Figure 4.1.3.1. *Plausible reaction mechanism for the synthesis of hydroxamic acids by* ***II*** *as noticed using* C^{13} *NMR experiments*



Scheme 4.1.3.1. *Plausible mechanism for the involvement of DMAP*

4.2. 4-NBsOXY mediated racemization free synthesis of ureas via Lossen Rearragement

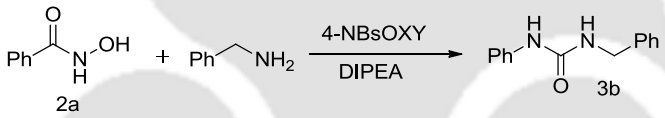
4.2.1. Development of a new method for urea synthesis using 4-NBsOXY

The major disadvantage of most of the coupling reagents (Chapter 1, section 1.6) invented to date is the generation of undesired byproducts and chemical waste as well as racemization. We have developed a new method for the preparation of ureas *via* Lossen rearrangement of hydroxamic acids (which was prepared by 4-NBsOXY in section 4.1) into corresponding isocyanate by the reaction of 4-NBsOXY.

At first, we prepared the 1,3-Diphenylurea by the reaction of benzhydroxamic acid (1 mmol) with coupling reagent 4-NBsOXY (1 mmol) with DIPEA in DMF at room temperature for

90 min followed by the addition of benzylamine (1 mmol) and the reaction was continued for 4 h. The yield of the product was 80%. To improve the yield, we optimized the reaction conditions. We performed the reaction in various solvents such as $CHCl_3$, CH_3CN, ethyl acetate, acetone, THF, and DMF (*Table 4.2.1.1*). In case of THF the yield of the product went up to 92%, hence THF was accepted as the best solvent among all the solvents tested. Use of the other solvents, $CHCl_3$, CH_3CN, ethyl acetate, acetone, and DMF also gave good results. In case of water, yield of the product was very less and 74% (82 mg) of the starting material was recovered back.

Table 4.2.1.1. *Optimisation of the solvents*[a]

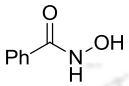
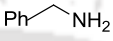
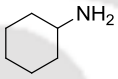
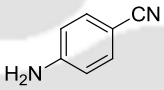
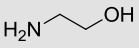
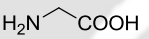
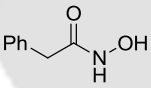
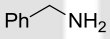
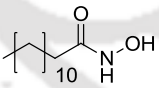
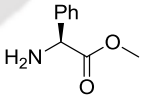
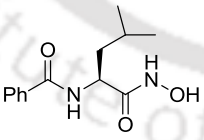
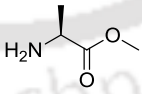
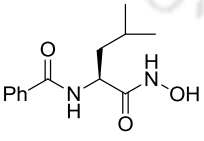
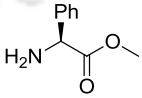
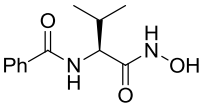
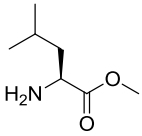


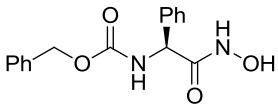
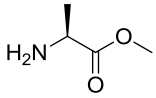
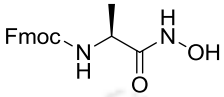
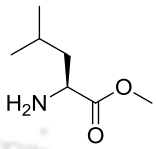
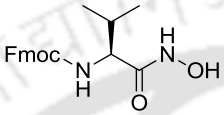
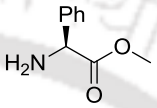
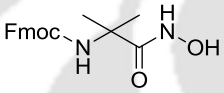
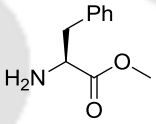
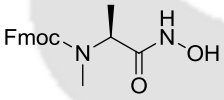
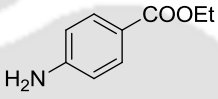
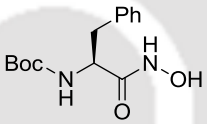
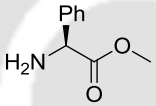
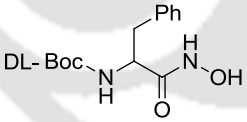
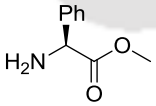
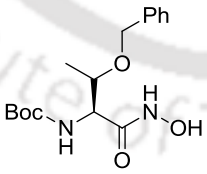
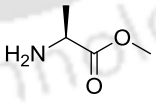
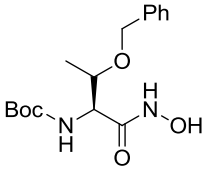
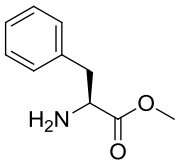
Entry	Solvent	Isolated yield %
1	$CHCl_3$	87
2	CH_3CN	85
3	THF	91
4	EtOAc	87
5	Acetone	82
6	DMF	80
7	H_2O	20

[a]*Reaction conditions: substrate 2a (127 mg, 1 mmol), 4-NBsOXY (327 mg, 1 mmol.), DIPEA (193.5 mg, 1.5 mmol), amine (107 mg, 1 mmol) and solvent (2 mL), temperature (first 90 min at 0 ºC and then 4 h at room temperature) total reaction time 5.5 h.*

Under the above optimized conditions, we examined various aliphatic, aromatic, long chain carboxylic acid and *N*-protected amino acids derived hydroxamic acids with various amines, in all the cases we got very good yield (up to 92%, *Table 4.2.1.2*). This method worked well with all kinds of amines, such as aniline, secondary amine, ethanolamine, and free glycine. In case of ethanolamine amino group was attached selectively. The yields were found to be good to excellent in all the cases including sterically hindered amino acids, e.g., leucine, valine, phenylalanine, and phenylglycine. This method was also compatible with all common amino protecting groups, such as Boc, Fmoc, Cbz, and benzyl, as well as various side chain alcohol protecting groups of amino acids, such as, ^{t}Bu and Bzl. All the products were characterized using ^{1}H NMR, ^{13}C NMR, HRMS and IR spectroscopy. The product **3g** was characterized using XRD data also (*Figure 4.2.1.1*). Most importantly, by-products generated in our method, Oxyma and 4-nitrobenzenesulfonic acids, were easily recovered by simple acid-base work-up or a column chromatographic separation after acid wash of the reaction mixture. Chlorination of the recovered 4-nitrobenzenesulfonic acid with sulfonyl chloride at 60 °C in toluene gave 4-nitrobenzenesulfonyl chloride, which was treated with the recovered Oxyma in presence of DIPEA to regenerate 4-NBsOXY. NMR spectra of the recovered byproducts were as good as the commercial sample.

Table 4.2.1.2. *Wide scope of the synthesis of ureas using* ***II***

Entry	Acid		Amine	Product Id	Product Yield[a]
1		A		**3a**	83
		B		**3b**	91
		C		**3c**	90
		D		**3d**	82
		E		**3e**	74
		F		**3f**	91
		G		**3g**	81
2				**3h**	88
3				**3i**	92
4				**3j**	87
5				**3k**	90
6				**3l**	89

7			**3m**	82
8			**3n**	80
9			**3o**	79
10			**3p**	81
11			**3q**	70
12			**3r**	89
13			**3s**	88
14			**3t**	85
15			**3u**	82

[a]*Isolated yield, the compounds were characterized by NMR, ESI-MS and IR; all amino acids were L-amino acids except 13, which was with DL configuration*

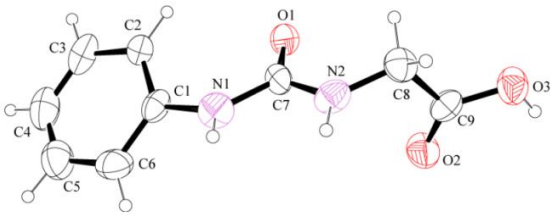


Figure 4.2.1.1. *ORTEP diagram of 2-(3-phenylureido)acetic acid **3g** with 50% ellipsoid. CCDC NO 971230*

4.2.2. A probable mechanism

A possible reaction mechanism is shown in Figure 4.2.2.1. At first, the carboxylic acid/*N*-protected amino acid reacts with **II** to form unstable O-sulfonylated intermediate (**V)** at 0 °C. In second step, **V** undergoes Lossen rearrangement to form stable intermediate isocynate **VI**, which was characterized by ^{1}H, ^{13}C NMR, and HRMS. Finally, the nucleophile reacts with isocynate **VI** to produce urea **3a**. To confirm the above possible reaction mechanism, we performed a reaction between benzhydroxamic acid and 4-NBsOXY in presence of DIPEA in an NMR tube using $CDCl_3$ as a solvent and ^{13}C NMR was recorded at specified time interval. Initially, at 0 min three peaks were present; one at 166.8 ppm (**1**) corresponding to the carbonyl carbon of the hydroxamic acid (**2a**) and the other two peaks at 155.5 and 151.9 ppm (**2** and **3**) corresponding to the carbonyl carbons of 4-NBsOXY. At 90 min, the peaks **1**, **2** and **3** were replaced by **4**, **5** and **6**, respectively. One peak at 162.2 ppm (**4**) corresponds to the carbonyl carbon of Oxyma and the other two peaks at 151.4 and 148.1 ppm (**5** and **6**) correspond to 4-nitrobenzenesulfonic acid. Finally, one new peak appeared at 155.7 ppm (**7**) after the addition of the cyclohexyl amine, which corresponds to the carbonyl

carbon of final product urea. These observations support the proposed mechanism depicted in Figure 4.2.2.1.

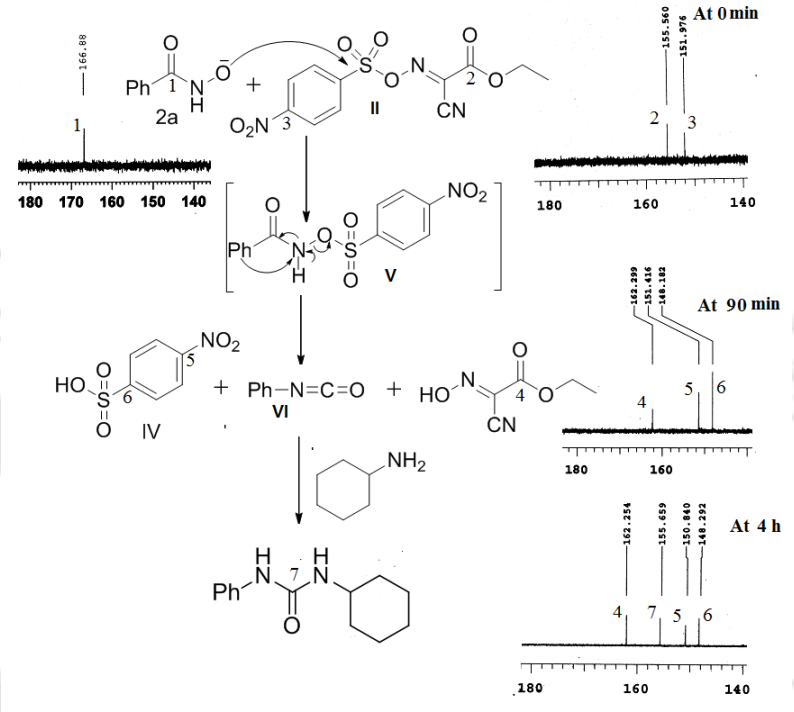
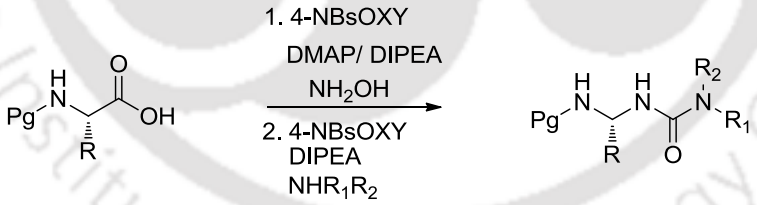


Figure 4.2.2.1. *Plausible reaction mechanism for synthesis of urea with supporting evidences from ^{13}C NMR data*

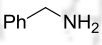
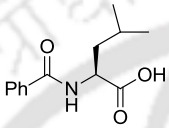
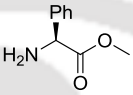
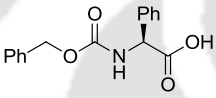
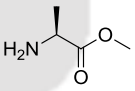
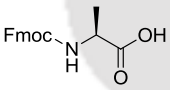
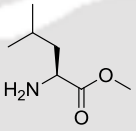
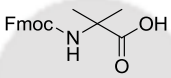
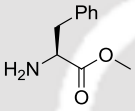
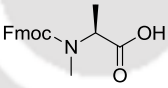
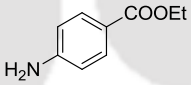
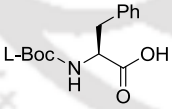
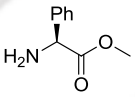
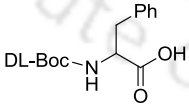
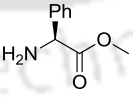
4.3. 4-NBsOXY mediated single pot synthesis of ureas from carboxylic acids

Finally, we have designed a one-pot protocol for urea synthesis direct from carboxylic acids/*N*-protected amino acids using 4-NBsOXY using the reactions described above. The one-pot protocol requires 2 equiv of 4-NBsOXY; one equiv for activation of the carboxylic acid into Oxyma ester (IV) which converts into hydroxamic acid followed by the addition of hydroxylamine hydrochloride, further one equiv of 4-NBsOXY is necessary for conversion of hydroxamic acid into urea via isocynate (Lossen rearrangement). The scope of the one pot protocol was investigated with a variety of *N*-protected amino acids. All of the amino acids, even with bulky side chains, produced good yield (*Table 4.3.1*).



Scheme 4.3.1. *Synthesis of ureas direct from carboxylic acids*

Table 4.3.1*. One pot synthesis of ureas from carboxylic acids*

Entry	Acid	Amine	Time	Product Id	Product yield[a]
1			8 h	**3b**	68
2			8 h	**3k**	69
3			8 h	**3m**	62
4			8 h	**3n**	61
5			8 h	**3p**	63
6			8 h	**3q**	45
7			8 h	**3r**	71
8			8 h	**3s**	69

[a]*Reaction condition, acid (1 mmol), hydroxamic acid (1.5 mmol), 4-NBsOXY (2 mmol), Amine (1 mmol), DMAP (0.1 mmol) and DIPEA (3 mmol), temperature (at first 2.5 h at room temperature, 90 min at 0 °C and room temp for 4 h).*

4.3.1. Investigation on racemization involved in the reaction

We have studied the racemization probability of our method of urea synthesis by comparing the HPLC profiles (Symmetry C8, 5 µm 3.0× 150 mm analytical column, CH_3CN in H_2O with 0.1% formic acid, linear gradient from 0 to 100% CH_3CN in H_2O upto 10 min, then 10 to 20 min 100% CH_3CN) of product of DL-Boc-phenyl alanine (**3s**) and L-Boc-phenyl alanine (**3r**) with methyl ester of phenylglycine. We observed two peaks with retention times of 10.73 min and 11.17 min in HPLC for product of DL-Boc-phenyl alanine, these two peaks corresponds to the two diastereomers, i.e two diastereomers were present in DL-Boc-phenylalanine. On the other hand, a single peak at 10.89 min was observed in the HPLC profile of L-Boc-phenylalanine **3r**, which corresponds to the single stereoisomeric product. This implies that no signal of racemization could be detected. The singlet at δ 3.71 ppm for methoxy proton of **3r** and two singlets at δ 3.70 ppm and δ 3.68 ppm for the same of **3s** in ^{1}H NMR confirm that (*Figure 4.3.1.1*). From the above data, we can conclude that this method does not cause any detectable racemization (*Figure 4.3.1.1*).

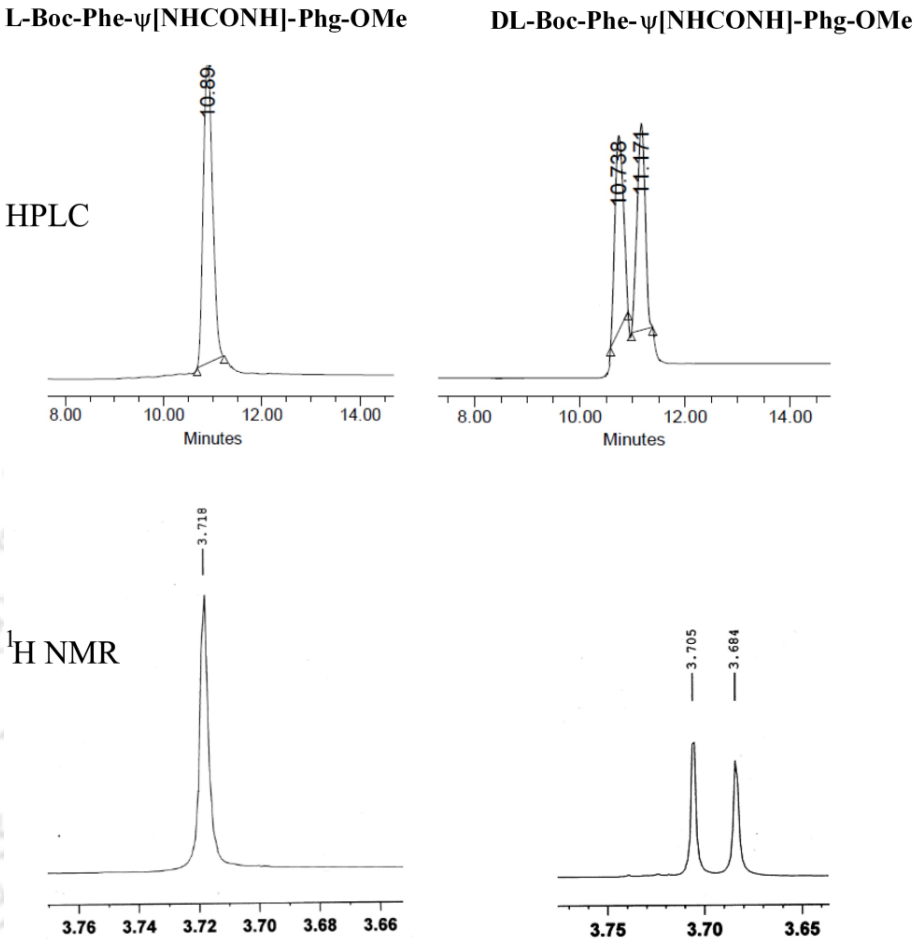


Figure 4.3.1. *HPLC profile diagrams of products* ***3s*** *&* ***3t****and* ^{1}*H NMR of products* ***3r*** *&* ***3s****, (Phe = Phenylalanine, Phg = Phenylglycine)*

4.4. Conclusion

In summary, we have developed an efficient method for the racemization free synthesis of ureas and hydroxamic acids under milder reaction conditions utilizing Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY) as a recyclable reagent. The reaction is compatible with common *N*-protecting groups, such as Boc, Fmoc, Cbz, benzyl, and various side chain alcohol protecting groups of amino acids, such as, ^{t}Bu and OBzl.

4.5. Experimental Section

4.5.1. Materials and methods

As described in chapter 2 section 2.4.1

4.5.2. General procedure for the synthesis of Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate(4-NBsOXY, I):

4-Nitro benzenesulfonyl chloride (1 mmol) was added to a stirred solution of Oxyma (1 mmol), DIPEA (1 mmol) in DCM (2 mL) under nitrogen at 0 °C. Then the reaction mixture was stirred at room temperature for 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the resulting solution was extracted with DCM (2 x 10 mL) and 5% citric acid (2 x 5 mL) washed with brine (2 x 5 mL), dried over anhydrous $CaCl_2$. Then it was dried in *vacuum* and purified by recrystallization using hexane. Characterization data including XRD structure is reported.

4.5.3. General procedure for the synthesis of hydroxamic acids 2a-m:

4-NBsOXY (**II,** 1 mmol) was added to a stirred solution of carboxylic acid (1 mmol), DIPEA (1 mmol) and DMAP (0.1 mmol) in THF (2 mL) at room temperature. The reaction mixture was stirred for 30 min followed by the addition of hydroxylamine hydrochloride in DMF (0.5 mL), DIPEA (1.5 mmol). The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary evaporator and then diluted with 15 mL of ethyl acetate and washed with 5% HCl (2×10

mL), 5% $NaHCO_3$ (2×10 mL), saturated NaCl solution (2×10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the organic layer gave a residue that was purified on silica gel column chromatography using hexane and ethyl acetate.

4.5.4. General procedure for the synthesis of urea 3a-r:

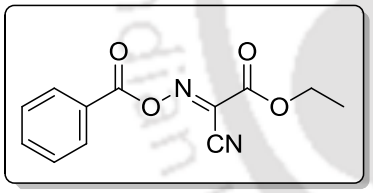
4-NBsOXY (1 mmol) was added to a stirred solution of hydroxamic acid (1 mmol), DIPEA (1.5 mmol) in THF (2 mL) at 0 °C. Then the reaction mixture was stirred at the same temperature for 90 min followed by the addition of amine (1 mmol) and stirring at room temperature for more 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary evaporator and then diluted with 15 mL of ethyl acetate and washed with 5% HCl (2×10 mL), 5% $NaHCO_3$ (2×10 mL), saturated NaCl solution (2×10 mL) and dried over anhydrous Na_2SO_4 and the evaporation of the solvent gave a residue that was purified on silica gel column chromatography using hexane and ethyl acetate.

4.5.5. General procedure for the synthesis of urea from carboxylic acids (one-pot protocol): 4-NBsOXY (1 mmol) was added to a stirred solution of carboxylic acid (1 mmol), DIPEA (1 mmol) and DMAP (0.1 mmol) in THF (2 mL) at room temperature. Then the reaction mixture was stirred for 30 min followed by the addition of hydroxylamine hydrochloride in DMF (0.5 mL), DIPEA (1.5 mmol) and stirred for more 2 h at room temperature. 4-NBsOXY (1 mmol) and DIPEA (1 mmol) was added again to the above reaction mixture at 0 °C and stirring continued for 90 min at the same temperature followed

by addition of amine (1 mmol) and stirring at room temperature for further 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary evaporator and then diluted with 15 mL of ethyl acetate, washed with 5% HCl (2×10 mL), 5% $NaHCO_3$ (2×10 mL), saturated NaCl solution (2×10 mL) and dried over anhydrous Na_2SO_4. The obtained residue was purified on silica gel column chromatography using hexane and ethyl acetate.

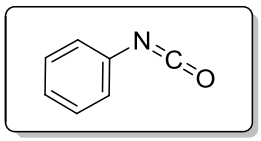
4.6. Characterization data

Bz-Oxyma (intermediate IV).



White solid, Yield 92%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20–8.18 (d, *J* = 7.8 Hz, 2H), 7.72-7.70 (t, *J* = 7.8 Hz, 1H), 7.56-7.53 (t, *J* = 7.8 Hz, 2H), 4.53–4.49 (q, *J* =7.2 Hz, 2H), 1.45–1.43 (t, *J* =7.2, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8, 157.1, 135.3, 131.8, 130.7, 129.3, 125.9, 107.2, 64.7, 14.1; HRMS (ESI) m/z: $[M+Na]^+$calculated for $C_{12}H_{10}N_2O_4Na$ is 269.0538 found 269.0555.

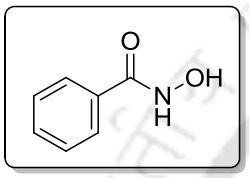
Isocyanatobenzene (intermediate VI).



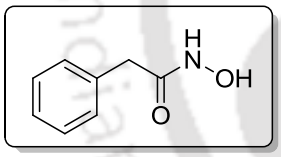
White solid, Yield 90%, Mp. 125-127 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.36 (d, *J* = 7.8 Hz, 2H), 7.28-7.26 (t, *J* = 7.2 Hz, 2H), 7.03-7.01 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 132.6, 128.7, 127.6, 127.0, 124.1; HRMS (ESI) m/z: $[M+H]^+$ calculated for C_7H_6NO is 120.0449 found 120.0456.

1) *N*-Hydroxybenzamide (2a, Table 4.1.1.2)

White solid, Yield 82%; Mp. 125-127 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72-7.71 (d, *J* = 7.8 Hz, 2H), 7.50-7.48 (t, *J* = 7.2 Hz, 1H), 7.41-7.38 (t, *J* = 7.8 Hz, 2H), 3.20 (br, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 166.8, 131.9, 131.5, 128.6, 127.0; FT-IR (KBr) 3296, 3058, 2758, 1643, 1612, 1573 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_7H_8NO_2$ is 138.0555 found 138.0553.

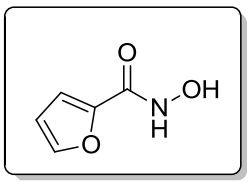


2) *N*-Hydroxy-2-phenylacetamide (2b, Table 4.1.1.2)



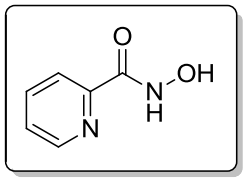
White solid, Yield 79%; Mp. 130-132 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.24 (m, 5H), 3.49 (s, 2H), 2.30 (br, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 169.36, 134.25, 126.3, 129.1, 128.7, 128.5, 127.2, 40.0; FT-IR (KBr) 3190, 3029, 2923, 1685, 1630, 1545 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_8H_{10}NO_2$ is 152.0712 found 152.0718.

3) *N*-hydroxyfuran-2-carboxamide (2c, Table 4.1.1.2)



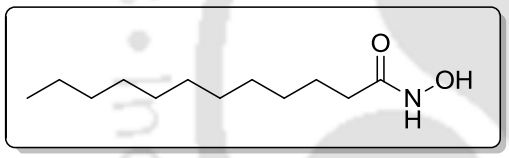
White solid, Yield 76%; Mp. 122-123 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (br, 2H), 7.63-7.62 (d, *J* = 7.2 Hz, 1H), 7.31-7.30 (d, *J* = 7.2 Hz, 1H), 6.54-6.53 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 147.0, 144.3, 119.3, 112.1; FT-IR (KBr) 3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_5H_6NO_3$ is 128.0348 found 128.0348.

4) *N*-hydroxypicolinamide (2d, Table 4.1.1.2)



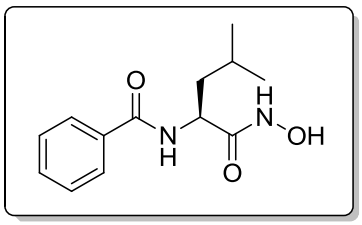
White solid, Yield 74%; Mp. 125-126 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.5 (br, 2H), 8.48-8.47 (d, *J* = 4.2 Hz, 1H), 8.07-8.06 (d, *J* = 7.8 Hz, 1H), 7.78-7.75 (m, 1H), 7.37-7.34 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2, 149.5, 148.4, 137.6, 126.7, 122.5; FT-IR (KBr) 3324, 3166, 2961, 1683, 1626, 1550 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_6H_7N_2O_2$ is 139.0508 found 139.0510.

5) *N*-Hydroxydodecanamide (2e, Table 4.1.1.2)



White solid, Yield 81%; Mp. 75-76 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.46-2.44 (t, *J* = 7.8 Hz, 2H), 1.61-1.57 (m, 2H), 1.28-1.23 (m, 16H), 0.86-0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 171.7, 33.1, 32.0, 29.7, 29.6, 29.4, 29.4, 29.3, 25.6, 22.7, 14.1; FT-IR (KBr) 3258, 2915, 2847, 1663, 1623, 1469, 1423, 720, 649 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{12}H_{26}NO_2$ is 216.1964 found 216.1964.

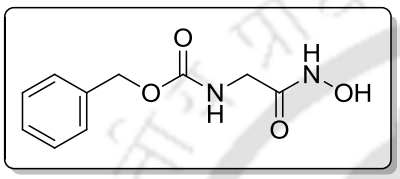
6) *N*-(1-(hydroxyamino)-4-methyl-1-oxopentan-2-yl)benzamide (2f, Table 4.1.1.2)



White solid, Yield 81%; Mp. 122 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.76-7.75 (d, *J* = 7.8 Hz, 2H), 7.47-7.46 (t, *J* = 7.2 Hz, 1H), 7.38-7.37 (t, *J* = 5.4 Hz, 2H), 4.53-4.51 (m, 1H), 2.9 (br, 1H), 1.69-1.66 (m, 2H), 1.26-1.23 (m, 1H), 0.91-0.90 (d, *J* = 6 Hz, 3H), 0.88-0.87 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 169.7, 168.2, 133.3, 131.8,

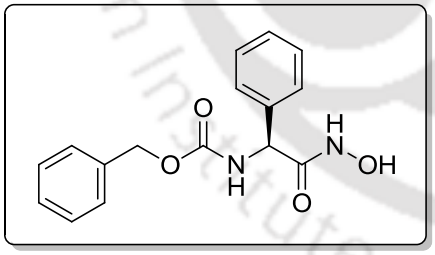
128.4, 128.1, 127.1, 126.8, 49.5, 40.9, 24.7, 22.4, 21.8; FT-IR (KBr) 3301, 2956, 2461, 1642, 1612, 1535, 1425, 866, 693 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{13}H_{19}N_2O_3$ is 251.1396 found 251.1376. $[\alpha]^{27}{}_D$ = + 6.40 (c = 0.2, THF).

7) Benzyl (2-(hydroxyamino)-2-oxoethyl)carbamate (2g, Table 4.1.1.2)



White solid, Yield 78%; Mp. 119-121 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.17 (m, 5H), 5.16 (s, 2H), 3.95-3.94 (d, *J* = 5.4 Hz, 2H), 0.9 (br, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 167.4, 157.2, 136.1, 128.5, 128.2, 128.0, 67.2, 41.9; FT-IR (KBr) 3318, 2943, 1705, 1672, 1536, 770, 697 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{10}H_{13}N_2O_4$ is 225.0875 found 225.0886.

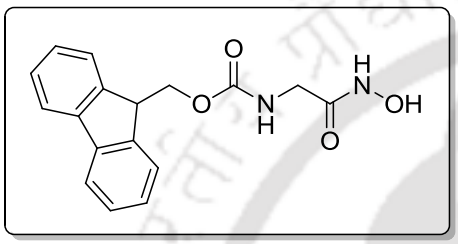
8) Benzyl (2-(hydroxyamino)-2-oxo-1-phenylethyl)carbamate (2h, Table 4.1.1.2)



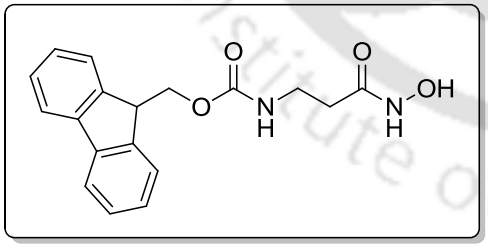
White solid, Yield 78 %; Mp. 157-158 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.33-7.16 (m, 10H), 5.18 (s, 1H), 5.08 (s, 2H), 3.74 (br, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 167.8, 156.1, 137.2, 136.0, 128.8, 128.7, 128.4, 128.3, 128.1, 127.9, 127.0, 126.8, 67.1, 56.1; FT-IR (KBr) 3348, 3266, 2915, 1715, 1647, 1497, 751, 697 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{16}H_{17}N_2O_4$ is 301.1188 found 301.1209. $[\alpha]^{27}{}_D$ = + 16.00 (c = 1, THF).

9) (9H-Fluoren-9-yl)methyl (2-(hydroxyamino)-2-oxoethyl)carbamate (2i, Table 4.1.1.2)

White solid, Yield 80%; Mp 127-128 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75(d, *J* = 6.6 Hz, 2H), 7.58-7.57 (d, *J* = 6.6 Hz, 2H), 7.40-7.38 (t, *J* = 6.6 Hz, 2H), 7.32-7.30 (t, *J* = 5.4 Hz, 2H), 4.39-4.38 (d, *J* = 7.2 Hz, 2H), 4.19-4.16 (t, *J* = 6.6 Hz, 1H), 3.71-3.70 (d, *J* = 6 Hz, 2H), 3.37 (br, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 167.4, 157.3, 143.7,141.2, 127.7, 127.1, 125.0, 119.9, 67.2, 47.0, 41.9; FT-IR (KBr) 3342, 3259, 2922, 1695, 1667, 1551, 1281, 737, 698 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{17}H_{16}N_2O_4Na$ is 335.1008 found 335.0999.

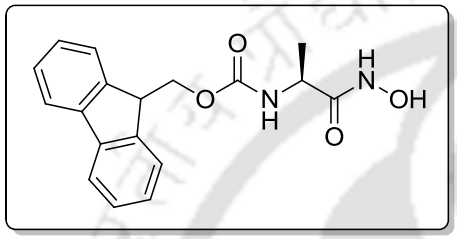


10) (9H-Fluoren-9-yl)methyl (3-(hydroxyamino)-3-oxopropyl)carbamate (2j, Table 4.1.1.2)



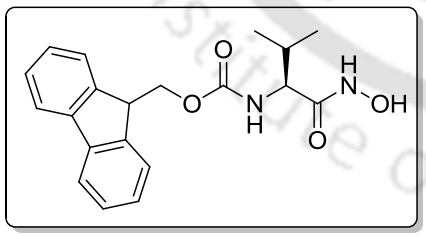
White solid, Yield 81%; Mp. 170-171 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66-7.65 (d, *J* = 7.2 Hz, 2H), 7.51-7.48 (d, *J* = 7.2 Hz, 2H), 7.31-7.28 (t, *J* = 7.2 Hz, 2H), 7.22-7.20 (t, *J* = 7.2 Hz, 2H), 4.26-4.25 (d, *J* = 6.6 Hz, 2H), 4.11-4.09 (t, *J* = 6.6 Hz, 1H), 3.67 (br, 1H), 3.30-3.28 (t, *J* = 6 Hz, 2H), 2.21-2.19 (t, *J* = 6 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 156.4, 143.8, 141.4, 127.8, 127.2, 125.2, 120.1, 67.4, 53.2, 47.2, 27.4 cm^{-1}; FT-IR (KBr) 3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated $C_{18}H_{19}N_2O_4$ is 327.1345 found 327.1346.

11) (9H-Fluoren-9-yl)methyl (1-(hydroxyamino)-1-oxopropan-2-yl)carbamate (2k, Table 4.1.1.2)



White solid, Yield 80%; Mp. 128-129 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.778-7.77 (d, *J* = 4.8 Hz, 2H), 7.61-7.59 (d, *J* = 7.8 Hz, 2H), 7.46-7.44 (t, *J* = 6 Hz, 2H), 7.31-7.29 (t, *J* = 7.8 Hz, 2H), 4.41-4.40 (d, *J* = 6.6 Hz, 2H), 4.31-4.27 (m, 1H), 4.12-4.11 (t, *J* = 6.6 Hz, 1H), 2.5 (br, 1H), 1.39-1.38 (d, *J* = 6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 170.3, 156.3, 143.6, 141.1, 127.6, 127.6, 124.9, 119.8, 66.9, 49.1, 46.9, 18.0; FT-IR (KBr) 3426, 3306, 2925, 1685, 1618, 1533 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{18}H_{19}N_2O_4$ is 327.1345, found 327.1331. $[\alpha]^{27}{}_D$ = - 8.00 (c = 0.5, DMF).

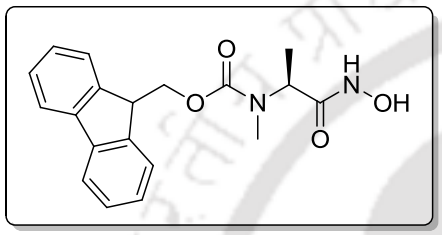
12) (9H-fluoren-9-yl)methyl (1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)carbamate (2l, Table 4.1.1.2)



White solid, Yield 75%; Mp. 173-174 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.74-7.73 (d, *J* = 7.2 Hz, 2H), 7.55-7.51 (d, *J* = 6.6 Hz, 2H), 7.38-7.36 (t, *J* = 7.2 Hz, 2H), 7.29-7.27 (t, *J* = 7.8 Hz, 2H), 5.9 (br, 1H), 4.41-4.38 (d, *J* = 7.2 Hz, 2H), 4.33-4.30 (m, 1H), 4.18-4.16 (t, *J* = 7.2 Hz, 1H), 2.15-1.93 (m, 1H), 0.92-0.90 (d, *J* = 6.6 Hz, 3H), 0.89-0.87 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 168.8, 156.8, 143.8, 141.3, 127.8, 127.1, 125.0, 120.0, 67.2, 58.3, 47.1, 30.9, 19.0, 18.4; FT-IR (KBr) 3305,

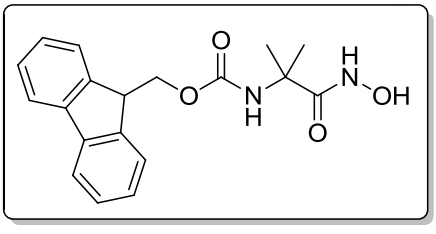
3208, 2925, 2467, 1686, 1647, 1536 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{20}H_{23}N_2O_4$ is 355.1658, found 355.1650. $[\alpha]^{27}{}_D$ = - 77.20 (c = 0.5, DMF).

13) (9H-fluoren-9-yl)methyl (1-(hydroxyamino)-1-oxopropan-2-yl)(methyl)carbamate (2m, Table 4.1.1.2)



White solid, Yield 77%; Mp. 160-161 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.67 (d, *J* = 7.6 Hz, 2H), 7.48-7.46 (d, *J* = 7.6 Hz, 2H), 7.34-7.30 (t, *J* = 7.2 Hz, 2H), 7.25-7.21 (t, *J* = 7.6Hz, 2H), 4.61-4.59 (m, 1H), 4.36-4.34 (d, *J* = 6.6 Hz, 2H), 4.15-4.12 (t, *J* = 6.6 Hz, 1H), 2.72 (s, 3H), 1.26-1.25 (t, *J* = 7.6Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 157.0, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 68.0, 51.8, 47.2, 29.7, 14.2; FT-IR (KBr) 3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{19}H_{20}N_2NaO_4$ is 363.1321 found 363.1320. $[\alpha]^{27}{}_D$ = - 33.60 (c = 0.5, DMF).

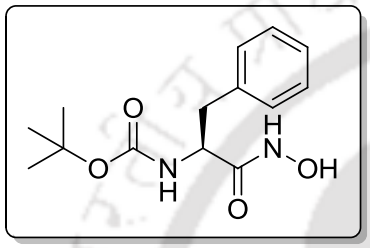
14) (9H-fluoren-9-yl)methyl (1-(hydroxyamino)-2-methyl-1-oxopropan-2-yl)carbamate (2n, Table 4.1.1.2)



White solid, Yield 75%; Mp. 172-174 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.58-7.57 (d, *J* = 7.2 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 4.43-4.42 (d, *J* = 6.6 Hz, 2H), 4.18-4.17 (t, *J* = 6.6 Hz, 1H), 1.44 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 155.6, 143.8, 141.4, 127.9, 127.2, 125.0, 120.1, 66.5, 55.6, 47.2, 25.3; FT-IR (KBr)

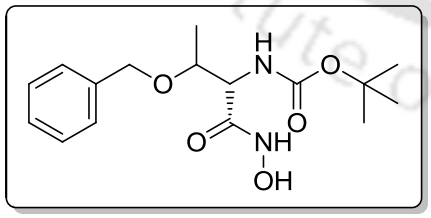
3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{19}H_{20}N_2NaO_4$ is 363.1321 found 363.1324.

15) Tert-butyl (1-(hydroxyamino)-1-oxo-3-phenylpropan-2-yl)carbamate (2o, Table 4.1.1.2)



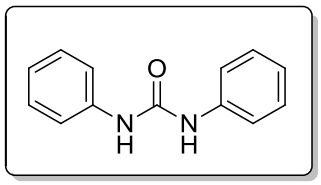
White solid, Yield 80%; Mp. 135 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.29 (d, *J* = 6.6 Hz, 2H), 7.24-7.22 (d, *J* = 7.2 Hz, 1H), 7.14-7.13 (t, *J* = 7.2 Hz, 2H), 4.24-4.20 (m, 1H), 3.07-3.0 (dd, *J* = 6.6 Hz, 1H), 2.97-2.94 (dd, *J* = 6.6 Hz, 1H), 2.03 (br, 1H), 1.40 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 168.9, 155.8, 136.4, 129.3, 128.5, 126.9, 80.4, 53.4, 38.7, 28.2; FT-IR (KBr) 3350, 3309, 2918, 2927, 1672, 1662, 1513 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{14}H_{21}N_2O_4$ is 281.1501 found 281.1542. $[\alpha]^{27}{}_D$ = + 5.00 (c = 1, THF).

16) Tert-butyl (3-(benzyloxy)-1-(hydroxyamino)-1-oxobutan-2-yl)carbamate (2q, Table 4.1.1.2)



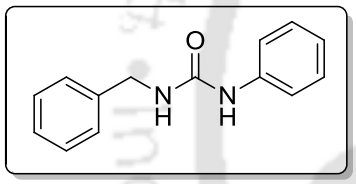
White solid, Yield 80%; Mp. 168-169 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 5.4 (br, 1H) 4.57 (s, 2H), 4.52-4.50 (m, 1H), 4.30 (br, 1H), 4.11 (m, 1H), 1.42 (s, 9H),), 1.17-1.16 (d, *J* = 6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.4, 156.0, 137.8, 128.6, 128.0, 80.6, 74.4, 71.8, 56.5, 28.4, 15.8; FT-IR (KBr) 3331, 3260, 2983, 1679, 1637, 1525 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{16}H_{24}N_2O_5Na$ is 347.1583 found 347.1584. $[\alpha]^{27}{}_D$ = + 32.40 (c = 0.2, THF).

17) 1,3-Diphenylurea (3a, Table 4.2.1.2)



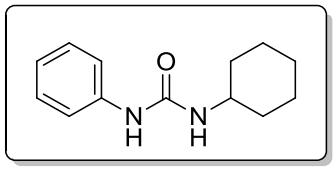
White solid, Yield 83%; Mp. 225-226 ºC; ^{1}H NMR (400 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.90 (s, 2H), 7.33-7.31 (d, *J* = 7.6 Hz, 4H), 7.22-7.18 (t, *J* = 8 Hz, 4H), 6.96-6.92 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 152.7, 138.3, 129.0, 128.9, 123.0; FT-IR (KBr) 3336, 2891, 2870, 1685, 1600, 1534 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{13}H_{13}N_2O$ is 213.1028 found 213.1018.

18) 1-Benzyl-3-phenylurea (3b, Table 4.2.1.2)



White solid, Yield 91%; Mp. 173-174 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.22 (m, 10H), 4.85 (br, 1H), 4.39-4.38 (d, *J* = 7.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 139.3, 137.6, 128.7, 128.5, 127.7, 127.6, 127.2, 122.7, 43.8 cm^{-1}; FT-IR (KBr) 3226, 2971, 2830, 1666, 1630, 1555; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{14}H_{15}N_2O$ is 227.1184, found 227.1185.

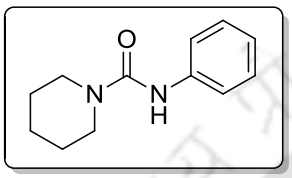
19) 1-Cyclohexyl-3-phenylurea (3c, Table 4.2.1.2)



White solid, Yield, 90%; Mp 168-169 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.31-7.26 (m, 4H), 7.07-7.06 (t, *J* = 6.8 Hz, 1H), 6.63 (d, *J* = 6.8 Hz, 1H), 4.88-4.87 (s, 1H), 3.65-3.62 (m, 1H), 1.96-1.94 (m, 2H); 1.69-1.67 (m, 2H), 1.59-1.57 (m, 1H),1.38-1.34 (m, *J*= 4.8 Hz, 2H), 1.17-1.14 (m, 3H);^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 156.0, 139.4, 129.1, 122.9, 120.2, 48.9, 33.7, 25.7, 25.0;

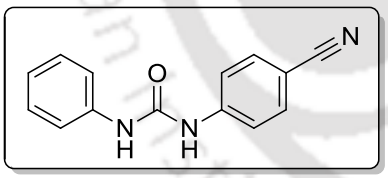
FT-IR (KBr) 3236, 2961, 2810, 1685,1620, 1585 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{13}H_{19}N_2O$ is 219.1497 found 219.1497.

20) *N*-Phenylpiperidine-1-carboxamide (3d, Table 4.2.1.2)



White solid, Yield 82%; Mp. 170-171 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50-7.48 (d, *J* = 7.2 Hz, 2H), 7.35-7.34 (t, *J* = 7.2 Hz, 2H), 7.04-7.05 (t, *J* = 7.6 Hz, 1H), 3.40-3.34 (m, 4H), 2.6 (br, 1H), 1.45-1.41 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 156.7, 131.8, 131.6, 128.6, 127.0, 45.0, 28.4, 24.4; FT-IR (KBr) 3126, 2971, 2790, 1635, 1625, 1555 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{12}H_{17}N_2O$ is 205.1341 found 205.1333.

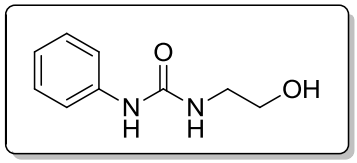
21) 1-(4-cyanophenyl)-3-phenylurea (3e, Table 4.2.1.2).



White solid, Yield 81%; Mp. 170-172 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85-7.83 (d, *J* = 7.8, 1H), 7.60-7.55 (m, 4H),, 7.40-7.38 (d, *J* = 7.6 Hz, 2H), 7.31-7.27 (t, *J* = 7.8 Hz, 2H), 7.04-7.03 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.8, 143.8, 138.4, 133.2, 128.7, 123.4, 119.6, 119.5, 118.4, 104.6; FT-IR (KBr) 3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{14}H_{12}N_3O$ is 238.0980 found 238.0978.

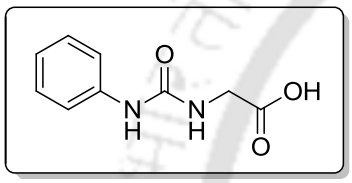
22) 1-(2-hydroxyethyl)-3-phenylurea (3f, Table 4.2.1.2)

White solid, Yield 91%; Mp. 120-121 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (br, 1H), 7.27-7.17 (m, 4H), 6.98-6.96 (t, *J* = 7.4 Hz, 1H), 5.9 (br, 1H), 3.58-3.55 (t, *J* = 5.2 Hz, 2H), 3.26-



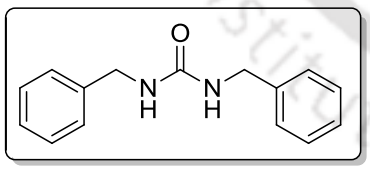
3.24 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 157.2, 139.2, 139.1, 128.8, 122.6, 119.5, 119.3, 61.7, 42.3; FT-IR (KBr) 3116, 2991, 2872, 1695, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_9H_{13}N_2O_2$ is 181.0977 found 181.0974.

23) 2-(3-Phenylureido)acetic acid (3g, Table 4.2.1.2)



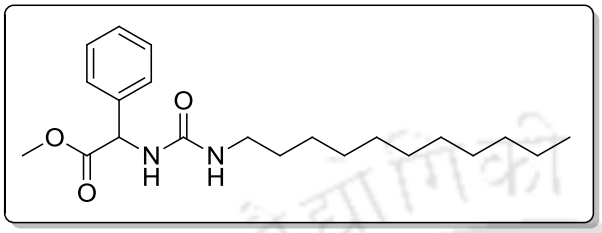
White solid, Yield 81%; Mp. 96-97 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ 7.38-7.40 (d, *J* = 8 Hz, 2H), 7.21-7.19 (d, *J* = 8.4 Hz, 2H), 7.10-7.06 (t, *J* = 8 Hz, 2H), 6.84-6.80 (t, *J* = 7.6 Hz, 2H), 3.7 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.6, 158.3, 140.8, 129.9, 129.7, 128.5, 123.8, 120.5, 42.6; FT-IR (KBr) 3216, 2951, 2892, 1772, 1665, 1645, 1544 cm^{-1}; HRMS (ESI) m/z: $[M-H]^-$ calculated for $C_9H_9N_2O_3$ is 193.0613 found 193.0616.

24) 1,3-Dibenzylurea (3h,Table 4.2.1.2).



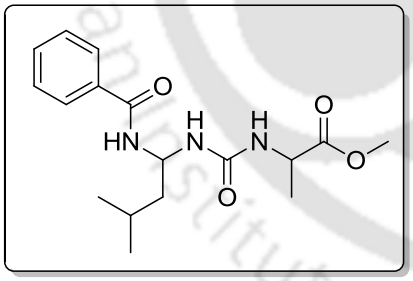
White solid, Yield 88%; Mp. 172-173 ºC; ^{1}H NMR (400 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.32-7.22 (m, 10H), 6.82 (br, 2H), 4.37-4.36 (d, *J* = 7.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 161.5, 137.7, 128.7, 127.7, 127.0, 42.0; FT-IR (KBr) 3296, 3058, , 2092, , 1643, 1612, 1573 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{15}H_{17}N_2O$ is 241.1341 found 241.1336.

25) Methyl 2-phenyl-2-(3-undecylureido)acetate (3i, Table 4.2.1.2)



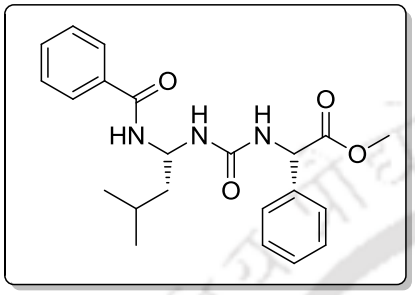
White solid, Yield 92%; Mp. 78-80 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.32-7.27 (m, 5H), 5.42(s, 1H), 3.72 (s, 3H), 3.08-3.06 (t, *J* = 7.2 Hz, 2H), 1.58-1.53 (m, 2H), 1.44-1.39 (m, 2H), 1.25-1.18 (m, 14H), 0.84-0.82 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 172.9, 158.1, 137.1, 128.8, 128.3, 127.3, 127.1, 57.2, 52.5, 40.1, 35.0, 31.9, 30.0, 29.5, 29.3, 26.8, 25.3, 22.6, 13.9; FT-IR (KBr) 3326, 2921, 2850, 1732, 1634, 1568 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{21}H_{35}N_2O_3$ is 363.2648, found 363.2650.

26) Methyl 2-(3-(1-benzamido-3-methylbutyl)ureido)propanoate (3j, Table 4.2.1.2)



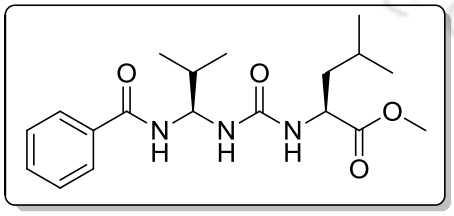
White solid, Yield 87%; Mp. 196-197 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.36-7.21 (m, 3H), 5.64 (br, 1H), 5.13 (m, 1H), 4.30-4.25 (m, 1H), 3.65 (s, 3H), 1.63-1.59 (m, 2H), 1.36-1.25 (m, 3H), 0.85-0.84 (d, *J* = 5.4 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.3, 167.5, 157.6, 134.0, 131.5, 128.9, 128.3, 127.4, 56.4, 52.0, 49.2, 43.6, 27.6, 22.2, 18.5; FT-IR (KBr) 3365, 3317, 2956, 1744, 1660, 1637, 1567 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{17}H_{26}N_3O_4$ is 336.1923 found 336.1924.

27) Methyl 2-(3-(1-benzamido-3-methylbutyl)ureido)-2-phenylacetate (3k, Table 4.2.1.2)



White solid, Yield 90%; Mp. 202-203 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.80-7.80 (d, *J* = 7.8 Hz, 1H), 7.73-7.71(d, *J* = 7.2 Hz, 1H), 7.51-7.27(m, 8H), 5.42-5.40 (d, *J* = 7.2 Hz, 1H), 5.34-5.31 (m, 1H), 3.71 (s, 3H), 1.81-1.74 (m, 2H), 1.69-1.65 (m, 1H), 0.93-0.91 (d, *J* = 6.6 Hz, 3H), 0.90-0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 172.1, 168.4, 157.6, 136.7, 136.4, 133.8, 131.7, 128.7, 128.6, 128.3, 127.2, 127.1, 57.5, 57.3, 52.3, 42.8, 24.8, 22.1, 22.0; FT-IR (KBr) 3365, 3317, 2956, 1744, 1660, 1637, 1567 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{22}H_{27}N_3O_4Na$ is 420.1899 found 420.1896. $[\alpha]^{27}{}_D$ = + 64.40 (c = 1, MeOH:$CHCl_3$).

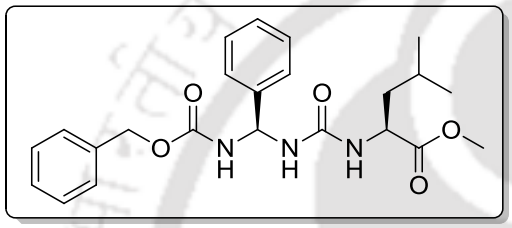
28) Methyl 2-(3-(1-benzamido-2-methylpropyl)ureido)-4-methylpentanoate (3l, Table 4.2.1.2)



White solid, Yield 89%; Mp. 200 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09-8.07 (d, *J* = 7.8 Hz, 1H), 7.74-7.73 (d, *J* = 7.2 Hz, 2H), 7.43-7.28 (m, 3H), 4.88-4.86 (m, 1H), 4.28-4.25 (m, 1H), 3.65 (s, 3H), 2.18-2.16 (m, 1H), 1.65-1.50 (m, 2H), 1.28-1.24 (m, 1H), 0.96-0.95 (d, *J* = 6.6 Hz, 3H), 0.92-0.91 (d, *J* = 6.6 Hz, 3H), 0.86-0.84 (d, *J* = 6.6 Hz, 3H), 0.79-0.68 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.7, 168.2, 158.2, 134.0, 131.7, 128.5, 127.4, 63.7, 52.0, 49.5,

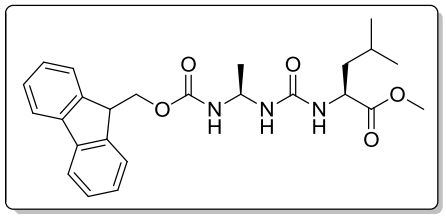
41.2, 32.2, 28.3, 24.8, 22.9, 21.8, 19.0; FT-IR (KBr) 3345, 2960, 1748, 1637, 1625, 1560 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{19}H_{30}N_3O_4$ is 364.2236 found 364.2235. $[\alpha]^{27}{}_D$ = - 23.40 (c = 0.5, MeOH:$CHCl_3$).

29) Methyl 9-isobutyl-3,7-dioxo-1,5-diphenyl-2-oxa-4,6,8-triazadecan-10-oate (3m, Table 4.2.1.2)



White solid, Yield 82%; Mp. 189-190 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.21 (m, 5H), 6.27-6.26 (d, *J* = 6 Hz, 1H), 5.86-5.85 (br, 2H), 5.08 (s, 2H), 4.50-4.46 (m, 1H), 3.68 (s, 3H), 1.67-1.63 (m, 2H), 1.54-1.52 (m, 1H), 0.92-0.90 (d, *J* = 8.4 Hz, 3H), 0.89-0.88 (d, *J* = 8.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.0, 157.3, 156.3, 136.2, 128.9, 128.7, 128.6, 128.2, 128.1, 127.4, 127.2, 126.0, 125.8, 67.1, 61.3, 52.2, 51.7, 49.7, 41.6, 24.9, 22.9, 22.0; FT-IR (KBr) 3299, 3034, 2958, 1726, 1689, 1636, 1571 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{23}H_{29}N_3O_5Na$ is 450.2005 found 450.2006. $[\alpha]^{27}{}_D$ = + 14.40 (c = 0.25, THF).

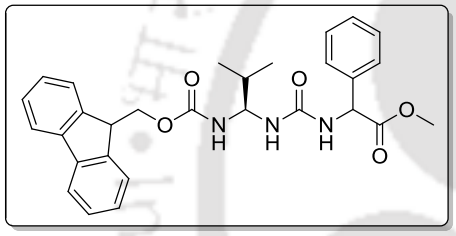
30) Methyl 1-(9H-fluoren-9-yl)-9-isobutyl-5-methyl-3,7-dioxo-2-oxa-4,6,8-triazadecan-10-oate (3n, Table 4.2.1.2)



White solid, Yield 80%; Mp. 201-203 °C; ^{1}H NMR (600 MHz, $CDCl_3$, , few drops of CD_3OD for solubility) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.56-7.55 (d, *J* = 7.2 Hz, 4H); 7.41-7.39 (t, *J* = 7.4 Hz, 2H), 7.32-7.30 (t, *J* = 7.4 Hz, 2H), 5.55 (br, 1H), 5.22-5.20 (m, 1H), 4.41-4.35 (m, 3H), 4.24-4.20 (t, *J* = 7.4 Hz, 1H), 3.73 (s, 3H), 1.63-1.43 (m, 6H), 0.95-0.94 (d, *J* = 6.6 Hz, 3H),

0.91-0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 174.8, 157.5, 156.6, 143.9, 141.3, 127.8, 127.2, 125.1, 120.0, 67.0, 55.9, 51.6, 49.5, 47.1, 41.6, 24.8, 22.9, 21.8, 21.2; FT-IR (KBr) 3294, 2955, 2926, 1730, 1694, 1637, 1573 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{25}H_{31}N_3O_5Na$ is 476.2161 found 476.2159.

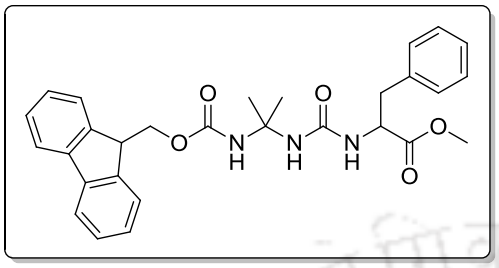
31) Methyl 1-(9H-fluoren-9-yl)-5-isopropyl-3,7-dioxo-9-phenyl-2-oxa-4,6,8-triazadecan-10-oate (3o, Table 4.2.1.2)



White solid, Yield 79%; Mp. 158-160 ºC; ^{1}H NMR (600 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 7.77-7.75 (d, *J* = 6.6 Hz, 2H), 7.63-7.62 (d, *J* = 7.8 Hz, 2H), 7.43-7.27 (m, 9H), 6.9 (br, 1H), 5.48-5.45 (m, 2H), 4.86 (m, 1H), 4.33-4.19 (m, 3H), 3.66 (s, 3H), 2.06-2.03 (m, 1H), 0.92-0.89 (d, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 170.6, 155.0, 154.0, 142.5, 139.4, 136.3, 127.2, 126.6, 126.1, 125.6, 123.8, 118.4, 64.1, 62.0, 55.3, 50.7, 45.5, 31.0, 16.8; FT-IR (KBr) 3365, 3298, 2952, 1724, 1692, 1636, 1564 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{29}H_{32}N_3O_5$is 502.2342, found 502.2346. $[\alpha]^{27}{}_D$ = + 36.80 (c = 0.5, DMF).

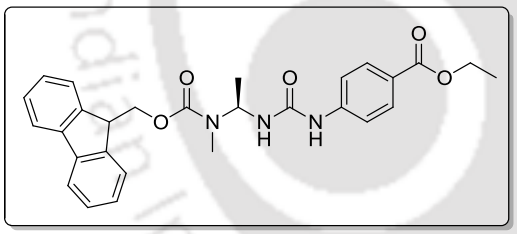
32) Methyl 9-benzyl-1-(9H-fluoren-9-yl)-5,5-dimethyl-3,7-dioxo-2-oxa-4,6,8-triazadecan-10-oate (3p,Table 4.2.1.2)

White solid, Yield 81%; Mp. 180-182 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94-7.93 (d, *J* = 7.2 Hz, 2H), 7.82-7.80 (d, *J* = 7.8 Hz, 2H), 7.59-7.56 (t, *J* = 7.2 Hz, 3H), 7.51-7.45 (m, 5H), 7.36-7.34 (t, *J* = 7.2 Hz, 1H), 6.3 (s, 1H), 5.49-5.47 (d, *J* = 8.4 Hz, 1H), 4.85-4.80 (m,1H),



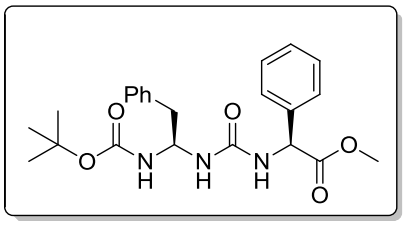
4.59-4.58 (d, *J* = 5.2 Hz, 2H), 4.41-4.39 (t, *J* = 6.4Hz, 1H), 3.88 (s, 3H), 3.36-3.33 (dd, *J* = 6 Hz, 1H), 3.27-3.22 (dd, *J* = 6.4 Hz, 1H), 1.70 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 155.1, 152.1, 143.6, 141.0, 135.9, 129.1, 128.3, 128.2, 127.5, 126.9, 126.8, 125.0, 119.7, 66.7, 60.3, 56.0, 52.0, 46.9, 38.0, 28.1; FT-IR (KBr) 3304, 3066, 2972, 1693, 1646, 1540 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{29}H_{31}N_3NaO_5$ is 524.2161 found 524.2164.

33) Ethyl 4-(3-(1-((((9H-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)ethyl)ureido) benzoate (3q, Table 4.2.1.2)



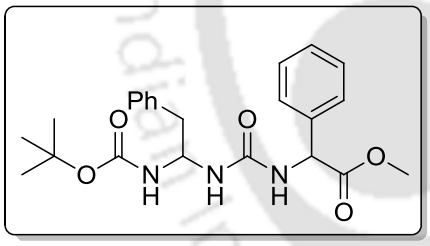
White solid, Yield, 74%; Mp. 174-175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.92-7.90 (d, *J* = 7.2 Hz, 1H), 7.75-7.72 (d, *J* = 7.2 Hz, 3H), 7.59-7.25 (m, 8H), 5.60 (s, 1H), 5.30 (s, 1H), 4.41-4.33 (m, 4H), 4.21-4.18 (t, *J* = 6.6 Hz, 1H), 2.75 (s, 3H), 1.38-1.36 (t, *J* = 7.2 Hz, 3H), 1.19-1.17- (t, *J* = 6.8 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 158.9, 156.6, 143.7, 141.4, 139.4, 130.9, 127.9, 127.3, 125.2, 125.1, 124.4, 120.2, 118.3, 114.2, 67.8, 63.2, 60.9, 47.3, 31.7, 18.8, 14.5; FT-IR (KBr) 3257, 2966, 2872, 1699, 1653, 1590 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{28}H_{29}N_3NaO_5$ is 510.2005 found 510.2004.

34) Methyl 6-benzyl-10,10-dimethyl-4,8-dioxo-2-phenyl-9-oxa-3,5,7-triazaundecan-1-oate (3r, Table 4.2.1.2)



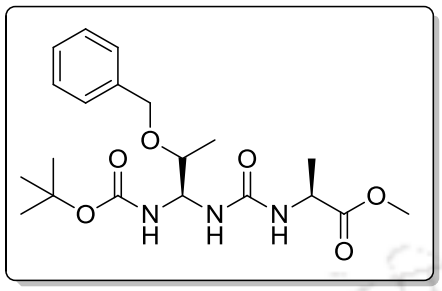
White solid, Yield 89%; Mp. 129-130 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.18 (m, 10H), 5.55 (br, 1H), 5.46-5.45 (d, *J* = 6 Hz, 1H), 5.32-5.31 (m, 1H), 3.71 (s, 3H), 3.07-3.03 (m, 2H), 1.32 (s, 9H), ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 171.7, 156.8, 155.5, 136.6, 136.3, 129.1, 128.6, 128.5, 128.2, 128.1, 126.9, 126.3, 79.5, 58.7, 57.0, 52.1, 40.2, 27.9 ; FT-IR (KBr) 3388, 3341, 3061, 2983, 1738, 1681, 1635, 1504 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{23}H_{30}N_3O_5$is 428.2185 found 428.2193. $[\alpha]^{27}{}_D$ = + 94.40 (c = 1, THF).

35) DL-Methyl 6-benzyl-10,10-dimethyl-4,8-dioxo-2-phenyl-9-oxa-3,5,7-triazaundecan-1-oate (3s, Table 4.2.1.2)



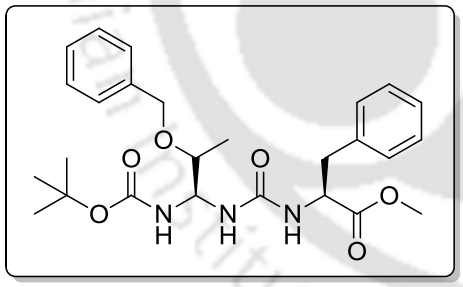
White solid, Yield 88%; Mp. 129-130 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.16 (m, 10H), 5.42-5.41 (d, *J* = 6 Hz, 1H), 5.09-5.05 (m, 1H), 3.70 (s, 3H), 3.68 (s, 3H), 3.08-3.05 (m, 2H), 2.84-2.81 (m, 2H), 1.39 (s, 9H), 1.34 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 172.3, 157.0, 155.9, 136.9, 129.4, 128.9, 128.8, 128.5, 127.3, 127.2, 126.7, 80.1, 60.3, 60.1, 57.6, 57.3, 52.7, 52.6, 40.4, 28.3, 28.2; FT-IR (KBr) 3388, 3341, 3061, 2983, 1738, 1681, 1635, 1504 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{23}H_{30}N_3O_5$ is 428.2185 found 428.2193.

36) Methyl 6-(1-(benzyloxy)ethyl)-2,10,10-trimethyl-4,8-dioxo-9-oxa-3,5,7-triazaundecan-1-oate (3t, Table 4.2.1.2)



White solid, Yield 85%; Mp. 140-142 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.29 (m, 5H), 5.41-539 (m, 1H), 5.02 (br, 1H), 4.62-4.60 (d, *J* = 6 Hz, 2H), 4.44-4.37 (m, 2H), 3.72 (s, 3H), 1.44 (s, 9H), 1.36-1.34 (d, *J* = 7.2 Hz, 3H), 1.21-1.20 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.3, 156.8, 155.2, 137.7, 128.4, 127.8, 80.2, 71.0, 61.8, 52.1, 49.1, 27.8, 18.4, 15.8; FT-IR (KBr) 3327, 3030, 2979, 1733, 1688, 1641, 1569 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{20}H_{31}N_3O_6Na$ is 432.2111 found 432.2123. $[\alpha]^{27}{}_D$ = -12.20 (c = 1, THF).

37) Methyl 2-benzyl-6-(1-(benzyloxy) ethyl)-10,10-dimethyl-4,8-dioxo-9-oxa-3,5,7-triazaundecan-1-oate (3u, Table 4.2.1.2)



White solid, Yield 82%; Mp. 157-159 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.11 (m, 10H), 5.35 (br, 1H), 5.29-5.27 (m, 1H), 5.04 (br, 1H), 4.70-4.68 (m, 1H), 4.63-4.61 (d, *J* = 6 Hz, 2H), 4.38-4.36 (d, *J* = 11.4 Hz, 1H), 3.62 (s, 3H), 3.11-3.07 (dd, *J* = 6.6 Hz, 1H), 3.03-3.0 (dd, *J* = 6.6 Hz, 1H), 1.40 (s, 9H), 1.22-1.21 (d, *J* = 6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 156.6, 155.6, 137.8, 136.7, 129.3, 128.6, 128.4, 128.0, 126.8, 80.4, 76.3, 71.2, 62.2, 54.6, 52.0, 38.6, 28.4, 16.0; FT-IR (KBr) 3326, 2981, 2933, 1723, 1666, 1627, 1527 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{36}N_3O_6$ is 486.2604 found 486.2602. $[\alpha]^{27}{}_D$ = + 26.80 (c = 0.5, THF).

4.7. Selected spectra

4.7.1. NMR (^{1}H and ^{13}C) and HRMS spectra of compounds

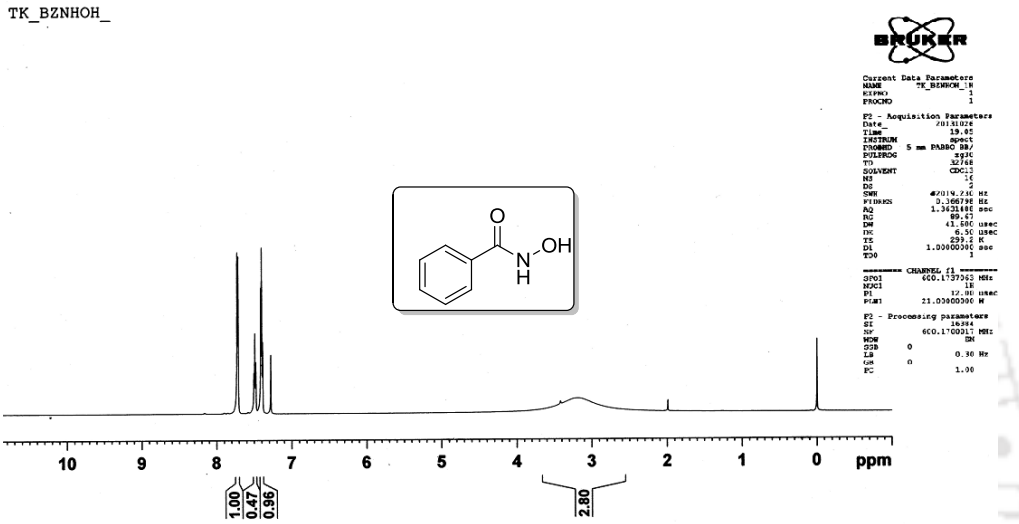


Figure S1. ^{1}H NMR Spectra of compound **2a**, Table 4.1.1.2

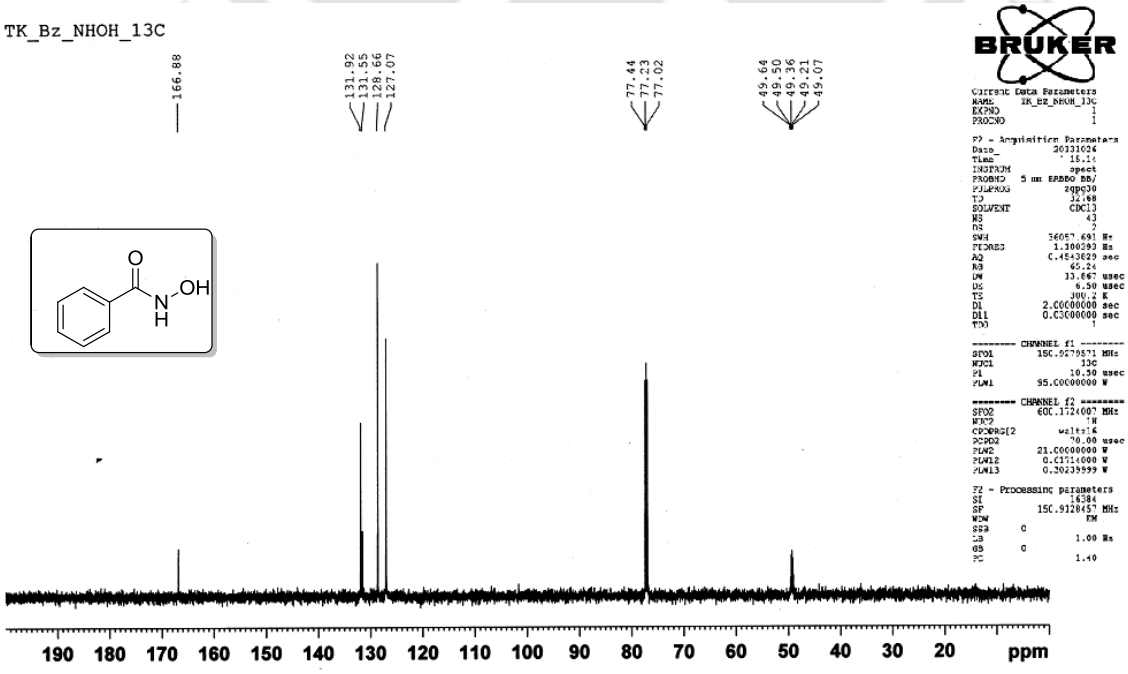


Figure S2. ^{13}C NMR spectra of compound **2a**, Table 4.1.1.2

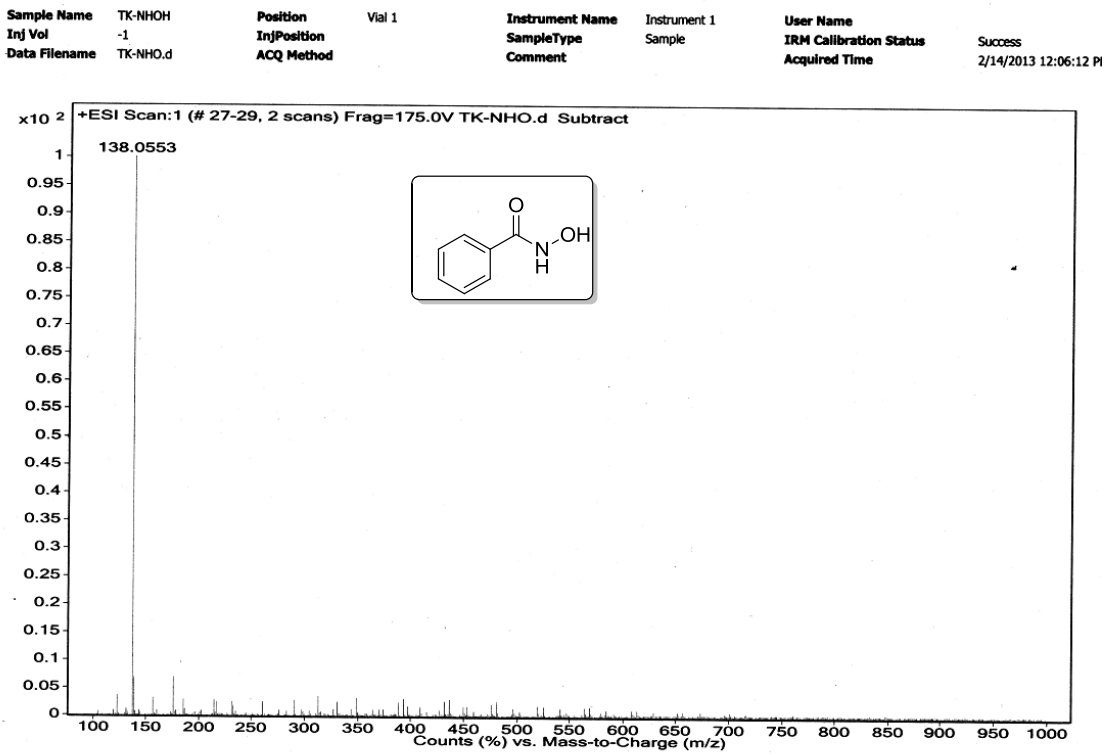


Figure S3. HRMS spectra of compound **2a**, Table 4.1.1.2

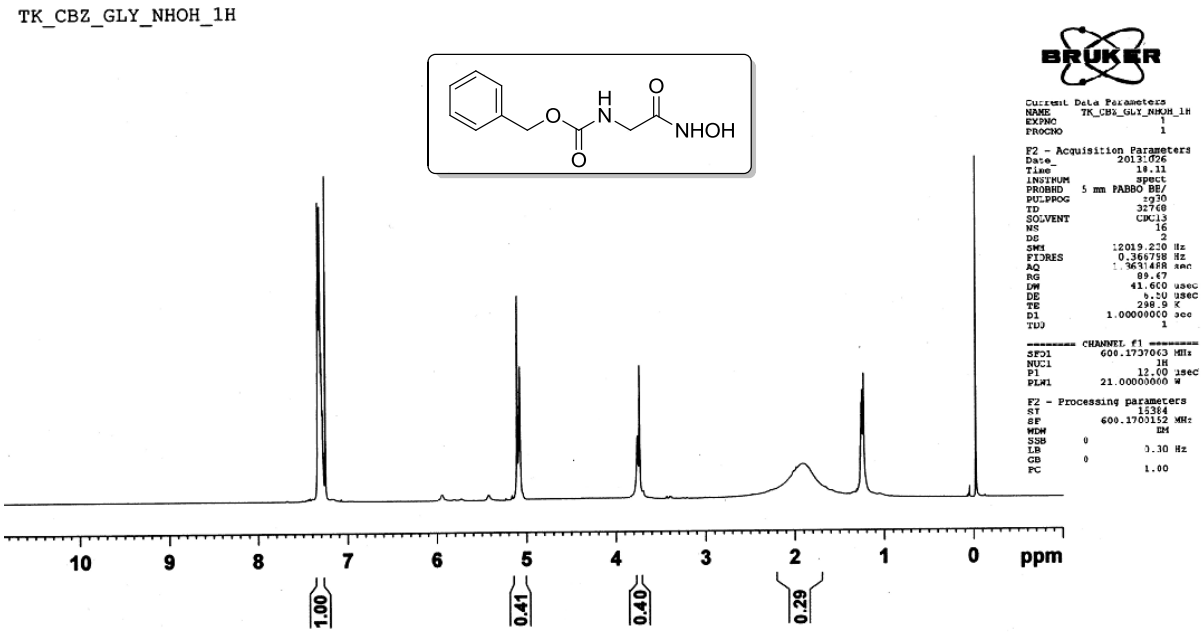


Figure S4. ^{1}H NMR spectra of compound **2g**, Table 4.1.1.2

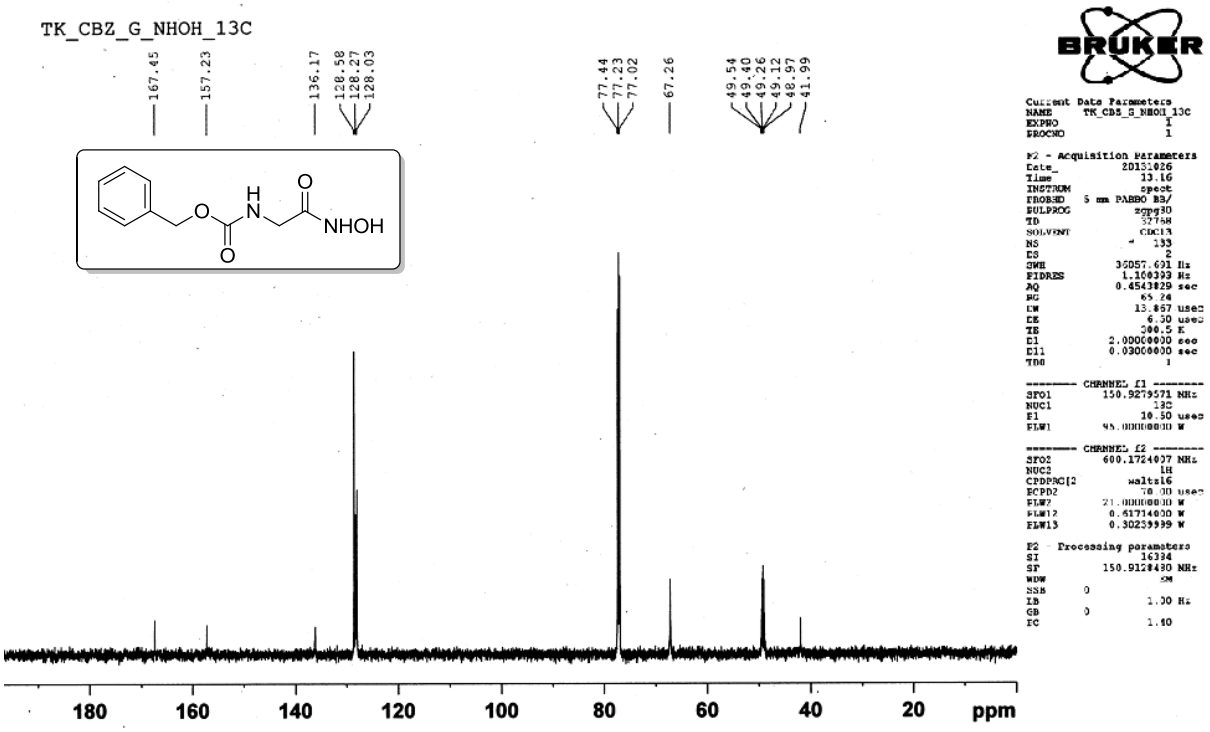


Figure S5. ^{13}C NMR spectra of compound **2g**, Table 4.1.1.2

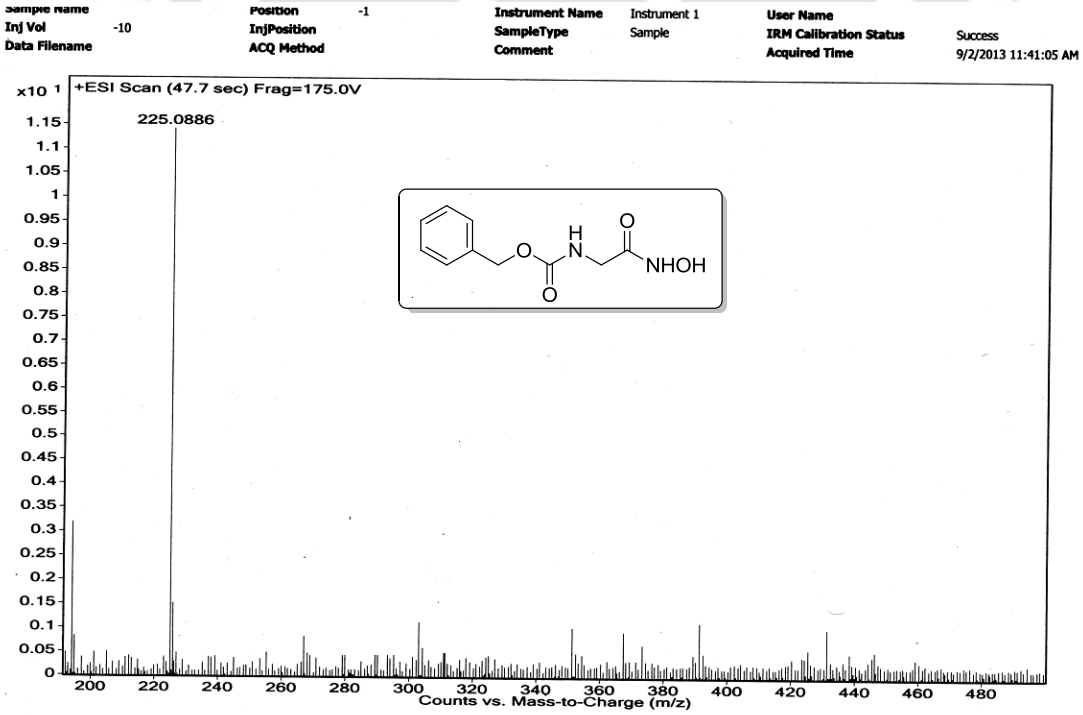


Figure S6. HRMS spectra of compound **2g**, Table 4.1.1.2

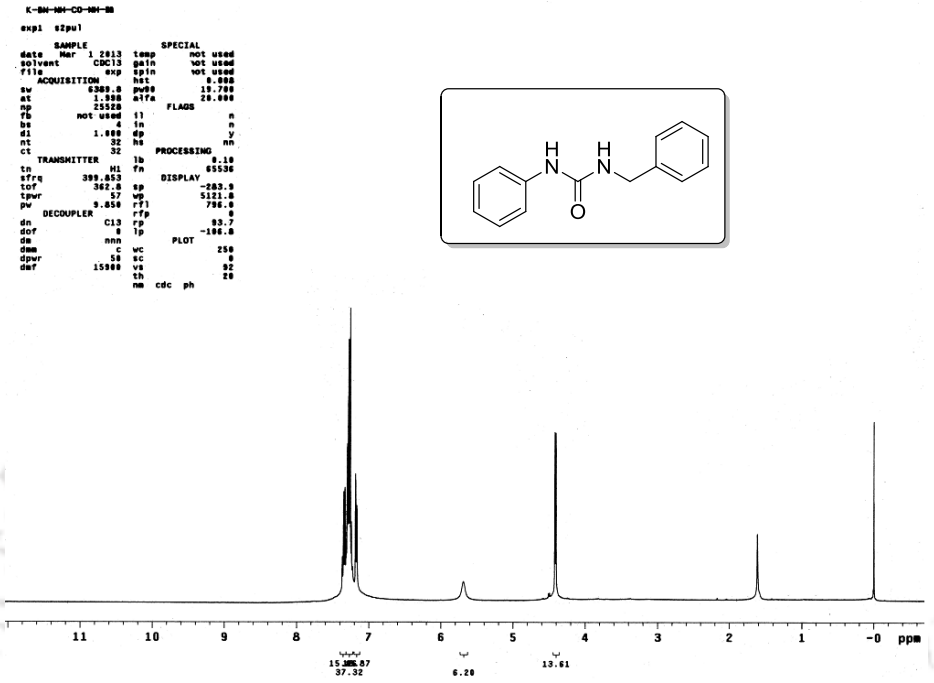


Figure S7. ^{1}H NMR spectra of compound **3b**, Table 4.2.1.2

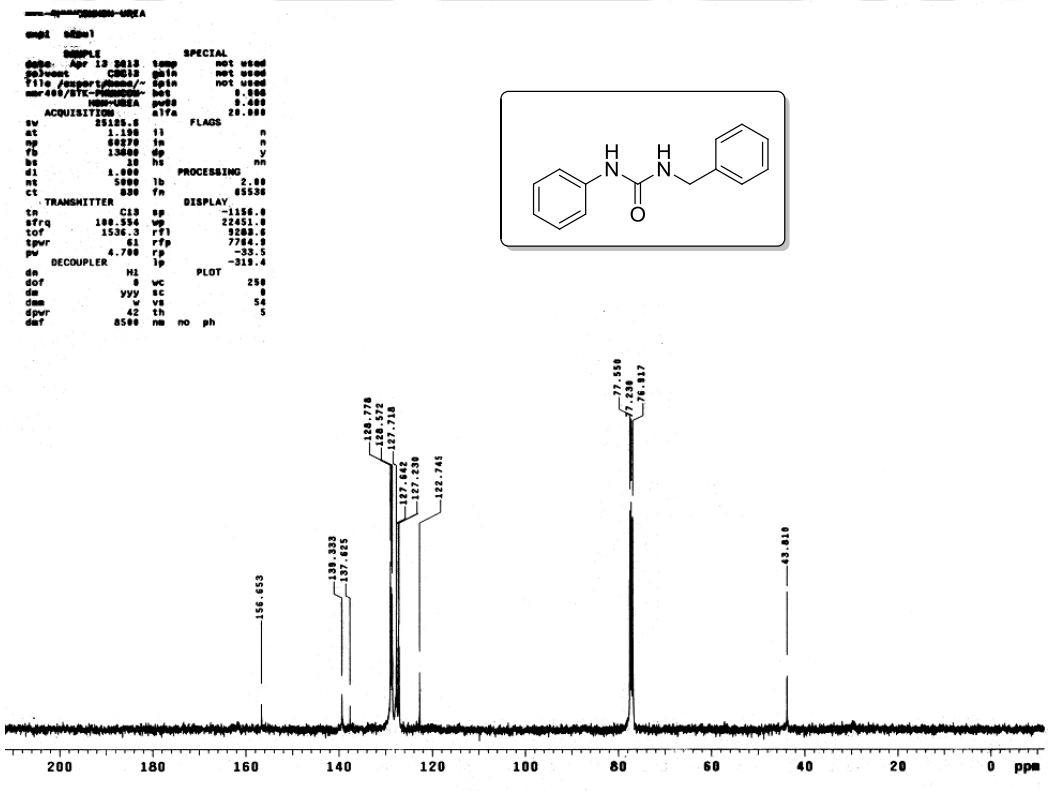


Figure S8. ^{13}C NMR spectra of compound **3b**, Table 4.2.1.2

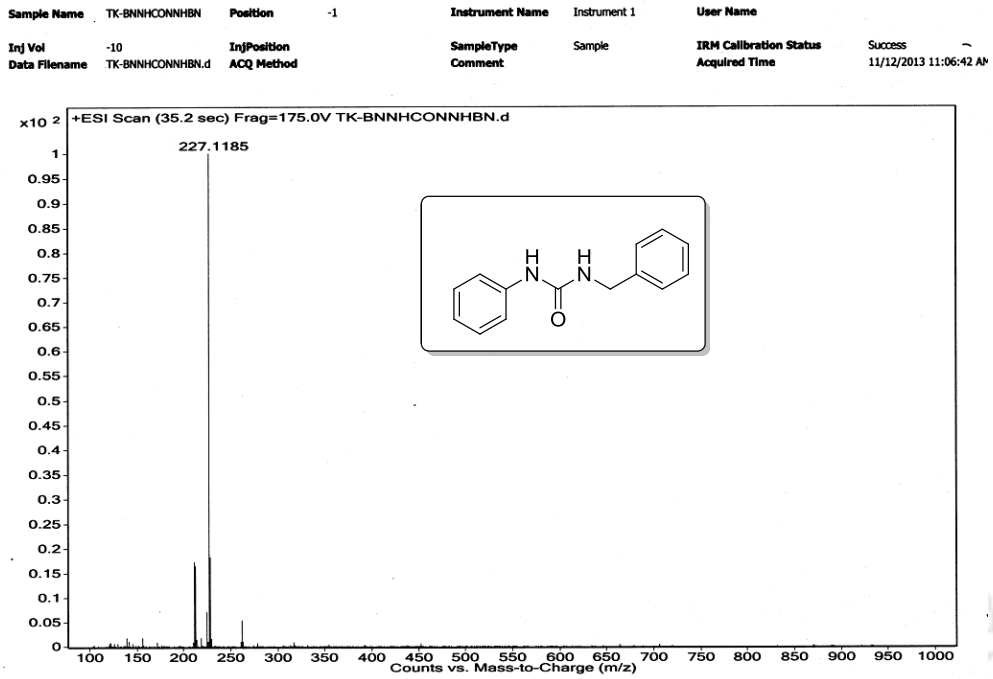


Figure S9. HRMS spectra of compound **3b**, Table 4.2.1.2

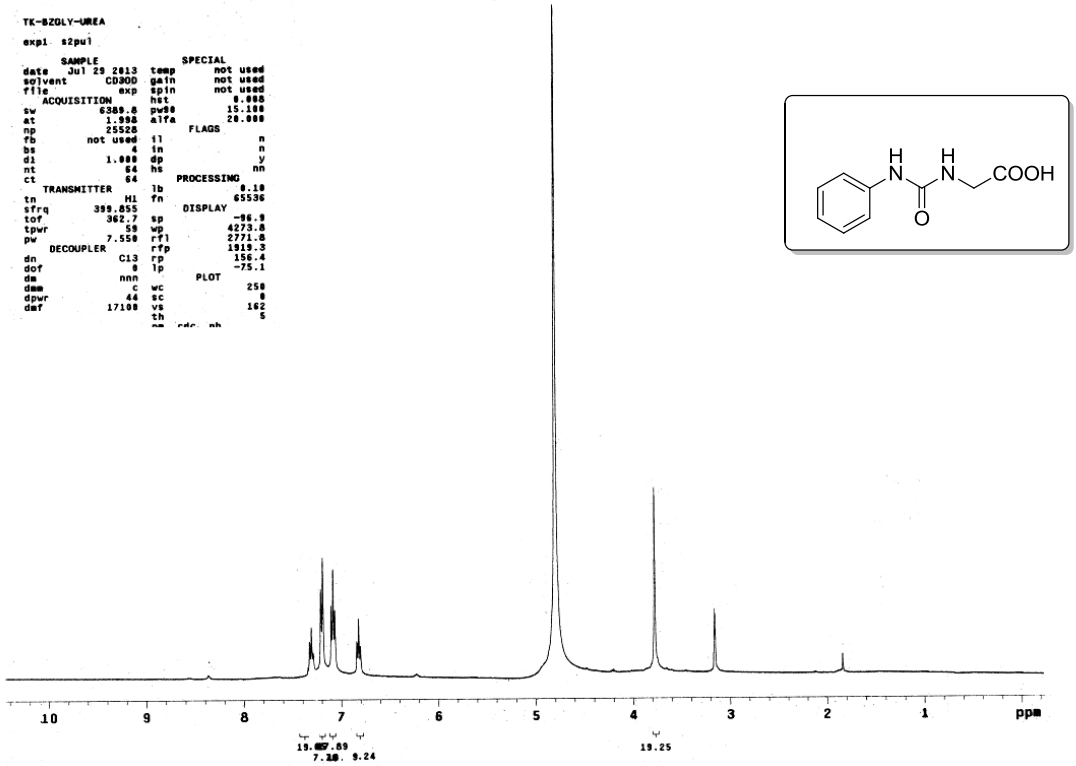


Figure S10. ^{1}H NMR spectra of compound **3g**, Table 4.2.1.2

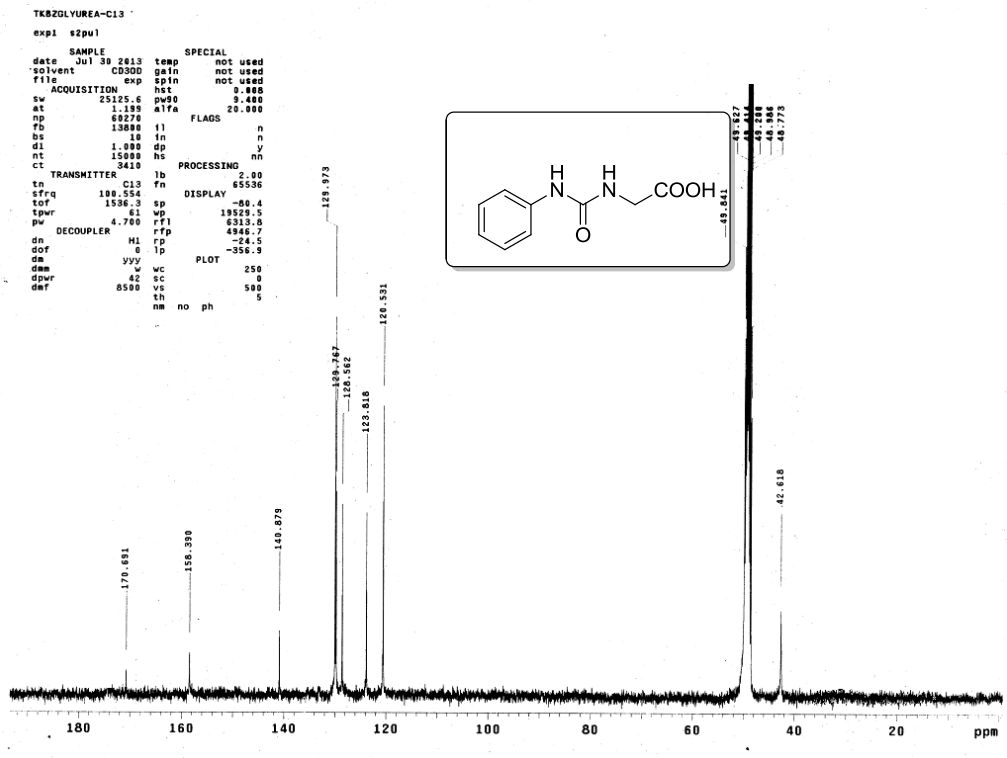


Figure S11. ^{13}C NMR spectra of compound **3g**, Table 4.2.1.2

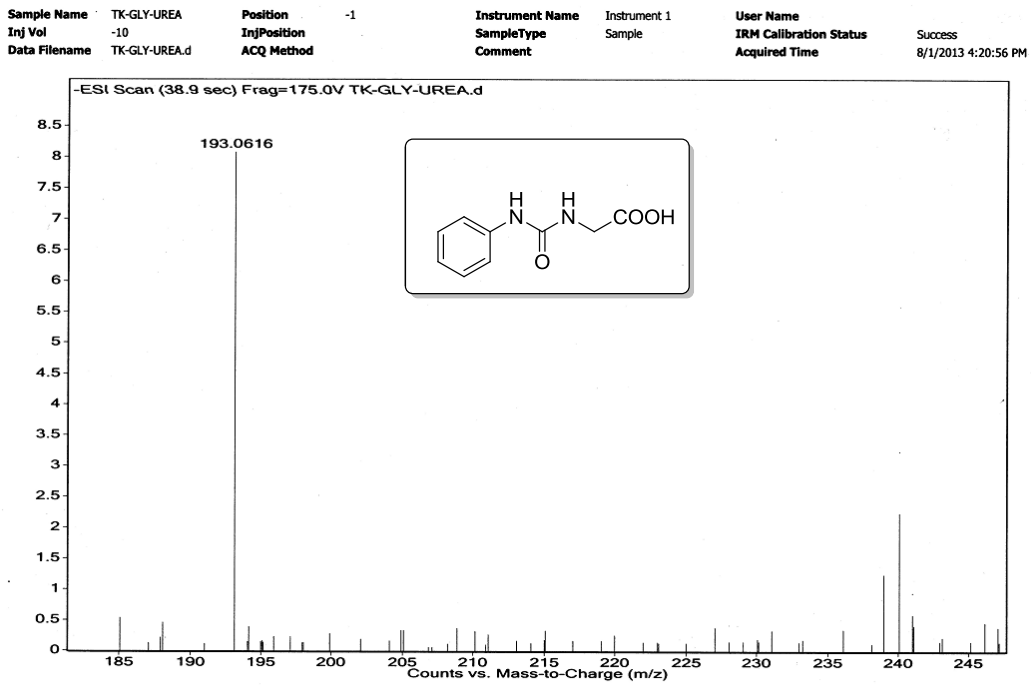


Figure S12. HRMS spectra of compound **3g**, Table 4.2.1.2

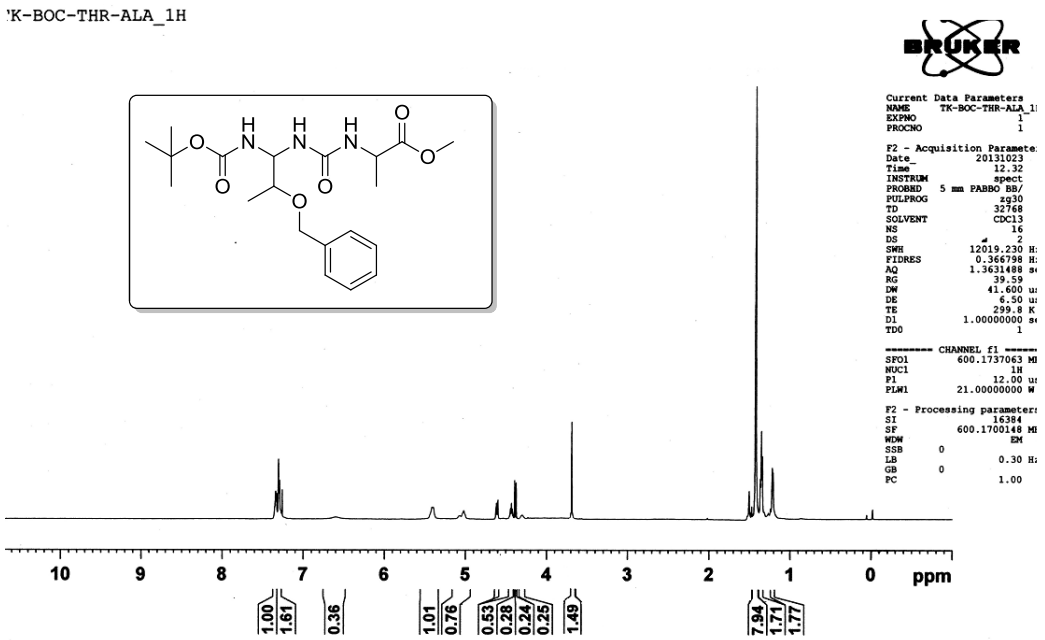


Figure S13. ^{1}H NMR spectra of compound **3t**, Table 4.2.1.2

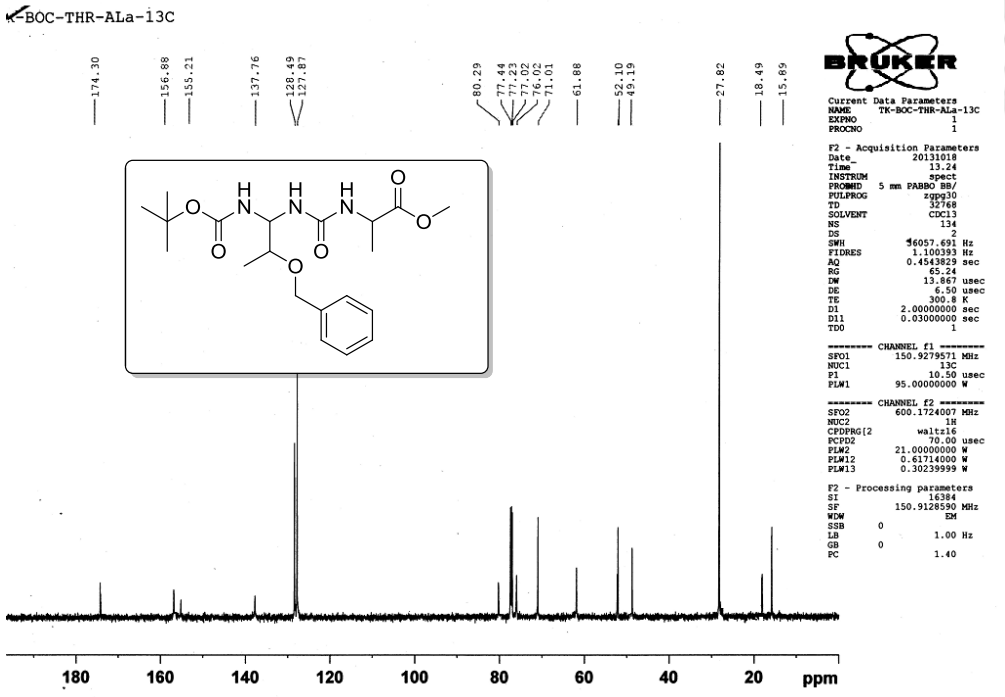


Figure S14. ^{13}C NMR spectra of compound **3t**, Table 4.2.1.2

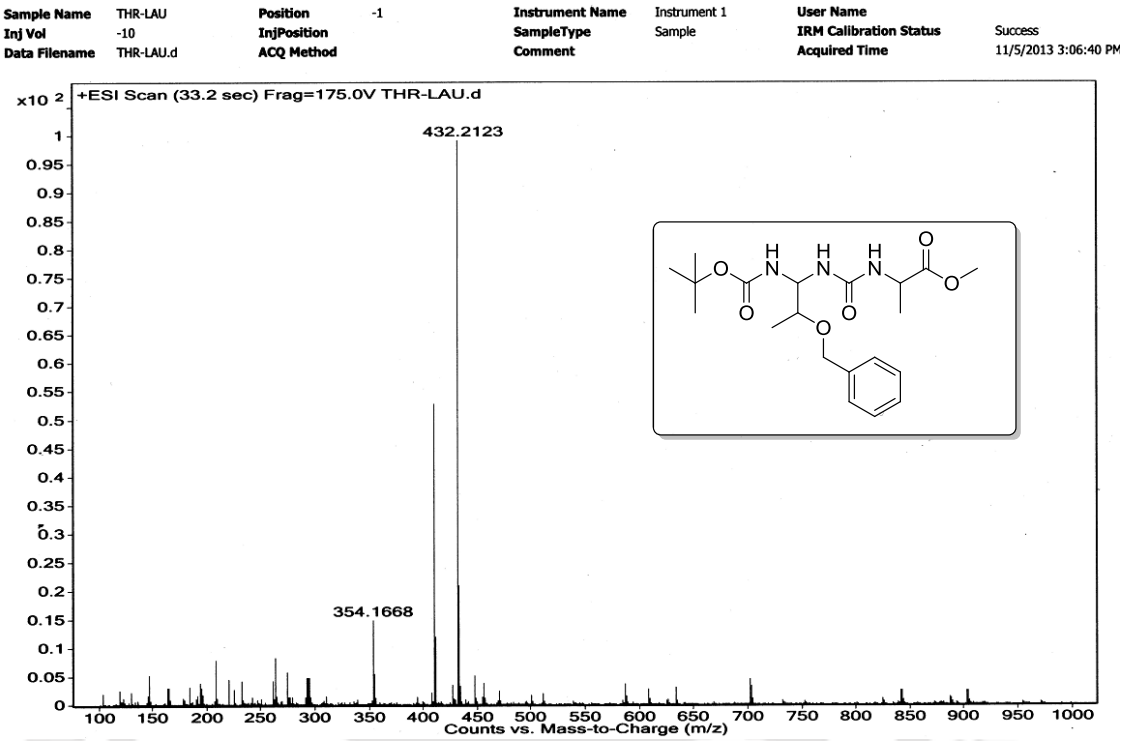


Figure S15. HRMS spectra of compound **3t**, Table 4.2.1.2

4.7.2. HPLC profiles for racemization studies

HPLC profiles of hydroxamic acids, run time 20 min, isocratic gradient of 10 % isopropanol in hexane.

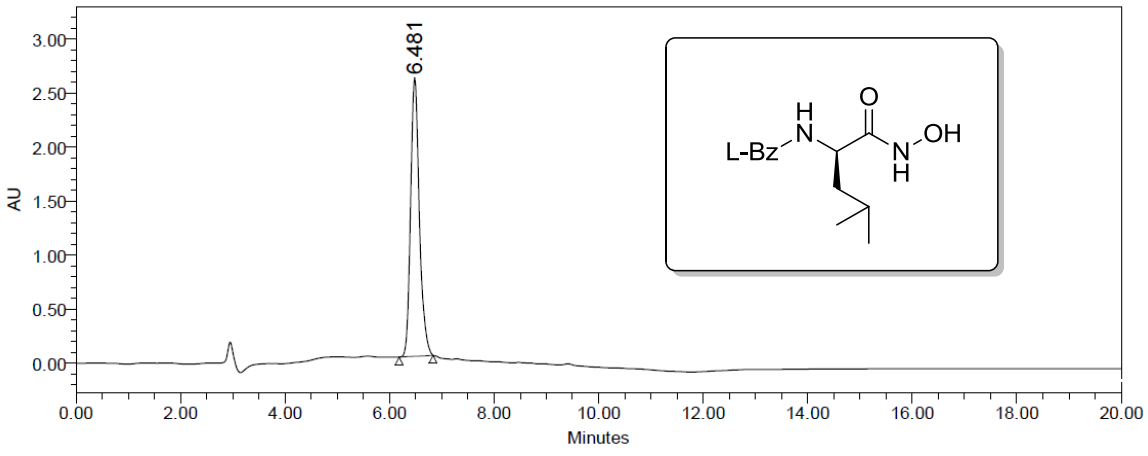


Figure S16. HPLC profile diagram of compound **2f**

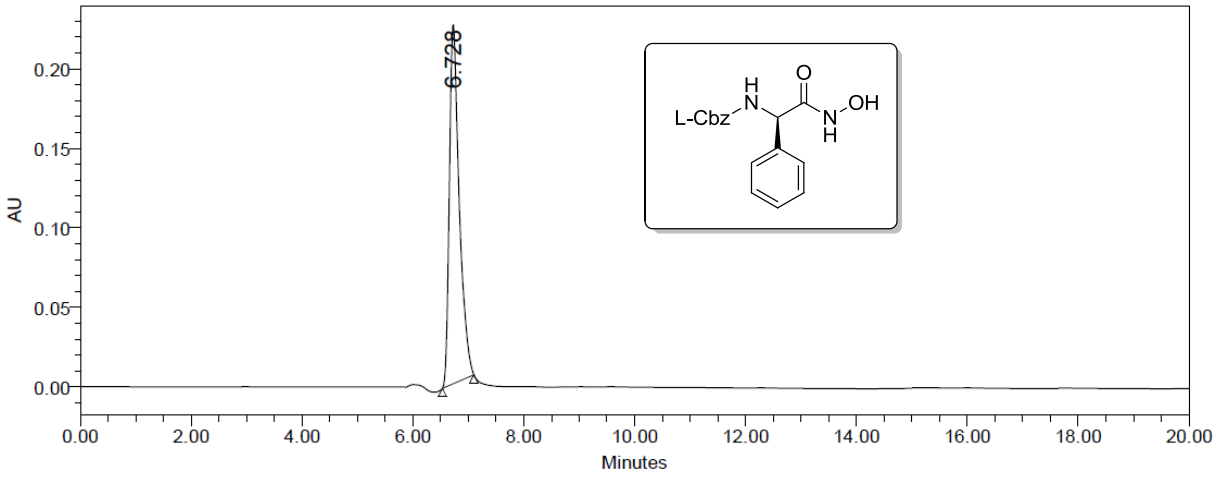


Figure S17. HPLC profile diagram of compound **2h**

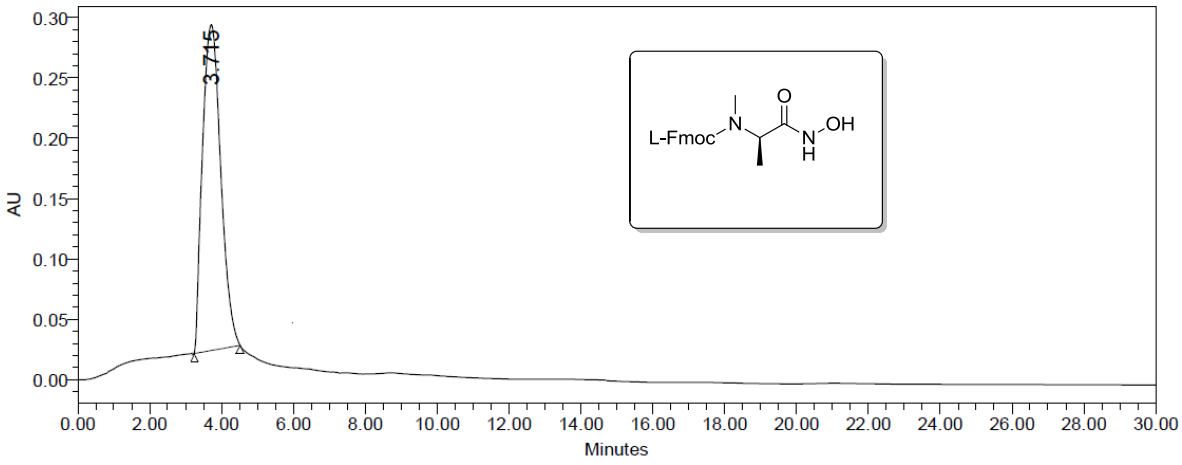


Figure S18. HPLC profile diagram of compound **2m**

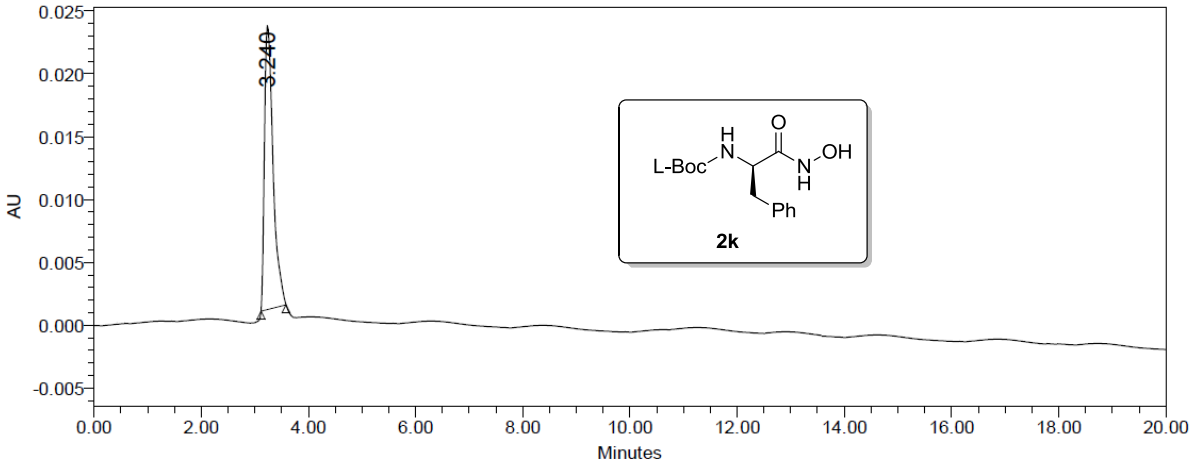


Figure S19. HPLC profile diagram of compound **2o**

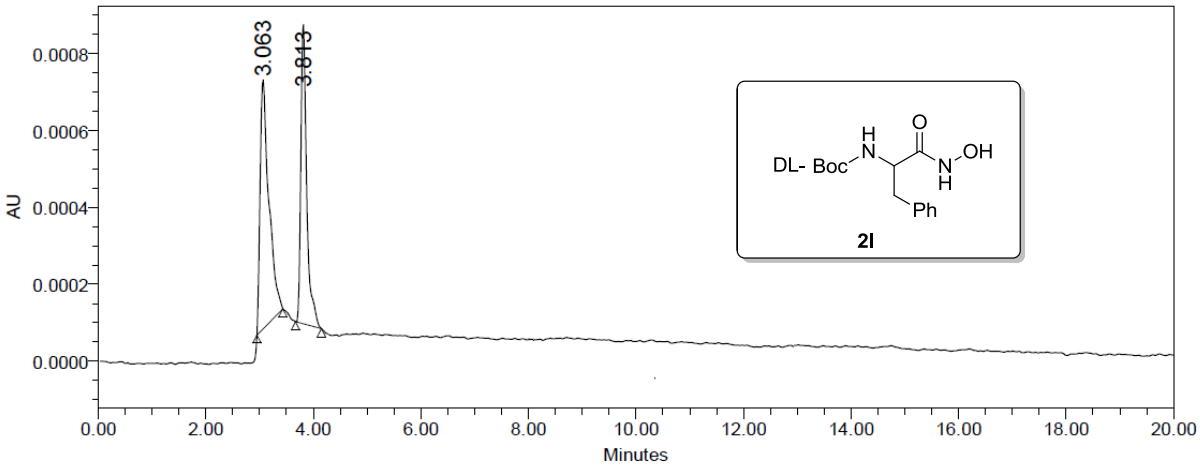


Figure S20. HPLC profile of compound **2p**

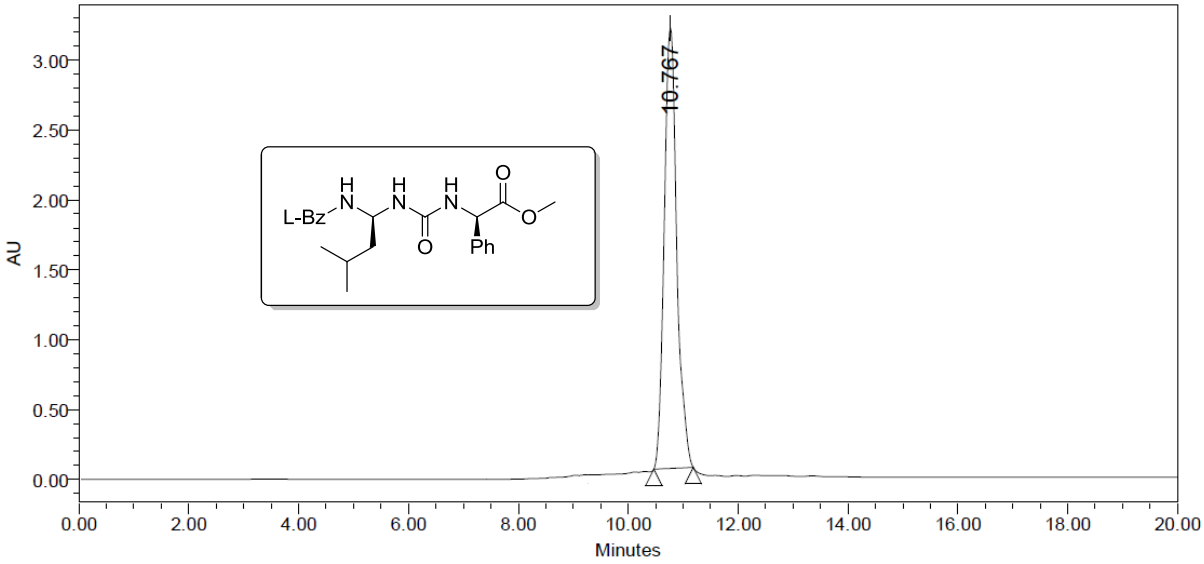


Figure S21. HPLC profile diagram of compound **3k**

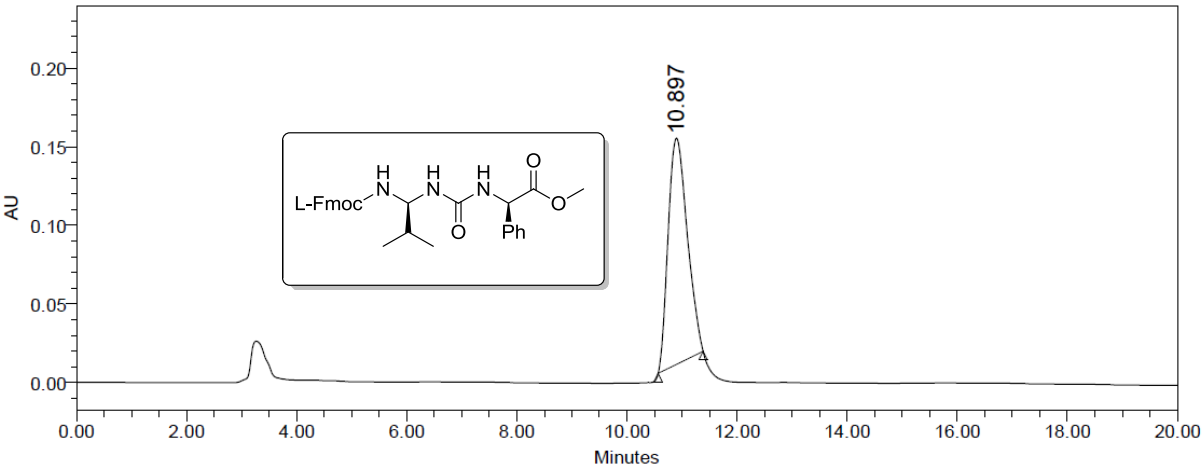


Figure S22. HPLC profile diagram of compound **3o**

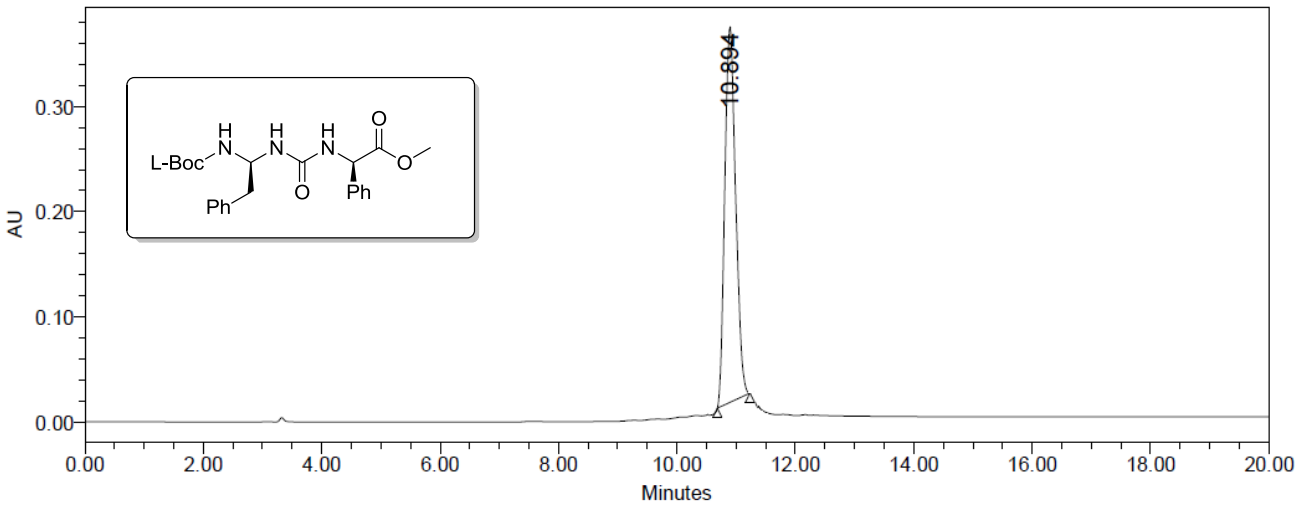


Figure S23. HPLC profile of compound **3r**

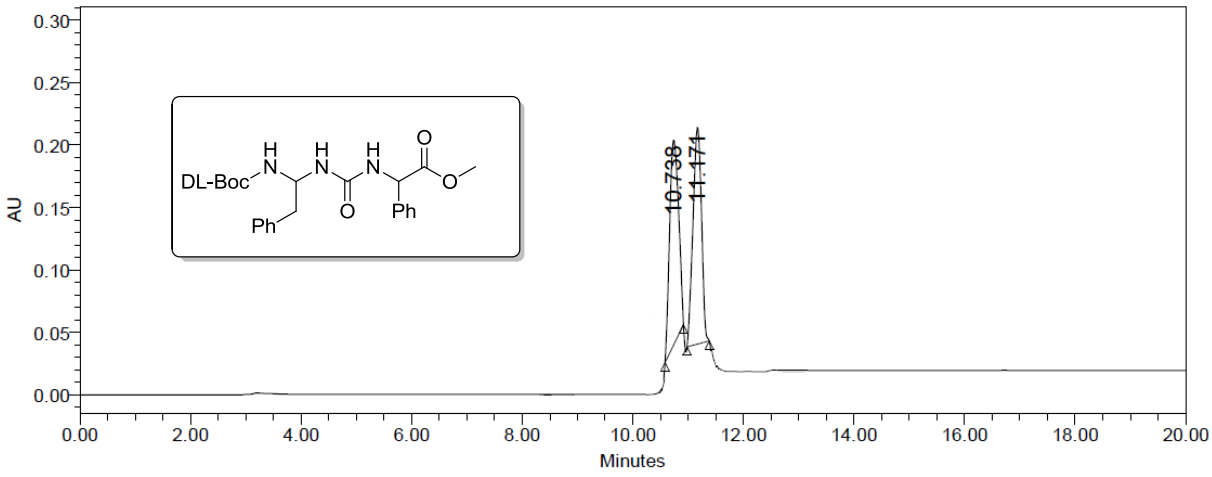


Figure S24. HPLC profile diagram of compound **3s**

Chapter 5: Chemoselective and Stereoselective Synthesis of Peptide Alcohols from Amides via Transamidation

After successful completion of the stereoselective synthesis of amides, peptides, hydroxamates, and peptide ureas, further we wanted to demonstrate the synthesis of another important class of organic compounds, peptide alcohols. Peptides containing a *C*-terminal alcohol function (*C*-terminal peptide alcohols) exhibit a range of biological activities (Chapter 1, section 1.3.3).

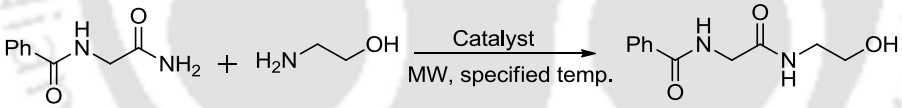
5.1. Synthesis of peptide alcohols

Peptaibols or *C*-terminal peptide alcohols have been synthesized from *C*-terminal carboxylic acids using various reagents (Chapter 1, section 1.7). Although *C*-terminal amides are readily available in usual SPPS, there are no methods in literature on the synthesis of peptide alcohols from amides. We are interested to develop a new methodology for the synthesis of peptide alcohols from *C*-terminal amides.

At first, we performed a reaction between benzoylglycinamide and ethanol amine in presence of PTSA under microwave at 60 °C for 5 h. The upper limit of microwave irradiation was fixed at 150 Watts in a dedicated CEM microwave synthesizer. That resulted the desired product in 58% yield. To improve the yield, the reaction was performed with various solvents and catalysts. PTSA (1 equiv) in methanol or acetonitrile solvents at 80 °C for 3 h was found to be the best combination. Reaction did not progress in water and starting

materials were recovered back. HOBt worked well (*entry 9 and 10, Table 5.1.1*) but not preferred for its explosive nature.[118] Next, we went for optimization of temperature and it was found that the reaction becomes faster at 80 °C and 150 watts, (*entry 15, 18, and 19*). However, 90 min was necessary for completion of the reaction. The catalytic nature was investigated using varied amount of PTSA and found that although it could be recovered back, stoichiometric amount of PTSA was necessary (*entry 15, 16, and 17*). Presence of TFA or acetic acid did not yield any product. Absence of PTSA also did not produce any product. When all the reagents were mixed together, the reaction gave reasonably good yield, but when the amino alcohol was added after the pre-activation (optimized time: 6 min) of the amide with PTSA in methanol or acetonitrile, the yield increased up to 6%.

Table 5.1.1. *Optimization of the reaction conditions*[a]



Entry	Catalyst (mmol)	Solvent	Temp (°C)	Yield (%)
1	PTSA (1.0)	CH_3CN	100	80
2	PTSA (1.0)	Toulene	100	77
3	PTSA (1.0)	Xylene	100	50
4	PTSA (1.0)	H_2O	100	0
5	PTSA (1.0)	DMF	100	0
6	PTSA (1.0)	THF	100	40
7	PTSA (1.0)	MeOH	100	87
8	PTSA (1.0)	MeOH	80	87
9	HOBt (1.0)	CH_3CN	80	73

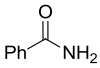
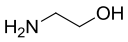
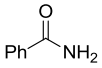
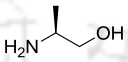
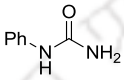
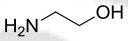
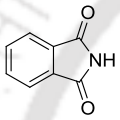
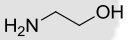
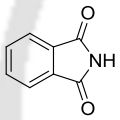
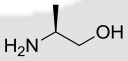
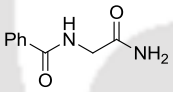
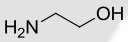
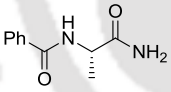
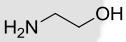
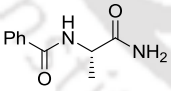
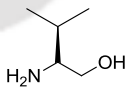
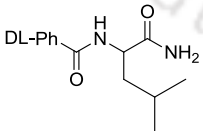
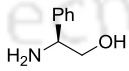
10	HOBt (1.0)	MeOH	80	77
11	Oxyma (1.0)	MeOH	80	10
12	TFA (1.0)	MeOH	80	0
13	1,2,3-Triazole (1.0)	MeOH	80	0
14	PTSA (1.0)	CH_3CN [b]	80	84
15	**PTSA (1.0)**	**MeOH [b]**	**80**	**90**
16	PTSA (0.5)	MeOH [b]	80	47
17	PTSA (0.2)	MeOH [b]	80	20
18	PTSA (1.0)	MeOH [b]	60	58[c]
19	PTSA (1.0)	MeOH [b]	50	32[c]
20	—	CH_3CN	80	0

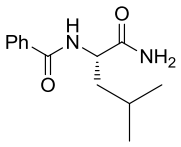
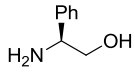
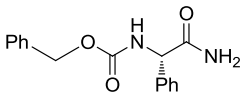
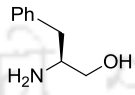
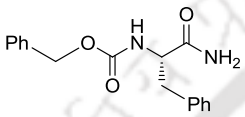
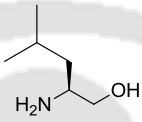
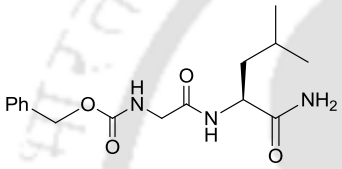
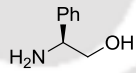
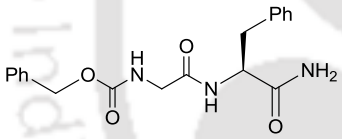
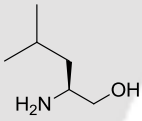
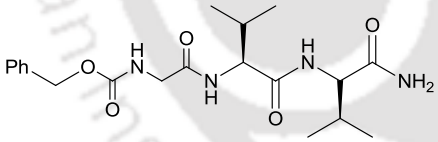
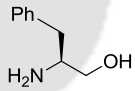
[a]In each case, benzoylglycinamide (1 mmol), aminoalcohol (3 mmol), catalyst (1 mmol), was heated at 80 [o]C (150 watts was fixed as upper limit) for 90 min. Otherwise stated. [b]Aminoalcohol was added after pre-activation of amide (condition for pre-activation: microwave irradation on amide (1 mmol), PTSA (1 mmol) in MeOH or CH_3CN at 80 °C, 150 watts for 6 min). [c]Reaction continued for 6 h.

The above result encouraged us to explore the scope of the reaction with various amino alcohols and with various amides. At first, we synthesized the peptide alcohols from phenyl carboxamide (*entry 1 and 2, Table 5.1.2*) with different amino alcohols; these hydroxyalkyl amides afford a facile route to a variety of heterocycles which are present in many biologically active natural products. We also evaluated the scope of this method under the optimized reaction conditions by reacting with a variety of amino alcohols with ureas (*entry 3*) and secondary amides (*entry 4 and 5*). All the reactions produced good to excellent yields. However, in some cases, reaction temperature was necessary to elevate (80-100 °C).

Finally we successfully applied our method for the preparation of peptide alcohols (*entry 6-12*), dipeptides (*entry 13 and 14*), and a tripeptide alcohol (*entry 15*) starting from various *N*-protected amino acids by solution phase synthesis with good yields (up to 90%) as shown in table 5.1.2.

Table 5.1.2. *Substrate scope for the synthesis of peptide alcohols from amides*

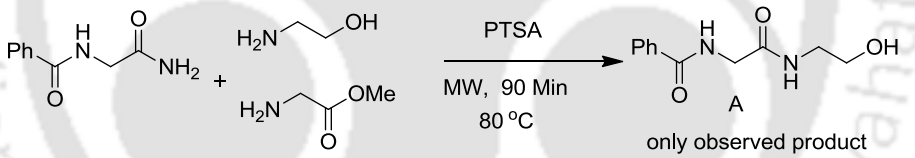
Entry	Amide	Aminoalcohol	Time, temp °C	Racemization %	Yield[a] %
1			2.5 h, 100	-	75
2			3 h, 100	-	77
3			4 h, 100	-	72
4			1.5 h, 80	-	85[b]
5			1.5 h, 80	-	81[b]
6			1.5 h, 80	-	90
7			2.5 h, 80	-	79
8			3 h, 90	n. d.[c]	75
9			3 h, 90	50	71

10			3 h, 90	nd[c]	70
11			3 h, 90	nd[c]	73
12			3 h, 90	nd[c]	71
13			3 h, 90	0.29	70
14			3 h, 90	0.49	70
15			3 h, 90	0.96	68

[a]*In each case, amide (1 mmol), aminoalcohol (3 mmol), catalyst (1 mmol), and methanol was used as solvent.* [b]*Acetonitrile was used as the solvent.* [c]*nd implies "no racemization could be detected by HPLC,* ^{1}H *and* ^{13}C *NMR".*

We have also checked the chemoselectivity of this current protocol by performing a competition experiment, with equivalent amount of both ethanolamine and glycinemethyl ester with benzoylglucinamide (*Scheme 5.2.1*). We observed selectively of one probable product (A). There was no reaction with glycine methylester as shown in Scheme 5.2.1. This

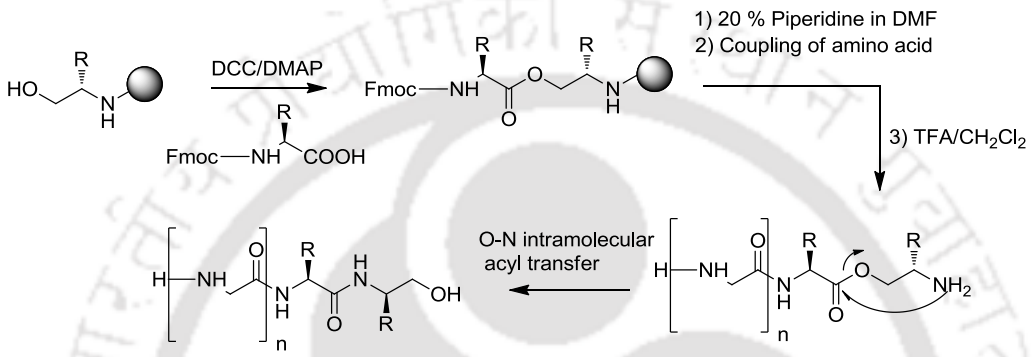
indicates the β-hydroxy group of ethanolamine is involved in the reaction. To be sure of the chemoselectivity, we performed another competition experiment with equivalent amount of both ethanolamine and propyl amine at the same condition. Bz-Gly-$NHCH_2CH_2OH$ was the only observed product also in this case (*Scheme 5.1.1*). Nevertheless, we checked the reaction between benzoylglycinenamide (1 mmol) and glycinemethyl ester (3 mmol) in presence of PTSA (1 mmol) under microwave irradiation. It worked, but low (30%) yield of the product (benzoylglycylglycine methyl ester, Bz-Gly-Gly-OMe) was obtained after 15 h under microwave irradiation (upper limit 150 W) at 80 °C. Prolonged treatment did not improve the yield of the reaction (checked until 24 h).



Scheme 5.1.1. *Chemoselective synthesis of peptide alcohols*

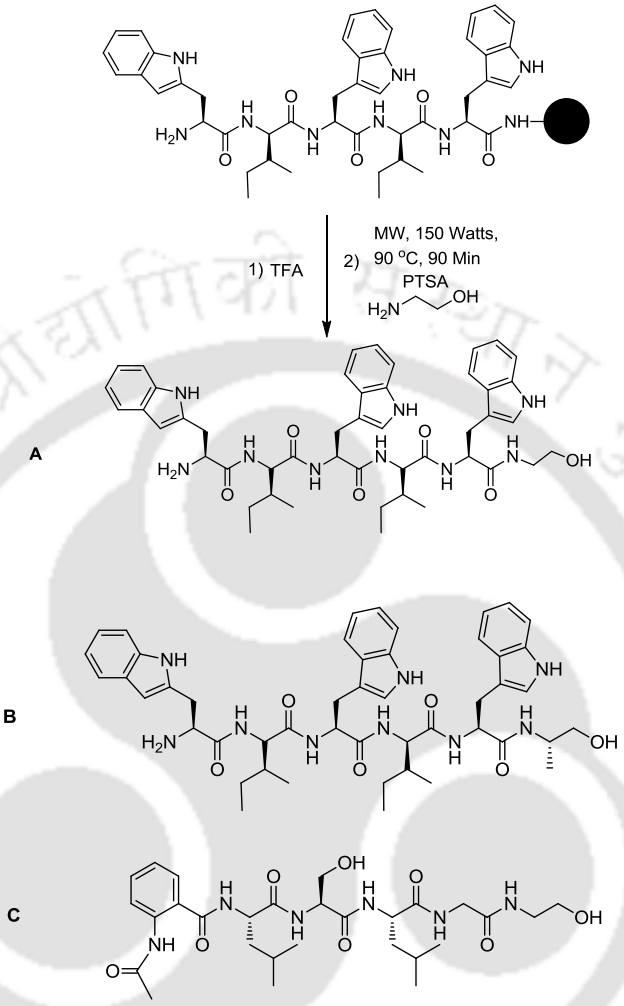
The synthesis of larger *C*-terminal peptide alcohols is difficult, because of non avaliblity of *C*-terminal free carboxylic acid group. Some of the reported SPPS based methods involve the reductive cleavage of a peptide ester linkage,[119] the use of redox-sensitive resins,[120] aminolysis of a peptide ester linkage with a β-amino alcohol,[121] cleavage of standard peptide alcohol ester linkage,[122] tetrahydropyranyl based linkers,[123] hemisuccinate linkers[124] and polymeric diphenyldiazomethane.[125] Recently, Muriel Amblard and co-workers synthesized

peptide alcohols using an *O* to *N* acyl transfer reaction by a modified resin[126] as shown in (*Scheme 5.1.2*).



Scheme 5.1.2. *Preparation of peptide alcohols*

The scope of this method was extended for the synthesis of larger peptide alcohol from *C*-terminal amide with amino alcohols under the above optimized reaction condition. *C*-terminal amides are the readily available *C*-terminal functionality in usual SPPS, at first we synthesized *C*-terminal amides on Rink amide resin following Fmoc/^{t}Bu orthogonal protection strategy and cleaved from the resine using TFA to obtain a *C*-terminal amide. Amino alcohols were attached to this *C*-terminal amide. We have prepared three peptide alcohols. Those are the fragments of gramicidine derivatives (A and B in *Scheme 5.1.3*) and an anthranilic acid containing α/β-hybrid peptide alcohol (C). The peptides were then purified using preparative HPLC and reacted with suitable beta amino alcohols under microwave irradiation in presence of PTSA to generate good quality peptide alcohols.



Scheme 5.1.3. *Synthesis of peptide alcohols* ***A***, ***B*** *and* ***C****.*

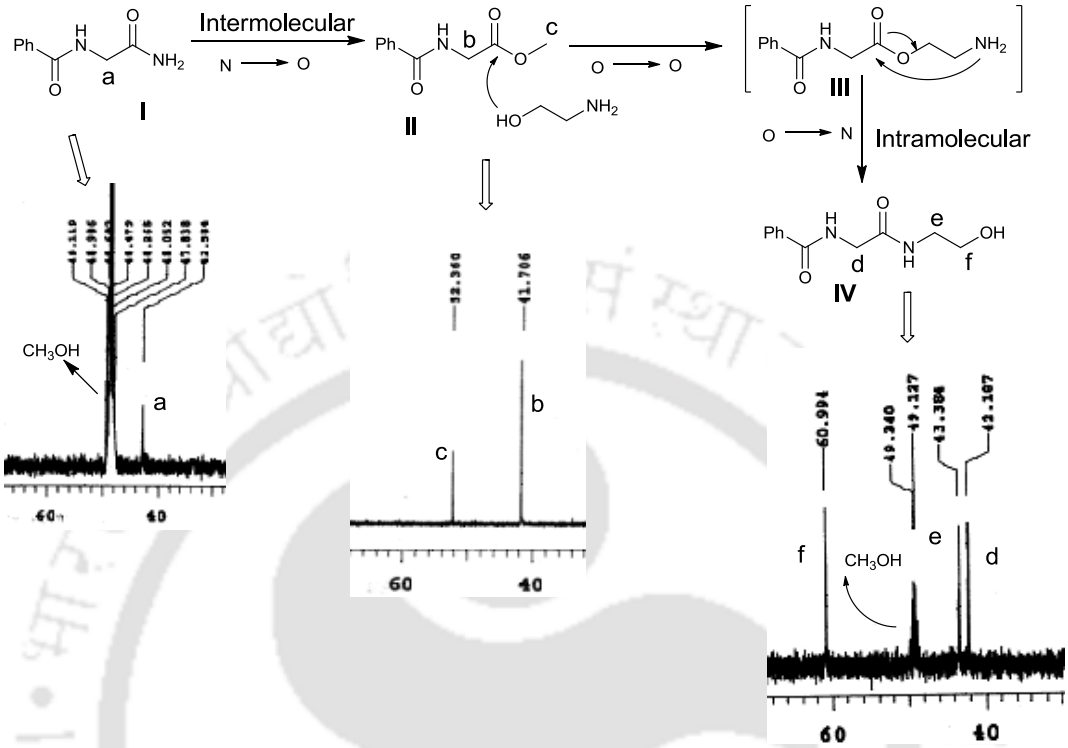
5.1.1. Racemization

We have checked the racemization probability of the current protocol during the peptide alcohol synthesis using HPLC. From the HPLC profiles (Symmetry C8, 5 mm 3.0× 15.0 mm analytical column, linear gradient of 0 to 100% CH_3CN in H_2O with 0.1% formic acid, run for 20 min) of the products revealed no detectable racemization in case of single amino acid derived peptide alcohols (*entries 8, 11, and 12 in Table 5.1.2*). Whereas, in case of peptide alcohols derived from dipeptides (*entry 13 and 14*), the tripeptide (*entry 15*), gramicidine A

derivatives (A and B in *Scheme 5.1.3*), and anthranilic acid containing peptide (C), less than 1.5% racemization were observed.

5.1.2. Plausible reaction mechanism

The plausible reaction mechanism can be depicted as shown in the Scheme 5.2.1.1, based on products. The desired product formation involves the intermolecular *N* to *O* acyl migration followed by subsequent intramolecular *O* to *N* acyl migration. It was confirmed from the data of NMR by the reaction of benzoylglycinamide in methanol in presence of PTSA. We have recorded ^{13}C NMR spectra of benzoylglycinamide (**VII**) and observed the peak at 42.5 ppm (**a**), which corresponds to the -CH_2- of benzoylglycinamide. We initiated the reaction with benzoylglycinamide and PTSA in absence of ethanolamine in methanol at 80 °C and recorded the ^{13}C NMR of reaction mixture after 6 min, we observed two new peaks at 52.3 and 41.7 (**b** and **c**), which corresponds to the methylester of benzoylglycine (V**III**). Finally ethanolamine was added to the above reaction mixture and C^{13} NMR was recorded after 90 min. In this case, we observed **b** and **c** peaks are replaced by three new peaks at 42.1, 43.3 and 60.9 ppm (**d**, **e,** and **f**) which corresponds to the expected product (**X**, *Scheme 5.1.2.1*). However, in absence of methanol, the intermediate III may get generated by intermolecular *N* to *O* acyl migration directly.



Scheme 5.1.2.1. *Plausible reaction mechanism.*

5.2. Conclusion

In summary, we have developed a novel, efficient, chemoselective, and racemization free method for the preparation of peptide alcohols under milder reaction conditions in presence of commercially available and cost effective PTSA from usually available *C*-terminal functional group in SPPS, amide, under microwave conditions. This method obviates the need of specially modified resin and may find application in industries as well as in academia.

5.3. Experimental section

5.3.1. Materials and methods

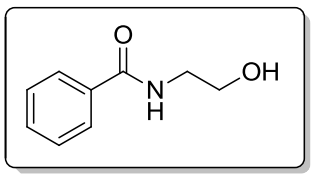
As described in chapter 2 section 2.4.1

5.3.2. General procedure for the preparation of peptide alcohols.

Primary amide (1 mmol) was taken in a pressure glass tube (5 mL) and dissolved in acetonitrile/ methanol (2 mL), para-toluenesulfonic acid (1 mmol) was added and irradiated using a CEM discover microwave reactor at 150 W, 80 °C for 6 min for pre-activation. Then, the amino alcohol (3 mmol) was added to the same vial and again irradiated at 80 ºC for 90 min keeping the upper limit of microwave energy at 150 W. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary evaporator and the product was purified on silica gel column chromatography using hexane and ethyl acetate.

5.4. Characterization data

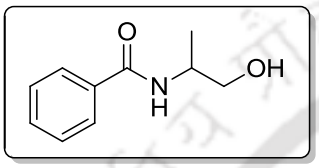
1) *N*-(2-hydroxyethyl)benzamide (product of entry 1, Table 5.1.2)



White solid, Yield 75%; Mp. 61-62 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.73 (d, *J* = 7.6 Hz, 2H), 7.44-7.42 (t, *J* = 6.8 Hz, 1H), 7.36-7.32 (t, *J* = 7.2 Hz, 2H), 3.74-3.72 (t, *J* = 4.8 Hz, 2H),

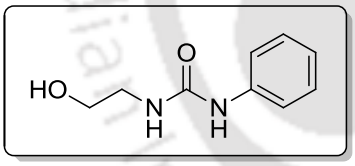
3.55-3.53 (t, *J* = 4.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 168.9, 134.1, 131.7, 128.6, 127.1, 61.8, 42.9; FT-IR (KBr) 3378, 2975, 1695, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$calculated for $C_9H_{12}NO_2$ is 166.0868, found 166.0863.

2) *N*-(1-hydroxypropan-2-yl)benzamide (product of entry 2, Table 5.1.2)



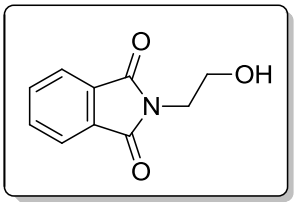
White solid, Yield 77%; Mp. 101-102 °C; ^{1}H NMR (400MHz, MeOH-D_4) δ 7.73-7.25 (m, 5H), 6.77 (br, 1H), 4.20-4.14 (m, 1H), 3.67-3.63 (dd, J = 4.8 Hz, 1H), 3.56-3.51 (dd, *J* = 5.6 Hz, 1H), 1.16-1.15 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, MeOH-D_4) δ 170.3, 133.3, 131.9, 128.5, 127.1, 66.0, 48.0, 17.0; FT-IR (KBr) 3405, 2989, 1669, 1565 cm^{-1}; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{10}H_{14}NO_2$ is 180.1025 found 180.1032.

3) 1-(2-hydroxyethyl)-3-phenylurea (product of entry 3, Table 5.1.2)



White solid, Yield 72%; Mp. 121-122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (br, 1H), 7.27-7.17 (m, 4H), 6.98-6.96 (t, *J* = 7.2 Hz, 1H), 5.9 (br, 1H), 3.58-3.55 (t, *J* = 5.2 Hz, 2H), 3.26-3.24 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, few drops of CD_3OD for solubility) δ 157.2, 139.2, 139.1, 128.8, 122.6, 119.5, 119.3, 61.7, 42.3; FT-IR (KBr) 3116, 2991, 2872, 1695, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$calculated for $C_9H_{13}N_2O_2$is 181.0977 found 181.0974.

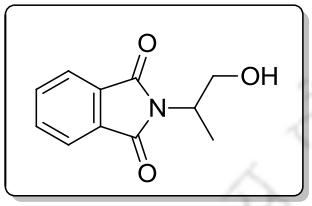
4) 2-(2-hydroxyethyl)isoindoline-1,3-dione (product of entry 4, Table 5.1.2)



White solid, Yield 85%, Mp. 122-123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84-7.82 (m, 2H), 7.71-7.69 (m, 2H), 3.89-3.84 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 134.2, 132.1, 123.5,

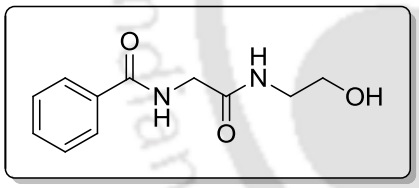
61.0, 40.9; FT-IR (KBr) 3452, 2991,1719, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$calculated for $C_{10}H_{10}NO_3$is 192.0661 found 192.0662.

5) 2-(1-hydroxypropan-2-yl)isoindoline-1,3-dione (product of entry 5, Table 5.1.2)



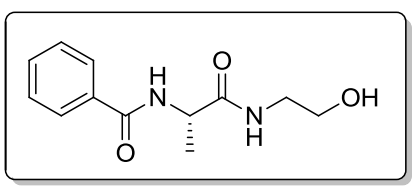
White solid, Yield 81%; Mp. 110-112 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.72 (m, 2H), 7.68-7.61 (m, 2H), 4.45-4.40 (m, 1H), 4.00-3.96 (dd, *J* = 8 Hz, 1H), 3.80-3.76 (dd, *J* = 8.4 Hz, 1H), 1.36-1.34 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 134.1 131.9, 123.3, 63.9, 49.6; FT-IR (KBr) 3487, 2945, 1769, 1689, 1597 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$calculated for $C_{11}H_{12}NO_3$ is 206.0817 found 206.0985.

6) *N*-(2-((2-hydroxyethyl)amino)-2-oxoethyl)benzamide (product of entry 6, Table 5.1.2)



White solid, Yield 90%; Mp. 125-127 °C; ^{1}H NMR (400 MHz, $CDCl_3$ and few drops of MeOH-D_4) δ 7.80-7.78 (d, *J* = 8 Hz, 2H), 7.49-7.46 (t, *J* = 7.2 Hz, 1H), 7.41-7.38 (t, *J* = 7.6 Hz, 2H), 4.05 (s, 2H), 3.65-3.64 (t, *J* = 4.8 Hz, 2H), 3.37-3.36 (t, *J* = 3.6 Hz, 2H); ^{13}C NMR (100MHz, $CDCl_3$ and few drops of MeOH-D_4) δ 170.2, 168.6, 133.2, 132.1, 128.6, 127.3, 60.9, 43.3, 42.1; FT-IR (KBr) 3437, 2925, 1640, 1630, 1538 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{11}H_{14}N_2NaO_3$ is 245.0902 found 245.0902.

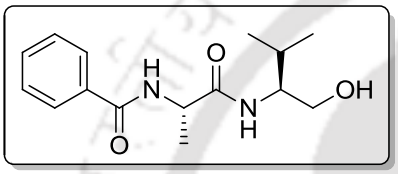
7) (S)-*N*-(1-((2-hydroxyethyl)amino)-1-oxopropan-2-yl)benzamide (product of entry 7, Table 5.1.2)



White solid, Yield, 79%; Mp. 128-129 °C; ^{1}H NMR (400 MHz, MeOH-D_4) δ 7.78-7.34 (m, 5H), 4.49-4.43 (m, 1H), 3.51-3.49 (d, *J*= 5.6 Hz, 2H), 3.23-3.16 (m,

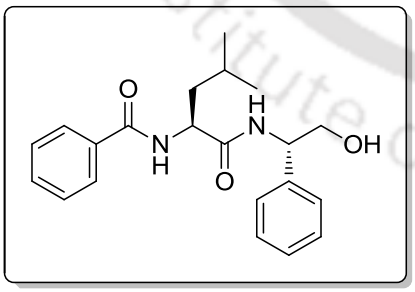
2H), 1.37-1.35 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, MeOH-D_4) δ 175.6, 170.1, 135.3, 133.0, 129.6, 128.7, 61.6, 51.3, 43.1, 18.4; FT-IR (KBr) 3438, 3005, 1696, 1645, 1554 cm^{-1}; FT-IR (KBr, ν/cm^{-1}) 1622, 3300, 3425; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_{12}H_{17}N_2O_3$ is 237.1239 found 237.1250. $[\alpha]^{27}{}_D$ = - 18.00 (c = 1, $CHCl_3$).

8) *N*-((S)-1-(((S)-1-hydroxy-3-methylbutan-2-yl)amino)-1-oxopropan-2-yl)benzamide (product of entry 8, Table 5.1.2)



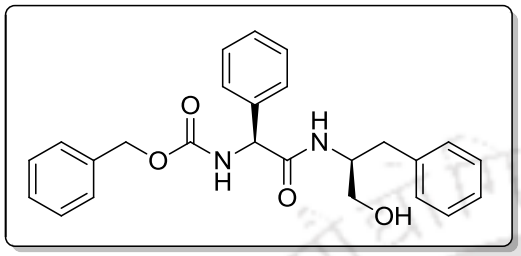
White solid, Yield 75%; Mp. 102-103 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J* = 7.2 Hz, 2H), 7.42-7.30 (m, 3H), 4.78-4.74 (m, 1H), 3.64-3.57 (m, 3H), 1.83-1.80 (m, 1H), 1.42-1.41 (d, *J* = 7.2 Hz, 3H), 0.83-0.79 (dd, *J* = 6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 167.7, 133.7, 131.9, 128.6, 127.3, 63.2, 57.4, 49.8, 29.0, 18.9, 18.8; FT-IR (KBr) 3303, 2962, 1639, 1651, 1538 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$calculated for $C_{15}H_{22}N_2NaO_3$is 301.1528 found 301.1531. $[\alpha]^{27}{}_D$ = - 41.80 (c = 1, THF).

10) *N*-((S)-1-(((S)-2-hydroxy-1-phenylethyl)amino)-4-methyl-1-oxopentan-2-yl)benzamide (product of entry 10, Table 5.1.2)



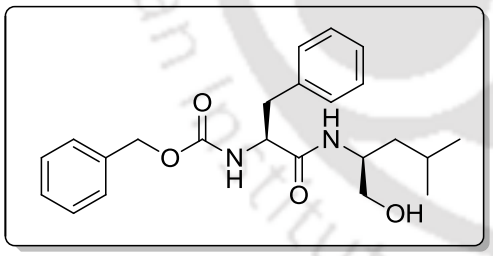
White solid, Yield 70%; Mp. 120-121 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.12 (m, 15H), 6.03-6.01 (s, 1H), 5.92 (s, 1H), 5.09-5.07 (d, *J* = 8 Hz, 1H), 4.13-4.10 (m, 1H), 3.57-3.56 (dd, *J* = 8.4 Hz, 1H), 3.50-3.48 (dd, *J* = 7.2 Hz, 1H), 2.88-2.80 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ and few drops of MeOH-D_4) δ 172.8, 168.1, 139.0, 133.6, 131.8, 128.5, 127.6, 127.4, 127.3, 127.2, 126.8, 65.6, 56.1, 52.3, 40.9, 24.8, 22.7, 22.2; FT-IR (KBr) 3424, 2926, 1666, 1635, 1541 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$calculated for $C_{21}H_{27}N_2O_3$ is 355.2022 found 355.2024. $[\alpha]^{27}{}_D$ = - 10.20 (c = 1, THF).

11) Benzyl ((S)-2-(((S)-1-hydroxy-3-phenylpropan-2-yl)amino)-2-oxo-1-phenylethyl)carbamate (product of entry 11, Table 5.1.2)



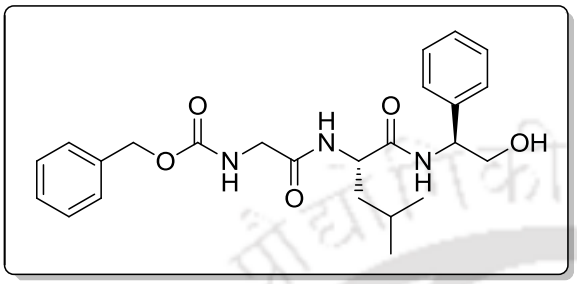
White solid, Yield 73%; Mp. 142-143 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.09 (m, 15H), 6.31 (s, 1H), 6.05-6.03 (d, *J* = 6 Hz, 1H), 5.06-5.04 (d, *J* = 8 4Hz, 2H), 4.11-4.07 (m, 1H), 3.51-3.45 (m, 2H), 2.86-2.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ and few drops of MeOH-D_4), δ 170.4, 156.1, 137.8, 137.7, 136.1, 129.3, 129.2, 129.0, 128.6, 128.5, 128.4, 128.3, 128.1, 127.2, 126.5, 67.2, 62.9, 60.6, 53.3, 36.7; FT-IR (KBr) 3389, 2965, 1685, 1644, 1575 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{25}H_{27}N_2O_4$ is 419.1971 found 419.1970. $[\alpha]^{27}{}_D$ = + 23.40 (c = 1, THF).

12) Benzyl ((S)-1-(((S)-1-hydroxy-4-methylpentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (product of entry 12, Table 5.1.2)



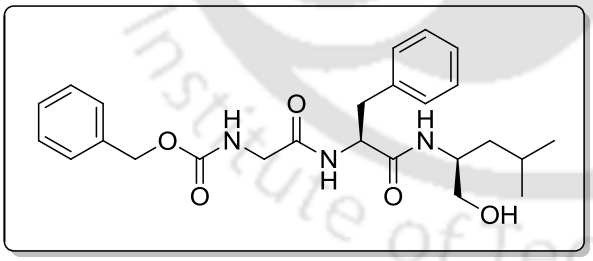
White solid, Yield 71%; Mp. 130-131 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.18 (m, 10H), 5.83 (s, 1H), 5.50 (s, 1H), 5.01 (s, 2H), 4.36-4.33 (m, 1H), 3.91-3.88 (m, 1H), 3.41-3.38 (dd, *J* = 7.2 Hz, 1H), 3.31-3.29 (dd, *J* = 8 Hz, 1H), 3.13-3.08 (dd, *J* = 6 Hz, 1H), 2.99-2.94 (dd, *J* = 8 Hz, 1H), 1.46-1.41 (m, 2H), 1.22-1.18 (m, 1H), 0.83-0.82 (d, *J* = 6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 156.2, 136.7, 136.1, 129.4, 128.7, 128.6, 128.2, 128.0, 127.0, 67.0, 65.0, 56.6, 49.9, 39.8, 38.9, 24.7, 23.0, 22.2; FT-IR (KBr) 3312, 2955, 1692, 1651, 1531 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{23}H_{31}N_2O_4$ is 399.2284 found 399.2282. $[\alpha]^{27}{}_D$ = - 19.80 (c = 1, THF).

13) Benzyl (2-(((S)-1-(((S)-2-hydroxy-1-phenylethyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-oxoethyl)carbamate (product of entry 13, Table 5.1.2)



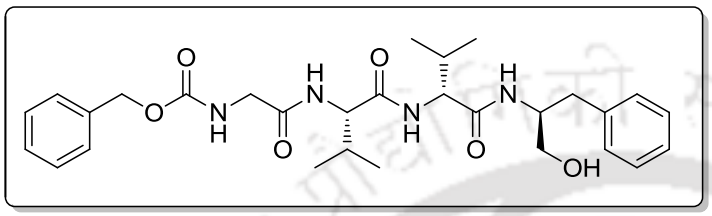
White solid, Yield, 70%; Mp. 150-151 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.22 (m, 10H), 5.08 (s, 2H), 4.95-4.94(d, *J* = 8 Hz, 1H), 4.48-4.46 (m, 1H), 3.85-3.69 (m, 4H), 1.63-1.53 (m, 3H), 0.93-0.89 (d, *J* = 7.6 Hz, 6H), ^{13}C NMR (100 MHz, $CDCl_3$ and few drops of MeOH-D_4) δ 171.1, 170.1, 157.1, 136.3, 136.0, 129.1, 128.4, 128.1, 128.0, 127.8, 126.8, 67.0, 64.4, 54.6, 49.8, 44.1, 39.7, 24.4, 22.8, 22.0; FT-IR (KBr) 3116, 2991, 2872, 1695, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{24}H_{32}N_3O_5$ is 442.2342 found 442.2342. $[\alpha]^{27}{}_D$ = + 1.80 (c = 1, THF).

14) Benzyl (2-(((S)-1-(((S)-1-hydroxy-4-methylpentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamate (product of entry 14, Table 5.1.2)



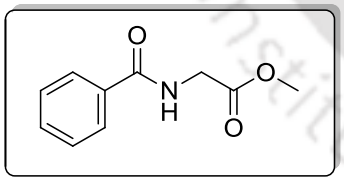
White solid, Yield, 70%; Mp. 142-143 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.15 (m, 10H), 6.78-6.76 (d, *J* = 7.6 Hz, 1H), 6.14 (br, 1H), 5.05 (s, 2H), 4.69-4.67 (m, 1H), 3.92-3.75 (m, 3H), 3.40-3.34 (m, 2H), 3.04-2.96 (m, 4H), 1.50-1.47 (m, 1H), 1.27-1.21 (m, 2H), 0.83-0.82 (d, *J* = 7.6 Hz, 6H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 169.8, 156.9, 136.5, 136.1, 129.2, 128.5, 128.4, 128.2, 128.1, 126.9, 67.0, 64.8, 54.8, 49.9, 39.9, 38.3, 24.7, 23.0, 22.3; FT-IR (KBr) 3116, 2991, 2872, 1695, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{25}H_{33}N_3NaO_5$ is 478.2318 found 478.2317. $[\alpha]^{27}{}_D$ = - 24.60 (c = 1, THF).

15) Benzyl (2-(((S)-1-(((R)-1-(((S)-1-hydroxy-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)carbamate (product of entry 15, Table 5.1.2)



White solid, Yield, 68%; Mp. 120-121 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.16 (m, 10H), 6.48-6.47 (d, *J* = 8 Hz, 1H), 5.59 (s, 1H), 5.09 (s, 2H), 4.19-4.15 (m, 2H), 3.81-3.52 (m, 5H), 2.82(m, 2H), 2.04-2.02 (m, 1H), 1.85-1.80 (m, 2H). 1.25-1.22 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 172.7, 169.7, 157.0, 137.7, 136.2, 129.3, 128.7, 128.7, 128.6, 128.4, 126.7, 67.3, 63.4, 60.3, 57.0, 52.9, 44.6, 37.1, 31.2, 19.8, 19.0, 17.9, 16.6; FT-IR (KBr) 3116, 2991, 2872, 1695, 1635, 1524 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{29}H_{41}N_4O_6$ is 541.3026 found 541.3036. $[\alpha]^{27}{}_D$ = - 20.00 (c = 0.25, THF).

33) Benzoylamino-acetic acid methyl ester (9b, Table 3.2.1)



White solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80-7.78 (d, *J* = 7.6 Hz, 2H), 7.50-7.47 (t, *J* = 7.2 Hz, 1H), 7.41-7.39 (t, *J* = 7.6 Hz, 2H), 6.56 (br, 1H), 4.20-4.19 (d, *J* = 5.2 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 166.2, 132.6, 130.2, 128.2, 127.0, 51.7, 44.0; FT-IR (KBr) 3421, 2910, 1731, 1642 cm^{-1}; HRMS (ESI) m/z $[M+Na]^+$ calculated for $C_{10}H_{11}NNaO_3$ is 216.0637 found 216.0633.

5.5. Selected spectra

5.5.1. Copies of NMR (^{1}H and ^{13}C) and HRMS spectra:

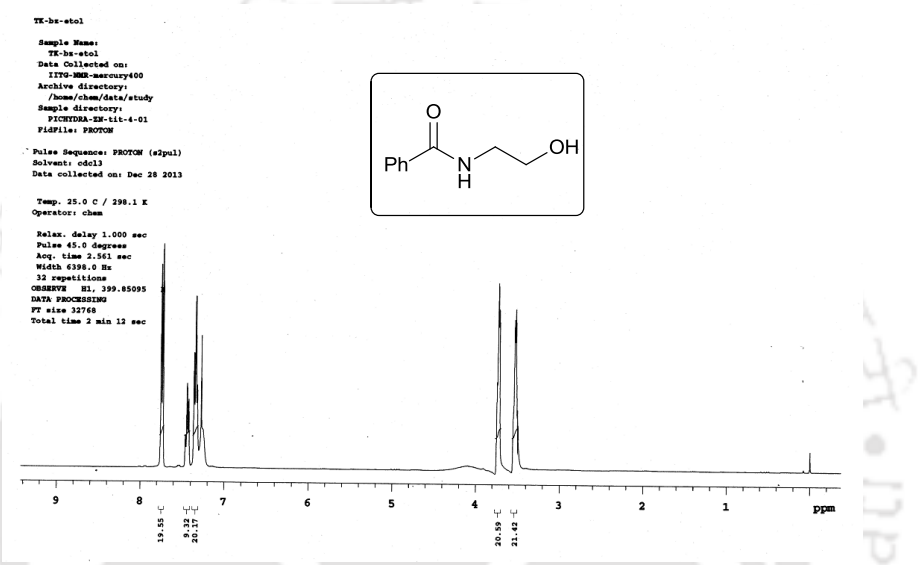


Figure S1. ^{1}H NMR spectra of the product of entry 1 in Table 5.3.2

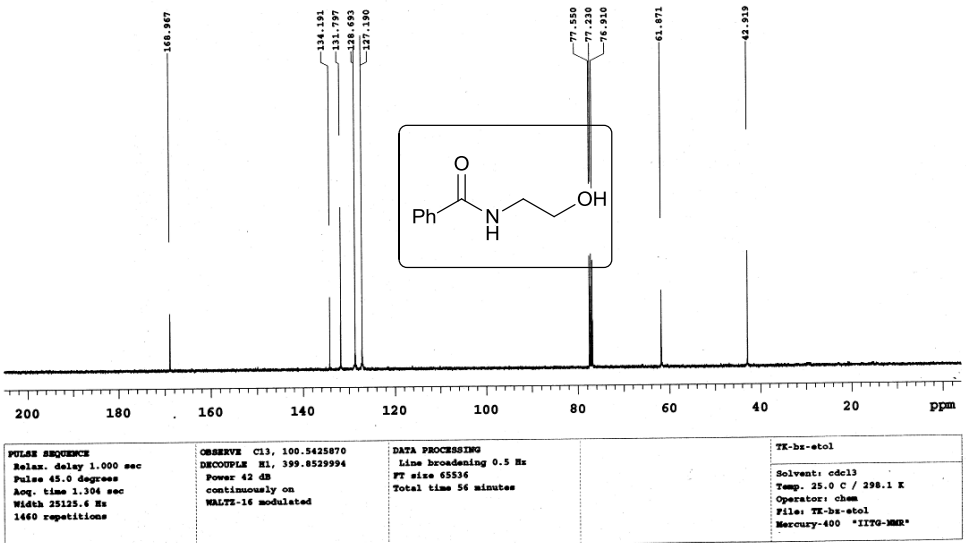


Figure S2. ^{13}C NMR spectra of the product of entry 1 in Table 5.3.2

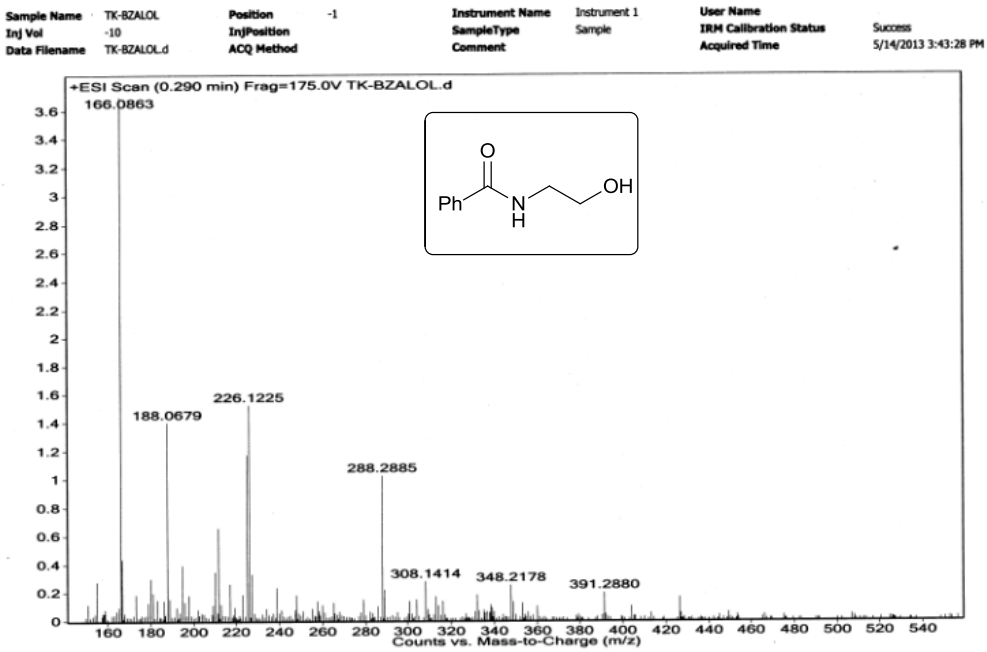


Figure S3. HRMS spectra of the product of entry 1in Table 5.3.2

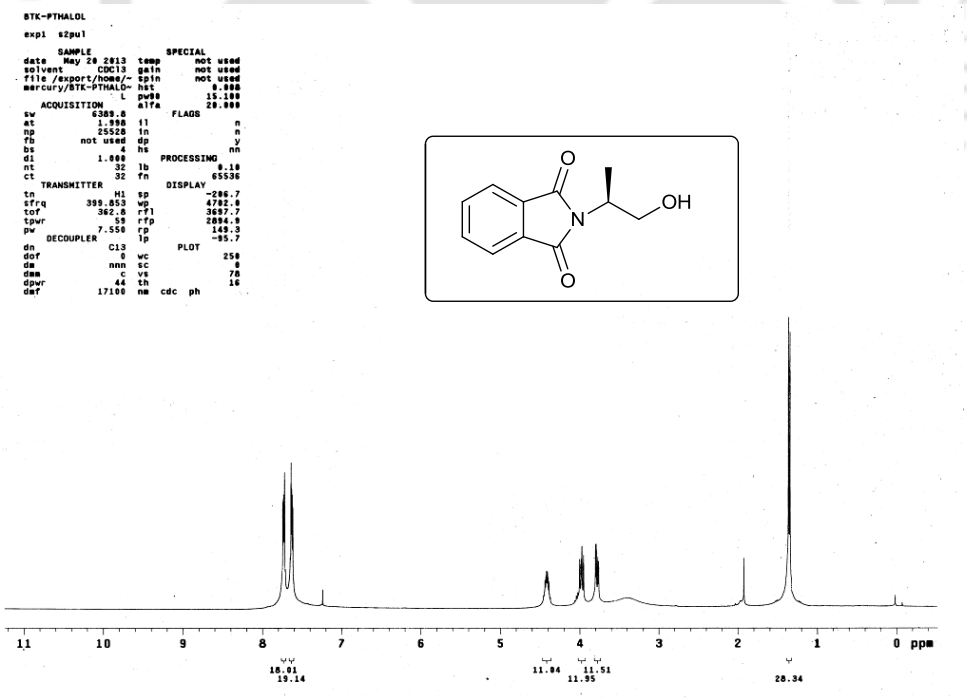


Figure S4. ^{1}H NMR spectra of the product of entry 5 in Table 5.3.2

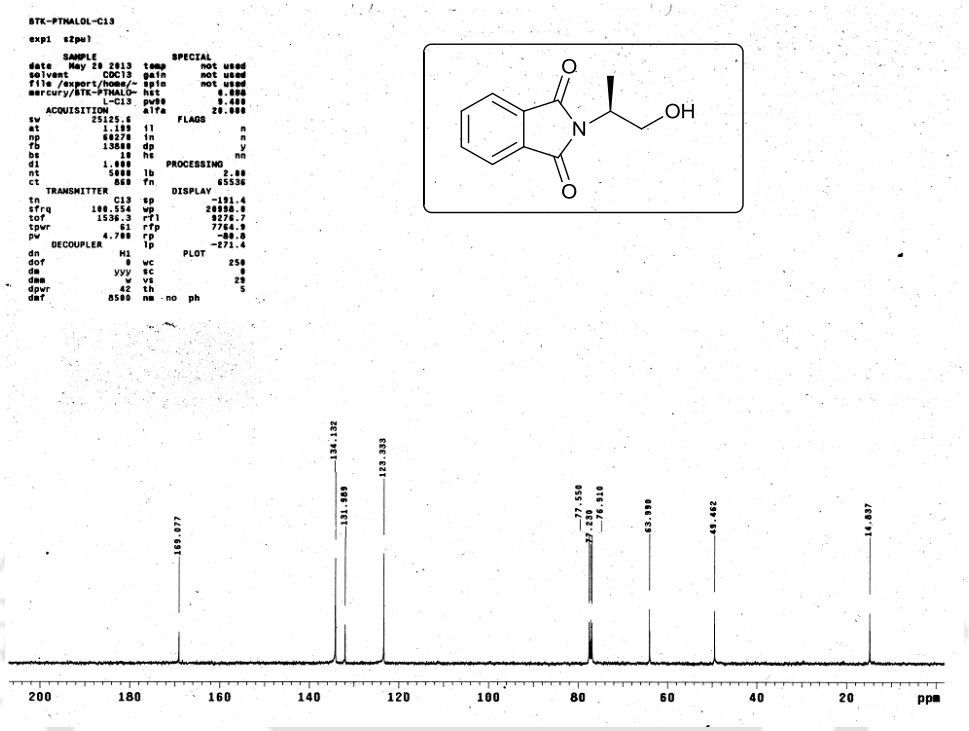


Figure S5. ^{13}C NMR spectra of the product of entry 5 in Table 5.3.2

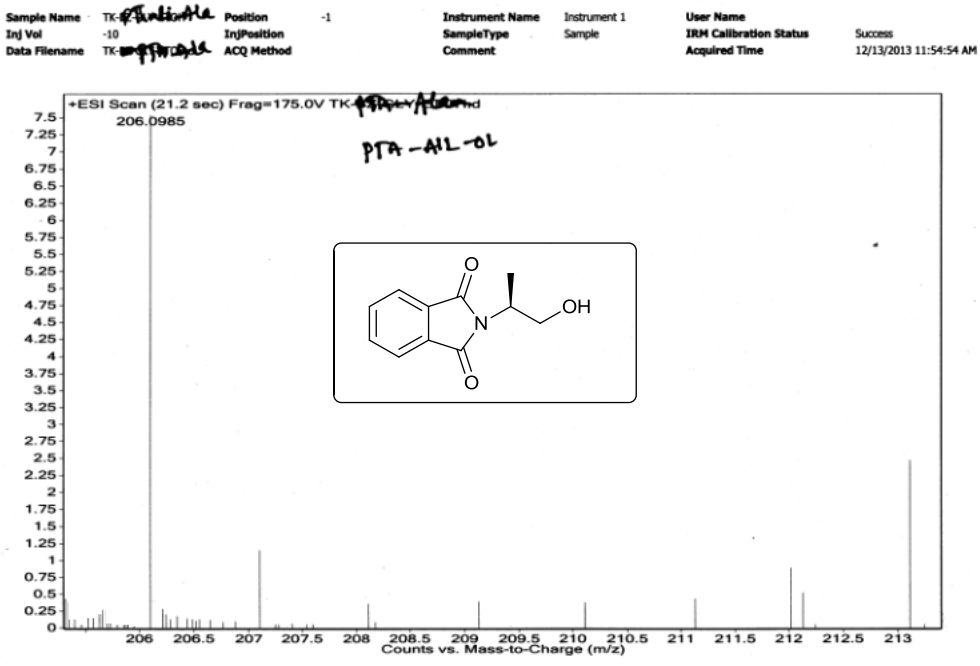


Figure S6. HRMS spectra of the product of entry 5 in Table 5.3.2

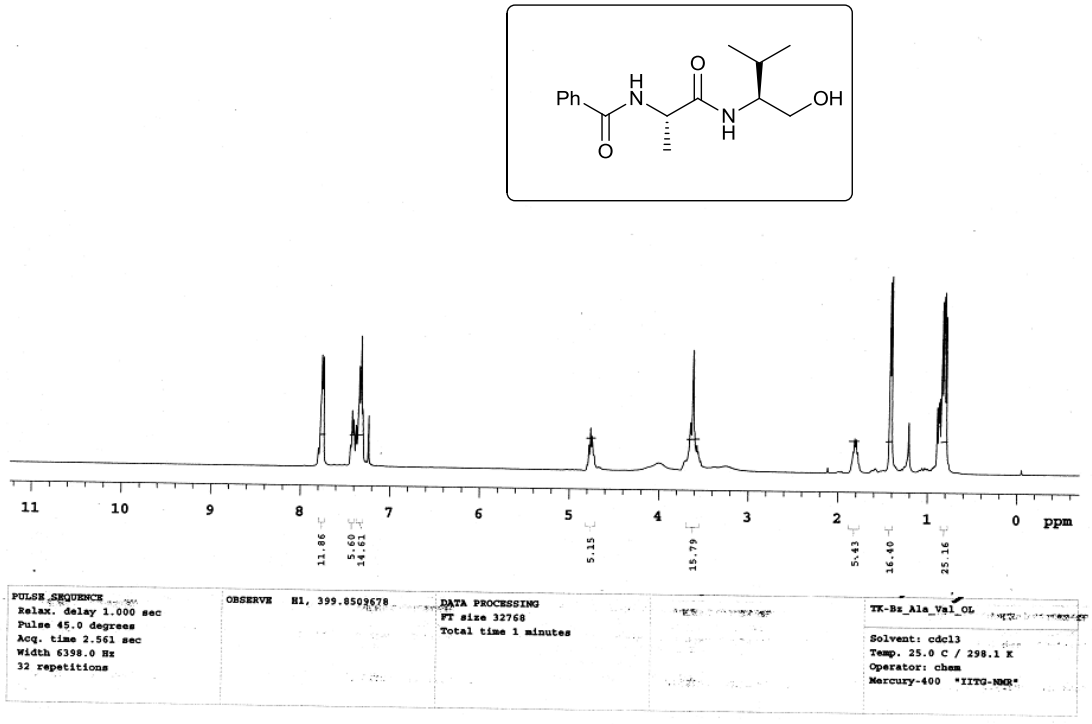


Figure S7. ^{1}H NMR spectra of the product of entry 8 in Table 5.3.2

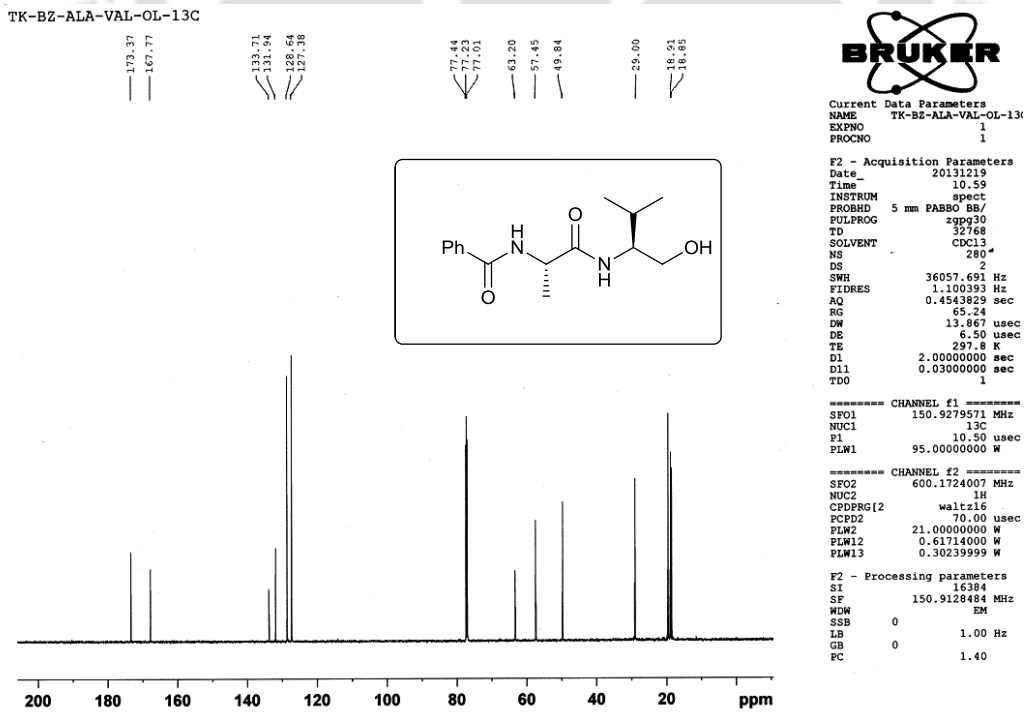


Figure S8. ^{13}C NMR spectra of the product of entry 8 in Table 5.3.2

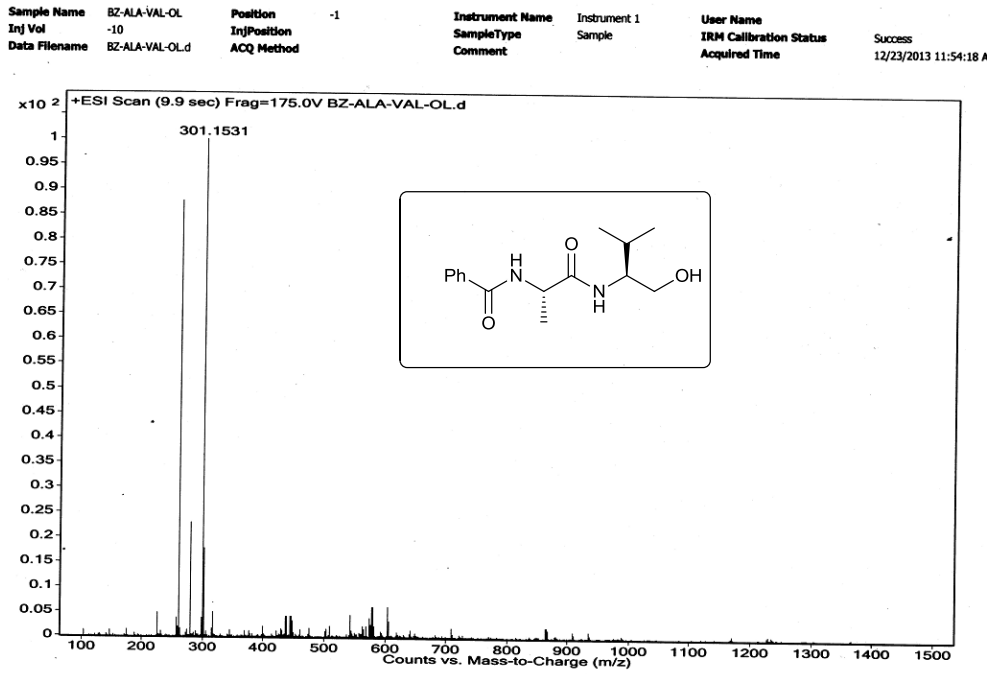


Figure S9. HRMS spectra of the product of entry 8 in Table 5.3.2

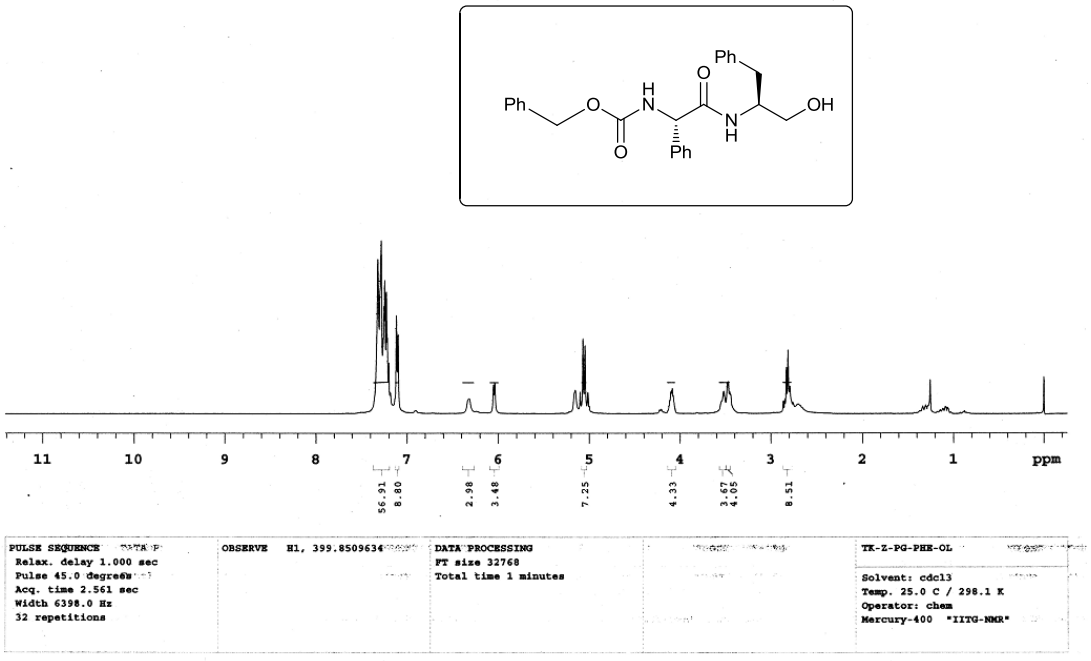


Figure S10. ^{1}H NMR spectra of the product of entry 11 in Table 5.3.2

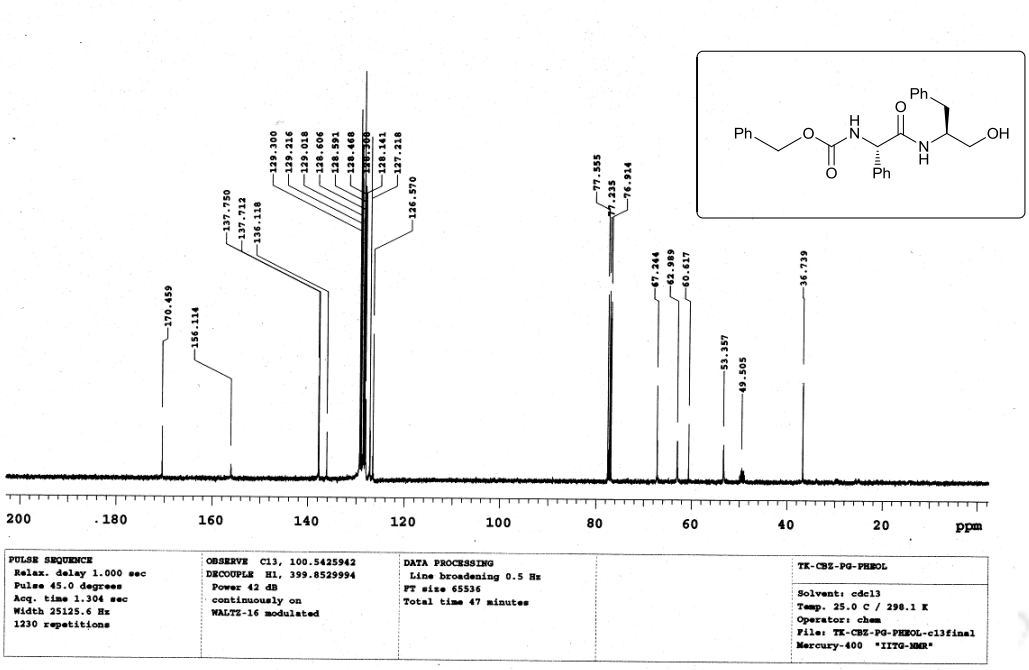


Figure S11. ^{13}C NMR spectra of the product of entry 11 in Table 5.3.2

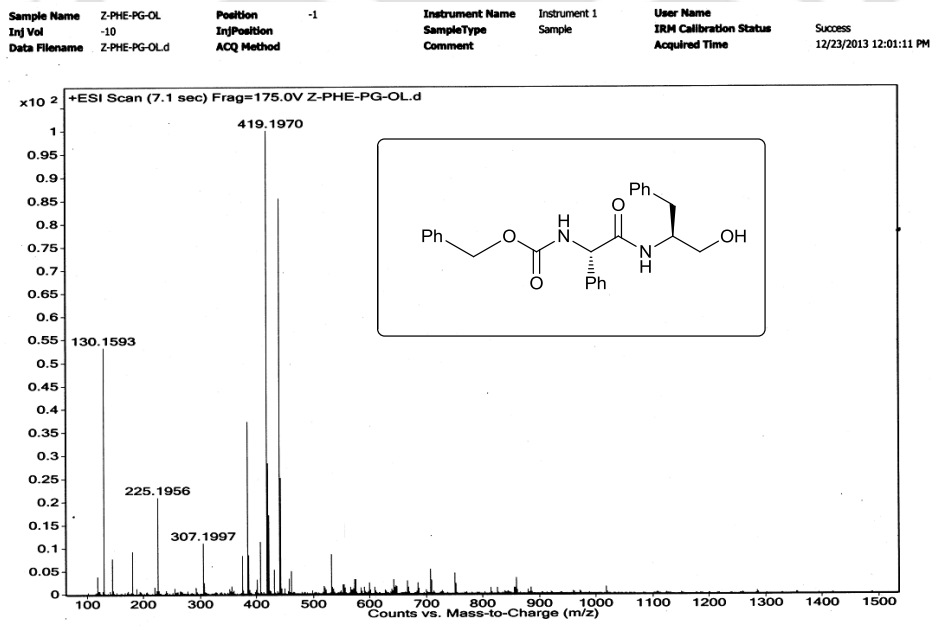


Figure S12. HRMS spectra of the product of entry 11 in Table 5.3.2

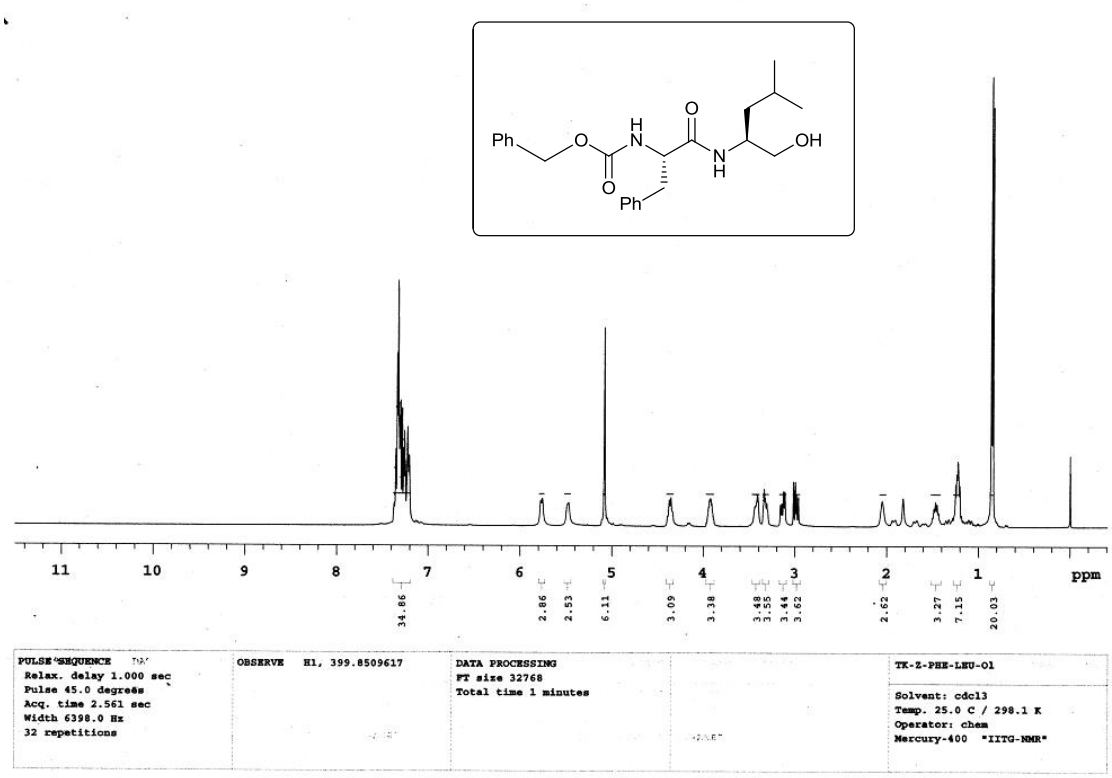


Figure S13. ^{1}H NMR spectra of the product of entry 12 in Table 5.3.2

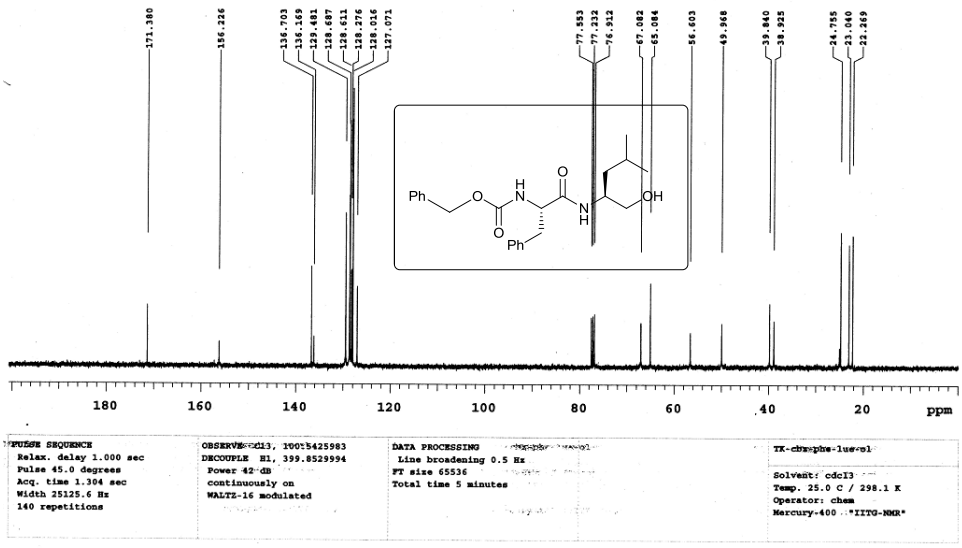


Figure S14. ^{13}C NMR spectra of the product of entry 12 in Table 5.3.2

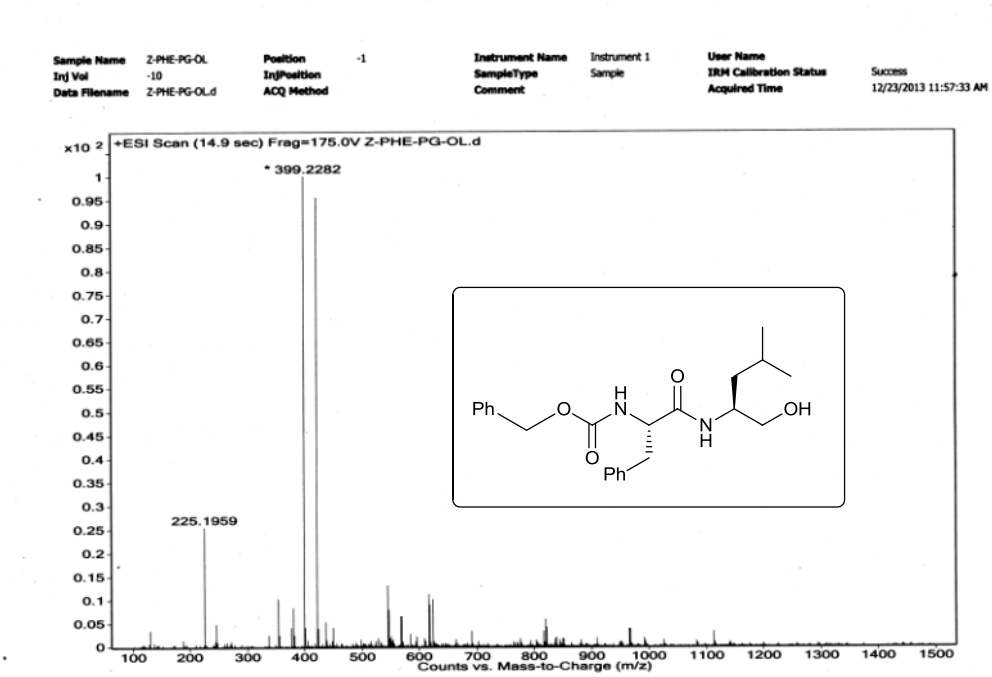


Figure S15. HRMS spectra of the product of entry 12 in Table 5.3.2

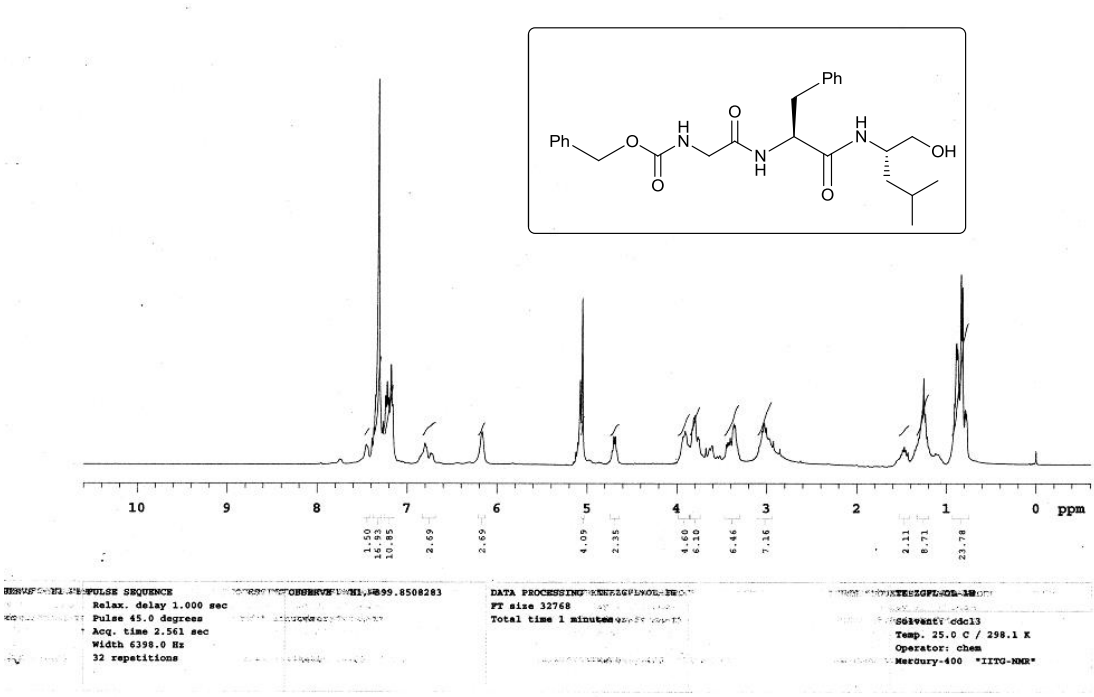


Figure S16. ^{1}H NMR spectra of the product of entry 14 in Table 5.3.2

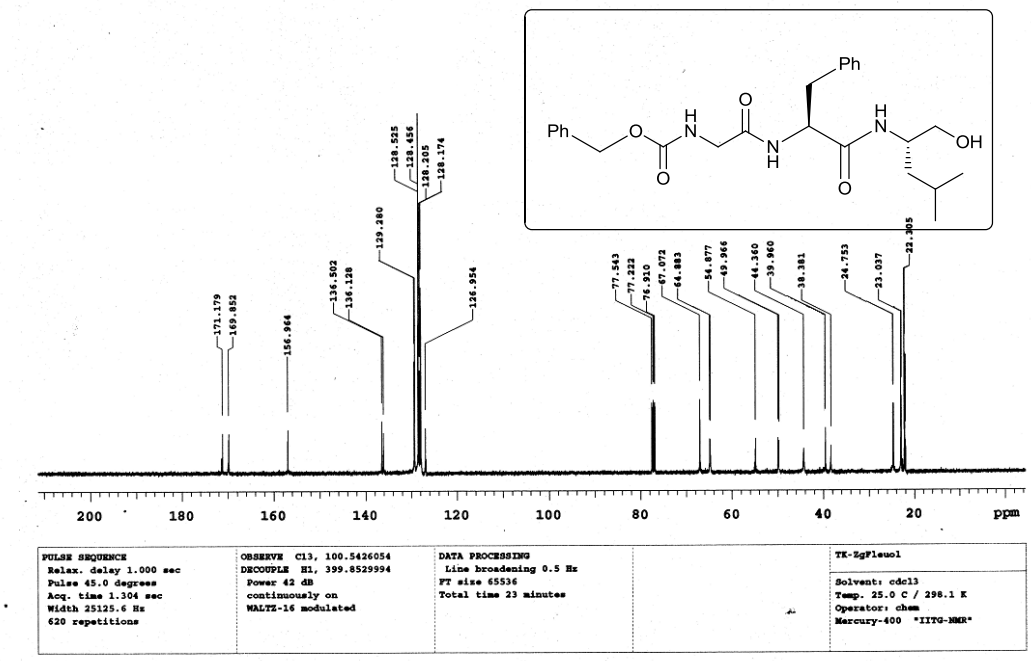


Figure S17. ^{13}C NMR spectra of the product of entry 14 in Table 5.3.2

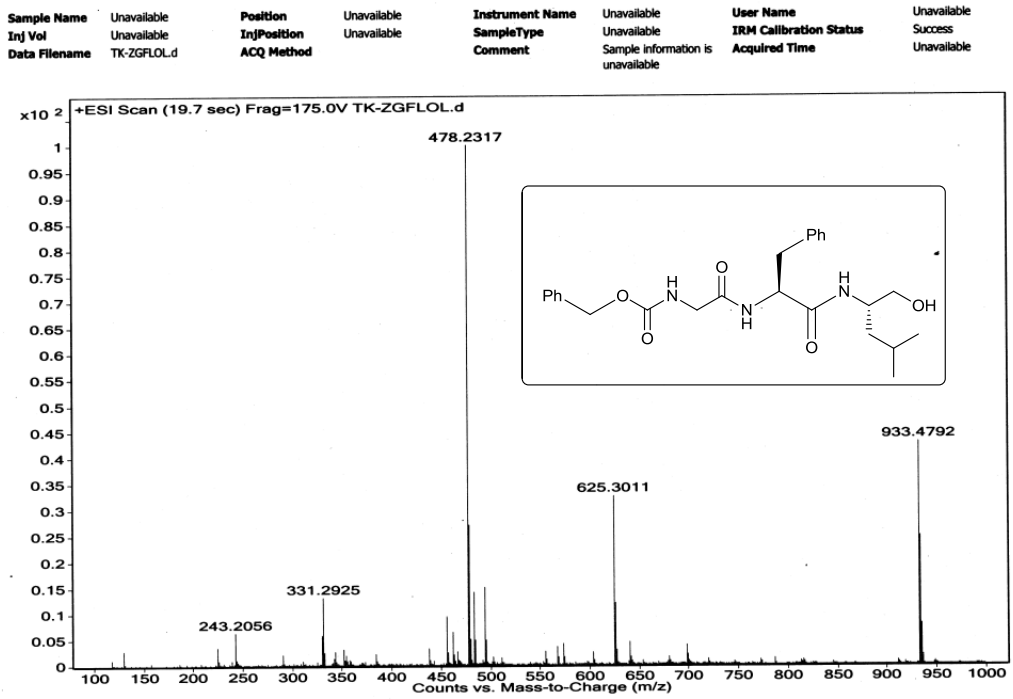


Figure S18. HRMS spectra of the product of entry 14 in Table 5.3.2

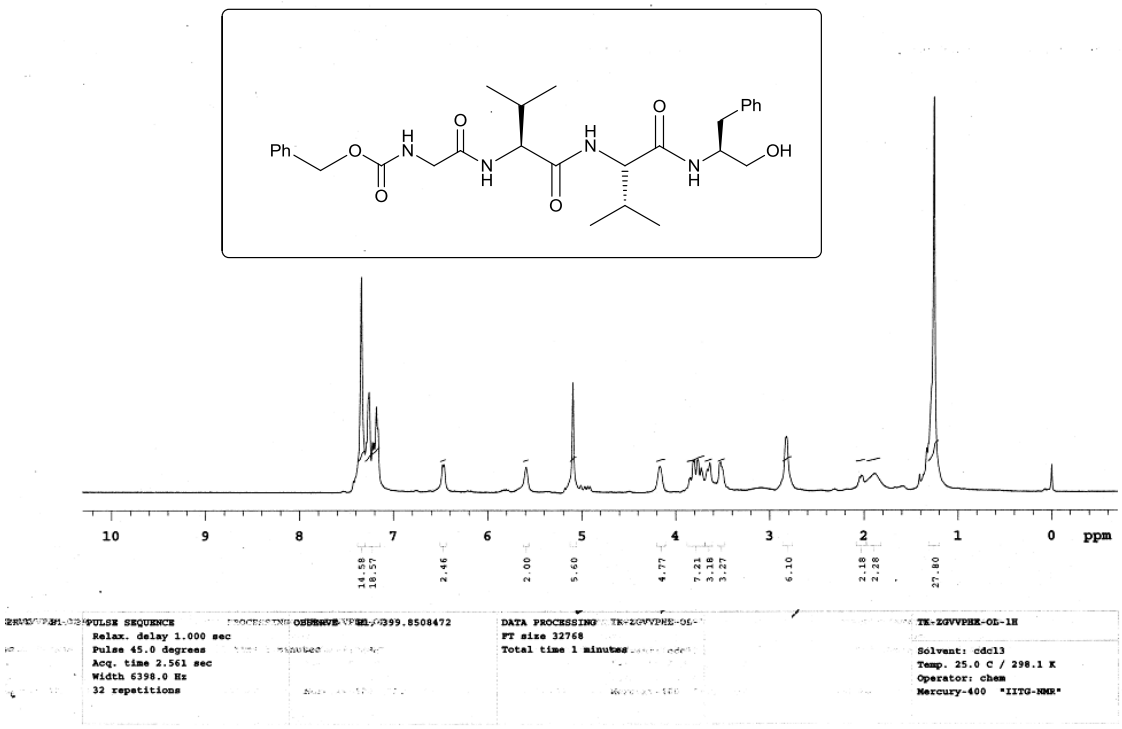


Figure S19. ^{1}H NMR spectra of the product of entry 15 in Table 5.3.2

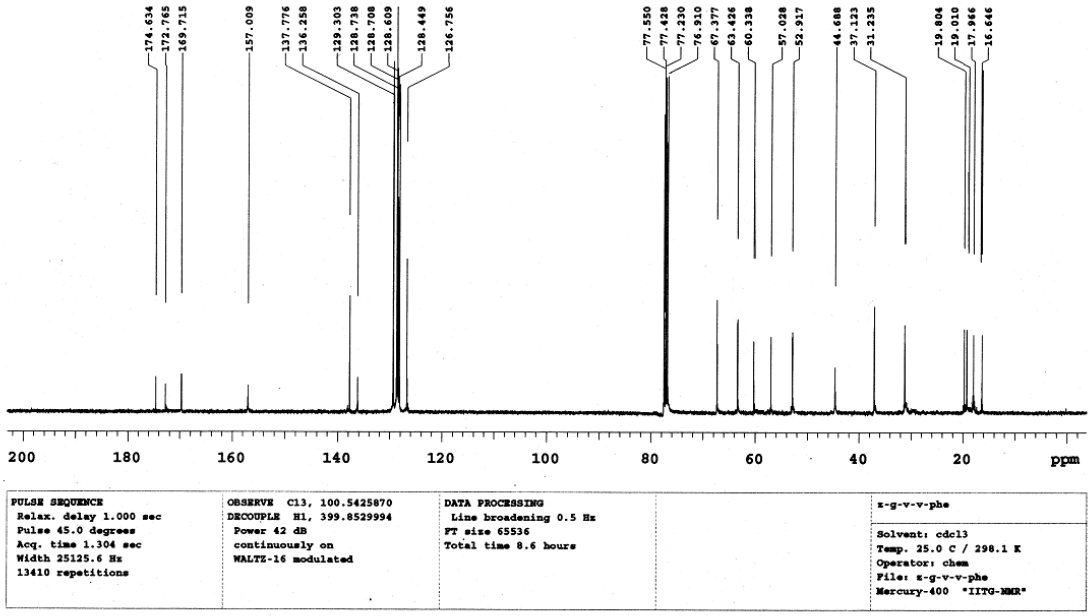


Figure S20. ^{13}C NMR spectra of the product of entry 15 in Table 5.3.2

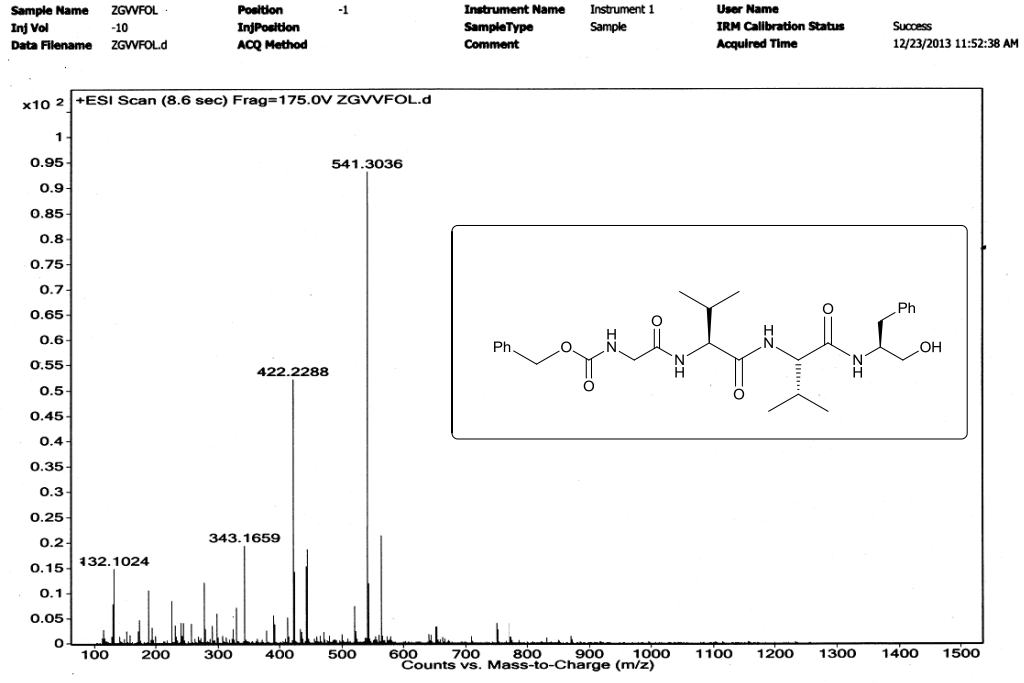


Figure S21. HRMS spectra of the product of entry 15 in Table 5.3.2

5.5.2. HPLC data for racemization studies:

HPLC profiles of peptide alcohols, reverse phase, run time 20 min (linear gradient of 0 to 100% CH_3CN in H_2O with 0.1% formic acid)

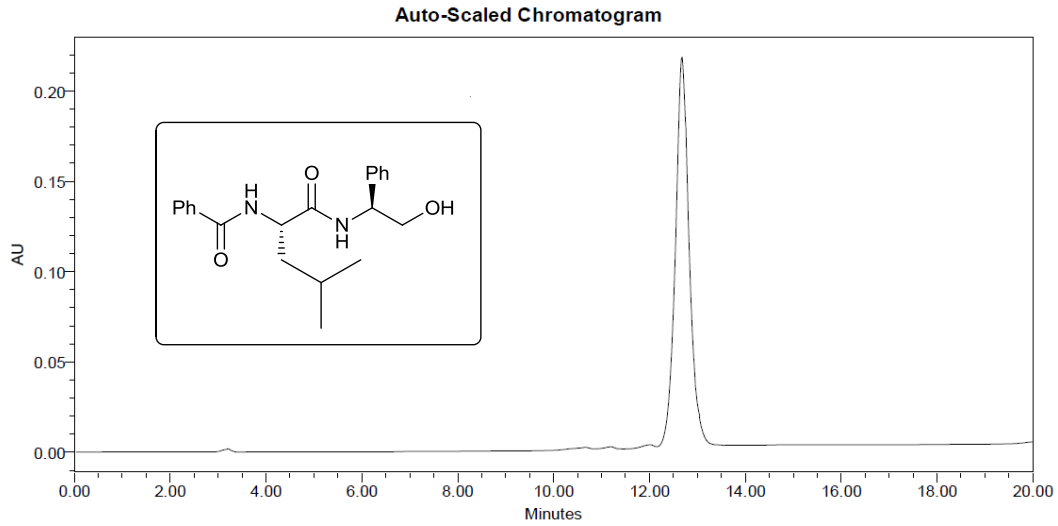
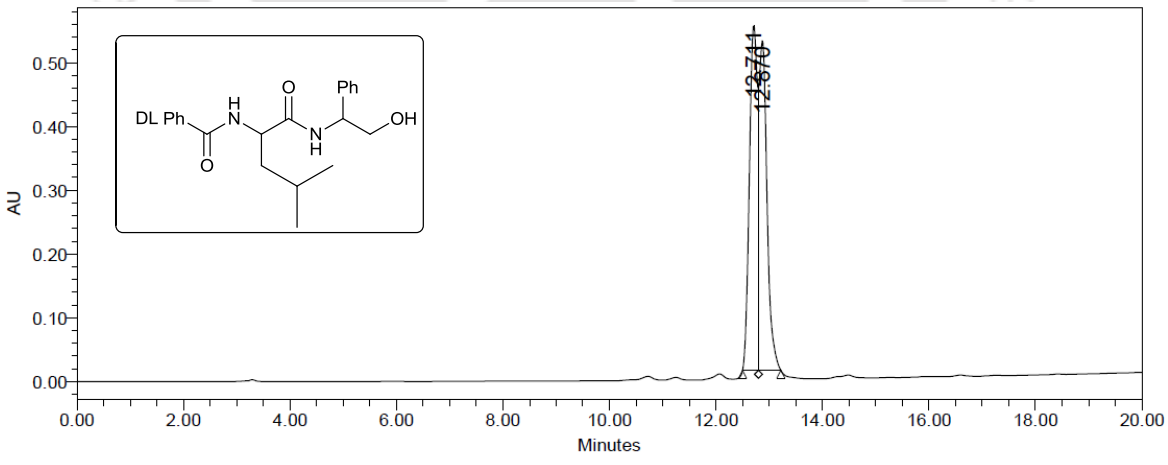


Figure S22 HPLC profile diagram of the product of entry 9 in Table 5.3.2



	RT	Area	% Area	Height
1	12.711	5711231	51.75	541199
2	12.870	5323968	48.25	516790

Figure S23 HPLC profile diagram of the product of entry 10 in Table 5.3.2

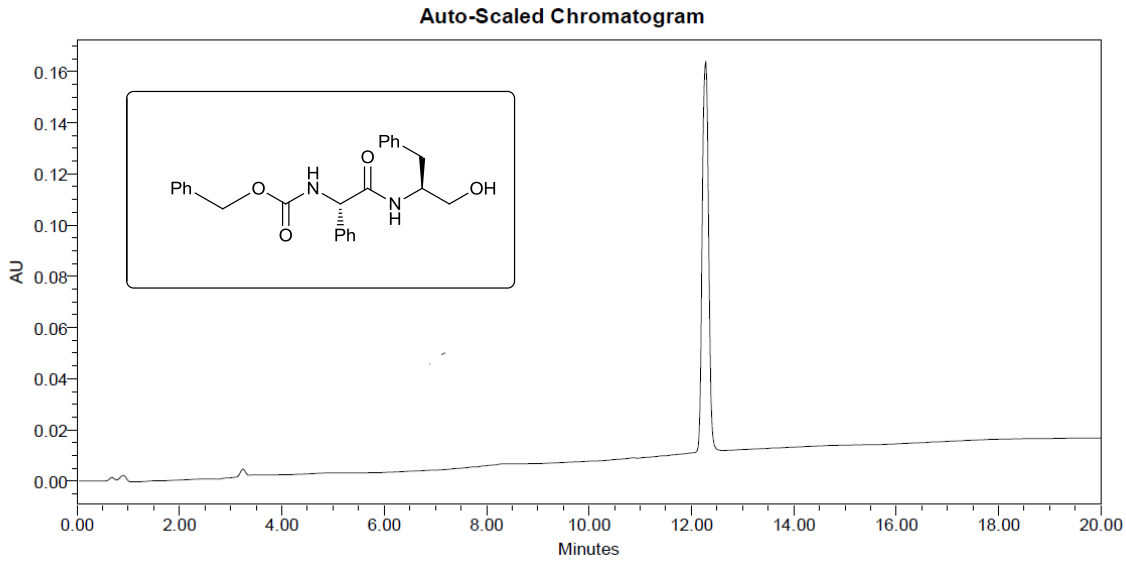


Figure S24 HPLC profile diagram of the product of entry 11 in Table 5.3.2

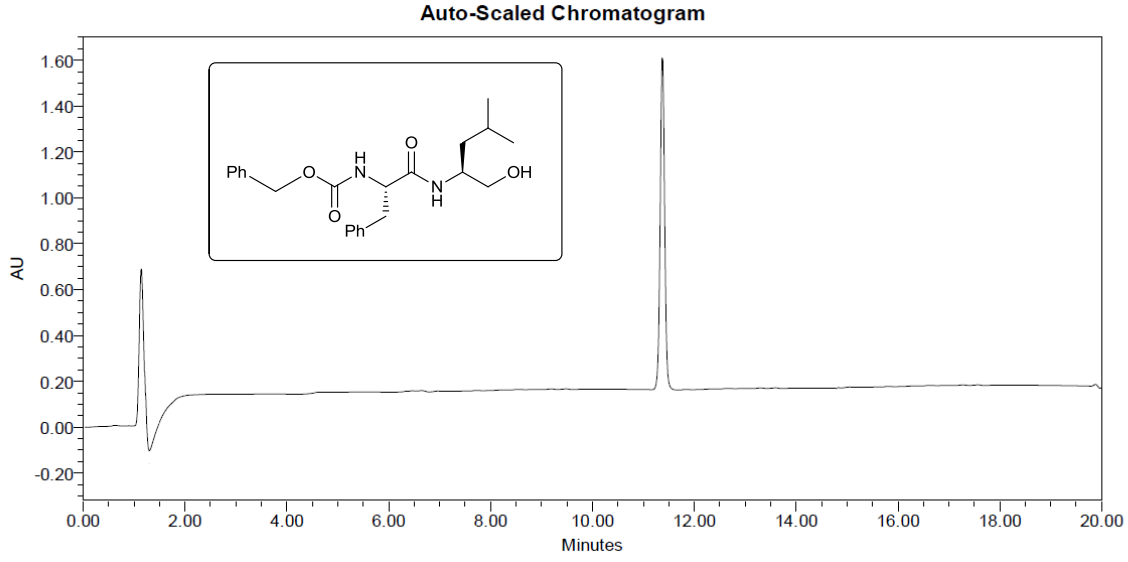
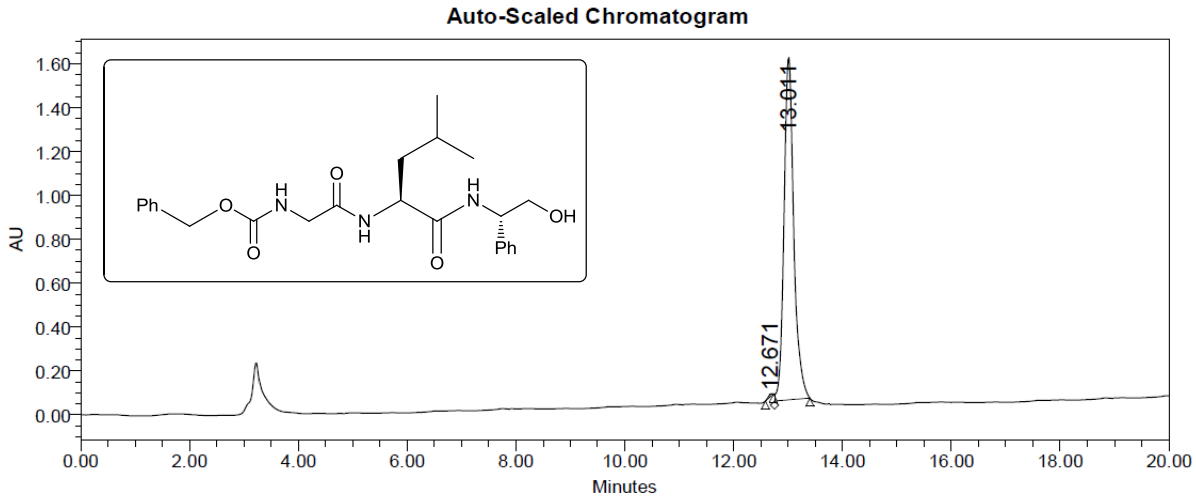
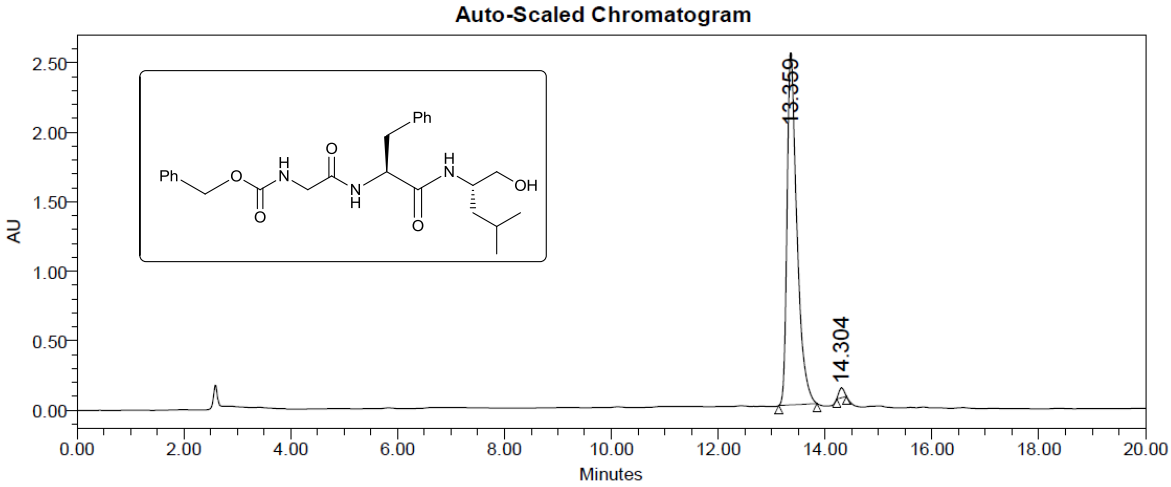


Figure S25 HPLC profile diagram of the product of entry 12 in Table 5.3.2



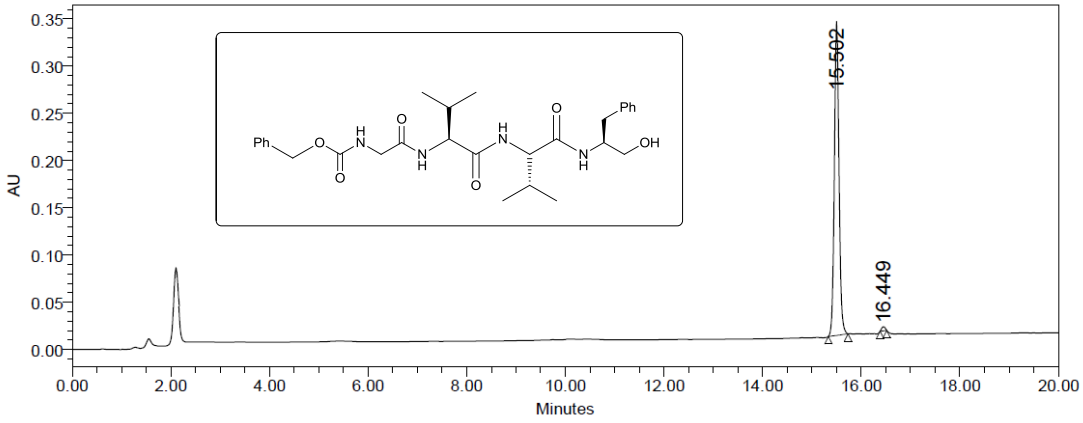
	RT	Area	% Area	Height
1	12.671	55936	0.29	10882
2	13.011	19481136	99.71	1563010

Figure S26 HPLC profile diagram of the product of entry 13 in Table 5.3.2



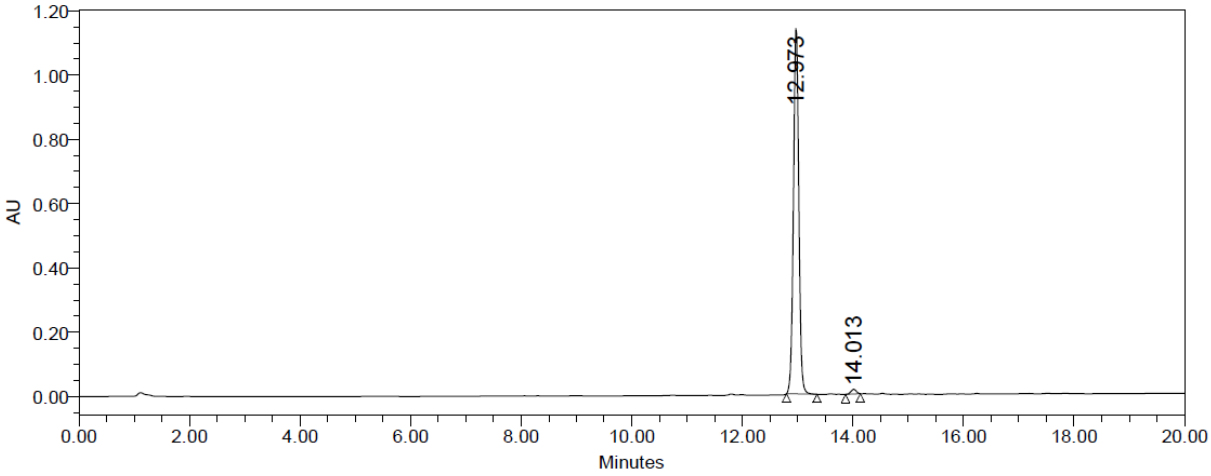
	RT	Area	% Area	Height
1	13.359	32560748	99.60	2549534
2	14.304	463261	0.40	70573

Figure S48 HPLC profile diagram of the product of entry 14 in Table 2



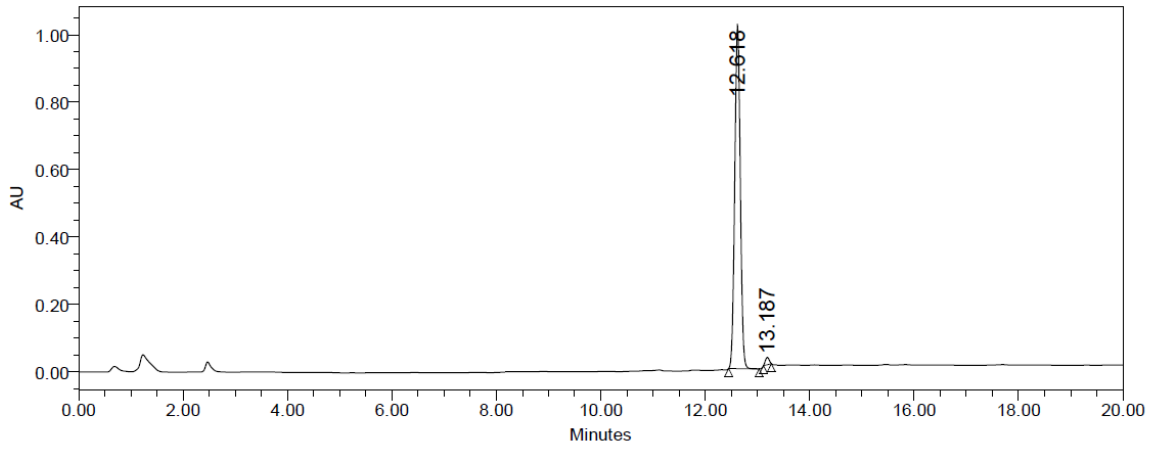
	RT	Area	% Area	Height
1	15.502	2169156	99.04	330702
2	16.449	21026	0.96	4354

Figure S27 HPLC profile diagram of the product of entry 15 in Table 5.3.2



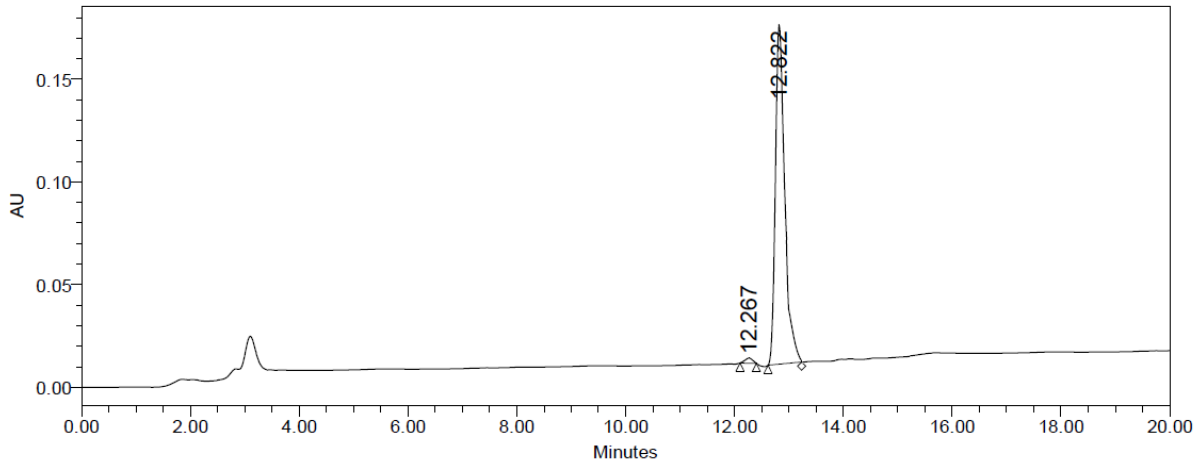
	RT	Area	% Area	Height
1	12.973	7644662	98.74	1140857
2	14.013	97751	1.26	14754

Figure S28 HPLC profile diagram of peptide alcohol **A**in Scheme 5.3.3



	RT	Area	% Area	Height
1	12.618	7524790	98.57	1018656
2	13.187	109328	1.43	21627

Figure S29 HPLC profile diagram of peptide alcohol **B** in Scheme 5.3.3



	RT	Area	% Area	Height
1	12.267	24372	1.09	2478
2	12.822	2221749	98.91	165780

Figure S30 HPLC profile diagram of peptide alcohol **C** in Scheme 5.3.3

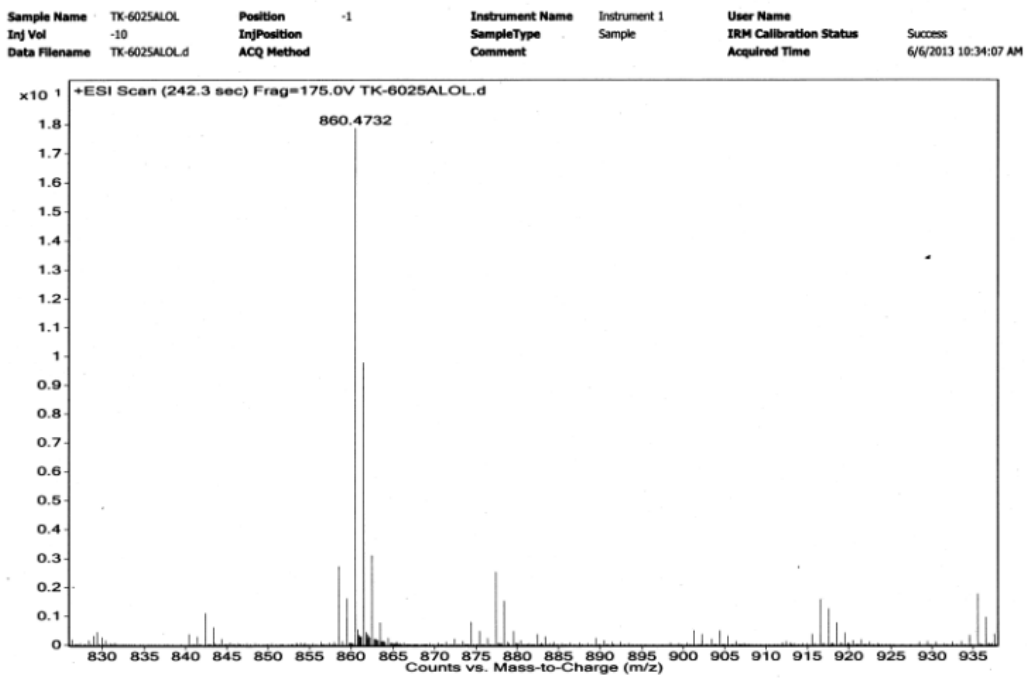


Figure S31 Mass spectra of peptide alcohol**A** in Scheme 5.3.3

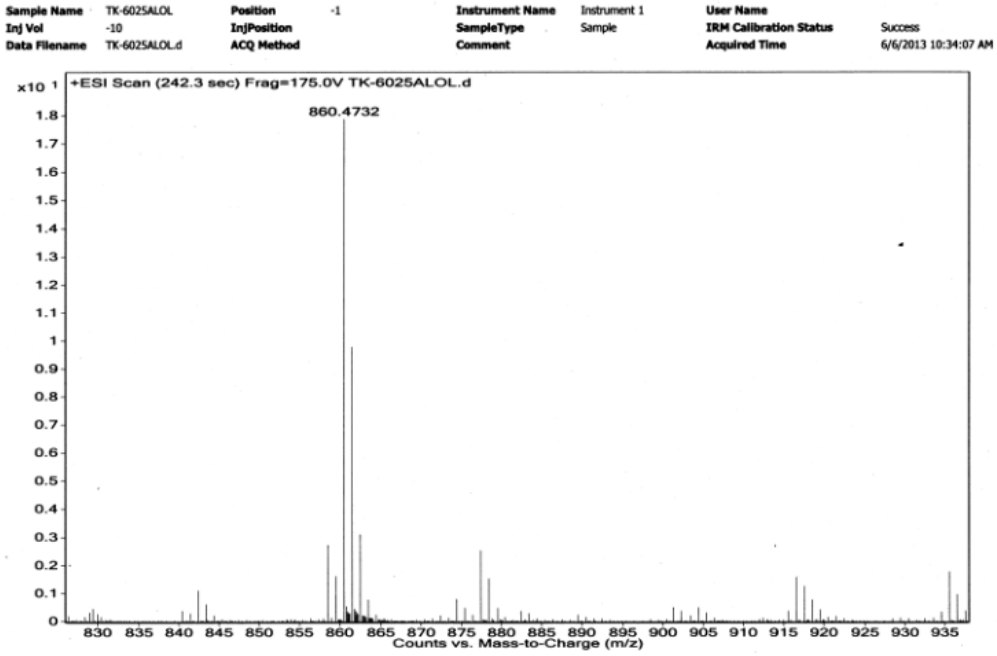


Figure S32ESI-MS spectrum of the peptide alcohol**B** in Scheme 5.3.3

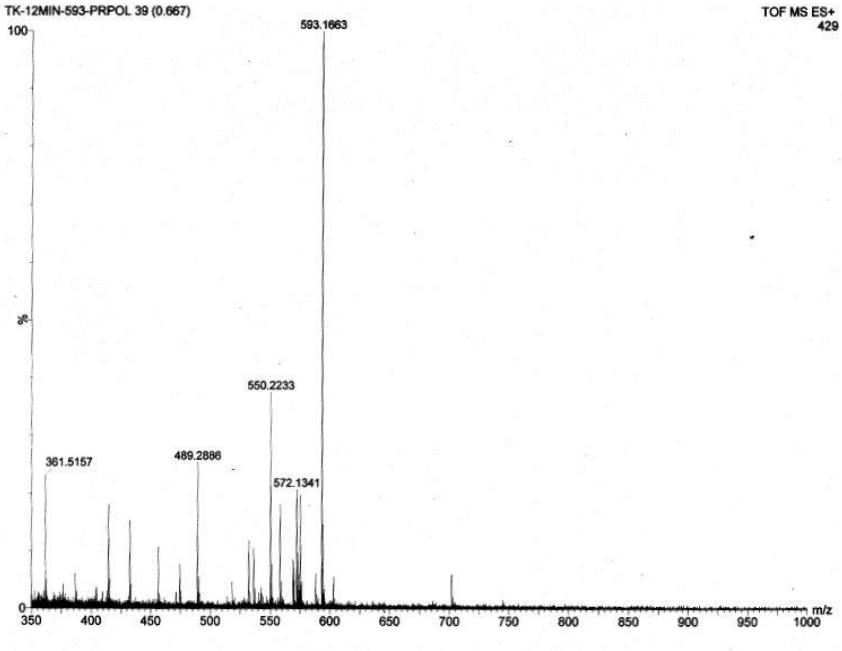
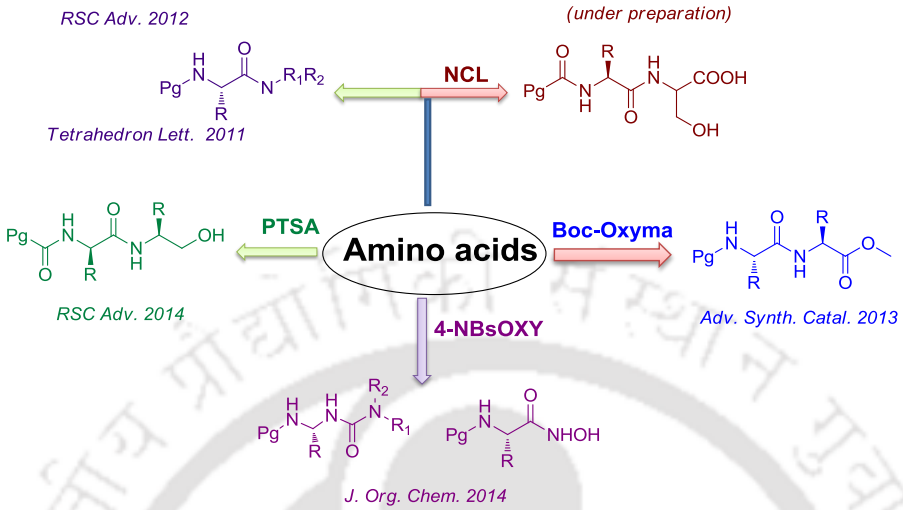


Figure S33ESI-MS spectrum of peptide alcohol **C** in Scheme 5.3.3

Conclusions and Future Directions

Conclusions

The work presented in this thesis is mainly centred on the development of new strategies for the synthesis of amides, esters, peptides, peptide hydroxamic acids, peptide ureas, and peptide alcohols. The whole structure of the thesis is depicted in Scheme 1. We have developed a solvent free and catalyst free synthesis of amides, peptide synthesis by a novel chemical ligation strategy from benzyl ester of *N*-protected amino acids, and the synthesis of amides direct from carboxylic acids. These are described in chapter 2 of this thesis. In chapter 3, we have described the development of a new coupling reagent (Boc-Oxyma) for racemization free synthesis of amides, peptides, esters and thioesters. The 4-NBsOXY, another new reagent, mediated racemization free synthesis of hydroxamic acids and ureas (via Lossen rearrangement) from carboxylic acids is described in chapter 4. In chapter 5, we described the biologically active peptide alcohol synthesis from amides.



Scheme 1. *Thesis overview*

Future directions

The new coupling reagents and the methods that we developed in my Ph. D. tenure can further be used for the synthesis of cyclic peptides, oxa-peptides, aza-peptides, and many other organic transformations. One can explore the native chemical ligation with serine, threonine/cysteine sites direct from *C*-terminal amides (*C*-terminal amides are readily available in usual SPPS) for the synthesis of biologically active polypeptides and cyclic peptides. In literature most of the reported native chemical ligations was found with esters. To the best of our knowledge till date there was no report on native chemical ligation direct from amides. Thus, it would be a challenging task to synthesize polypeptide/cyclic peptide direct from amides via NCL type ligation.

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Research Outcome

Publications:

1) **Thalluri Kishore**, Ashim Paul, Manne Srinivasa Rao, Dharm Dev and Bhubaneswar Mandal. "Microwave assisted chemoselective organocatalytic peptide alcohol synthesis from *C*-terminal amide. *RSC Advances*. **2014**, *4*, 47841–47847.

2) Dharm Dev, Nani Babu Palakurthy, **Thalluri Kishore,** Jyothi Chandra and Bhubaneswar Mandal. "Ethyl 2-cyano-2-(2-nitrophenylsulfonyloxyimino)acetate (2-NBsOXY): A recyclable coupling reagent for racemization free synthesis of peptide, amide, hydroxamates, and ester "*The Journal of Organic Chemistry*. **2014,** *79*, 5420–5431.

3) **Thalluri Kishore**, Manne Srinivasa Rao, Dharm Dev and Bhubaneswar Mandal. "Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (4-NBsOXY) mediated Lossen rearragement: Single pot racemization free synthesis of hydroxamic acids and ureas from carboxylic acids" *The Journal of Organic Chemistry*. **2014**, *79*, 3765−3775.

4) **Thalluri Kishore**, Nadimpally Krishna Chaitanya, Maharishi Parasar Chakravarty, Ashim Paul and Bhubaneswar Mandal. "Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-Cyanoacetate (Boc-Oxyma) as coupling reagent for racemization free esterification, thioesterification, amidation and peptide synthesis." *Advanced Synthesis and Catalysis*. **2013**, *355*, 448–462.

5) **Thalluri Kishore**, Nadimpally Krishna Chaitanya, Ashim Paul and Bhubaneswar Mandal. "Waste reduction in amide synthesis by a continuous method based on recycling of the reaction mixture." *RSC Advances*. **2012**, *2*, 6838–6845.

6) Nadimpally Krishna Chaitanya, **Thalluri Kishore**, Nani Babu Palakurthy, Abhijit Saha and Bhubaneswar Mandal. "Catalyst and solvent-free amidation of inactive esters of *N*-protected amino acids." *Tetrahedron Letters*. **2011**, *52*, 2579–2582.

7) Ashim Paul, Krishna Chaitanya Nadimpally, Tanmay Mondal, **Thalluri Kishore** and Bhubaneswar Mandal. "Inhibition of amyloid formation by β-sheet breaker hybrid peptide." (Under rivision).

8) Dharm Dev, Nani Babu Palakurthy, Jyothi Chandra, **Thalluri Kishore** and Bhubaneswar Mandal. "Metal free synthesis of benzoxazole and benzothiazole from carboxylic acids using (Ethyl 2-cyano-2-(2-nitrophenylsulfonyloxyimino) acetate (2-NBsOXY) and PTSA. (Communicated):

9) **Thalluri Kishore**, Manne Srinivasa Rao and Bhubaneswar Mandal. " Boc-Oxyma mediated epimerization free synthesis of peptide hydroxamic acids." (Communicated)

10) **Thalluri Kishore**, Manne Srinivasa Rao and Bhubaneswar Mandal. "Native chemical ligation of inactive esters of *N*-protected amino acids at Serine." (Manuscript under preparation)

11) Book Chapter: **Thalluri Kishore**, Dharm Dev, Srinivasa Rao Manne, Nani Babu Palakurthy, Jyothi Chandra and Bhubaneswar Mandal. The coupling reagents the world in need indeed. *New dimensions in chemistry & chemical technologies- Applications in pharma industry* (NDCT-2014), 23-25th June, JNTU Hyderabad, India, page no 348–351.

Conference abstracts:

1) **Thalluri Kishore** and Bhubaneswar Mandal, "Ethyl 2-(tert-Butoxycarbonyloxyimino)-2-Cyanoacetate (Boc-Oxyma) as Coupling Reagent for Racemization free Peptide Synthesis" , *Abstract Book, 20th ISCB International Conference (ISCBC-2014)*, 1st-4th March 1-4, University of Delhi, Delhi, India, (poster) P 114, page: 20.

2) **Thalluri Kishore**, Nadimpally Krishna Chaitanya and Bhubaneswar Mandal, "Reagent economized and waste minimized approach of amide syntheses by one pot", *Abstract book National symposium on recent trends in chemical science and technology (RTCST-2012),* 3rd – 4th March, 2012, IIT Patna, P 26, page 78.

3) Ashim Paul, Krishna C. Nadimpally, **Kishore Thalluri**, and Bhubaneswar Mandal*, "One pot protocol for amide synthesis of unprotected amino acids", *Abstract book National symposium on recent trends in chemical science and technology (RTCST-2012)*, 3rd – 4th March, 2012, IIT Patna, P 28, page 80.

4) Nadimpally Krishna Chaitanya, **Kishore Thalluri**, Ashim Paul, Nani babu Palakurthy and Bhubaneswar Mandal*; "Catalyst and solvent free amidation of inactive esters of N-protected amino acids: A green chemistry perspective", *Abstract Book CRSI-2012 (national symposium), held at IISER Trivandrum & NIIST Trivandrum*, 3rd – 5th February, 2012, P 28, Page 68.

5) Krishna Chaitanya Nadimpally, **Kishore Thalluri**, Santu Mandal, Bhubaneswar Mandal*; "Facile Synthesis of Water Soluble Peptide Based Polymers*" Abstract book frontiers in chemcial sciences-2010 (national symposium), IIT Guwahati*, December 3-4, 2010, P 83, page 113.

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- Qualified Graduate Aptitude Test (GATE) with **All India Rank - 110**, held on March, 2009 in CHEMISTRY, organized by Ministry of Human Resource Development, Government of India.

Research Experience:

- **2012-Present**: **Senior Research Fellow** at the Department of Chemistry, Indian Institute of Technology Guwahati, India.
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Research and Instrumental skills:

- I have developed a two new coupling reagents Boc-Oxyma and 4-NBsOXY. Boc-Oxyma helps calmer pathway to synthesize amides and peptides by solution phase and solid phase peptide synthesis. Similarly, we synthesized the racemization free hydroxamates and urea using 4-NBsOXY direct from acids. We synthesized amide and peptide from inactive esters and acids via native chemoselective ligation at serine site. Finally we successfully synthesized the peptide alcohols direct from amide.
- Peptide synthesis by both solution and solid phase
- Conformational analysis of chiral compounds by 2D-NMR (COSY, NOESY), time dependent NMR, variable temperature NMR, HPLC, HPLC-MS, Polari meter, FT-IR spectroscopy, mass spectrometry (MS/LC-MS (kinetic study)), gas Chromatography, *etc*.

Main Skills:

Teaching Experiences:

Since 2009: Both Tutorial and Teaching Assistantship at IIT Guwahati, India (B.Tech and M. Sc).

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