SOME APPLICATIONS OF BENZOTRIAZOLE COMPOUNDS IN SYNTHETIC CHEMISTRY

İlker AVAN

Ph.D Dissertation

Department of Chemistry

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ANADOLU ÜNİVERSİTESİ

JÜRİ VE ENSTİTÜ ONAYI

İlker Avan'ın **"Some Applications of Benzotriazole Compounds in Synthetic Chemistry"** başlıklı **Kimya** Anabilim Dalındaki, Doktora Tezi 26.12.2011 tarihinde, aşağıdaki jüri tarafından Anadolu Üniversitesi Lisansüstü Eğitim Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca değerlendirilerek kabul edilmiştir.

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ABSTRACT

Ph.D. Dissertation

SOME APPLICATIONS OF BENZOTRIAZOLE COMPOUNDS IN SYNTHETIC CHEMISTRY

İlker AVAN

Anadolu University
Graduate School of Sciences
Chemistry Program

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2012, 116 pages

Nowadays, therapeutic applications of peptides and small peptide-like chains (peptidomimetics) have gained much attention in pharmacological sciences.

In the present study, peptidomimetic compounds including α-aminoxy peptides, depsipeptides and their hybrid analogs were prepared by using novel synthetic methodologies. Many new synthetically useful intermediates including *N*-(PG-aminoacyl)benzotriazoles, *N*-(PG-α-aminoxyacyl)benzotriazoles, *O*-PG(α-hydroxyacyl)benzotriazoles were prepared from their carboxylic acid analogs and their synthetic utilities were demonstrated by the preparation of some novel biologically active compounds depsipeptides, depsides, chiral oligoesters, α-aminoxy peptides and α-aminoxy hybrid peptides in high yield without causing racemization.

Keywords: Peptidomimetic, pseudo peptides, depside, depsipeptide, aminoxy peptide, *N*-acylbenzotriazole

ÖZET

Doktora Tezi

BENZOTRİAZOL BİLEŞİKLERİNİN SENTETİK KİMYADAKİ BAZI UYGULAMALARI

İlker AVAN

Anadolu Üniversitesi
Fen Bilimleri Enstitusü
Kimya Anabilim Dalı

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Peptit ve peptit benzeri yapıların (peptidomimetikler) tedavi edici uygulamaları son zamanlarda farmakolojik bilim dallarında büyük ilgi uyandırmıştır.

Bu tez çalışmasında, depsipeptitler, α-aminoksipeptitler ve onların hibrit türlerini içeren peptidomimetik bileşikler yeni sentetik yöntemler kullanılarak hazırlanmıştır. Sentetik olarak faydalı birçok yeni ara ürün olan *N*-(PG-aminoasil)benzotriazoller, *N*-(PG-α-aminoksiasil)benzotriazoller, *O*-PG(α-hidroksiasil)benzotriazoller karboksilik asit analoglarından hazırlanmıştır. Bunların sentetik kullanımlarındaki faydaları depsipeptitler, depsitler, kiral oligoesterler, α-aminoksi peptitler ve α-aminoksi hibrit peptitleri de içeren birçok biyolojik aktif bileşiğin yüksek verimde ve rasemizasyona neden olmadan hazırlanmasıyla gösterilmiştir.

Anahtar Kelimeler: Peptidomimetik, psödo peptit, depsit, depsipeptit, aminoksi peptit, *N*-asilbenzotriazol

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LIST OF ABBREVIATIONS

$[\alpha]_D^{23}$	: Specific rotation at 23 ^oC in (deg mL)/(g dm)
Ala	: Alanine ($CH_3CH(NH_2)COOH$)
Alk	: Alkyl
AO	: Aminoxy
Appx	: Appendix
Ar	: Aryl
Asp	: Aspartic acid
Boc	: *tert*-Butyloxycarbonyl
BOP	: Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
br	: Broad (spectral)
Bt	: Benzotriazol-1-yl
BtH	: 1*H*-Benzotraizole
C	: Carbon
CaH_2	: Calcium hyride
Calcd.	: Calculated
Cbz	: Benzyloxycarbonyl
$CDCl_3$	: Deuterated chloroform
CDI	: 1,1'-Carbonyldiimidazole
Cys	: Cysteine ($HO_2CCH(NH_2)CH_2SH$)
D	: Dextrorotary (right)
d	: Doublet
DBU	: 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	: *N*,*N*'-Dicyclohexylcarbodiimide
DCM	: Dichloromethane

DIC	: *N*,*N*′-Diisopropylcarbodiimide
DIEA	: *N*,*N*-Diisopropylethylamine, or Hünig's base
DMAP	: 4-Dimethylaminopyridine
DMF	: Dimethylformamide
DMSO	: Dimethylsulfoxide
DMSO-*d6*	: Deutoreted dimethylsulfoxide
EDCI	: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	: Enantiomeric excess
equiv.	: Equivalent(s)
Et	: Ethyl
et al.	: and others
Et_3N	: Triethylamine
EtOAc	: Ethyl acetate
Eur	: Euro (money currency)
FDA	: Food and Drug Administration
Fe	: Iron
Fmoc	: 9-Fluorenylmethoxycarbonyl
g	: Gram(s)
Gln	: Glutamine
Glu	: Glutamic acid
Gly	: Glycine (NH_2CH_2COOH)
h	: Hour
H	: Hydrogen
H_2O_2	: Hydrogen peroxide
HBTU	: *O*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate

HCl	: Hydrochloric acid
HIV	: Human Immunodeficiency virus
HMPA	: Hexamethylphosphoramide
HOAt	: 1-Hydroxy-7-azabenzotriazole
HOBt	: *N*-Hydroxybenzotriazole
HPLC	: High-performance liquid chromatography
HPyOPfp	: *N*,*N*,*N*′,*N*′-bis(tetramethylene)-O-pentafluorophenyluronium hexafluorophosphate (PfPyU)
HRMS	: High resolution mass spectrometry
Hz	: Hertz
Ile	: Isoleucine ($HO_2CCH(NH_2)CH(CH_3)CH_2CH_3$)
i-Pr	: Isopropyl
J	: Coupling constant (in NMR spectroscopy)
K_2CO_3	: Potassium carbonate
L	: Levorotary (left)
LC-MS	: Liquid chromatography–mass spectrometry
Leu	: Leucine ($HO_2CCH(NH_2)CH_2CH(CH_3)_2$)
lit.	: Literature
Lys	: Lysine ($HO_2CCH(NH_2)(CH_2)_4NH_2$)
m	: Multiplet (spectral); metre(s); milli
m/z	: Mass-to-charge ratio
MBHA	: Methylbenzhydrylamine
Me	: Methyl
MeOH	: Methyl alcohol
Met	: Methionine ($HO_2CCH(NH_2)CH_2CH_2SCH_3$)
$MgSO_4$	: Magnessium sulphate

Min.	: Minute(s)
mol	: Mole(s)
mp	: Melting point
N	: Nitrogen
N	: Normality
Na	: Sodium
Na_2SO_4	: Sodium sulphate
NaH	: Sodium hydride
$NaHCO_3$	: Sodium bicarbonate
$NaHSO_3$	: Sodium bisulfite
NEM	: *N*-Ethylmorpholine
NMM	: *N*-Methylmorpholine
NMR	: Nuclear magnetic resonance
O	: Oxygen
^oC	: Degree Celcius
OEt	: Ethoxy
OMe	: Methoxy
p	: para
PG	: Protecting group
Ph	: Phenyl
Phe	: Phenylalanine ($C_6H_5CH_2CH(NH_2)COOH$)
Phth	: Phthalimide
PPh_3	: Triphenyl phosphine
ppm	: Parts per million
Pro	: Proline
PS	: Polysyrene resine

p-TSA	: para-Toluene sulphonic acid
PyBOP	: (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBrop	: Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
q	: Quartet
R	: Rectus (right)
rt	: Room temperature
s	: Singlet (spectral)
S	: Sinister (left)
$SOCl_2$	: Thionyl chloride
SPPS	: Solid phase peptide synthesis
t	: Tertiary
t	: Triplet (spectral)
TBAP	: tetra-*n*-Butylammonium per-ruthenate
TBTU	: 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TCBC	: 2,4,6-Trichlorobenzoylchloride
TEA	: Triethylamine
TFA	: Trifluoroacetic acid
THF	: Tetrahydrofuran
TIPS	: Triisopropylsilane
TLC	: Thin-layer chromatography
TMS	: Tetramethylsilane
Trp	: Tryptophan
Tyr	: Tyrosine
USD	: United States Dollars (money currency)

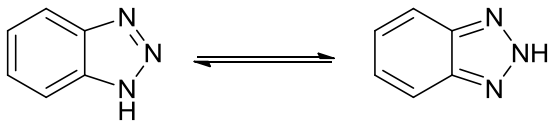
Val	: Valine ($HO_2CCH(NH_2)CH(CH_3)_2$)
Z	: Cbz, (Benzyloxycarbonyl) protection group
δ	: Chemical shift in parts per million downfield from tetramethylsilane
α	: Alpha
β	: Beta

1. BENZOTRIAZOLE AND ITS SYNTHETIC UTILITIY IN ORGANIC SYNTHESES

1.1. Benzotriazole

1*H*-Benzotriazole ($C_6H_5N_3$) **1.1** is a fused heterocyclic system, which consist of benzene and 1,2,3-triazole moiety (Figure 1.1). Benzotriazole **1.1** is a fascinating compound that has a pioneering role in both applied and synthetic chemistry. 1*H*-Benzotriazole **1.1** is used as a corrosion inhibitor, exclusively for copper and its alloys by preventing undesirable surface reactions [1]. For the last two decades, the synthetic utility of benzotriazole has been extensively explored by Katritzky and co-workers at the University of Florida [2]. Moreover, benzotriazole mediated synthetic chemistry has become an attractive tool for many chemical processes, including multistep drug preparations, biologically active compounds, natural products, and peptide analogs [2-5].

Benzotriazole **1.1** is a readily available compound that is less expensive (100 g, 51 EUR in Turkey and 38 USD in USA [6]) and a stable non-toxic solid compound [2]. Moreover it is well soluble material in most of the organic solvent, such as benzene, diethyl ether, chloroform, ethanol, acetone and DMF and it is slightly soluble in water [2]. 1*H*-Benzotriazole **1.1** is a weak acid (pK_a=8.3); that makes it soluble in basic media and it is also a weak base (pK_{aH}=1.6); that allows it soluble in acidic media [7-8].



1.1a **1.1b**

1*H*-tautomer 2*H*-tautomer

Figure 1.1. Benzotriazole **1.1** and its tautomeric equilibrium

The secret of multipronged utility of benzotriazole **1.1** and its intermediates is hidden in its structural and electronic features. The prolonged resonance enhancement of benzotriazole allows it to behave like an electron-donating group, as well an electron-withdrawing group, depending on its substitutions (Figure 1.2). These electronic features of benzotriazole let it also to be a good leaving

group in many reactions [2]. The leaving ability of benzotriazole is comparable with cyano and sulfonyl groups [9].

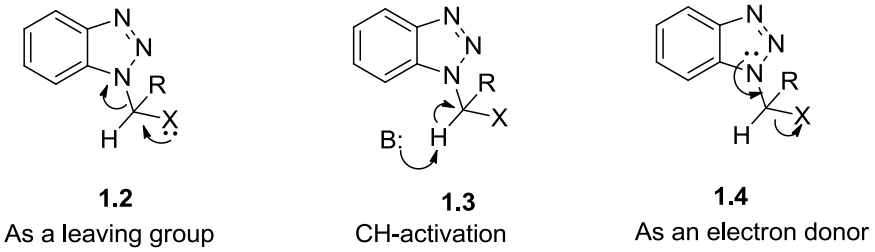


Figure 1.2. Activity of benzotriazole

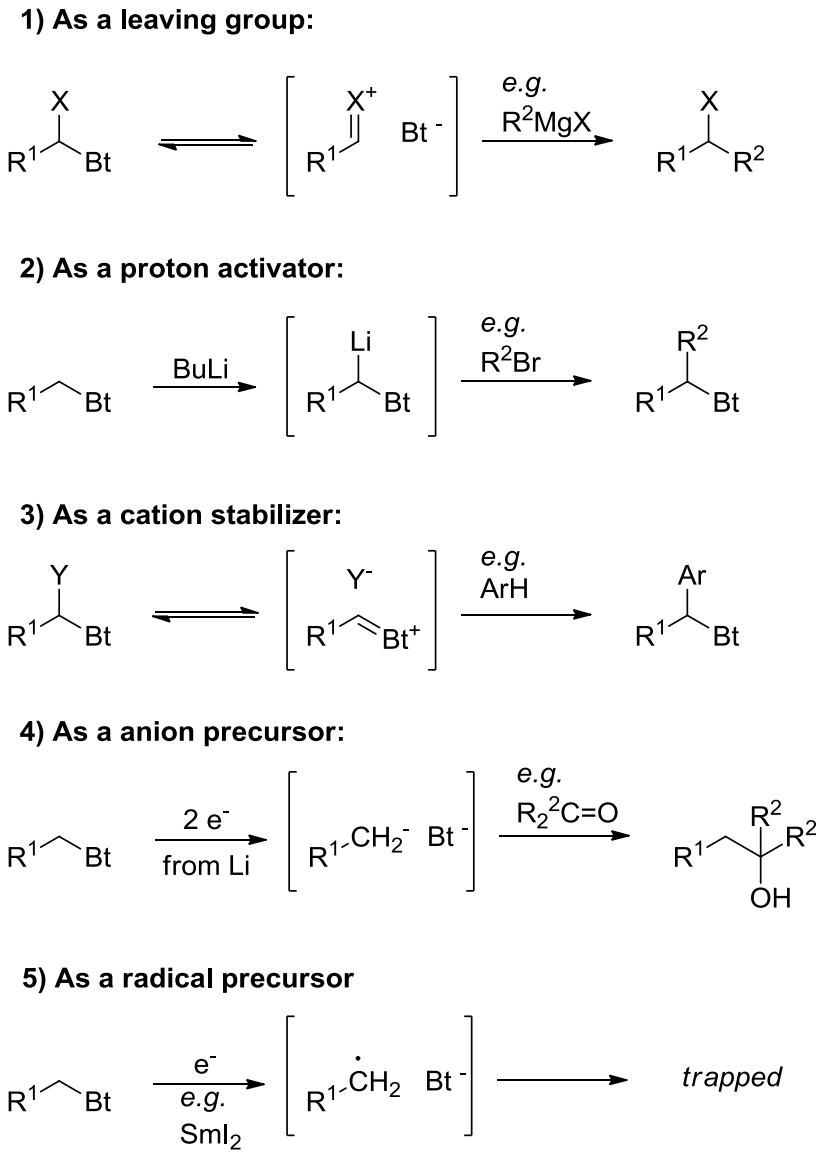


Figure 1.3. Reactivity profile of benzotriazole.

Therefore, benzotriazole **1.1** has been considered to be an excellent synthetic auxiliary in many publications because of its unique physical and chemical properties outlined above. As a good auxiliary, benzotriazole **1.1** can be simply first introduced with the substance, then it activates substance to react, and last it can be easily eliminated during work-up. Recently, the use of benzotriazole for Heterocyclic chemistry was reviewed by Katritzky and Rachwal and outlined under five major title [3, 10]; 1) a leaving group, 2) a proton activator, 3) a cation stabilizer, 4) an anion precursor, and 5) a radical precursor (Figure 1.3).

1.2. *N*-Acylbenzotriazoles

N-Acylbenzotriazoles **1.5** have recently flourished for the C-, S-, N- and O-acylation of proton labile compounds including activated –CH, thiols, amines, and alcohols.

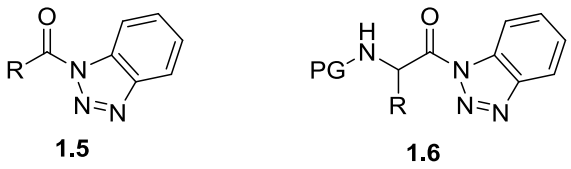


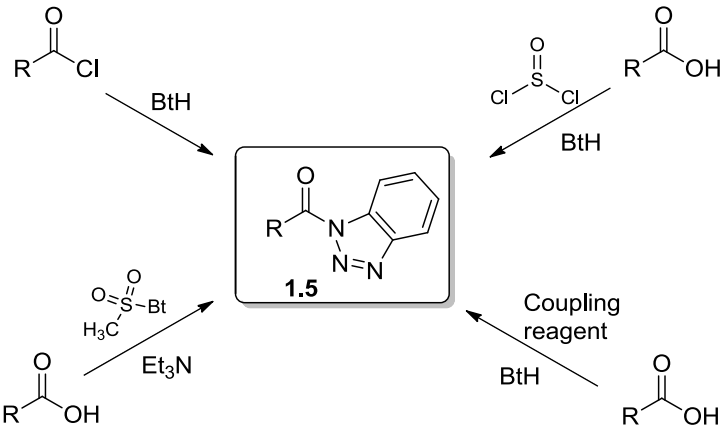
Figure 1.4. *N*-Acylbenzotriazole 1.5 and *N*-(protected-α-aminoacyl)benzotriazoles **1.6**

N-Acylbenzotriazoles **1.5** are powerful acylation reagents that can be used for synthesizing many new synthetic intermediates, drug candidates, natural products and especially new building blocks for peptide conjugates [5]. Particularly, *N*-(protected-α-aminoacyl)benzotriazoles **1.6** allow rapid preparation of biologically active peptides and peptide conjugates in high yields and purity under mild reaction conditions with full retention of original chirality [5]. Moreover, this developed methodology can be applied for both solution and solid-phase preparation of complex or difficult peptides [5, 11-13]. *N*-Acylbenzotriazoles **1.5** have some major advantages among the other common acylation reactions, those are i) easy preparation and less cost ii) crystalline compounds which relatively resistant to hydrolysis. So they can easily handle and can be stored for longer time in lab iii) give rapid coupling reaction, iv) retain the original chirality, v) most reactions can be done in water based solvent, green

chemistry, vi) the reactions can be easily monitored by TLC, and vii) the reactions can be easy worked up and purified by simply aqueous washing with acid or base [5].

1.2.1. Preparation of *N*-acylbenzotriazoles

Most of the *N*-acylbenzotriazoles **1.5** are stable crystalline compounds that can be easily prepared from its acid analogs and can be safely handle and store in laboratory [5]. *N*-Acylbenzotriazoles **1.5** can be easily prepared from the corresponding acid chlorides by replacing chloride with excess amount of benzotriazole (Scheme 1.1). Avoiding preparation of less stable acid chlorides, *N*-acylbenzotriazoles **1.5** are prepared directly from acid analogs by treating with thionyl chloride in the presence of excess benzotriazole at room temperature or less [14, 15]. *N*-Acylbenzotriazoles **1.5** derived from less soluble carboxylic acids can be obtained by treating those acids with methyl sulfonylbenzotriazole in the presence of triethylamine under heat [16]. Another preparation of *N*-acylbenzotriazoles **1.5** is to use coupling reagents such as DCC and EDCI. Generally Boc protected amino acids can be converted to their acylbenzotriazoles by the use of coupling reagents, since Boc group is unstable under acidic work up conditions [17].



Scheme 1.1. Preparation of *N*-acylbenzotriazoles **1.5**

1.3. Synthetic utility of *N*-acylbenzotriazoles

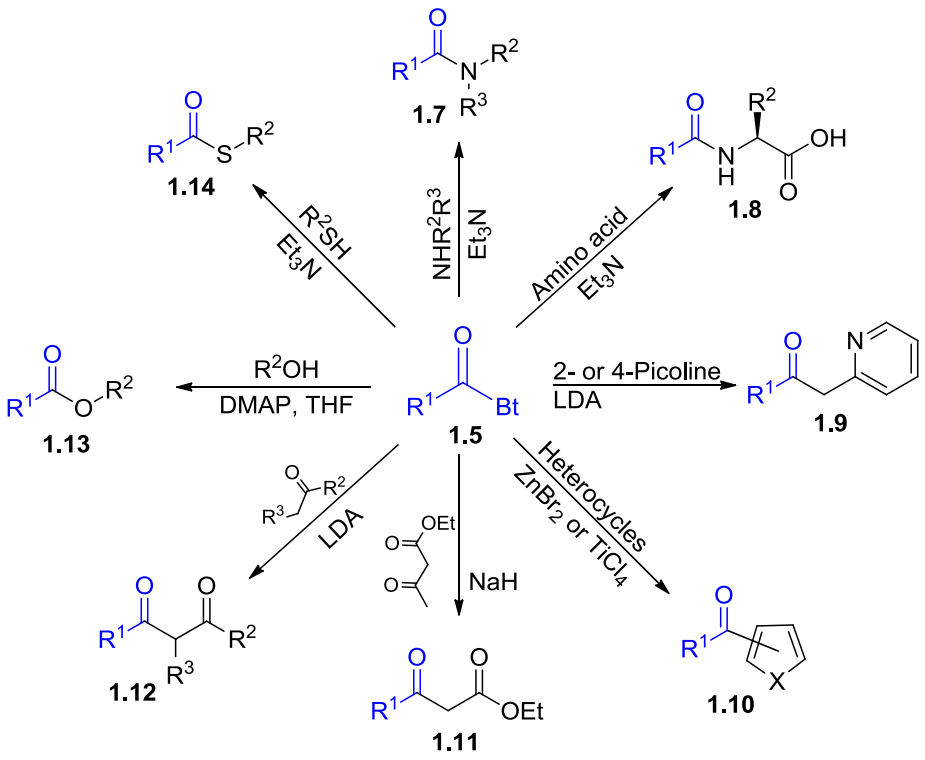
N-Acylbenzotriazoles **1.5** have been successfully employed for N-, C-, S-, and O-acylation of proton labile compounds including activated –CH, thiols, amines, and alcohols [18] (Scheme 1.2). Especially;

i) N-Acylation of amines (ammonia, primary and secondary) to form amides [16] including hydrazine [19, 20], hydroxyl amine [21, 22], nucleosides [23], and amino acids [5, 24].

ii) C-acylation of activated –CH groups including ketones [25], β-ketoesters and β-diketones [26], friedel-craft acylation of heterocycles [27-29], 2-, 4-picolines or 2-methylquinoline [30], nitroalkanes [31].

iii) S-Acylation of thiols to form thioesters including thiophenol [32] and cysteine [33], glutathione [34].

iv) O-acylation of alcohols (primary and secondary) to form esters including sugars [35, 36], stereoids [37], terpenes [38, 39] and α-hydroxycarboxylic acids [17].



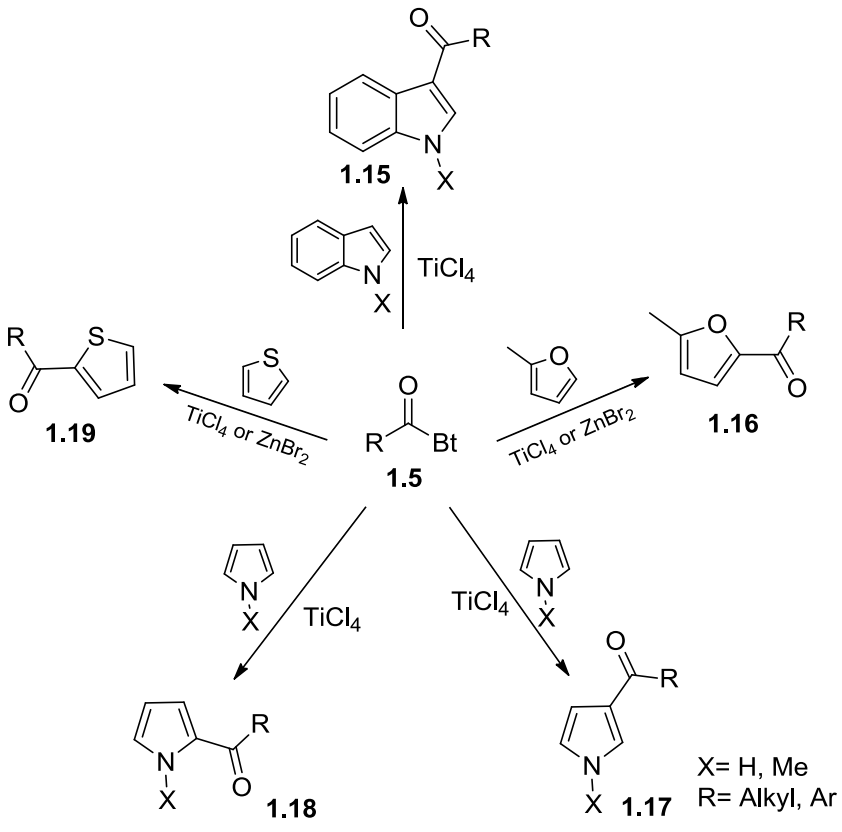
Scheme 1.2. Various uses of *N*-acylbenzotriazoles **1.5** for N-, C-, S-, and O-acylation

1.3.1. C-Acylation with *N*-acylbenzotriazoles

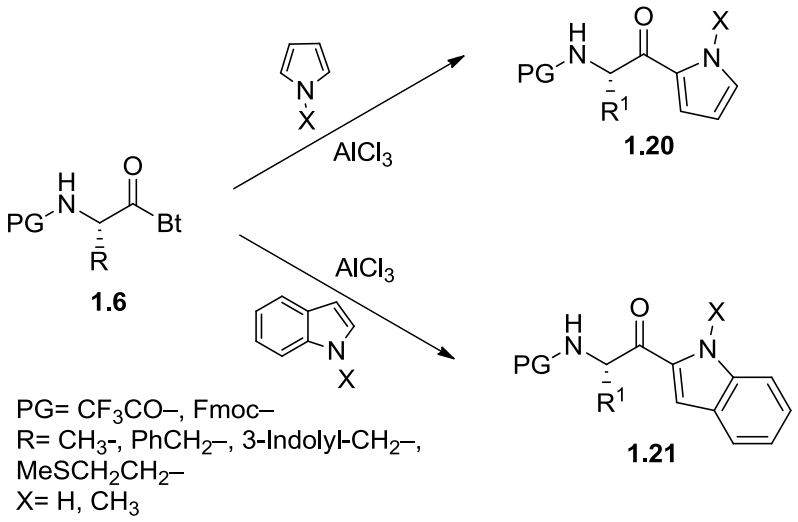
1.3.1.1. C-Acylation of heterocycles

C-Acylations of furan, thiophene, pyrroles, and indoles with *N*-acylbenzotriazoles **1.5** under the Friedel–Crafts reaction conditions in the presence of $TiCl_4$ (at 23 °C) or $ZnBr_2$ (at 110 °C), can provide C-acylated heterocycles in high yields with high regioselectivity (Scheme 1.3) [18, 27, 28].

Moreover, indoles and pyrroles can be acylated under Friedel–Crafts conditions in the presence of $AlCl_3$ with *N*-(Tfa- and Fmoc-α-aminoacyl) benzotriazoles to obtain α-aminoalkyl *N*-heteroaryl ketones with complete retention (>99%) of chirality (Scheme 1.4) [29].



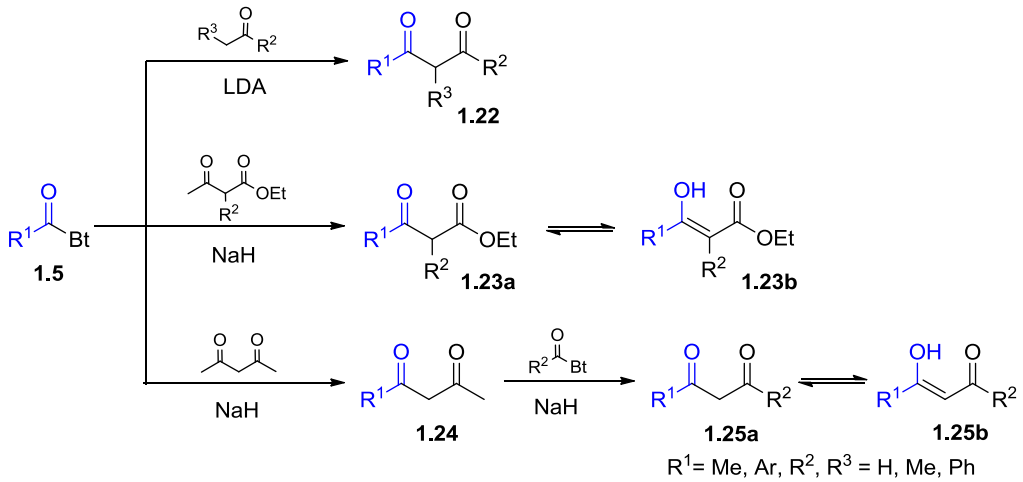
Scheme 1.3. Friedel–Crafts acylation with heterocycles



Scheme 1.4. α-Aminoalkyl *N*-heteroaryl ketones

1.3.1.2. C-Acylation of ketones, β-diketones, β-ketoesters

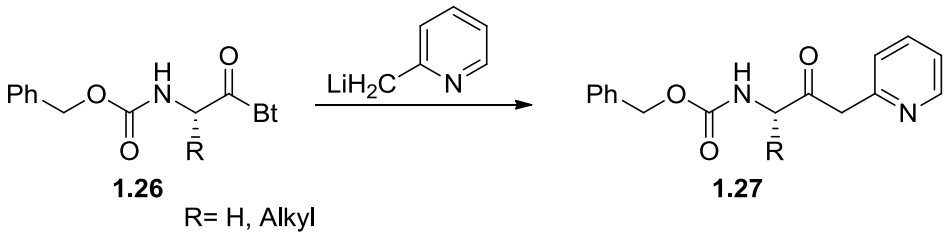
Ketones, β-diketones, β-ketoesters, cyanides and sulfones can be acylated with *N*-acylbenzotriazoles **1.5** under basic conditions [18]. C-acylation of ethyl acetoacetate and acetylaceton with aromatic *N*-acylbenzotriazoles **1.5** undergoes the deacetylation to afford β-ketoesters and β-diketones. C-acylative deacetylation of acetylacetone by successive reactions with 2 mol of different *N*-acylbenzotriazoles gives an unsymetric β-diketone **1.25** [18, 26] (Scheme 1.5).



Scheme 1.5. C-Acylation of ketones, β-diketones, β-ketoesters

1.3.1.3. C-Acylation of 2-picoline, 4-picolines or 2-methylquinoline

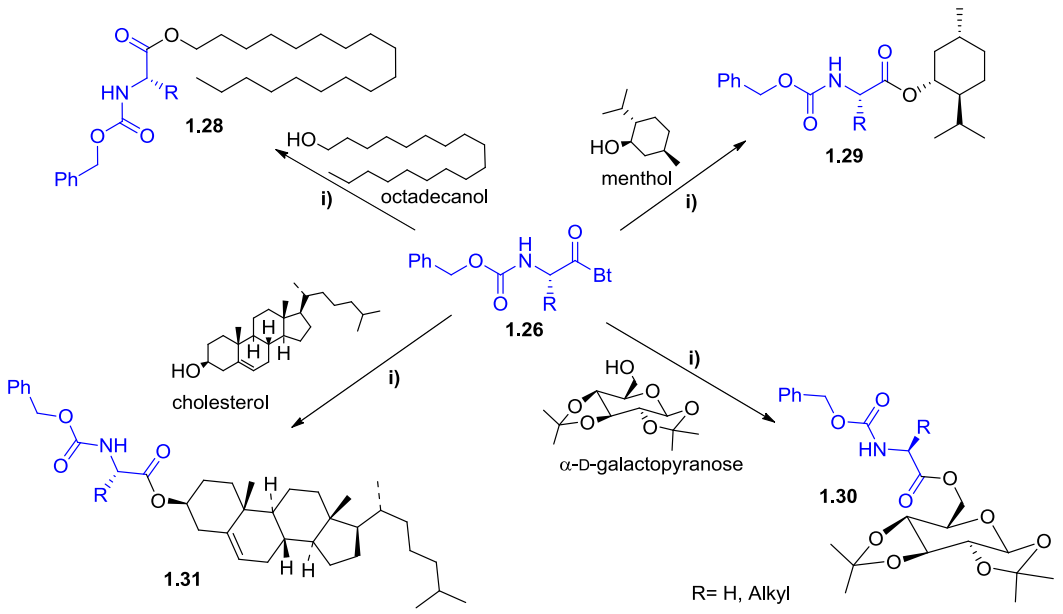
2-picoline, 4-picoline and 2-methylquinoline are acylated by *N*-(Cbz)-α-aminoacylbenzotriazoles **1.26** to afford novel aminoacyl conjugates **1.27** in moderate yields (33-53%) with retention of original chirality (Scheme 1.6) [30].



Scheme 1.6. C-Acylation of 2-picoline

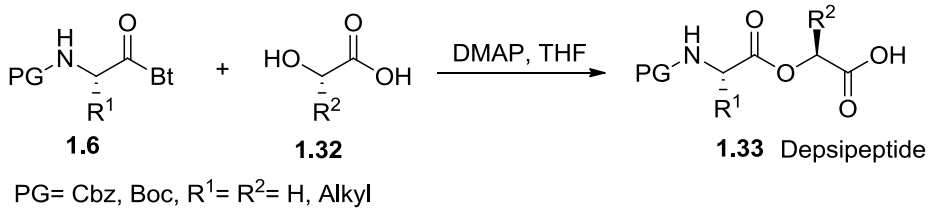
1.3.2. O-Acylation with *N*-acylbenzotriazoles

N-acylbenzotriazoles **1.5** were treated with naturally occurring alcohols such as sugars [35, 36], stereoids [37], terpenes [38] and long chain alcohols [39] under microwave conditions to form *O*-(α-protected-aminoacyl)esters **1.28-1.31** with full retention of original chirality [5, 40].

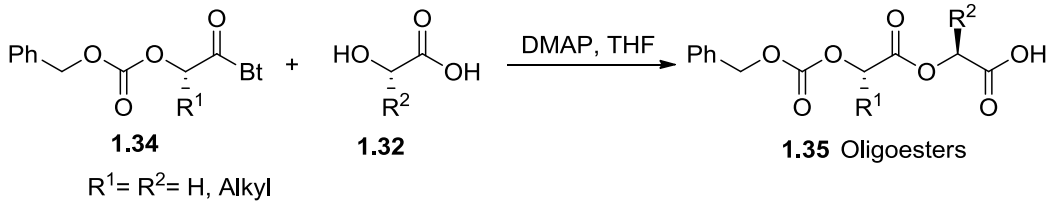


Scheme 1.7. Preparation of *O*-(protected α-aminoacyl)ester **1.28-1.31**
i) DMAP (0.1 equiv), THF, MW, 65 °C, 15 min

Recently, depsipeptides and oligoesters were derived from α-hydroxycarboxylic acids by treating with *N*-acylbenzotriazoles in the presence of DMAP in THF (Scheme 1.8 and 1.9) [17]. The detailed preparation of depsipeptides and chiral oligoesters are described in Chapter 3.



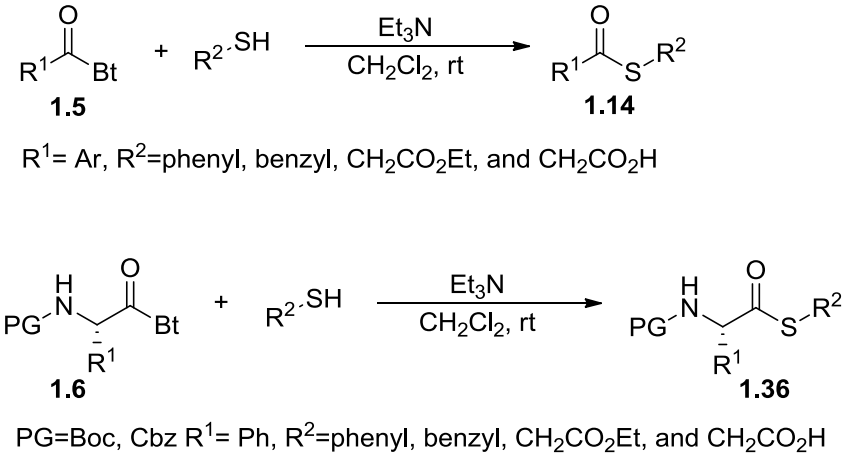
Scheme 1.8. Preparation of depsipeptides



Scheme 1.9. Preparation of chiral oligoesters

1.3.3. S-Acylation with *N*-acylbenzotriazoles

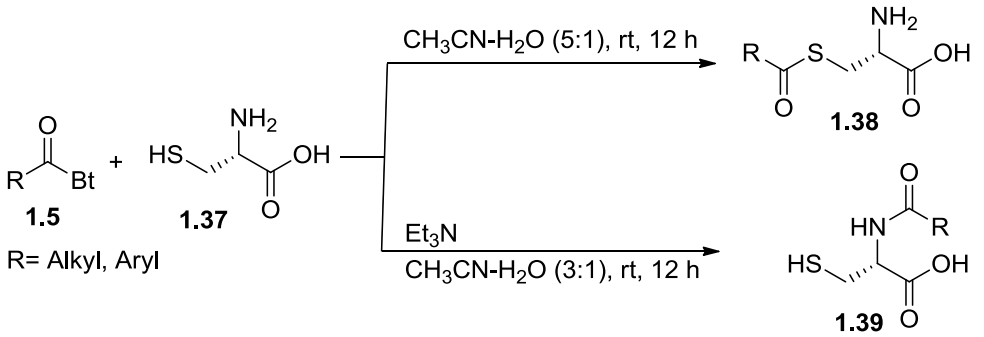
Various thioesters can be synthesized by reactions of various thiols with *N*-acylbenzotriazoles **1.5** under mild conditions [32] (Scheme 1.10).



Scheme 1.10. Synthesis of thioesters

1.3.3.1. Selective S and N-Acylation

Recently, Katritzky and coworkers described a new methodology which allows selective synthesis of *S*-Acyl- and *N*-Acylcysteines [33]. L-Cysteine **1.37** can be selectively acylated with *N*-acylbenzotriazoles **1.5** in the presence of triethylamine in CH_3CN-H_2O (3:1) to form *N*-acyl-L-cysteines **1.39**, whereas it gives exclusively *S*-acyl-L-cysteines **1.38** in the absence of base in CH_3CN-H_2O (5:1) at 20 ^oC (Scheme 1.8). Same methodology was later on effectively applied for selective acylation of glutathione [34].

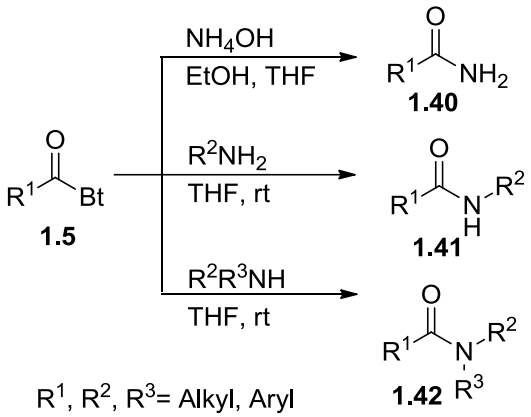


Scheme 1.11. Selective acylation of L-cysteine

Moreover, the selectively S-acylated cysteine peptides derived from *N*-(PG-aminoacyl)-benzotriazoles **1.6** were successfully used for native chemical ligation [41] [42].

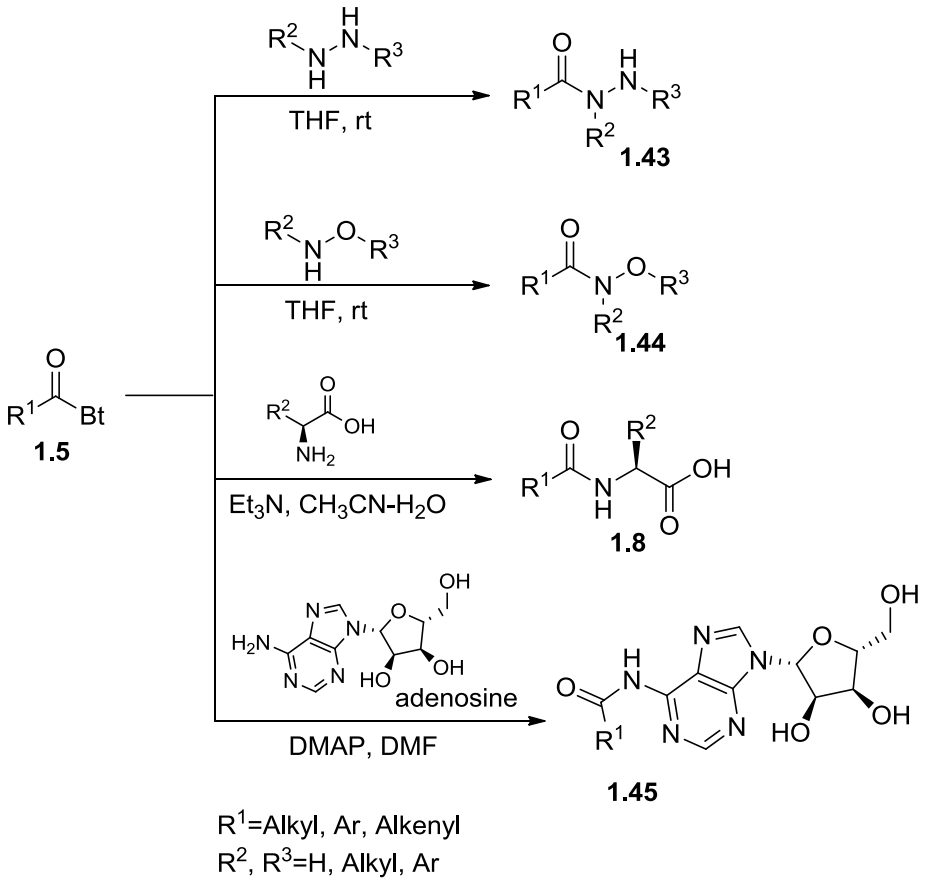
1.3.4. N-Acylation with *N*-acylbenzotriazoles

Acylation of amines is one of the fundamental reactions that forms amide bond in many organic compounds such as peptides, polymers, drugs, and etc. Among the other acylation methods (featuring with acyl chlorides, acid anhydrides, activated esters, carboxylic acids with coupling reagents); *N*-acylbenzotriazoles **1.5** have now a significant importance for N-acylation. Ammonia, primary amines, and secondary amines can be acylated under mild condition with *N*-acylbenzotriazoles **1.5** to form primary, secondary, and tertiary amides in high yields [16, 18].



Scheme 1.12. Synthesis of primary, secondary, and tertiary amides

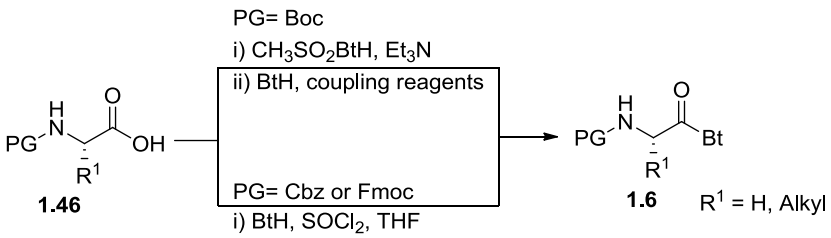
Furthermore, hydrazines, hydroxyl amines, nucleosides, and amino acids were converted to the corresponding hydrazides **1.43** [19, 20], *O*,*N*-dialkyl hydroxamic acids **1.44** (Weinreb amides) [21, 22], dye labelled nucleosides **1.45** [23] and oligopeptides **1.8** [5, 24], respectively.



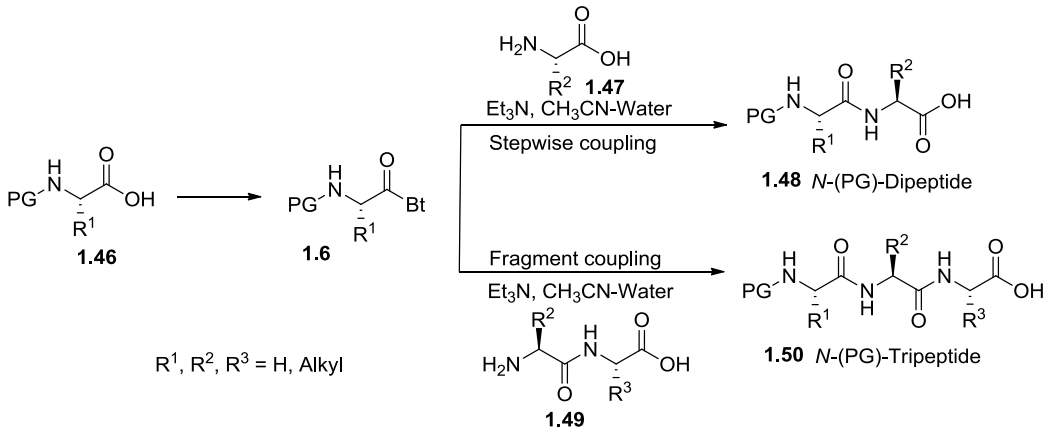
Scheme 1.13. Synthesis of hydrazides, *O*,*N*-dialkyl hydroxamic acids, peptide linked nucleosides and oligopeptides

1.3.4.1. Peptide synthesis with *N*-Acylbenzotriazoles

N-Acylation of amines with benzotriazole methodology has been successfully employed for peptide coupling [18]. The stable intermediates *N*-(PG-aminoacyl)benzotriazoles **1.6** can be derived from corresponding *N*-protected amino acids. *N*-(Boc)protected-aminoacylbenzotriazoles are obtained from corresponding amino acids; i) by treatment of methanesulfonyl-benzotriazole ($MeSO_2Bt$) in the presence of triethylamine or ii) by treating with coupling reagents such as DCC, EDCI in the presence of excess benzotriazole. *N*-(Cbz) and *N*-(Fmoc)-protected aminoacylbenzotriazoles extensively are derived from corresponding amino acids by treatment of thionly chloride ($SOCl_2$) in the presence of excess benzotriazole (Scheme 1.14).



Scheme 1.14. Preparetion of *N*-(Boc)protected-aminoacylbenzotriazoles

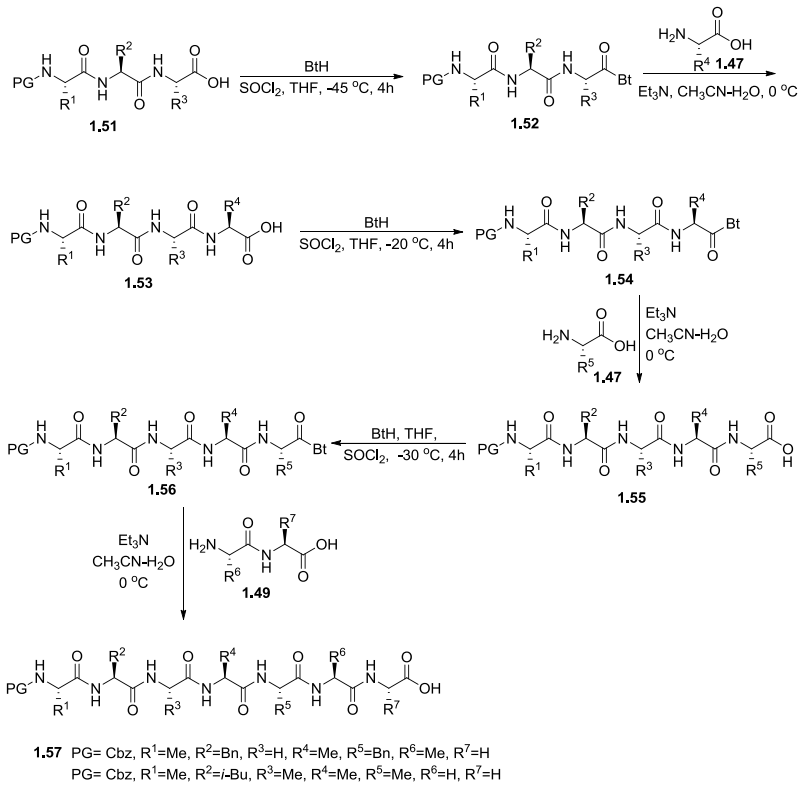


Scheme 1.15. General scheme for peptide synthesis using benzotriazole methodology

Later, *N*-(PG-aminoacyl)benzotriazoles **1.6** are treated with free amino acids **1.47** in the presence of triethylamine in CH_3CN-water (7:3) [18, 24] resulting in a new peptide conjugate with full retention of chirality in high yields [18, 24] (Scheme 1.15). This methodology enables to use a wide range of un-protected

amino acids including Glycine, Alanine, Phenylalanine, Leucine, Valine, Isoleucine, Tyrosine, Tryptophan, Serine, Threonine, Cysteine, and Methionine and also protected amino acids such as one side benzyl protected of Aspartic and Glutamic acid, $^{\omega}NO_2$-Arginine, and $^{\omega}$Cbz-Lysine [5]. While the coupling reactions using other peptide coupling reagents such as DCC, DIC, EDCI, HOBt, HOAt, PyBOP, HPyOPfp, CDI and etc suffer the requirement of prior protection and subsequent de-protection of amino acids in multistep peptide preparations, peptide coupling with benzotriazole methodology does not require the protection of amino acids **1.47** [5, 24]. In addition, one advantageous of the benzotriazole methodology is that all the reactions are carried out in the presence of water which is inexpensive and environmentally friendly solvent [5, 24].

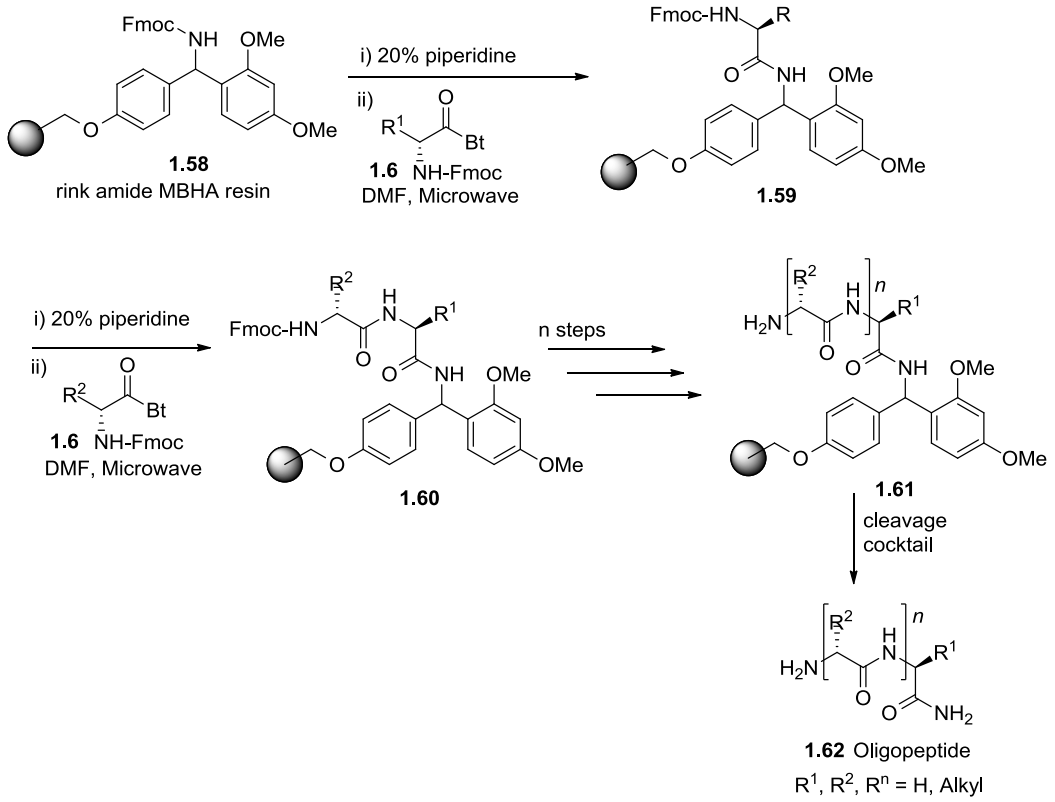
Katritzky and co-workers recently reported the solution phase preparation of penta-, hexa-, and hepta peptides in quantitative yields from *N*-(protected α-tri-, tetra- and pentapeptidoyl)benzotriazoles [43]. The preparation of *N*-(Cbz-protected)heptapeptides **1.57** from published article [43] are outlined in Scheme 1.16.



Scheme 1.16. Preparation of *N*-(Cbz)protected-heptapeptides **1.57**

1.3.4.2. Solid phase peptide synthesis (SPPS) with *N*-Acylbenzotriazoles

Solid-phase peptide synthesis (SPPS) is one of the major techniques for the rapid synthesis of potentially bioactive peptides. [44] Recently, *N*-(Fmoc)protected-aminoacylbenzotriazoles **1.6** have been used for the synthesis of some oligopeptides **1.62** on the Rink amide MBHA solid support [11, 12]. The general preparation of *n*-oligopeptides **1.62** using benzotriazole methodology on solid phase is outlined on Scheme 1.17.



Scheme 1.17. Solid Phase Peptide Synthesis (SPPS) using benzotriazole methodology

1.4. Peptidomimetics

Peptidomimetics are small protein-like chains designed to mimic peptides. As potential drugs, peptidomimetics are devoid of the undesirable properties of natural peptides [45-47]. Peptidomimetics benefit from conformational constraints and exhibit better pharmacokinetic properties. In contrast to their natural analogues, peptidomimetics are usually characterized by; (i) high affinity for

specific receptors , (ii) good metabolic stability towards endogenous proteases, (iii) greater oral bio-availability , (iv) more rapid excretion. [45-47]

Peptidomimetic compounds can be built up by cyclization of linear peptides [48] and coupling of stable unnatural amino acids (small peptidic scaffolds) [45-47] [49]. Unnatural amino acids can be generated from their native analogs after having several types of intellectual modifications. Amine alkylation [50], R group substitution [51] [52], structural bond extension [53] [54], cyclization [55], and isosteric replacements of atom or functional groups on amino acid backbone are most favored modifications in peptidomimetic chemistry (Figure 1.4).

Isosteric replacements of peptide backbone with a heteroatom constitute an important aspect of peptide chemistry because of its implications in the design of peptidomimetics. Even a single atom is replaced by its isostere brings diverse electrostatic properties and new secondary conformation to peptidomimetic chain, resulting in new pharmacokinetic properties [45-47].

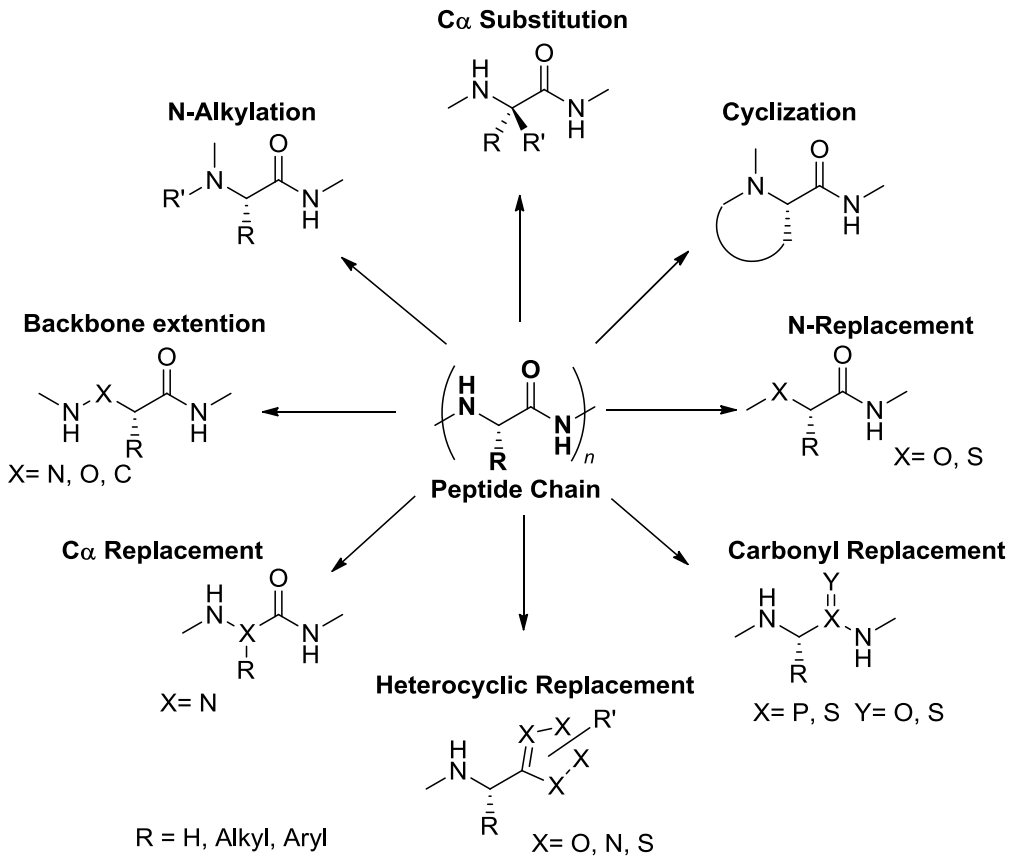


Figure 1.5. Various modifications to generate peptidomimetics

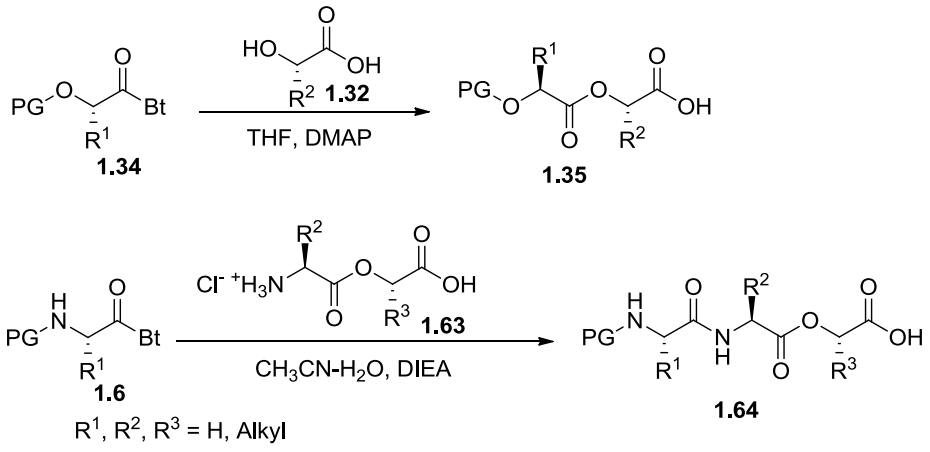
1.5. Scope

The therapeutic applications of peptides and small peptide-like chains have gained much attention in pharmacological science for the last decades. Nevertheless, low bioavailability and enzymatic degradation of peptides in living organism limits the utilities of peptides for therapeutic applications. Therefore, a new field of chemistry, peptidomimetic, has been revealed to improve pharmacokinetic properties of peptides. Novel peptide-like compounds have been designed and synthesized to investigate their biological importance.

Benzotriazole methodology, recently developed by Katritzky, A. R. leads to synthesize organic materials including valuable synthetic intermediates, drugs, natural products, and bio-conjugates, peptides in efficient way.

Herein, peptidomimetics, those oligoesters **1.35**, depsipeptides **1.64**, aminoxypeptides **1.68** and their hybrid analogs were successfully synthesized by using benzotriazole methodology. Free depsipeptides and aminoxy-acids were proved to be useful intermediates for synthesizing new peptide conjugates.

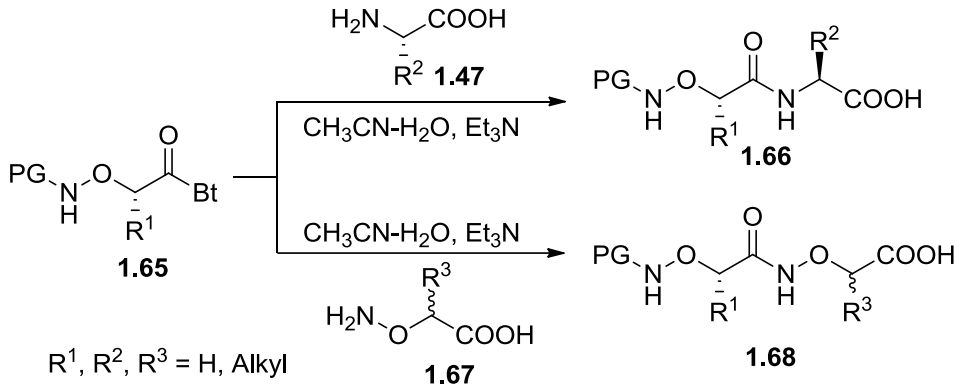
In chapter 2, depsipeptides **1.64** and chirally pure oligoesters **1.35**, a class of peptidomimetics, were prepared from *O*-PG(α-hydroxyacyl)benzotriazoles **1.34** and *N*-PG(α-Aminoacyl)benzotriazoles **1.6** by treatment with unprotected α-hydroxycarboxylic acids **1.32**, and amino acids **1.47** (Scheme 1.18).



Scheme 1.18. Preparation of depsipeptides and chiral oligoesters

In chapter 3, *N*-(PG)-α-aminoxy acids are converted to *N*-PG(α-aminoxyacyl)benzotriazoles **1.65**, which react with amines, α-amino acids **1.47**, α-

dipeptides **1.49** and α-aminoxy acids **1.67** to give aminoxyacyl amides, aminoxy hybrid peptides **1.66** and α-aminoxy peptides **1.68** in good yields under mild conditions with retention of chirality in the case of chiral compounds (Scheme 1.19).



Scheme 1.19. Preparation of aminoxy and aminoxy hybrid peptides

In chapter 4, aminoxy hybrid peptides and aminoxy peptides were attempted to prepare by using *N*-PG(α-aminoxyacyl)benzotriazoles **1.65** with microwave assisted solid-phase synthesis, nevertheless, attempts were not satisfactory to prepare them.

Chapter 5 provides a brief summary and achievements of this study.

2. EFFICIENT SYNTHESIS OF DEPSIPEPTIDES AND OLIGOESTERS BY USING BENZOTRIAZOLE METHODOLOGY[1]

2.1. Biological Importance of Depsipeptides

Depsipeptides are analogs of natural peptides containing both amino acids and α-hydroxy acids linked by amide and ester bonds [17]. Natural depsipeptides have significant biological activities (antimicrobial, antifungal, anti-inflammatory) and possess therapeutic properties including anticancer and anti-HIV activity [56, 57].

Romidepsin (FR228) is a cyclic depsipeptide, originally extracted from the bacterium *Chromobacterium violaceum* [58-60] (Figure 2.1). Romidepsin (FK228) is an antineoplastic antitumor agent as histone deacetylase inhibitor and an FDA approved anticancer drug, marketed under the trade name **Istodax** for treatment of cutaneous T-cell lymphoma [61]. Dolastine-10 and didemnin B (Figure 2.1) are cytotoxic depsipeptides, which were subjected to extensive phase II studies with their anti-tumor activities [62, 63]. Callipeltins and Papuamide A have promising inhibitory activity against HIV [64]. The cyclic depsipeptide, Valinomycin (Figure 2.1) is a natural ionophore, which is highly selective for potassium [65]; Nonactin (Figure 2.1) is another natural ionophore which has also anti-microbial effects [66].

Depsipeptides contain at least one ester bond in place of an amide link in their structure. The isosteric replacement of N-H group which is H-donor by the O atom of an ester causes depsipeptides to have poorer H-bonding. The decreased resonance of esters relative to amides leads to have lower rotational barriers for cis-trans isomerization in depsipeptides than in native peptide conjugates, and also induces to form more flexible structures [67]. Replacing an amino acid residue with a α-hydroxy acid causes structural perturbation in α-helix and β-sheet peptide structures [68-70].

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[1] Reprinted (adapted) with permission from *Journal of Organic Chemistry*, **2011**, *76*, 4884–4893 [17]. Copyright © 2011 American Chemical Society.

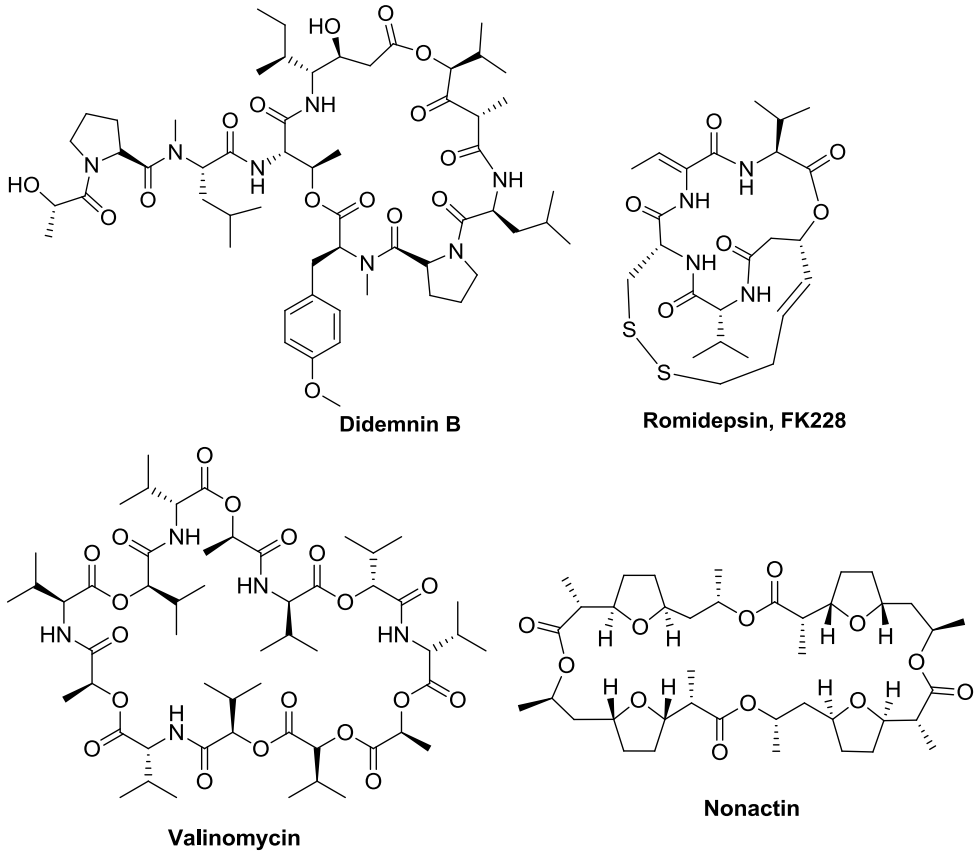


Figure 2.1. Structures of Didemnin B, Romidepsin FK228, Valinomycin and Nonactin

In the literature, methods for the preparation of depsipeptides require the use of coupling reagents in such combination as DIC-DMAP [71], DCC-DMAP [72]; EDCl-DMAP [73], PyBrop-DIEA [74] or Yamaguchi coupling conditions (TCBC, DIEA, DMAP) [75]. Riguera did a study to improve depsipeptide coupling with several coupling reagents. Coupling reactions resulted in variable yields (2–92%) with variable coupling times (2–20 h). Best results (92%, 2h) were obtained by DIC in the presence of DMAP [71]. Hence, there is still need to be developed more efficient strategies for the preparation of depsipeptides and peptide analogs in high yields.

In the present work, *O*-PG(α-hydroxyacyl)benzotriazoles **2.6** were prepared and their synthetic utility was shown in the preparation of both depsipeptides via N-acylation and chiral oligoesters via O-acylation without causing racemization. Unprotected depsidipeptides (depsides) were readily obtained from *N*-PG(α-aminoacyl)benzotriazoles and used for the preparation of longer depsipeptide conjugates.

2.2. Preparation of Depsipeptides and Oligoesters by Using Benzotriazole Methodology

The synthetic utility of *N*-PG(α-aminoacyl)benzotriazoles **2.5** has been explained in Chapter 1. To prepare depsipeptides and oligoesters, required *O*-PG(α-hydroxyacyl)benzotriazoles **2.6** were also prepared from corresponding acid analogues. α-Hydroxy acids **2.2** were prepared from α-amino acids via diazotization reaction. α-Hydroxy acids **2.2** were protected by common peptide protection groups, Cbz and Fmoc. *O*-PG(α-hydroxyacyl)benzotriazoles **2.6** were prepared to react with α-hydroxy **2.2** and α-amino acids **2.1** to form depsipeptides **2.7**, **2.10** and oligoesters **2.9**, respectively (Figure 2.2).

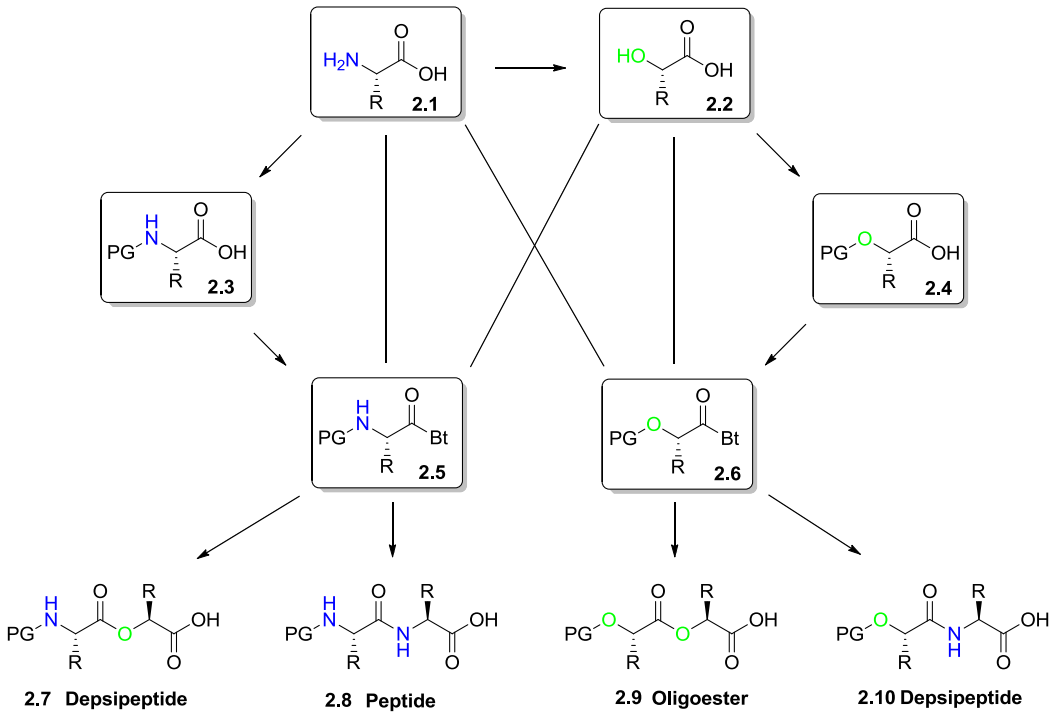
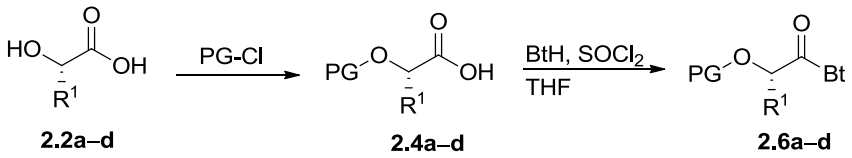


Figure 2.2. Synthetic route for preparation of depsipeptides, oligoesters and peptides

2.2.1 Synthesis of *O*-protected-α-hydroxycarboxylic acids 2.4a–d

α-Hydroxycarboxylic acids **2.2a–d** were treated with Cbz-Cl or Fmoc-Cl in basic conditions to form *O*-protected-α-hydroxycarboxylic acids **2.4a–d** (51–62%) (Scheme 2.1 and Table 2.1).



2.2a–d **2.4a–d** **2.6a–d**

Scheme 2.1. Synthesis of *O*-protected-α-hydroxycarboxylic acids **2.4a–d** and *O*-PG(α-hydroxyacyl)benzotriazoles **2.6a–d**

Table 2.1. *O*-protected-α-hydroxycarboxylic acids **2.4a–d**

Entry	**PG**:	R^1	Yield (%)	$[\alpha]_D^{23}$
a	Cbz	CH_2Ph (Phe)	**2.4a,** 56	-25.6
b	Cbz	$CH_2CH(CH_3)_2$ (Leu)	**2.4b,** 62	-29.2
c	Fmoc	$CH_2CH(CH_3)_2$ (Leu)	**2.4c,** 62	-8.9
d	Fmoc	CH_2Ph (Phe)	**2.4d,** 51	-12.4

2.2.2 Synthesis of *O*-PG(α-Hydroxyacyl)benzotriazoles 2.6a–d

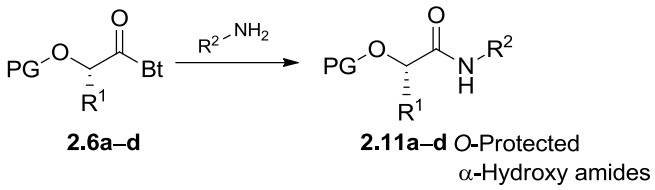
O-PG(α-Hydroxyacyl)benzotriazoles **2.6a–d** (72–86%) were prepared, by the treatment of the corresponding carboxylic acids **2.4a–d** with 4 equiv. of 1*H*-benzotriazole and 1 equiv. of $SOCl_2$ in THF at 10 ^oC for 4 h (Scheme 2.1, Table 2.2).

Table 2.2. *O*-PG(α-Hydroxyacyl)benzotriazoles **2.6a–d**

Entry	PG	R^1	Yield (%)	$[\alpha]_D^{23}$
a	Cbz	CH_2Ph (Phe)	**2.6a,** 76	-4.3
b	Cbz	$CH_2CH(CH_3)_2$ (Leu)	**2.6b,** 86	-61.6
c	Fmoc	$CH_2CH(CH_3)_2$ (Leu)	**2.6c,** 72	-79.2
d	Fmoc	CH_2Ph (Phe)	**2.6d,** 80	-16.6

2.2.3 *O*-PG(α-Hydroxyacyl)amides 2.11a–d

Reaction of *O*-PG(α-hydroxyacyl)benzotriazoles **2.6a–d** with amines and amino acids affords the corresponding amide derivatives **2.11a–d** (56–88%). (Scheme 2.2 and Table 2.3)



2.6a–d **2.11a–d** *O*-Protected α-Hydroxy amides

Scheme 2.2. Synthesis of depsiamides **2.11a–d**.

Retention of the original chirality of depsiamides **2.11** was supported by chiral HPLC analysis using a (*S*,*S*) Welk-O1 column (MeOH (100%), flow rate 1.0 mL/min, detection at 254 nm). The diastereomer **2.11a** showed a single retention-time peak at 5.93 min. in chiral HPLC, while its corresponding diastereomeric mixture (**2.11a+2.11a'**) showed two peaks at 4.24 and 5.95 min. Compound **2.11b** has a single retention-time peak at 10.02 min. in chiral HPLC, while its corresponding diastereomeric pair (**2.11b+2.11b'**) showed two peaks at 7.81 and 10.03 min (MeOH (100%), flow rate 0.5 mL/min, detection at 254 nm) (See Appx-1 and 2).

Table 2.3. *O*-PG(α-hydroxyacyl)amides **2.11a–d**

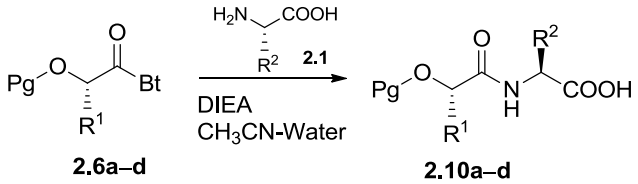
Entry	**2.6**	Amine	Yield (%)	$[\alpha]_D^{23}$
a	Cbz-(*O*Phe)-Bt, **2.6a**	L-α-methylbenzylamine	**2.11a**, 88	-12.9
b	Cbz-(*O*Phe)-Bt, **2.6a**	DL-α-methylbenzylamine	**(2.11a+2.11a')**, 76	-37.2
c	Cbz-(*O*Leu)-Bt, **2.6b**	L-α-methylbenzylamine	**2.11b**, 65	-66.0
d	Cbz-(*O*Leu)-Bt, **2.6b**	DL-α-methylbenzylamine	**(2.11b+2.11b')**, 56	-41.0
e	Cbz-(*O*Leu)-Bt, **2.6b**	*p*-methoxyaniline	**2.11c**, 71	-53.3
f	Fmoc-(*O*Leu)-Bt, **2.6c**	L-α-methylbenzylamine	**2.11d**, 67	-54.6

2.2.4 Preparation of depsipeptides

2.2.4.1. Preparation of depsipeptides via N-Acylation

O-PG(α-hydroxyacyl)benzotriazoles **2.6** were treated with the appropriate natural amino acids in aqueous acetonitrile-diisopropylethylamine (DIEA) at 10 ^{o}C for 0.5–2 h, to afford depsidipeptides **2.10a–d** (74–94%) (Scheme 2.3 and Table 2.4). In attempts to show the retention of chirality, diastereoisomeric analogs of **2.10a** and **2.10d** were prepared from DL-amino acids. Chiral HPLC

analyses were performed by using several HPLC columns including Chirobiotic T, (S,S) Whelk-O1 and Chiracell OD-H. Diastereoisomeric separations for **(2.10a**+**2.10a')**, and **(2.10d**+**2.10d')** were not observed on HPLC analysis using a variety of solvent system and flow rates; however the absence of racemization in the depsidipeptide conjugates **(2.10a**+**2.10a')** and **(2.10d**+**2.10d')** was checked by ^{1}H NMR spectra, where the methyl signal showed two separated doublets split in the DL-alanine moiety. While **2.10a** has a clear doublet at 1.27 ppm (J = 7.2 Hz), **(2.10a**+**2.10a')** has two separated doublets 1.24 and 1.33 ppm (J = 7.2 Hz). A similar result was also showed that there is no detectible racemization on ^{1}H NMR spectra for the compounds **2.10d** and **(2.10d**+**2.10d')** (See Appx-7-10).

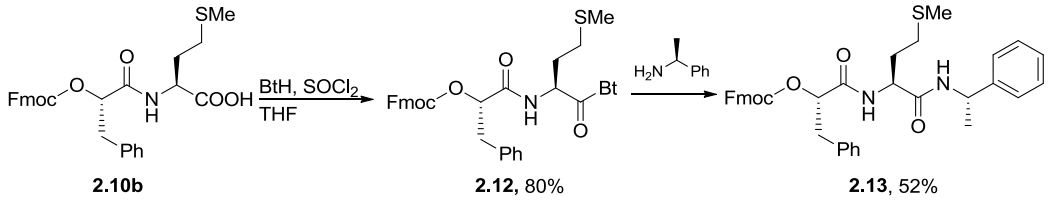


Scheme 2.3. Preparation of depsipeptides **2.10a–d** via N-acylation.

Table 2.4. Depsidipeptides **2.10a–d**

Entry	PG	R^1	Amino acid, **2.1**	Yield (%)	$[\alpha]_D^{23}$
a	Cbz	CH_2Ph (Phe), **2.6a**	L-Ala-OH, **2.1**[a]	**2.10a,** 86	-17.6
b	Cbz	CH_2Ph (Phe), **2.6a**	DL-Ala-OH, **(2.1a**+**2.1a')**	**(2.10a**+**2.10a')**, 80	-50.6
c	Fmoc	CH_2Ph (Phe), **2.6c**	L-Met-OH, **2.1b**	**2.10b,** 74	-16.9
d	Fmoc	CH_2Ph (Phe), **2.6c**	L-Glu-OH, **2.1c**	**2.10c,** 82	-23.8
e	Fmoc	CH_2Ph (Phe), **2.6c**	L-Ala-OH, **2.1a**	**2.10d,** 87	-30.0
f	Fmoc	CH_2Ph (Phe), **2.6c**	DL-Ala-OH, **(2.1a+2.1a')**	**(2.10d+2.10d')**, 94	-26.8

Compound **2.10b** was further treated with $SOCl_2$ and 1*H*-benzotriazole in THF to obtain benzotriazole activated conjugate **2.12** (Scheme 2.4). *O*-Fmoc(depsidipeptidoyl)benzotriazole **2.12** was reacted with L-methylbenzylamine in THF to afford the corresponding amide derivative **2.13** in 52% yield (Scheme 2.4, Appx-11,12).

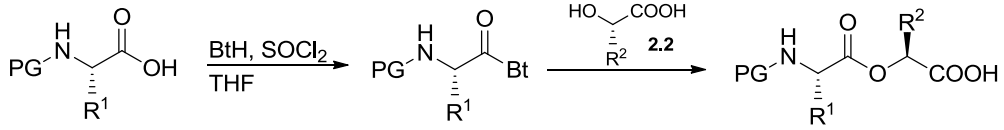


2.10b **2.12,** 80% **2.13**, 52%

Scheme 2.4. Preparation of amide derivative **7**

2.2.4.2. Preparation of depsipeptides via O-Acylation

Treatment of *N*-PG(α-Aminoacyl)benzotriazoles **2.5a–d** with α-hydroxycarboxylic acids **2.2** in the presence of DMAP in THF gave depsidipeptides **2.7a–d** (Scheme 2.5, Table 2.5).



2.3 **2.5** **2.7a–d**

Scheme 2.5. Preparation of depsidipeptides **11** and **13** via O-acylation.

Table 2.5. Depsidipeptides **2.7a–d**

Entry	PG	R^1	**2.2**	Yield (%)	$[\alpha]_D^{23}$
a	Cbz	$CH_2CH(CH_3)_2$ (Leu)	L-(*O*Phe)-OH, **2.2a**	**2.7a,** 76	-21.0
b	Cbz	$CH_2CH(CH_3)_2$ (Leu)	DL-(*O*Phe)-OH, **(2.2a+2.2a')**	**(2.7a+2.7a'),** 78	-11.3
c	Cbz	Me (Ala)	L-(*O*Phe)-OH, **2.2a**	**2.7b**[a]**,** 76	-25.9
d	Cbz	CH_2Ph (Phe)	L-(*O*Leu)-OH, **2.2b**	**2.7c**[a]**,** 47	-34.5
e	Cbz	$CH_2CH_2SCH_3$ (Met)	L-(*O*Phe)-OH, **2.2a**	**2.7d,** 54	-18.6

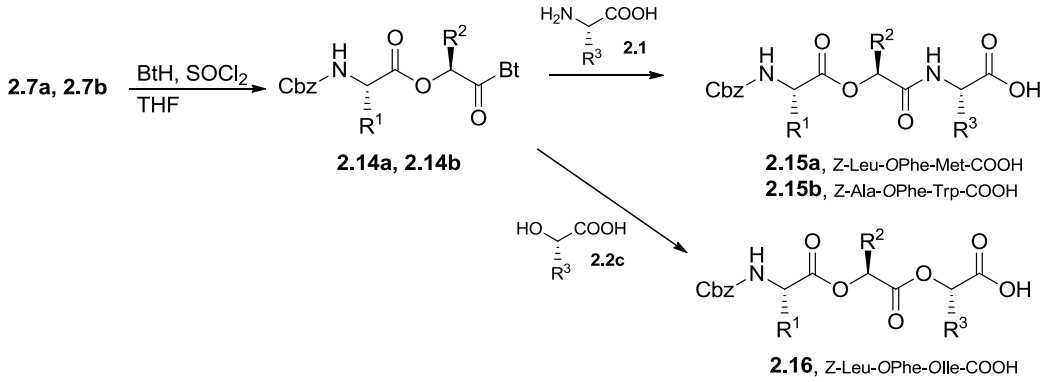
[a] Isolated as dicyclohexylamine salt

In our preliminary results, DBU, TEA and DIEA were used for *O*-acylation. Nevertheless, O-acylated products showed racemizations when DBU, TEA and DIEA were used since coupling reactions require longer times. DMAP mostly used in coupling reactions was tried for O-acylation. Since DMAP acts as a base and also activates the carboxyl group via the acyl pyridinium salt [76] gave better results for O-acylation. The retention of original chirality of depsidipeptide products **2.7a** and **(2.7a+2.7a')** was confirmed by chiral HPLC analysis using a Chirobiotic T column (MeOH (100%), flow rate 0.5 mL/min, detection at 254

nm.). Compound **11a** showed a single retention-time peak in chiral HPLC analysis at 6.46 min., while the corresponding diastereomixture **(2.7a+2.7a')** showed two peaks at 6.33 and 6.84 min (Appx-13 and 14).

2.2.4.3. Preparation of depsitripeptides

N-Cbz-(Depsidipeptidoyl)benzotriazoles **2.14a** and **2.14b** were obtained by treatment of **2.7a** and **2.7b** with benzotriazole in the presence of $SOCl_2$ in THF. Reaction of **2.14a** and **2.14b** with amino acids **2.1a, 2.1b** in CH_3CN-water in the presence of DIEA afforded depsitripeptides **2.15a, 2.15b**, while treatment with α-hydroxycarboxylic acid **2.2c** in THF in the presence of DMAP gave depsitripeptide **2.16** by O-acylation (Scheme 2.6, Table 2.6).



Scheme 2.6. Preparation of depsitripeptides **2.15a, 2.15b** and **2.16**

Table 2.6. Depsitripeptides **2.15a, 2.15b** and **2.16**

Entry	Sequence	Yield, (%)	$[\alpha]_D^{23}$
a	Cbz-Leu-*O*Phe-Met-OH	**2.15a**, 78	-34.7
b	Cbz-Ala-*O*Phe-Trp-OH	**2.15b**, 63	-87.3
c	Cbz-Leu-*O*Phe-*O*Ile-OH	**2.16**, 55	-24.2

The structure and absolute configuration of **2.15a** was clearly recognized by X-ray crystallography (Figure 2.3).

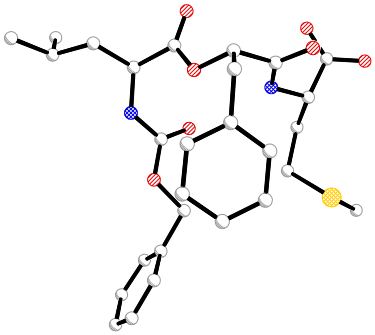
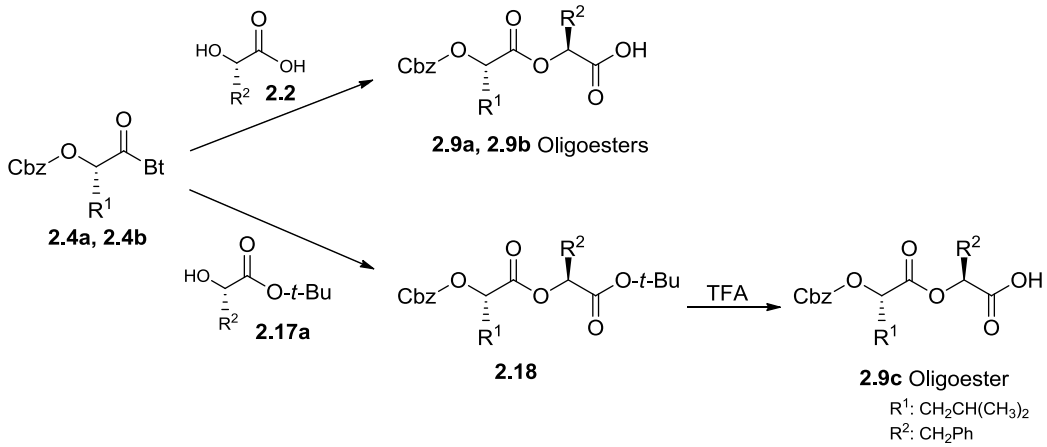


Figure 2.3. X-ray crystal structure of Cbz-Leu-*O*Phe-Met-OH **2.15a**. (Hydrogen atoms are not shown.)

2.2.5 Preparation of chiral oligoesters 2.9a–c

Chiral oligoesters **2.9a**, **2.9b** were prepared from Cbz-*O*Phe-Bt **2.6a** by reacting it with unprotected α-hydroxy acids **2.2b** and **2.2d** in THF in the presence of DMAP for 4–6 h (75–85%). Partial racemization was observed on ^{1}H NMR studies; while **2.9c** was prepared from Cbz-*O*Leu-Bt **2.6b** since the coupling reaction was completed after 10 h. This problem was overcome by the coupling reaction was carried out using the *t*-butyl ester of α-hydroxyacid **2.17**. Once product **2.18** was formed, it was deprotected treating with a mixture of TFA/CH_2Cl_2 (1:1) to afford **2.9c** in 67% as overall yield (Scheme 2.7 and Table 2.7).



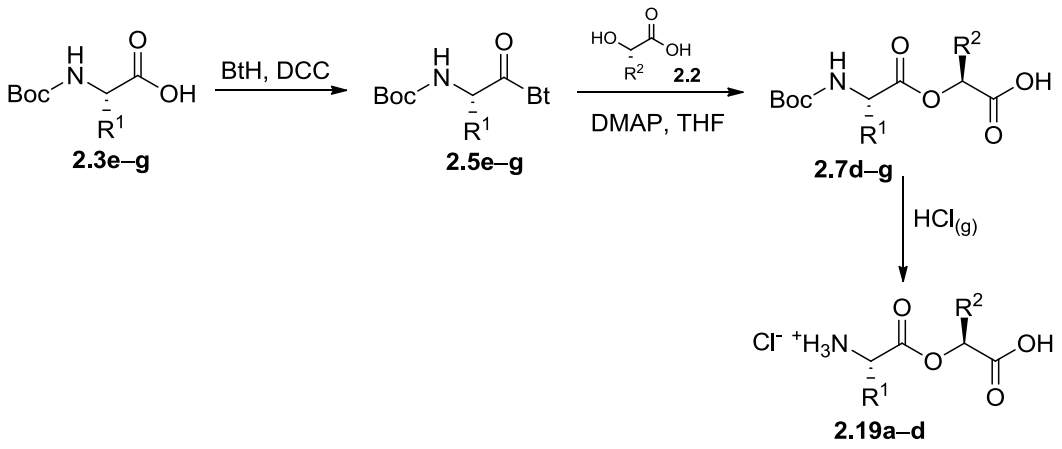
Scheme 2.7. Preparation of oligoesters **2.9a–c** via O-Acylation

Table 2.7. Oligoesters **2.9a–c**

Entry	R^1	R^2	Yield (%)	$[\alpha]_D^{23}$
a	CH_2Ph (Phe), **2.4a**	$CH(CH_3)_2$ (Val), **2.2d**	**2.9a**, 85	-30.0
b	CH_2Ph (Phe), **2.4a**	$CH_2CH(CH_3)_2$ (Leu), **2.2b**	**2.9b**, 75	-34.8
c	$CH_2CH(CH_3)_2$ (Leu), **2.4b**	CH_2Ph (Phe), **2.18a**	**2.9c**, 69[a]	-38.9

2.2.6. Preparation of unprotected depsidipeptides (free depsides) 2.19

L-α-Dipeptides (dipeptides) are helpful intermediates to obtain longer peptide analogs. Nevertheless, the functions and applications of dipeptides have been poorly examined compared with proteins or amino acids, due to the lack of an efficient preparation of dipeptides [77]. Herein, a new milder strategy for preparation of unprotected depsidipeptides is developed (Scheme 7, 8). *N*-Boc(α-aminoacyl)benzotriazoles **2.5e–g** were obtained from Boc-protected amino acids **2.3e–g** in DCC-coupling conditions with 1*H*-benzotriazole. *N*-Boc(α-aminoacyl)benzotriazoles **2.5e–g** were reacted with α-hydroxy acids **2.2a**, **2.2b** in the presence of DMAP in THF to obtain Boc-protected-depsidipeptides **2.7d–g**. Then without isolation of **2.7d–g**, Boc deprotection was achieved by $HCl_{(g)}$ in dry CH_2Cl_2 for 1 h to afford unprotected depsidipeptides **2.19a–d** as hydrochloride salts (Scheme 2.8, Table 2.8).

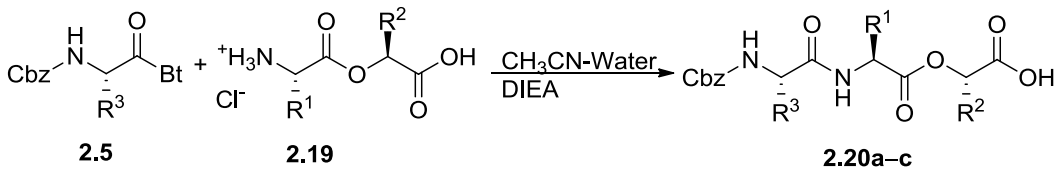


Scheme 2.8. Synthesis of unprotected depsides **2.19**.

Table 2.8. Free depsidipeptides (depsides) **2.19a–d**

Entry	R^1	R^2	Yield (%)	Mp (°C)	$[\alpha]_D^{23}$
a	H	CH_2Ph	**2.19a,** 48	150–155	-31.4
b	CH_3	$CH_2CH(CH_3)_2$	**2.19b**, 36	165–168	-25.8
c	H	$CH_2CH(CH_3)_2$	**2.19c**, 62	65–70	-37.5
d	CH_2Ph	$CH_2CH(CH_3)_2$	**2.19d**, 56	139–140	-24.4

N-PG(α-aminoacyl)benzotriazoles **2.5c**, **2.5d** were reacted with unprotected depsidipeptides (free depsides) **2.19a**, **2.19d** in the presence of 2 equiv. DIEA in MeCN-H_2O (7:1) to afford *N*-PG-depsitripeptides **2.20a–c** (Scheme 2.9, Table 2.9).



2.5 **2.19** **2.20a–c**

Scheme 2.9. Preparation of depsitripeptides **2.20a–c**.

Table 2.9. Depsitripeptides **2.20a–c**

Entry	R^3	R^1	R^2	**2.20**, Yield (%)	Mp (°C)	$[\alpha]_D^{23}$
a	CH_2Ph	H	$CH_2CH(CH_3)_2$	**2.20a**, 71	52–55	-15.7
b	$CH_2CH_2SCH_3$	CH_2Ph	$CH_2CH(CH_3)_2$	**2.20b**, 56	140–142	-36.3
c	$CH_2CH_2SCH_3$	H	$CH_2CH(CH_3)_2$	**2.20c**, 68	113–115	-22.5

2.3. Conclusion

In conclusion, novel *O*-PG(α-hydroxyacyl)benzotriazoles have been prepared and employed for the synthesis of depsipeptides and depsiamides by N-acylation of unprotected amino acids in aqueous condition. *O*-PG(α-hydroxyacyl)benzotriazoles were also used for the preparation of chiral oligoesters by *O*-acylation of unprotected α-hydroxy acids in dry THF The retention of chirality in products after *O*-acylation and *N*-acylation reactions was

examined by chiral HPLC, which showed no detectable racemization. In addition, novel free depsides were prepared in good yields and their synthetic utility was showed in the preparation of longer depsipeptide analogs. These synthetic methodologies are expected to be useful for the syntheses of other biologically active depsipeptides.

2.4 Experimental Section

Melting points are uncorrected. ^{1}H (300MHz) and ^{13}C (75MHz) NMR spectra were recorded on 300MHz apparatus in $CDCl_3$ or DMSO-d_6 with using TMS as internal standard. DMF was dried by distilling over CaH_2, whereas THF was used after distillation over Na/benzophenone. Unprotected amino acids **2.1**, and *N*-(protected)-amino acids **2.3** were purchased from commercial sources. *N*-(Acylbenzotriazoles) **2.5a–d** were prepared by previously reported methods [78]. L-α-Hydroxycarboxylic acids **2.2** [79], and **2.17a**-b [80] were prepared using literature methods.

2.4.1. Synthesis of *O*-Cbz and *O*-Fmoc protected α-hydroxycarboxylic acids analogs 2.4a–d

2.4.1.1. General Synthesis of 2.4a and 2.4b

Pyridine (18 mmol) was added to a stirred solution of α-hydroxycarboxylic acids **2.2** (18 mmol) in THF (30 mL) dropwise at 4 ^{o}C in 10 minutes. After stirred 10 min on an ice bath, benzylchloroformate (21.7 mmol) was added to the mixture at same temperature in 30 min. Then the mixture was stirred overnight at room temperature. The solvent was removed and the residue taken in to EtOAc (50 mL) and washed with 2 N HCl (3 x 15 mL) and brine (15 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude was purified with flush column chromatography (EtOAc-hexanes (15%)) to afford **2.4a–b** as colorless oil.

Z-L-(*O*Phe)-OH (2.4a). Colorless oil (56%); $[\alpha]_D^{23}$ = –25.6 (*c* 1.5, CH_3OH), (lit. [81] $[\alpha]_D^{23}$ = –18.2 ^{o} (*c* 1.2, $CHCl_3$)); ^{1}H NMR ($CDCl_3$) δ 3.15 (dd, *J* = 14.3 Hz, 8.7 Hz, 1H), 3.26 (dd, *J* = 14.4 Hz, 4.2 Hz, 1H), 5.10–5.20 (m, 2H), 5.19 (dd, *J* = 8.4 Hz, 3.9 Hz, 1H), 5.40 (br s, 1H), 7.20–7.40 (m, 10H); ^{13}C NMR

($CDCl_3$) δ 37.4, 70.4, 75.8, 127.4, 128.5, 128.8, 129.6, 134.9, 135.4, 154.7, 174.4. Anal. Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.71; H, 5.50.

Z-L-(*O*Leu)-OH (2.4b). Colorless oil (62%); $[\alpha]_D^{23}$ = –29.2 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.95 (dd, *J* = 6.0 Hz, 3.3 Hz, 6 H), 1.62–1.76 (m, 1H), 1.78–1.91 (m, 2H), 5.00 (dd, *J* = 9.6 Hz, 3.9 Hz, 1H), 5.20 (s, 2H), 7.30–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 21.6, 23.1, 24.7, 39.9, 70.9, 73.4, 128.5, 128.8, 135.0, 154.9, 176.2; Anal. Calcd. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 62.93; H, 7.14.

2.4.1.2. Synthesis of 2.4c and 2.4d

A solution of pyridine (8.2 mmol) in THF (8 mL) was added to a stirred solution of Fmoc-Cl (8.0 mmol) and α-hydroxycarboxylic acids **2.2** (8.2 mmol) at -10 ^{o}C in THF (30 mL) at same temperature. The reaction was allowed to get room temperature and stirred overnight. The reaction was monitored by TLC EtOAc-hexanes (1:4). The mixture was taken into separation funnel and washed with 2N HCl (3 x 20 mL) and brine (20 mL). After it was dried over the $MgSO_4$, the solvent was removed under reduced pressure. The crude was purified by column chromatograph [EtOAc-hexanes (15%)] to afford **2.4c–d** as white microcrystals.

Fmoc-L-(*O*Leu)-OH (2.4c) [82] White microcrystals (1.76 g, 62%), mp 112–113 °C; $[\alpha]_D^{23}$ = –8.9 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.99 (dd, *J* = 7.2 Hz, 6.6 Hz, 6H), 1.66–1.78 (m, 1H), 1.80–1.98 (m, 2H), 4.26–4.40 (m, 2H), 4.53 (dd, *J* = 9.9 Hz, 6.9 Hz, 1H), 5.02 (dd, *J* = 9.6 Hz, 3.6 Hz, 1H), 7.28–7.36 (m, 2H), 7.36–7.44 (m, 2H), 7.62 (t, *J* = 6.6 Hz, 2H), 7.76 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.6, 23.2, 24.8, 39.8, 46.9, 70.6, 73.8, 120.3, 125.3, 125.4, 127.4, 128.1, 141.5, 143.2, 143.5, 154.9, 176.2; Anal. Calcd. for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 71.26; H, 6.22.

Fmoc-L-(*O*Phe)-OH (2.4d) White microcrystals (1.6 g, 51%), mp 101–103 °C; $[\alpha]_D^{23}$ = –12.4 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 3.18 (dd, *J* = 14.4 Hz, 9.0 Hz, 1H), 3.30 (dd, *J* = 14.4 Hz, 3.9 Hz, 1H), 4.20–4.34 (m, 2H), 4.38–4.46 (m, 1H), 5.21 (dd, *J* = 8.7 Hz, 4.1 Hz, 1H), 7.20–7.42 (m, 9H), 7.54 (dd, *J* = 7.5 Hz,

3.3 Hz, 2H), 7.73 (d, *J* = 7.5 Hz, 2H), 9.35 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.4, 46.7, 70.6, 75.8, 120.2, 125.3, 125.4, 127.4, 127.5, 128.1, 128.8, 129.6, 135.5, 141.4, 143.2, 143.6, 154.6, 175.1; Anal. Calcd. for $C_{24}H_{20}O_5$: C, 74.21.14; H, 5.19. Found: C, 74.02; H, 5.42.

2.4.2. General preparation of *O*-PG(α-hydroxyacyl)benzotriazole (2.6)

Thionyl chloride (1.2 mmol) was added to a solution of benzotriazole (4.16 mmol) in freshly distilled CH_2Cl_2 (10 mL) at 5 ^{o}C, and the reaction mixture stirred for 20 min at the same temperature. *O*-(PG)-hydroxycarboxylic acid **2.4** (1.0 mmol) dissolved in CH_2Cl_2 (2 mL) was added dropwise to the mixture. After stirring for 3 h at 10 ^{o}C, the reaction mixture was allowed to warm to room temperature. After 1 h, the white precipitate was filtered off and discarded. The solution was diluted with more CH_2Cl_2 (10 mL) and washed with saturated Na_2CO_3 solution (3 x 10 mL), then saturated brine solution and finally dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford *O*-PG(hydroxyacyl)benzotriazole **2.6**.

Cbz-L-(*O*Phe)-Bt (2.6a). White microcrystals (76%), mp 97–98 °C; $[\alpha]_D^{23}$ = –4.3 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 3.34 (dd, *J* = 14.1 Hz, 9.0 Hz, 1H), 3.50 (dd, *J* = 14.1 Hz, 3.6 Hz, 1H), 5.15 (s, 2H), 6.49 (dd, *J* = 9.0 Hz, 3.6 Hz, 1H), 7.20–7.42 (m, 10H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 8.26 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 38.0, 70.6, 114.5, 120.6, 126.9, 127.6, 128.5, 128.8, 129.7, 131.1, 131.2, 134.8, 135.2, 146.1, 154.7, 168.4; Anal. Calcd. for $C_{23}H_{19}N_3O_4$·: C, 68.82; H, 4.77; N, 10.47. Found: C, 68.88; H, 4.77; N, 10.39.

Cbz-L-(*O*Leu)-Bt (2.6b). Colorless oil (86%); $[\alpha]_D^{23}$ = –61.6 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.98 (d, *J* = 6.3 Hz, 3H), 1.08 (d, *J* = 6.3 Hz, 3H), 1.82–2.10 (m, 3H), 5.12 (s, 2H), 6.33 (dd, *J* = 10.2 Hz, 3.0 Hz, 1H), 7.30–7.42 (m, 5H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 6.9 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.5, 23.4, 25.2, 40.4, 70.6, 74.9, 114.6, 120.6, 126.8, 128.5, 128.8, 131.0, 131.3, 134.9, 146.1, 154.9, 169.6. Anal. Calcd. for $C_{20}H_{21}N_3O_4$: C, 65.38; H, 5.76; N, 11.44. Found: C, 65.24; H, 6.10; N, 11.07.

Fmoc-L-(*O*Leu)-Bt (2.6c). Colorless oil (72%); $[\alpha]_D^{23}$ = –79.2 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.03 (d, *J* = 5.1 Hz, 3H), 1.12 (d, *J* = 5.1 Hz, 3H), 1.86–2.28 (m, 3H), 4.28–4.42 (m, 2H), 4.50–4.58 (m, 1H), 6.33 (d, *J* = 10.8 Hz, 1H), 7.28–7.46 (m, 4H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.60–7.80 (m, 5H), 8.15 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.5, 23.4, 25.3, 40.3, 46.9, 70.8, 75.0, 114.6, 120.3, 120.6, 125.4, 125.5, 126.8, 127.4, 128.2, 131.0, 131.3, 141.5, 143.2, 143.5, 146.2, 155.0, 169.6. Anal. Calcd. for $C_{27}H_{25}N_3O_4$: C, 71.19; H, 5.53; N, 9.22. Found: C, 70.35; H, 5.58; N, 9.75.

Fmoc-L-(*O*Phe)-Bt (2.6d). White microcrystals (80%), mp 132–134 °C; $[\alpha]_D^{23}$ = –12.4 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.37 (dd, *J* = 14.1 Hz, 9.3 Hz, 1H), 3.55 (dd, *J* =14.1 Hz, 3.6 Hz, 1H), 4.20–4.36 (m, 2H), 4.38–4.50 (m, 1H), 6.49 (dd, *J* = 9.3 Hz, 3.6 Hz, 1H), 7.20–7.44 (m, 9H), 7.46–7.60 (m, 3H), 7.60–7.76 (m, 3H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.25 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 38.0, 46.7, 70.8, 76.8, 114.5, 120.2, 120.6, 125.3, 125.5, 126.9, 127.4, 127.6, 128.1, 128.9, 129.7, 131.1, 131.2, 135.4, 141.4, 143.1, 143.4, 146.1, 154.6, 168.3. Anal. Calcd. for $C_{30}H_{23}N_3O_4$: C, 73.61; H, 4.74; N, 8.58. Found: C, 73.47; H, 5.01; N, 8.40.

2.4.3. General preparation of *O*-PG(α-hydroxyacyl) amides (2.11a–d)

Amine (1.2 equiv.) and pyridine (1.2 equiv. mol) in THF (2 mL) were added to a stirred solution of *O*-PG(hydroxyacyl)benzotriazole **2.5** (1 equiv. mol) in THF (4 mL) dropwise at 10 ^{o}C and the mixture was stirred for 2 h at room temperature. After evaporation of THF, EtOAc (15 mL) was added to the solution, which was washed with 2N HCl (2 x 10 mL), saturated Na_2CO_3 solution (3 x 10 mL) and brine (10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave the crude amides **2.11a–d**.

Benzyl ((S)-1-oxo-3-phenyl-1-(((S)-1-phenylethyl)amino)propan-2-yl) carbonate (2.11a). The crude product was recrystallized from diethyl ether-hexanes to give white microcrystals (88%), mp 121–122 °C; $[\alpha]_D^{23}$ = –12.9 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.9 Hz, 3H), 3.18 (dd, *J* = 14.4 Hz, 6.3 Hz, 1H), 3.28 (dd, *J* = 14.1 Hz, 4.5 Hz, 1H), 5.00–5.10 (m, 1H), 5.10 (d, *J* = 5.7

Hz, 2H), 5.29 (dd, *J* = 6.0 Hz, 4.8 Hz, 1H), 6.12 (d, *J* = 7.8 Hz, 1H), 7.12–7.40 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 21.5, 38.0, 48.5, 70.5, 77.9, 126.3, 127.2, 127.6, 128.6, 128.7, 128.8, 128.9, 129.0, 130.0, 134.8, 135.4, 142.5, 153.7, 167.6; Anal. Calcd. for $C_{25}H_{25}N_1O_4$·: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.36; H, 6.44; N, 3.34.

Benzyl ((2S)-1-oxo-3-phenyl-1-((1-phenylethyl)amino)propan-2-yl) carbonate (2.11a+2.11a'). Diastereoisomeric mixture. White microcrystals (76%), mp 88–92 °C; $[\alpha]_D^{23}$ = –37.2 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.9 Hz, 2H), 1.37 (d, *J* = 6.9 Hz, 1H), 3.10–3.22 (m, 2H), 5.00–5.16 (m, 3H), 5.26–5.36 (m, 1H), 6.10–6.18 (m, 1H), 7.00–7.40 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 21.5, 21.6, 37.8, 38.0, 48.5, 70.5, 77.9, 126.3, 127.1, 127.2, 127.5, 127.6, 128.6, 128.7 (2C) 128.8, 128.9, 129.0, 130.0, 134.8, 135.4, 142.5, 153.7, 167.6. Anal. Calcd. for $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.25; H, 6.45; N, 3.35.

Benzyl ((S)-4-methyl-1-oxo-1-(((S)-1-phenylethyl)amino)pentan-2-yl) carbonate (2.11b). White microcrystals (65%), mp 66–67 °C; $[\alpha]_D^{23}$ = –66.0 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, *J* = 6.3 Hz, 6H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.72–1.82 (m, 3H), 5.10 (dd, *J* = 7.2 Hz, 5.4 Hz, 1H), 5.16 (s, 2H), 6.31 (d, *J* = 7.8 Hz, 1H), 7.20–7.44 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.0, 23.3, 24.7, 41.1, 48.6, 70.5, 126.3, 127.6, 128.6, 128.9, 129.0, 134.9, 142.7, 154.2, 169.2. Anal. Calcd. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.15; H, 7.67; N, 3.54.

Benzyl ((2S)-4-methyl-1-oxo-1-((1-phenylethyl)amino)pentan-2-yl) carbonate (2.11b+2.11b'). Diastereoisomeric mixture. White microcrystals (56%), mp 38–42 °C; $[\alpha]_D^{23}$ = –41.0 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.84–0.98 (m, 6H), 1.38–1.52 (m, 3H), 1.66–1.82 (m, 3H), 5.04–5.26 (m, 4H), 6.24–6.36 (m, 1H), 7.20–7.42 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.03, 23.3, 24.7, 41.1, 48.6, 70.5, 126.3, 127.6, 128.6, 128.7, 128.9, 134.9, 142.7, 154.2, 169.1. Anal. Calcd. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.20; H, 7.52; N, 3.60.

(S)-Benzyl (1-((4-methoxyphenyl)amino)-4-methyl-1-oxopentan-2-yl) carbonate (2.11c). White microcrystals (71%), mp 117–118 °C; $[\alpha]_D^{23}$ = –53.3 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, *J* = 6.0 Hz, 3.3 Hz, 6H), 1.72–1.86 (m, 3H), 3.79 (s, 3H), 5.20–5.28 (m, 3H), 6.85 (d, *J* = 6.9 Hz, 2H), 7.36–7.42 (m, 7H), 7.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.2, 24.7, 41.3, 55.7, 70.7, 77.0, 114.4, 122.2, 128.7, 129.0, 129.1, 130.1, 134.8, 154.2, 157.0, 167.9. Anal. Calcd. for $C_{21}H_{25}NO_5$: C, 67.91; H, 6.78; N, 3.77. Found: C, 67.83; H, 6.98; N, 3.67.

(9*H*-Fluoren-9-yl)methyl ((2S)-4-methyl-1-oxo-1-((1-phenylethyl)amino) pentan-2-yl) carbonate (2.11d). White microcrystals (67%), mp 155–158 °C; $[\alpha]_D^{23}$ = –54.6 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.95 (dd, *J* = 6.3 Hz, 3.0 Hz, 6H), 1.51 (d, *J* = 6.9 Hz, 3H), 1.66–1.82 (m, 3H), 4.18–4.26 (m, 1H), 4.38–4.54 (m, 2H), 5.06–5.20 (m, 2H), 6.31 (d, *J* = 7.5 Hz, 1H), 7.22–7.34 (m, 7H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.52–7.58 (m, 2H), 7.77 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.0, 23.4, 24.7, 41.2, 46.9, 48.7, 70.4, 120.3, 125.1, 126.3, 127.4, 127.7, 128.2, 128.9, 141.5, 143.2, 143.3, 154.3, 169.1. Anal. Calcd. for $C_{29}H_{31}NO_4$: C, 76.12; H, 6.83; N, 3.06. Found: C, 76.13; H, 6.79; N, 2.71.

2.4.5. General preparation of depsidipeptides (2.10) and depsitripeptide (2.15)

The unprotected amino acids **2.1** (1.5 mmol) and DIEA (1.5 mmol) were dissolved in the minimum amount of water. Acetonitrile (3 mL) was added to the solution which was cooled to 10 ^{o}C. A solution of *O*-PG(hydroxyacyl)benzotriazole **2.5** or **2.14** (1 mmol) in acetonitrile (4 mL) was added dropwise over 10 min at 10 ^{o}C and stirred for 0.5–2 h at 10 ^{o}C. The reaction mixture was monitored by TLC [EtOAc-hexanes (1:2)]. After completion of reaction, the solvent was evaporated, EtOAc (20 mL) was added and the mixture was washed with 4N HCl solution (3 x 15 mL), brine (15 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give crude product **2.10** or **2.15**.

Cbz-L-(*O*Phe)-L-Ala-OH (2.10a). The crude product was recrystallized from CH_2Cl_2-hexanes to give white microcrystals (86%), mp 137–139 °C; $[\alpha]_D^{23}$ = –17.6 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.27 (d, *J* = 7.2 Hz, 3H), 3.16 (dd, *J*

= 14.4 Hz, 6.0 Hz, 1H), 3.26 (dd, *J* = 14.4 Hz, 4.2 Hz, 1H), 4.50–4.60 (m, 1H), 5.11 (d, *J* = 12.0 Hz, 1H), 5.17 (d, *J* = 12.0 Hz, 1H), 5.33 (t, *J* = 5.7 Hz, 1H), 6.48 (d, *J* = 7.5 Hz, 1H), 7.10–7.18 (m, 2H), 7.20–7.30 (m, 3H), 7.30–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 18.1, 37.9, 47.9, 70.6, 77.5, 127.3, 128.6, 128.7, 128.9, 129.0, 129.9, 134.8, 135.2, 153.7, 168.7, 176.4; Anal. Calcd. for $C_{20}H_{21}N_1O_6\cdot$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.52; H, 5.82; N, 3.36.

Cbz-L-(*O*Phe)-DL-Ala-OH (2.10a+2.10a'). The crude product was recrystallized from CH_2Cl_2-hexanes to give white microcrystals (80%), diastereoisomeric mixture, mp 135–137 °C; $[\alpha]_D^{23}$ = –50.6 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, *J* = 7.2 Hz, 1.5H), 1.33 (d, *J* = 7.2 Hz, 1.5H), 3.02–3.18 (m, 1H), 3.20–3.28 (m, 1H), 4.46–4.56 (m, 1H), 5.04–5.14 (m, 2H), 5.24–5.34 (m, 1H), 6.47 (d, *J* = 7.2 Hz, 1H), 7.08–7.16 (m, 2H), 7.17–7.24 (m, 3H), 7.26–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 17.9, 18.1, 37.9, 38.1, 47.9, 48.0, 70.6, 127.3, 128.6, 128.9, 129.0, 129.8, 130.0, 134.8, 135.2, 135.3, 153.7, 168.6, 169.1, 176.5; Anal. Calcd. for $C_{20}H_{21}N_1O_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.78; H, 5.80; N, 3.65.

Cbz-L-(*O*Phe)-L-Met-OH (2.10b). The crude product was recrystallized from CH_2Cl_2-hexanes to give white microcrystals (74%), mp 130–132 °C; $[\alpha]_D^{23}$ = –16.9 (*c* 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.84–1.96 (m, 1H), 1.98 (s, 3H), 2.00–2.12 (m, 1H), 2.15–2.28 (m, 2H), 3.19 (d, *J* = 4.8 Hz, 2H), 4.21 (t, *J* = 6.9 Hz, 1H), 4.40 (dd, *J*= 10.5 Hz, *J* = 6.9 Hz, 1H), 4.50 (dd, *J* = 10.8 Hz, 7.5 Hz, 1H), 4.64–4.80 (m, 1H), 5.34 (t, *J* = 4.8, 1H), 6.78 (d, *J* = 7.8 Hz, 1H), 7.08–7.14 (m, 2H), 7.20–7.36 (m, 5H), 7.36–7.44 (m, 2H), 7.56 (t, *J* = 7.2 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 15.4, 29.7, 30.9, 37.7, 46.9, 51.4, 70.5, 77.5, 120.3, 125.2, 127.4, 128.2, 128.6, 130.1, 135.1, 141.5, 143.1, 143.4, 153.7, 168.8, 175.4; Anal. Calcd. for $C_{29}H_{29}N_1O_6S$: C, 67.03; H, 5.63; N, 2.70. Found: C, 67.25; H, 5.93; N, 2.50.

Fmoc-L-(*O*Phe)-L-Glu-(OMe)-OH (2.10c). The crude product was recrystallized from EtOAc-hexanes to give white microcrystals (82%), mp 155–157 °C; $[\alpha]_D^{23}$ = –23.8 (*c* 1.0, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 1.76–1.90 (m, 1H), 1.98–2.10 (m, 1H), 2.28–2.38 (m, 2H), 2.94 (dd, *J* = 14.4 Hz, 9.6 Hz, 1H),

3.08 (dd, *J* = 14.7 Hz, 3.9 Hz, 1H), 3.55 (s, 3H), 4.20–4.32 (m, 2H), 4.34–4.48 (m, 2H), 5.07 (dd, *J* = 9.6 Hz, 3.6 Hz, 1H), 7.20–7.36 (m, 6H), 7.41 (t, *J* = 7.8 Hz, 2H), 7.50–7.60 (m, 2H), 7.89 (d, *J* = 7.8 Hz, 2H), 8.51 (d, *J* = 8.1 Hz, 1H), 12.82 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.3, 29.7, 37.2, 46.2, 51.0, 51.4, 69.0, 76.9, 120.3, 125.1, 126.8, 127.2, 127.9, 128.4, 129.3, 136.6, 140.8, 143.1, 143.4, 153.8, 168.6, 172.7, 172.8; Anal. Calcd. for $C_{30}H_{29}N_1O_8$: C, 67.79; H, 5.50; N, 2.63. Found: C, 67.43; H, 5.47; N, 2.57.

Fmoc-L-(*O*Phe)-L-Ala-OH (2.10d). The crude product was recrystallized from EtOAc-hexanes to give white microcrystals (87%), mp 195–196 °C; $[\alpha]_D^{23}$ = –30.0 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 1.29 (d, *J* = 7.2 Hz, 3H), 3.92 (dd, *J* = 14.7 Hz, 9.9 Hz, 1H), 3.10 (dd, *J* = 14.4 Hz, 3.3 Hz, 1H), 4.20–4.30 (m, 2H), 4.32–4.44 (m, 2H), 5.05 (dd, *J* = 9.6 Hz, 3.3 Hz, 1H), 7.20–7.36 (m, 6H), 7.38–7.46 (m, 2H), 7.50–7.60 (m, 2H), 7.89 (d, *J* = 7.8 Hz, 2H), 8.52 (d, *J* = 7.5 Hz, 1H), 12.6 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 17.2, 37.3, 46.1, 47.5, 69.0, 76.8, 120.3, 125.0, 125.1, 126.7, 127.2, 127.8, 128.3, 129.3, 136.6, 140.8, 143.1, 143.3, 153.8, 168.2, 173.8; Anal. Calcd. for $C_{27}H_{25}N_1O_6$: C, 70.58; H, 5.48; N, 3.05. Found: C, 70.20; H, 5.51; N, 2.86.

Fmoc-L-(*O*Phe)-DL-Ala-OH (2.10d+2.10d'). The crude product was recrystallized from EtOAc-hexanes to give white microcrystals (94%), diastereoisomeric mixture, mp 203–205 °C; $[\alpha]_D^{23}$ = –26.8 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 1.23 (d, *J* = 7.2 Hz, 1.5 H), 1.29 (d, *J* = 7.2 Hz, 1.5 H), 2.88–3.00 (m, 1H), 3.02–3.16 (m, 1H), 4.20–4.30 (m, 2H), 4.32–4.48 (m, 2H), 5.02–5.12 (m, 1H), 7.20–7.38 (m, 6H), 7.38–7.46 (m, 2H), 7.50–7.60 (m, 2H), 7.89 (d, *J* = 7.5 Hz, 2H), 8.52 (d, *J* = 7.5 Hz, 1H), 12.65 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 17.2, 17.4, 37.5, 46.2, 47.5, 69.0, 76.8, 76.9, 120.3, 125.0, 125.1, 126.7, 127.2, 127.9, 128.3, 129.3, 129.4, 136.5, 136.7, 140.8, 143.1, 143.4, 153.8, 168.0, 168.3, 173.8.; Anal. Calcd. for $C_{27}H_{25}N_1O_6$: C, 70.58; H, 5.48; N, 3.05. Found: C, 70.27; H, 5.55; N, 2.89.

Fmoc-L-(*O*Phe)-L-Met-Bt (2.12). Compound **2.12** was prepared according to the given procedure for **2.6**. The crude product was recrystallized from CH_2Cl_2-hexanes to give white microcrystals (80%), mp 160–162 °C; $[\alpha]_D^{23}$ = –56.9 (*c* 1.5,

$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3H), 2.02–2.18 (m, 1H), 2.22–2.40 (m, 3H), 3.25 (d, *J* = 5.1 Hz, 2H), 4.28 (t, *J* = 6.9 Hz, 1H), 4.47 (dd, *J* = 10.2 Hz, 6.9 Hz, 1H), 4.55 (dd, *J* = 10.5 Hz, 6.9 Hz, 1H), 5.39 (t, *J* = 4.8, 1H), 5.98–6.08 (m, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.14–7.46 (m, 9H), 7.52 (t, *J* = 6.9 Hz, 1H), 7.60–7.70 (m, 3H), 7.78 (d, *J* = 7.5 Hz, 2H), 8.13 (d, *J* = 8.1 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 15.4, 29.9, 32.0, 37.7, 46.9, 52.5, 70.6, 77.7, 114.5, 120.3, 120.6, 125.3, 126.9, 127.4, 127.5, 128.2, 128.7, 130.1, 131.3, 131.2, 135.2, 141.6, 143.2, 143.4, 146.2, 153.8, 168.6, 170.5; Anal. Calcd. for $C_{35}H_{32}N_4O_5S$: C, 67.72; H, 5.20; N, 9.03. Found: C, 67.48; H, 5.23; N, 8.85.

(9H-Fluoren-9-yl)methyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-(((S)-1-phenylethyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl) carbonate. Monohydrate (2.13). Compound **2.13** was prepared according to the given procedure for **2.11**. The crude product was recrystallized from EtOAc-hexanes to give white microcrystals (52%), mp 176–178 °C; $[\alpha]_D^{23}$ = –60.0 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.45 (d, *J* = 6.9 Hz, 3H), 1.78–1.88 (m, 2H), 1.94 (s, 3H), 2.08–2.18 (m, 1H), 2.24–2.34 (m, 1H), 3.18–3.22 (m, 2H), 4.24 (t, *J* = 7.2 Hz, 1H), 4.38–4.56 (m, 3H), 5.00–5.10 (m, 1H), 5.29 (t, *J* = 5.4 Hz, 1H), 6.56 (d, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 7.14–7.20 (m, 2H), 7.22–7.38 (m, 10H), 7.40–7.46 (m, 2H), 7.59 (t, *J* = 7.2 Hz, 2H), 7.78 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 15.1, 22.1, 30.0, 30.8, 37.9, 46.9, 49.4, 52.1, 70.6, 120.3, 125.3, 126.2, 127.5, 127.7, 128.2, 128.7, 128.9, 130.0, 135.3, 141.5, 143.1, 143.3 154.1, 168.6, 169.4; Anal. Calcd. for $C_{37}H_{38}N_2O_5S.H_2O$: C, 69.35; H, 6.29; N, 4.37. Found: C, 69.09; H, 6.15; N, 4.25.

2.4.6. General preparation of O-acylated depsidipeptides (2.7)

DMAP (0.75 mmol) in dry THF (2 mL) was added to a stirred solution of *N*-PG(α-aminoacyl)benzotriazole **2.5** (0.5 mmol) and α-hydroxycarboxylic acid (0.75 mmol) in dry THF (10 mL) at 4 ^{o}C. Then the reaction mixture was stirred for 4–6 h at room temperature until shown completed by TLC [EtOAc-hexanes (1:2)]. The solvent was evaporated under reduced pressure and the residue was dissolved in diethyl ether (25 mL), washed with 3 N HCl (4 x 5 mL), water (3 x

10 mL), brine (5 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give crude product **2.7**.

Cbz-L-Leu-L-(*O*Phe)-OH (2.7a). The residue was purified by column chromatograph [EtOAc-hexanes, (from 15 to 30%)] to obtain a sticky oil, (76%); $[\alpha]_D^{23}$ = –21.0 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76–1.05 (m, 6H), 1.40–1.55 (m, 1H), 1.55–1.80 (m, 2H), 3.13 (dd, *J* = 13.8 Hz, 7.8 Hz, 1H), 3.26 (dd, *J* = 14.4 Hz, 4.2 Hz, 1H), 4.30–4.40 (m, 1H), 5.00–5.20 (m, 2H), 5.31 (dd, *J* = 8.4 Hz, 5.1 Hz, 1H), 7.18–7.52 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.9, 23.0, 24.8, 37.2, 41.6, 52.5, 67.3, 73.2, 127.3, 128.2, 128.4, 128.7, 129.5, 135.5, 136.3, 156.3, 172.5, 173.7; Anal. Calcd. for $C_{23}H_{27}N_1O_6$·: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.54; H, 6.87; N, 3.29.

Cbz-L-Leu-DL-(*O*Phe)-OH (2.7a+2.7a'). The crude was purified by column chromatograph [EtOAc-hexanes, (from 15 to 30%)] to obtain a sticky oil, (78%); diastereoisomeric mixture, $[\alpha]_D^{23}$ = –11.3 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76–1.00 (m, 6H), 1.30–1.53 (m, 2H), 1.56–1.76 (m, 1H), 3.04–3.20 (m, 1H), 3.20–3.36 (m, 1H), 3.34–3.44 (m, 1H), 5.02–5.18 (m, 3H), 5.33 (dd, *J* = 9.9 Hz, 3.6 Hz, 1H), 7.18–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.8, 23.0, 24.7, 24.8, 37.2, 41.4, 41.6, 52.5, 52.6, 67.4, 73.2, 73.4, 127.4, 128.3, 128.4, 128.7, 129.5, 129.6, 135.5, 135.8, 136.3, 156.2, 156.3, 172.5, 173.7; Anal. Calcd. for $C_{23}H_{27}N_1O_6$: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.40; H, 6.75; N, 3.60.

Cbz-L-Ala-L-(*O*Phe)-N(Cy)$_2$ (2.7b). The crude was taken into in diethyl ether (6 mL) in test tube and dicyclohexylamine (110 mg, 0.60 mmol) was added dropwise. Hexane (2 mL) was added and the mixture was kept at room temperature until it gave white crystals. Then the crystals were collected and washed with excess of hexanes to give pure Cbz-L-Ala-L-(*O*phe)-N(Cy)$_2$ **2.7b** as a white crystals (76%), mp 155–156 ^{o}C; $[\alpha]_D^{23}$ = –25.9 (*c* 1.2, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.00–1.25 (m, 7H), 1.30–1.45 (m, 6H), 1.59 (s, 2H), 1.73 (s, 4H), 1.91 (s, 4H), 2.80–2.95 (m, 2H), 3.04 (dd, *J* = 14.4 Hz, 9.9 Hz, 1H), 3.24 (dd, *J* = 14.4 Hz, 3.6 Hz, 1H), 4.20–4.40 (m, 1H), 4.95–5.10 (m, 3H), 5.30–5.41 (m, 1H), 7.10–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 25.0, 25.3, 29.0, 29.1, 38.2, 50.0, 52.7, 67.0,

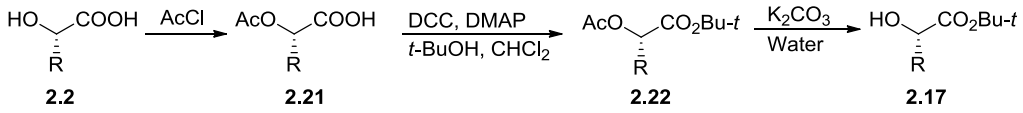
77.0, 126.5, 128.5, 128.7, 129.4, 136.6, 138.4, 155.6, 172.2, 173.6; Anal. Calcd. for $C_{32}H_{44}N_2O_6$: C, 69.54; H, 8.02; N, 5.07. Found: C, 69.25; H, 8.21; N, 4.90.

Cbz-L-Phe-L-(*O*Leu)-N(Cy)$_2$ (2.7c). The crude was taken into diethyl ether (6 mL) in a test tube and dicyclohexylamine (110 mg, 0.60 mmol) was added dropwise. Hexane (2 mL) was added and the mixture was kept at room temperature until it gave white crystals. Then the crystals were collected and washed with excess of hexanes to give pure Cbz-L-Phe-L-(*O*leu)-N(Cy)$_2$ **11c** as a white crystals (47%), mp 127–128 ^{o}C; $[\alpha]_D^{23}$ = –34.5 (*c* 1.2, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, *J* = 6.2 Hz, 6H), 1.06–1.86 (m, 19H), 1.90–2.06 (m, 4H), 2.82–3.00 (m, 2H), 3.09 (dd, *J* = 14.4 Hz, 6.6 Hz, 1H), 3.31 (dd, *J* = 13.5 Hz, 5.7, 1H), 4.62–4.74 (m, 1H), 4.90–5.16 (m, 3H), 5.29 (d, *J* = 7.8 Hz, 1H), 7.18–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.9, 23.6, 25.0, 25.1, 25.3, 29.5, 38.4, 41.0, 52.8, 55.2, 67.0, 75.1, 127.0, 128.3, 128.5, 128.7, 129.9, 136.6, 155.8, 171.2, 175.0.; Anal. Calcd. for $C_{35}H_{50}N_2O_6$: C, 70.68; H, 8.47; N, 4.71. Found: C, 70.78; H, 8.92; N, 4.75.

Cbz-L-Met-L-(*O*Phe)-OH (2.7d). The crude was recrystallized from $CHCl_3$-hexanes to give white microcrystals (54%), mp 114–116 ^{o}C; $[\alpha]_D^{23}$ = –18.6 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.88–2.00 (m, 1H), 2.03 (s, 3H), 2.06–2.20 (m, 1H), 2.44–2.58 (m, 2H), 3.14 (dd, *J* = 14.4 Hz, 7.8 Hz, 1H), 3.26 (dd, *J* = 14.7 Hz, 4.5 Hz, 1H), 4.50–4.60 (m, 1H), 5.09 (s, 2H), 5.32 (dd, *J* = 7.8 Hz, 3.9 Hz, 1H), 5.42 (d, *J* = 7.8 Hz, 1H), 7.18–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 15.4, 29.7, 31.9, 37.2, 53.1, 67.4, 73.4, 127.5, 128.3, 128.5, 128.7, 129.5, 135.3, 136.2, 156.1, 171.6, 173.7; Anal. Calcd. for $C_{22}H_{25}N_1O_6S$: C, 61.24; H, 5.84; N, 3.25. Found: C, 60.98; H, 5.85; N, 3.08.

2.4.7. Synthesis of *t*-butyl (L)-α-hydroxycarboxlates 2.17a and 2.17b

Compounds **2.17a, 2.17b** were prepared using reported procedure by Yang *et al* [80] (Scheme 2.10). α-Hydroxycarboxlic acid **2.2** was treated with acetyl chloride to afford *O*-acetyl-(L)-α-hydroxycarboxlic acid **2.21**, which was treated with *t*-BuOH in the presence of DCC-DMAP to afford *t*-butyl *O*-acetyl-(L)-α-hydroxycarboxlate **2.22** Treatment of *t*-butyl *O*-acetyl-(L)-α-hydroxycarboxlate **2.22** with K_2CO_3 in water gave *t*-butyl (L)-α-hydroxycarboxlate **2.17.**



Scheme 2.10. Synthesis of *t*-butyl (L)-α-hydroxycarboxlates

2.4.8. General preparation of depsitripeptides (15, 16) and starting materials (2.14)

Cbz-L-Leu-L-(*O*Phe)-Bt (2.14a). Compound **2.14a** was prepared according to the given procedure for **2.6**. Yellowish oil (83%) was used without purification.

Cbz-L-Ala-L-(*O*Phe)-Bt (2.14b). Compound **2.14b** was prepared according to the given procedure for **2.6**. Yellowish oil (78%) was used without purification.

Cbz-L-Leu-L-(*O*Phe)-L-Met-OH (2.15a). Compound **2.15a** was prepared according to the given procedure for **2.10**. White microcrystals (78%), mp 116–118 °C; $[\alpha]_D^{23}$ = –37.4 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.80–0.98 (m, 6H), 1.40–1.52 (m, 1H), 1.52–1.70 (m, 2H), 1.84–2.16 (m, 4H), 2.22 (q, *J* = 7.2 Hz, 2H), 3.08–3.25 (m, 2H), 4.25–4.30 (m, 1H), 4.50–4.62 (m, 1H), 4.90–5.04 (m, 1H), 5.05–5.20 (s, 2H), 5.40–5.50 (m, 1H), 6.40–6.65 (br s, 2H), 7.00–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 15.3, 21.7, 23.0, 24.9, 29.9, 30.7, 37.6, 40.5, 51.6, 53.0, 67.6, 74.9, 127.3, 128.2, 128.6, 128.8, 129.9, 135.6, 136.0, 156.9, 169.5, 171.3, 174.8; Anal. Calcd. for $C_{28}H_{36}N_2O_7S$: C, 61.75; H, 6.66; N, 5.14. Found: C, 61.73; H, 6.62; N, 5.06.

Cbz-L-Ala-L-(*O*Phe)-L-Trp-OH (2.15b). Compound **2.15b** was prepared according to the given procedure for **2.10**. White microcrystals (63%), mp 193–195 °C; $[\alpha]_D^{23}$ = –87.3 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 1.20 (d, *J* = 7.2 Hz, 3H), 2.85–3.20 (m, 4H), 4.00–4.15 (m, 1H), 4.51 (q, *J* =7.2 Hz, 1H), 4.85–5.05 (m, 2H), 5.10–5.25 (m, 1H), 6.90–7.40 (m, 11H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 6.9 Hz, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 10.87 (s, 1H), 12.71 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 16.7, 26.9, 37.2, 49.1, 52.6, 65.5, 74.0, 109.3, 111.3, 118.2, 118.4, 120.9, 123.6, 126.5, 127.2, 127.8, 127.8, 128.1, 128.3, 129.3, 136.0, 136.5, 136.8, 155.9, 168.3, 172.0, 172.8; Anal. Calcd. for $C_{31}H_{31}N_3O_7$: C, 66.77; H, 5.60; N, 7.54. Found: C, 66.49; H, 5.48; N, 7.47.

Cbz-L-Leu-L-(*O*Phe)-L-(*O*Ile)-OH (2.16). Compound **2.16** was prepared according to the given procedure for **2.7**. Colorless oil (55%); $[\alpha]_D^{23}$ = –24.2 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 12H), 1.20–1.40 (m, 1H), 1.40–1.60 (m, 2H), 1.60–1.78 (m, 2H), 1.94–2.08 (m, 1H), 3.14 (dd, *J* = 14.4 Hz, 8.1 Hz, 1H), 3.30 (dd, *J* = 14.7 Hz, 4.2 Hz, 1H), 4.36–4.48 (m, 1H), 5.00–5.12 (m, 3H), 5.14–5.40 (m, 2H), 7.10–7.40 (m, 10H), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.7, 15.4, 21.8, 23.1, 24.5, 24.8, 36.7, 37.2, 41.8, 52.4, 67.3, 73.4, 127.3, 128.3, 128.4, 128.7 (2C), 129.5, 129.6, 135.6, 136.4, 156.2, 169.0, 172.7, 174.2.; Anal. Calcd. for $C_{29}H_{37}NO_8$: C, 66.02; H, 7.07; N, 2.65. Found: C, 65.79; H, 7.53; N, 2.42.

2.4.9. General preparation of oligoesters (2.9)

O-PG(α-hydroxyacyl)benzotriazole **2.4** (0.5 mmol) in dry THF (2 mL) was added to a stirred solution of DMAP (0.6 mmol) and α-hydroxycarboxylic acid (0.6 mmol) in dry THF (10 mL) at 4 ^{o}C. Then the reaction mixture was stirred for 4–6 h at room temperature until shown completed by TLC [EtOAc-hexanes (1:2)]. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (15 mL), washed with 3 N HCl (4 x 5 mL), brine (5 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give crude oily product **2.9a, 2.9b**. The solution of crude in CH_2Cl_2 (1 mL) was loaded onto a short silica column (1 cm dia x 2 cm length) and eluted with EtOAc-hexanes (1:1). Evaporation of solvent gave pure **2.9a, 2.9b**.

Cbz-L-(*O*Phe)-L-(*O*Val)-OH (2.9a). colorless oil, (85%); $[\alpha]_D^{23}$ = –30.0 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ; 0.98 (t, *J* = 6.9 Hz, 6H), 2.24–2.36 (m, 1H), 3.14 (dd, *J* = 14.7 Hz, 9.0 Hz, 1H), 3.32 (dd, *J* = 14.7 Hz, 3.9 Hz, 1H), 5.00 (d, *J* = 4.2 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 5.16 (d, *J* = 12.3 Hz, 1H), 5.23 (dd, *J* = 9.0 Hz, 3.6 Hz, 1H), 7.20–7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 17.1, 18.8, 30.3, 37.4, 70.3, 76.0, 77.1, 127.3, 128.5, 128.7, 128.8, 129.6, 135.0, 135.8, 154.7, 169.2, 174.5; Anal. Calcd. for $C_{22}H_{24}O_7$·: C, 65.99; H, 6.04. Found: C, 65.61; H, 6.23.

Cbz-L-(*O*Phe)-L-(*O*Leu)-OH (2.9b). Colorless oil (75%); $[\alpha]_D^{23}$ = –34.8 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (dd, *J* = 8.1 Hz, 5.7 Hz, 6H), 1.64–1.86 (m,

3H), 3.12 (dd, *J* = 14.4, *J* = 9.3 Hz, 1H), 3.31 (dd, *J* = 14.7 Hz, 3.6 Hz, 1H), 5.04–5.22 (m, 4H), 7.20–7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.7, 23.1, 24.8, 37.3, 39.7, 70.3, 71.5, 76.1, 127.3, 128.5, 128.7, 128.8, 129.7, 135.0, 135.8, 154.7, 169.2, 175.3; Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 65.01; H, 6.62.

Cbz-L-(*O*Leu)-L-(*O*Phe)-OBu-*t* (2.18). DMAP (0.05 g, 0.41 mmol) was added to a stirred solution of Cbz-(*O*Leu)-Bt **2.4b** (0.15 g, 0.41 mmol) and HO-(*O*Phe)-OBu-*t* **2.17a** (0.091 g, 0.41 mmol) in THF (10 mL) at 4 ^{o}C. Then the reaction mixture was stirred for 5 h at room temperature until it completed on TLC (EtOAc-hexanes (1:2)). Then the solvent was evaporated under reduced pressure. The residue was taken into EtOAc (15 mL) and washed with saturated 3N HCl (3 x 5 mL), saturated Na_2CO_3 (2 x 5 mL) and brine (5 mL). After dried over $MgSO_4$, the solvent was evaporated to give pure Cbz-L-(*O*Leu)-L-(*O*Phe)-OBu-*t* **2.18** as colorless oil (86%); $[\alpha]_D^{23}$ = –39.0 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, *J* = 6.3 Hz, 6H), 1.36 (s, 9H), 1.64–1.86 (m, 3H), 3.14 (d, *J* = 5.7 Hz, 2H), 5.00 (dd, *J* = 9.9, *J* = 4.2 Hz, 1H), 5.14–5.20 (m, 3H), 7.20–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.6, 23.2, 24.6, 28.1, 37.4, 39.9, 70.24, 74.1, 82.7, 127.2, 128.5 (2C), 128.8 (2C), 129.7, 135.2, 135.8, 154.8, 168.0, 169.9; Anal. Calcd. for $C_{27}H_{34}O_7$: C, 68.92; H, 7.28. Found: C, 69.14; H, 7.62.

Cbz-L-(*O*Leu)-L-(*O*Phe)-OH (2.9c). The solution of Cbz-L-(*O*Leu)-L-(*O*Phe)-OBu-*t* **2.18** (0.10 g, 0.21 mmol) in CH_2Cl_2-TFA [6 mL, (1:1)] was stirred for 2 h at 4 ^{o}C. Then the volatile part was removed under reduced pressure. The residue oil which was taken into EtOAc (10 mL) was washed with 1N HCl (3 x 5 mL) and brine (5 mL). After it was dried over $MgSO_4$, the solvent was removed to give oily crude. The solution of crude in CH_2Cl_2 (1 mL) was loaded onto a short silica column (1 cm dia x 2 cm length) and eluted with EtOAc-hexanes (1:1). Evaporation of solvent gave pure Cbz-L-(*O*Leu)-L-(*O*Phe)-OH **2.9c** as colorless oil (80%); $[\alpha]_D^{23}$ = –38.9 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90 (d, *J* = 6.3 Hz, 6H), 1.56–1.84 (m, 3H), 3.14 (dd, *J* = 14.4 Hz, 7.8 Hz, 1H), 3.25 (dd, *J* = 14.4 Hz, 4.5 Hz, 1H), 4.97 (dd, *J* = 9.3 Hz, 4.2 Hz, 1H), 5.16 (s, 2H), 5.36 (dd, *J* = 7.8 Hz, 4.5 Hz, 1H), 6.02 (s, 1H), 7.20–7.42 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.7, 23.1, 24.5, 37.2, 39.7, 70.3, 73.0, 74.1, 127.5, 128.5, 128.7, 128.8, 129.6, 135.1, 135.3,

154.9, 169.8, 173.8; Anal. Calcd. for $C_{23}H_{24}O_7$: C, 66.65; H, 6.32. Found: C, 66.95; H, 6.54.

2.4.10. General preparation of unprotected depsides (2.19)

DMAP (6.0 mmol) was added to a stirred solution of *N*-Boc(α-aminoacyl)benzotriazole **2.5e-g** (5.0 mmol) and α-hydroxycarboxylic acid **2.2** (0.6 mmol) in THF (10 mL) at 4 ^{o}C. The reaction mixture was stirred for 6 h at room temperature until reaction was completed by TLC [EtOAc-hexanes (1:2)]. The solvent was evaporated under reduced pressure and the residue was taken into EtOAc (25 mL), washed with saturated citric acid solution (3 x 15 mL), and brine (10 mL) then dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield crude product as oil. The crude was dissolved in 5.0 N $HCl_{(g)}$ in dry EtOAc (20 mL) and the solution was stirred for 15 min at room temperature. A white precipitate was formed, filtered off and discarded. The solvent was removed. The residue was dissolved in dry CH_2Cl_2 (25 mL). Dry HCl gas was bubbled into the flask for 1–2 h at room temperature while stirring. The precipitate which formed was collected and washed with dry CH_2Cl_2 to give pure free depside as hydrochloride salt **2.19**.

Gly-L-(*O*Phe).HCl (2.19a). White microcrystals (48%); mp 150–155 °C; $[\alpha]_D^{23}$ = –31.4 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 3.09 (dd, *J* = 14.4 Hz, 7.8 Hz, 1H), 3.20 (dd, *J* = 14.7 Hz, 4.5 Hz, 1H), 3.79 (s, 2H), 5.25 (dd, *J* = 7.5 Hz, 4.2 Hz, 1H), 7.20–7.40 (m, 5H), 8.57 (br s, 3H); ^{13}C NMR (DMSO-d_6) δ 36.4, 39.4, 74.0, 126.9, 128.5, 129.5, 136.1, 167.4, 169.8; Anal. Calcd. for $C_{11}H_{14}ClNO_4$·: C, 50.88; H, 5.43; N, 5.19. Found: C, 50.41; H, 5.93; N, 5.53.

L-Ala-L-(*O*Leu).HCl (2.19b). White microcrystals (36%), mp 165–168 ^{o}C; $[\alpha]_D^{23}$ = –25.8 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 0.91 (t, *J* = 6.6 Hz, 6H), 1.48 (d, *J* =7.2 Hz, 3H), 1.58–1.82 (m, 3H), 4.14 (q, *J* = 7.2 Hz, 1H), 4.98 (dd, *J* = 8.7 Hz, 3.6 Hz, 1H), 8.69 (br s, 3H); ^{13}C NMR (DMSO-d_6) δ 15.8, 21.5, 22.9, 24.1, 47.7, 71.8, 170.0, 170.8; Anal. Calcd. for $C_9H_{18}ClNO_4$: C, 45.10; H, 7.57; N, 5.84. Found: C, 45.16; H, 7.84; N, 5.63.

Gly-L-(*O*Leu).HCl (2.19c). White hydroscopic microcrystals (62%), mp 65–70 °C; $[\alpha]_D^{23}$ = –37.5 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 0.90 (t, *J* = 6.9 Hz, 6H), 1.52–1.82 (m, 3H), 3.87 (d, *J* = 9.0 Hz, 2H), 4.96 (dd, *J* = 9.6 Hz, 3.6 Hz, 1H), 8.58 (br s, 3H), 13.20 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.5, 22.9, 24.1, 39.1, 71.9, 167.5, 170.8; Anal. Calcd. for $C_8H_{16}ClNO_4$: C, 42.58; H, 7.15; N, 6.21. Found: C, 42.96; H, 7.15; N, 6.15.

L-Phe-L-(*O*Leu).HCl (2.19d). White microcrystals (56%), mp 139–140 °C; $[\alpha]_D^{23}$ = –24.4 (*c* 1.5, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 0.84 (dd, *J* = 5.1 Hz, 5.1 Hz, 6H), 1.45–1.65 (m, 3H), 3.19 (d, *J* = 6.6 Hz, 2H), 4.35 (t, *J* = 6.3 Hz, 1H), 4.84–5.00 (m, 1H), 7.20–7.42 (m, 5H), 9.00 (br s, 3H); ^{13}C NMR (DMSO-d_6) δ 21.4, 22.8, 23.9, 35.6, 52.9, 72.1, 127.1, 128.4, 129.7, 135.1, 168.9, 170.8; Anal. Calcd. for $C_{15}H_{22}ClNO_4.H_2O$: C, 53.97; H, 7.25; N, 4.20. Found: C, 54.53; H, 7.45; N, 4.08.

2.4.11. General procedure for the preparation of depsitripeptides (2.20)

The HCl salt of depside **2.19** (0.40 mmol) and DIEA (0.80 mmol) were dissolved in the minimum amount of cold water. Acetonitrile (3 mL) was added to this solution and cooled to 10 °C. A solution of *N*-PG(α-aminoacyl)-benzotriazoles **2.5** (0.20 mmol) in acetonitrile (3 mL) was added dropwise over 10 min at 10 °C and stirred for 0.5–2 h at 10 °C. The reaction mixture was monitored by TLC [EtOAc-hexanes (1:2)]. After completion of reaction, the solvent was evaporated. EtOAc (15 mL) was added and the mixture was washed with 4N HCl solution (3 x 10 mL), brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give crude product. The solution of crude in CH_2Cl_2 (1 mL) was loaded onto a short silica column (1 cm dia x 2 cm length) and eluted with EtOAc. The solvent was removed under reduced to give pure depsitripeptide **20a–c**.

Cbz-L-Phe-Gly-L-(*O*Leu)-OH (2.20a). White hydroscopic microcrystals (71%), mp 52–55 °C; $[\alpha]_D^{23}$ = –15.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.91 (t, *J* = 6.9 Hz, 6H), 1.60–1.84 (m, 3H), 2.84–3.00 (m, 1H), 3.02–3.22 (m, 1H), 3.90–4.20 (m, 2H), 4.40–4.60 (m, 1H), 4.90–5.10 (m, 3H), 5.72 (d, *J* = 8.1 Hz, 1H), 6.93 (br s, 1H), 7.10–7.34 (m, 10H), 7.95 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.7,

23.2, 24.8, 38.6, 39.7, 41.3, 56.2, 67.4, 72.0, 127.2, 128.1, 128.4, 128.7, 128.8, 129.5, 136.2, 136.3, 156.5, 169.5, 172.1, 173.7; Anal. Calcd. for $C_{25}H_{30}N_2O_7$: C, 63.82; H, 6.43; N, 5.95. Found: C, 63.76; H, 6.53; N, 5.90.

Cbz-L-Met-L-Phe-L-(*O*Leu)-OH (2.20b). White microcrystals (56%), mp 140–142 °C; $[\alpha]_D^{23}$ = –36.3 ^{o} (*c* 1.2, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.93 (dd, *J* = 7.5 Hz, 6.3 Hz, 6H), 1.64–1.96 (m, 5H), 2.01 (s, 3H), 2.49 (t, *J* = 6.9 Hz, 2H), 3.05 (dd, *J* = 14.1 Hz, 7.5 Hz, 1H), 3.28 (dd, *J* = 14.1 Hz, 5.4 Hz, 1H), 4.34 (q, *J* = 7.2 Hz, 1H), 4.82–4.94 (m, 1H), 5.04–5.14 (m, 3H), 5.64 (d, *J* = 7.5 Hz, 1H), 6.77 (d, *J* = 7.2 Hz, 1H), 7.14–7.28 (m, 5H), 7.30–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 15.2, 21.7, 23.2, 24.8, 30.0, 31.6, 37.7, 39.8, 53.3, 53.7, 67.4, 71.9, 127.4, 128.3, 128.5, 128.8 (2C), 129.6, 135.8, 136.2, 156.3, 171.1, 171.5, 174.1; Anal. Calcd. for $C_{28}H_{36}N_2O_7S$: C, 61.75; H, 6.66; N, 5.14. Found: C, 61.53; H, 7.02; N, 5.35.

Cbz-L-Met-Gly-L-(*O*Leu)-OH (2.20c). White microcrystals (68%), mp 113–115 °C; $[\alpha]_D^{23}$ = –22.5 (*c* 1.5, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.94 (dd, *J* = 8.4 Hz, 6.3 Hz, 6H), 1.64–1.90 (m, 3H), 1.90–2.00 (m, 1H), 2.00–2.16 (m, 1H), 2.06 (s, 3H), 2.56 (t, *J* = 7.2 Hz, 2H), 3.95 (dd, *J* = 18.0 Hz, 3.9 Hz, 1H), 4.28 (dd, *J* = 17.4 Hz, 5.7 Hz, 1H), 4.40–4.50 (m, 1H), 5.00–5.20 (m, 3H), 5.92 (d, *J* = 8.4 Hz, 1H), 7.15 (br s, 1H), 7.30–7.40 (m, 5H), 8.20 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.4, 21.7, 23.2, 24.8, 30.0, 32.0, 39.7, 41.3, 53.9, 67.6, 72.0, 128.3, 128.5, 128.7, 136.1, 156.7, 169.6, 172.3, 173.5; Anal. Calcd. for $C_{21}H_{30}N_2O_7S$: C, 55.49; H, 6.65; N, 6.16. Found: C, 55.71; H, 6.85; N, 5.95.

3. MILD AND EFFICIENT PREPARATION OF AMINOXY AMIDES, AMINOXY HYBRID PEPTIDES AND AMINOXY PEPTIDES USING *N*-(PG-AMINOXYACYL)BENZOTRIAZOLES[2]

3.1 α-Aminoxy Peptides

α-Aminoxy acids are analogs of β-amino acids in which the β-carbon atom is replaced by an oxygen atom [83, 84]. An α-aminoxy acid is more rigid than its corresponding β-amino acid [85], and aminoxy amide bonds are resistant to enzymatic degradation; therefore, α-aminoxy acids have been explored as peptidomimetics [86].

α-Aminoxy peptides have gain considerable interest as novel foldamers [87-88], because of their unusual conformations and remarkable bioactivities [89-91]. Aminoxy peptides may feature strong intramolecular hydrogen bonds between adjacent residues in peptidomimetic foldamers [92, 93]. For example, peptides containing α-aminoxy acids can possess eight-membered-ring through intramolecular hydrogen bonding (α N-O turns) [94, 95], and peptides containing β-aminoxy acids can possess nine-membered-ring through intramolecular hydrogen bonding (β N-O turns) [91]. Oligomers of homochiral α- or β-aminoxy acids can form helical structures consisting of consecutive N-O turns (1.8_8 and 1.7_9 helices, respectively) [96]. β-Sugar aminoxy peptides exhibited rigid ribbon-like secondary structures composed of 5/7 bifurcated intramolecular hydrogen bonds [92]. An α-aminoxy hybrid peptide acid provided robust 12/10-mixed helices [97].

Peptides consisting of α-aminoxy acids are good receptors for anions because of the acidity of their aminoxy amide protons [98]. α-Aminoxy acid derivative **3.1** is an effective chemical shift reagent for chiral carboxylic acids [99]; another analog **3.2** can form chloride channels to mediate chloride ion transportation across cell membranes [100] (Figure 3.1).

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[2] Reprinted (adapted) permission from *Journal of Organic Chemistry*, **2009**, *74*, 8690–8694 [83]. Copyright © 2009 American Chemical Society.

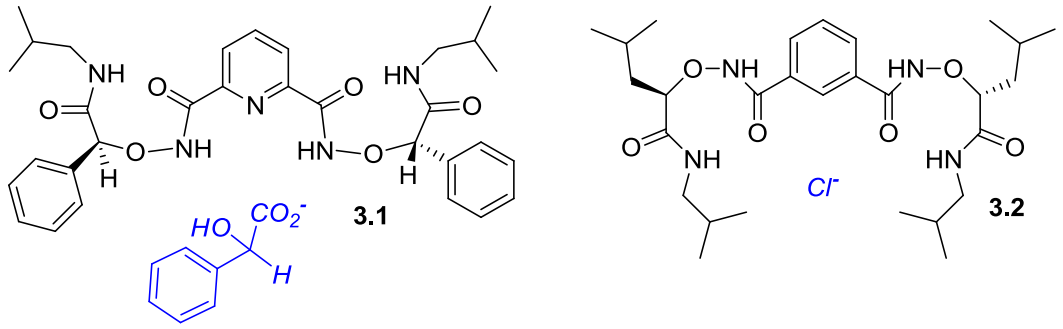


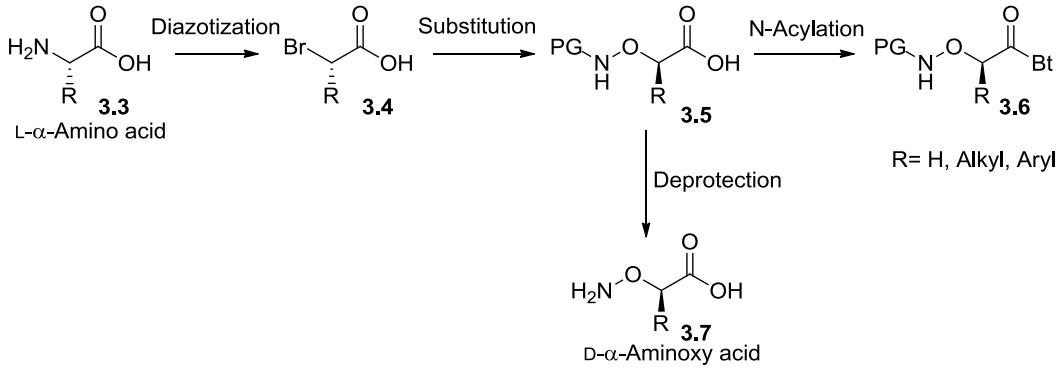
Figure 3.1. Special α-aminoxy acid derivatives

Published methods for the preparation of aminoxy acid derivatives and aminoxy peptides include alone or (i) combinations of coupling reagents such as BOP-HOBt-NEM, HBTU-HOBt-NEM, DIC-HOAt [101]; EDCl-HOBt, EDCl-HOAt [92]; TBTU-HOBt-DIEA [102], HOBt, BOP, DIEA [103]; *i*-BuOCOCl-NMM [90]; DIC-HOBt [104]; (ii) activated esters [105, 106]; (iii) α-amino diazoketone [107]. These methods often afford coupling products in low yields by longer reaction times [102, 104], and N-diacylated side products can be obtained [104]. Hence, there is a need for a mild and efficient general method to prepare aminoxyacyl amides**,** aminoxy hybrid peptides and aminoxy peptides.

In the present work, *N*-(PG-α-aminoxyacids) **3.5** are converted to *N*-(PG-α-aminoxyacyl)benzotriazoles **3.6**, which react under mild conditions with amines, α-amino acids **3.3**, α-dipeptides and α-aminoxy acids **3.7** to give aminoxyacyl amides **3.8**, aminoxy hybrid peptides **3.9** and α-aminoxy peptides **3.10** in high yields without causing racemization.

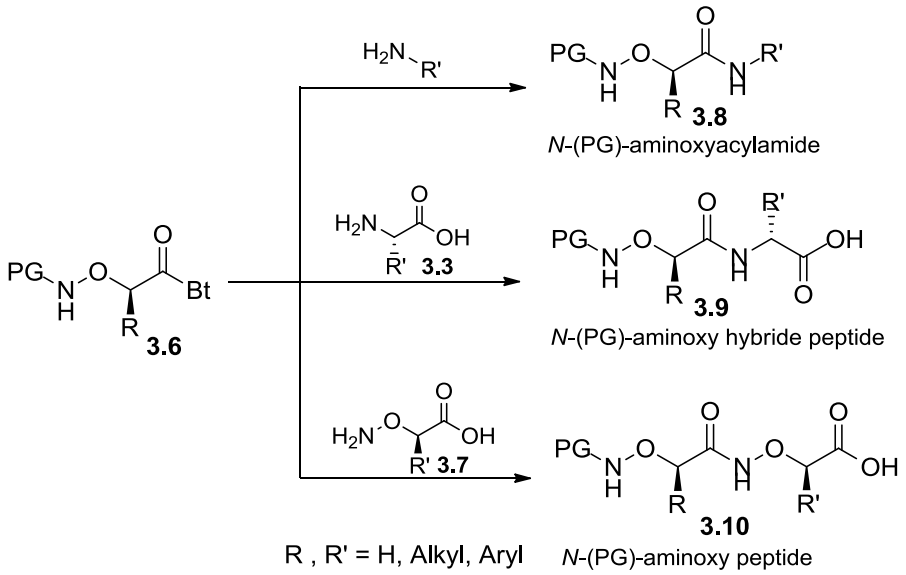
3.2. Preparation of α-Aminoxyacyl Amides, α-Aminoxy Hybrid Peptides, and α-Aminoxy peptides by Using Benzotriazole Methodology

α-Aminoxy acids **3.7** are analogs of β-amino acids in which the β-carbon atom in the β-amino acid backbone is replaced with an oxygen atom. α-Amino acids are underwent diazotization reaction to give α-bromo carboxylic acids **3.4** with full retention of chiral. *N*-(Protected)-α-aminoxyacids **3.5** are obtained from corresponding α-bromo carboxylic acids **3.4** via substitution reactions, outlined on Scheme 3.1.



Scheme 3.1. General synthetic route to prepare α-aminoxy acids

N-(PG-α-aminoxyacyl)benzotriazoles **3.6** derived from *N*-(PG-α-aminoxyacids) **3.5** were reacted with amines, α-amino acids **3.3**, α-dipeptides and α-aminoxy acids **3.7** to afford aminoxyacyl amides **3.8**, aminoxy hybrid peptides **3.9** and α-aminoxy peptides **3.10** (Scheme 3.1).



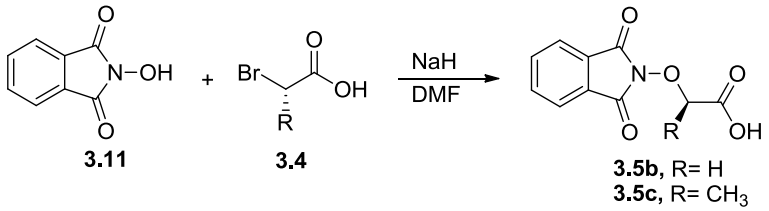
Scheme 3.2. General synthetic route to prepare target compounds

3.2.1. Preparation of α-aminoxy acids (3.7) and their *N*-(Phth) and *N*-(Cbz) protected analogs (3.5)

N-Protected(α-aminoxy)acids **3.5b–g** were prepared from either corresponding α-bromocarboxylic acids **3.4** or α-hydroxycarboxylic acids **3.14** and they were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis. *N*-Boc(α-aminoxy)acid **3.5a** was obtained from a commercial source.

3.2.1.1. Preparation of *N*-(Phth)-protected-aminoxy acids 3.5b and 3.5c

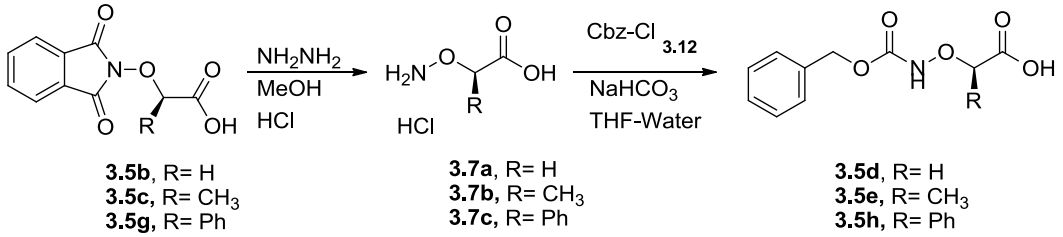
Treatment of *N*-hydroxy phthalimide **3.11** with α-bromo acids **3.4b, 3.4c** in the presence of NaH in DMF gave *N*-(Phth)-protected-aminoxy acids **3.5b**, **3.5c** in good yields (Scheme 3.3).



Scheme 3.3. Preparation of *N*-(Phth)-protected-aminoxy acids **3.5b** and **3.5c**

3.2.1.2. Preparation of *N*-(Cbz)-protected-aminoxy acids 3.5d, 3.5e and 3.5h

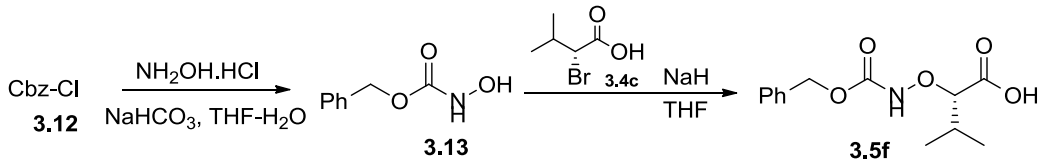
N-(Phth)-protected-aminoxy acids **3.5b**, **3.5c** and **3.5g** were treated with equimolar amount of hydrazine in methanol to give free aminoxy acids **3.7a**, **3.7b** and **3.7c**. These crude aminoxy acids were acylated with benzyl chloroformate **3.12** in the presence of $NaHCO_3$ in THF-H_2O to afford *N*-(Cbz) protected-α-aminoxy acids **3.5d**, **3.5e** and **3.5h** (Scheme 3.4).



Scheme 3.4. Preparation of *N*-(Cbz)-protected-aminoxy acids **3.5d**, **3.5e** and **3.5h**

3.2.1.3. Preparation of *N*-(Cbz)-protected aminoxy acid 3.5f

Benzyl chloroformate **3.12** was treated with hydroxylamine hydrochloride in the presence of sodium bicarbonate in THF-water to give *N*-carbobenzoxyhydroxylamine **3.13**. It was then reacted with (D)-2-bromo-3-methylbutanoic acid **3.4c** in the presence of NaH to afford *N*-(Cbz) protected aminoxy acid Cbz-L-AOVal-OH **3.5f** (Scheme 3.5).

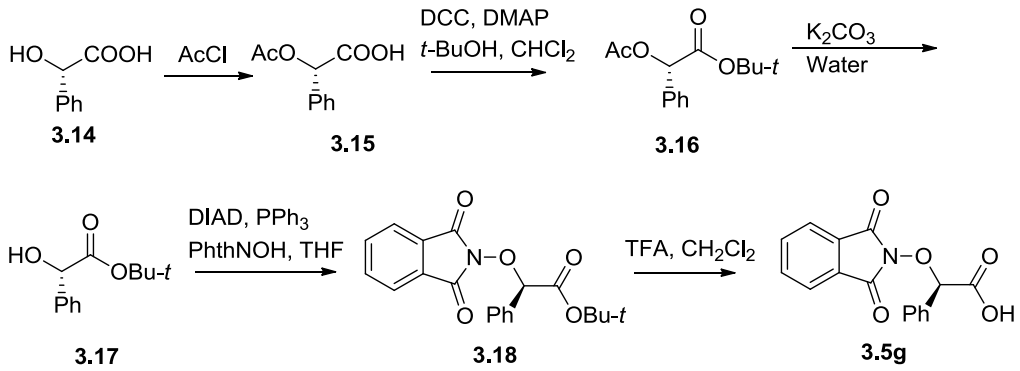


Scheme 3.5. Preparation of *N*-(Cbz)-protected aminoxy acid **3.5f**

3.2.1.4. Preparation of *N*-(Phth)-D-aminoxymandelic acid 3.5g

Commonly used method, including Mitsunobu reaction, was investigated by Yang and et al for preparation of *N*-(Phth)-protected aminoxy acids [80]. *N*-(Phth)-aminoxy acids are prepared from chiral α-hydroxy acids in good yields.

Mandelic acid **3.14** was protected with acetyl chloride to afford *O*-acetyl-(L)-mandelic acid **3.15**, which was reacted with *t*-BuOH in the presence of DCC-DMAP to afford *t*-butyl *O*-acetyl-(L)-mandelate **3.16**. Deprotection of *t*-butyl *O*-acetyl-(L)-mandelate **3.16** with K_2CO_3 in water gave *t*-butyl (L)-mandelate **3.17**, which was then treated with *N*-hydroxy phthalimide **3.11** in Mitsunobu conditions in the presence of DIAD and PPh_3 to give *t*-butyl *N*-(Phth)-(D)-aminoxymandelate **3.18**. Deprotection of *t*-butyl *N*-(Phth)-(D)-aminoxymandelate **3.18** with trifluoroacetic acid gave *N*-(Phth)-(D)-aminoxymandelic acid **3.5g** (Scheme 3.6). Later *N*-(Cbz) protected aminoxy acid **3.5h** was prepared from *N*-(Phth)-(D)-aminoxymandelic acid **3.5g** according to outlined procedure for **3.5d**, **3.5e** and **3.5h** in Scheme 3.6.



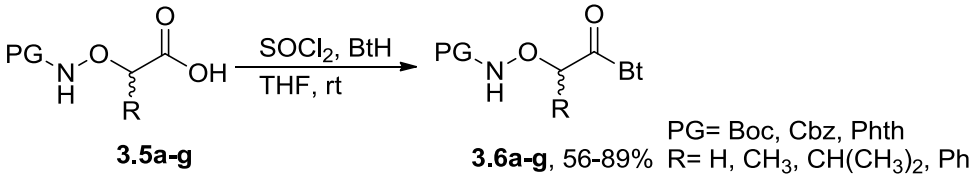
Scheme 3.6. Preparation of *N*-(Phth)-D-aminoxymandelic acid **3.5g**

Table 3.1. Preparation of *N*-(Phth) and *N*-(Cbz) protected α-aminoxy acid analogs **3.5b–h**

Entry	*N*-(PG)amino acid, **3.5**	R	Yield, %	Mp (°C)
a	*N*-(Phth)-AOGly-OH **3.5b**	H	**3.5b,** 43	163–165
b	*N*-(Phth)-D-AOAla-OH **3.5c**	Me	**3.5c,** 72	123–125
c	Cbz-AOGly-OH **3.5d**	H	**3.5d,** 74	69-70
d	Cbz-D-AOAla-OH **3.5e**	Me	**3.5e,** 68	Oil
e	Cbz-L-AOVal-OH **3.5f**	$CH(CH_3)_2$	**3.5f,** 61	Oil
f	*N*-(Phth)-D-AOMan-OH **3.5g**	Ph	**3.5g,** 71	105–106
g	Cbz-D-AOMan-OH **3.5h**	Ph	**3.5h,** 56	86–87

3.2.2. Preparation of *N*-PG(α-aminoxyacyl)benzotriazole 3.6a–g

N-PG(α-aminoxyacyl)benzotriazoles **3.6a**–**g** were prepared by treatment of *N*-PG(α-aminoxy)acids **3.5a**–**g** with 4 equivalents of 1*H*-benzotriazole and 1 equivalent of thionyl chloride in THF at room temperature in 56-89% yields (Scheme 3.7, Table 3.2).



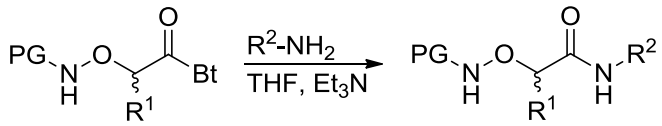
Scheme 3.7. Preparation of *N*-PG(α-aminoxyacyl)benzotriazoles **3.6a**–**g**

Table 3.2. Preparation of *N*-PG(α-aminoxyacyl)benzotriazoles **3.6a**–**g**

Entry	*N*-(PG)amino acid, **3.5**	R	Yield (%)	Mp (°C)
a	*N*-(Boc)-AOGly-OH **3.5a**	H	**3.6a**, 67	114–115
b	*N*-(Phth)-AOGly-OH **3.5b**	H	**3.6b**, 75	155–157
c	*N*-(Phth)-D-AOAla-OH **3.5c**	Me	**3.6c**, 56	145–147
d	Cbz-AOGly-OH **3.5d**	H	**3.6d**, 66	86–87
e	Cbz-D-AOAla-OH **3.5e**	Me	**3.6e**, 58	Oil
f	Cbz-L-AOVal-OH **3.5f**	$CH(CH_3)_2$	**3.6f**, 89	Oil
g	Cbz-D-AOMan-OH **3.5h**	Ph	**3.6g**, 77	Oil

3.2.3. Preparation of *N*-PG(α-aminoxyacyl) amides 3a–f

α-Aminoxyacyl amides also have intramolecular hydrogen bonds between adjacent residues (α N-O turns) in peptidomimetic foldamers [93, 94]. *N*-PG-(α-aminoxyacyl)amides **3a**–**f** and **(3f+3f′)** were prepared by a reaction between *N*-PG(α-aminoxyacyl)benzotriazole **2a**–**f** and the corresponding amines in THF at room temperature in the presence of triethylamine in 38-78% yields (Scheme 3.8, Table 3.3). Products **3a**–**f** and **(3f+3f′)** were well characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis. Retention of original chiralty of product **3f** was supported by chiral HPLC using a Whelk-O1 column ((MeOH (100%), flow rate 1.0 mL/min, detection at 254 nm). The diastereomer **3f** showed single retention-time peak in chiral HPLC at 3.46, while its corresponding diastereomeric mixture **(3f+3f′)** showed two peaks at 3.46 and 5.68 (See Appx-27 and 28).



3.6a-f **3.8a–f** and **(3.8f+3.8f')**, 38–78%

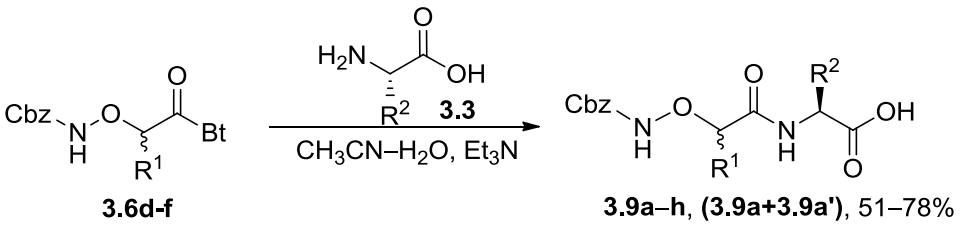
Scheme 3.8. Preparation of *N*-PG-(α-aminoxyacyl)amides **3.8a**–**f**, and **(3.8f+3.8f′)**

Table 3.3. Preparation of *N*-PG-(α-aminoxyacyl)amides **3.8a**–**f,** (**3.8f+3.8f′).**

Entry	**3.6**	Amine	Yield (%)	Mp (°C)
a	Boc-AOGly-Bt **3.6a**	*i*-propylamine	**3.8a**, 74	67–68
b	Boc-AOGly-Bt **3.6a**	*c*-hexylamine	**3.8b**, 62	137–139
c	Cbz-AOGly-Bt **3.6d**	*c*-hexylamine	**3.8c**,78	69–70
d	Phth-AOGly-Bt **3.6b**	*p*-methoxyaniline	**3.8d**, 38	210–211
e	Cbz-D-AOAla-Bt **3.6e**	*p*-methoxyaniline	**3.8e**, 56	31–32
f	Cbz-L-AOVal-Bt **3.6f**	(L)-2-methylbenzylamine	**3.8f**, 67	96–97
g	Cbz-L-AOVal-Bt **3.6f**	(DL)-2-methylbenzylamine	(**3.8f+3.8f′**), 58	Oil

3.2.4. Preparation of α-AO hybrid peptides 3.9a–h

α-Aminoxy hybrid peptides are defined as compunds that have at least one α-aminoxy acid residue and at least one natural amino acid residue. α-AO Hybrid dipeptides **3.9a**–**h, (3.9a+3.9a′)** were prepared by reaction of *N*-(PG-aminoxyacyl)benzotriazoles **3.6d**–**f** with corresponding amino acids in CH_3CN-H_2O in the presence of triethylamine at room temperature in 51–78% yields (Scheme 3.9, Table 3.4). Products **3.9a**–**h** were well characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis (See from Appx-33 to Appx-36). Retention of of original chiralty of α-AO hybrid dipeptides **3.9a, (3.9a+3.9a′)** was supported by chiral HPLC analysis using a Chirobiotic T column ((MeOH (100%), flow rate 1.0 mL/min, detection at 254 nm). α-AO Hybrid dipeptide, **3.9a** showed a single retention-time peak in chiral HPLC analysis at 3.15, while the corresponding enantiomeric mixture (**3.9a+3.9a′)** showed two peaks at 2.89, 3.69.



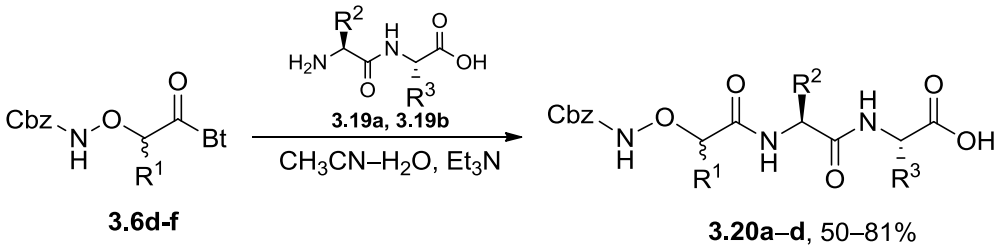
Scheme 3.9. α-AO-Hybrid dipeptides **3.9a–h**, (**3.9a+3.9a**′)

Table 3.4. α-AO-Hybrid dipeptides **3.9a–h**, (**3.9a+3.9a′**)

Entry	**3.6**	Amino acid	Product **3.9**,	Yield (%)
a	Cbz-AOGly-Bt **3.6d**	L-Phe-OH	Cbz-AOGly-L-Phe-OH **3.9a**	51
b	Cbz-AOGly-Bt **3.6d**	DL-Phe-OH	Cbz-AOGly-DL-Phe-OH (**3.9a+3.9a′**)	56
c	Cbz-D-AOAla-Bt **3.6e**	L-Phe-OH	Cbz-D-AOAla-L-Phe-OH **3.9b**	61
d	Cbz-D-AOAla-Bt **3.6e**	L-Trp-OH	Cbz-D-AOAla-L-Trp-OH **3.9c**	72
e	Cbz-D-AOAla-Bt **3.6e**	L-Leu-OH	Cbz-D-AOAla-L-Leu-OH **3.9d**	78
f	Cbz-L-AOVal-Bt **3.6f**	L-Phe-OH	Cbz-L-AOVal-L-Phe-OH **3.9e**	69
g	Cbz-L-AOVal-Bt **3.6f**	L-Trp-OH	Cbz-L-AOVal-L-Trp-OH **3.9f**	66
h	Cbz-AOGly-Bt **3.6d**	L-Cys-OH	Cbz-AOGly-L-Cys-OH **3.9g**	66
i	Cbz-D-AOAla-Bt **3.6e**	L-Cys-OH	Cbz-D-AOAla-L-Cys-OH **3.9h**	61

3.2.5. Preparation of α-AO-hybrid tripeptides 3.20a–d

α-AO-Hybrid tripeptides **3.20a**–**d** were prepared a reaction between *N*-PG(α-aminoxyacyl)benzotriazoles **3.6d**–**f** and unprotected dipeptides **3.19a, 3.19b** in CH_3CN-H_2O in the presence of triethyl amine in 50–81% yields (Scheme 4, Table 4).



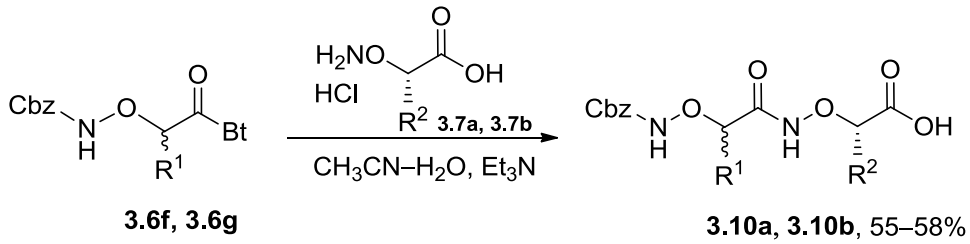
Scheme 3.10. Preparation of α-AO-hybrid tripeptides **3.20a**–**d**

Table 3.5. Preparation of α-AO-hybrid tripeptides **3.20a**–**d**

Entry	**3.6**	Dipeptide, **3.19**	Product, **3.20**	Yield (%)
a	Cbz-AOGly-Bt **3.6d**	Gly-L-Phe-OH	Cbz-AOGly-Gly-L-Phe-OH **3.20a**	81
b	Cbz-AOGly-Bt **3.6d**	Gly-L-Leu-OH	Cbz-AOGly-Gly-L-Leu-OH **3.20b**	70
c	Cbz-D-AOAla-Bt **3.6e**	Gly-L-Phe-OH	Cbz-D-AOAla-Gly-L-Phe-OH **3.20c**	50
d	Cbz-L-AOVal-Bt **3.6f**	Gly-L-Phe-OH	Cbz-L-AOVal-Gly-L-Phe-OH **3.20d**	67

3.2.6. Preparation of α-Aminoxy Dipeptides 3.10a, 3.10b

α-Aminoxy dipeptides **3.10a, 3.10b** were prepared in 55–58% yields by reaction between *N*-PG(α-aminoxyacyl)benzotriazoles **3.6** and the corresponding aminoxy acids **3.7a, 3.7b** in CH_3CN-H_2O (3:1) in the presence of triethylamine at room temperature (Scheme 3.11, Table 3.6). Products **3.10a, 3.10b** were well characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.



Scheme 3.11. Preparation of α-aminoxy dipeptides **3.10a, 3.10b**

Table 3.6. Preparation of α-aminoxy dipeptides **3.10a**, **3.10b**

Entry	**3.6**	**3.7**	Product **3.10**	Yield(%)
a	Cbz-L-AOVal-Bt **3.6f**	AOGly-OH **3.7a**	Cbz-L-AOVal-AOGly-OH **3.10a**	58
b	Cbz-D-AOMan-Bt **3.6g**	D-AOAla-OH **3.7b**	Cbz-D-AOMan-D-AOAla-OH **3.10b**	52

3.3. Conclusions

In conclusion, a mild and effective method for the preparation of α-aminoxyacyl amides, α-aminoxy hybrid peptides and α-aminoxy peptides has been developed by reacting *N*-PG(α-aminoxyacyl)benzotriazoles **3.6** with amines, α-amino acids, peptides and α-aminoxy acids. All the α-aminoxy derivatives were obtained under mild reaction conditions in good yields and without racemization.

In comparison with literature methods, this method has following the advantages i) milder reaction conditions, ii) shorter reaction times, iii) better yields, iv) inexpensive and easily synthesizable *N*-PG(α-aminoxyacyl)benzotriazoles **3.6** reagents for peptide coupling v) does not require C part protection of amino acid, vi) avoids N-diacylation products and vii) avoids to give racemization products.

3.4. Experimental Section

Melting points are uncorrected and were recorded on a capillary point apparatus equipped with a digital thermometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a 300 MHz NMR spectrometer in $CDCl_3$ or DMSO-d_6 with using TMS as internal standard. DMF was dried over CaH_2, whereas THF was used after distillation over Na/benzophenone. 2-(tert-Butoxycarbonylaminoxy)acetic acid **3.5a** and 2-aminoxyacetic acid-hemi hydrochloride salt **3.7a** were purchased from commercial sources.

3.4.1. Synthesis of α-aminoxy acids 3.7 and their *N*-(Phth) and *N*-(Cbz) protected analogs 3.5

3.4.1.1. Synthesis of 3.5b and 3.5c

NaH in mineral oil (2.4 equiv. mmol, 60%) was portion wise added to a stirred solution of *N*-hydroxyphthalimide **3.11** (1.2 equiv. mmol) in DMF (4 mL) at 0 ^{o}C under nitrogen atmosphere. After the mixture was stirred for 10 min. α-Bromo acid **3.4b** and **3.4c** (1.0 equiv. mmol) in DMF (2 mL) was added dropwise over 20 min. at 0 ^{o}C then allowed to rise to room temperature and stirred overnight. The mixture was poured into the crushed ice-water and acidified with 4 N HCl. The residue was filtered off and washed with hexanes to give *N*-(Phth)-protected-aminoxy acids **3.5b** and **3.5c**.

Phth-AOGly-OH (3.5b). The crude product was recrystallized from EtOAc to give yellowish microcrystals (43%), mp 163–165 °C, (lit. [108] 166–168°C); ^{1}H NMR (DMSO-d_6) δ 4.76 (s, 2H), 7.88 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 73.0, 123.4, 128.7, 134.9, 162.7, 168.1.

Phth-D-AOAla-OH (3.5c). The crude product was recrystallized from the EtOAc-hexanes to give yellowish microcrystals (72%), mp 123–125 °C, (lit. [108] 125–130°C); $[\alpha]_D^{23}$ = +52^{o} (*c* 1.00, CH_3OH, lit. $[\alpha]_D^{23}$ = +89.5^{o} (*c* 1.00, $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.47 (d, *J* = 6.6 Hz, 3H), 4.76 (q, *J* = 6.7 Hz, 1H), 7.88 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 16.7, 80.7, 123.4, 128.6, 134.9, 163.2, 170.7.

3.4.2. Synthesis of 3.5d, 3.5e and 3.5h

3.4.2.1. General procedure for deprotection of *N*-hydroxy phthalimide

Hydrazine hydrate (1.2 equiv.) was added to a solution of *N*-(Phth)-aminoxy acid **3.5b, 3.5c** and **3.5g** (1 equiv. mol) in MeOH (30 mL). After stirring at room temperature for 3 hour, $NaHCO_3$ (3 equiv.) was added. The solvent was evaporated under vacuum. Water (5 mL) and 6 N HCl (5 mL) were added into the remaining white precipitate. Then water was evaporated to produce its hydrochloride salt under vacuum. Remaining salt was taken into methyl alcohol (20 mL) or isopropyl alcohol (20 mL), filtered the solid, which was discarded and

the solution was evaporated to give α-aminoxy acid-hydrochloride salt. This salt was used for further reactions without further purification.

3.4.2.2. General procedure for Cbz protection of α-aminoxy acids

Benzylchloroformate **14** (1.2 equiv. mol) in THF (8 mL) was added to a stirred solution of α-aminoxy acid-hydrochloride salt (1 equiv. mol) and $NaHCO_3$ (3 equiv. mol) in THF-water (20:1, 30 mL) at 0 ^oC over 15 min. After the reaction mixture was stirred overnight, most of the solvent was removed under vacuum. Then 10% NaOH (30 mL) was added and washed with diethyl ether (2 x 15 mL). Aqueous phase was acidified with 6 N HCl and extracted with EtOAc (3 x 20 mL). Organic phase was dried over Na_2SO_4; solvent was removed under vacuum to give *N*-(Cbz) protected-α-aminoxy acids **3.5d, 3.5e** and **3.5g**.

Cbz-AOGly-OH (3.5d). The crude product was recrystallized from CH_2Cl_2-hexanes to give white microcrystals (74%), mp 69–70 °C; ^1H NMR ($CDCl_3$) δ 4.49 (s, 2H), 5.21 (s, 2H), 7.36 (s, 5H), 8.37 (s, 1H), 8.56 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 68.7, 73.7, 128.7, 128.9, 129.0, 134.9, 158.7, 172.8.; Anal. Calcd. for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.53; H, 4.85; N, 6.16.

Cbz-D-AOAla-OH (3.5e). Oil (68%); $[\alpha]_D^{23}$ = +59.3^o (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.47 (d, *J* = 7.2 Hz, 3H), 4.49 (q, *J* = 7.0 Hz, 1H), 5.18 (s, 2H), 7.35 (s, 5H), 8.45 (s, 1H), 8.91 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.4, 68.3, 80.4, 128.5, 128.8, 135.2, 158.3, 176.0; Anal. Calcd. for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.85. Found: C, 54.89; H, 5.70; N, 6.01.

3.4.3. Synthesis of 3.5f

Cbz-NHOH (**3.13).** Benzyl chloroformate **3.12** (0.5 ml, 3.3 mmol) in CH_2Cl_2 (8 ml) was added dropwise to a stirred mixture of hydroxylamine hydrochloride (0.24 g, 3.5 mmol) and sodium bicarbonate (2.1 g, 25 mmol) in THF-water (40 ml, 20:1) over 15 min. The mixture was stirred overnight, and then most of the solvent was evaporated under vacuum. 10% NaOH (10 mL) was added and the mixture was extracted with diethylether (2 x 10 mL), which was discarded. Aqueous phase was acidified with 6 N HCl and extracted with CH_2Cl_2

(3 x 20 mL). Organic phase was dried over Na_2SO_4, solvent was removed under vacuum to give crude product and recrystallized from CH_2Cl_2-hexanes to give *N*-carbobenzoxyhydroxylamine **3.13** as white microcrystals (86%), mp 61–62 °C (lit. [109] mp 62–64 °C); ^{1}H NMR ($CDCl_3$) δ 5.18 (s, 2H), 6.81 (br s, 1H), 7.28 (s, 1H), 7.36 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 68.1, 128.6, 128.8, 128.8, 135.5, 159.4.

Cbz-L-AOVal-OH (3.5f). NaH in mineral oil (0.24 g, 6 mmol, 60%) was added portion wise to a stirred solution of benzyl hydroxycarbamate (0.44 g, 2.65 mmol) in anhydrous THF (3 mL), at -10 ^{o}C under nitrogen. After the mixture was stirred 10 min. (*R*)-2-bromo-3-methylbutanoic acid (0.50 g, 2.65 mmol) in anhydrous THF (2 mL) was added dropwise over 20 min at –10 ^{o}C. Then it was allowed to rise to room temperature and stirred overnight. 1 N cold HCl (25 mL) was added. The most of the solvent was evaporated under vacuum and aqueous phase was extracted with EtOAc (3 x 20 mL). The organic phase was dried over Na_2SO_4 and solvent was evaporated under vacuum. The oily crude was purified by column chromatography using EtOAc-hexanes as eluent (1:3 to1:1) to give an oil (45%); $[\alpha]_D^{23}$ = –82.7^o (*c* 1.00, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.98 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 2.18–2.30 (m, 1H), 4.24 (d, *J* = 4.2 Hz, 1H), 5.20 (s, 2H), 7.36 (s, 5H), 8.40 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.2, 19.0, 30.6, 68.5, 89.5, 128.6, 128.9, 135.2, 158.7, 174.5; Anal. Calcd. for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.27; H, 6.43; N, 5.44.

3.4.4. Synthesis of 3.5g

***O*-Acetyl-(L)-Mandelic acid (3.15).** A solution of acetylchloride (8 mL) and (L)-mandelic acid **16** (2.6 g, 17 mmol) was stirred at room temperature for 12 h. After evaporation of the volatile material, *O*-acetyl-(L)-mandelic acid **3.15** was obtained as white microcrystals (95%), mp 83–85 °C (lit. [110] mp 95–97.5 °C); $[\alpha]_D^{23}$ = +152^o (*c* 1.00, CH_3OH); (lit. [110]) $[\alpha]_D^{23}$ = +148^o (*c* 1.87, acetone)]; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H), 5.94 (s, 1H), 7.38–7.44 (m, 3H), 7.46–7.52 (m, 2H), 11.00 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.8, 74.3, 126.8, 127.8, 129.1, 129.7, 133.3, 170.6, 174.6; Anal. Calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.75; H, 5.16.

***t*-Butyl *O*-Acetyl-(L)-Mandelate (3.16).** DCC (4.15 g, 20 mmol) in CH_2Cl_2 (10 mL) was added to a solution of *O*-acetyl (L)-mandelic acid **3.15** (2.35 g, 15 mmol), tert-butanol (2.60 g, 35 mmol) and DMAP (0.61 g, 5 mmol) in CH_2Cl_2 (30 mL) at 0 ^oC. After stirring at room temperature overnight, urea was precipitated and filtered off. Organic layer was washed 10% Na_2CO_3 (2 x 15 mL) and 1N HCl (2 x 15 mL) and water (10 mL), dried over Na_2SO_4 and evaporated to give compound **3.16** as white microcrystals (86%), mp 24–28 °C [lit. [111] oil]; $[\alpha]_D^{23}$ = +30.5^o (*c* 1.00, CH_3OH); [lit. [111] $[\alpha]_D^{23}$ = +65.1^o (*c* 1.0, CH_2Cl_2)]; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H), 2.18 (s, 3H), 5.79 (s, 1H), 7.34–7.42 (m, 2H), 7.42–7.54 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 20.9, 28.0, 75.2, 82.6, 127.7, 128.8, 129.1, 134.5, 168.0, 170.5; Anal. Calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.75. Found: C, 67.02; H, 7.56.

***t*-Butyl (L)-Mandelate (3.17).** t-Butyl *O*-acetyl-(L)-mandelate **3.16** (2.52 g, 10 mmol) in methyl alcohol (10 mL) was added to a solution of K_2CO_3 (4.14 g, 30 mmol) in water (15 mL) and methyl alcohol (5 mL) and the mixture was stirred for 12 h at room temperature. Then, methanol was removed under reduced pressure and aqueous solution was extracted with CH_2Cl_2 (3 x 15 mL). Organic layer was dried over Na_2SO_4 and evaporated to give compound **3.17** as white microcrystals (78%), mp 53–54 °C [lit. [112] 69–70 ^oC]; $[\alpha]_D^{23}$ = +35.8^o (*c* 1.00, CH_3OH); [lit. [112] $[\alpha]_D^{23}$ = +97.4^o (*c* 1.0, CH_3OH)]; ^{1}H NMR δ 1.41 (s, 9H), 3.52 (s, 1H), 5.04 (s, 1H), 7.30–7.60 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 27.0, 72.2, 82.3, 125.6, 127.3, 127.6, 138.2, 172.1; Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74 Found: C, 69.33; H, 8.13.

***t*-Butyl *N*-(Phth)-(D)-Aminoxymandelate (3.18).** DIAD (1.4 mL, 7.5 mmol) dropwise was added to a stirred solution of *N*-hydroxy phthalimide **3.11** (0.94 g, 5.76 mmol), PPh_3 (1.64 g, 6.24 mmol) and t-butyl (L)-mandelate **3.17** (1.09 g, 4.8 mmol) in THF (10 mL) over 20 min. at 0 ^oC. After the solution was stirred for 8 h at room temperature, solvent was removed under vacuum. And the crude was purified by column chromatography (EtOAc-hexanes, 1:5) to give compound **3.18** as white microcrystals (89%), mp 91–92 °C; $[\alpha]_D^{23}$ = –42.8^o (*c* 1.00, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 5.75 (s, 1H), 7.32–7.38 (m, 3H),

7.56–7.62 (m, 2H), 7.66–7.72 (m, 2H), 7.72–7.80 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.0, 83.1, 86.1, 123.7, 128.7, 128.8, 128.9, 129.9, 133.4, 134.6, 163.2, 167.0; Anal. Calcd. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.64; H, 5.38; N, 3.95.

***N*-(Phth)-(D)-Aminoxymandelic acid (3.5g).** trifluoroacetic acid (15 mL) was added dropwise to a solution of *t*-butyl *N*-(Phth)-(D)-aminoxymandelate **20** (1.34 g, 3.8 mmol) in CH_2Cl_2 (15 mL) at 0 ^{o}C. After stirred at 10 ^{o}C for 3 h, the solvent was evaporated completely to give a solid, which was then washed with water (10 mL) and ethyl ether (10 mL) to give pure compound **3.5g** as white microcrystals (71%), mp 105–106 °C; $[\alpha]_D^{23} = -123.3^{o}$ (*c* 1.00, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 5.76 (s, 1H), 7.36–7.44 (m, 3H), 7.52–7.58 (m, 2H), 7.83 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 85.1, 123.3, 128.4, 128.5, 129.6, 133.9, 134.9, 162.8, 168.9; Anal. Calcd. for $C_{16}H_{11}NO_5.H_2O$: C, 60.95; H, 4.16; N, 4.44. Found: C, 61.23; H, 4.08; N, 4.39.

Cbz-D-AOMan-OH (3.5h). *N*-(Cbz) protected aminoxy acid **3.5h** was prepared from *N*-(Phth)-(D)-aminoxymandelic acid **3.5g** according outlined procedure for **3.5d**, **3.5e** and **3.5h** in Scheme 3.4. as white microcrystals (56%), mp 86–87 °C; $[\alpha]_D^{23} = +44^{o}$ (*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 5.10-5.24 (m , 2H), 5.40 (s, 1H), 7.28-7.52 (m, 10H), 8.69 (br s, 1H), 9.22 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 67.3, 84.8, 127.3, 127.5, 127.7, 127.8, 127.9, 128.8, 132.0, 134.3, 157.2, 172.8; Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.02; N, 4.65. Found: C, 63.73; H, 4.85; N, 5.08.

3.4.5. General preparation of *N*-(PG-aminoxyacyl)benzotriazole (3.6)

Thionyl chloride (1.2 mmol) was added to a solution of benzotriazole (4.16 mmol) in anhydrous THF (5 mL) at 0 ^{o}C, and the reaction mixture stirred for 20 min at same temperature. *N*-(PG)-Aminoxyacetic acid (1.0 mol) dissolved in anhydrous THF (3 mL) was added dropwise to the mixture. After stirring for 4 h at 0 ^{o}C, the reaction mixture was allowed to warm to room temperature. After 1 h, the white precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (25 mL) and the solution washed with saturated Na_2CO_3 solution (3 x 10 mL), then saturated brine solution

and finally dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to afford *N*-(PG-aminooxyacyl)benzotriazoles (**3.6**).

Boc-AOGly-Bt (**3.6a**). White microcrystals (67%), mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 5.54 (s, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.96 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 28.3, 74.6, 82.7, 114.1, 120.6, 126.8, 130.9, 131.0, 146.0, 156.4, 168.3; Anal. Calcd. for $C_{13}H_{16}N_4O_4$·: C, 53.42; H, 5.52; N, 19.17. Found: C, 53.07; H, 5.69; N, 18.59.

Phth-AOGly-Bt (**3.6b**). White microcrystals (75%), mp 155–157 °C; ^{1}H NMR ($CDCl_3$) δ 5.93 (s, 2H), 7.56 (t, J = 8.1 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.71–7.81 (m, 2H), 7.82–7.94 (m, 2H), 8.15 (d, J = 8.4 Hz, 1H,) 8.31 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 74.3, 114.1, 120.5, 123.9, 126.8, 128.8, 131.1, 134.9, 163.0, 165.2; Anal. Calcd. for $C_{16}H_{10}N_4O_4$·: C, 59.63; H, 3.13; N, 17.38. Found: C, 59.24; H, 3.02; N, 17.10.

Phth-D-AOAla-Bt (**3.6c**). White microcrystals (56%), mp 145–147 °C; ^{1}H NMR ($CDCl_3$) δ 1.96 (d, J = 6.9 Hz, 3H), 6.34 (q, J = 6.9 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.64–7.78 (m, 3H), 7.78–7.88 (m, 2H), 8.13 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 17.2, 81.1, 114.8, 120.5, 124.0, 127.0, 128.9, 131.2, 131.3, 134.9, 146.3, 163.5, 168.8.; Anal. Calcd. for $C_{17}H_{12}N_4O_4$·: C, 60.71; H, 3.60; N, 16.66. Found: C, 60.56; H, 3.46; N, 16.79.

Cbz-AOGly-Bt (**3.6d**). White microcrystals (66%), mp 86–87 °C; ^{1}H NMR ($CDCl_3$) δ 5.22 (s, 2H), 5.56 (s, 2H), 7.30–7.40 (m, 5H), 7.55 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 7.2 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.24-8.27 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 68.0, 74.6, 114.0, 120.5, 126.8, 128.5, 128.6, 128.7, 130.7, 131.0, 135.2, 145.9, 157.0, 168.0, 169.9; Anal. Calcd. for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 58.82; H, 4.07; N, 16.78.

Cbz-D-AOAla-Bt (**3.6e**). Colorless oil, (58%); $[\alpha]_D^{23}$ = +74.2^o (*c* 1.00, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.76 (d, J = 7.2 Hz, 3H), 5.18 (s, 2H), 5.94 (q, J = 6.9 Hz, 1H), 7.33 (s, 5H), 7.53 (t, J = 7.2 Hz, 1H), 7.67 (t, J = 7.2 Hz, 1H), 8.13 (d, J = 8.1 Hz, 1H), 8.18 (s, 1H), 8.26 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 17.2, 29.8, 67.9, 80.7, 114.4, 114.5, 120.5, 126.8, 128.4, 128.6, 128.7, 131.0,

131.2, 135.4, 145.9, 157.5, 171.2. HRMS, $[M+Na]^+$: Found: 363.1084, Theoretical for $C_{17}H_{16}N_4O_4.Na^+$: 363.1064.

Cbz-L-AOVal-Bt (**3.6f**). Oil, (89%); $[\alpha]_D^{23} = -130.8^o$ (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.05 (d, *J* = 6.9 Hz, 3H), 1.20 (d, *J* = 6.9 Hz, 3H) , 2.42–2.62 (m, 1H), 5.17 (s, 2H), 5.77 (d, *J* = 4.2 Hz, 1H), 7.33 (s, 5H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.87 (s, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 17.0, 19.4, 31.4, 68.0, 88.8, 114.6, 120.6, 126.8, 128.5, 128.7, 128.8, 131.0, 131.2, 135.4, 146.0, 157.5, 170.6. HRMS, $[M+Na]^+$: Found: 391.1391, Theoretical for $C_{19}H_{20}N_4O_4.Na^+$: 391.1377.

Cbz-D-AOMan-Bt (**3.6g**). Oil (77%); $[\alpha]_D^{23} = -19.8^o$ (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 5.20 (dd, *J* = 17.9, *J* = 12.0 Hz, 2H), 5.94 (s, 1H), 7.30–7.45 (m, 8H), 7.50 (t, *J* = 8.1 Hz, 1H), 7.60–7.70 (m, 3H), 7.96 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 8.29 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 68.2, 86.0, 114.5, 120.6, 126.9, 128.6, 128.7, 128.8, 129.2 (2C), 130.2, 131.0, 132.4, 135.4, 146.1, 157.4, 168.6. HRMS, $[M+Na]^+$: Found: 425.1230, Theoretical for $C_{22}H_{18}N_4O_4.Na^+$: 425.1220.

3.4.6. General preparation of *N*-(PG)-aminoxy acid amides (3.8)

Amine (1 equiv.) and triethylamine (1 equiv. mol) in THF (2 mL) were added to a stirred solution of *N*-(Pg-aminoxyacyl)benzotriazole **3.6** (1 equiv. mol) in THF (4 mL) dropwise at 0 ^oC and the mixture was stirred for 2 h at room temperature. After evaporation of THF, EtOAc (20 mL) was added to the solution, which was washed with saturated Na_2CO_3 solution (3 x 10 mL), brine (10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave the crude amides.

tert-Butyl 2-(isopropylamino)-2-oxoethoxycarbamate (**3.8a**) [102]. The crude product was recrystallized from diethyl ether-hexanes to give white microcrystals (74%), mp 67–68 °C; ^1H NMR ($CDCl_3$) δ 1.19 (d, *J* = 6.6 Hz, 6H), 1.49 (s, 9H), 4.00–4.20 (m, 1H), 4.27 (s, 2H), 7.51 (s, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.5, 28.1, 41.0, 76.1, 83.0, 157.8, 167.8; Anal. Calcd. for $C_{10}H_{20}N_2O_4$·: C, 51.71; H, 8.68; N, 12.06. Found: C, 52.09; H, 8.90; N, 12.12.

tert-Butyl 2-(cyclohexylamino)-2-oxoethoxycarbamate (**3.8b**). The crude product was recrystallized from diethyl ether-hexanes to give white microcrystals (62%), mp 137–139 °C; ^{1}H NMR ($CDCl_3$) δ 1.14–1.41 (m, 6H), 1.48 (s, 9H), 1.70–1.80 (m, 2H), 1.84–1.95 (m, 2H), 3.70–3.85 (m, 1H), 4.28 (s, 2H), 7.48 (s, 1H), 7.99 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 25.6, 28.1, 32.8, 47.9, 76.2, 83.1, 157.7, 167.6; Anal. Calcd. for $C_{13}H_{24}N_2O_4$·: C, 57.33; H, 8.88; N, 10.29. Found: C, 57.25; H, 9.17; N, 10.15.

Benzyl 2-(cyclohexylamino)-2-oxoethoxycarbamate (**3.8c**). The crude product was recrystallized from diethyl ether-hexanes to give white microcrystals (78%), mp 69–70 °C; ^{1}H NMR ($CDCl_3$) δ 1.11–1.42 (m, 5H), 1.50–1.78 (m, 3H), 1.80–2.00 (m, 2H), 3.65–3.87 (m, 1H), 4.29 (s, 2H), 5.19 (s, 2H), 7.25–7.40 (m, 5H), 7.75 (br s, 1H), 8.14 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.9, 25.6, 32.8, 48.1, 68.3, 76.3, 128.6, 128.8, 128.8, 135.1, 158.5, 167.5; Anal. Calcd. for $C_{16}H_{22}N_2O_4$·: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.97; H, 7.48; N, 9.06.

2-(1,3-Dioxoisoindolin-2-yloxy)-*N*-(4-methoxyphenyl)acetamide (**3.8d**). The crude product was recrystallized from diethyl ether-hexanes to give white microcrystals (38%), mp 210–211 °C; ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H), 4.84 (s, 2H), 6.90–7.00 (m, 2H), 7.65–7.75 (m, 2H), 7.80–8.00 (m, 4H), 9.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.5, 76.9, 114.2, 121.5, 124.2, 128.5, 130.5, 135.2, 156.6, 163.9, 164.7; Anal. Calcd. for $C_{17}H_{14}N_2O_5$: C, 62.57; H, 4.32; N, 8.58. Found: C, 62.40; H, 3.96; N, 8.12.

(D)-Benzyl 1-(4-methoxyphenylamino)-1-oxopropan-2-yloxycarbamate (**3.8e**). The residue was purified by column chromatography [EtOAc-hexanes (from 1:3 to 1:2)] to give white microcrystals (56%), mp 121–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.51 (d, J = 7.2 Hz, 3H), 3.79 (s, 3H), 4.39 (q, J = 7.2 Hz, 1H), 5.20 (d, J = 1.8 Hz, 2H), 6.85 (d, J = 9.0 Hz, 2H), 7.34 (s, 5H), 7.55 (d, J = 9.0 Hz, 2H), 7.97 (s, 1H), 9.62 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.4, 55.7, 68.6, 83.8, 114.2, 121.6, 128.7, 128.9, 129.0, 131.2, 135.0, 156.4, 158.9, 169.6; Anal. Calcd. for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.89; H, 6.01; N, 8.00.

Benzyl (*S*)-3-methyl-1-oxo-1-((*R*)-1-phenylethylamino)butan-2-yloxycarbamate (**3.8f**). The crude product was recrystallized from diethyl ether-

hexanes to give white microcrystals (67%), mp 96–97 °C; $[\alpha]_D^{23}$ = –39.7° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 0.82 (d, *J* = 6.9 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3H), 1.39 (d, *J* = 7.2 Hz, 3H), 2.14–2.26 (m, 1H), 4.02 (d, *J* = 4.2 Hz, 1H), 5.04–5.16 (m, 1H), 5.18 (d, *J* = 1.8 Hz, 2H), 7.30–7.40 (m, 10H), 7.99 (s, 1H), 8.18 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.7, 19.4, 22.2, 30.9, 48.7, 68.2, 92.0, 126.3, 127.2, 128.6, 128.7, 128.9, 135.3, 143.8, 158.5, 169.8; Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 67.85; H, 7.36; N, 7.38.

Benzyl (2S)-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-yloxycarbamate (**3.8f+3.8f′**). The residue was purified by column chromatography [EtOAc-hexanes (from 1:4 to 1:2)] to give an oil (58%); $[\alpha]_D^{23}$ = –6.8° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ (data from diastereomeric mixture): 0.95 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.9 Hz, 3H), 1.52 (d, *J* = 6.9 Hz, 3H), 2.14–2.32 (m, 1H), 4.00–4.10 (m, 1H), 5.04 (s, 1H), 5.08-5.16 (m, 1 H), 5.19 (d, *J* = 3.3 Hz, 1H), 7.20–7.42 (m, 10H), 7.80 (s, 1H), 8.18 (br s, 1H); Other diastereomer: 0.81 (d, *J* = 6.9 Hz), 0.98 (d, *J* = 6.9 Hz), 1.39 (d, *J* = 7.2 Hz), 7.88 (s); ^{13}C NMR ($CDCl_3$) δ 16.7, 19.4, 22.2, 30.7, 30.9, 48.8, 68.2, 91.6, 92.0, 126.3, 126.5, 127.2, 128.6, 128.8, 135.2, 143.5, 143.8, 158.4 (2C), 169.7 (2C); Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 67.71; H, 7.04; N, 7.32.

3.4.7. General preparation of α-AO hybrid dipeptides (3.9) and α-AO hybrid tripeptides (3.20)

The unprotected amino acids (1.5 mmol) and triethylamine (2.0 mmol) were dissolved in minimum amount of water. Acetonitrile (3 mL) was added to this solution and cooled to 0 °C. A solution of *N*-(PG-aminooxyacyl)benzotriazole **3.6** (1 mmol) in acetonitrile (4 mL) was added dropwise over 10 min at 0 °C and stirred for 4 h at 10 °C. After evaporation of THF, EtOAc (20 mL) was added and the mixture was washed with 4N HCl solution (3 x 15 mL), brine (15 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give crude product **3.9** or **3.20**.

Cbz-AOGly-L-Phe-OH (3.9a). The residue was purified by column chromatography [EtOAc-hexanes (from 1:3 to 1:1)] to give an oil (51%); $[\alpha]_D^{23}$ = –9.0° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 3.02 (dd, *J* = 14.0 Hz, 8.3 Hz, 1H),

3.25 (dd, J = 14.0 Hz, 4.9 Hz, 1H), 4.28 (s, 2H), 4.76–4.89 (m, 1H), 5.11 (s, 2H), 7.14-7.30 (m, 5H), 7.33 (s, 5H), 7.72 (br s, 1H), 8.08 (s, 1H), 8.23 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.3, 53.6, 68.5, 75.8, 127.2, 128.6, 128.8, 128.9, 129.4, 135.1, 136.2, 158.5, 169.8, 174.5; Anal. Calcd. for $C_{19}H_{20}N_2O_6.H_2O$·: C, 58.46; H, 5.16; N, 7.18. Found: C, 58.76; H, 5.42; N, 7.38.

Cbz-AOGly-DL-Phe-OH (3.9a+3.9a′). The residue was purified by column chromatography [EtOAc-hexanes (from 1:3 to 1:1)] to give an oil (56%); (data from enantiomeric mixture) ^{1}H NMR ($CDCl_3$) δ 3.05 (dd, J = 14.1 Hz, 8.4 Hz, 1H), 3.26 (dd, J = 14.1 Hz, 5.1 Hz, 1H), 4.30 (s, 2H), 4.77–4.86 (m, 1H), 5.12 (s, 2H), 6.08 (br s, 1.5 H), 7.16–7.30 (m, 5H), 7.34 (s, 5H), 8.04 (br s, 0.5 H), 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.2, 53.7, 68.5, 75.9, 127.3, 128.7, 128.8, 128.9, 128.9, 129.4, 135.1, 136.2, 158.5, 169.7, 174.4; Anal. Calcd. for $C_{19}H_{20}N_2O_6$·: C, 61.28; H, 5.41; N, 7.52. Found: C, 60.89; H, 5.59; N, 7.69.

Cbz-D-AOAla-L-Phe-OH (3.9b). The residue was purified by column chromatography [EtOAc-hexanes (from 1:4 to 1:2)] to give white microcrystals, (61%), mp 33–34 °C; $[\alpha]_D^{23}$ = +39.3^o (c 1.00, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.9 Hz, 3H), 3.06 (dd, J = 14.6, 7.7 Hz, 1H), 3.28 (dd, J = 14.0, 5.6 Hz, 1H), 4.30 (q, J = 7.0 Hz, 1H), 4.75–4.90 (m, 1H), 5.05–5.20 (m, 2H), 7.15–7.44 (m, 10H), 7.62 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.9, 37.1, 53.5, 68.3, 82.6, 127.3, 128.6, 128.8, 128.9, 129.4, 135.2, 136.3, 158.2, 172.4, 174.5; Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.06; H, 5.92; N, 7.73.

Cbz-D-AOAla-L-Trp-OH (3.9c). The residue was recrystallized from EtOAc-hexanes to give white microcrystals, (72%), mp 128–130 °C; $[\alpha]_D^{23}$ = +62.0^o (c 1.00, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 1.14 (d, J = 6.6 Hz, 3H), 3.04 (dd, J = 14.4, 8.4 Hz, 1H), 3.18 (dd, J = 14.7, 5.1 Hz, 1H), 4.23 (q, J = 6.9 Hz, 1H), 4.45–4.52 (m, 1H), 5.04 (s, 2H), 6.92–7.14 (m, 4H), 7.28–7.44 (m, 6H), 7.53 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 8.1 Hz, 1H), 10.55 (s, 1H), 10.85 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 16.6, 27.1, 52.4, 66.1, 80.5, 109.5, 111.2, 118.1, 118.2, 120.8, 123.4, 127.1, 127.8, 128.0, 128.3, 136.0, 136.1, 157.3, 170.4, 172.9; Anal. Calcd. for $C_{22}H_{23}N_3O_6$: C, 62.11; H, 5.45; N, 9.88. Found: C, 62.02; H, 5.48; N, 9.80.

Cbz-D-AOAla-L-Leu-OH (3.9d). The residue was washed with hexanes to give a colorless oil, (78%); $[\alpha]_D^{23}$ = +21.8° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 0.93 (d, *J* = 3.9 Hz, 6H), 1.43 (d, *J* = 6.6 Hz, 3H), 1.58–1.78 (m, 3H), 4.38 (q, *J* = 6.9 Hz, 1H), 4.51–4.63 (m, 1H), 5.17 (s, 2H), 7.35 (s, 5H), 7.88 (br s, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.0, 21.9, 23.1, 25.1, 40.7, 50.9, 68.4, 82.7, 128.5, 128.9, 135.2, 158.5, 172.5, 176.5; Anal. Calcd. for $C_{17}H_{24}N_2O_4$·: C, 57.94; H, 6.86; N, 7.95. Found: C, 58.15; H, 7.29; N, 7.89.

Cbz-L-AOVal-L-Phe-OH (3.9e). The residue was recrystallized from CH_2Cl_2-hexanes to give white microcrystals, (69%), mp 126–127 °C; $[\alpha]_D^{23}$ = –64.4° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 0.67 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 1.85–2.02 (m, 1H), 3.02 (dd, *J* = 14.0, 9.8 Hz, 1H), 3.31 (dd, *J* = 14.3, 4.7 Hz, 1H), 3.98 (d, *J* = 4.8 Hz, 1H), 4.73-4.88 (m, 1H), 5.13 (d, *J* = 3.6 Hz, 2H), 7.16–7.28 (m, 5H), 7.30–7.42 (m, 5H), 8.10 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 17.1, 18.8, 30.6, 37.2, 53.5, 68.4, 91.7, 127.2, 128.7, 128.8 (2C), 129.4, 135.3, 136.4, 158.5, 172.2, 175.4; Anal. Calcd. for $C_{22}H_{26}N_2O_6$·: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.79; H, 6.43; N, 6.75.

Cbz-L-AOVal-L-Trp-OH (3.9f). The residue was washed with diethylether-hexanes mixture to give white microcrystals, (66%), mp 27-28 °C; $[\alpha]_D^{23}$ = –64.2° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 0.71 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.9 Hz, 3H), 1.82–1.96 (m, 1H), 3.29 (dd, *J* = 14.7, 7.8 Hz, 1H), 3.39 (dd, *J* = 14.7, 4.8 Hz, 1H), 3.96 (d, *J* = 5.4 Hz, 1H), 4.86–4.96 (m, 1H), 5.09 (s, 2H), 7.04–7.12 (m, 2H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.28–7.40 (m, 6H), 7.59 (d, *J* = 8.1 Hz, 1H), 8.11 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 17.4, 18.8, 27.3, 30.6, 53.0, 68.3, 91.6, 110.1, 111.5, 118.7, 119.8, 122.3, 123.3, 127.6, 128.6, 128.8, 135.3, 136.3, 158.3. 172.1, 175.1; Anal. Calcd. for $C_{24}H_{27}N_3O_6$.1/2 H_2O: C, 62.33; H, 6.10; N, 9.09. Found: C, 62.23; H, 6.15; N, 8.48.

Cbz-AOGly-L-Cys-OH (3.9g). The residue was purified by column chromatography [EtOAc-hexanes (from 1:3 to 1:1)] to give an oil (66%); $[\alpha]_D^{23}$ = 0.0° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.62 (t, *J* = 9 Hz, 1H), 2.88–3.00 (m, 2H), 4.42 (s, 2H), 4.76–4.84 (m, 1H), 5.14 (s, 2H), 7.28–7.40 (m, 5H), 8.30 (s, 1H), 8.66 (s, 1H), 8.70 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.3, 54.2, 68.5, 75.8, 128.6,

128.9, 135.2, 158.7, 170.1, 172.5. HRMS, $[M+Na]^+$: Found: 351.0613, Theoretical for $C_{13}H_{16}N_2O_6S.Na^+$: 351.0621.

Cbz-D-AOAla-L-Cys-OH (3.9h). The residue was purified by column chromatography [EtOAc-hexanes (from 1:3 to 1:1)] to give an oil (61%); $[\alpha]_D^{23}$ = +1.8° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.44 (d, *J* = 6.9 Hz, 3H), 1.63 (t, *J* = 9 Hz, 1H), 2.86–3.10 (m, 2H), 4.41(q, *J* = 6.9 Hz, 1H), 4.70–4.86 (m, 1H), 5.17 (s, 2H), 6.41 (br s, 1H), 7.26–7.40 (m, 5H), 8.07 (s, 1H), 8.21 (s, 1H).; ^{13}C NMR ($CDCl_3$) δ 17.1, 26.3, 54.2, 68.5, 82.8, 128.6, 128.9, 135.2, 158.5, 172.9 (2C). HRMS, $[M+Na]^+$: Found: 365.0764, Theoretical for $C_{14}H_{18}N_2O_6S.Na^+$: 365.0778.

Cbz-AOGly-Gly-L-Phe-OH (3.20a). The residue was recrystallized from diethylether-hexanes to give white hydroscopic microcrystals, (81%), mp 29–31 °C; $[\alpha]_D^{23}$ = +7.1° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 2.92 (br s, 1H), 3.04 (br s, 1H), 3.76 (br s, 1H), 3.83 (br s, 1H), 4.22 (br s, 2H), 4.67 (br s, 1H), 5.00 (br s, 2H), 6.92–7.36 (m ,12H), 8.04 (s, 1H), 8.73 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.4, 42.8, 53.8, 68.4, 75.9, 127.3, 128.3, 128.6, 128.8, 128.9, 129.6, 135.3, 136.1, 158.6, 169.8, 170.5, 173.8; Anal. Calcd. for $C_{21}H_{23}N_3O_7$: C, 58.74; H, 5.40; N, 9.79. Found: C, 58.57; H, 5.28; N, 9.60.

Cbz-AOGly-Gly-L-Leu-OH (3.20b). The residue was recrystallized from CH_2Cl_2-hexanes to give white hydroscopic microcrystals, (70%), mp 28-30 °C; $[\alpha]_D^{23}$ = –23.3° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 0.89 (t, *J* = 5.3 Hz, 6H), 1.46–1.74 (m, 3H), 3.92 (dd, *J* = 16.2, 5.1 Hz, 1H), 4.07 (dd, *J* = 16.5, 5.7 Hz, 1H), 4.39 (s, 2H), 4.44–4.58 (m, 1H), 5.14 (s, 2H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.28–7.40 (m, 6H), 8.15 (s, 1H), 8.86 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.9, 23.0, 25.0, 40.7, 42.8, 51.3, 68.3, 75.9, 128.5, 128.6, 128.8, 128.9, 135.3, 158.6, 170.1, 170.5, 175.5; Anal. Calcd. for $C_{18}H_{25}N_3O_7$·: C, 54.68; H, 6.37; N, 10.63. Found: C, 54.84; H, 6.69; N, 10.02.

Cbz-D-AOAla-Gly-L-Phe-OH (3.20c). The residue was recrystallized from EtOAc-hexanes to give white microcrystals, (50%), mp 63–64 °C; $[\alpha]_D^{23}$ = +52.2° (*c* 1.00, CH_3OH); ^1H NMR (DMSO-d_6) δ 1.25 (d, *J* = 6.3 Hz, 3H), 2.87 (dd, *J* = 13.2, 9.0 Hz, 1H), 3.05 (dd, *J* = 13.7, *J* = 4.1 Hz, 1H), 3.72 (s, 2H), 4.23 (q, *J* = 6.6 Hz, 1H), 4.35–4.50 (m, 1H), 5.09 (s, 2H), 7.15–7.42 (m, 10H), 8.03 (s, 1H),

8.19 (d, J = 7.8 Hz, 1H), 10.52 (s, 1H), 12.81 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 16.7, 36.8, 41.4, 53.5, 66.2, 80.9, 126.5, 127.9, 128.1, 128.2, 128.4, 129.1, 136.2, 137.4, 157.1, 168.4, 170.9, 172.7; Anal. Calcd. for $C_{22}H_{25}N_3O_7$: C, 59.59; H, 5.68; N, 9.48. Found: C, 59.24; H, 5.91; N, 9.20.

Cbz-L-AOVal-Gly-L-Phe-OH (3.20d). The residue was recrystallized from CH_2Cl_2-hexanes to give white microcrystals, (67%), mp 123–124 °C; $[\alpha]_D^{23}$ = –47.5^o (c 1.00, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.87 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 7.2 Hz, 3H), 2.04–2.20 (m, 1H), 3.05 (dd, J = 13.7, 6.5 Hz, 1H), 3.20 (dd, J = 15.3, 5.4 Hz, 1H), 3.82–3.98 (m, 2H), 4.06 (d, J = 4.5 Hz, 1H), 4.71–4.82 (m, 1H), 5.02 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 11.7 Hz, 1H), 6.93 (d, J = 7.5 Hz, 1H) 7.12–7.28 (m, 5H), 7.30–7.38 (m, 5H), 7.99 (br s, 1H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.2, 19.1, 30.8, 37.5, 42.9, 53.8, 68.3, 91.7, 127.2, 128.6, 128.8, 129.6, 135.2, 136.1, 158.4, 169.6, 172.5, 173.8; Anal. Calcd. for $C_{24}H_{29}N_3O_7$: C, 61.14; H, 6.20; N, 8.91. Found: C, 61.00; H, 6.19; N, 8.84.

3.4.8. General preparation of α-aminoxy dipeptides (3.10)

α-Aminoxy acid hydrochloric acid salts **3.7** (1.5 mmol) and triethylamine (3.5 mmol) were dissolved in a minimum amount of water. Acetonitrile (3 mL) was added to the solution and cooled to 0 ^{o}C. A solution of *N*-(PG-aminoxyoacyl)benzotriazole **3.5** (1 mmol) in acetonitrile (3 mL) was added drop wise over 10 min. at 0 ^{o}C and stirred for 4 h at 10 ^{o}C. After the solvent was evaporated under vacuum, EtOAc (20 mL) was added and the mixture was washed with 3 N HCl solution (3 x 15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give crude product **3.10**.

Cbz-L-AOVal-AOGly-OH (3.10a). The residue was an oily mixture of **3.10a** (80%) and Cbz-L-AOVal-OH (20%); $[\alpha]_D^{23}$ = –56.0^o (c 1.00, CH_3OH); (Data from the mixture) ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 6.9 Hz, 3H), 1.04 (d, J = 6.9 Hz, 3H), 2.18–2.30 (m, 1H), 4.19 (d, J = 4.2 Hz, 1H), 4.43 (d, J = 17.4 Hz, 1H), 4.54 (d, J = 17.4 Hz, 1H), 5.17 (d, J = 11.7 Hz, 1H), 5.24 (d, J = 12.0 Hz, 1H), 7.32–7.42 (m, 5H), 8.17 (s, 1H), 11.64 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.7, 18.9, 30.9, 69.0, 75.1, 91.2, 128.6, 128.7, 128.8, 128.9, 129.0, 134.7, 159.5, 171.1,

172.0. HRMS, $[M+Na]^+$: Found: 363.1175, Theoretical for $C_{15}H_{20}N_2O_7.Na^+$: 363.1163.

Cbz-D-AOMan-D-AOAla-OH (3.10b). The residue was a hydroscopic solid (52%), mp 27–28 °C; $[\alpha]_D^{23}$ = +8.6° (*c* 1.00, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.50 (d, *J* = 6.9 Hz, 3H), 4.30-4.45 (m, 1H), 5.17 (s, 2H), 5.30 (s, 1H), 6.90-7.60 (m, 10H), 8.27 (br s, 1H), 11.11(br s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.5, 16.7, 68.8, 81.5, 81.8, 87.4, 128.0 (2C), 128.3, 128.6, 128.9, 129.0 (2C), 129.9, 131.9, 133.6, 134.9, 158.9, 159.1, 169.1, 170.1, 173.8, 174.1; HRMS, $[M+Na]^+$: Found: 411.1171, Theoretical for $C_{19}H_{20}N_2O_7.Na^+$: 411.1163.

4. SOLID PHASE SYNTHESIS OF AMINOXY HYBRIDE PEPTIDES AND AMINOXY PEPTIDES

4.1. Benzotriazole Mediated Solid-phase peptide synthesis (SPPS)

Solid-phase peptide synthesis (SPPS) is one of the major technique for the rapid synthesis of potentially bioactive peptides [44]. The increased efficiency of SPPS with improved coupling reagents has led to its near-exclusive use for peptide preparation [113, 114].

Recently, Katritzky and coworkers reported that the preparation of tri-, tetra-, penta-, hexa-, and heptapeptides in 71% average crude yields by microwave-assisted benzotriazole mediated solid phase peptide synthesis utilizing *N*-Fmoc-(aminoacyl)benzotriazoles and *N*-Fmoc-(dipeptidoyl)benzotriazoles [11, 12, 115]**.**

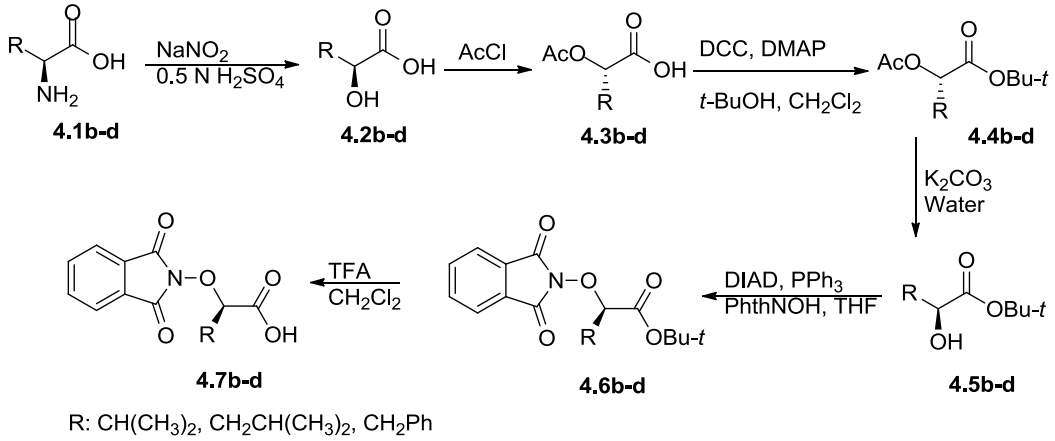
Reported solid-phase syntheses of aminoxy peptides using diverse coupling reagents can suffer from *N*-overacylation [106], coupling of 6 hours [101], low yields [101], and require acid sensitive protecting groups reducing the generality of the method. Hence, there is a need for mild and efficient method to prepare difficult aminoxyacyl hybrid peptides and aminoxy peptides using solid-phase peptide synthesis.

Herein, microwave assisted solid-phase syntheses of aminoxy peptides using *N*-Phth-(α-aminoxyacyl)benzotriazoles **4.8** was investigated. Nevertheless a general method for the preparation of aminoxy peptides by using *N*-Phth protected-(α-aminoxyacyl)benzotriazoles **4.8** could not be developed due to the less stability of *N*-(Phth)-protection against nucleophilic attacks in basic media.

4.2. The preparation of *N*-(Phth)-aminoxy acids 4.7

N-(Phth)-aminoxyglycine **4.7a** was prepared by substitution reactions of α-bromocarboxylic acid with *N*-hydroxyphthalimide as previously reported in Chapter 3. *N*-(Phth)-protected AOVal **4.7b**, AOLeu **4.7c**, and AOPhe **4.7d** analogs were prepared according to the literature procedures [80, 108] as shown in Scheme 1. (L)-α-Hydroxycarboxylic acids **4.2b-d** were obtained by diazotization of the corresponding amino acids **4.1b-d.** Then **4.2b-d** were treated with acetyl

chloride to give *O*-acetyl-(L)-α-carboxylic acids **4.3b-d**, which were reacted with *t*-BuOH in the presence of DCC/DMAP to produce *t*-butyl *O*-acetyl-(L)-α-carboxylates **4.4b-d**. Treatment of **4.4b-d f** with K_2CO_3 in water gave *t*-butyl (L)-α-hydroxycarboxylates **4.5b-d**. *t*-Butyl (L)-α-hydroxycarboxylates **4.5b-d** underwent Mitsunobu reactions with *N*-hydroxyphthalimide to give *t*-butyl *N*-(Phth)-(D)-aminoxycarboxylates **4.6b-d** in the presence of DIAD and PPh_3. Treatment of **4.6b-d** with trifluoroacetic acid gave *N*-(Phth)-(D)-aminoxycarboxylic acids **4.7b-d** in yields of 76-78% (Table 4.1).



Scheme 4.1. Preparation of *N*-(Phth)-aminoxy acids **4.7b-d**

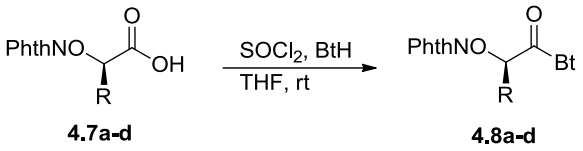
Table 4.1. Preparation of *N*-(Phth)-aminoxy acids **4.7**

Entry	R	Aminoxy acid, **4.7**	**4.7,** Yield %*	Mp (°C)
a	H	*N*-(Phth)-AOGly-OH, **4.7a**	43	163-165
b	*i*-Pr	*N*-(Phth)-AOVal-OH , **4.7b**	78	86-87
c	*i*-Bu	*N*-(Phth)-AOLeu-OH , **4.7c**	76	76-77
d	Bn	*N*-(Phth)-AOPhe-OH , **4.7d**	76	133-135

*Yields for last steps

4.3. Preparation of *N*-Phth(α-aminoxyacyl)benzotriazole 4.8a-d

N-Phth(α-aminoxyacyl)benzotriazoles **4.8a-d** were prepared by treatment of *N*-Phth(α-aminoxy) acids **4.7a-d** with 4 equivalents of 1*H*-benzotriazole and 1 equivalent of thionyl chloride in THF at room temperature in 75-87% yields (Scheme 4.2, Table 4.2), characterizing products by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.



4.7a-d **4.8a-d**

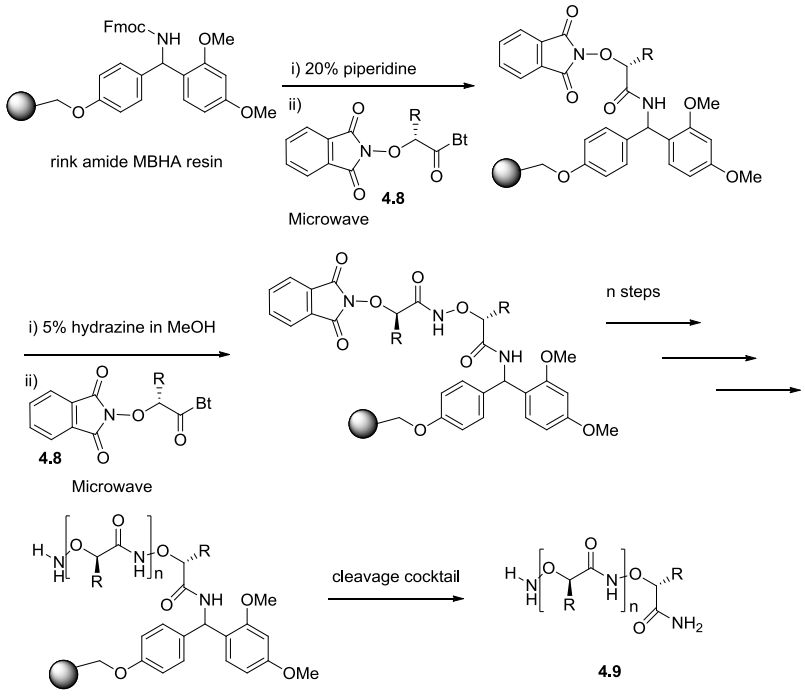
Scheme 4.2. Preparation of *N*-Phth(α-aminoxyacyl)benzotriazoles **4.8a-d**

Table 4.2. Preparation of *N*-Phth(α-aminoxyacyl)benzotriazoles **4.8a-d**

Entry	R	**4.8a-d**	Yield %	Mp (°C)
a	H	*N*-(Phth)-AOGly-Bt, **4.8a**	75	155-157
b	*i*-Pr	*N*-(Phth)-AOVal-Bt, **4.8b**	78	137-138
c	*i*-Bu	*N*-(Phth)-AOLeu-Bt, **4.8c**	76	128-129
d	Bn	*N*-(Phth)-AOPhe-Bt, **4.8d**	87	126-127

4.4 Attempted preparation of aminoxy peptides 4.9 on solid phase

Aminoxy peptides **4.9** were tried to prepare using microwave assisted solid-phase synthesis shown in Scheme 4.3. By using rink amide MBHA and Clear amide resin as solid support, *N*-Phth-(α-aminoxyacyl)benzotriazoles **10** were treated with resin under microwave conditions to give aminoxy peptides as shown in Scheme 4.3.



Scheme 4.3. Attempted preparation of aminoxy peptides **4.9** on solid phase

Table 4.3. Attempted preparation of aminoxy peptides **4.9**

Entry	Aminoxy peptides **4.9 (**Sequence N-C terminus)	**4.9,** Yield %
a	Phth-AOGly-AOPhe-AOGly-AOPhe-NH_2, **4.9a**	
b	Phth-AOVal-AOGly-AOLeu-AOPhe-NH_2, **4.9b**	-
c	Phth-AOGly-AOGly-AOGly-NH_2, **4.9c**	-

In this approach, Phth-AOGly-AOGly-AOGly-NH_2, **4.9c** was tried to prepare by using Clear amide resin (PS-PEG resin) or using rink amide MBHA (PS resin). LC-MS analysis confirmed the formation **4.9c** by using Clear amide resin, in addition of result, the formation of some possible side products has been observed. Nevertheless, those side products were not been able to characterize tetrapeptides Phth-AOGly-AOPhe-AOGly-AOPhe-NH_2, **4.9a** and Phth-AOVal-AOGly-AOLeu-AOPhe-NH_2, **4.9b** was attempted to prepare by using rink amide MBHA (PS) resin, unfortunately, none of the formation of those peptides were observed by LC-MS.

4.5 Attempted preparation of α-aminoxy hybrid peptides 4.10

α-Aminoxy hybrid peptides include at least one α-aminoxy acid residue. Aminoxy hybrid peptides **4.10** were tried to prepare by using a combination of *N*-Fmoc(α-aminoacyl)benzotriazoles **4.11**, and *N*-Phth(α-aminoxyacyl)benzotriazoles **4.8** similar to Scheme 4.3. To optimize the synthesis condition for α-aminoxy peptides, hybrid tetrapeptide Phth-AOGly-AOGly-Phe-Phe-NH_2 **4.10a** was tried to prepare. Nevertheless, none of the attempts showed the product formation.

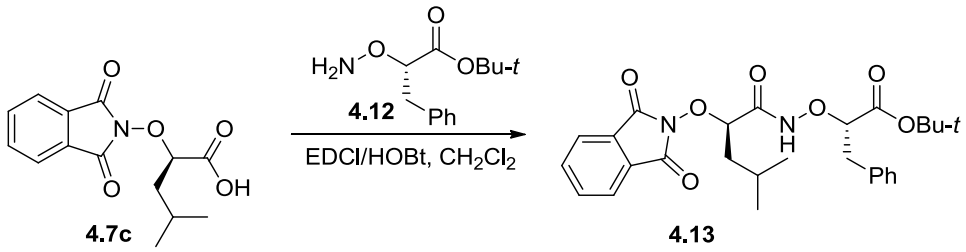
Table 4.4. The preparation of aminoxy hybrid peptides **4.10**

Entry	Aminoxy peptides **4.10** (Sequence N-C terminus)	**4.10**, Yield %
a	Phth-AOGly-AOGly-Phe-Phe-NH2, **4.10a**	-
b	Phth-Ala-AOGly-Ala-AOGly-NH2, **4.10b**	-

4.6 Result and Discussion

In the literature [101, 116] coupling reagents (such as BOP-HOBt-NEM, EDCl-HOBt, TBTU/HOBt/DIEA, DIC/HOBt) have been used for constructing the aminoxy peptide chain. Among of the many amine protecting groups, phthalimide has great attention due to its stability in acidic media and easily deprotection in basic media, especially with hydrazine, for solid phase peptide synthesis.

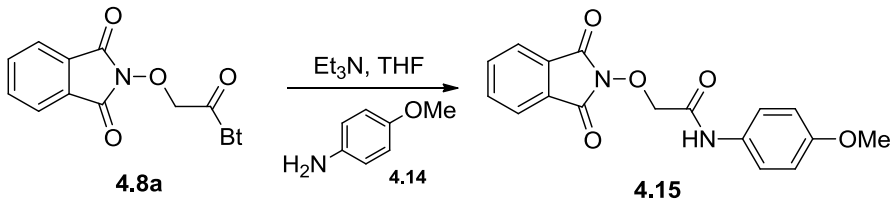
Dan Yang et al have prepared an *N*-Phth protected α-aminoxydipeptide **4.13** by using coupling reagent EDCl/HOBt in CH_2Cl_2 [116] (Scheme 4.4).



Scheme 4.4. Synthesis of *N*-Phth protected α-aminoxydipeptide **4.13**

Shin and coworkers also generated the first solid phase synthesis of oligomeric α-aminoxy peptides with phthalimide protection using BOP-HOBt-NEM coupling reagent in 40-65% HPLC yields [101].

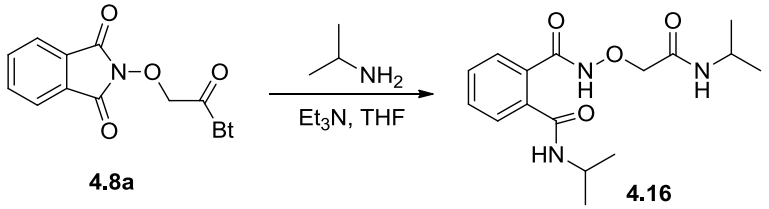
Moreover, in previous chapter, an *N*-Phth protected α-aminoxy acid amide **4.15** was prepared in 20% yield from *N*-Phth(α-aminoxyacyl)benzotriazole **4.8a** [83] (Scheme 4.5).



Scheme 4.5. Synthesis of *N*-Phth protected α-aminoxy acid amide

While *N*-Phth(α-aminoxyacyl)benzotriazoles **4.8** were treated with primary amines such as isopropylamine, deprotection products were also obtained. The

coupling product could not be observed (Scheme 4.6). Hence, peptide chain could not be lengthened in solid phase synthesis since the ring opening of phthalimide takes place with primary and secondary amines in basic media.



Scheme 4.6. Ring opening of phthalimide with primary and secondary amines

In conclusion, *N*-phthalimide protection is not stable against strong nuclephiles like primer amines in basic media, whereas it forms coupling products in low yields (20%) with aromatic amines like *p*-methoxyaniline **4.14**. In acidic media when coupling reagents (BOP-HOBt-NEM or EDCl-HOBt) were used, *N*-phthalimide protection is stable to form coupling products (Scheme 4.4 and Figure 4.1).

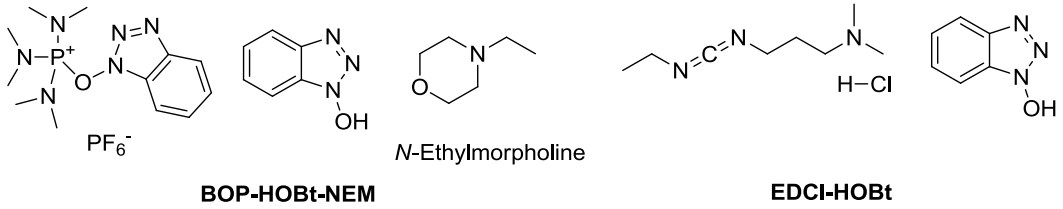
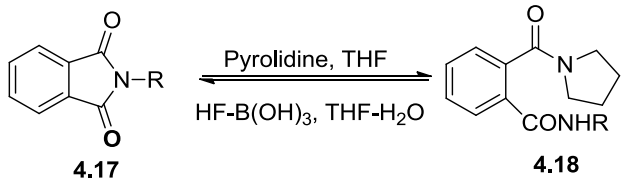


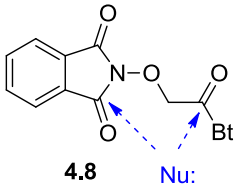
Figure 4.1. Structures of coupling reagents (BOP-HOBt-NEM and EDCl-HOBt)

The ring opening and closing of phthalimide was examined in aprotic solvents resulting that phthalimide ring is opened by the nucleophilic addition of pyrolidine in basic media whereas the reverse reaction recloses phthalimide ring under acidic condition (Scheme 4.7) [117].



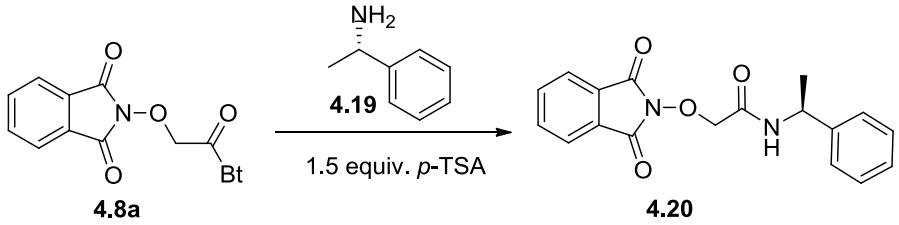
Scheme 4.7. The ring opening and closing of phthalimide in aprotic solvents

As outlined above, it is obvious that there is a competitive reaction between ring opening and *N*-acylation depending on reaction condition, especially pH (Scheme 4.8).



Scheme 4.8. Competitive reaction of nucleophile between ring opening and *N*-acylation

Therefore coupling reactions of *N*-Phth(α-aminoxyacyl)benzotriazole **4.8** were attempted in the presence of *p*-toluenesulphonic acid. The coupling product was obtained with 20% yield (without isolation, yield is based on NMR) when 1.5 equivalent of *p*-toluenesulphonic acid (*p*-TSA) was used (Scheme 4.9). Several attempts were done to improve the product yields by changing the amount of catalyst and temperature. Nevertheless, the yield of product formation could not be improved.



Scheme 4.9. Coupling reaction of *N*-Phth(α-aminoxyacyl)benzotriazole **4.8** with primary amines by acid catalyst

4.7. Conclusion

Treatment of *N*-Phth(α-aminoxyacyl)benzotriazoles **4.8** with primary, secondary and aromatic amines in basic solution media gave low yields of coupling products due to ring opening of phthalimide protection. Several attempts were done to improve product yield in solution phase, nevertheless, none of them was successful. Since the solid phase peptide synthesis requires more efficient coupling reactions, benzotriazole mediated solid phase synthesis of aminoxy peptides could not be attained with phthalimide protection under microwave condition.

4.8. Experimental Section

4.8.1. Method and Instrumentation

The Rink amide MBHA resin (0.37 meq/g), CLEAR-amide resin (0.52 meq/g) and ninhydrin test kit commercially were obtained from Peptides International, Louisville, KY, USA. A Discover BenchMate peptide synthesizer from CEM (Mathews, NC, USA) was used, while monitoring of temperature and irradiation power using an internal fiber optic probe. Compound **4.7a** was prepared from corresponding α-bromo carboxylic acid by substitution reaction as Chapter 3, Scheme 3.3. (*S*)-*t*-butyl 2-hydroxy-carboxylates **4.5b-d** was prepared according to literature procedure [80] (same procedure is described in Chapter 3 for mandelic acid derivatives) and they were used for the preparation of (*R*)-*t*-butyl 2-phthalimidoxy-carboxylates **4.6b-d** without characterization.

4.8.1.1. Solid Phase Peptide Synthesis (SPPS) Protocol

Standard stepwise solid phase synthesis was performed manually in a 25 mL Discover SPPS reaction vessel. Peptides were tried to prepare on Rink-amide MBHA resin or on CLEAR-amide resin.

The resin (0.1 mM) was swelled in DCM (5 mL) for 1 h. After treatment of resin with 20% piperidine-DMF (5 mL) for 20 min twice, the free-base amide resin was filtered off, washed with DMF (3 x 5 mL), MeOH (3 x 5 mL) and DCM (3 x 5 mL), dried under vacuum. A solution of the *N*-Phth-(α-aminoxyacyl)benzotriazole **4.8** (3 equiv) or *N*-Fmoc-(α-aminoacyl)benzotriazole (3 equiv) in DMF (3 mL) was added. The coupling reaction was carried out under microwave irradiation (75-80 ^{o}C, 50 Watt, 10-30 min) and the completion of the coupling reaction was evaluated by a negative ninhydrin test. Resin was washed with DMF (3 x 5 mL), MeOH (3 x 5 mL) and DCM (3 x 5 mL), dried under vacuum. Deprotection of the *N*-Fmoc-protection was achieved for each stage with 20% piperidine-DMF. Deprotection of the *N*-Phth-protection was achieved for each stage with 5% hydrazine in MeOH (2 x 10 mL). After deprotection, resin was washed with DMF (3 x 5 mL), MeOH (3 x 5 mL) and DCM (3 x 5 mL), dried under vacuum. Finally, the resulting peptidyl resin was cleaved with cleavage

cocktail (88% TFA, 5 % phenol, 5% water, 2% TIPS) for 30 min. Following cleavage, the peptide was precipitated in diethyl ether-hexanes at -70 ^{o}C, the mixture incubated for 24 h at 4 ^{o}C and decanted to afford the crude peptides **4.9** or **4.10**.

4.8.2. General synthesis of (*R*)-*t*-butyl 2-phthalimidoxy-carboxylates 4.6b-d

To a solution of (*S*)-*t*-butyl 2-hydroxy-carboxylate **4.5b-d** (10 mmol), *N*-hydroxyphthalimide (14 mmol), and triphenylphosphine (15 mmol) in anhydrous THF (40 mL) at 0 °C under nitrogen was added diethylazodicarboxylate (15 mmol) via a syringe. The mixture was stirred overnight, and the solvent was removed in vacuo. The residue was taken up with EtOAc. The organic layer was washed sequentially with 10% H_2O_2 twice, 5% $NaHSO_3$ and water (20 mL). Organic layer was dried over anhydrous $MgSO_4$ and evaporated off under vacuum. The resulting residue was purified by flash column chromatography (10% EtOAc in n-hexane) to provide compound (*R*)-*t*-butyl 2-phthalimidoxy-carboxylates **4.6b-d** as white solid.

(*R*)-tert-butyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-methylbutanoate (4.6b) White microcrystals (83%), mp 79–80 °C [lit. [80] mp 79–80 °C]; ^{1}H NMR ($CDCl_3$) δ 1.07 (d, J = 6.9 Hz, 3H), 1.21 (d, J = 6.9 Hz, 3H), 1.49 (s, 9H), 2.20–2.35 (m, 1H), 4.33 (d, J = 7.5 Hz, 1H), 7.72–7.78 (m, 2H), 7.80–7.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.3, 18.6, 28.1, 30.6, 82.5, 91.7, 123.7, 134.7.

(*R*)-tert-butyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-4-methylpentanoate (4.6c) White microcrystals (85%), mp 85–87 °C [lit. [80] mp 85–87 °C]; ^{1}H NMR ($CDCl_3$) δ 0.79 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.3 Hz, 3H), 1.26 (s, 9H), 1.40–1.55 (m, 1H), 1.70–1.85 (m, 2H), 4.53 (dd, J = 8.4, J = 5.4 Hz, 1H), 7.50–7.60 (m, 2H), 7.60–7.70 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 22.2, 23.1, 24.7, 28.1, 40.2, 82.6, 85.1, 123.7, 134.7, 163.4, 169.3.

(*R*)-tert-butyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate (4.6d) White microcrystals (85%), mp 70–72 °C [lit. [80] mp 68–70 °C]; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 3.27 (dd, J = 14.4, J = 7.5 Hz, 1H), 3.35 (dd, J = 14.1, J = 6.9 Hz, 1H), 4.90-4.96 (m, 1H), 7.20-7.38 (m, 5H), 7.72–7.76 (m, 2H), 7.78–7.84

(m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9, 37.4, 82.9, 86.5, 123.8, 127.2, 128.6, 129.1, 129.7, 134.7, 135.3, 163.3, 168.0.

4.8.3. General synthesis of (*R*)-2-phthalimidoxy-carboxylic acids 4.7b-d

A mixture of (*R*)-*t*-butyl 2-phthalimidoxy-carboxylates **4.6** (9 mmol) in CH_2Cl_2 (20 mL) and TFA (8 mL) was stirred at 0 °C for 1 h. Then solvent was removed under vacuum. The crude was recrystallized from CH_2Cl_2-hexanes to give (*R*)-2-phthalimidoxy-carboxylic acids **4.7**.

(*R*)-2-((1,3-dioxoisoindolin-2-yl)oxy)-3-methylbutanoic acid (**4.7b)** White microcrystals (78%), mp 86–87 °C [lit. [108] mp 85–87 °C]; ^{1}H NMR ($CDCl_3$) δ 1.10–1.30 (m, 6H), 2.38–2.60 (m, 1H), 4.58 (d, *J* = 5.1 Hz, 1H), 7.60–8.20 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 17.6, 18.7, 31.4, 91.0, 124.3, 128.8, 135.3, 164.1, 171.8.

(*R*)-2-((1,3-dioxoisoindolin-2-yl)oxy)-4-methylpentanoic acid (**4.7c)** White microcrystals (76%), mp 76–77 °C [lit. [108] mp 76–77 °C]; ^{1}H NMR ($CDCl_3$) δ 1.02 (d, *J* = 6.6 Hz, 3H),1.09 (d, *J* = 6.6 Hz, 3H), 1.70–1.85 (m, 1H), 1.90–2.05 (m, 1H), 2.05-2.20 (m, 1H), 4.87 (dd, *J* = 9.3, *J* = 4.2 Hz, 1H), 7.76-7.83 (m, 2H), 7.83–7.90 (m, 2H), 8.10 (br s, 1H).; ^{13}C NMR ($CDCl_3$) δ 21.9, 23.2, 24.8, 40.6, 84.9, 124.3, 128.9, 135.3, 164.1, 173.0.

(*R*)-2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoic acid (4.7d) White microcrystals (81%), mp 133–135 °C [lit. [108] mp 135 °C]; ^{1}H NMR ($CDCl_3$) δ 3.39 (dd, *J* = 15.6, *J* = 6.3 Hz, 1H), 3.53 (dd, *J* = 14.7, *J* = 5.1 Hz, 1H), 5.00 (dd, *J* = 6.3, *J* = 5.1 Hz, 1H), 7.20–7.40 (m, 5H), 7.80–7.98 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 37.4, 86.1, 124.1, 127.3, 128.6, 129.7, 134.8, 135.1, 163.7, 172.0.

4.8.4. General synthesis of (*R*)-2-phthalimidoxy-acyl-benzotriazole 4.8a-d

Thionyl chloride (1.10 mmol) was added to a solution of BtH (4.0 mmol) in anhydrous THF (3 mL) at 0 C, and the reaction mixture stirred for 20 min at same temperature. (*R*)-2-Phthalimidoxy-carboxylic acids **4.7** (1.0 mmol) dissolved in anhydrous THF (5 mL) was added dropwise to this mixture. After stirring for 2 h at 0 ^{o}C, the reaction mixture allowed to heat to room temperature. After stirred 1 h, a white precipitate formed during the reaction was filtered off, and the filtrate

was concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and solution washed with saturated Na_2CO_3 solution (3 x 10 mL) and then brine, and dried over anhydrous $MgSO_4$. Solvent was evaporated under reduced pressure to afford (*R*)-2-phthalimidoxy-acyl-benzotriazole **4.8a-d** as white microcrystals.

Phth-AOGly-Bt (4.8a) Characterization data was given in Chapter 3 for compound **3.6b**.

Phth-AOVal-Bt (4.8b) White microcrystals (78%), mp 137–138 °C; ^{1}H NMR ($CDCl_3$) δ 1.16 (d, J = 6.9 Hz, 3H), 1.39 (d, J = 6.9 Hz, 3H), 2.60–2.80 (m, 1H), 5.93 (d, J = 7.5, 1H), 7.55 (t, J = 7.2 Hz, 1H), 7.70–7.85 (m, 5H), 8.11 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 18.2, 18.8, 31.7, 90.1, 114.8, 120.4, 123.9, 126.9, 128.8, 131.0, 134.8, 146.2, 163.2, 168.7; Anal. Calcd. for $C_{19}H_{16}N_4O_4$·: C, 62.63; H, 4.43; N, 15.38. Found: C, 62.56; H, 4.28; N, 15.27.

Phth-AOLeu-Bt (4.8c) White microcrystals (76%), mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (d, J = 6.6 Hz, 3H), 1.20 (d, J = 6.6 Hz, 3H), 1.90–2.05 (m, 1H), 2.20–2.40 (m, 2H), 6.33 (dd, J = 8.7, J = 3.9 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.68–7.90 (m, 5H), 8.11 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 22.0, 23.3, 24.9, 40.6, 83.9, 114.8, 120.5, 123.4, 123.9, 126.8, 128.9, 131.0, 131.3, 134.8, 146.3, 163.3, 169.0; Anal. Calcd. for $C_{20}H_{18}N_4O_4$·: C, 63.48; H, 4.79; N, 14.81. Found: C, 63.74; H, 5.10; N, 14.76.

Phth-AOPhe-Bt (4.8d) White microcrystals (87%), mp 126–127 °C; ^{1}H NMR ($CDCl_3$) δ 3.65 (dd, J = 69, J = 3.3 Hz, 2H), 6.50 (t, J = 6.9 Hz, 1H), 7.16–7.30 (m, 3H), 7.36–7.42 (m, 2H), 7.52 (t, J = 6.9 Hz, 1H), 7.64–7.80 (m, 5H), 8.08 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 37.6, 85.1, 114.7, 120.5, 123.9, 126.9, 127.5, 128.8, 129.8, 131.1, 134.3, 134.9, 146.2, 163.2, 167.8; Anal. Calcd. for $C_{23}H_{16}N_4O_4$: C, 66.99; H, 3.91; N, 13.59. Found: C, 66.72; H, 3.96; N, 13.52.

5. SUMMARY OF ACHIEVEMENTS

1*H*-Benzotriazole and its substituted derivatives have essential utilities in many organic syntheses. Benzotriazole chemistry enables fast and efficient preparation of many of organic materials including drugs, synthetic intermediates and natural products. Recently *N*-acylbenzotriazoles have gained much attention on the racemization free preparation of peptide analogs in shorter time comparing coupling reagents.

In this present work, Chapter 1 provides a short review about benzotriazole methodology in synthetic organic chemistry. Some of recent applications of *N*-acyl benzotriazoles especially for peptide chemistry were briefly outlined in the same chapter. Importance and aspects of peptidomimetic compounds in biological systems was also enlightened in Chapter 1.

A new synthetic preparation of depsipeptides, depsides and chiral oligoesters by using benzotriazole methodology were presented in Chapter 2. *O*-PG(α-hydroxyacyl)benzotriazoles were elegantly prepared and their synthetic utility was proven by the preparation of both depsipeptides via N-acylation and chiral oligoesters via O-acylation without racemization. Unprotected depsidipeptides (depsides) were readily prepared from *N*-PG(α-aminoacyl)benzotriazoles and utilized for obtaining longer depsipeptide conjugates.

A new class of peptidomimetic compounds, α-aminoxy and α-aminoxy hybrid peptides, was effectively prepared in solution phase by using benzotriazole methodology in Chapter 3. *N*-(PG-α-aminoxyacyl)benzotriazoles derived from *N*-(PG-α-aminoxyacids) were reacted under mild conditions with amines, α-amino acids, α-dipeptides and α-aminoxy acids to give aminoxyacyl amides, α-aminoxy hybrid peptides and α-aminoxy peptides in high yields without causing racemization.

α-Aminoxy and α-aminoxy hybrid peptides were also attempted to prepare on solid phase in Chapter 4. Nevertheless, all trials for the preparation of α-aminoxy peptides did not give good results due to the less stability of phthalimide protecting group in basic condition. The ring opening-closing reaction of

phthalimide protection group was also elaborately examined in Chapter 4 and impropriety of phthalimide protection for benzotriazole mediated solid phase peptide synthesis was discussed in Chapter 4.

Herein, novel preparation of peptidomimetic compounds including α-aminoxy peptides and depsipeptides were advanced by using benzotriazole methodology. Many new synthetically useful intermediates were synthesized and utilized for the preparation of biologically active compounds. All experimental details for each compound and product characterization data for new compounds were completely given at the end of the each chapter.

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APPENDIXES

Appx-1. HPLC analysis of benzyl ((S)-4-methyl-1-oxo-1-(((S)-1-phenylethyl)amino)pentan-2-yl) carbonate **2.11b**

6/29/2010 14:14:09 1 / 1

==== Shimadzu LCsolution Analysis Report ====

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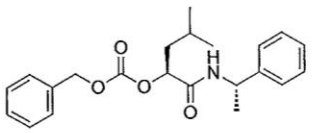
Acquired by	: Admin
Sample Name	: Z-OLeu-(L)-MBA
Sample ID	: Z-OLeu-(L)-MBA
Vail #	:
Injection Volume	: 20 uL
Data File Name	: l.pome.194.lcd
Method File Name	: test.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 6/29/2010 1:49:39 PM
Data Processed	: 6/29/2010 2:12:20 PM



HPLC data of **2.11b**

<Chromatogram>

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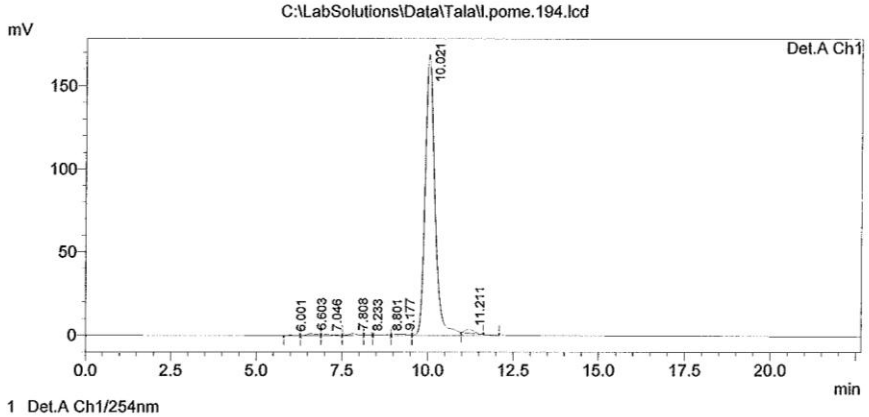

1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.001	2344	205	0.067	0.117
2	6.603	19488	992	0.557	0.566
3	7.046	12896	539	0.369	0.308
4	7.808	17693	1001	0.506	0.572
5	8.233	3615	263	0.103	0.150
6	8.801	9774	428	0.279	0.244
7	9.177	18831	743	0.538	0.424
8	10.021	3372255	168783	96.424	96.336
9	11.211	40406	2247	1.155	1.283
Total		3497302	175202	100.000	100.000

Coulmn: Whelk-O1 10/100 FI
Flow rate: 0.5 mL/min
Solvent: MeOH (100%)

Appx-2. HPLC analysis of benzyl ((2S)-4-methyl-1-oxo-1-((1-phenylethyl)amino)pentan-2-yl) carbonate **(2.11b+2.11b')**

6/29/2010 14:14:19 1 / 1

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Tala\l.pome.193.lcd

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Sample ID	: Z-OLeu-(DL)-MBA
Vail #	:
Injection Volume	: 20 uL
Data File Name	: l.pome.193.lcd
Method File Name	: test.lcm
Batch File Name	:
Report File Name	: Default.lcr
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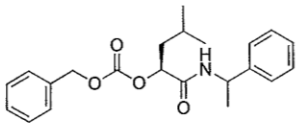
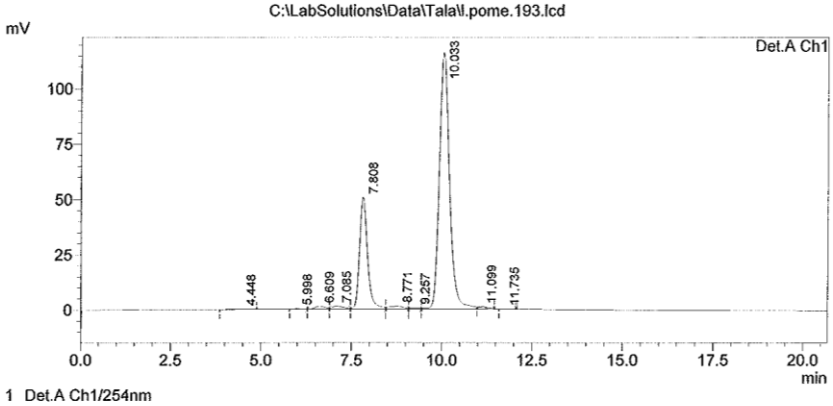


HPLC data of **(2.11b+2.11b')**

<Chromatogram>

C:\LabSolutions\Data\Tala\l.pome.193.lcd



1 Det.A Ch1/254nm

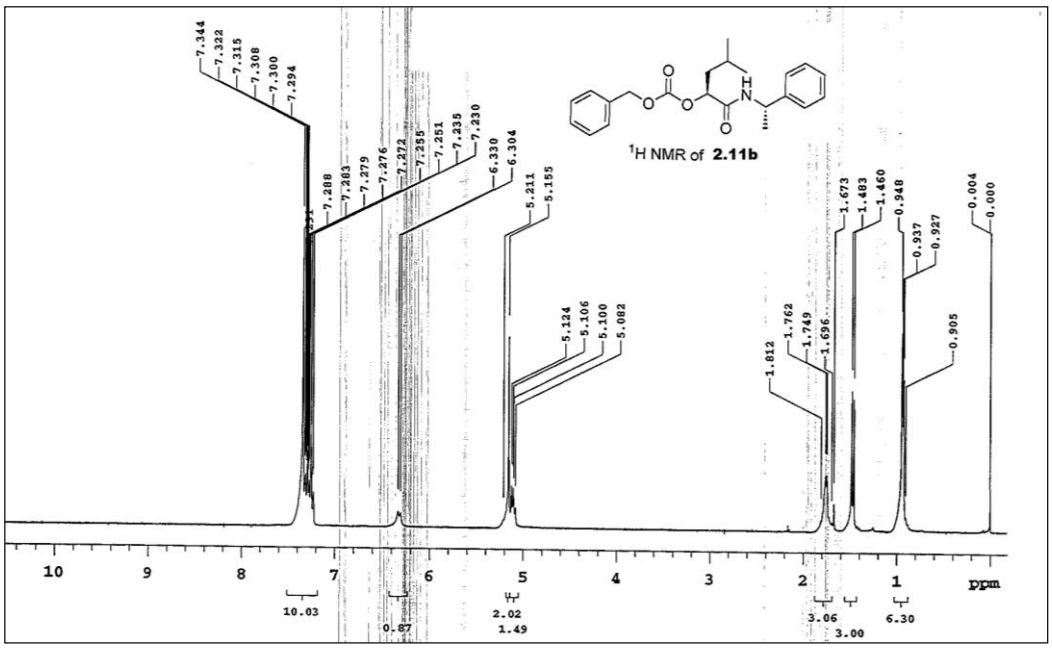
PeakTable

Detector A Ch1 254nm

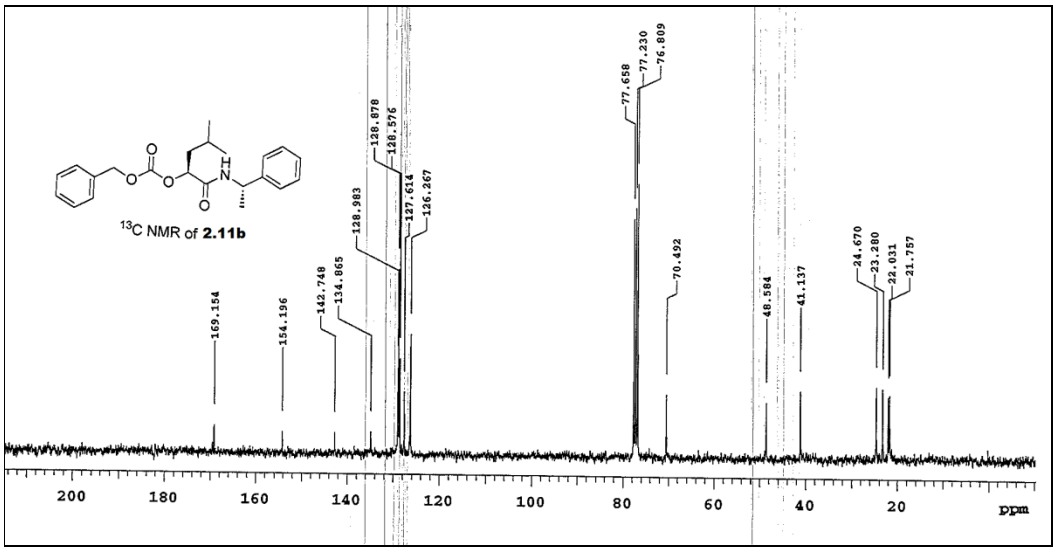
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.448	3951	141	0.123	0.082
2	5.998	2853	211	0.089	0.122
3	6.609	26089	1311	0.814	0.761
4	7.085	31556	1495	0.984	0.867
5	7.808	777896	50647	24.264	29.381
6	8.771	39767	1428	1.240	0.828
7	9.257	12185	641	0.380	0.372
8	10.033	2305678	116123	71.917	67.365
9	11.099	4725	287	0.147	0.166
10	11.735	1321	94	0.041	0.054
Total		3206020	172379	100.000	100.000

Coulmn: Whelk-O1 10/100 FEC
Flow rate: 0.5 mL/min
Solvent: MeOH (100%)

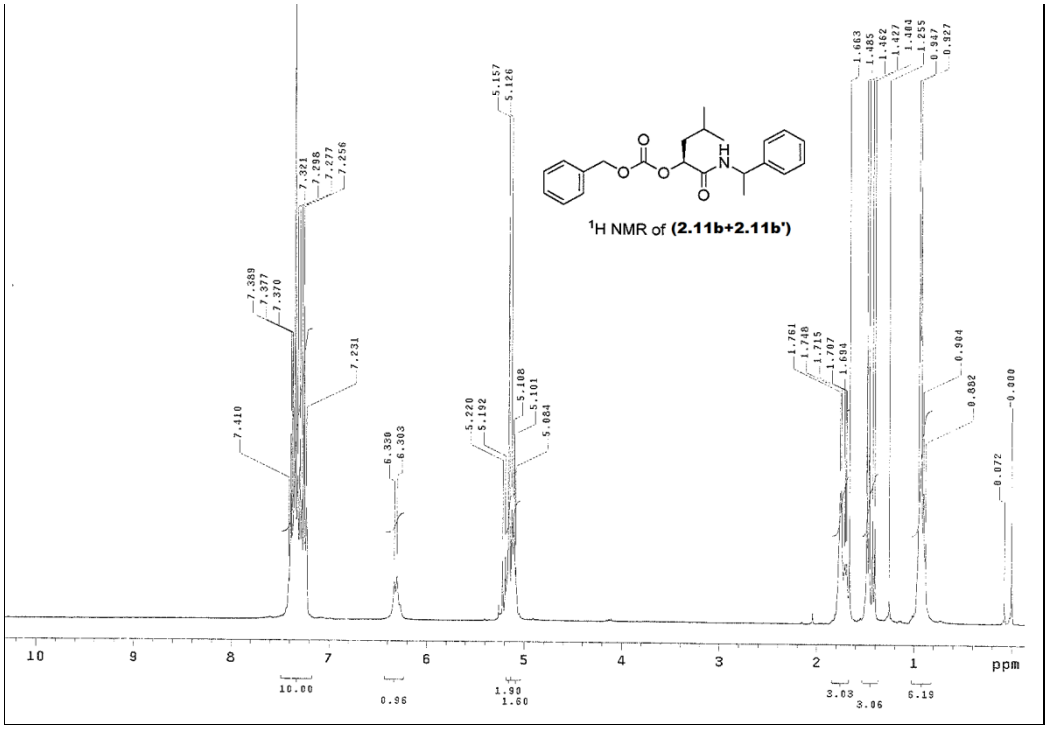
Appx-3. ^{1}H NMR spectra of benzyl ((S)-4-methyl-1-oxo-1-(((S)-1-phenylethyl)amino)pentan-2-yl) carbonate **2.11b**



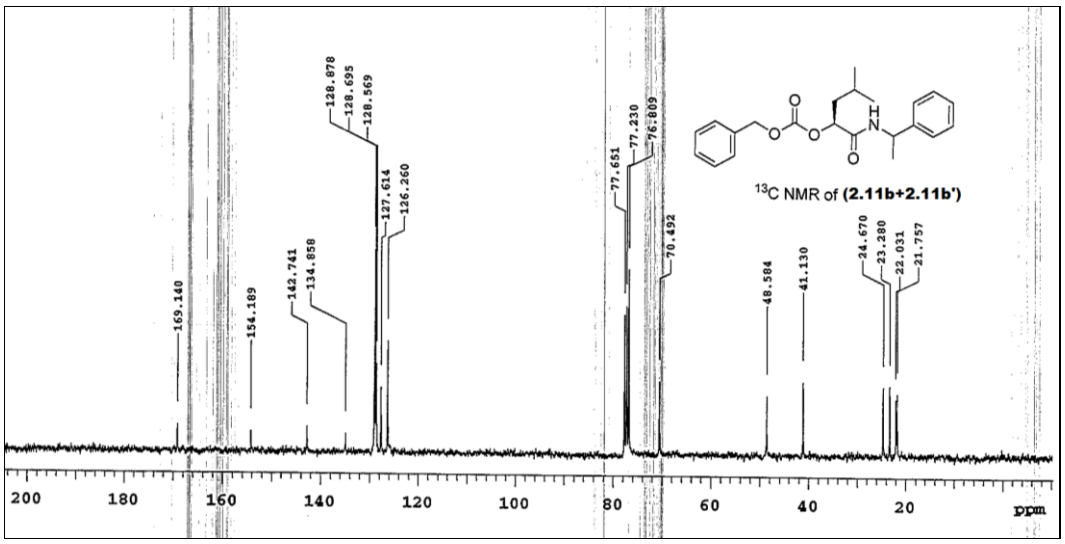
Appx-4. ^{13}C NMR spectra of benzyl ((S)-4-methyl-1-oxo-1-(((S)-1-phenylethyl)amino)pentan-2-yl) carbonate **2.11b**



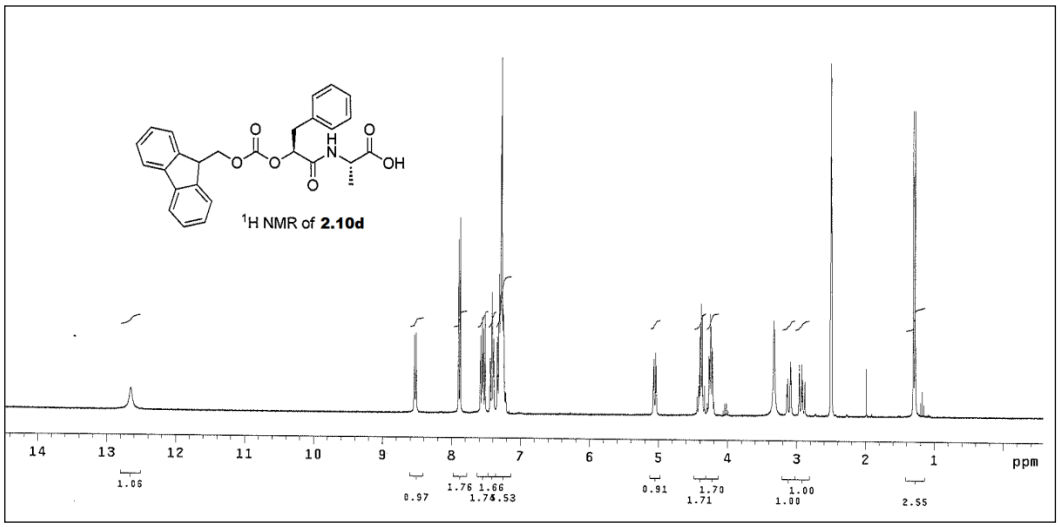
Appx-5. ^{1}H NMR spectra of benzyl ((2S)-4-methyl-1-oxo-1-((1-phenylethyl)amino)pentan-2-yl) carbonate **(2.11b+2.11b')**



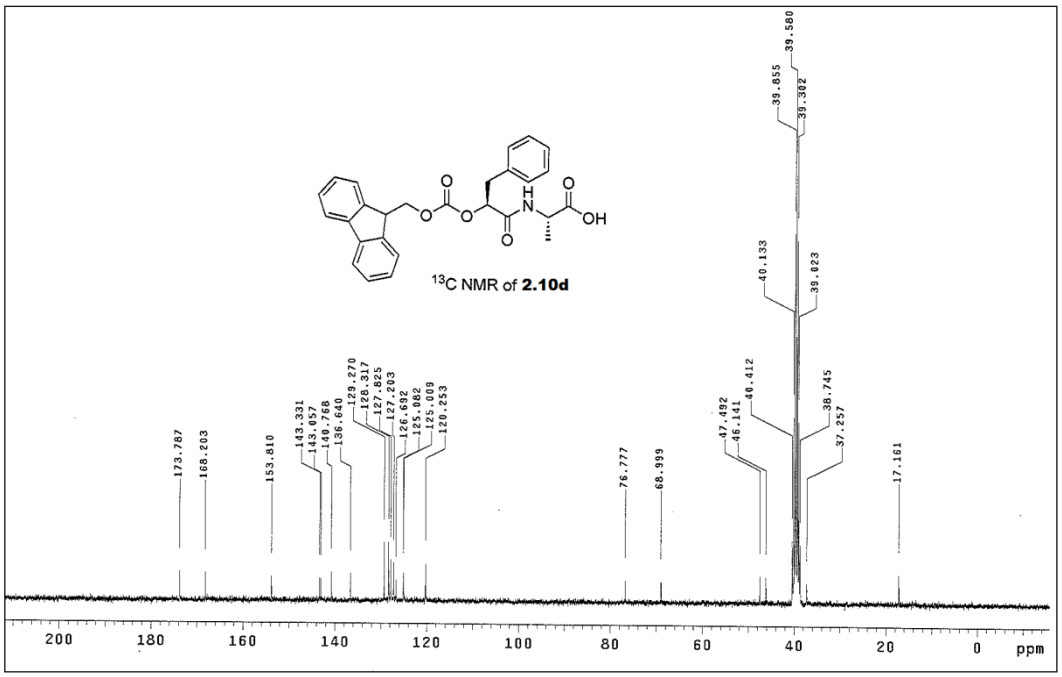
Appx-6. ^{13}C NMR spectra of benzyl ((2S)-4-methyl-1-oxo-1-((1-phenylethyl)amino)pentan-2-yl) carbonate **(2.11b+2.11b')**



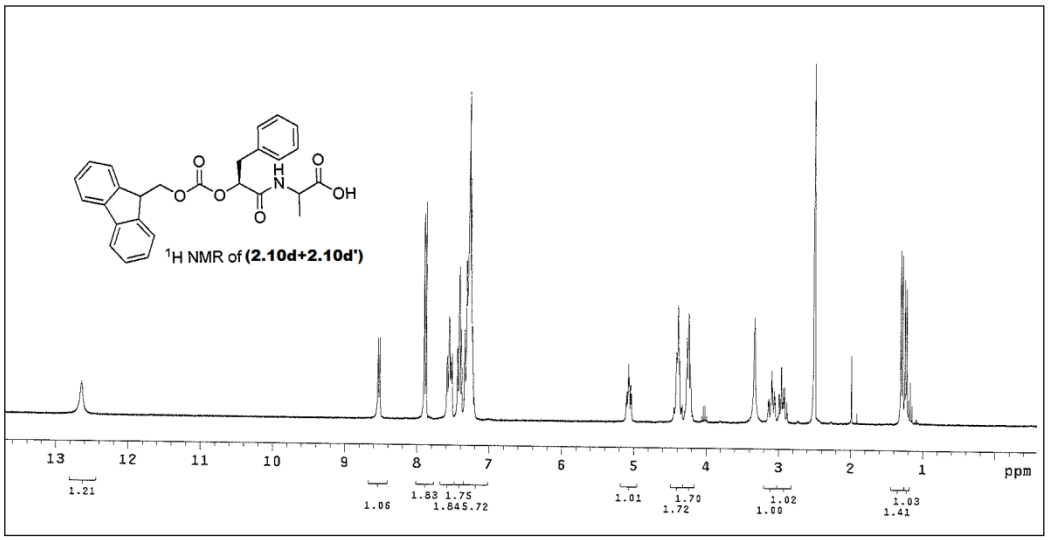
Appx-7. ^{1}H NMR spectra of Fmoc-L-(*O*Phe)-L-Ala-OH **2.10d**



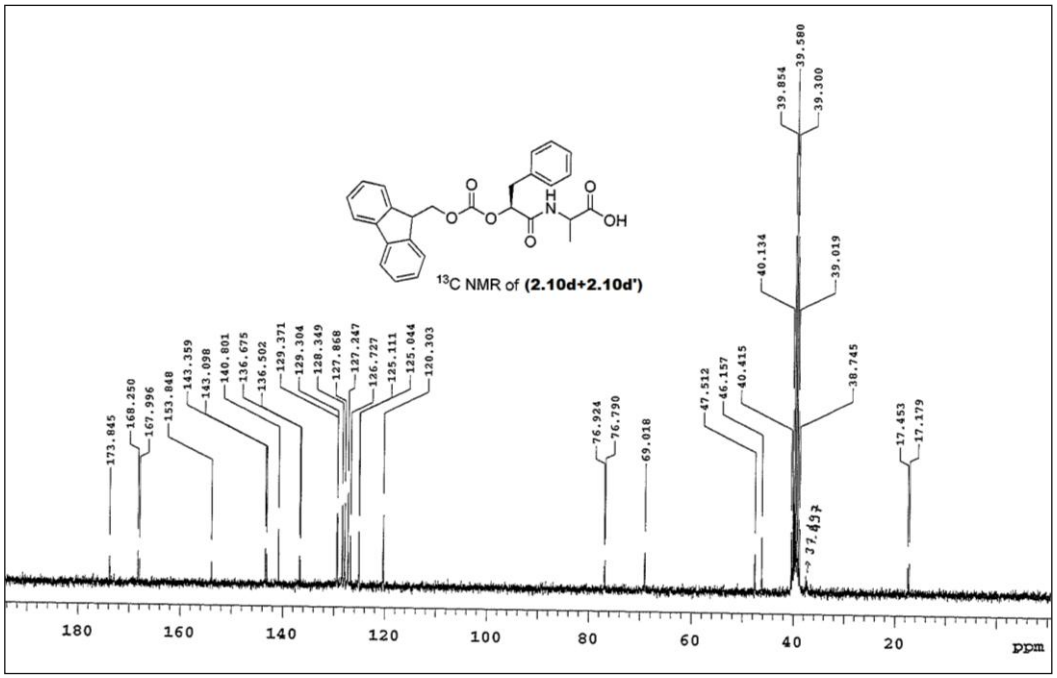
Appx-8. ^{13}C NMR spectra of Fmoc-L-(*O*Phe)-L-Ala-OH **2.10d**



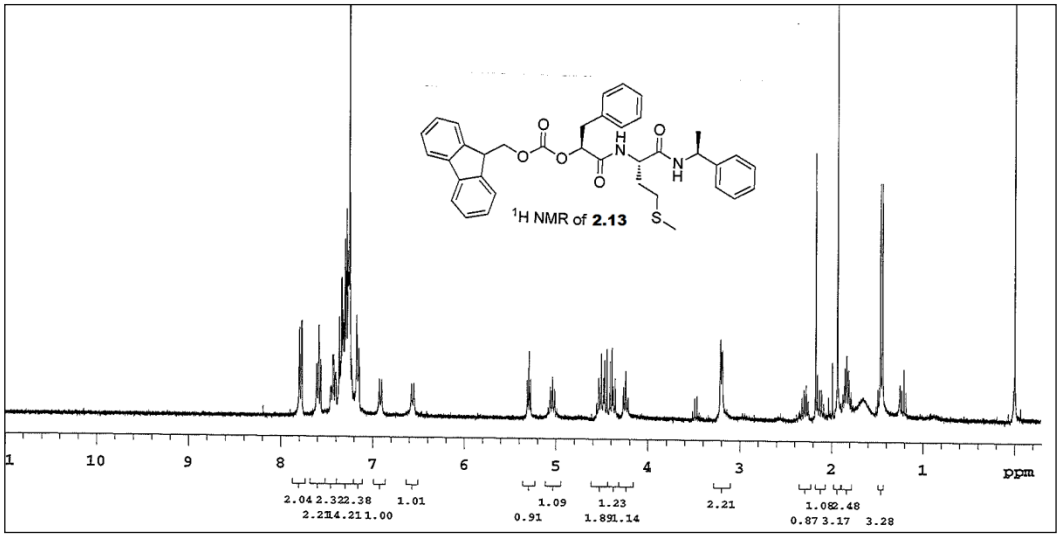
Appx-9. ^{1}H NMR spectra of Fmoc-L-(*O*Phe)-DL-Ala-OH **(2.10d+2.10d')**



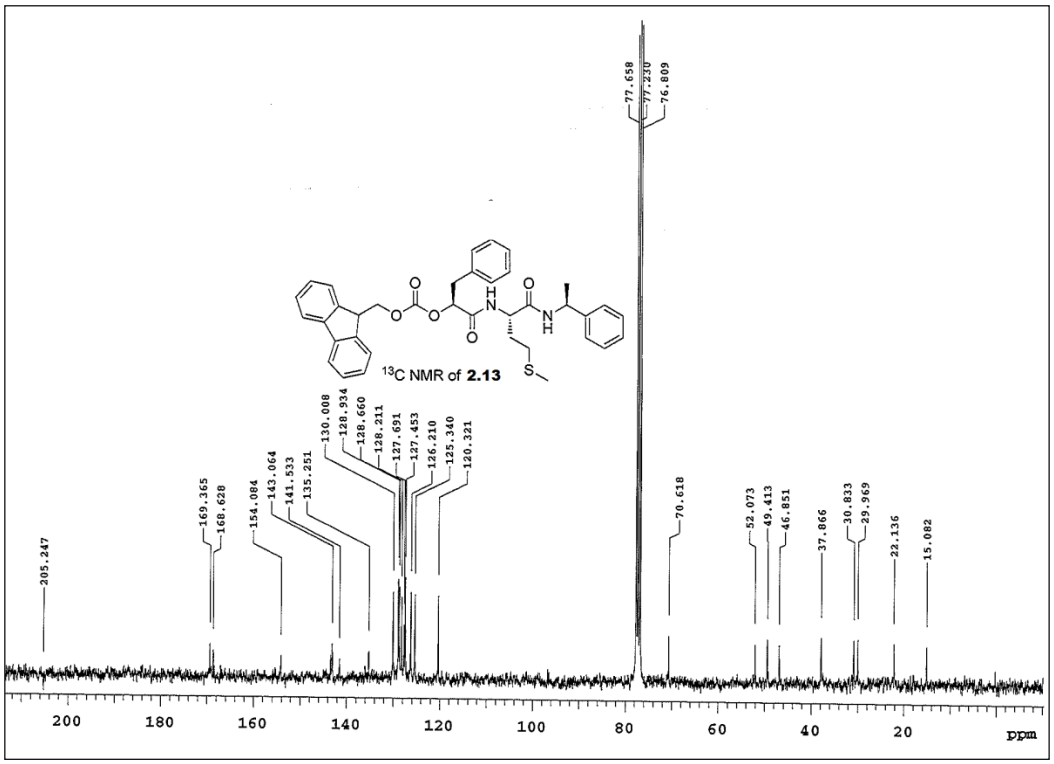
Appx-10. ^{13}C NMR spectra of Fmoc-L-(*O*Phe)-DL-Ala-OH (**2.10d+2.10d'**)



Appx-11. ^{1}H NMR spectra of (9H-Fluoren-9-yl)methyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-(((S)-1-phenylethyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl) carbonate **2.13**



Appx-12. ^{13}C NMR spectra of (9H-Fluoren-9-yl)methyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-(((S)-1-phenylethyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl) carbonate **2.13**



Appx-13. HPLC analysis of Z-L-Leu-L-(*O*Phe)-OH **2.7a**

6/29/2010 13:11:11 1 / 1

==== Shimadzu LCsolution Analysis Report ====

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Vail #	:
Injection Volume	: 20 uL
Data File Name	: E134.lcd
Method File Name	: test.lcm
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Report File Name	: Default.lcr
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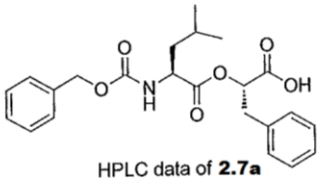

HPLC data of **2.7a**

<Chromatogram>

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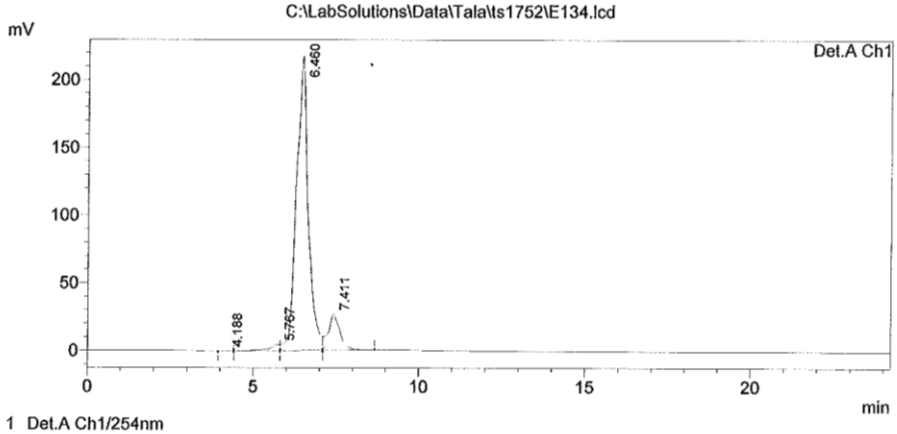

1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.188	1460	80	0.024	0.032
2	5.767	88443	4477	1.438	1.802
3	6.460	5383976	217120	87.561	87.365
4	7.411	674958	26844	10.977	10.801
Total		6148837	248522	100.000	100.000

Column: Chirobiotic T
Flow rate: 0.5 mL/min
Solvent: MeOH (100%)

Appx-14. HPLC analysis of Z-L-Leu-DL-(*O*Phe)-OH **(2.7a+2.7a')**

6/29/2010 13:11:19 1 / 1

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Tala\ts1752\E135.lcd

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Vail #	:
Injection Volume	: 20 uL
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Method File Name	: test.lcm
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Report File Name	: Default.lcr
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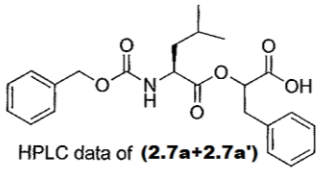

HPLC data of **(2.7a+2.7a')**

<Chromatogram>

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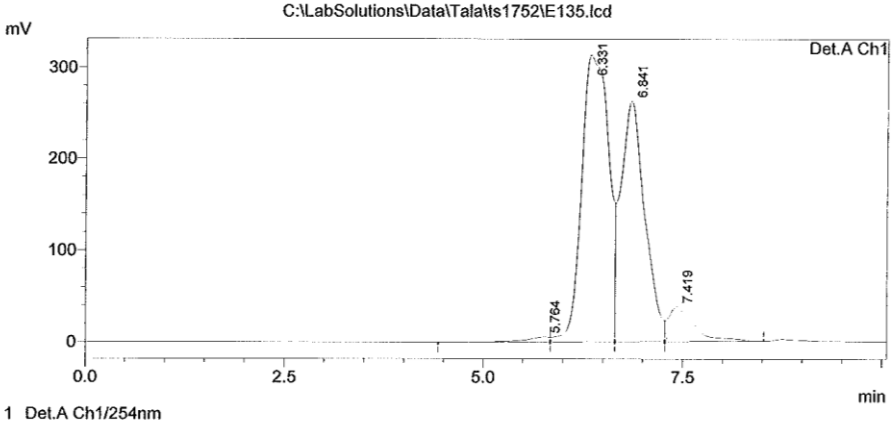


1 Det.A Ch1/254nm

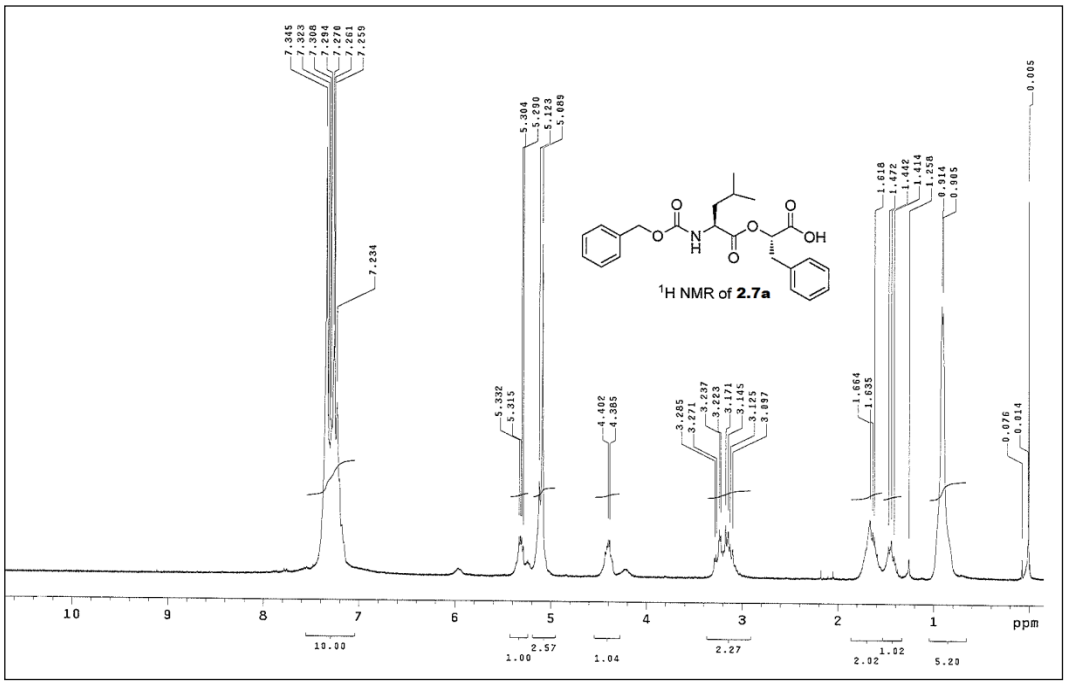
PeakTable

Detector A Ch1 254nm

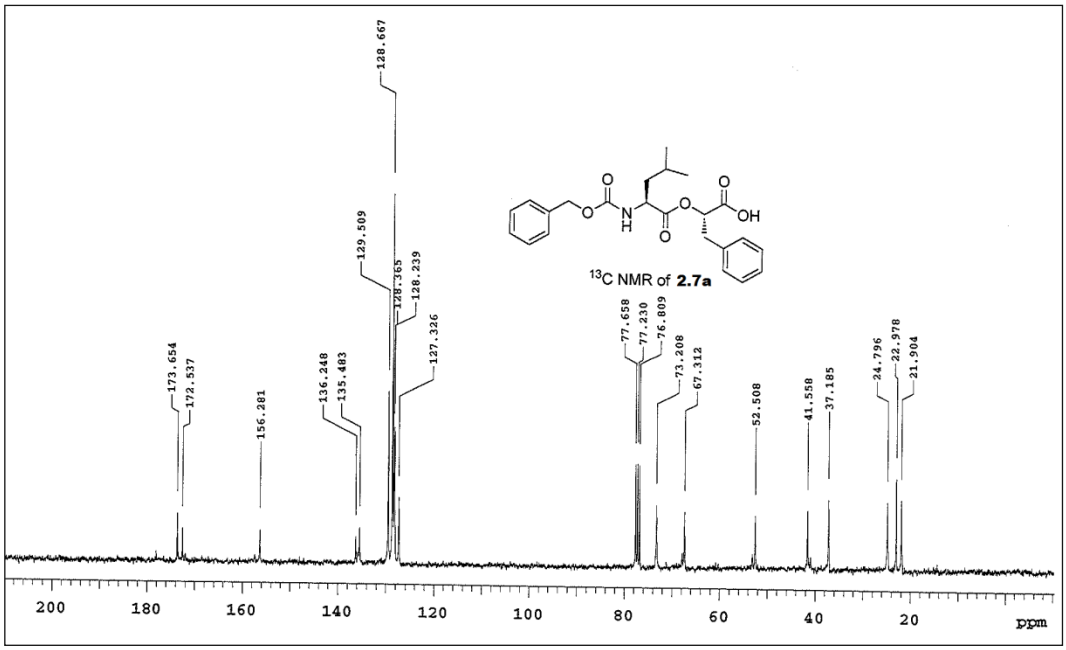
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.764	105972	5328	0.768	0.861
2	6.331	7353276	313109	53.297	50.578
3	6.841	5477170	262290	39.699	42.369
4	7.419	860355	38332	6.236	6.192
Total		13796774	619060	100.000	100.000

Column: Chirobiotic T
Flow rate: 0.5 mL/min
Solvent: MeOH (100%)

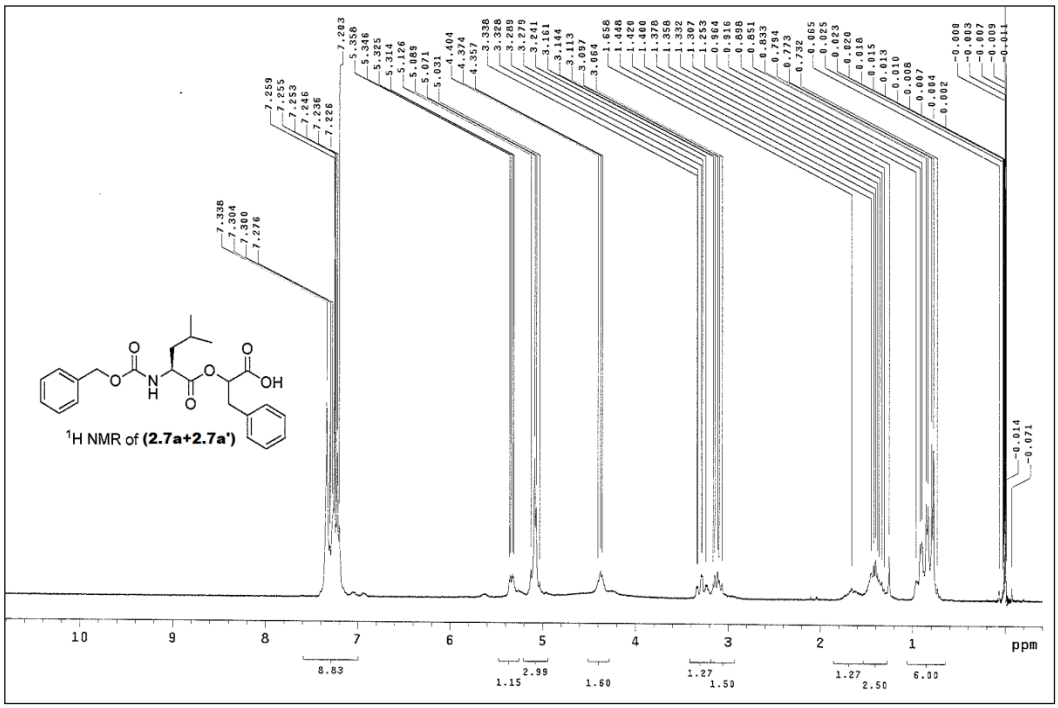
Appx-15. ^{1}H NMR spectra of Z-L-Leu-L-(*O*Phe)-OH **2.7a**



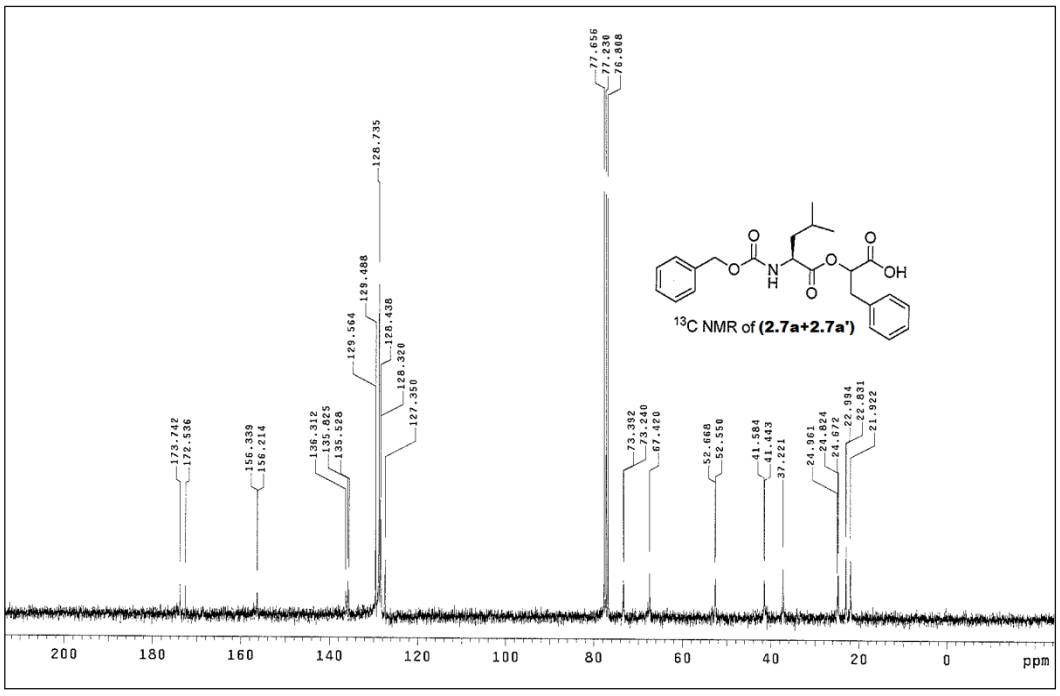
Appx-16. ^{13}C NMR spectra of Z-L-Leu-L-(*O*Phe)-OH **2.7a**



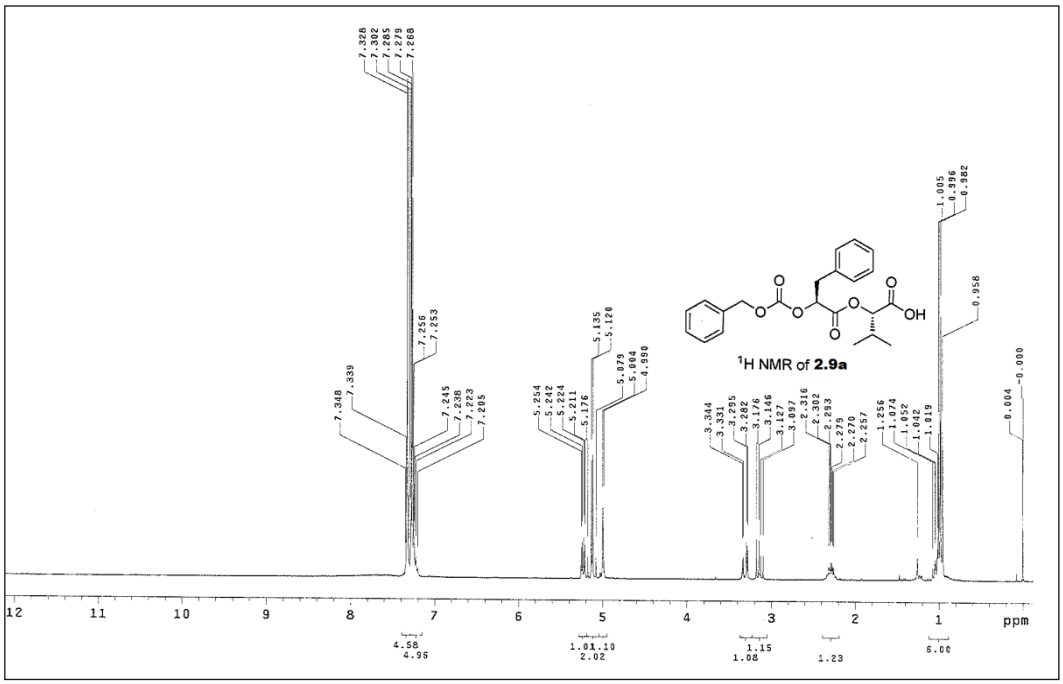
Appx-17. ^{1}H NMR spectra of Z-L-Leu-DL-(*O*Phe)-OH (**2.7a+2.7a'**)



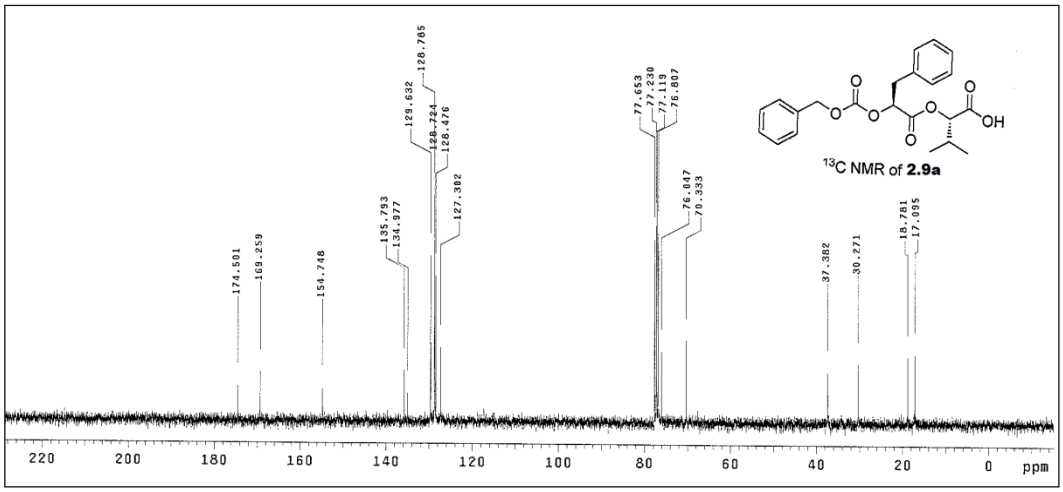
Appx-18. ^{13}C NMR spectra of Z-L-Leu-DL-(*O*Phe)-OH (**2.7a+2.7a'**)



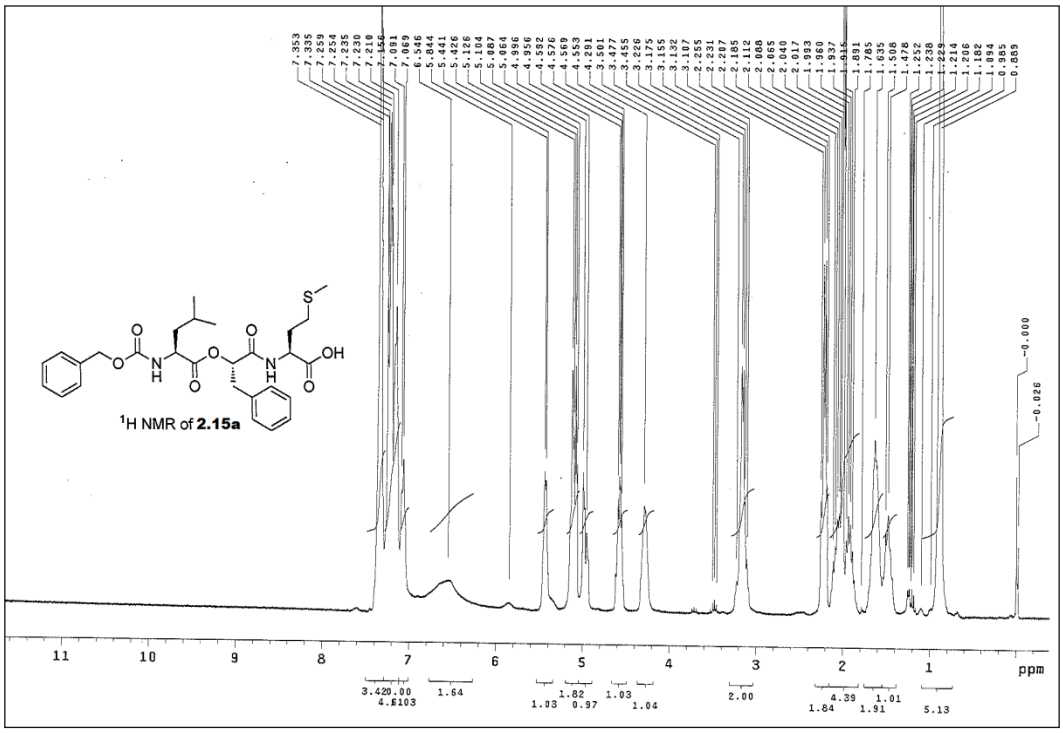
Appx-19. ^{1}H NMR spectra of Z-L-(*O*Phe)-L-(*O*Val)-OH **2.9a**



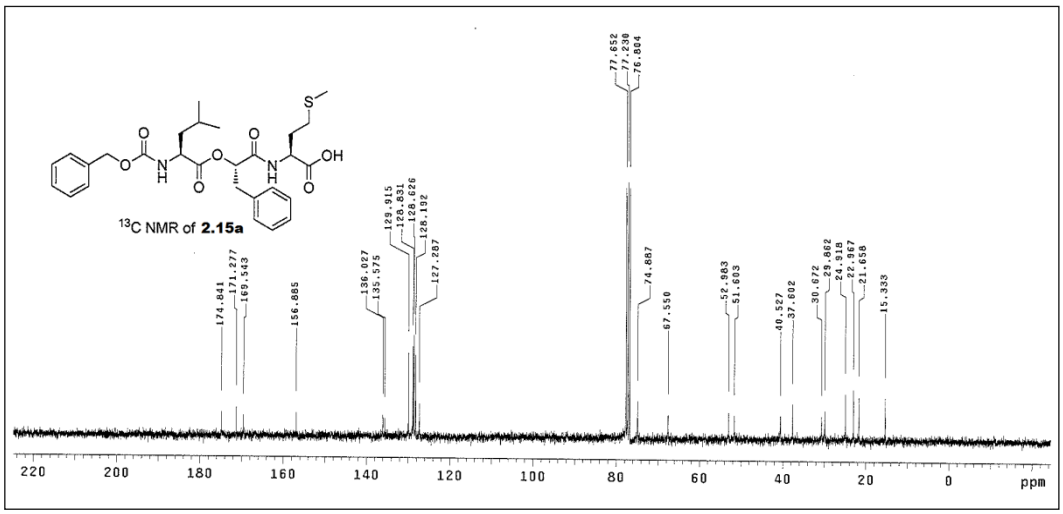
Appx-20. ^{13}C NMR spectra of Z-L-(*O*Phe)-L-(*O*Val)-OH **2.9a**



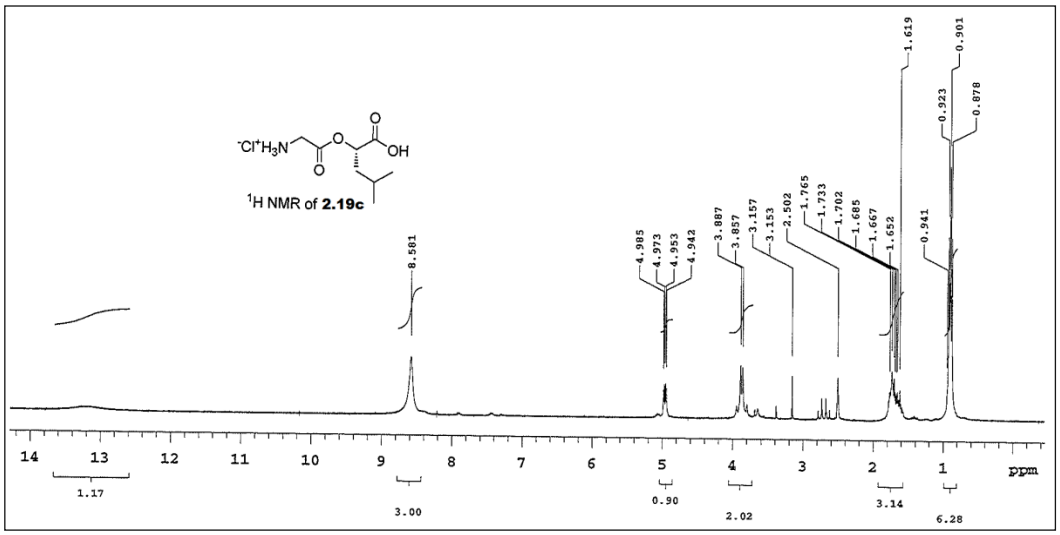
Appx-21. ^{1}H NMR spectra of Z-L-Leu-L-(*O*Phe)-L-Met-OH **2.15a**



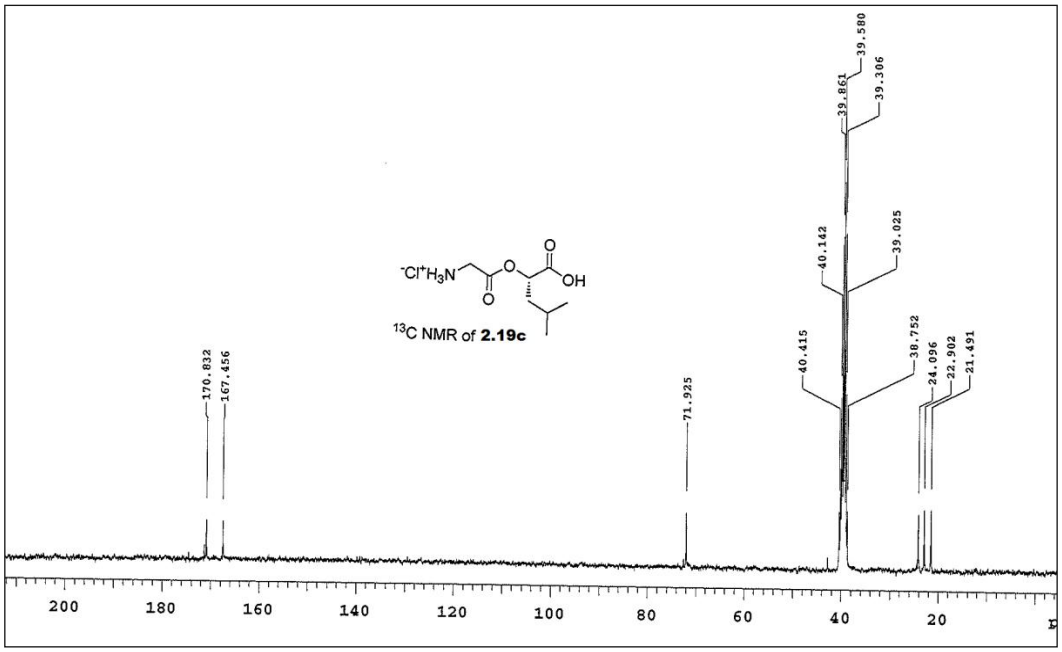
Appx-22. ^{13}C NMR spectra of Z-L-Leu-L-(*O*Phe)-L-Met-OH **2.15a**



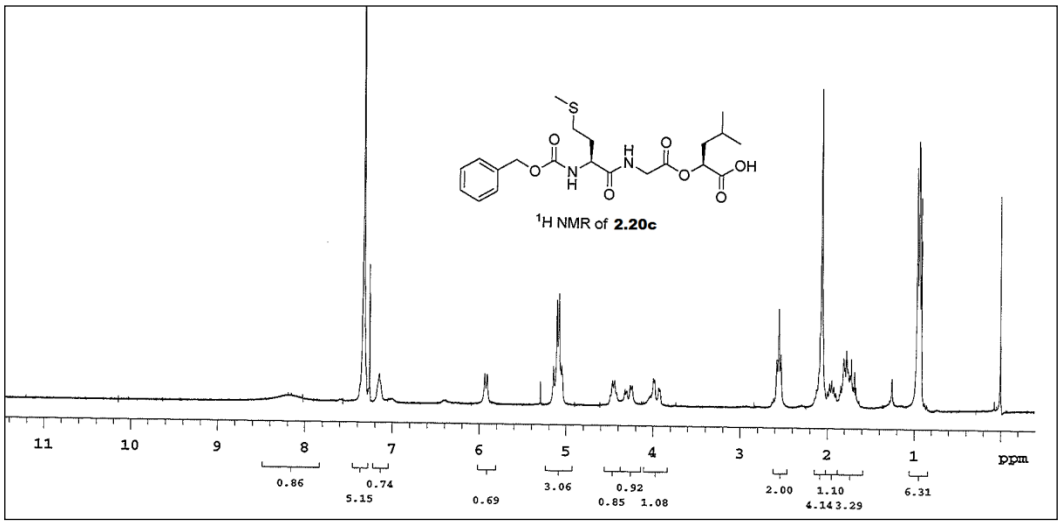
Appx-23. ^{1}H NMR spectra of Gly-L-(*O*Leu)-OH **2.19c**



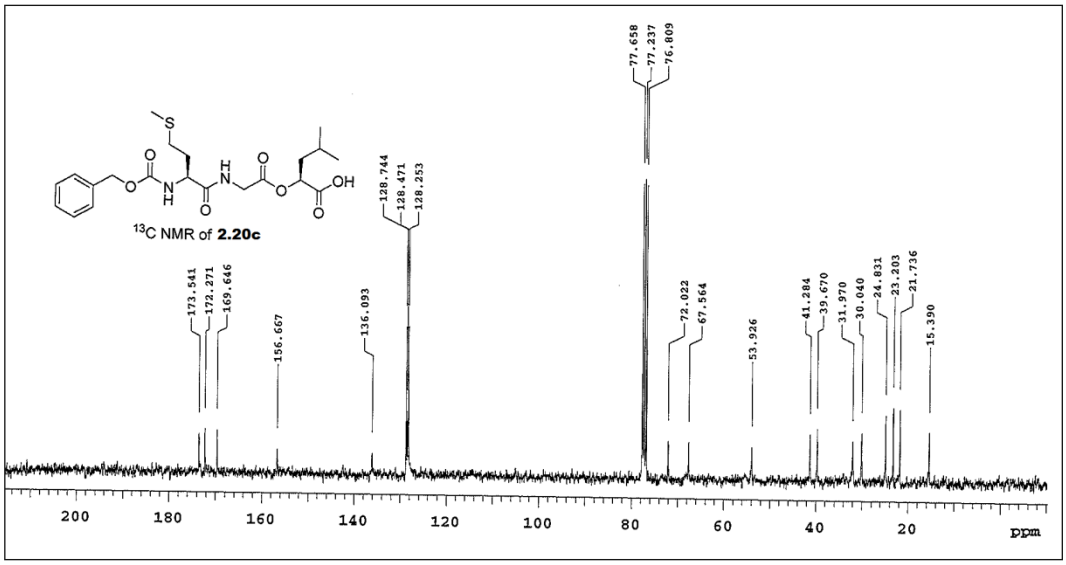
Appx-24. ^{13}C NMR spectra of Gly-L-(*O*Leu)-OH **2.19c**



Appx-25. ^{1}H NMR spectra of Z-L-Met-Gly-L-(*O*Leu)-OH **2.20c**



Appx-26. ^{13}C NMR spectra of Z-L-Met-Gly-L-(*O*Leu)-OH **2.20c**



Appx-27. HPLC analysis of benzyl (*S*)-3-methyl-1-oxo-1-((*R*)-1-phenylethylamino)butan-2-yloxycarbamate **3.8f**

9/14/2009 14:54:28 1 / 1

==== Shimadzu LCsolution Analysis Report ====

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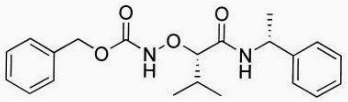

HPLC data of **3.8f**

<Chromatogram>

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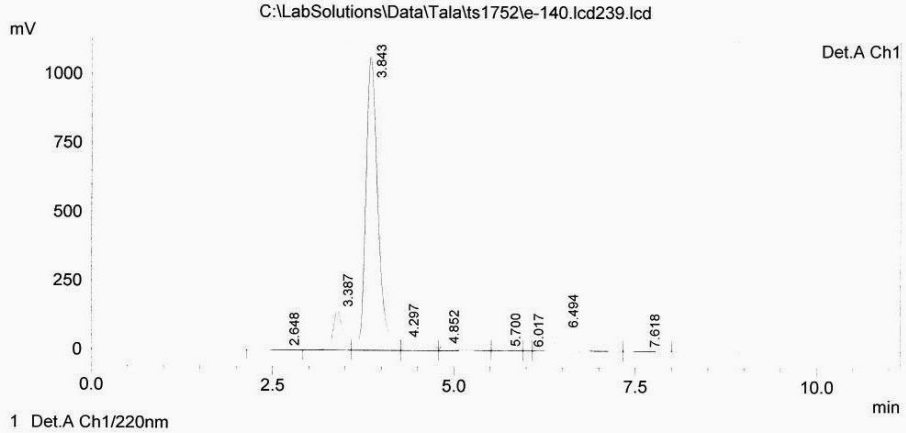

1 Det.A Ch1/220nm

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.648	39782	314	0.250	0.024
2	3.387	1352457	143933	8.515	10.842
3	3.843	12468763	1063830	78.505	80.136
4	4.297	681599	33961	4.291	2.558
5	4.852	191639	11670	1.207	0.879
6	5.700	26773	1474	0.169	0.111
7	6.017	4092	518	0.026	0.039
8	6.494	1104614	71149	6.955	5.360
9	7.618	13083	683	0.082	0.051
Total		15882802	1327532	100.000	100.000

Column: (S,S) WHELK-O1 10/100 FEC
Solvent: MeOH (100%)
Flow rate: 1.0 mL/min

Appx-28. HPLC analysis of benzyl (2S)-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-yloxycarbamate (**3.8f+3.8f'**)

9/14/2009 14:38:41 1 / 1

==== Shimadzu LCsolution Analysis Report ====

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Sample ID	: 3g1-2
Vail #	:
Injection Volume	: 20 uL
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Method File Name	: test.lcm
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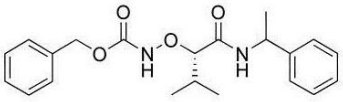
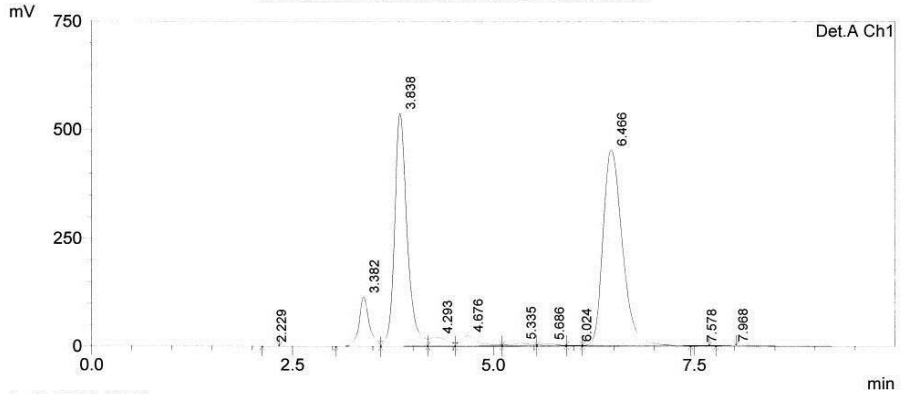


HPLC data of **(3.8f+3.8f')**

<Chromatogram>

C:\LabSolutions\Data\Tala\ts1752\e-140.lcd237.lcd



1 Det.A Ch1/220nm

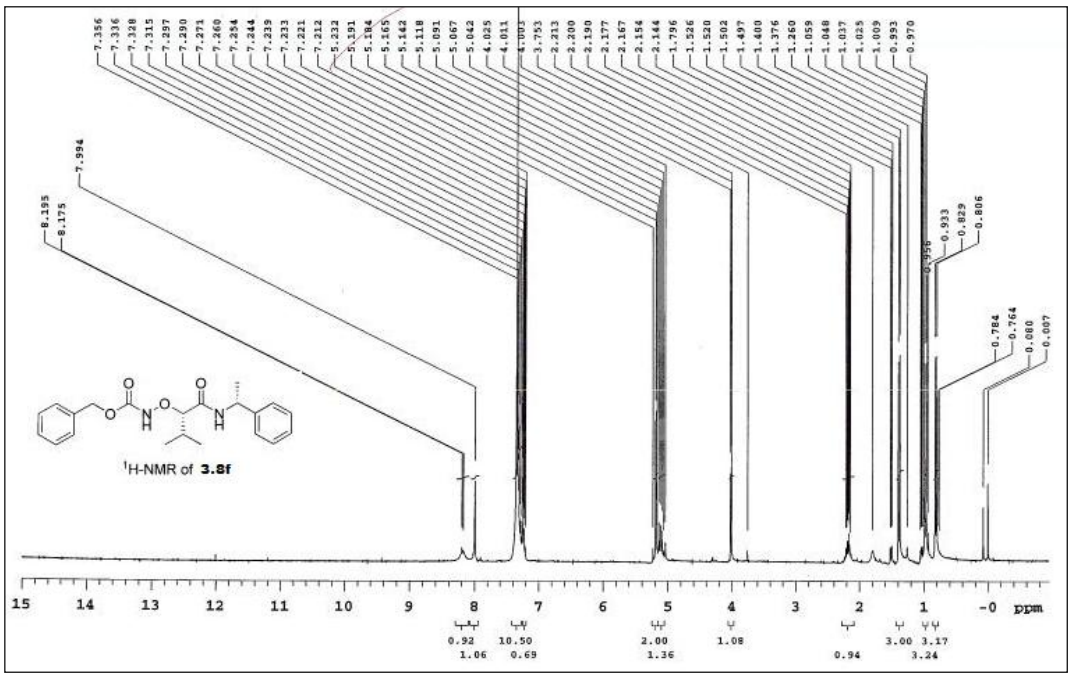
PeakTable

Detector A Ch1 220nm

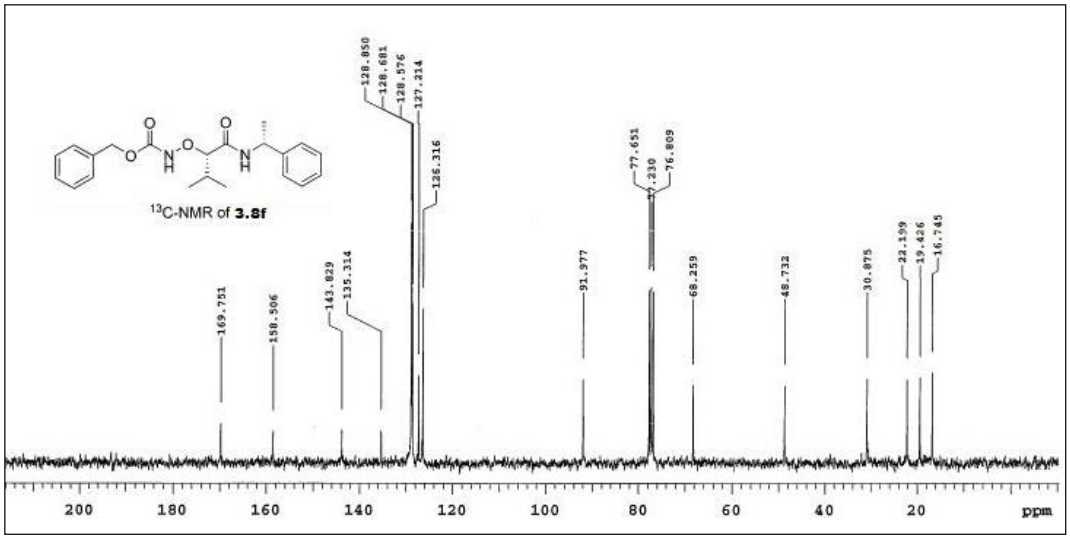
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.229	1842	294	0.012	0.025
2	3.382	1019434	114525	6.697	9.861
3	3.838	5844787	537250	38.398	46.257
4	4.293	312272	20452	2.052	1.761
5	4.676	337733	23657	2.219	2.037
6	5.335	101351	6245	0.666	0.538
7	5.686	65399	4753	0.430	0.409
8	6.024	11922	1146	0.078	0.099
9	6.466	7522871	452738	49.422	38.981
10	7.578	768	100	0.005	0.009
11	7.968	3193	278	0.021	0.024
Total		15221570	1161438	100.000	100.000

Column: (S,S) WHELK-O1 10/100 FEC
Solvent: MeOH (100%)
Flow rate: 1.0 mL/min

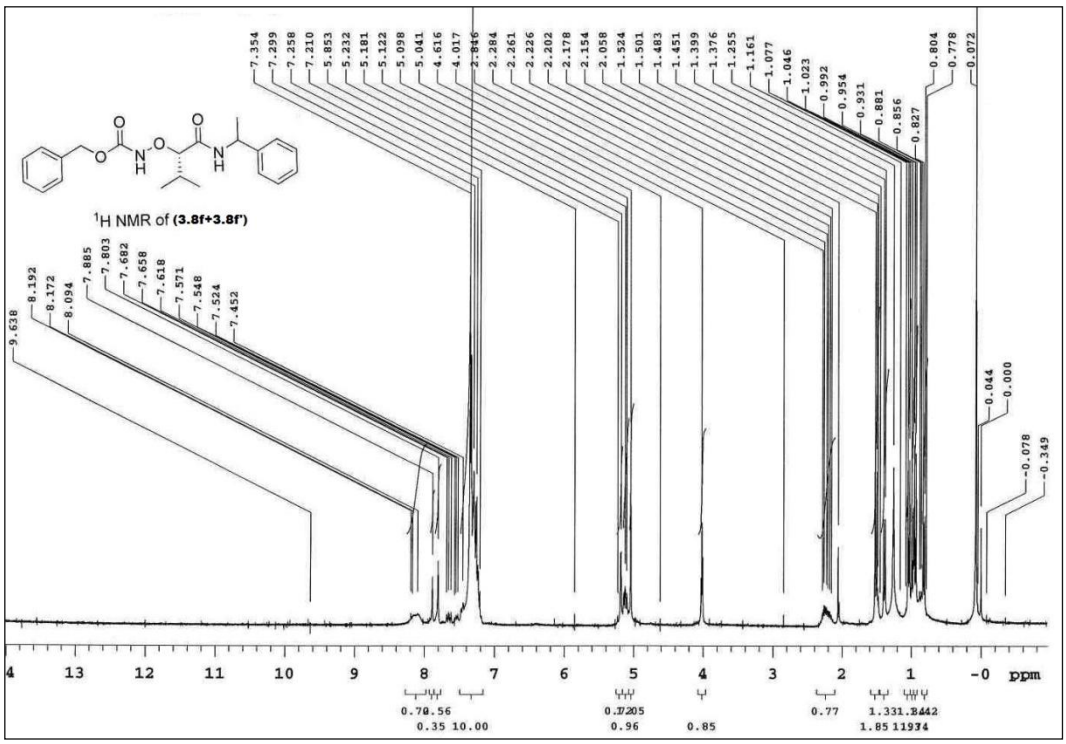
Appx-29. ^{1}H NMR spectra of benzyl (*S*)-3-methyl-1-oxo-1-((*R*)-1-phenylethylamino)butan-2-yloxycarbamate **3.8f**



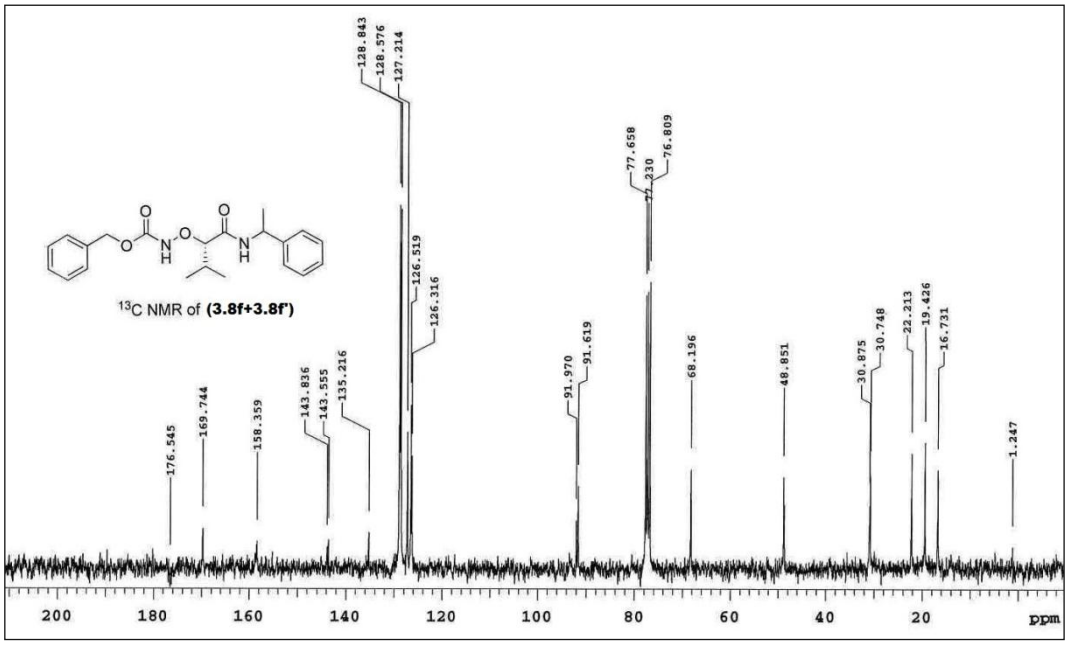
Appx-30. ^{13}C NMR spectra of benzyl (*S*)-3-methyl-1-oxo-1-((*R*)-1-phenylethylamino)butan-2-yloxycarbamate **3.8f**



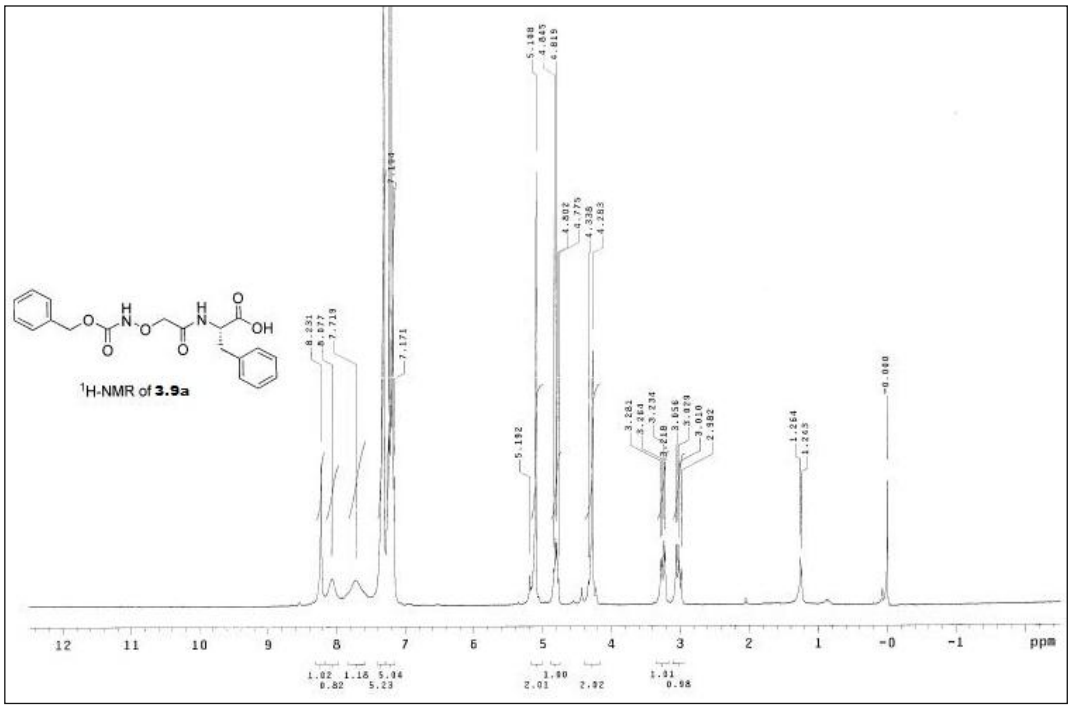
Appx-31. ^{1}H NMR spectra of benzyl (2S)-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-yloxycarbamate **(3.8f+3.8f')**



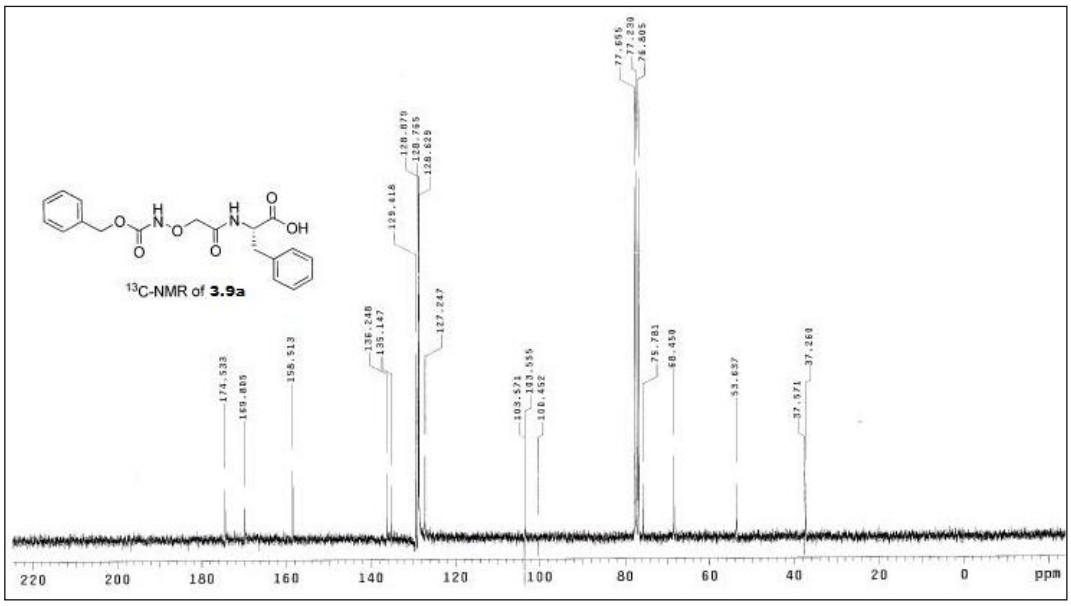
Appx-32. ^{13}C NMR spectra of benzyl (2S)-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-yloxycarbamate **(3.8f+3.8f')**



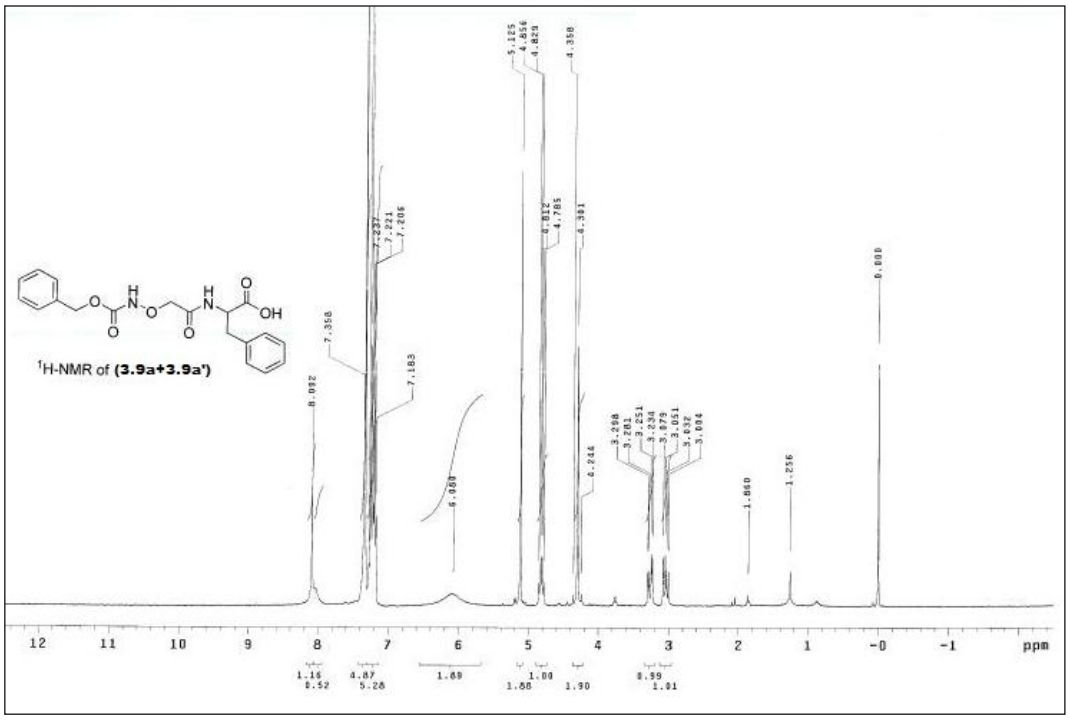
Appx-33. ^{1}H NMR spectra of Z-AOGly-L-Phe-OH **3.9a**



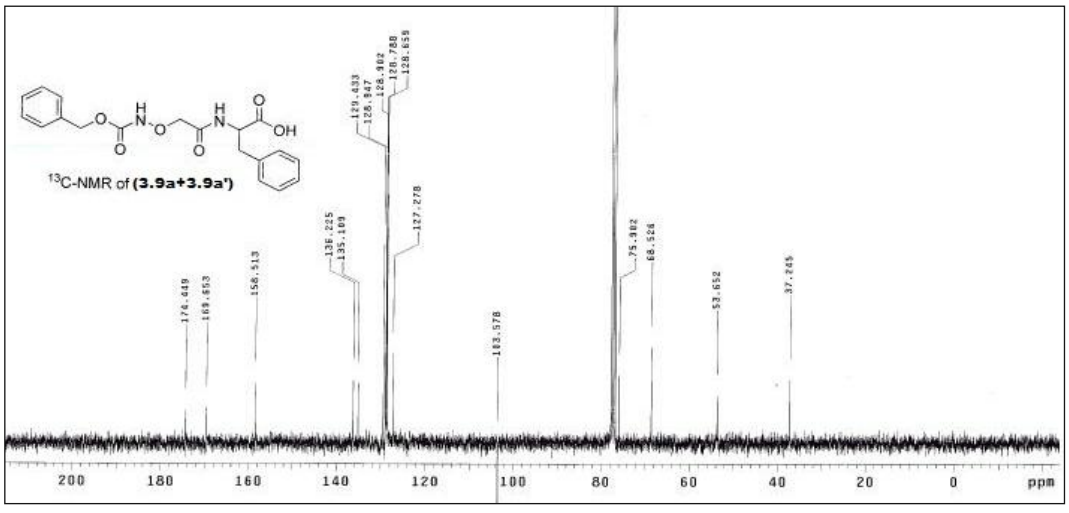
Appx-34. ^{13}C NMR spectra Z-AOGly-L-Phe-OH of **3.9a**



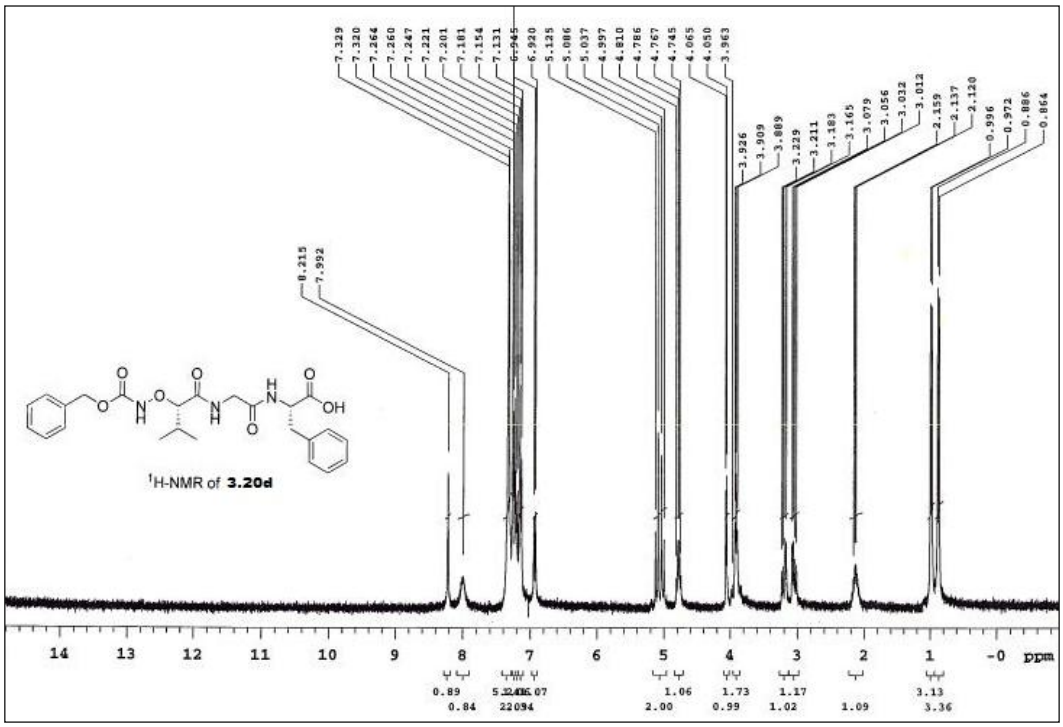
Appx-35. ^{1}H NMR spectra of Z-AOGly-DL-Phe-OH **(3.9a+3.9a')**



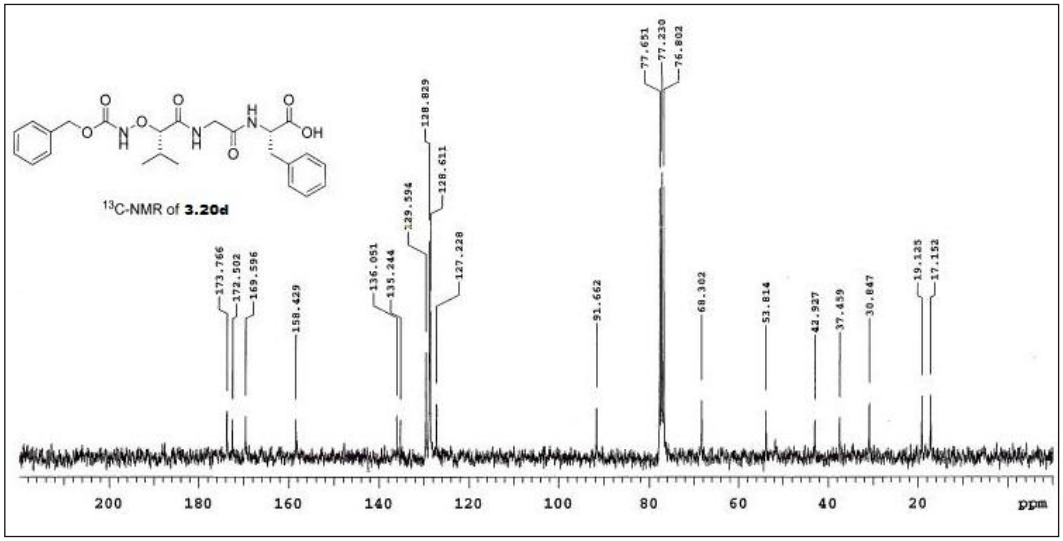
Appx-36. ^{13}C NMR spectra of Z-AOGly-DL-Phe-OH **(3.9a+3.9a')**



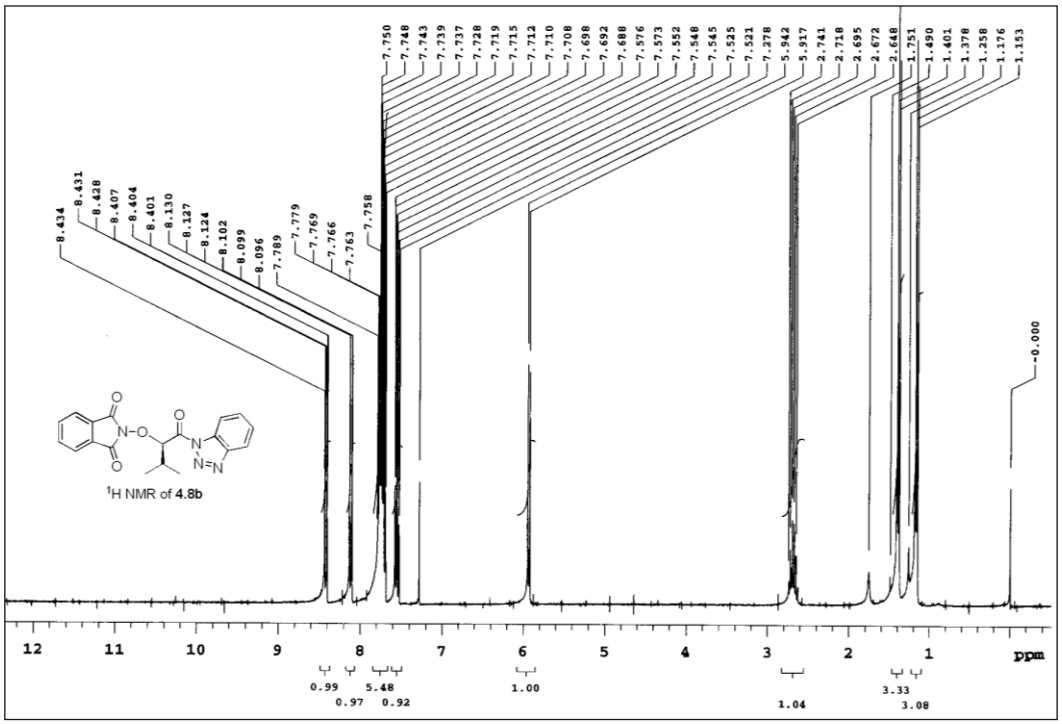
Appx-37. ^{1}H NMR spectra of Cbz-L-AOVal-Gly-L-Phe-OH **3.20d**



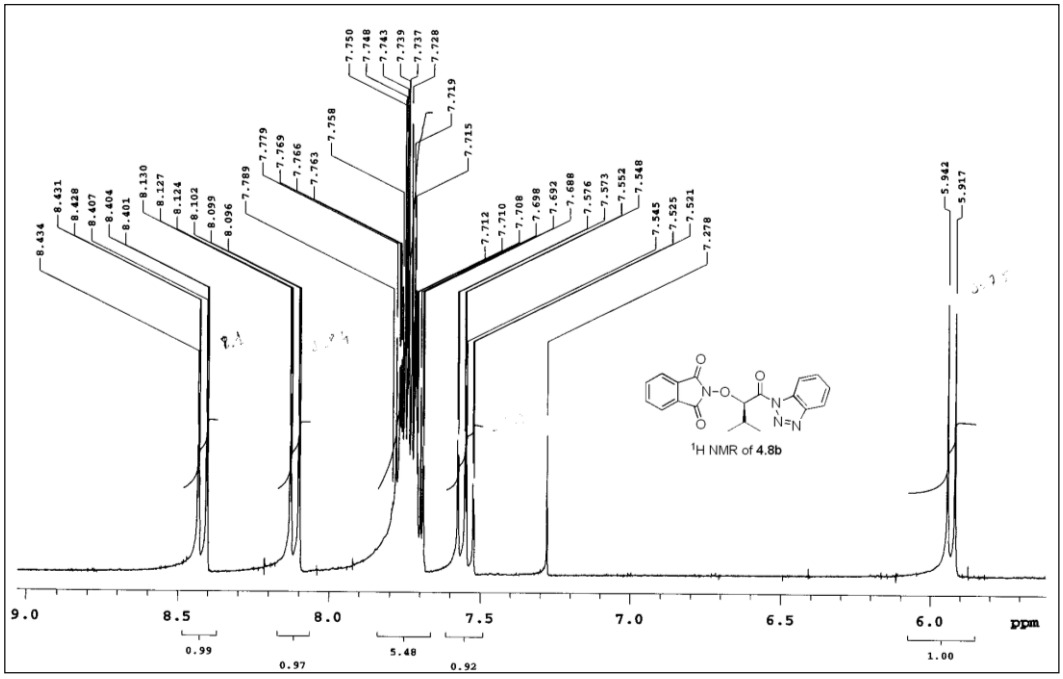
Appx-38. ^{13}C NMR spectra of Cbz-L-AOVal-Gly-L-Phe-OH **3.20d**



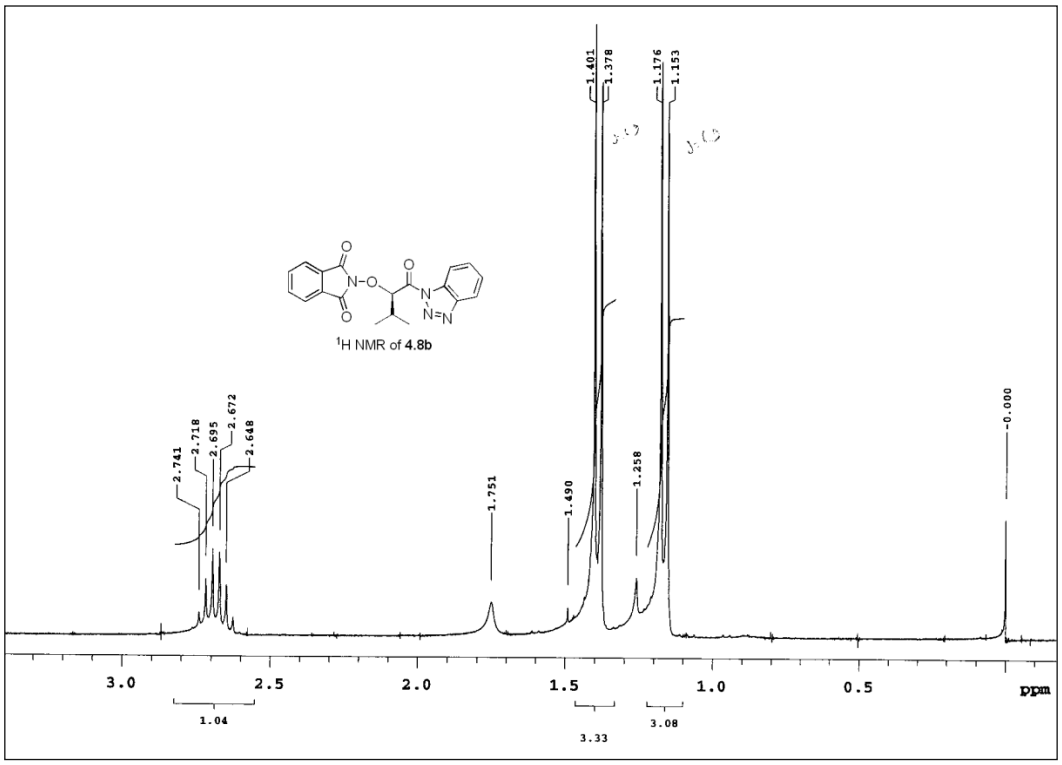
Appx-39. ^{1}H NMR spectra of *N*-(Phth)-AOVal-Bt **4.8b**



Appx-40. Expansion of ^{1}H NMR spectra of *N*-(Phth)-AOVal-Bt **4.8b**



Appx-41. Second expansion of ^{1}H NMR spectra of *N*-(Phth)-AOVal-Bt **4.8b**



Appx-42. ^{13}C NMR spectra of *N*-(Phth)-AOVal-Bt **4.8b**