

Development of Novel Recyclable Coupling Reagents and Investigation on Chirality Controlled Self-Assembly of Dipeptides

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Doctor of Philosophy in Chemistry*



Submitted by

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*Dedicated
To
My Family*



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

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STATEMENT

I do hereby declare that the matter embodied in this thesis entitled “**Development of Novel Recyclable Coupling Reagents and Investigation on Chirality Controlled Self-Assembly of Dipeptides**” is the result of experiments carried out by me in the Department of Chemistry, Indian Institute of Technology Guwahati, India, under the supervision of Prof. Bhubaneswar Mandal.

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

Date: 30.08.2024

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CERTIFICATE

This is to certify that **Mr. Sayanta Roy** (Roll No. 176122102) has been working under my supervision since January 2018 as a regular registered Ph.D. student. I am forwarding his thesis entitled “**Development of Novel Recyclable Coupling Reagents and Investigation on Chirality Controlled Self-Assembly of Dipeptides**” to be submitted for the Ph.D. (Science) Degree of this Institute. I certify that he has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in his thesis and this work has not been submitted elsewhere for a degree.

Date: 30.08.2024

Place: IIT Guwahati

Prof. Bhubaneswar Mandal

Thesis Supervisor

Department of Chemistry

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Sayanta Roy

List of abbreviations

A β	Amyloid beta
ACN	Acetonitrile
AFM	Atomic force microscopy
Aib	2-Aminoisobutyric acid
AIDS	Acquired immunodeficiency syndrome
Alloc	Allyloxycarbonyl
Ant	Anthranilic acid
AOP	(7-Azabenzotriazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate
BDDC	Bis[[4-(2,2-dimethyl-1,3-dioxolyl)]-methyl]-carbodiimide
BDMP	5-(1 <i>H</i> -benzotriazol-1-yloxy)-3,4-dihydro-1-methyl-2 <i>H</i> -pyrrolium hexachloroantimonate
BEC	<i>N</i> - <i>tert</i> -butyl- <i>N'</i> -ethylcarbodiimide
Boc	<i>tert</i> -Butyloxycarbonyl
Boc-Oxyma	Ethyl 2-(<i>tert</i> -butoxycarbonyloxyimino)-2-cyanoacetate
BOI	2-(Benzotriazole-1-yl)-oxy-1,3-dimethylimidazolidinium hexafluorophosphate
BOMI	(1 <i>H</i> -benzotriazol-1-yloxy)- <i>N,N</i> -dimethylmethaniminium hexachloroantimonate
BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
Bzl	Benzyl
Cbz	Benzyloxycarbonyl
CETP	Cholesteryl ester transfer protein
CD	Circular dichroism
CHCl ₃	Chloroform
CIC	<i>N</i> -cyclohexyl, <i>N'</i> -isopropyl carbodiimide

(COCl) ₂	Oxalyl chloride
COCl ₂	Phosgene
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate
CsF	Cesium fluoride
Cs ₂ CO ₃	Cesium carbonate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
deg	degree
DEPBT	Diethyl (4-oxobenzo[d][1,2,3]triazin-3(4 <i>H</i>)-yl) phosphate
DIC	<i>N,N'</i> -diisopropylcarbodiimide
DIPEA	<i>N,N</i> -Diisopropylethyl amine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
DNBs	1 <i>H</i> -benzo[d][1,2,3]triazol-1-yl 2,4-dinitrobenzenesulfonate
DPPP	1,3-Bis(diphenylphosphino)propane
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI-MS	Electrospray Ionization Mass Spectrometry
EtOAc	Ethyl Acetate
Equiv	Equivalent
FDA	Food and Drug Administration
Fmoc	9-Fluorenylmethoxycarbonyl
FT-IR	Fourier Transformation Infrared
FESEM	Field Emission Scanning Electron Microscopy
HAPyU	1-(1-Pyrrolidinyl-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]-pyridin-1-yl)methylene)pyrrolidinmium hexafluorophosphate <i>N</i> -oxide
HATU	<i>N</i> -[(Dimethylamino)-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridin-1-yl)methylene)- <i>N</i> -methylmethanaminium hexafluorophosphate <i>N</i> -oxide

HBPyU	O-(benzotriazol-1-yl)oxybis(pyrrolidino)-uronium hexafluorophosphate
HBTU	2-(1 <i>H</i> -benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU	O-(6-Chlorobenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
HIV	Human immunodeficiency virus
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
HOMO	Highest occupied molecular orbital
HPLC	High performance Liquid Chromatography
HRMS	High-resolution Mass Spectrometry
KOAc	Potassium acetate
LHRH	Luteinizing hormone–releasing hormone
LiHMDS	Lithium bis(trimethylsilyl)amide
LiOH	Lithium hydroxide
LUMO	Lowest unoccupied molecular orbital
MALDI	Matrix-assisted laser desorption/ionization
MeOH	Methanol
mL	Milli Liter
4-MP	4-Methyl piperidine
MW	Microwave
mM	Millimolar
μM	Micromolar
MS	Mass spectra
MYMsA	<i>N</i> -methylnemethylsulfonamide
MYTsA	<i>N</i> -methylnetoluenesulfonamide
NaHCO ₃	Sodium bicarbonate
Na ₂ SO ₄	Sodium sulfate

NBs	1-((naphthalen-2-ylsulfonyl)methyl)-1 <i>H</i> -benzo-[<i>d</i>][1,2,3]triazole
2-NBs	1 <i>H</i> -Benzo[<i>d</i>][1,2,3]triazol-1-yl 2-nitrobenzenesulfonate
4-NBs	1 <i>H</i> -Benzo[<i>d</i>][1,2,3]triazol-1-yl 4-nitrobenzenesulfonate
4-NBsOXY	Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate
NEt ₃	Triethylamine
nm	Nanometre
NMI	<i>N</i> -Methyl imidazole
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
<i>o</i> -NosylOXY	Ethyl 2-cyano-2-(2-nitrophenylsulfonyloxyimino)
ORTEP	Oak Ridge Thermal Ellipsoid Plot
Oxyma	Ethyl 2-cyano-2-(hydroxyimino)acetate
PCy ₃	Tricyclohexylphosphine
PdCl ₂	Palladium(II) chloride
PdCl ₂ (PPh ₃) ₂	Bis(triphenylphosphine)palladium(II) dichloride
Pd(COOCH ₃) ₂	Palladium(II) trifluoroacetate
Pd ₂ (dba) ₃	Tris(dibenzylideneacetone)dipalladium(0)
Pd(OAc) ₂	Palladium(II) acetate
Pd(PPh ₃) ₄	Tetrakis(triphenylphosphine)palladium(0)
PIC	<i>N</i> -phenyl, <i>N</i> -isopropylcarbodiimide
PPh ₃	Triphenylphosphine
PS-DCC	Polymer cyclohexylcarbodiimide
PS-EDC	Polymer 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide
PS-TBTU	<i>N</i> -[(1 <i>H</i> -benzotriazol-1-yl)(dimethylamino)-methylene]- <i>N</i> -methylmethanaminium tetrafluoroborate <i>N</i> -oxide
PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyOxm	O-[(cyano(ethoxycarbonyl)methyliden)-amino]-yloxytri(pyrrolidino) phosphonium hexafluorophosphate
Phg	Phenylglycine
PXRD	Powder X-Ray Diffraction
SC-XRD	Single Crystal X-Ray Diffraction
SOCl ₂	Thionyl chloride
SPPS	Solid Phase Peptide Synthesis
TATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TBTU	2-(1 <i>H</i> -Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TBs	1 <i>H</i> -benzo[<i>d</i>][1,2,3]triazol-1-yl 4-methylbenzenesulfonate
<i>t</i> Bu	<i>tert</i> -Butyl
TCBOXY	(<i>E</i>)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
TGA	Thermogravimetric analysis
ThT	Thioflavin T
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
<i>t_R</i>	Retention time
UV	Ultraviolet

Abbreviations for intensities of ¹H-NMR signals

s	singlet	q	quartet
d	doublet	m	multiplet
dd	doublet of doublet	Hz	Hertz
t	triplet	MHz	Mega-Hertz

List of 22 natural amino acids

<i>R group</i>	<i>Amino acids</i>	<i>Abbreviation</i>	
		<i>Three letter code</i>	<i>One letter code</i>
Non-polar	Glycine	Gly	G
	Alanine	Ala	A
	Proline	Pro	P
	Valine	Val	V
	Leucine	Leu	L
	Isoleucine	Ile	I
	Methionine	Met	M
	Selenocysteine	Sec	U
Aromatic	Phenylalanine	Phe	F
	Tyrosine	Tyr	Y
	Tryptophan	Trp	W
Polar, uncharged	Serine	Ser	S
	Threonine	Thr	T
	Cysteine	Cys	C
	Asparagine	Asn	N
	Glutamine	Gln	Q
Positively charged	Lysine	Lys	K
	Arginine	Arg	R
	Histidine	His	H
	Pyrrolysine	Pyl	O
Negatively charged	Aspartic acid	Asp	D
	Glutamic acid	Glu	E

Abstract

The contents of the thesis entitled, “**Development of Novel Recyclable Coupling Reagents and Investigation on Chirality Controlled Self-Assembly of Dipeptides**” are divided into seven chapters based on the results of the experimental work performed during the complete course of the doctoral studies.

Chapter 1: Contains the general introduction of peptide coupling reagents, supramolecular self-assembly of small peptides, and metal ion sensing.

Chapter 2: This chapter demonstrates the rapid generation of Oxyma-based novel recyclable coupling reagent and its applications.

Chapter 3: This chapter discusses the development of heterocyclic recyclable coupling reagents and applications.

Chapter 4: In this chapter, we demonstrate a novel coupling reagent mediated construction of C-N, C-O and C-C bonds.

Chapter 5: This chapter describes the influence of amino acids side-chain and chirality on the supramolecular assemblies of dipeptides.

Chapter 6: This chapter describes chirality controlled self-assembly of dipeptides and selective sensing of Fe^{3+} .

Chapter 7: This chapter describes the self-assembly of enantiomeric dipeptides and applications.

The chapter-wise summaries of the mentioned research works are described below.

Chapter 1: Introduction

Amide, ester, nitrile, and ketone moieties are widespread in various natural products, pharmaceuticals, and dyes. Various approaches have been developed for constructing amide, ester, nitrile, and ketone bonds, among which coupling reagents play a significant role. Over the last few decades, many coupling reagents based on carbodiimides, phosphonium salts, uronium/aminium salts, etc, have been developed and commercialized. Dicyclohexylcarbodiimide (DCC) is an efficient reagent compatible with solution phase peptide synthesis with *t*Bu chemistry but not with Fmoc solid phase peptide synthesis. DCC produces insoluble dicyclohexyl urea (DCU) by-product, which is very difficult to remove even after passing through column chromatography. Several coupling reagents encounter critical challenges, complex synthesis and difficult purification, poor racemization suppressing capacity, low yields, poor self-stability, use of excess reagents, limited versatility, compatibility issues with solvents, etc. Modern coupling reagents based on Oxyma, HOBt, and HOAt have been developed and are widely utilized to address such challenges.

Construction of ketones using conventional methods, including oxidation of an alcohol, amide to ketone, aldehyde to ketone, and acid chloride to ketone, has been well-established. However, the direct conversion of ketones from carboxylic acids has intrigued synthetic chemists for decades. While excess organometallic reagents can achieve this transformation, their practical application in organic synthesis remains limited. Recently, metal-catalyzed Suzuki-Miyaura coupling of carboxylic derivatives with boronic acids has enabled the synthesis of regioselective ketones.

Small peptide-based self-assemblies are significant because of their extensive applications in nanomedicine, drug delivery, organic electronics, biosensing, and nanobiomaterials. Their importance stems from their readily accessible nature, specific binding, biocompatibility, molecular recognition, and functional adaptability. Self-assembly involves arranging molecules into a stable and well-defined structure under equilibrium conditions, primarily through various non-covalent interactions like hydrophobic interactions, hydrogen bonding, π - π stacking, and electrostatic interactions. Peptide assemblies can exhibit a variety of morphologies, such as nanotubes, nanospheres, nanofibrils, nanorods, nanotapes, gels, nanovesicles, droplets, and many more under different conditions.

Chapter 2: Rapid Generation of Oxyma-Based Novel Recyclable Coupling Reagent and its Applications

Amide and esters are prevalent functionalities in nature, existing in pharmaceuticals, cosmetics, and drugs. The sustainable construction of amide or ester bonds is a hot research topic. The reaction involves activating the carboxylic acid group using a recyclable coupling reagent and subsequently adding a coupling partner. As of now, various coupling reagents have been developed and commercialized. Oxyma-based coupling reagents have recently been widely used due to their effectiveness, low risk of explosion, and ability to suppress racemization compared to other coupling reagents.

Drawing inspiration from the unique reactivity of Oxyma-based coupling reagents, we focused on developing thiophene-ring-containing Oxime-based novel coupling reagents to address critical amidation, esterification, and peptide synthesis sustainably. We synthesized various Oxime-based reagents; among them, Oxyma-based reagents showed better amidation reactivity. The DFT calculations for 5-H, 5-Cl, and 5-NO₂ substituted thiophene ring-containing Oxyma-based reagents revealed that the chloro-substituted reagent (*o*-CTSXY) exhibited greater reactivity compared to the others. The reagent was synthesized rapidly, straightforwardly, and in an eco-friendly manner, offering 99–100% yield within a few minutes. The reagent offered racemization-free synthesis of amides, hydroxamates, esters, thioesters, and peptides in an environmentally friendly manner. The coupling reagent is easily up-scalable, equally effective in solution and solid-phase peptide synthesis, and recyclable.

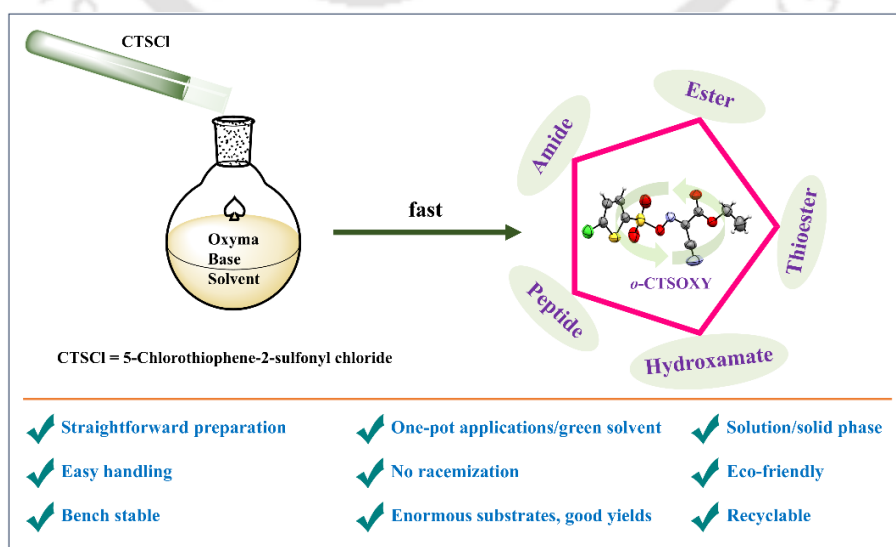


Figure 1. A schematic representation of the synthesis of *o*-CTSXY and its applications.

Chapter 3: Development of Heterocyclic Recyclable Coupling Reagents and Applications

Amide, ester, thioester, and nitrile functionalities are present in many biologically active molecules. Although numerous coupling reagents have been reported for constructing the mentioned functionalities, several encounter many difficulties, including harsh synthesis, excess reagent use, guanidylolation, racemization of chiral centers, and more. To prevent racemization, HOBt-based coupling reagents are widely used. Furthermore, HOBt typically accelerates the reaction rate and suppresses side reactions. To date, very few sulfonate esters containing HOBt-based coupling reagents have been reported, and they also suffer many drawbacks. To our knowledge, a 5-member heterocyclic ring containing HOBt and polymeric HOBt-based coupling reagent has not been reported yet.

Here, we developed quick, straightforward protocols for synthesizing HOBt and polymeric HOBt-based new-generation coupling reagents. We found their application for racemization-free amidation, esterification, peptide synthesis, and other reactions in a sustainable manner. Coupling reagent (CTSOBt) crystallized in the O-substituted desmotropic form. DFT studies also favored the formation of the O-substituted form over the N-substituted. The formation of a polymeric coupling reagent (P-CTSOBt) was confirmed by IR and EDX analysis. The reagents are compatible with a broad range of substrates and are easily recyclable. The reagent (CTSOBt) is equally effective for both solution and solid-phase peptide synthesis. Synthesis of several linear as well as cyclic peptides in the solid phase was done efficiently with satisfactory yields.

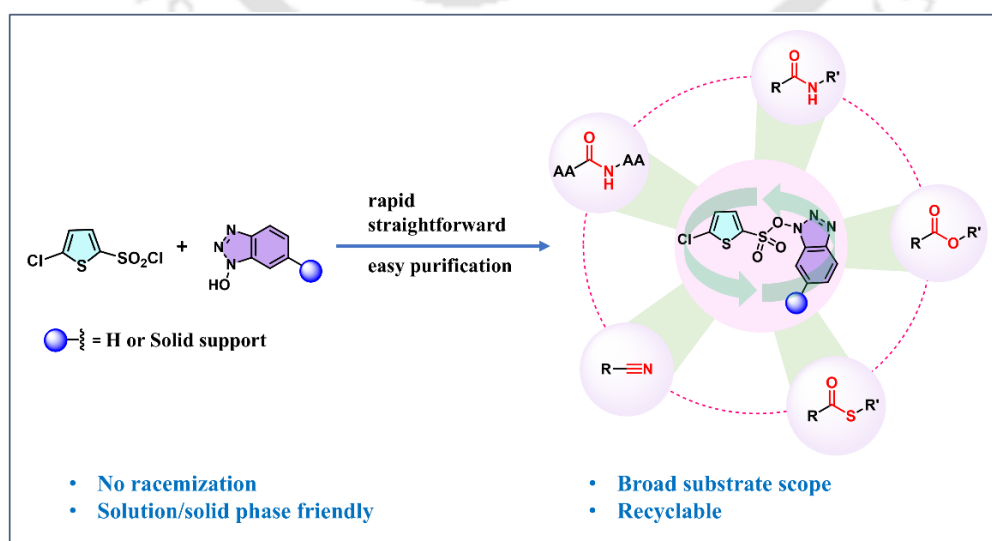


Figure 2. Synthesis of CTSOBt and P-CTSOBt and their diverse applications.

Chapter 4: A Novel Coupling Reagent Mediated Construction of C-N, C-O and C-C Bonds

The formation of amide, ester, and ketone bonds remains an active research area. Although several HOBt-based reagents have replaced the carbodiimide method, the challenge of racemization persists when using many of these reagents. Recently, several HOAt-based reagents, such as HATU, HAPyU, and PyAOP, have offered effective coupling by suppressing racemization and enhancing yield production. The unique properties of HOAt, particularly its 7th position N-atom, enhance leaving group quality and reactivity and help preserve the configurational integrity of chiral centers. Activated carboxylic esters have been crucial in carbonyl-retention Suzuki–Miyaura reactions. However, efficient ketone synthesis from acyl-OAt through Suzuki–Miyaura reactions has not been explored yet.

To date, thiophene ring-containing HOAt-based coupling reagent has not been established. Here, we efficiently synthesized CTSOAt with an excellent yield (99–100%). The reagent offers racemization-free synthesis of amides, peptides, and esters with outstanding yields. The reagent is equally effective in solution and solid-phase peptide synthesis. Beyond amidation and esterification, this reagent also participates in synthesizing ketones from carboxylic acids. The activation of carboxylic acids using CTSOAt led to the formation of an acyl-OAt intermediate. Subsequent treatment with aryl boronic acids in the presence of a Pd-catalyst offered the desired ketones with excellent yields.

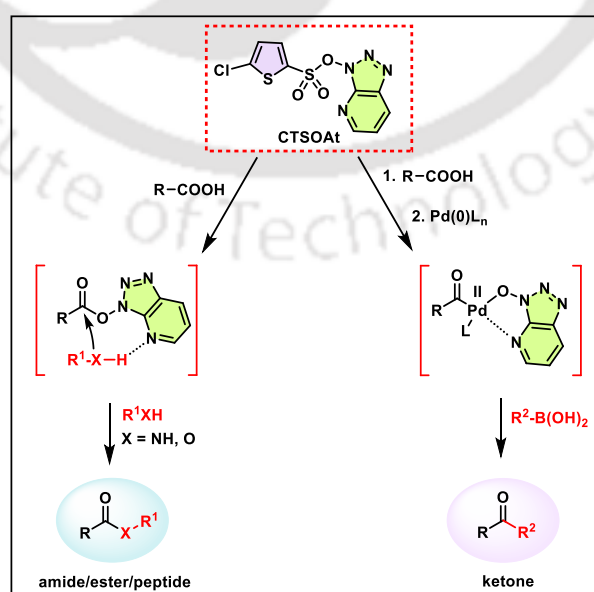


Figure 3. CTSOAt mediated the construction of C-N, C-O, and C-C bonds.

Chapter 5: Influence of Amino Acids Side-Chain and Chirality on the Supramolecular Assemblies of Dipeptides

In previous chapters, we explored peptide synthesis. Interestingly, some of the synthesized peptides formed crystals. Subsequently, we designed, synthesized, and crystallized some new peptides, investigating the self-assembly applications of all the crystalline peptides. Molecular self-assembly plays a crucial role in nanotechnology and biomedical sciences. Peptide-based self-assembly manifests diverse morphologies. The three-dimensional structure formation of peptides and proteins is predominantly influenced by interactions between the side chains of amino acids and facilitated by non-covalent interactions. While L/L amino acid-bearing dipeptides have been extensively studied for self-assembly, exploring L/D amino acid-bearing dipeptides remains limited.

We studied the self-assembly of dipeptides Boc-L-Ile-L-Phe-OMe (**5A**), Boc-L-Ile-D-Phe-OMe (**5B**), Boc-L-Ile-L-Phg-OMe (**5C**), and Boc-L-Ile-D-Phg-OMe (**5D**) to investigate the impact of side-chain and chirality on self-assembly. The presence of the extra methylene (-CH₂-) group on the side-chain of Phe in **5A** led to distinct molecular arrangements to those observed in **5C**. In the crystalline state, **5A** adopted a single helix-like architecture, whereas **5C** self-associated around a water molecule to form a hexagonal hollow tube-like structure. Conversely, the molecular arrangements in **5B** and **5D** exhibited similarities but deviated from **5A** and **5C** due to differences in chirality. Peptides **5B** and **5D** displayed discontinuous double helix-like structures. In solution, Peptides **5A** and **5C** were self-associated to form helical ribbon-like and nanotube-like structures, respectively. Conversely, Peptides **5B** and **5D** adopted nanorod-like structures.

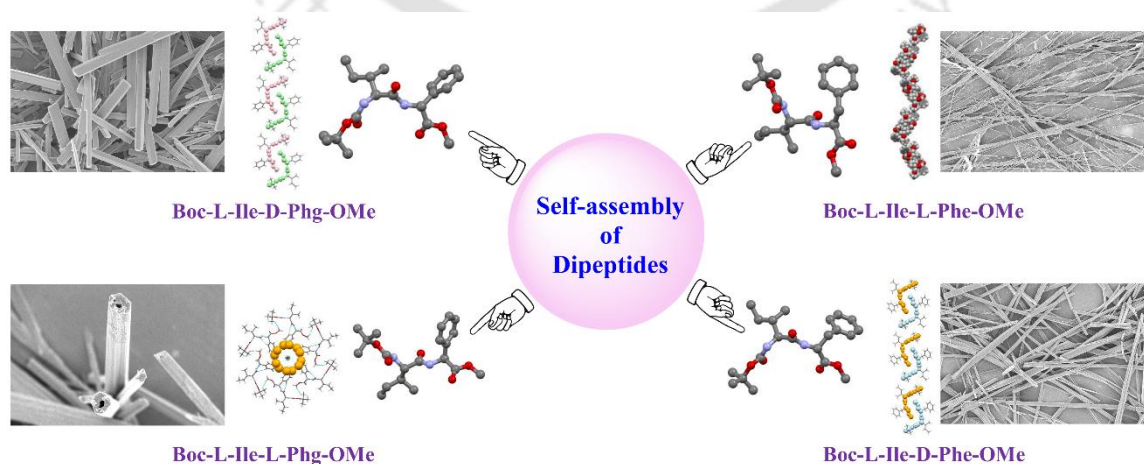


Figure 4. Self-assembly of L/L and L/D amino acid-containing peptides.

Chapter 6: Chirality Controlled Self-Assembly of Dipeptides and Selective Sensing of Fe^{3+}

Molecular self-assembly is a fundamental process for creating nanostructures. Peptides containing D-amino acid in sequences find extensive applications in medicinal chemistry, biological chemistry, and nanotechnology. Moreover, D-amino acids within peptide sequences enhance therapeutic effects, antimicrobial activity, and enzymatic stability. While the supramolecular self-assembly of peptides with L/L amino acid sequences is well understood, exploring peptides containing alternating L/D amino acids remains limited. Additionally, metal ion sensing using peptides is a crucial research area. Although peptides containing amino acids like histidine, tryptophan, and tyrosine have been extensively studied for metal ion sensing, the potential of peptides with other amino acids has not been explored enough. We are intrigued to investigate the supramolecular self-assembly and structural diversity of Fmoc peptides containing alternating L/L and L/D amino acids and their metal ion sensing properties.

Here, we have described the supramolecular self-assembly of both N-(Fmoc) and C-(OMe) protected dipeptides, Fmoc-L-Ala-L-Val-OMe (**6A**) and Fmoc-L-Ala-D-Val-OMe (**6B**). In the crystalline state, peptide **6A** formed a porous channel-like architecture, while peptide **6B** adopted a dimeric form. In solutions, peptide **6A** formed rod-like or tube-like structures, whereas peptide **6B** exhibited fibrillar structures. These self-assembled structures demonstrated significant thermal stability during dry heating. Moreover, both peptides could effectively bind with various bioactive dyes and selectively sense Fe^{3+} ions.

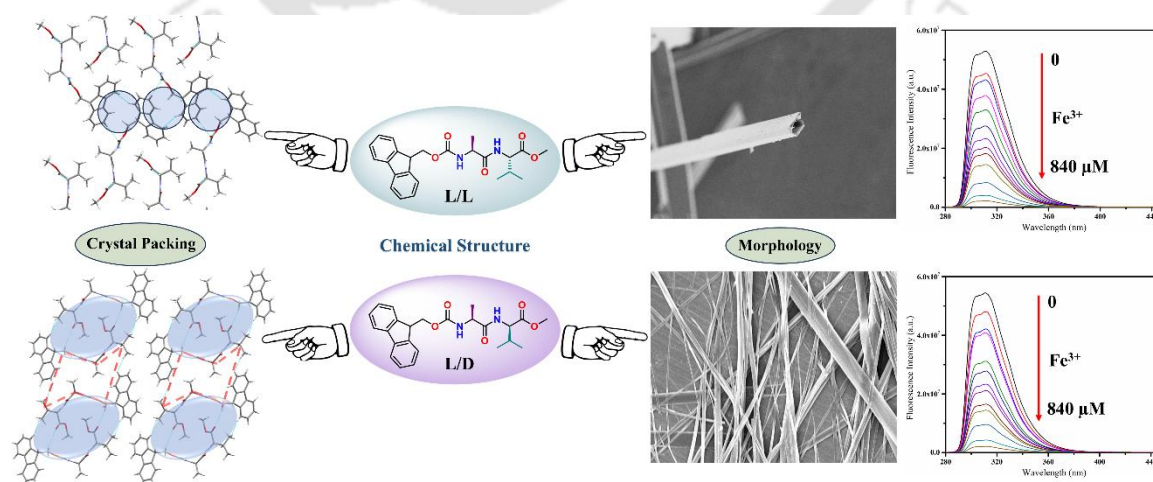


Figure 5. Self-assembly of alternating L/L and L/D amino acids containing dipeptides and Fe^{3+} sensing.

Chapter 7: Self-Assembly of Enantiomeric Dipeptides and Applications

Small peptide-based self-assembly gives rise to diverse nanostructures. We have already discussed the self-assembly of L/L and L/D amino acids containing diastereomeric peptides. Self-assembly of enantiomeric peptides containing one unnatural chiral amino acid has not been well explored. Recently, peptide nanovesicles have been widely used as potential drug-delivery vehicles due to excellent target specificity, biocompatibility, and low toxicity. Amino acids such as histidine, tryptophan, and tyrosine-based peptides have already been discovered for effective metal ion sensing. However, peptides other than these amino acids for selective sensing of toxic metal ions have not been well explored.

In this study, we explored the self-assembly of enantiomeric dipeptides (Fmoc-Gly-L-Phg-OMe (**7A**) and Fmoc-Gly-D-Phg-OMe (**7B**)) containing one unnatural chiral (L-Phg or D-Phg) amino acid. Remarkably, both peptides **7A** and **7B** formed well-ordered hollow vesicles in solution. Interestingly, in the crystalline state, peptides **7A** and **7B** self-assembled into parallel β -sheet structures and formed right-handed and left-handed helix-like arrangements, respectively. Furthermore, for both peptides, two helical strands running in opposite directions did not create a double helix; rather, they created void spaces. The formation of such void spaces in the crystalline state may be responsible for the vesicle formation in solution. These self-assembled structures exhibited significant thermal stability upon dry heating. Additionally, the peptides (**7A**, **7B**) demonstrated the ability to bind with bioactive drugs/dyes. Notably, they also functioned as effective fluorescent probes for Cr^{6+} ions.

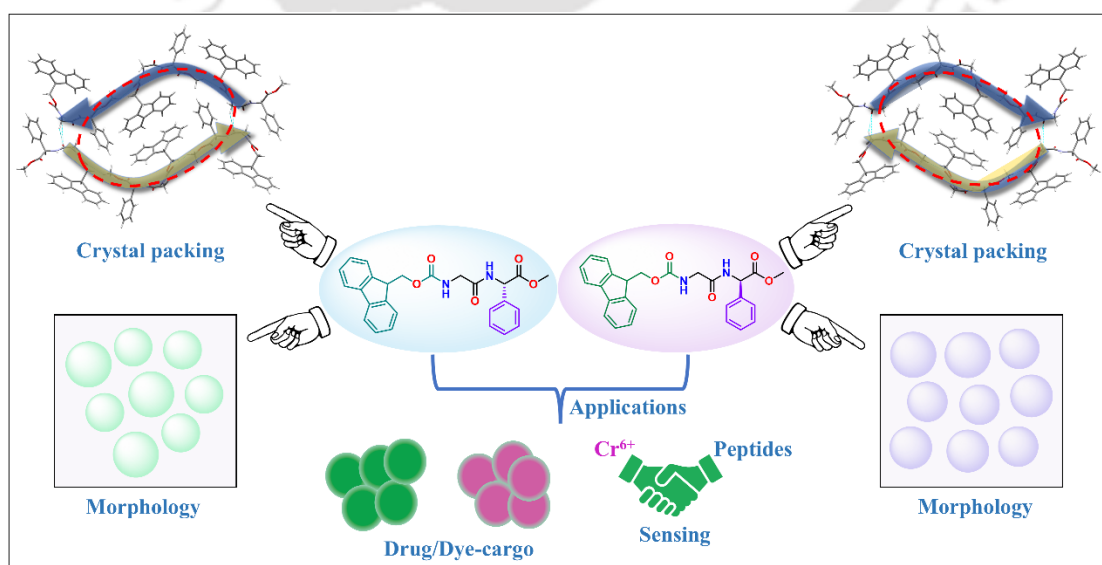


Figure 6. Self-assembly of enantiomeric dipeptides and their applications.

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Chapter 1

Introduction and Objectives



1.1. Introduction

The thesis describes the development of recyclable novel coupling reagents for constructing amides, hydroxamates, esters, thioesters, peptides, nitriles, and ketones. Additionally, the supramolecular self-assembly of dipeptides and dipeptide-based metal ion sensing is also explored. The introductory chapter provides a brief discussion on the importance of these cited molecules, existing synthesis methodologies, and various features related to self-assembly.

1.2. Amino acids

Amino acids are essential components of peptides and proteins, serving an important role in regulating the proper function of living organisms. Amino acids have L and D isomeric forms, but proteins exclusively contain L-isomers. Naturally occurring 22 L-amino acids commonly known as coded or proteinogenic amino acids. When amino acids link together, they form amide (peptide) bonds. Each α -amino acid contains an α -carbon linked with a $-\text{NH}_2$ group, a $-\text{COOH}$ group, a hydrogen (H) atom, and a side chain (R group) (Figure 1.1a). The characteristics of amino acids are significantly influenced by their side chains, containing various functional groups. Amino acids can be categorized as acidic, basic, or neutral based on side-chain properties. At physiological pH (7.4), amino acids are present in a zwitterionic form (Figure 1.1b). In addition to the natural amino acids, some unnatural variants are also present in biological systems, such as β -alanine, γ -aminobutyric acid (GABA), Aib, ornithine, and various D-amino acids. Unnatural amino acids enhance the proteolytic stability of peptides.

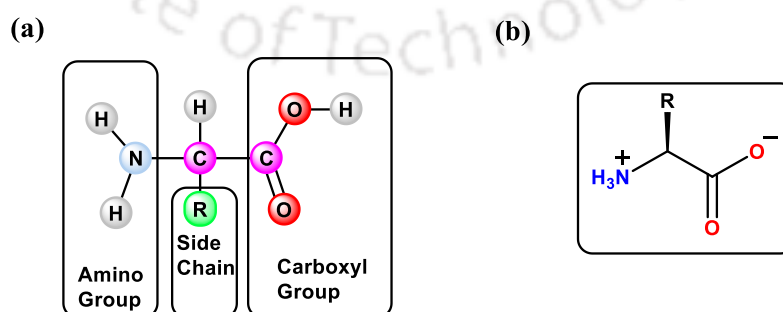


Figure 1.1. (a) Chemical formula and (b) zwitterionic form of α -amino acid.

1.3. Importance of some biologically relevant compounds

1.3.1. Importance of amides and peptides

Amide bonds are ubiquitous in nature. They are found in numerous natural products, pharmaceuticals, cosmetics, fine chemicals, and drugs.¹ Paracetamol, a well-known drug, treats mild pain and fever.² Penicillin, a class of molecules, serves as an antibiotic for treating bacterial infections.³ Apixaban is an anticoagulant used to treat blood clots and prevent strokes.⁴ Valsartan, an angiotensin II receptor type, is employed to manage heart failure and reduce blood pressure.⁵ Ponatinib, a multikinase inhibitor, treats leukemia patients.⁶

Peptides are essential for various physiological processes within the body. They play important roles in human cells, including cell signaling and functioning as immune modulators. Peptide-derived medications are used to treat numerous diseases. Insulin was the first peptide drug to manage blood sugar levels.⁷ Carnosine (β -alanyl-L-histidine), a natural antioxidant, is found in the human brain, gastrointestinal tissues, and muscle.⁸ Goserelin,⁹ a synthetic decapeptide and analog of LHRH, is used to treat prostate and breast cancers (Figure 1.2). Macimorelin serves as a medication for testing adult growth hormone deficiency.¹⁰

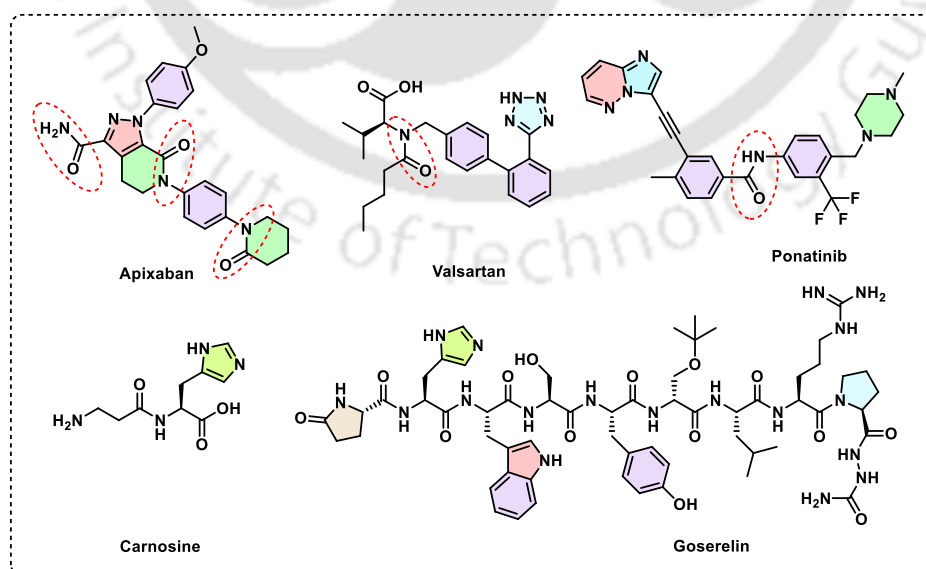


Figure 1.2. Therapeutically important amides and peptides.

1.3.2. Importance of esters, thioesters and hydroxamates

Ester, thioester, and hydroxamate-based compounds are essential in drug discovery, medicinal chemistry, and cosmetics.¹¹ Aceclofenac, an NSAID, is employed for treating musculoskeletal disorders and relieving pain.¹² Beclometasone, a corticosteroid, is prescribed for asthma management.¹³ Oseltamivir is an antiviral medication utilized to treat symptoms caused by influenza A and B viruses.¹⁴ Fluticasone furoate is employed in the treatment of allergic rhinitis.¹⁵ Dalcetrapib, a CETP inhibitor, increased high-density lipoprotein (HDL) cholesterol levels in the patient body.¹⁶ Trichostatin A (TSA)¹⁷ functions as a histone deacetylase inhibitor, with applications as an antifungal, antibiotic, and a potential anti-cancer agent (Figure 1.3).

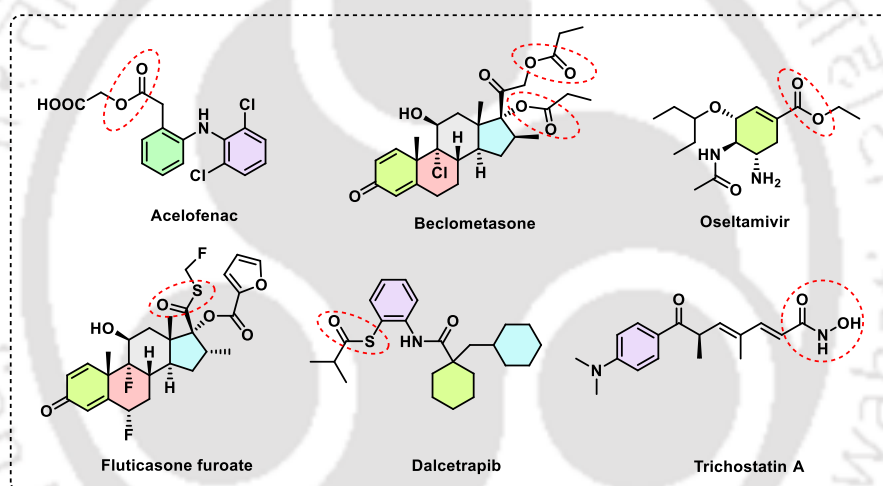


Figure 1.3. Therapeutically important esters, thioesters, and hydroxamates.

1.3.3. Importance of nitriles and ketones

Nitriles and ketones are crucial functional groups in biologically active compounds, pharmaceuticals, and dyes.^{18, 19} Nitriles are key intermediates for transforming into amines, aldehydes, and carboxylic acids. Ketones are compounds produced by the liver during the breakdown of fats. Enzalutamide, a nonsteroidal antiandrogen, is prescribed for managing prostate cancer.²⁰ Rilpivirine is an antiretroviral medication employed in the treatment of AIDS.²¹ Warfarin, an anticoagulant, aids in preventing blood clot formation.²² Tiaprofenic acid²³ is an NSAID prescribed for managing inflammation and pain (Figure 1.4).

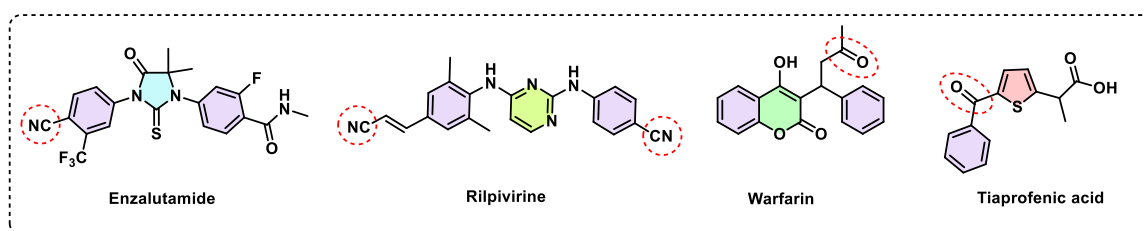
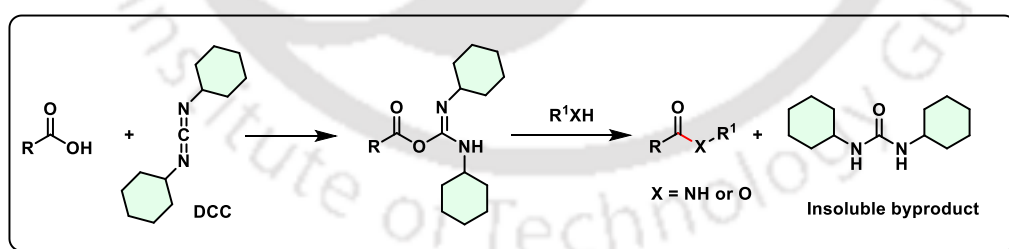


Figure 1.4. Therapeutically important nitriles and ketones.

1.4. Existing methods for synthesizing amides, hydroxamates, esters, thioesters, and peptides

Amidation and esterification are reactions that occur between carboxylic acids and amines, alcohols, or thiols. While amides can be directly formed by reacting a carboxylic acid and an amine, this approach is infrequently used due to the harsh reaction conditions.²⁴ Alternatively, various strategies for amide bond formation have been developed, including acyl halides, acyl azides, anhydrides, carbodiimides (Scheme 1.1), and active ester, etc.²⁵ Despite various synthetic methods for the cited compounds, challenges like harsh reaction conditions, side product formation, and substrate epimerization/racemization persist. Modern coupling reagents-based approaches address these issues, enabling efficient synthesis of the cited compounds in both solution and solid phase.



Scheme 1.1. DCC-based synthesis of amides and esters.

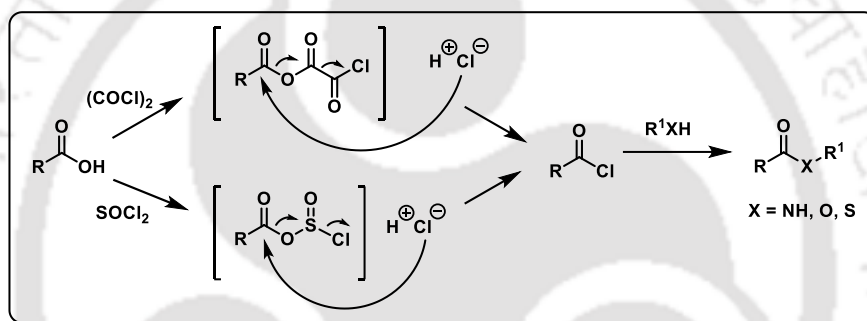
1.4.1. Solution phase synthesis

Synthesis of peptides initially relied on solution-phase methods until Merrifield's solid-phase peptide synthesis (SPPS) was developed.²⁶ Despite SPPS's dominance, solution-phase synthesis remains valuable for producing large-scale amides, esters, and small

peptides. During solution-phase synthesis, reactions take place within a single phase. Curtius²⁷ synthesized the first protected peptide in 1882, while Emil Fischer²⁸ achieved the first free peptide synthesis in 1901.

1.4.1.1. Acid halides method

Amidation and esterification can be simply achieved using the acyl halides method (Scheme 1.2). Various acylating agents, such as SOCl_2 , $(\text{COCl})_2$, COCl_2 , and cyanuric fluoride, are used.²⁹⁻³² Acyl halide methods are faster and proceed via highly reactive intermediates, but they can lead to unwanted side products and may result in the loss of configuration in chiral products.

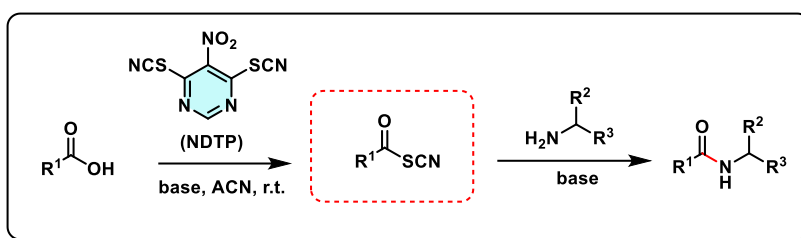


Scheme 1.2. Acyl-chloride mediated amidation and esterification.

1.4.1.2. Active esters method

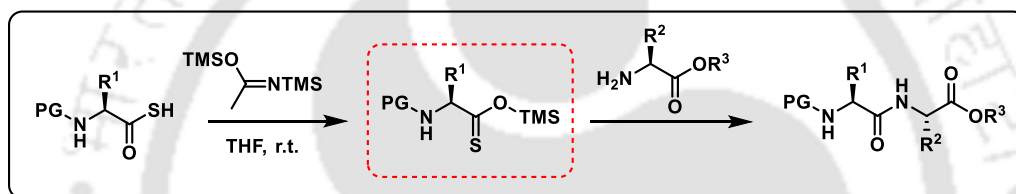
The active ester method, which uses a moderately reactive ester as the reaction intermediate, plays a vital role in minimizing racemization and epimerization during amidation or esterification. Before the widespread use of coupling reagents, this strategy was quite effective for synthesizing peptides. Various active ester methods have been developed, including p-nitrophenyl, 1,4,5-trichlorophenyl, N-hydroxyphthalimide, N-succinimide, and pentafluoro active esters.³³⁻³⁷ Notably, the first oxytocin synthesis was employed using the p-nitrophenyl (PNP) ester method.³⁸

Wang and coworker employed 5-nitro-4,6-dithiocyanatopyrimidine (NDTP) as an activator for carboxylic acids for synthesizing amides and peptides (Scheme 1.3).³⁹



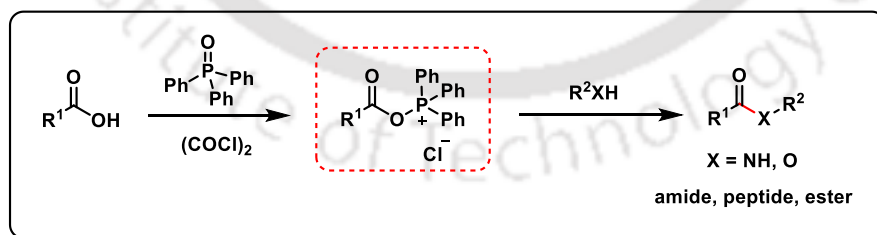
Scheme 1.3. Amide/peptide synthesis using NDTP as an acid activator.

In 2011, Liebeskind and colleagues demonstrated the activation of thioacids using bistrimethylsilylacetamide (BSA). Notably, the subsequent reaction between O-silylthionoesters and amines resulted in the exclusive amide bond formation (Scheme 1.4).⁴⁰



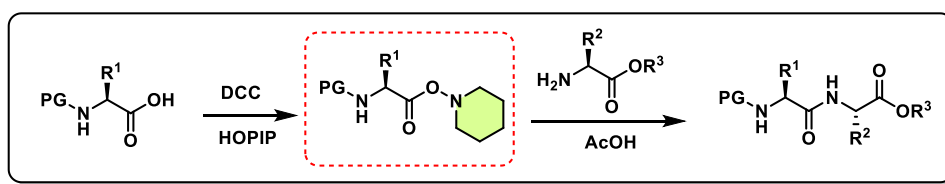
Scheme 1.4. Peptide synthesis using BSA as an acid activator.

Ni and colleagues developed an amidation and esterification method using a Ph_3PO (catalyst)/ $(\text{COCl})_2$ system. The acyl phosphonium salt generated in situ acted as an active ester, facilitating the synthesis of peptides, amides, and esters (Scheme 1.5).⁴¹



Scheme 1.5. Ph_3PO (catalyst)/ $(\text{COCl})_2$ assisted peptide synthesis.

Young and colleagues also developed 1-hydroxypiperidine (PIP) as an active ester for peptide synthesis (Scheme 1.6).⁴²



Scheme 1.6. 1-Hydroxypiperidine (PIP) active esters mediated peptide synthesis.

Despite reported active ester methods, they still encounter thermodynamic and kinetic barriers. Moreover, their applicability remains restricted to basic dipeptide or tripeptide substrates, and overactivated carboxylic acid derivatives often cause unwanted side reactions and racemization/epimerization. In addition, the use of HOXt (HOBt, HOAt)-based active ester methods enhances reaction yields by minimizing side product formation and reducing racemization of chiral centers in both solution and solid phases.

1.4.1.3. Coupling reagent-based method

The most successful and widely used method for amides, peptides, hydroxamates, and esters synthesis involves coupling reagents. Over time, various well-known coupling reagents, including carbodiimides, uronium/aminium salts, and phosphonium salts, have been reported.⁴³ In 1955, Sheehan introduced DCC as the first and most extensively used coupling reagent for amidation and esterification.⁴⁴ However, due to issues like insoluble by-products and incompatibility with Fmoc SPPS, DCC has been replaced by other coupling reagents such as DIC, CIC, and EDC (Figure 1.5),⁴⁵⁻⁴⁷ which are more suitable for SPPS and do not produce insoluble by-products. Additionally, other coupling reagents based on carbodiimides, such as BEC, BDDC, and PIC, are well-established.⁴⁸⁻⁵⁰

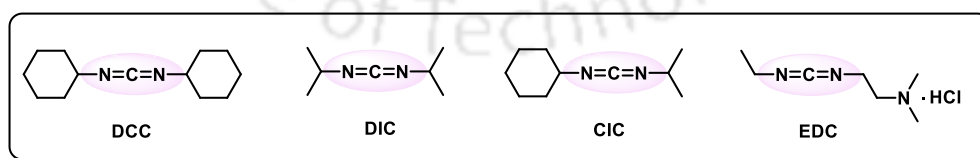


Figure 1.5. Carbodiimide-based coupling reagents.

Recently, in peptide synthesis, benzotriazole and azabenzotriazole have gained extensive use due to their improved coupling efficiency and strong racemization-suppressing capacity. Notably, HOBt-based several phosphonium, uronium/iminium salts such as

BOP, PyBOP, HBTU, HCTU, BOI, TBTU, HBPYU, BOMI, and BDMP are well-established.^{25, 51, 52} HOAt-based coupling reagents show higher efficiency than HOBt-based reagents in suppressing racemization and improving yield production. This enhanced effectiveness is due to the 7th position nitrogen atom in the benzotriazole ring. The electron-accepting property of the N-atoms of the ring enhances the leaving group quality, resulting in increased reactivity. Moreover, the 7th N-atom of the ring facilitates classic neighboring group participation, enhancing reactivity and maintaining configurational integrity. HOAt-based several phosphonium and uronium reagents, including HATU, TATU, HAPyU, AOP, and PyAOP, are well established (Figure 1.6).⁵³⁻⁵⁷ Recently, several polymer-bound coupling reagents such as PS-EDC, PS-DCC, and PS-TBTU have emerged for efficient peptide synthesis.⁵⁸⁻⁶⁰ Besides using uronium and phosphonium derivatives of HOBt and HOAt as coupling reagents, several other HOBt-based sulfonate and carbonyl ester coupling reagents have been developed, such as NBs, 4-NBs, TBs, 2-NBs, and DNBs.²⁵ While various HOBt and HOAt-based coupling reagents find widespread application, many are often restricted due to their explosive nature.

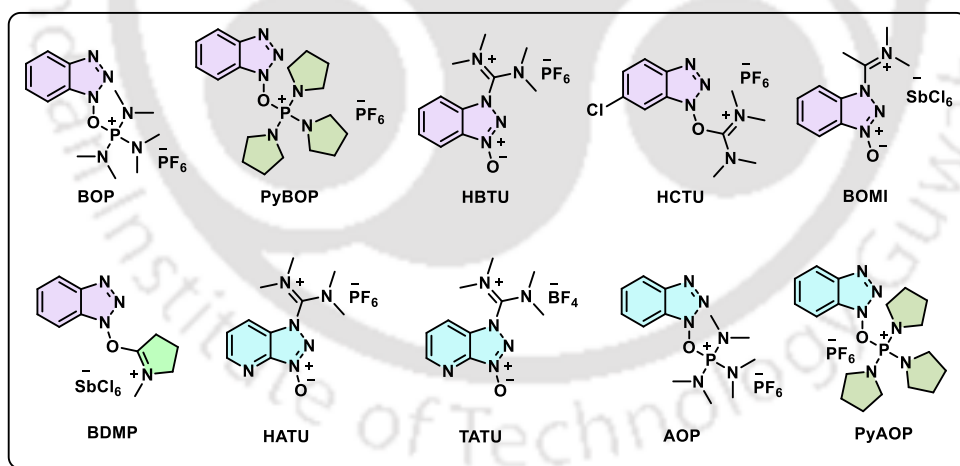


Figure 1.6. HOBT and HOAt-based coupling reagents.

El-Faham and Albericio introduced Oxyma, a highly efficient and safe additive as a replacement for benzotriazole or azabenzotriazole in peptide synthesis.⁶¹ Oxyma suppresses racemization and exhibits impressive coupling efficiency, and it also has a low risk of explosion. Albericio group also reported COMU (uronium salt) as a safe alternative to benzotriazole-based coupling reagents.⁶² Additionally, they highlighted

PyOxm, a phosphonium salt of Oxyma, for hindered peptide assembly.⁶³ Oxyma performs comparably to HOAt and outperforms HOBt. Our research group introduced other Oxyma-based reagents (*o*-NosylOXY, 4-NBsOXY, TCBOXY, and Boc-Oxyma),⁶⁴⁻⁶⁷ which excel in racemization-free amidation and esterification (Figure 1.7).

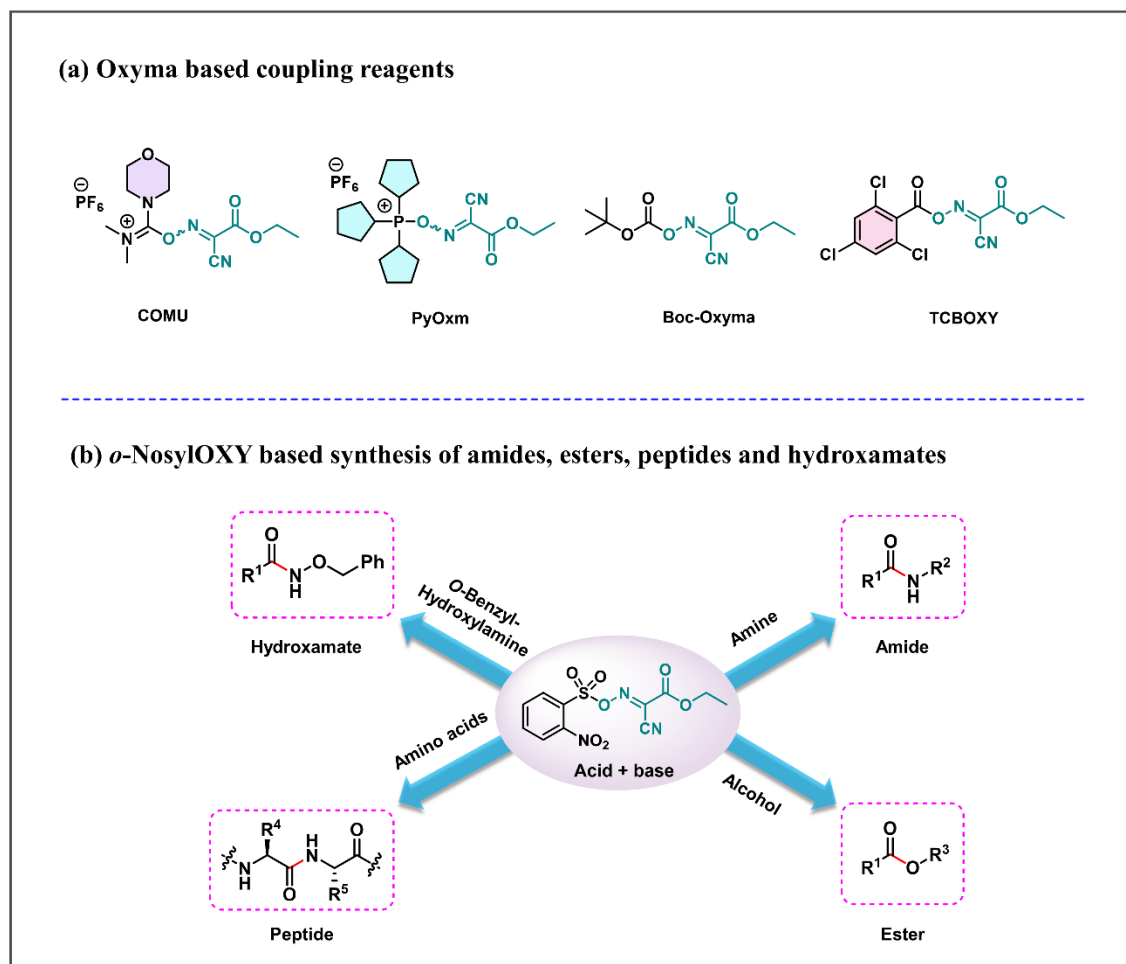


Figure 1.7. (a) Oxyma-based coupling reagents and (b) *o*-NosylOXY based synthesis of amides, esters, peptides and hydroxamates.

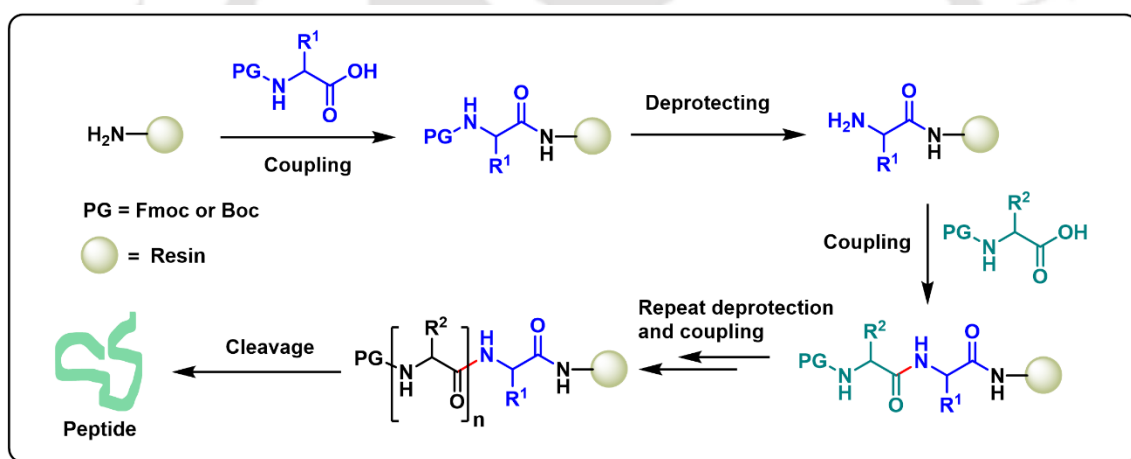
1.4.2. Solid phase peptide synthesis

Solid phase peptide synthesis (SPPS) constructs a long-chain peptide step by step while it is linked to an insoluble polymer. This technique allows for the removal of unreacted reagents through washing without losing any product. Consequently, the final peptide product's purity is improved. In 1963, the groundbreaking work of Prof. Robert Bruce Merrifield led to the first successful implementation of solid-phase peptide synthesis,

marking a significant milestone in peptide chemistry.²⁶ Solid-phase peptide synthesis comprises the following steps:

- (i) Loading of activated N-protected (Fmoc or Boc) amino acid onto the resin.
- (ii) Deprotection of the N-protection from the resin-bound amino acid.
- (iii) Coupling of the next activated amino acid
- (iv) Chain elongation by repeating steps (ii) and (iii).
- (v) Finally, cleavage of the expected peptide from the solid-support.

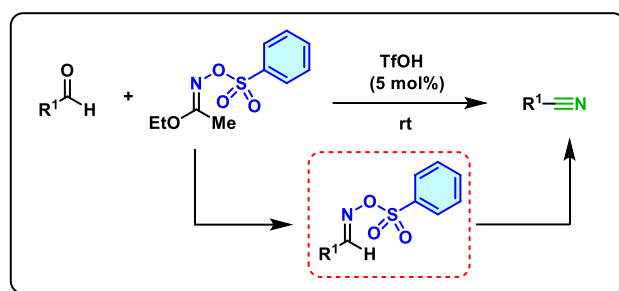
SPPS follows two standard strategies: (a) Fmoc/*t*Bu⁶⁸ and (b) Boc/Bzl⁶⁹. The activation of amino acids during SPPS is primarily carried out by coupling reagents like BOP, HBTU, PyBOP, HATU, and COMU. These methods play a crucial role in building peptide chains efficiently (Scheme 1.7).



Scheme 1.7. Schematic representation of SPPS.

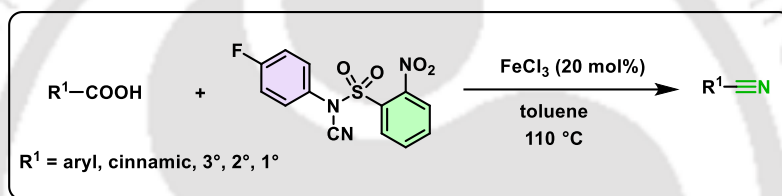
1.5. Existing methods for synthesizing nitriles

Nitrile synthesis can be achieved through several methods. Notably, the dehydration of amides, commonly known as the Pinner method, is one approach.⁷⁰ Additionally, Hyodo et al. demonstrated a Brønsted acid-catalyzed synthesis of nitriles using O-protected aldoximes derived from aldehydes and O-protected oximes via transoximation (Scheme 1.8).⁷¹



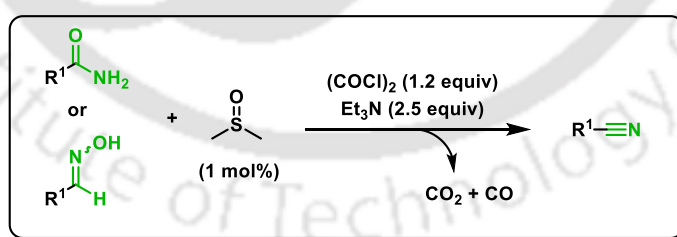
Scheme 1.8. Nitrile synthesis from O-protected aldoximes.

Zhang and colleagues devised a method to synthesize nitriles by reacting electron-deficient N-cyano-N-aryl-arylsulfonamide with common carboxylic acids utilizing an affordable Fe catalyst (Scheme 1.9).⁷²



Scheme 1.9. Fe-catalyzed nitrile synthesis from acids.

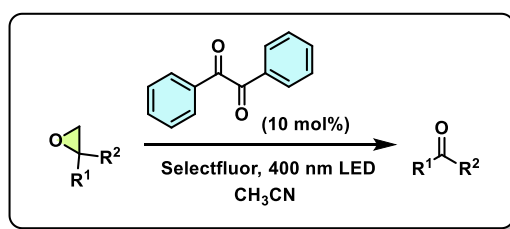
Ding and colleagues developed aldoxime or primary amide-based nitrile synthesis method employing oxalyl chloride, catalytic dimethyl sulfoxide, and Et₃N base (Scheme 1.10).⁷³



Scheme 1.10. Catalytic Swern oxidation for synthesizing nitriles from amides or aldoximes.

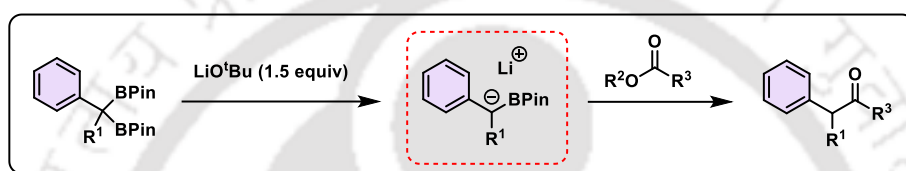
1.6. Existing methods for synthesizing ketones

Dehydrogenation of alcohol is the most common method for synthesizing ketones.⁷⁴ Holt et al. reported a radical dication-based C-C bond breaking of 1,1-disubstituted epoxides, which was subsequently followed by demethylation, providing ketones (Scheme 1.11).⁷⁵



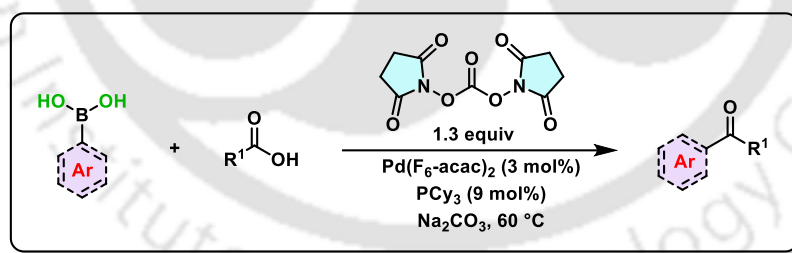
Scheme 1.11. Epoxide demethylation-based formation of ketones.

Lee et al. found an effective method to convert esters into ketones using benzyldiboronates (Scheme 1.12).⁷⁶



Scheme 1.12. Reaction of activated benzyldiboronates with esters for ketone synthesis.

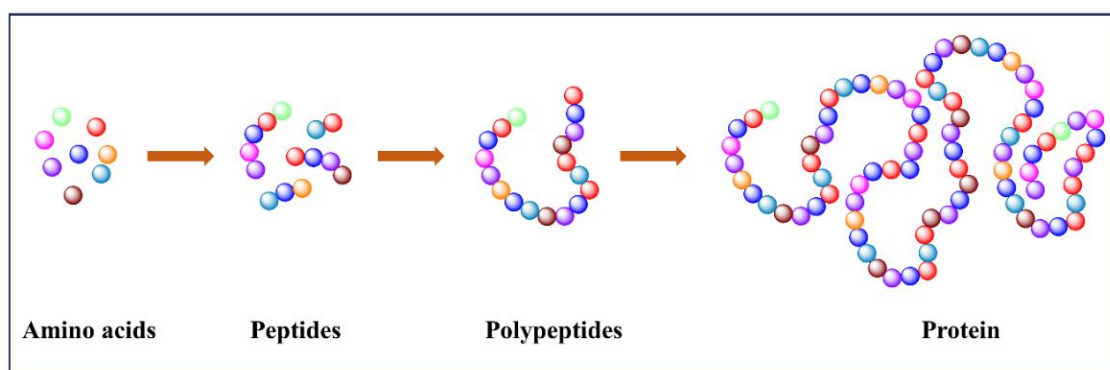
Gooßen and Ghosh reported a ketone synthesis method using palladium-catalyzed reactions of carboxylic acids, employing di(N-succinimidyl) carbonate (DSC) as an acid activator (Scheme 1.13).⁷⁷



Scheme 1.13. Ketone formation from the cross-coupling of boronic acid and activated carboxylic acids.

1.7. Peptides and proteins

Proteins are biomacromolecules present in living organisms and serve diverse functions. Proteins facilitate essential chemical reactions necessary for life. Polypeptides are the fundamental unit of a protein and contain over 50 subunits (Scheme 1.14). Proteins function as hormones, receptors, growth factors, and neurotransmitters.^{78, 79}



Scheme 1.14. Schematic representation of the process from amino acids to protein formation.

The peptide bond (C-N) exhibits a partial double-bond character resulting from N-lone pair delocalization, leading to a planar arrangement with restricted rotation (Figure 1.8a). This bond can exist in two geometric isomeric forms: cis and trans (Figure 1.8b).

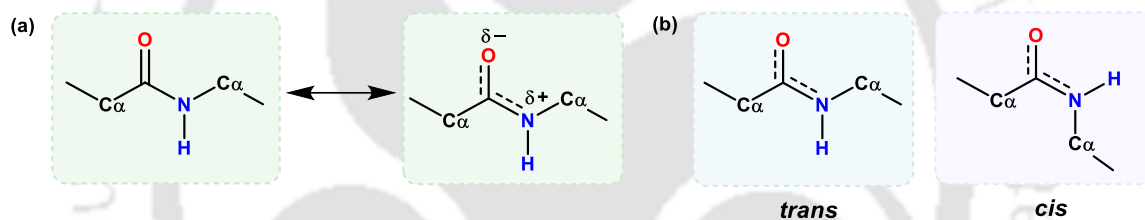


Figure 1.8. (a) Resonating structures and (b) cis-trans isomeric form of peptide bond.

1.7.1. Torsion angles

Torsion angles phi, psi, and omega (φ , ψ , ω) are crucial for predicting protein conformation. These angles describe the chain's rotation around the central bond: φ around N-C $_{\alpha}$, ψ around C $_{\alpha}$ -C, and ω around the peptide bond (C-N). φ and ψ range from -180° to $+180^{\circ}$, while ω is nearly 180° because of the partial double-bond character of the peptide bond (Figure 1.9).⁸⁰

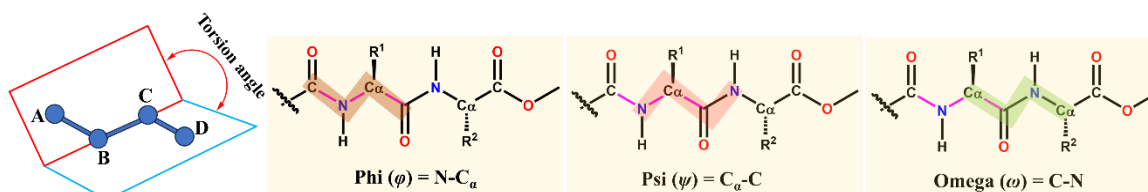


Figure 1.9. Graphical diagram of torsion angles.

1.7.2. Structure of proteins

Proteins have four distinct structures: primary, secondary, tertiary, and quaternary. Amino acids linked polypeptides refer to the protein's primary structure. The secondary structure includes α -helix, β -sheet, and β -turn, primarily formed by hydrogen bonds. The tertiary structure is the 3D folding arrangement of the polypeptide chain, while the quaternary structure is the closed-packed arrangement of protein subunits.

α -helix: An α -helix is a coiled configuration of polypeptides maintained by intramolecular hydrogen bonds between the i^{th} C=O and $(i+4)^{\text{th}}$ N-H groups of residues.⁸¹ It contains 3.6 amino acid residues per turn, with a distance of 5.4 Å between helical turns (Figure 1.10).

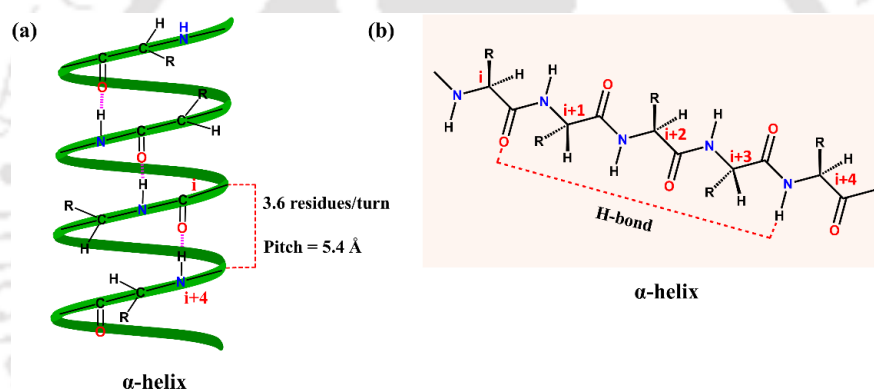


Figure 1.10. (a) Graphical diagram and (b) H-bond of α -helix.

β -sheet: β -sheets form between two polypeptide chains linked through hydrogen bonds (C=O and NH of neighboring strands). They can be parallel (strands align in the same direction) or anti-parallel (strands align in reverse directions) (Figure 1.11).⁸²

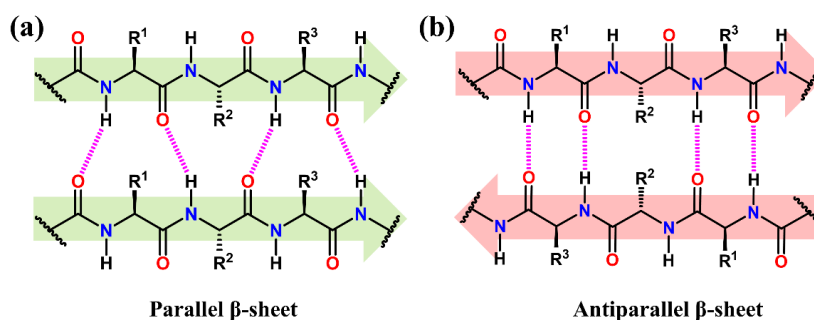


Figure 1.11. (a) Graphical diagram of parallel β -sheet and (b) antiparallel β -sheet structures.

β -turn: The most frequently observed turn in proteins is β -turn. It involves four consecutive amino acids and is stabilized through an intramolecular hydrogen bond between i^{th} C=O and $(i+3)^{\text{th}}$ NH groups of residues (Figure 1.12).⁸³

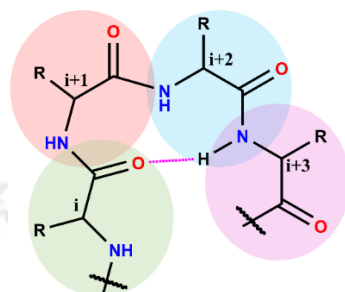
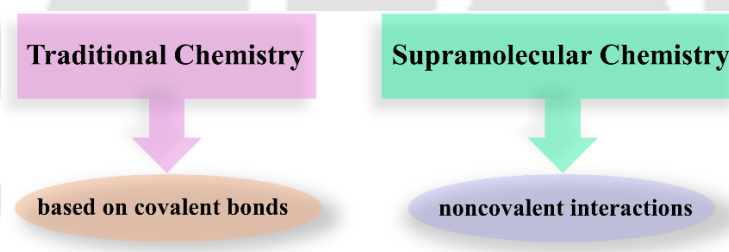


Figure 1.12. Diagram of β -turn structure.

1.8. Supramolecular assemblies

Supramolecular chemistry, a concept introduced by Jean-Marie Lehn⁸⁴, refers to “chemistry beyond the molecule,” focusing on the chemistry of molecular assemblies through noncovalent interactions. While traditional chemistry emphasizes covalent bonds, supramolecular chemistry explores noncovalent interactions between molecules.



The noncovalent interactions that are significantly crucial in forming and stabilizing supramolecular assemblies include:

- Electrostatic interactions
- Hydrogen bonding interactions
- Van der Waals interactions
- Hydrophobic interactions
- π - π stacking interactions

1.9. Self-assembly of small peptides

Molecular self-assembly forms diverse supramolecular structures through noncovalent interactions. The concept of forming supramolecular structures originates from nature, where biological systems create complex structures from amino acids, nucleic acids, and lipids. Recently, self-assembling peptides have gained significant attention for their applications in nanobiotechnology, nanomedicine, and nanomaterials. Peptide self-assembly offers several advantages, such as high chemical and biological diversity, excellent biocompatibility and biodegradability, molecular recognition abilities, target specificity, low toxicity, and effective membrane penetration. Peptides, including aliphatic, aromatic, cyclic, amphiphilic, and surfactant-like peptides, can self-assemble into numerous nanostructures, including nanowires, nanofibrils, nanoribbons, nanotapes, nanospheres, nanobelts, nanotubes and nanovesicles, depending on internal and external factors (Figure 1.13). These nanostructures are widely used in drug delivery, biomedical applications, tissue engineering, cell culture, biosensors, and nanoelectronics.

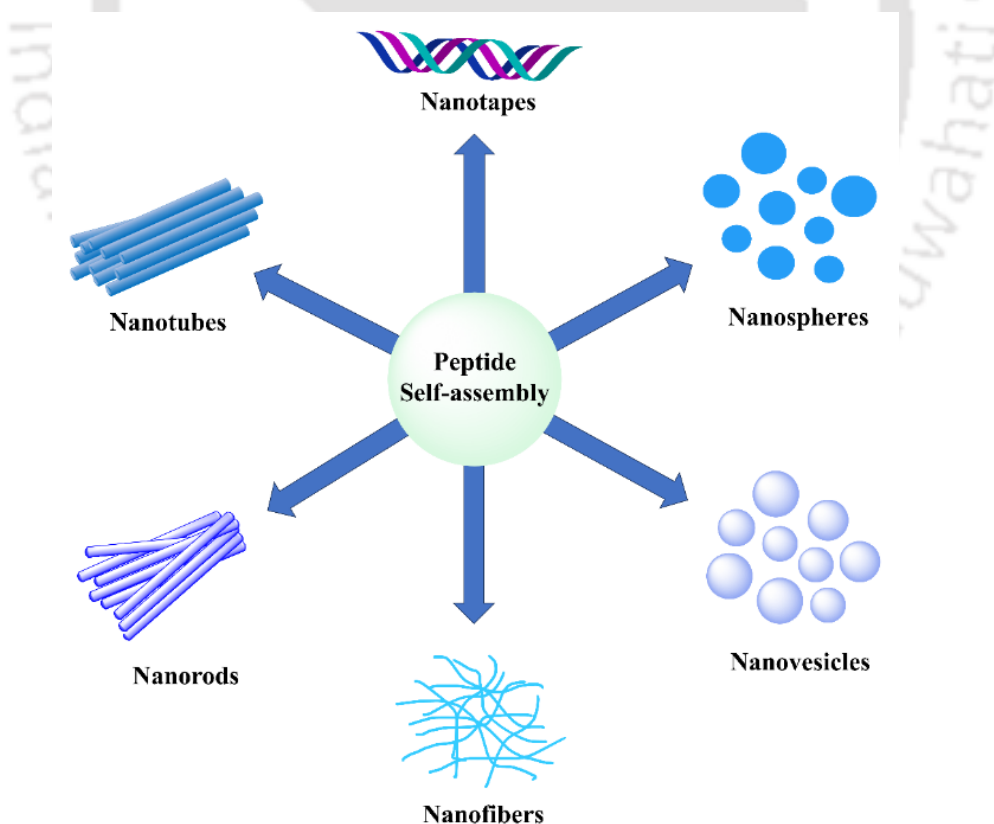


Figure 1.13. Peptide-based supramolecular nanostructures.

1.9.1. Self-assembly of dipeptides

A dipeptide represents the simplest form of a self-assembling peptide structure. Its compact size and straightforward synthesis make it an excellent model for studying peptide self-assembly mechanisms. Gazit et al. first studied diphenylalanine (FF)⁸⁵ based self-assembly, an important amyloid β (A β) motif, which formed ordered nanotubes,⁸⁶ nanofibril,⁸⁷ and nanoforests⁸⁸. These nanotubes were utilized for casting silver nanowires, templating magnetic nanoparticles, electrochemical biosensing, and creating low molecular-mass organogels.^{86, 89-91} Birkedal et al. demonstrated that TrpGly·H₂O-based nanotubes containing water molecules exhibited negative thermal expansion.⁹² Sozzani and coworkers demonstrated that dipeptides AV, VA, IV, and VI formed nanochannel pores in the crystalline state through hydrogen bonding, which could store gases like carbon dioxide, methane, and hydrogen.⁹³ Zelenovskiy and colleagues showed the enantiomeric dipeptides-based formation of microtubes. Notably, the L-FF isomer produced right-handed helices, whereas the D-FF isomer generated left-handed helices.⁹⁴ Halder and colleagues investigated the self-assembly behavior of aliphatic-aromatic backbone dipeptides, specifically Boc-Phe-x-aminobenzoic acid (where x = o/m/p). They discovered that these dipeptides have potential in N₂ gas absorption.⁹⁵ Görbitz and colleagues explored the parallel β -sheet structure formation using a protected dipeptide (Boc-VV-OMe).⁹⁶ Moretto et al. discovered the formation of hollow rod and organogel using hydrophobic dipeptide Boc-Cys(Me)-Leu-OMe. Additionally, the peptide also formed narrow lumen in the crystalline state.⁹⁷ Halder and coworkers reported that the dipeptide Boc-Val-Phe-OMe formed amyloid fibril-like morphologies. Additionally, it displayed parallel β -sheet aggregation and a cross β -sheet structure in crystalline state.⁹⁸ Our group recently showed the self-assembly of dipeptides resembling A β _{39-40/41-42} peptide fragments. Boc-Val-Val-OMe (**1**) and Boc-Ile-Ala-OMe (**2**) formed parallel β -sheets, while peptide **2** formed cross β -sheets in crystal packing. In solution, peptide **1** created a two-sided spear-like structure, and peptide **2** formed hollow tubes.⁹⁹ We also reported the self-assembly of both terminal-protected hydrophobic dipeptides with L/L, L/D, and D/L amino acids. In solution, L-Val-L-Ile and L-Ile-L-Val formed rod-like structures, while D-Val-L-Ile and L-Ile-D-Val formed hexagonal hollow tubes. In the crystalline state, L-Val-L-Ile and D-Val-L-Ile formed helix-like architectures, with D-Val-L-Ile adopting a hollow tube-like structure.¹⁰⁰ Additionally, our group examined the

dipeptide self-assembly containing unnatural amino acids (Ant and Aib) with different protecting groups. These peptides formed rod-like fibers and vesicular morphologies in solution. Some exhibited N₂ gas absorption capacity, while some demonstrated drug and dye encapsulation abilities.¹⁰¹ We also examined Boc-X-Trp-OMe (X = Val, Leu, Ile, and Phe) peptides self-assembly. All peptides exhibited spherical morphologies in solution and diverse self-assembly patterns in the crystalline state.¹⁰²

1.10. Small peptide-based metal ion detection

Small peptides aid in detecting heavy metal ions through various techniques. Excessive accumulation of these ions in the environment poses significant threats to both the ecosystem and human health. Additionally, an overabundance of essential metal ions in the body can lead to serious health issues. Lee and coworkers developed a fluorescence sensor based on the dipeptide dansyl-methionine-histidine, which selectively detected metal ions like Ag (I), Hg (II), and Cu (II).¹⁰³ Hwang and colleagues demonstrated the use of a dipeptide sensor (Py-Ser-Asp-COOH) for detecting Al (III) ions. This peptide-based probe effectively detected Al (III) ions in groundwater, tap water, and urine.¹⁰⁴ Jang and coworkers developed a pyrene-based dipeptide sensor (Pyr-WH) for ratiometric selective sensing of Ag (I) ions and Ag nanoparticles in aqueous solutions. Pyr-WH successfully entered living HeLa cells and exhibited a ratiometric detection of Ag(I) ions within the cell.¹⁰⁵ Neupane and colleagues developed a dipeptide fluorescence probe TPE-Ser-His-CONH₂ (TPE = tetraphenylethylene) to selectively detect Hg²⁺ and Al³⁺ ions. The buffer solution was essential for the aggregation-induced emission of the peptide sensor, influenced by different metal ions.¹⁰⁶

1.11. Green and sustainable peptide synthesis

Chemical waste management is crucial for mitigating the environmental impact. The majority of peptide synthesis methods rely heavily on non-recyclable and toxic coupling reagents and hazardous solvents, such as dimethylformamide (DMF), 1-methyl-2-pyrrolidinone (NMP), and dichloromethane (DCM), which exacerbate serious environmental and safety concerns. Solvents contribute to 80-90% of the total waste mass in a chemical process. Since the synthetic process does not eliminate the use of solvents,

researchers have attempted to replace these hazardous solvents with greener alternatives. Over the past 10 years, Albericio and his co-workers have significantly contributed to green peptide synthesis by finding less hazardous alternative solvents (water, acetonitrile, ethyl acetate, 2-methyltetrahydrofuran) to replace DMF and DCM.¹⁰⁷ Reducing the use of hazardous solvents with greener ones and recovering and recycling the same could be viable alternatives to minimize environmental impact. To overcome the problem of hazardous solvents, several novel alternative-green solvent mixtures such as dimethyl carbonate (DMC)/anisole, NBP/ethyl acetate (EtOAc), dimethyl sulfoxide (DMSO)/EtOAc, and DMSO/2-MeTHF, were found to have comparable polarity and viscosity to replace DMF in SPPS.¹⁰⁸

1.12. Knowledge gap

Although several coupling reagents based on Oxyma, benzotriazole, and azabenzotriazole have been reported, many of them exhibit significant drawbacks, such as harsh reaction conditions, poor stability, and solubility, limited versatility, poor racemization suppressing capacity, use of excess reagent, generation of toxic and unwanted by-products, etc. Therefore, developing new-generation coupling reagents is crucial to overcome the problem mentioned above. Sustainable peptide synthesis is also important to minimize the environmental impact.

While several ketone synthesis procedures have been established, the conversion of ketones from carboxylic acids remains limited. Organometallic reagents can achieve this transformation but require excess reagents, thus limiting their applicability.

While the self-assembly of L/L amino acid-containing peptides has been extensively studied, the specific roles of side chains and chirality in peptides containing L/L and L/D amino acids are largely unexplored. Additionally, a comparative analysis of their structural properties is lacking.

Meanwhile, peptides with amino acids such as histidine, tryptophan, and tyrosine have been widely investigated for metal ion sensing. However, the investigation of small peptides containing L/L and L/D amino acids, instead of histidine, tryptophan, and tyrosine, for heavy metal ion sensing, and the influence of the chirality of amino acids on the self-assembly of peptides remains largely unexplored.

Supramolecular self-assembly of enantiomeric Fmoc dipeptides containing one unnatural chiral amino acid has not been well explored. Their comparative studies and sensing capability for toxic heavy metal ions remain largely unexplored. Therefore, there is a need to investigate the supramolecular self-assembly of dipeptides containing one achiral and one unnatural chiral amino acid, focusing on heavy metal ion sensing.

1.13. Objectives of the thesis

The following objectives are proposed in my Ph.D. thesis work to address the aforementioned problems.

1. Developing Oxyma-based novel recyclable coupling reagent in a mild, straightforward, and sustainable manner and investigating its applications.
2. Developing novel heterocyclic recyclable coupling reagents and exploring their diverse applications in both solution and solid phases.
3. Development of a thiophene ring-containing HOAt-based novel coupling reagent aims to advance synthetic methodologies for creating C-N, C-O, and C-C bonds.
4. Investigation on self-assembly of dipeptides containing L/L and L/D amino acids, focusing on the influence of side chains and chirality of the amino acids.
5. Investigation of chirality controlled self-assembly of dipeptides and their applications in dye binding and selective sensing of Fe^{3+} .
6. Examining the supramolecular self-assembly of enantiomeric dipeptides containing one chiral unnatural amino acid and their applications in drug and dye binding and selective sensing of Cr^{6+} .

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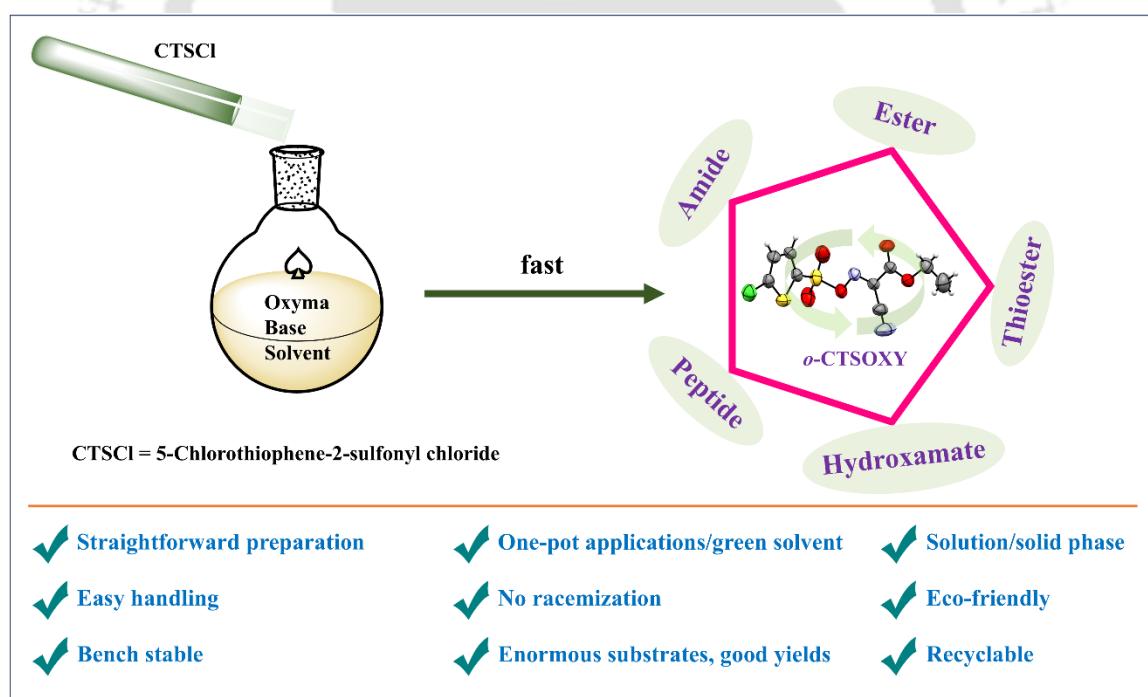
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Chapter 2

Rapid Generation of Oxyma-Based Novel Recyclable Coupling Reagent and its Applications



2.1. Background

Amide and ester bonds are prevalent in nature and are widely present in pharmaceuticals, polymers, and cosmetics.^{1, 2} Constructing these bonds typically involves activating the carboxylic acid group with a coupling reagent and introducing an amine or alcohol. Reagents like carbodiimides, phosphonium salts, and uronium salts are used,³ but challenges such as harsh conditions, excess base, toxic byproducts, and racemization persist. DCC, a common carbodiimide reagent, produces an insoluble byproduct (DCU), complicating final product purification and also causing racemization.⁴ Benzotriazole and azabenzotriazole-based reagents are often used to prevent racemization but can be explosive and toxic (e.g., BOP⁵). Recently, Oxyrna has been found to be a safer alternative to HOBt or HOAt but needs additives or support for effectiveness.⁶

Industrial chemical waste harms the environment and public health. To reduce waste, recovery and recycling of chemicals are trending. Avoiding metal catalysts and using recyclable organic reagents promote sustainability. Pharmaceutical companies are developing FDA-approved therapeutic amides, esters, and peptides,⁷ but peptide synthesis faces challenges due to hazardous coupling reagents and solvents.

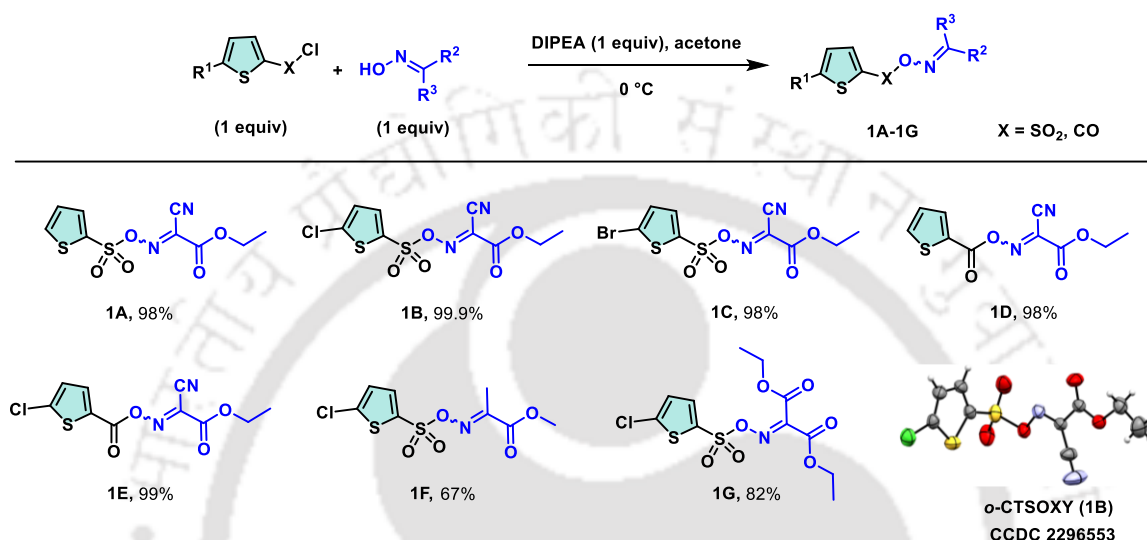
In this chapter, we developed an Oxyrna-based recyclable coupling reagent for the racemization-free synthesis of amides, hydroxamates, esters, thioesters, and peptides using green solvents in both solution and solid-phase. This new reagent and protocol offer the chemist a sustainable amidation and esterification method.

2.2. Design and synthesis of coupling reagent

Oxime such as ethyl-2-cyano-2-(hydroxyimino)acetate performs comparably to HOAt and outperforms HOBt in preventing racemization and facilitating challenging couplings in both manual and automated synthesis. Inspired by its unique reactivity, our interest focused on exploring sulfonate or carbonyl ester-containing Oxime-based new-generation coupling reagents to address critical amidation, esterification, and peptide synthesis. We envisaged that thiophene ring-containing Oxime-based coupling reagent could eliminate aforementioned limitations. We designed and synthesized a series of new reagents based on Oximes and thiophene sulfonyl or carbonyl chlorides with satisfactory yields (Table

2.1). The purification of the synthesized reagents did not require column chromatography; washing the organic part with mild acid and base was sufficient to achieve pure reagents. All the reagents were characterized using NMR (^1H , ^{13}C) and HRMS.

Table 2.1. Synthesis of Oxime-based reagents^a

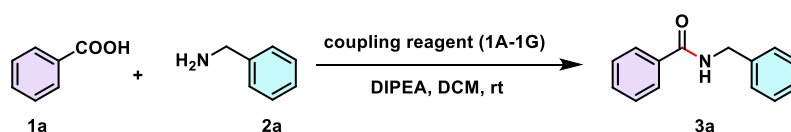


^aReaction conditions: Thiophene derivative (1 mmol), Oxime (1 mmol), DIPEA (1 mmol), acetone (2 mL), 0 °C, 5–10 min (**1A–1E**), 12 h (**1F** and **1G**).

2.3. Amidation using reagents 1A-1G

Next, the coupling efficiency of the synthesized reagents (**1A–1G**) was evaluated through amidation of benzoic acid and benzylamine in DCM with DIPEA at room temperature. Reagent *o*-CTSOXY (**1B**) yielded the highest product of **3a**, while **1F** was inactive (entries 2, 6, Table 2.2). Other reagents produced different yields of amide **3a** (Table 2.2). The results indicated that the sulfonate ester-containing Oxyma-based reagent was more effective than the carbonyl ester variant, likely due to its higher electrophilic character.

Table 2.2. Amidation using 1A-1G reagents



Entry ^a	Reagent	Yield ^b (%)
1	1A	34
2	1B	73
3	1C	53
4	1D	30
5	1E	68
6	1F	---
7	1G	21

^a1a (0.5 mmol), 2a (0.5 mmol), 1A-1G (0.5 mmol), DIPEA (0.6 mmol), DCM (1 mL), rt, 12 h (time after the addition of amine). ^bIsolated yields.

2.4. DFT study

We anticipated that the electronic factors within a substituted thiophene ring could significantly influence reagent reactivity. We hypothesized that an electron-withdrawing group at the 5th position would enhance reactivity. To compare the reactivity of 5-H (**1A**), 5-Cl (**1B**), and 5-NO₂ (**1X**, designed) substituted thiophene ring-containing Oxyma-based reagents, we performed a density functional theory (DFT) study using Gaussian 09W software.⁸ We computed HOMO and LUMO energies using the B3LYP/6-31G(d,p) basis set. A larger HOMO–LUMO energy gap indicates higher stability and lower reactivity.⁹ Reagent **1B** had a smaller HOMO–LUMO gap (4.462 eV) compared to **1A** (4.871 eV) and **1X** (4.727 eV), indicating higher reactivity (Figure 2.1).

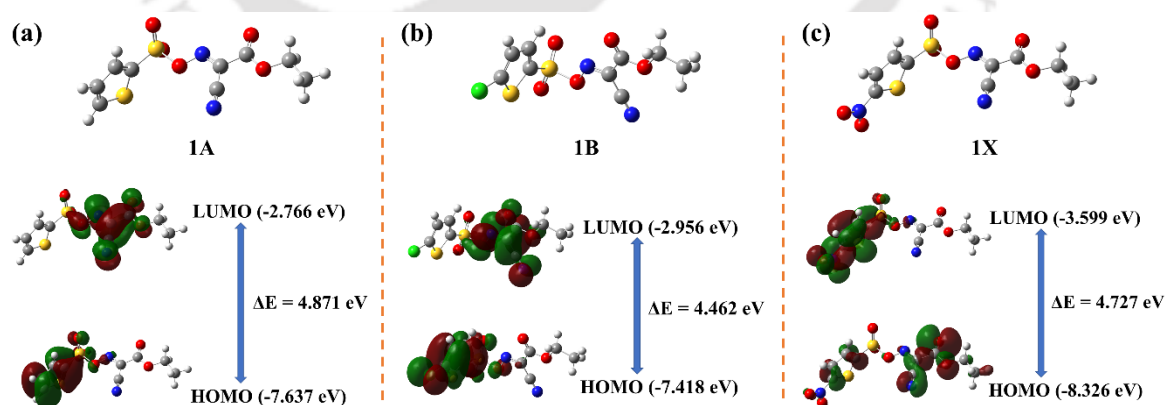
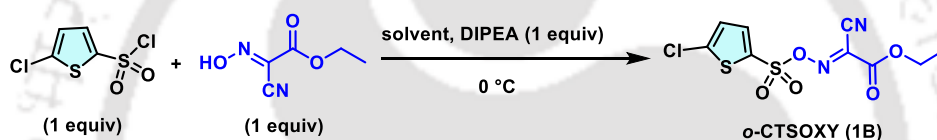


Figure 2.1. Gas phase optimized structure of **1A**, **1B**, and **1X**. ΔE (HOMO-LUMO) of (a) **1A** (4.871 eV), (b) **1B** (4.462 eV), (c) **1X** (4.727 eV).

2.5. Solvent screening and gram-scale synthesis of *o*-CTSOXY (**1B**)

Based on the positive experimental and theoretical results, we disclosed quick generation *o*-CTSOXY (**1B**) under mild, straightforward, and eco-friendly conditions and explored its applications in organic transformations. We screened several solvents, all of which provided excellent yields of **1B**. Acetone was selected as the optimal solvent due to its cost-effectiveness and eco-friendly nature. The synthetic strategy was effective on both small and large scales, achieving a 99.9% yield (14.85 g) of reagent **1B** on a large scale (entry 7, Table 2.3), demonstrating its industrial suitability. The stepwise scale-up processes are summarized in Table 2.3.

Table 2.3. Optimization of *o*-CTSOXY (1B**)**



Entry	Solvent	Y (mmol)	Time (min)	Yield ^b (%)
1 ^a	Acetone	1	5	99.9
2 ^a	EtOAc	1	5	99.7
3 ^a	DCM	1	5	99.5
4 ^a	ACN	1	5	99.6
5	Acetone	9.21	5	99.9
6	Acetone	23.03	5	99.9
7	Acetone	46.06	5	99.9

^a5-Chlorothiophene-2-sulfonyl chloride (1 mmol), ethyl (*E*)-2-cyano-2-(hydroxyimino)acetate (1 mmol), DIPEA (1 mmol), solvent (2 mL), 0 °C. Y: mmol of 5-chlorothiophene-2-sulfonyl chloride. ^bIsolated yields by workup.

2.6. Stability test of *o*-CTSOXY (**1B**)

No erosion was observed after exposing the reagent **1B** to air at room temperature for a week or storing it in a sealed container for a month. The stability of reagent **1B** was monitored for six months to one year under refrigerated conditions (0–4 °C) using analytical HPLC (Figure 2.2). The results showed excellent stability.

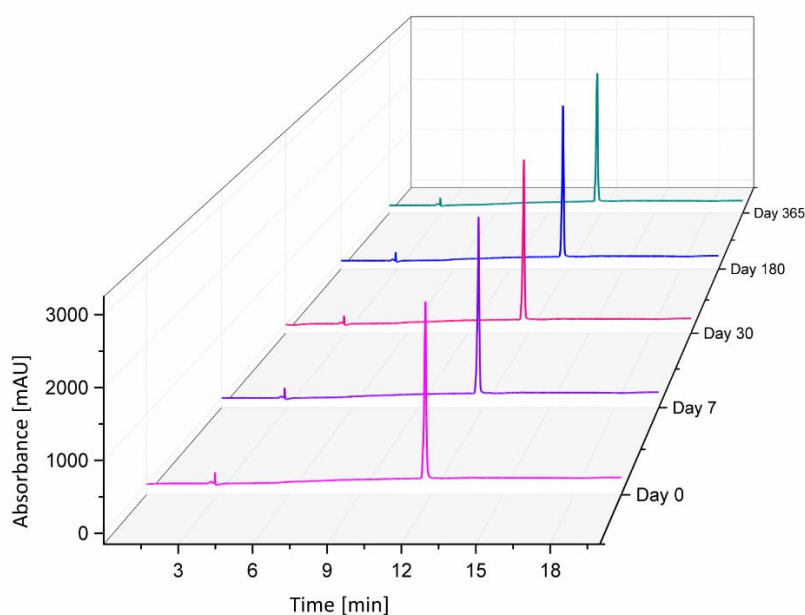


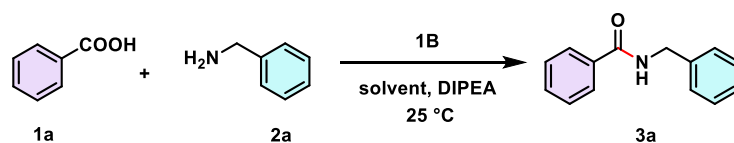
Figure 2.2. HPLC profile of *o*-CTSOXY (**1B**) from day zero to one year with constant retention time: 11.7 min. Thermo Scientific Vanquish system, Eclipse Plus C18 Agilent column (4.6 × 250mm, 5 μm), flow rate 1 mL.min⁻¹, linear gradient 5–100% acetonitrile in water, run time 20 min.

2.7. Reaction optimization, racemization study, and substrate scope

2.7.1. Optimization of amidation

The effective coupling efficiency of reagent **1B** provides a versatile platform for amidation and esterification. Our research focused on environmentally friendly amidation, esterification, and peptide synthesis. Reaction optimization revealed that solvents play a significant role in amidation (Table 2.4). Notably, DMF and acetone led to the highest yields of **3a** in amidation (entries 6, 8, Table 2.4). However, due to the hazardous nature of DMF, we chose acetone as the best solvent for its cost-effectiveness and eco-friendliness. Thus, acetone was selected as the optimal solvent for all processes.

Table 2.4. Optimization of amidation



Entry ^a	Solvent	Time ^b	Yield ^c (%)
1	DCM	12 h	73
2	THF	12 h	48
3	EtOAc	12 h	78
4	ACN	12 h	77
5	DMSO	3 h	70
6	DMF	30 min	96
7	CHCl ₃	12 h	54
8	Acetone	40 min	95
9	H ₂ O	28 h	---

^aReaction conditions: **1a** (0.5 mmol), **1B** (0.5 mmol), DIPEA (0.6 mmol), solvent (1 mL), **2a** (0.5 mmol), 25 °C. ^bTime after the addition of amine. ^cIsolated yields.

2.7.2. Racemization study

Racemization is an important factor in drug discovery. Many coupling reagents cause racemization during reactions. We investigated this by synthesizing Boc-DL-Phe-L-Phg-OMe and Boc-L-Phe-L-Phg-OMe using reagent **1B** and comparing their chiral HPLC profiles. Boc-DL-Phe-L-Phg-OMe showed two equally intense peaks with equal area, indicating a 1:1 mixture of diastereomers. In contrast, Boc-L-Phe-L-Phg-OMe showed a single peak, suggesting no racemization occurred during synthesis (Figure 2.3).

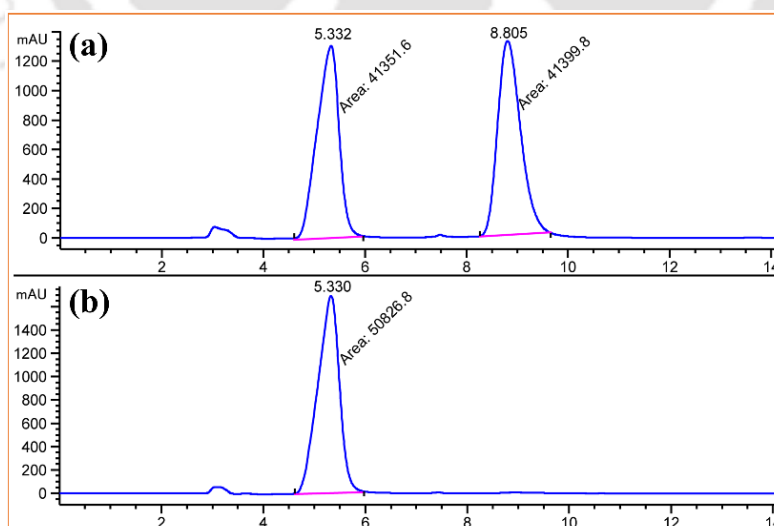


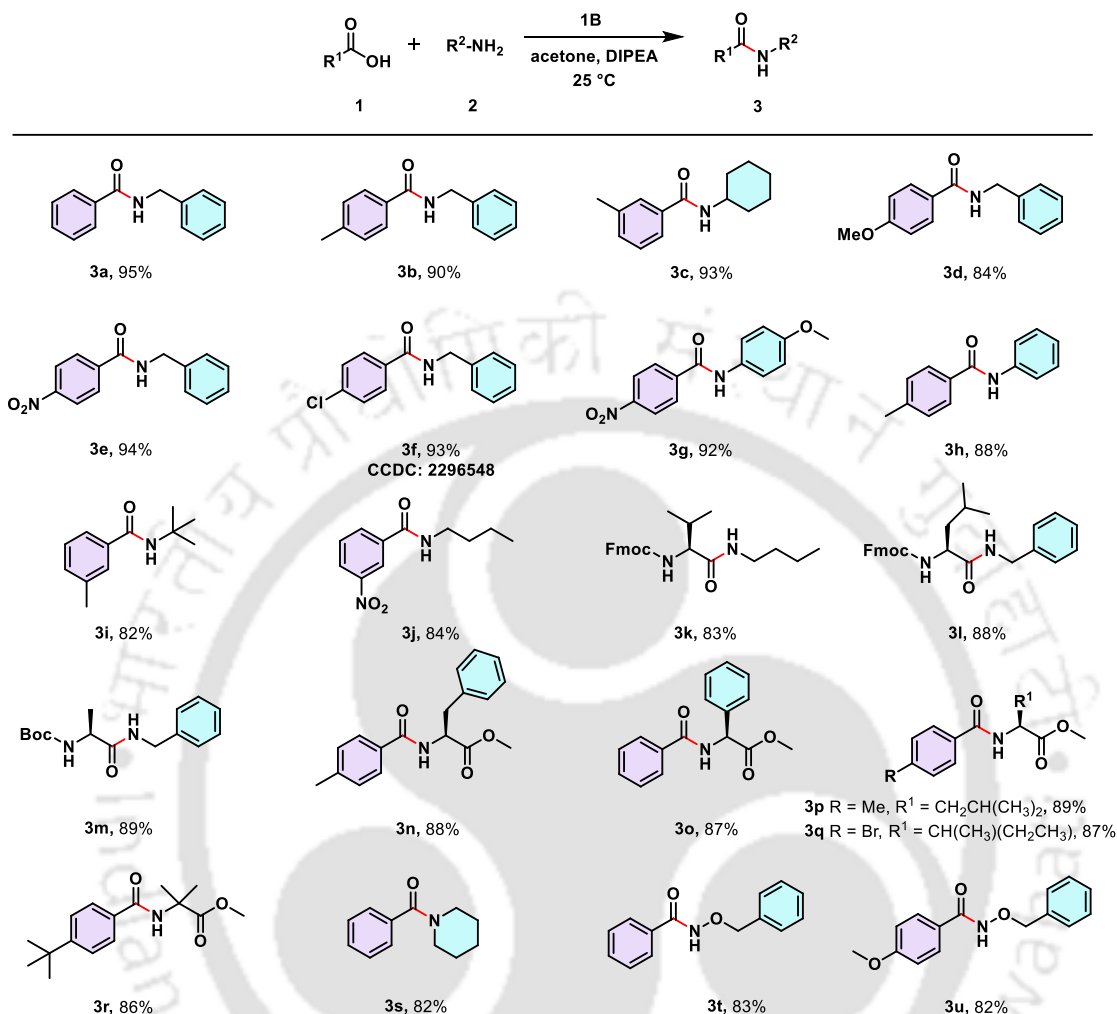
Figure 2.3. HPLC profile of (a) Boc-DL-Phe-L-Phg-OMe, (b) Boc-L-Phe-L-Phg-OMe. Agilent 1260 Infinity II prime LC system, CHIRALPAK® AS-H (5 μ m, 4.6 $\times$ 250 mm) column, 10% isopropanol in hexane, run time 20 min.

The results were also supported by 1D NMR (^1H and ^{13}C) spectroscopy. In ^1H NMR experiments, Boc-DL-Phe-L-Phg-OMe showed two peaks at δ 6.95, 6.86, 5.08, 5.00, 3.69, and 3.68 ppm with equal intensity and integration due to the presence of 1:1 diastereomers (Boc-D-Phe-L-Phg-OMe and Boc-L-Phe-L-Phg-OMe). Only one peak appeared for Boc-L-Phe-L-Phg-OMe at the same δ positions, indicating no racemization occurred during synthesis (Figure 2.7, 2.8). Similarly, in ^{13}C NMR, Boc-DL-Phe-L-Phg-OMe showed four peaks near δ 170 ppm and two peaks near 38 ppm, while Boc-L-Phe-L-Phg-OMe showed only two and one peaks at the same δ positions, respectively (experimental data).

2.7.3. Scope of amides and hydroxamates

After successfully completing the racemization test, we explored the scope of amidation under optimized conditions with various carboxylic acids and amines (Table 2.5). Both electron-donating and electron-withdrawing groups containing aromatic acids, as well as different side chains and protecting groups containing amino acids, participated actively in amide formation. Notably, reactions with 4- and 3-toluic acid produced the corresponding amides (**3b**, **3c**) with excellent yields of 90% and 93%, respectively. Benzoic acids with electron-donating (p-OMe) and electron-withdrawing (p-NO₂, p-Cl) groups reacted smoothly, yielding amides **3d–3f** with impressive yields (84%–94%). Even electron-deficient aromatic amines reacted efficiently under the optimized conditions, resulting in desired amides **3g** and **3h** with high yields (92% and 88%). This versatile transformation accommodated both sterically hindered and moderately long-chain hydrophobic amines, producing the desired amides **3i** and **3j** with satisfactory yields (82% and 84%). Additionally, Fmoc and Boc-protected amino acids were compatible under optimized conditions, yielding the desired amides **3k–3m** with excellent yields (83%–89%). C-protected hydrophobic and sterically hindered hydrophobic amino acids also participated actively in amide (**3n–3r**) formation under optimized conditions. Interestingly, secondary amine was effective, yielding the desired product **3s** with an 82% yield. Furthermore, even the poorly nucleophilic *O*-benzylhydroxylamine responded well under the optimized conditions, resulting in satisfactory yields of the desired hydroxamate products (**3t**, **3u**).

Table 2.5. Scope of amides and hydroxamates^a



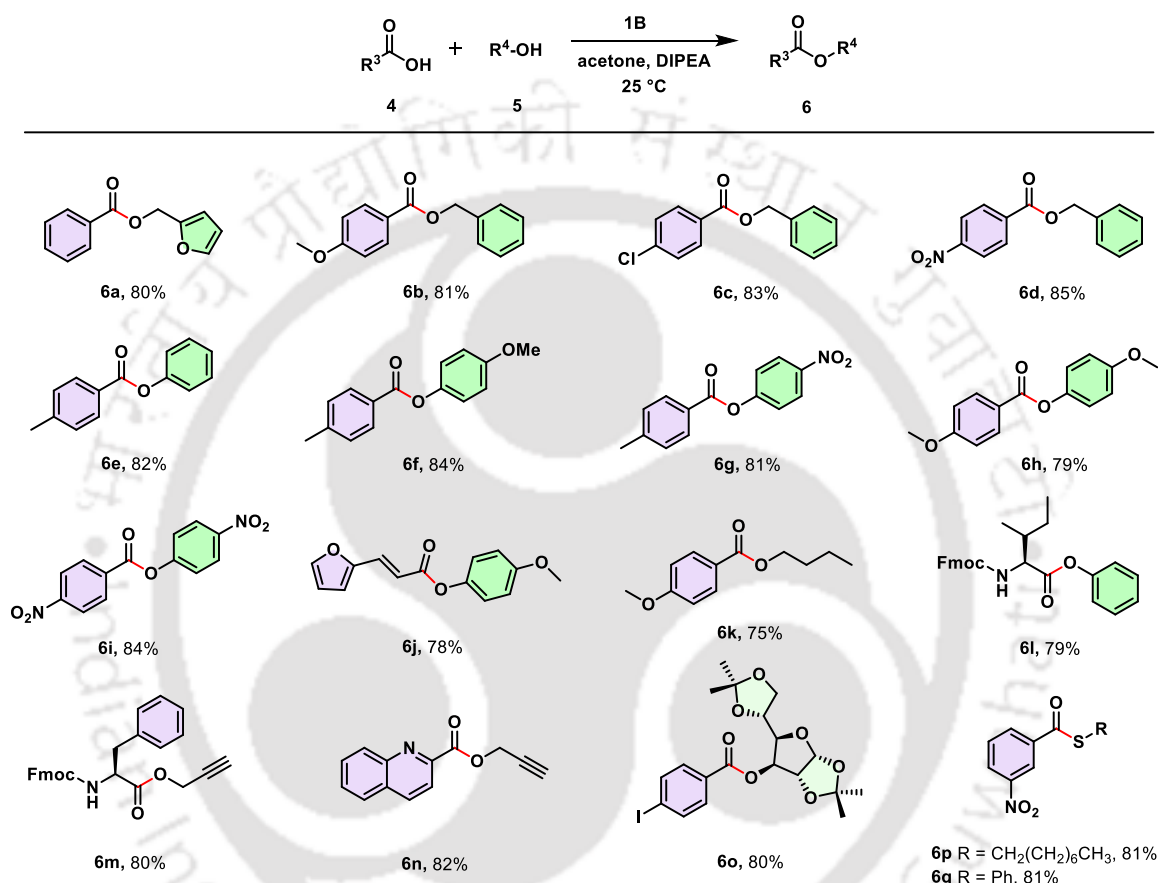
^aReaction conditions: **1** (0.5 mmol), **1B** (0.5 mmol), DIPEA (0.6 mmol), acetone (1 mL), **2** (0.5 mmol), 25 °C, reaction time: 40 min–3 h (time after the addition of nucleophile). Isolated yields.

2.7.4. Scope of esters and thioesters

The reaction generality was further examined by synthesizing various esters and thioesters under optimized conditions (Table 2.6). A heterocyclic alcohol yielded ester **6a** with 80%. Aromatic acids with electron-donating and withdrawing groups produced esters **6b–6d** with 81–85%. Poor nucleophilic aromatic alcohols also reacted well, yielding esters **6f–6i** with 79–84%. The α , β -unsaturated aromatic acid reacted efficiently, producing ester **6j** with a 78% yield. Moderately long-chain alcohols met the reaction criteria, yielding **6k** with a 75% yield. Fmoc amino acids were compatible with the

reaction conditions, yielding esters **6l** and **6m** with yields of 79% and 80%. Heteroaryl carboxylic acids formed ester **6n** with 82%. Acetonide-protected glucose yielded ester **6o** with 80%. Thioesters **6p** and **6q** were obtained with satisfactory yield.

Table 2.6. Scope of esters and thioesters^a



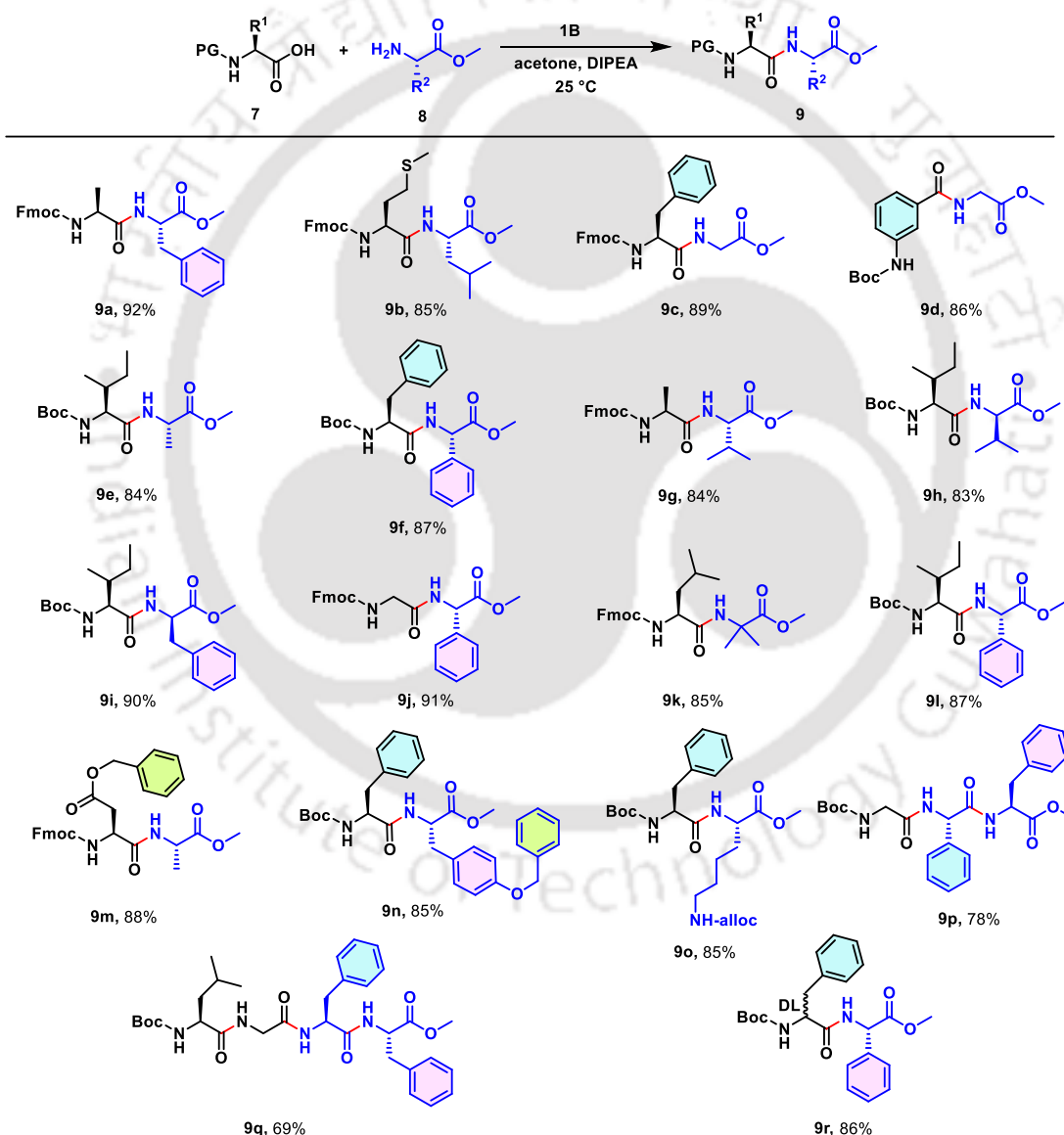
^aReaction conditions: **4** (0.5 mmol), **1B** (0.5 mmol), DIPEA (0.6 mmol), acetone (1 mL), **5** (0.5 mmol), 25 °C, reaction time: 30 min–5 h, 8 h for thioesters (time after the addition of nucleophile). Isolated yields.

2.7.5. Scope of peptides

Next, we employed this strategy for challenging peptide coupling reactions. All peptides were achieved with excellent yields without racemization at 25 °C (Table 2.7). Conventional protecting groups like Boc, Fmoc, Alloc, and Bzl were well tolerated. Fmoc and Boc-protected dipeptides **9a–9d** were synthesized with excellent yields (85–92%). Even sterically hindered amino acids such as Ile, Phg, Val, and Aib, which are typically challenging substrates for peptide coupling, efficiently yielded dipeptides **9e–9g**, and

9i–9k. Furthermore, installing sterically demanding amino acids at both C and N terminals of the dipeptides (**9h**, **9l**) was successfully achieved with good yields (83% and 87%). Side chain-protected amino acids also showed excellent coupling efficiency (**9m–9o**). We also successfully synthesized a tripeptide (**9p**) and a tetrapeptide (**9q**) with satisfactory yields (78% and 69%), demonstrating the versatility and effectiveness of our approach.

Table 2.7. Scope of peptides^a



^aReaction conditions: **7** (0.5 mmol), **1B** (0.5 mmol), DIPEA (0.6 mmol), acetone (1 mL), **8** (0.5 mmol), 25 °C, reaction time: 1 h–4 h (time after the addition of nucleophile). Isolated yields.

2.7.6. Gram scale synthesis and late-stage modification

The protocol successfully enabled the large-scale synthesis of amides and peptides and the late-stage modification of natural products and drugs. Notably, gram-scale synthesis of amide **3c** (using 7.34 mmol of acid) and peptide **9i** (using 4.32 mmol of amino acid) achieved excellent yields (**3c**: 94%, **9i**: 91%). Additionally, both the natural product and drug molecule offered excellent yields of desired products (**6r** and **3v**) (Figure 2.4).

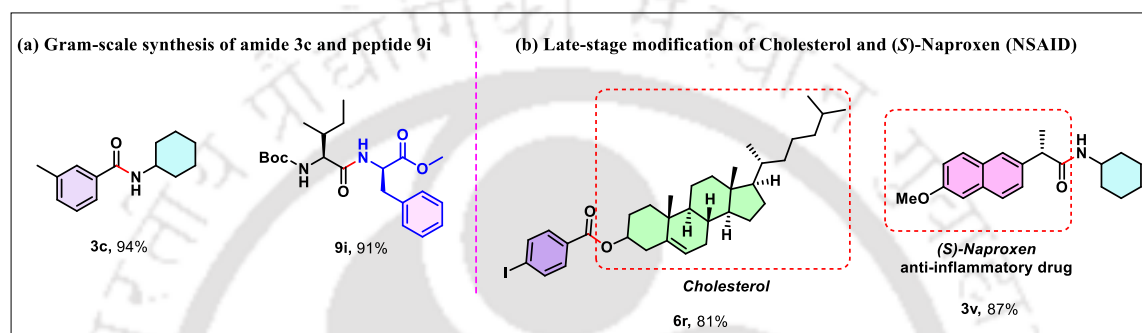


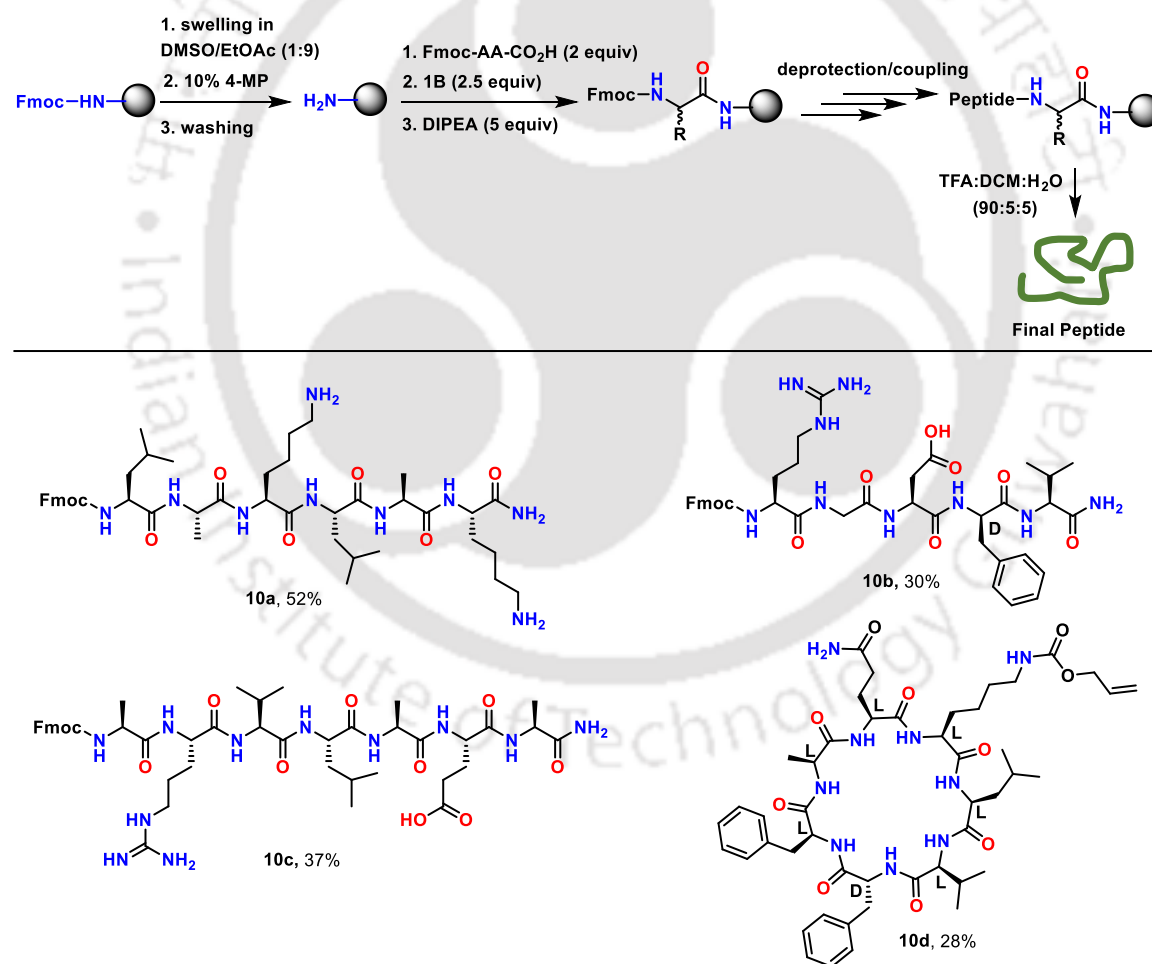
Figure 2.4. (a) Gram scale synthesis; (b) Late-stage modification of natural product and drug.

2.8. Solid phase peptide synthesis

Despite the establishment of various peptide synthesis methods, synthesizing long-chain peptides remains a significant challenge. This study aimed to replace hazardous solvents like DMF and DCM with environmentally friendly alternatives and develop a modified protocol using coupling reagent **1B** for sustainable SPPS. With this goal in mind, we initially targeted the synthesis of the Fmoc-LAKLAK-NH₂ peptide in solid-phase using a DMSO/EtOAc solvent mixture¹⁰ with reagent **1B**, following the Fmoc/*t*Bu¹¹ strategy. The successful synthesis of the Fmoc-LAKLAK-NH₂ peptide (**10a**) on rink amide resin offered 52% yield (step-wise synthesis of **10a** shown in Table 2.10). This peptide is a fragment of the apoptosis-inducing peptide KLA (KLAKLAK)₂.¹² In DMF and acetone, the peptide yield was 48% and 47%, respectively; interestingly, the DMSO/EtOAc mixture proved to be the most effective solvent. To examine the generality of the process in the DMSO/EtOAc mixture with reagent **1B**, we synthesized three biologically active moderately long-chain peptides. We synthesized the linear version (**10b**, 30%) of cyclo(-Arg-Gly-Asp-D-Phe-Val-); this cyclic pentapeptide demonstrates exceptional potency

and selectivity as an inhibitor of the $\alpha_v\beta_3$ integrin, making it a promising anticancer drug candidate.¹³ The synthesis of an HIV protease substrate and inhibitor-related peptide Fmoc-ARVLAEAA-NH₂ (**10c**) was also successfully done.¹⁴ The reagent is not limited to linear peptide synthesis but is also remarkably effective in synthesizing cyclic peptides. We synthesized a cyclic peptide Cy-K(alloc)LVfFAE (**10d**) by head-to-tail cyclization (Table 2.8), similar to the cyclic peptide Cy-KLVfFAE, which recognizes a part sequence of the A β peptide responsible for inhibiting A β_{1-40} aggregation.¹⁵ Therefore, the reagent demonstrated excellent potential in both solution and solid-phase applications in a sustainable manner.

Table 2.8. Scope of peptides^a



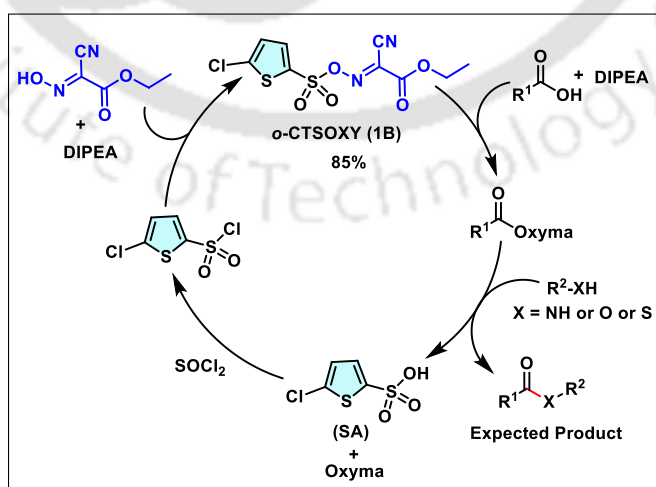
^aReaction conditions: Rink amide MBHA resin, loading: 0.7 mmol/g, 200 mg (0.14 mmol), amino acids (0.28 mmol), **1B** (0.35 mmol), DIPEA (0.7 mmol), solvent: DMSO/EtOAc (1:9), Fmoc deprotection: 10% 4-MP (4-Methylpiperidine) in DMSO/EtOAc, coupling time: 3 h. Isolated yields. Note, **AA** = amino acid.

2.9. Recovery of solvents and byproducts

Recovering and recycling solvents and reagents is essential for reducing environmental impact. We successfully recovered solvents after synthesizing reagent **1B** (46.06 mmol scale of 5-chlorothiophene-2-sulfonyl chloride) and amide **3c** (7.34 mmol scale of acid) using a rotary evaporator. Acetone was distilled off at 30 °C/350 mb pressure, achieving 95% (95 mL out of 100 mL) recovery for **1B** and 87% (13 mL out of 15 mL) recovery for amide **3c**. Byproducts like Oxyrna (90%, 939 mg) and 5-chlorothiophene-2-sulfonic acid (91%, 1.33 g) were efficiently retrieved through column chromatography after amidation (**3c**). We also recovered solvents from the SPPS waste stream after synthesizing the Fmoc-LAKLAK-NH₂ (**10a**) sequence using Rink amide MBHA resin (0.14 mmol). The total waste stream obtained was 180 mL, of which 162 mL was EtOAc and 18 mL was DMSO. We recovered 92% of EtOAc using 30 °C/140 mb pressure on the rotavapor and 83% of DMSO using the Kugelrohr Short-Path Distillation Apparatus.

2.10. Recyclability of reagent **1B**

Reagent **1B** was recycled from the recovered byproducts. The conversion of 5-chlorothiophene-2-sulfonic acid to its acid chloride was achieved by adding SOCl₂ in toluene along with a catalytic amount of DMF at 70 °C. Subsequently, it was readily converted to reagent **1B** (85% yield) by introducing Oxyrna and DIPEA (Scheme 2.1).



Scheme 2.1. Recycling of reagent **1B**

2.11. Yield comparison with *o*-NosylOXY

We conducted a yield comparison between compounds synthesized using reagent **1B** and the Oxyma-based coupling reagent *o*-NosylOXY. The comparison showed that reagent **1B** produced better yields in some cases than *o*-NosylOXY, while in others, it produced slightly lower yields (Table 2.9). This indicates that the newly synthesized reagent **1B** is nearly as efficient as our previously reported *o*-NosylOXY.

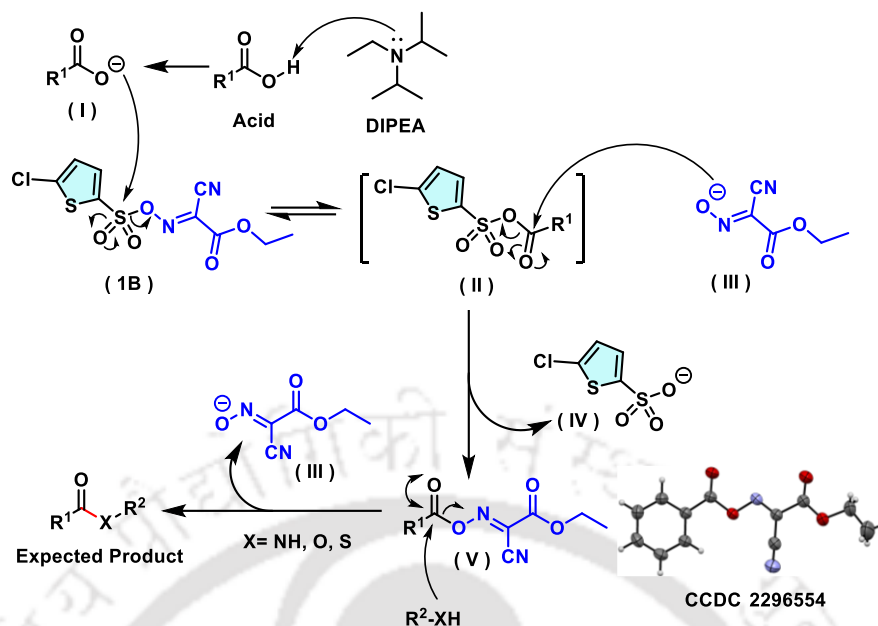
Table 2.9. Yield comparison

Entry	Compound	With reagent 1B	With <i>o</i> -NosylOXY ^a
1	Amide 3a	95%	88%
2	Ester 6d	85%	80%
3	Peptide 9a	92%	86%
4	Peptide 9b	85%	80%
5	Peptide 9g	84%	86%
6	Peptide 9h	83%	82%
7	Peptide 9i	90%	87%
8	Peptide 9j	91%	93%
9	Peptide 9k	85%	81%
10	Peptide 9l	87%	88%
11	Peptide 9m	88%	82%

^aYields using literature-reported optimized conditions.¹⁶

2.12. Plausible mechanism

The mechanism for synthesizing amides, esters, and peptides involves the following steps: Initially, DIPEA deprotonates the carboxylic acid, forming a carboxylate ion (I). The carboxylate ion (I) then attacks the sulfonyl center of **1B**, forming a mixed anhydride (II) with the elimination of Oxyma anion (III). The Oxyma anion (III) then attacks the carboxylic center of mixed anhydride (II), eliminating the sulfonate anion (IV) and forming intermediate (V). Intermediate (V) was obtained from a reaction between benzoic acid and reagent **1B**, characterized by NMR (¹H, ¹³C), HRMS, and SC-XRD. Reaction of intermediate (V) with nucleophiles (e.g., amine, amino acid, or alcohol) yields the desired product (amide, peptide, or ester) by eliminating the Oxyma anion (III) (Scheme 2.2).



Scheme 2.2. Plausible mechanistic pathway for amidation and esterification.

2.13. Conclusion

Sustainable chemical synthesis is essential for minimizing environmental impact. We have developed a novel, recyclable coupling reagent *o*-CTSOXY (**1B**) for the racemization-free synthesis of amides, hydroxamates, esters, thioesters, and peptides in a sustainable manner. Additionally, we have demonstrated solvent and byproduct recovery along with the recyclability of reagent **1B**, significantly reducing environmental impact. Reagent **1B** operates under mild and ambient conditions, ensuring complete retention of stereochemistry in chiral products. Other key advantages include (a) easy, straightforward synthesis of **1B** within a very short time (~5 min), (b) excellent bench stability of **1B**, (c) wide substrate compatibility, (d) use of environmentally friendly solvents, and (e) compatibility with both solution and solid-phase synthesis. This reagent and protocol adhere to green chemistry principles and are valuable additions to the peptide chemist's toolbox.

2.14. Experimental section

2.14.1. Materials and instrumentations

As described in chapter 8

2.14.2. General procedure for the synthesis of coupling reagent

DIPEA (1 mmol) was added to the Oxime (1 mmol) in acetone (2 mL). The reaction mixture was kept in ice-cold conditions (0 °C) with stirring. Substituted thiophene-2-sulfonyl or carbonyl chloride (1 mmol) was dropwise added to it. The reaction was continuously stirred at the same temperature. Next, TLC was performed to monitor the reaction progress. Upon reaction completion, acetone was evaporated, and ethyl acetate was added. The organic layer was subjected to washes with 10% citric acid and saturated NaHCO₃ solution. Finally, the organic part was dried over anhydrous Na₂SO₄, filtered, and the filtrate was evaporated using a rotavapor to yield the pure coupling reagent.

2.14.3. General procedure for synthesizing amides, hydroxamates, esters, and thioesters

Reagent **1B** (0.5 mmol) was introduced into the mixture of acid (0.5 mmol) and DIPEA (0.6 mmol) in acetone (1 mL). After pre-activation (for 15–20 minutes), a nucleophile (0.5 mmol) (such as an amine, O-benzylhydroxylamine, alcohol, or thiol) was introduced. The reaction progress was monitored by TLC. Post-reaction, acetone was evaporated, and ethyl acetate (EtOAc) was poured. The organic part was washed twice with 10% citric acid and then with saturated NaHCO₃ solution, respectively. The resulting organic portion was dried using anhydrous Na₂SO₄, filtered, and the filtrate was evaporated using a rotavapor to obtain the crude product. Column chromatography was performed to purify the crude product.

2.14.4. General procedure for synthesizing dipeptides

Reagent **1B** (0.5 mmol) was mixed with a stirred solution of N-protected amino acid (0.5 mmol) and DIPEA (0.6 mmol) in acetone (1 mL). After pre-activation (for 15–20 min), a neutralized solution of C-protected amino acid (0.5 mmol) was introduced. The depletion of the reactant was monitored by TLC. Post-reaction, acetone was evaporated, and EtOAc was poured. The organic layer was washed twice with 10% citric acid and saturated

NaHCO₃ solution, respectively. The resulting organic portion was dried using anhydrous Na₂SO₄, filtered, and the filtrate was evaporated using a rotavapor to obtain the crude peptide. The crude peptide was subsequently purified using column chromatography.

2.14.5. General procedure for synthesizing tri and tetrapeptides

Both N-Boc and C-methyl ester-protected dipeptides were synthesized using the described procedure. The Boc group was removed using a TFA/DCM (1:1) mixture, followed by evaporation. The mixture was washed with diethyl ether and neutralized using DIPEA in acetone. Simultaneously, another N-protected amino acid (0.5 mmol), reagent **1B** (0.5 mmol), and DIPEA (0.6 mmol) in acetone (1 mL) were pre-activated. The Boc-deprotected dipeptide solution was mixed with this mixture. Reaction progress was monitored by TLC. After completion, acetone was evaporated, and EtOAc was poured. The organic part was washed twice with 10% citric acid and a saturated NaHCO₃ solution, respectively. Next, the organic portion was dried with anhydrous Na₂SO₄, filtered, and the filtrate was evaporated to obtain the crude product. Purification of the crude was achieved through column chromatography.

2.14.6. General procedure for solid phase peptide synthesis (SPPS)

All designed peptides were manually synthesized within a frit-fitted plastic syringe using Fmoc/tBu solid-phase strategy. Rink amide MBHA resin was used as the solid support. The resin (200 mg, loading 0.7 mmol/g) was placed in a 2 mL frit-fitted plastic syringe and swollen in DMSO/EtOAc (1:9) for 3 hours on a Tarsons test tube rotator. Fmoc deprotection was achieved using 10% 4-methylpiperidine (4-MP)/(DMSO/EtOAc) (1:9) (3 times × 1.5 mL). After washing the resin with DMSO/EtOAc (1:9), a mixture of coupling reagent **1B** (0.35 mmol), acid (0.28 mmol), and DIPEA (0.7 mmol) in DMSO/EtOAc (1:9) was pre-activated for 5-10 minutes in a 25 mL beaker. This mixture was then added to the resin-filled syringe and rotated for 3 hours. Kaiser test was performed to check the complete coupling. The sequential Fmoc deprotection and amino acid coupling steps were continued until the desired final peptide sequence was obtained. The final peptide was detached from the resin using a TFA: DCM: H₂O (90:5:5) cocktail

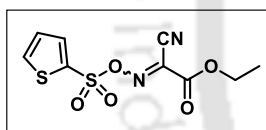
for 3 hours. The crude peptide was precipitated in cold diethyl ether and subsequently purified via reverse-phase HPLC.

2.14.6.1. Synthesis of Cy-K(alloc)LVfFAE

The solid-phase synthesis was started by attaching Fmoc-Glu-OMe to the resin via its side chain. Peptide coupling continued until the Fmoc-K(Alloc)LVfFAE-COOMe sequence was obtained using coupling reagent **1B**. The C-terminal methyl ester was removed with 5 equivalents of LiOH in THF/H₂O, followed by Fmoc-cleavage at the N-terminus using 10% 4-MP in DMSO/EtOAc. Head-to-tail cyclization was achieved with reagent **1B** (2 equiv) over 12 hours. The final peptide was cleaved from the resin, precipitated in cold ether, and then purified by reversed-phase HPLC.

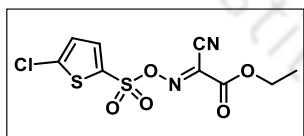
2.15. Characterization data

Ethyl-2-cyano-2-(((thiophen-2-ylsulfonyl)oxy)imino)acetate (**1A**):



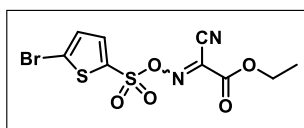
Yield: 98% (282 mg); light brown solid; ¹H NMR (600 MHz, CDCl₃) δ 7.95 (dd, J = 3.9, 1.3 Hz, 1H), 7.89 (dd, J = 5.0, 1.3 Hz, 1H), 7.24 (dd, J = 4.9, 4.0 Hz, 1H), 4.44 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 156.02, 137.95, 137.45, 131.94, 131.68, 128.44, 106.23, 64.90, 14.12; HRMS: (ESI) m/z : calcd for C₉H₉N₂O₅S₂, 288.9948 [M+H]⁺; found 288.9954.

Ethyl (E)-2-(((5-chlorothiophen-2-yl)sulfonyl)oxy)imino)-2-cyanoacetate (**1B**):

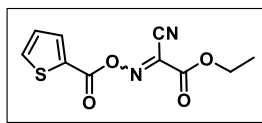


Yield: 99.9% (322.7 mg); faint yellowish solid; mp 58–60 °C; ¹H NMR (600 MHz, CDCl₃) δ 7.74 (d, J = 4.2 Hz, 1H), 7.07 (d, J = 4.2 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 155.88, 143.40, 137.58, 132.00, 129.52, 127.89, 106.13, 65.00, 14.11; HRMS: (ESI) m/z : calcd for C₉H₈ClN₂O₅S₂, 322.9558 [M+H]⁺; found 322.9556.

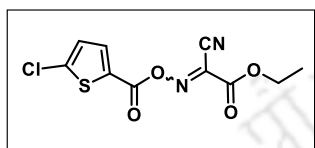
Ethyl-2-(((5-bromothiophen-2-yl)sulfonyl)oxy)imino)-2-cyanoacetate (**1C**):



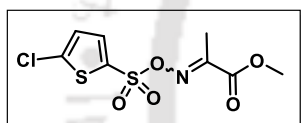
Yield: 98% (360 mg); faint brown solid; ¹H NMR (600 MHz, CDCl₃) δ 7.70 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.1 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 155.88, 138.16, 132.35, 131.96, 131.47, 126.31, 106.14, 65.02, 14.13; HRMS: (ESI) m/z : calcd for C₉H₇BrN₂NaO₅S₂, 388.8872 [M+Na]⁺; found 388.8870.

Ethyl-2-cyano-2-(((thiophene-2-carbonyl)oxy)imino)acetate (1D):

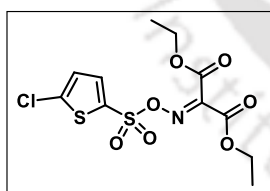
Yield: 98% (247 mg); light brown solid; ^1H NMR (600 MHz, CDCl_3) δ 8.09 (dt, $J = 3.7, 1.7$ Hz, 1H), 7.82 (d, $J = 4.9$ Hz, 1H), 7.24 (t, $J = 4.4$ Hz, 1H), 4.51 (q, $J = 7.1$ Hz, 2H), 1.44 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 157.09, 156.37, 136.87, 136.39, 131.55, 128.90, 128.21, 107.11, 64.77, 14.20; HRMS: (ESI) m/z : calcd for $\text{C}_{10}\text{H}_8\text{N}_2\text{NaO}_4\text{S}$, 275.0097 $[\text{M}+\text{Na}]^+$; found 275.0099.

Ethyl-2-(((5-chlorothiophene-2-carbonyl)oxy)imino)-2-cyanoacetate (1E):

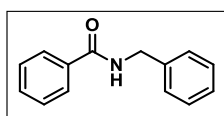
Yield: 99% (284 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.88 (d, $J = 4.1$ Hz, 1H), 7.07 (d, $J = 4.1$ Hz, 1H), 4.50 (q, $J = 7.1$ Hz, 2H), 1.43 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 156.92, 155.38, 141.92, 136.66, 131.82, 128.47, 126.30, 106.99, 64.85, 14.19; HRMS: (ESI) m/z : calcd for $\text{C}_{10}\text{H}_7\text{ClN}_2\text{NaO}_4\text{S}$, 308.9708 $[\text{M}+\text{Na}]^+$; found 308.9708.

Methyl-2-(((5-chlorothiophen-2-yl)sulfonyl)oxy)imino)propanoate (1F):

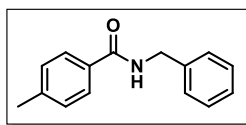
Yield: 67% (199 mg); light brown solid; ^1H NMR (600 MHz, CDCl_3) δ 7.66 (d, $J = 4.1$ Hz, 1H), 7.00 (d, $J = 4.1$ Hz, 1H), 3.88 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 162.56, 157.57, 141.18, 135.82, 131.77, 127.16, 53.69, 13.34; HRMS: (ESI) m/z : calcd for $\text{C}_8\text{H}_8\text{ClNNaO}_5\text{S}_2$, 319.9425 $[\text{M}+\text{Na}]^+$; found 319.9426.

Diethyl 2-(((5-chlorothiophen-2-yl)sulfonyl)oxy)imino)malonate (1G):

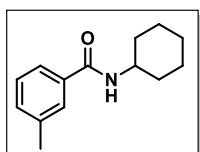
Yield: 82% (303 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.67 (d, $J = 4.1$ Hz, 1H), 7.02 (d, $J = 4.2$ Hz, 1H), 4.42 (q, $J = 7.1$ Hz, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.36 (q, $J = 7.4$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 158.00, 150.40, 142.02, 136.53, 134.64, 130.76, 127.45, 63.76, 14.11; HRMS: (ESI) m/z : calcd for $\text{C}_{11}\text{H}_{12}\text{ClKNO}_7\text{S}_2$, 407.9376 $[\text{M}+\text{K}]^+$; found 407.9376.

N-Benzylbenzamide (3a):

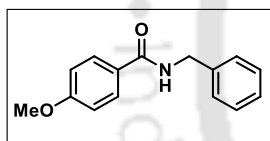
Yield: 95% (100 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, $J = 7.3$ Hz, 2H), 7.48 (t, $J = 7.4$ Hz, 1H), 7.40 (t, $J = 7.6$ Hz, 2H), 7.34 – 7.33 (m, 4H), 7.30 – 7.27 (m, 1H), 6.72 (s, 1H), 4.61 (d, $J = 4.8$ Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.59, 138.38, 134.55, 131.72, 128.95, 128.76, 128.07, 127.77, 127.16, 44.29; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{14}\text{NO}$, 212.1070 $[\text{M}+\text{H}]^+$; found 212.1084.

N-benzyl-4-methylbenzamide (3b):

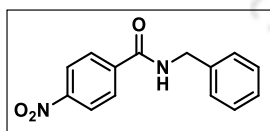
Yield: 90% (101 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.69 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 4.4 Hz, 4H), 7.30 – 7.27 (m, 1H), 7.21 (d, J = 7.9 Hz, 2H), 6.62 (s, 1H), 4.61 (d, J = 5.6 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.54, 142.10, 138.53, 131.67, 129.37, 128.89, 128.03, 127.67, 127.16, 44.18, 21.60; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{16}\text{NO}$, 226.1226 $[\text{M}+\text{H}]^+$; found 226.1221.

N-cyclohexyl-3-methylbenzamide (3c):

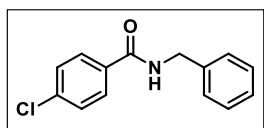
Yield: 93% (101 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.56 (s, 1H), 7.51 (d, J = 6.4 Hz, 1H), 7.31 – 7.27 (m, 2H), 5.97 (s, 1H), 3.97 (dtq, J = 11.7, 8.1, 3.8 Hz, 1H), 2.39 (s, 3H), 2.02 (dd, J = 12.7, 4.0 Hz, 2H), 1.75 (dt, J = 13.5, 3.9 Hz, 2H), 1.65 (dt, J = 13.1, 3.9 Hz, 1H), 1.47 – 1.38 (m, 2H), 1.22 (dtd, J = 16.5, 10.1, 8.5, 4.0 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.06, 138.56, 135.30, 132.15, 128.58, 127.79, 123.95, 48.82, 33.45, 25.79, 25.11, 21.55; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{19}\text{NNaO}$, 240.1359 $[\text{M}+\text{Na}]^+$; found 240.1387.

N-benzyl-4-methoxybenzamide (3d):

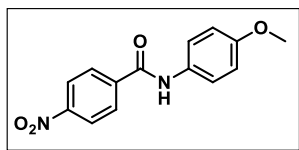
Yield: 84% (101 mg); colorless solid; ^1H NMR (600 MHz, CDCl_3) δ : 7.76 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 4.4 Hz, 4H), 7.29 (dt, J = 8.7, 4.1 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.47 (s, 1H), 4.61 (d, J = 5.8 Hz, 2H), 3.83 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.10, 162.40, 138.61, 128.98, 128.93, 128.08, 127.72, 126.82, 113.94, 55.59, 44.24; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{16}\text{NO}_2$, 242.1176 $[\text{M}+\text{H}]^+$; found 242.1181.

N-benzyl-4-nitrobenzamide (3e):

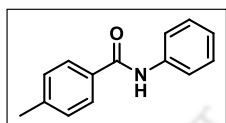
Yield: 94% (120 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.26 – 8.24 (m, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.38 – 7.30 (m, 5H), 6.62 (d, J = 27.1 Hz, 1H), 4.64 (d, J = 5.5 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.63, 149.74, 140.07, 137.64, 129.07, 128.40, 128.08, 128.07, 123.96, 44.58; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_3$, 257.0921 $[\text{M}+\text{H}]^+$; found 257.0916.

N-benzyl-4-chlorobenzamide (3f):

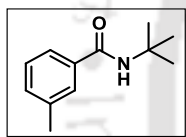
Yield: 93% (114 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.72 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.37 – 7.27 (m, 5H), 6.53 (d, J = 27.6 Hz, 1H), 4.61 (d, J = 5.7 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.56, 138.14, 137.97, 132.90, 129.00, 128.63, 128.09, 127.89, 44.38; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{ClNO}$, 246.0681 $[\text{M}+\text{H}]^+$; found 246.0677.

N-(4-methoxyphenyl)-4-nitrobenzamide (3g):

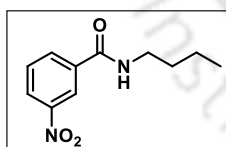
Yield: 92% (125 mg); yellowish solid; ^1H NMR (400 MHz, DMSO- d_6) δ 10.45 (s, 1H), 8.36 (d, J = 8.7 Hz, 2H), 8.17 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 163.41, 155.90, 149.07, 140.72, 131.74, 129.10, 123.55, 122.10, 113.85, 55.22; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_4$, 273.0870 $[\text{M}+\text{H}]^+$; found 273.0874.

4-methyl-N-phenylbenzamide (3h):

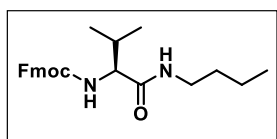
Yield: 88% (93 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.85 (s, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 7.7 Hz, 2H), 7.36 (t, J = 8.0 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 2.42 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.88, 142.57, 138.24, 132.31, 129.64, 129.27, 127.23, 124.63, 120.37, 21.71; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{14}\text{NO}$, 212.1070 $[\text{M}+\text{H}]^+$; found 212.1094.

N-(tert-butyl)-3-methylbenzamide (3i):

Yield: 82% (78 mg); faint pink solid; ^1H NMR (600 MHz, CDCl_3) δ 7.50 (s, 1H), 7.45 (d, J = 6.8 Hz, 1H), 7.24 (t, J = 6.1 Hz, 2H), 5.91 (s, 1H), 2.35 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.29, 138.47, 136.10, 131.95, 128.51, 127.67, 123.82, 51.73, 29.06, 21.54; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_{18}\text{NO}$, 192.1383 $[\text{M}+\text{H}]^+$; found 192.1397.

N-butyl-3-nitrobenzamide (3j):

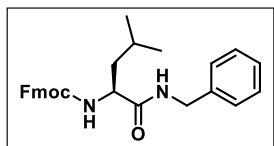
Yield: 84% (93 mg); faint yellowish solid; ^1H NMR (600 MHz, CDCl_3) δ 8.57 (t, J = 1.9 Hz, 1H), 8.32 (d, J = 8.2 Hz, 1H), 8.15 (d, J = 7.7 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 6.51 (s, 1H), 3.50 – 3.46 (m, 2H), 1.65 – 1.60 (m, 2H), 1.45 – 1.38 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.32, 148.32, 136.61, 133.45, 130.01, 126.09, 121.85, 40.37, 31.78, 20.34, 13.94; HRMS (ESI) m/z : calcd for $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3$, 223.1078 $[\text{M}+\text{H}]^+$; found 223.1097.

(9H-fluoren-9-yl)methyl (1-(butylamino)-3-methyl-1-oxobutan-2-yl)carbamate (3k):

Yield: 83% (164 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.89 (s, 1H), 5.44 (d, J = 8.3 Hz, 1H), 4.39 (dt, J = 17.0, 10.1 Hz, 2H), 4.21 (t, J = 6.9 Hz, 1H), 3.90 – 3.88 (m, 1H), 3.31 – 3.20 (m, 2H), 2.13 – 2.09 (m, 1H), 1.49 – 1.46 (m, 2H), 1.34 – 1.31 (m, 2H), 0.96 – 0.89 (m, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.24, 156.65, 144.01, 141.51, 127.93, 127.30, 125.25, 120.20, 67.22, 60.93,

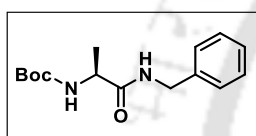
47.40, 39.48, 31.78, 31.26, 20.24, 19.44, 18.25, 13.91; HRMS (ESI) m/z : calcd for $C_{24}H_{30}N_2NaO_3$, 417.2149 $[M+Na]^+$; found 417.2126.

(9H-fluoren-9-yl)methyl (1-(benzylamino)-4-methyl-1-oxopentan-2-yl)carbamate (3l):



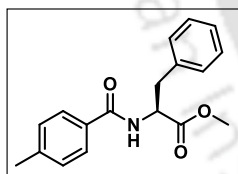
Yield: 88% (195 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, J = 7.3 Hz, 2H), 7.55 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.30 – 7.27 (m, 4H), 7.23 (t, J = 7.3 Hz, 3H), 6.45 (s, 1H), 5.29 (d, J = 7.9 Hz, 1H), 4.42 – 4.34 (m, 4H), 4.22 – 4.21 (m, 1H), 4.17 (t, J = 6.7 Hz, 1H), 1.68 – 1.64 (m, 2H), 1.56 (s, 1H), 0.93 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.27, 156.51, 143.94, 143.89, 141.51, 138.04, 128.90, 127.94, 127.82, 127.73, 127.30, 125.20, 120.20, 120.18, 67.17, 53.78, 47.36, 43.75, 41.61, 24.91, 23.15, 22.20; HRMS (ESI) m/z : calcd for $C_{28}H_{31}N_2O_3$, 443.2330 $[M+H]^+$; found 443.2338.

Tert-butyl (1-(benzylamino)-1-oxopropan-2-yl)carbamate (3m):



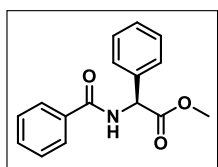
Yield: 89% (124 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.31 (t, J = 7.3 Hz, 2H), 7.25 (t, J = 7.3 Hz, 3H), 6.69 (s, 1H), 5.11 (s, 1H), 4.43 (s, 2H), 4.21 (s, 1H), 1.40 (s, 9H), 1.37 (d, J = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.79, 155.78, 138.27, 128.85, 127.75, 127.63, 80.35, 50.33, 43.57, 28.47, 18.48; HRMS (ESI) m/z : calcd for $C_{15}H_{22}N_2NaO_3$, 301.1523 $[M+Na]^+$; found 301.1532.

Methyl (4-methylbenzoyl)phenylalaninate (3n):

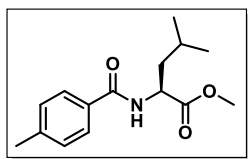


Yield: 88% (131 mg); faint pink solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, J = 8.1 Hz, 2H), 7.29 (t, J = 7.2 Hz, 2H), 7.25 (d, J = 7.9 Hz, 1H), 7.22 (d, J = 7.9 Hz, 2H), 7.13 (d, J = 6.9 Hz, 2H), 6.56 (d, J = 7.1 Hz, 1H), 5.09 (dt, J = 7.4, 5.6 Hz, 1H), 3.76 (s, 3H), 3.25 (ddd, J = 38.0, 13.9, 5.6 Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.33, 166.94, 142.47, 136.09, 131.22, 129.55, 129.47, 128.80, 127.36, 127.21, 53.66, 52.59, 38.13, 21.66; HRMS (ESI) m/z : calcd for $C_{18}H_{20}NO_3$, 298.1438 $[M+H]^+$; found 298.1435.

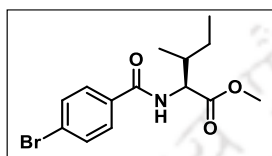
Methyl 2-benzamido-2-phenylacetate (3o):



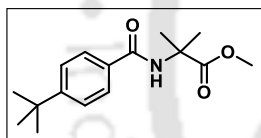
Yield: 87% (117 mg); faint yellowish solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 7.45 – 7.42 (m, 4H), 7.38 (t, J = 7.3 Hz, 2H), 7.35 – 7.32 (m, 1H), 7.18 (d, J = 6.0 Hz, 1H), 5.79 (d, J = 7.0 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.71, 166.73, 136.74, 133.78, 132.07, 129.23, 128.81, 128.79, 127.52, 127.34, 57.00, 53.12; HRMS (ESI) m/z : calcd for $C_{16}H_{16}NO_3$, 270.1125 $[M+H]^+$; found 270.1088.

Methyl (4-methylbenzoyl)leucinate (3p):

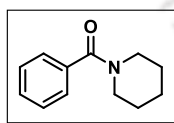
Yield: 89% (117 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (d, $J = 8.2$ Hz, 2H), 7.22 (d, $J = 7.9$ Hz, 2H), 6.55 (d, $J = 7.5$ Hz, 1H), 4.85 (td, $J = 8.4, 5.0$ Hz, 1H), 3.76 (s, 3H), 2.39 (s, 3H), 1.78 – 1.71 (m, 2H), 1.69 – 1.63 (m, 1H), 0.97 (d, $J = 6.8$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 174.01, 167.22, 142.38, 131.29, 129.42, 127.26, 52.54, 51.26, 42.12, 25.18, 23.03, 22.28, 21.64; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{22}\text{NO}_3$, 264.1595 $[\text{M}+\text{H}]^+$; found 264.1591.

Methyl 2-(4-bromobenzamido)-3-methylpentanoate (3q):

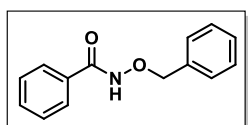
Yield: 87% (143 mg); colorless liquid; ^1H NMR (400 MHz, CDCl_3) δ 7.66 (d, $J = 8.5$ Hz, 2H), 7.56 (d, $J = 8.5$ Hz, 2H), 6.67 (d, $J = 8.0$ Hz, 1H), 4.78 (dd, $J = 8.4, 5.0$ Hz, 1H), 3.76 (s, 3H), 2.04 – 1.95 (m, 1H), 1.56 – 1.46 (m, 1H), 0.97 – 0.93 (m, 6H), 0.89 – 0.80 (m, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.75, 166.34, 133.13, 132.03, 128.87, 126.62, 57.04, 52.44, 38.41, 25.58, 15.66, 11.78; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{19}\text{BrNO}_3$, 328.0543 $[\text{M}+\text{H}]^+$; found 328.0560.

Methyl 2-(4-(tert-butyl)benzamido)-2-methylpropanoate (3r):

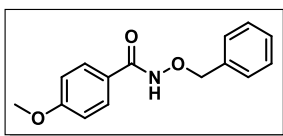
Yield: 86% (119 mg); faint pink solid; ^1H NMR (400 MHz, CDCl_3) δ 7.72 (d, $J = 8.4$ Hz, 2H), 7.44 (d, $J = 8.3$ Hz, 2H), 6.76 (s, 1H), 3.77 (s, 3H), 1.67 (s, 6H), 1.33 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 175.58, 166.73, 155.30, 131.85, 127.00, 125.68, 57.03, 52.95, 35.13, 31.37, 25.00; HRMS (ESI) m/z : calcd for $\text{C}_{16}\text{H}_{24}\text{NO}_3$, 278.1751 $[\text{M}+\text{H}]^+$; found 278.1751.

Phenyl(piperidin-1-yl)methanone (3s):

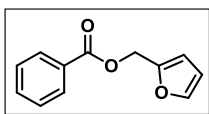
Yield: 82% (78 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.37 (s, 5H), 3.70 (s, 2H), 3.32 (s, 2H), 1.66 (s, 4H), 1.50 (s, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.50, 136.65, 129.52, 128.57, 126.94, 48.93, 43.29, 26.71, 25.78, 24.76; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_{16}\text{NO}$, 190.1226 $[\text{M}+\text{H}]^+$; found 190.1230.

N-(benzyloxy)benzamide (3t):

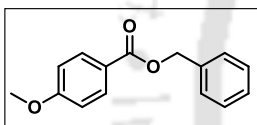
Yield: 83% (94 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.62 (s, 1H), 7.66 (d, $J = 7.3$ Hz, 2H), 7.51 (t, $J = 7.5$ Hz, 1H), 7.44 (d, $J = 6.4$ Hz, 2H), 7.42 – 7.36 (m, 5H), 5.03 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.66, 135.46, 132.30, 132.15, 129.57, 129.05, 128.92, 128.89, 127.26, 78.60; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{14}\text{NO}_2$, 228.1020 $[\text{M}+\text{H}]^+$; found 228.1014.

N-(benzyloxy)-4-methoxybenzamide (3u):

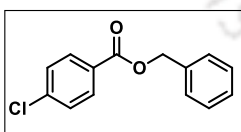
Yield: 82% (105 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.50 (s, 1H), 7.64 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 7.0 Hz, 2H), 7.38 (q, J = 7.7, 7.0 Hz, 3H), 6.89 (d, J = 8.7 Hz, 2H), 5.02 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 162.86, 135.61, 129.56, 129.14, 128.99, 128.87, 124.30, 114.15, 78.58, 55.62; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{16}\text{NO}_3$, 258.1125 $[\text{M}+\text{H}]^+$; found 258.1120; for $\text{C}_{15}\text{H}_{15}\text{NNaO}_3$, 280.0944 $[\text{M}+\text{Na}]^+$; found 280.0944.

Furan-2-ylmethyl benzoate (6a):

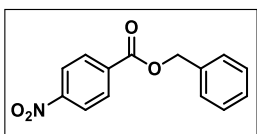
Yield: 80% (81 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.08 – 8.05 (m, 2H), 7.56 – 7.53 (m, 1H), 7.45 – 7.41 (m, 3H), 6.49 (d, J = 3.2 Hz, 1H), 6.39 (dd, J = 3.2, 1.9 Hz, 1H), 5.32 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.42, 149.74, 143.48, 133.26, 130.08, 129.93, 128.53, 110.97, 110.78, 58.70; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_{10}\text{NaO}_3$, 225.0523 $[\text{M}+\text{Na}]^+$; found 225.0527.

Benzyl 4-methoxybenzoate (6b):

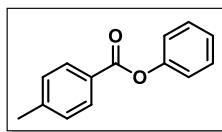
Yield: 81% (98 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.04 (d, J = 8.9 Hz, 2H), 7.45 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 6.92 (d, J = 8.9 Hz, 2H), 5.35 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.38, 163.63, 136.49, 131.93, 128.75, 128.33, 128.28, 122.73, 113.81, 66.58, 55.60; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{15}\text{O}_3$, 243.1016 $[\text{M}+\text{H}]^+$; found 243.1012.

Benzyl 4-chlorobenzoate (6c):

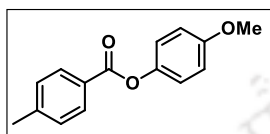
Yield: 83% (102 mg); colorless oil; ^1H NMR (600 MHz, CDCl_3) δ 8.05 – 7.99 (m, 2H), 7.48 – 7.34 (m, 7H), 5.37 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.76, 139.69, 135.98, 131.29, 128.92, 128.83, 128.77, 128.57, 128.44, 67.13; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{12}\text{ClO}_2$, 247.0521 $[\text{M}+\text{H}]^+$; found 247.0528.

Benzyl 4-nitrobenzoate¹⁷ (6d):

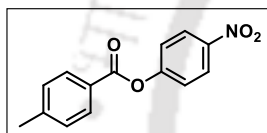
Yield: 85% (109 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.31 – 8.22 (m, 4H), 7.48 – 7.36 (m, 5H), 5.41 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 164.75, 150.80, 135.71, 135.44, 131.03, 128.96, 128.87, 128.65, 123.76, 67.86.

Phenyl 4-methylbenzoate (6e):

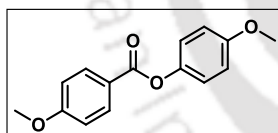
Yield: 82% (87 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.02 (d, J = 8.2 Hz, 2H), 7.36 – 7.33 (m, 2H), 7.23 (d, J = 8.0 Hz, 2H), 7.18 (t, J = 8.4 Hz, 1H), 7.13 (d, J = 7.6 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.45, 151.23, 144.60, 130.41, 129.65, 129.48, 127.02, 125.98, 121.96, 21.96; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{12}\text{NaO}_2$, 235.0730 $[\text{M}+\text{Na}]^+$; found 235.0746.

4-methoxyphenyl 4-methylbenzoate (6f):

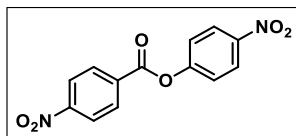
Yield: 84% (102 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.09 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.9 Hz, 2H), 6.94 (d, J = 9.0 Hz, 2H), 3.82 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.82, 157.44, 144.69, 144.50, 130.37, 129.45, 127.08, 122.69, 114.69, 55.80, 21.94; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{15}\text{O}_3$, 243.1016 $[\text{M}+\text{H}]^+$; found 243.1011.

4-Nitrophenyl 4-methylbenzoate¹⁸ (6g):

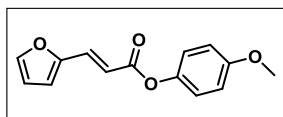
Yield: 81% (104 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.34 – 8.30 (m, 2H), 8.09 (d, J = 8.3 Hz, 2H), 7.44 – 7.39 (m, 2H), 7.34 (d, J = 7.9 Hz, 2H), 2.47 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 164.48, 156.05, 145.53, 145.49, 130.58, 129.72, 125.96, 125.45, 122.86, 22.04.

4-Methoxyphenyl 4-methoxybenzoate (6h):

Yield: 79% (102 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.15 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.9 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 3.89 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.47, 164.01, 157.39, 144.71, 132.42, 122.72, 122.13, 114.66, 113.99, 55.79, 55.68; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{15}\text{O}_4$, 259.0965 $[\text{M}+\text{H}]^+$; found 259.0971.

4-Nitrophenyl 4-nitrobenzoate¹⁹ (6i):

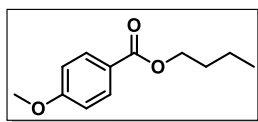
Yield: 84% (121 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.39 (s, 4H), 8.36 (d, J = 9.1 Hz, 2H), 7.46 (d, J = 9.1 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 162.65, 155.24, 151.43, 145.99, 134.10, 131.69, 125.66, 124.12, 122.68.

4-methoxyphenyl (E)-3-(furan-2-yl)acrylate (6j):

Yield: 78% (95 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.59 (d, J = 15.7 Hz, 1H), 7.53 (d, J = 1.3 Hz, 1H), 7.09 – 7.05 (m, 2H), 6.93 – 6.89 (m, 2H), 6.68 (d, J = 3.5 Hz, 1H), 6.51 – 6.47 (m, 2H), 3.81

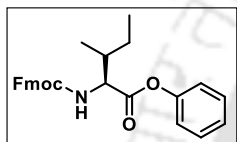
(s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.07, 157.39, 151.00, 145.37, 144.51, 132.69, 122.57, 115.77, 115.10, 114.65, 112.66, 55.80; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{O}_4$, 245.0809 $[\text{M}+\text{H}]^+$; found 245.0802.

Butyl 4-methoxybenzoate (6k):



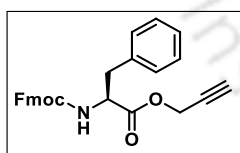
Yield: 75% (78 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.99 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.29 (t, J = 6.6 Hz, 2H), 3.85 (d, J = 1.3 Hz, 3H), 1.76 – 1.71 (m, 2H), 1.50 – 1.44 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.66, 163.44, 131.73, 123.18, 113.74, 64.73, 55.60, 31.03, 19.49, 13.97; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_{16}\text{NaO}_3$, 231.0992 $[\text{M}+\text{Na}]^+$; found 231.0999.

Phenyl 2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanoate (6l):



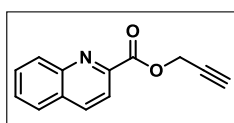
Yield: 79% (169 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.42 – 7.39 (m, 4H), 7.32 (t, J = 7.4 Hz, 2H), 7.27 – 7.25 (m, 1H), 7.10 (d, J = 7.9 Hz, 2H), 5.40 (d, J = 8.8 Hz, 1H), 4.61 (dd, J = 8.9, 4.8 Hz, 1H), 4.45 (d, J = 7.0 Hz, 2H), 4.26 (t, J = 7.0 Hz, 1H), 2.10 (d, J = 1.9 Hz, 1H), 1.37 – 1.29 (m, 2H), 1.08 (d, J = 6.8 Hz, 3H), 1.02 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.00, 156.36, 150.52, 143.93, 141.52, 129.74, 127.92, 127.29, 126.39, 125.27, 121.55, 120.21, 120.19, 67.29, 58.64, 47.41, 38.41, 25.40, 15.84, 11.90; HRMS (ESI) m/z : calcd for $\text{C}_{27}\text{H}_{28}\text{NO}_4$, 430.2013 $[\text{M}+\text{H}]^+$; found 430.2012.

Prop-2-yn-1-yl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninate (6m):



Yield: 80% (170 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.78 (d, J = 7.6 Hz, 2H), 7.57 (t, J = 8.0 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.33 – 7.27 (m, 5H), 7.14 (d, J = 7.1 Hz, 2H), 5.26 (d, J = 8.2 Hz, 1H), 4.80 – 4.70 (m, 3H), 4.44 (dd, J = 10.6, 7.3 Hz, 1H), 4.36 (dd, J = 10.5, 7.1 Hz, 1H), 4.22 (t, J = 7.0 Hz, 1H), 3.16 (ddd, J = 33.1, 13.9, 5.8 Hz, 2H), 2.53 (s, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.95, 155.72, 143.90, 141.51, 135.59, 129.62, 128.86, 127.92, 127.46, 127.26, 125.31, 120.20, 77.10, 75.82, 67.21, 54.84, 53.00, 47.33, 38.21; HRMS (ESI) m/z : calcd for $\text{C}_{27}\text{H}_{24}\text{NO}_4$, 426.1700 $[\text{M}+\text{H}]^+$; found 426.1697.

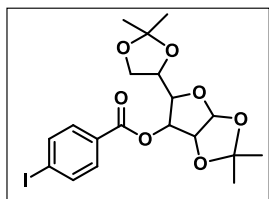
Prop-2-yn-1-yl quinoline-2-carboxylate (6n):



Yield: 82% (87 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ : 8.31 (d, J = 8.4 Hz, 2H), 8.21 (d, J = 8.4 Hz, 1H), 7.88 (dd, J = 8.1, 1.4 Hz, 1H), 7.80 (ddd, J = 8.5, 6.9, 1.3 Hz, 1H), 7.68 – 7.64 (m, 1H), 5.08 (d, J = 2.4 Hz, 2H), 2.55 (t, J = 2.4 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 164.83, 147.82, 147.47, 137.57,

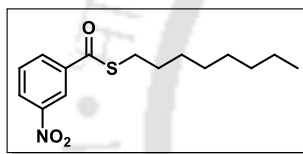
131.00, 130.59, 129.64, 129.03, 127.76, 121.33, 77.56, 75.69, 53.65; HRMS (ESI) m/z : calcd for $C_{13}H_{10}NO_2$, 212.0707 $[M+H]^+$; found 212.0706.

5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-6-yl-4-iodobenzoate (6o):



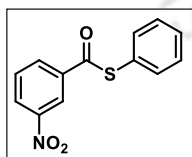
Yield: 80% (196 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 5.94 (d, J = 3.6 Hz, 1H), 5.48 (d, J = 2.4 Hz, 1H), 4.61 (d, J = 3.7 Hz, 1H), 4.35 – 4.27 (m, 2H), 4.10 (dd, J = 8.5, 5.5 Hz, 1H), 4.06 (dd, J = 8.7, 4.0 Hz, 1H), 1.55 (s, 3H), 1.40 (s, 3H), 1.31 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.95, 138.14, 131.25, 129.21, 112.62, 109.66, 105.33, 101.59, 83.54, 80.15, 77.07, 72.73, 67.54, 27.04, 26.92, 26.41, 25.40; HRMS (ESI) m/z : calcd for $C_{19}H_{23}I NaO_7$, 513.0381 $[M+Na]^+$; found 513.0386.

S-octyl 3-nitrobenzothioate (6p):



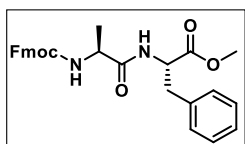
Yield: 81% (120 mg); colorless liquid; 1H NMR (600 MHz, $CDCl_3$) δ 8.79 (t, J = 1.8 Hz, 1H), 8.41 (ddd, J = 8.2, 2.2, 1.0 Hz, 1H), 8.27 (dt, J = 7.7, 1.2 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 3.12 (t, J = 7.4 Hz, 2H), 1.68 (p, J = 7.5 Hz, 2H), 1.42 (dt, J = 15.3, 7.2 Hz, 2H), 1.32 – 1.24 (m, 8H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.36, 148.44, 138.65, 132.89, 130.02, 127.60, 122.37, 31.97, 29.72, 29.51, 29.35, 29.28, 29.08, 22.84, 14.32; ESI-MS m/z : calcd for $C_{15}H_{21}NO_3S$, 295.3970 $[M]^+$; found 295.2866.

S-phenyl 3-nitrobenzothioate²⁰ (6q):



Yield: 81% (105 mg); yellowish solid; 1H NMR (600 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.33 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 8.0 Hz, 1H), 7.55 – 7.46 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 188.59, 148.62, 138.18, 135.16, 133.12, 130.25, 129.70, 128.01, 126.30, 122.64.

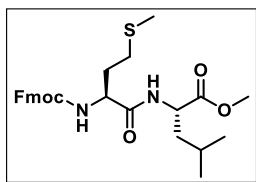
Methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanyl-L-phenylalaninate (9a):



Yield: 92% (217 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 6.3 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 7.23 (t, J = 7.0 Hz, 2H), 7.19 (d, J = 7.2 Hz, 1H), 7.07 (d, J = 7.3 Hz, 2H), 6.42 (s, 1H), 5.30 (s, 1H), 4.86 (q, J = 6.6 Hz, 1H), 4.40 (dd, J = 10.5, 7.2 Hz, 1H), 4.35 (t, J = 8.9 Hz, 1H), 4.21 (q, J = 8.2, 7.1 Hz, 2H), 3.72 (s, 3H), 3.12 (ddd, J = 48.1, 14.0, 5.9 Hz, 2H), 1.36 (d, J = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.96, 171.86, 156.08, 143.99, 141.52, 135.79, 129.44, 128.81, 127.97, 127.41, 127.31, 125.31,

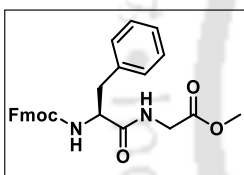
125.27, 120.23, 67.34, 53.39, 52.63, 50.60, 47.31, 38.00, 18.71; HRMS (ESI) m/z : calcd for $C_{28}H_{29}N_2O_5$, 473.2071 $[M+H]^+$; found 473.2064.

Methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-methionyl-L-leucinate (9b):



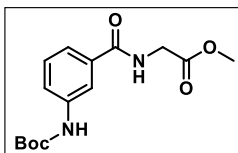
Yield: 85% (212 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ : 7.76 (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 7.5$ Hz, 2H), 7.40 (t, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.3$ Hz, 2H), 6.57 (d, $J = 6.8$ Hz, 1H), 5.60 (d, $J = 7.4$ Hz, 1H), 4.60 (td, $J = 8.7, 4.4$ Hz, 1H), 4.40 (dq, $J = 19.4, 10.2$ Hz, 3H), 4.21 (t, $J = 7.1$ Hz, 1H), 3.73 (s, 3H), 2.61 (d, $J = 7.6$ Hz, 2H), 2.13 (s, 3H), 2.07 (p, $J = 6.8$ Hz, 1H), 2.01 (dq, $J = 14.2, 7.2$ Hz, 1H), 1.68 – 1.62 (m, 2H), 1.55 (p, $J = 9.1, 7.7$ Hz, 1H), 0.94 – 0.90 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.21, 171.13, 156.19, 144.01, 143.87, 141.51, 141.50, 127.96, 127.29, 125.28, 125.25, 120.22, 120.20, 67.33, 53.58, 52.56, 51.08, 47.32, 41.46, 31.81, 30.06, 25.05, 22.96, 22.03, 15.20; HRMS (ESI) m/z : calcd for $C_{27}H_{35}N_2O_5S$, 499.2262 $[M+H]^+$; found 499.2262.

Methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-phenylalanylglycinate (9c):

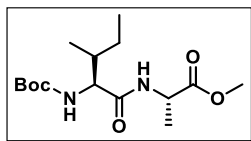


Yield: 89% (204 mg); white solid; 1H NMR (600 MHz, $DMSO-d_6$) δ 8.50 (t, $J = 5.7$ Hz, 1H), 7.87 (d, $J = 7.4$ Hz, 2H), 7.67 – 7.61 (m, 3H), 7.40 (q, $J = 6.6$ Hz, 2H), 7.32 (d, $J = 7.5$ Hz, 3H), 7.29 – 7.24 (m, 3H), 7.18 (t, $J = 7.3$ Hz, 1H), 4.31 – 4.27 (m, 1H), 4.18 – 4.09 (m, 3H), 3.89 (qd, $J = 17.4, 5.9$ Hz, 2H), 3.64 (s, 3H), 3.04 (dd, $J = 13.6, 3.2$ Hz, 1H), 2.81 – 2.77 (m, 1H); ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 172.20, 170.25, 155.84, 143.72, 140.66, 138.20, 129.24, 128.07, 127.65, 127.07, 126.27, 125.39, 120.10, 65.68, 56.03, 51.75, 46.55, 40.65, 37.43; HRMS (ESI) m/z : calcd for $C_{27}H_{27}N_2O_5$, 459.1915 $[M+H]^+$; found 459.1906.

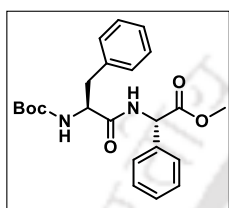
Methyl (3-((tert-butoxycarbonyl)amino)benzoyl)glycinate (9d):



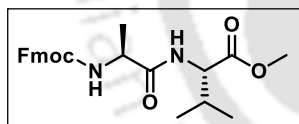
Yield: 86% (132 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.58 (s, 1H), 7.46 – 7.44 (m, 1H), 7.34 (t, $J = 7.8$ Hz, 1H), 6.80 (s, 2H), 4.23 (d, $J = 5.1$ Hz, 2H), 3.79 (s, 3H), 1.52 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.69, 167.46, 152.91, 139.14, 134.65, 129.52, 121.88, 121.65, 117.32, 81.12, 52.68, 41.94, 28.51; HRMS (ESI) m/z : calcd for $C_{15}H_{20}N_2NaO_5$, 331.1265 $[M+Na]^+$; found 331.1271.

Methyl (*tert*-butoxycarbonyl)-*L*-isoleucyl-*L*-alaninate (9e):

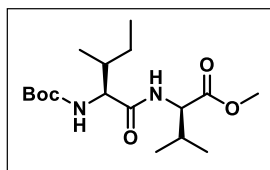
Yield: 84% (133 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 6.45 (s, 1H), 5.06 (s, 1H), 4.58 (p, $J = 7.3$ Hz, 1H), 3.95 (d, $J = 7.9$ Hz, 1H), 3.74 (s, 3H), 1.86 (s, 1H), 1.69 (d, $J = 22.7$ Hz, 1H), 1.44 (s, 9H), 1.41 (d, $J = 7.0$ Hz, 3H), 1.14 (ddq, $J = 14.3, 9.0, 7.3$ Hz, 1H), 0.95 – 0.89 (m, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.35, 171.32, 155.98, 80.15, 59.37, 52.66, 48.20, 37.57, 28.51, 24.93, 18.55, 15.67, 11.65; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{28}\text{N}_2\text{NaO}_5$, 339.1891 $[\text{M}+\text{Na}]^+$; found 339.1886.

Methyl (*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-phenylacetate (9f):

Yield: 87% (179 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.32 (d, $J = 5.6$ Hz, 3H), 7.28 – 7.21 (m, 5H), 7.16 (d, $J = 4.5$ Hz, 2H), 6.94 (s, 1H), 5.48 (d, $J = 5.3$ Hz, 1H), 4.99 (s, 1H), 4.42 (s, 1H), 3.69 (s, 3H), 3.11 (s, 1H), 3.03 (dd, $J = 13.8, 7.0$ Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.91, 170.77, 155.58, 136.64, 136.40, 129.59, 129.08, 128.90, 128.71, 127.41, 127.14, 80.51, 56.67, 55.70, 52.99, 38.30, 28.43; HRMS (ESI) m/z : calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_5$, 413.2071 $[\text{M}+\text{H}]^+$; found 413.2075; $\text{C}_{23}\text{H}_{28}\text{N}_2\text{NaO}_5$, 435.1890 $[\text{M}+\text{Na}]^+$; found 435.1890.

Methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-alanyl-*L*-valinate (9g):

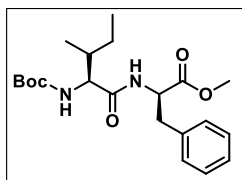
Yield: 84% (178 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 7.0$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.31 (t, $J = 7.3$ Hz, 2H), 6.54 (s, 1H), 5.44 (s, 1H), 4.54 (dd, $J = 7.8, 5.1$ Hz, 1H), 4.39 (d, $J = 6.2$ Hz, 2H), 4.36 – 4.28 (m, 1H), 4.21 (t, $J = 6.9$ Hz, 1H), 3.73 (s, 3H), 2.17 (h, $J = 6.7$ Hz, 1H), 1.41 (d, $J = 7.0$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.88 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.38, 156.20, 144.00, 143.94, 141.49, 127.94, 127.29, 125.26, 120.20, 67.35, 57.38, 52.41, 50.67, 47.29, 31.42, 19.12, 18.66, 17.90; HRMS (ESI) m/z : calcd for $\text{C}_{24}\text{H}_{29}\text{N}_2\text{O}_5$, 425.2071 $[\text{M}+\text{H}]^+$; found 425.2078.

Methyl (*tert*-butoxycarbonyl)-*L*-alloisoleucyl-*D*-valinate (9h):

Yield: 83% (143 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 6.61 (s, 1H), 5.08 (d, $J = 8.2$ Hz, 1H), 4.51 (dd, $J = 8.8, 4.9$ Hz, 1H), 4.02 (t, $J = 6.8$ Hz, 1H), 3.69 (s, 3H), 2.18 – 2.11 (m, 1H), 1.90 (dpd, $J = 10.4, 6.6, 3.7$ Hz, 1H), 1.52 – 1.43 (m, 1H), 1.41 (s, 9H), 1.14 – 1.05 (m, 1H),

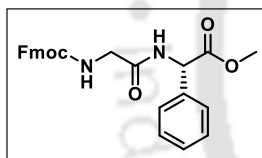
0.91 (t, $J = 7.2$ Hz, 6H), 0.87 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.41, 171.71, 155.90, 80.04, 59.55, 57.18, 52.27, 37.28, 31.26, 28.43, 24.75, 19.13, 17.89, 15.85, 11.70; HRMS (ESI) m/z : calcd for $\text{C}_{17}\text{H}_{32}\text{N}_2\text{NaO}_5$, 367.2204 $[\text{M}+\text{Na}]^+$; found 367.2208.

Methyl (tert-butoxycarbonyl)-L-isoleucyl-D-phenylalaninate (9i):



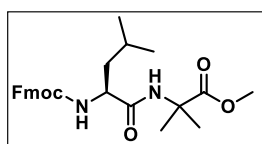
Yield: 90% (177 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ : 7.28 (t, $J = 8.1$ Hz, 2H), 7.23 (t, $J = 7.3$ Hz, 1H), 7.11 (d, $J = 7.2$ Hz, 2H), 6.43 (d, $J = 7.3$ Hz, 1H), 5.02 – 4.93 (m, 1H), 4.89 (q, $J = 6.6$ Hz, 1H), 4.00 (d, $J = 7.2$ Hz, 1H), 3.72 (s, 3H), 3.17 – 3.04 (m, 2H), 1.86 (dtd, $J = 10.3, 6.9, 4.1$ Hz, 1H), 1.43 (s, 9H), 1.35 (dtt, $J = 14.1, 6.7, 3.4$ Hz, 1H), 1.00 (dp, $J = 20.3, 6.9$ Hz, 1H), 0.85 (q, $J = 7.0$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.01, 171.42, 155.93, 135.95, 129.37, 128.86, 127.37, 80.14, 59.41, 53.21, 52.54, 38.21, 37.33, 28.49, 24.51, 15.67, 11.75; HRMS (ESI) m/z : calcd for $\text{C}_{21}\text{H}_{33}\text{N}_2\text{O}_5$, 393.2384 $[\text{M}+\text{H}]^+$; found 393.2390; $\text{C}_{21}\text{H}_{32}\text{N}_2\text{NaO}_5$, 415.2204 $[\text{M}+\text{Na}]^+$; found 415.2209.

Methyl (S)-2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-2-phenylacetate (9j):



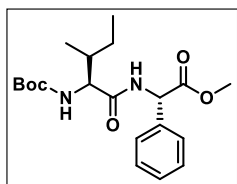
Yield: 91% (202 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.57 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.4$ Hz, 2H), 7.31 (dt, $J = 21.2, 6.4$ Hz, 7H), 7.14 – 7.06 (m, 1H), 5.56 (dd, $J = 25.1, 6.4$ Hz, 2H), 4.39 (d, $J = 7.2$ Hz, 2H), 4.21 (t, $J = 7.4$ Hz, 1H), 4.03 – 3.87 (m, 2H), 3.71 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.27, 168.51, 156.77, 143.94, 141.48, 136.19, 129.25, 128.93, 127.94, 127.45, 127.29, 125.27, 120.19, 67.52, 56.67, 53.14, 47.25, 44.54; HRMS (ESI) m/z : calcd for $\text{C}_{26}\text{H}_{24}\text{N}_2\text{NaO}_5$, 467.1577 $[\text{M}+\text{Na}]^+$; found 467.1581.

Methyl(S)-2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4methylpentanamido)-2-methylpropanoate (9k):



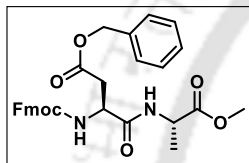
Yield: 85% (192 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.75 (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 7.4$ Hz, 2H), 7.39 (t, $J = 7.4$ Hz, 2H), 7.29 (t, $J = 7.4$ Hz, 2H), 6.74 (d, $J = 23.0$ Hz, 1H), 5.41 (dd, $J = 26.8, 8.6$ Hz, 1H), 4.46 – 4.33 (m, 2H), 4.20 (dt, $J = 12.9, 6.4$ Hz, 2H), 3.70 (s, 3H), 1.66 (dp, $J = 13.2, 7.9, 7.5$ Hz, 2H), 1.53 (s, 7H), 0.94 (t, $J = 5.6$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 174.88, 171.66, 156.47, 143.87, 141.46, 127.90, 127.24, 125.21, 120.16, 67.19, 56.65, 53.59, 52.78, 52.77, 47.30, 41.55, 24.95, 24.80, 23.09, 22.25; HRMS (ESI) m/z : calcd for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{O}_5$, 453.2384 $[\text{M}+\text{H}]^+$; found 453.2389.

Methyl (S)-2-(((2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methylpentanamido)-2-phenylacetate (9l):



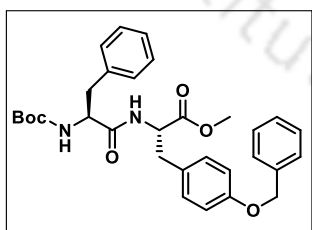
Yield: 87% (165 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.35 – 7.30 (m, 5H), 6.92 (s, 1H), 5.53 (d, J = 6.8 Hz, 1H), 5.03 (d, J = 6.6 Hz, 1H), 4.00 (s, 1H), 3.72 (s, 3H), 1.88 – 1.87 (m, 1H), 1.52 – 1.48 (m, 1H), 1.42 (s, 9H), 1.18 – 1.11 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.18, 171.17, 155.95, 136.38, 129.18, 128.79, 127.45, 80.19, 59.31, 56.69, 53.03, 37.41, 28.49, 24.98, 15.70, 11.61; HRMS (ESI) m/z : calcd for $\text{C}_{20}\text{H}_{31}\text{N}_2\text{O}_5$, 379.2227 $[\text{M}+\text{H}]^+$; found 379.2216; $\text{C}_{20}\text{H}_{30}\text{N}_2\text{NaO}_5$, 401.2047 $[\text{M}+\text{Na}]^+$; found 401.2035.

Benzyl (S)-3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(((S)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxobutanoate (9m):

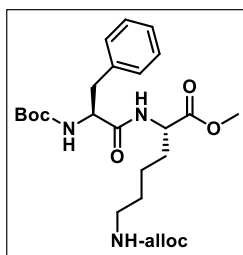


Yield: 88% (233 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.77 – 7.75 (m, 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.38 – 7.30 (m, 7H), 7.00 (d, J = 5.6 Hz, 1H), 5.95 (d, J = 7.7 Hz, 1H), 5.16 (q, J = 12.2 Hz, 2H), 4.63 (s, 1H), 4.54 (dt, J = 14.1, 7.0 Hz, 1H), 4.43 (d, J = 6.5 Hz, 2H), 4.22 (t, J = 6.9 Hz, 1H), 3.72 (s, 3H), 3.07 (d, J = 15.2 Hz, 1H), 2.75 (dd, J = 17.0, 6.0 Hz, 1H), 1.39 (d, J = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.02, 171.96, 170.09, 156.17, 143.91, 143.82, 141.51, 135.46, 128.82, 128.64, 128.49, 127.98, 127.30, 125.25, 125.21, 120.24, 120.22, 67.48, 67.21, 52.67, 51.02, 48.53, 47.30, 36.67, 18.21; HRMS (ESI) m/z : calcd for $\text{C}_{30}\text{H}_{31}\text{N}_2\text{O}_7$, 531.2126 $[\text{M}+\text{H}]^+$; found 531.2128.

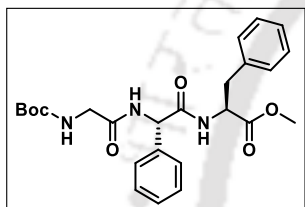
Methyl (S)-3-(4-(benzyloxy)phenyl)-2-(((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoate (9n):



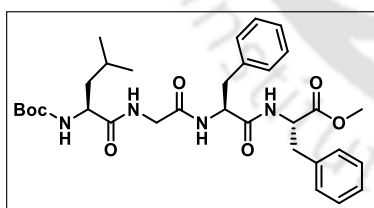
Yield: 85% (226 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.42 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.23 (t, J = 7.3 Hz, 1H), 7.19 (d, J = 7.1 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.25 (s, 1H), 5.02 (s, 2H), 4.95 (s, 1H), 4.73 (d, J = 4.8 Hz, 1H), 4.33 (s, 1H), 3.66 (s, 3H), 3.04 – 2.95 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.62, 170.90, 158.13, 137.14, 136.71, 130.47, 129.59, 128.88, 128.80, 128.20, 128.05, 127.71, 127.19, 115.10, 80.43, 70.19, 55.88, 53.61, 52.48, 38.49, 37.32, 28.46; HRMS (ESI) m/z : calcd for $\text{C}_{31}\text{H}_{37}\text{N}_2\text{O}_6$, 533.2647 $[\text{M}+\text{H}]^+$; found 533.2656.

Methyl *N*⁶-((allyloxy)carbonyl)-*N*²-((*tert*-butoxycarbonyl)-*L*-phenylalanyl)-*L*-lysinate (9o):

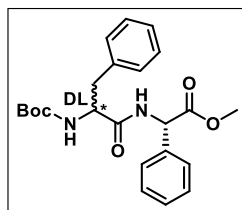
Yield: 85% (209 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.29 (t, *J* = 7.3 Hz, 2H), 7.24 (d, *J* = 7.1 Hz, 1H), 7.20 (d, *J* = 7.2 Hz, 2H), 6.54 (s, 1H), 5.91 (ddd, *J* = 22.2, 10.6, 5.4 Hz, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.20 (d, *J* = 10.4 Hz, 1H), 5.10 (s, 1H), 4.98 (s, 1H), 4.54 (d, *J* = 11.2 Hz, 3H), 4.37 (s, 1H), 3.70 (s, 3H), 3.18 – 3.02 (m, 4H), 1.83 – 1.77 (m, 1H), 1.53 – 1.45 (m, 2H), 1.40 (s, 9H), 1.29 – 1.25 (m, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 172.41, 171.44, 156.60, 136.78, 133.17, 129.54, 128.83, 127.13, 117.88, 80.46, 65.71, 55.95, 52.60, 52.10, 40.62, 38.31, 32.11, 29.35, 28.46, 22.23; HRMS (ESI) *m/z*: calcd for C₂₅H₃₈N₃O₇, 492.2705 [M+H]⁺; found 492.2710.

Methyl ((*S*)-2-(2-((*tert*-butoxycarbonyl)amino)acetamido)-2-phenylacetyl)-*L*-phenylalaninate (9p):

Yield: 78% (183 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.28 (d, *J* = 5.4 Hz, 1H), 7.22 – 7.15 (m, 8H), 7.00 (d, *J* = 7.0 Hz, 2H), 6.54 (s, 1H), 5.43 (d, *J* = 6.5 Hz, 1H), 5.20 (s, 1H), 4.73 (dd, *J* = 13.2, 6.2 Hz, 1H), 3.74 (ddd, *J* = 36.3, 17.0, 5.1 Hz, 2H), 3.57 (s, 3H), 3.03 (ddd, *J* = 60.1, 13.9, 5.9 Hz, 2H), 1.37 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 171.54, 169.58, 169.20, 156.19, 137.05, 135.73, 129.42, 129.18, 128.82, 128.71, 127.42, 127.37, 80.44, 57.11, 53.77, 52.60, 44.31, 37.79, 28.46; HRMS (ESI) *m/z*: calcd for C₂₅H₃₂N₃O₆, 470.2286 [M+H]⁺; found 470.2294.

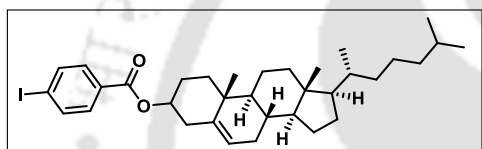
Methyl (*tert*-butoxycarbonyl)-*L*-leucylglycyl-*L*-phenylalanyl-*L*-phenylalaninate (9q):

Yield: 69% (206 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ: 7.25 – 7.14 (m, 7H), 7.12 (d, *J* = 7.2 Hz, 3H), 7.05 (d, *J* = 7.0 Hz, 2H), 6.92 (s, 1H), 5.34 – 5.23 (m, 1H), 4.78 (dq, *J* = 15.0, 6.9 Hz, 2H), 4.16 (d, *J* = 9.8 Hz, 1H), 3.83 (ddd, *J* = 63.5, 16.7, 5.1 Hz, 2H), 3.65 (s, 3H), 3.06 (dt, *J* = 14.2, 7.4 Hz, 2H), 2.97 (ddd, *J* = 30.3, 13.9, 7.0 Hz, 2H), 1.70 – 1.59 (m, 2H), 1.51 – 1.46 (m, 1H), 1.42 (s, 9H), 0.93 (t, *J* = 5.7 Hz, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 173.62, 171.86, 170.74, 168.99, 156.07, 136.58, 136.07, 129.54, 129.44, 128.72, 128.68, 127.21, 127.10, 80.30, 54.59, 53.61, 53.39, 52.52, 43.15, 41.53, 38.37, 38.15, 28.53, 24.94, 23.20, 22.09; HRMS (ESI) *m/z*: calcd for C₃₂H₄₅N₄O₇, 597.3283 [M+H]⁺; found 597.3295.

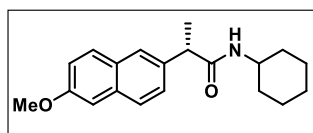
Boc-DL-Phe-L-Phg-OMe:

Yield: 86% (177 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.32 (q, J = 3.2 Hz, 6H), 7.28 – 7.25 (m, 3H), 7.25 – 7.13 (m, 9H), 7.13 – 7.09 (m, 2H), 6.95 (s, 1H), 6.86 (s, 1H), 5.49 (t, J = 8.3 Hz, 2H), 5.14 – 5.04 (m, 1H), 5.00 (s, 1H), 4.42 (s, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 3.11 (t, J = 9.8 Hz, 1H), 3.03 (q, J = 7.3, 6.9 Hz, 3H), 1.40 (s, 18H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.15, 170.92, 170.82, 170.78, 155.59, 155.58, 136.62, 136.39, 136.11, 129.59, 129.48, 129.13, 129.09, 128.90, 128.87, 128.73, 127.45, 127.42, 127.14, 127.09, 80.51, 80.48, 56.67, 56.57, 55.81, 55.68, 53.00, 38.54, 38.28, 28.43; HRMS (ESI) m/z : calcd for $\text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_5$, 413.2071 $[\text{M}+\text{H}]^+$; found 413.2045; $\text{C}_{23}\text{H}_{28}\text{N}_2\text{NaO}_5$, 435.1891 $[\text{M}+\text{Na}]^+$; found 435.1862.

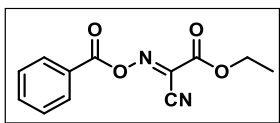
(8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl-4-iodobenzoate (6r)



Yield: 81% (250 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.81 (d, J = 8.5 Hz, 2H), 7.76 (d, J = 8.5 Hz, 2H), 5.44 (d, J = 4.2 Hz, 1H), 4.86 (dtd, J = 12.4, 8.4, 4.5 Hz, 1H), 2.47 (d, J = 7.6 Hz, 2H), 2.07 – 1.98 (m, 3H), 1.94 (dt, J = 13.5, 3.6 Hz, 1H), 1.86 (dtd, J = 13.3, 9.4, 5.9 Hz, 1H), 1.74 (dtd, J = 15.8, 12.5, 4.0 Hz, 1H), 1.56 – 1.47 (m, 4H), 1.42 – 1.33 (m, 3H), 1.32 – 1.24 (m, 3H), 1.24 – 1.10 (m, 7H), 1.08 (s, 3H), 1.06 – 0.99 (m, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (dd, J = 6.6, 2.7 Hz, 6H), 0.71 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.71, 139.73, 137.83, 131.25, 130.54, 123.13, 100.65, 75.16, 56.91, 56.36, 50.26, 42.54, 39.96, 39.74, 38.38, 37.22, 36.86, 36.41, 36.02, 32.15, 32.10, 28.45, 28.24, 28.06, 24.51, 24.05, 23.04, 22.78, 21.27, 19.59, 18.94, 12.09; HRMS (ESI) m/z : calcd for $\text{C}_{34}\text{H}_{50}\text{IO}_2$, 617.2851 $[\text{M}+\text{H}]^+$; found 617.2939.

(S)-N-cyclohexyl-2-(6-methoxynaphthalen-2-yl)propanamide (3v)

Yield: 87% (47 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.71 (dd, J = 8.7, 2.8 Hz, 2H), 7.65 (s, 1H), 7.37 (dd, J = 8.4, 1.8 Hz, 1H), 7.16 (dd, J = 8.9, 2.6 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 5.20 (d, J = 8.2 Hz, 1H), 3.92 (s, 3H), 3.74 (dtd, J = 10.9, 7.3, 4.0 Hz, 1H), 3.65 (q, J = 7.2 Hz, 1H), 1.88 – 1.80 (m, 1H), 1.77 (dd, J = 12.7, 4.3 Hz, 1H), 1.65 – 1.50 (m, 6H), 1.33 – 1.25 (m, 2H), 1.08 – 0.88 (m, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.52, 157.89, 136.97, 133.86, 129.43, 129.16, 127.67, 126.51, 126.26, 119.30, 105.82, 55.54, 48.39, 47.35, 33.17, 33.10, 25.65, 24.99, 24.93, 18.84; HRMS (ESI) m/z : calcd for $\text{C}_{20}\text{H}_{26}\text{NO}_2$, 312.1959 $[\text{M}+\text{H}]^+$; found 312.1954.

Ethyl (E)-2-((benzoyloxy)imino)-2-cyanoacetate (Bz-Oxyma, V):

Yield: 98% (121 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 8.20 (d, $J = 7.6$ Hz, 2H), 7.71 (t, $J = 7.4$ Hz, 1H), 7.56 (t, $J = 7.8$ Hz, 2H), 4.52 (q, $J = 7.2$ Hz, 2H), 1.45 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 160.87, 157.18, 135.37, 131.87, 130.80, 129.36, 125.97, 107.25, 64.76, 14.21; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{NaO}_4$, 269.0533 $[\text{M}+\text{Na}]^+$; found 269.0536.

Table 2.10. Stepwise synthesis of Fmoc-LAKLAK-NH₂ (10a)

Entry ^a	Peptide sequence	t_R /Total run time (min)	Expected mass (m/z)	Observed mass (m/z)
1	Fmoc-AK-NH ₂	6.9/15	$[\text{M}+\text{H}]^+$ 439.2340	439.2341
2	Fmoc-LAK-NH ₂	7.5/15	$[\text{M}+\text{H}]^+$ 552.3181	552.3184
3	Fmoc-KLAK-NH ₂	6.8/15	$[\text{M}+\text{H}]^+$ 680.4131	680.4138
4	Fmoc-AKLAK-NH ₂	6.9/15	$[\text{M}+\text{H}]^+$ 751.4502	751.4507
5	Fmoc-LAKLAK-NH ₂ (10a)	7.5/15	$[\text{M}+\text{H}]^+$ 864.5342	864.5343

HPLC: ^aThermo Scientific system, Poroshell 120 EC C18 Agilent column (4.6 × 100mm, 4 μm). t_R = HPLC retention time.

Table 2.11. HPLC retention time and observed mass of solid phase synthesized peptides

Entry	Peptide sequence	t_R /Total run time (min)	Expected mass (m/z)	Observed mass (m/z)
1 ^a	Fmoc-RGDfV-NH ₂ (10b)	10.2/15	$[\text{M}+3\text{H}]^+$ 816.4039	816.9070
2 ^a	Fmoc-ARVLAEA-NH ₂ (10c)	9.1/15	$[\text{M}+\text{H}]^+$ 950.5095	950.5095
3 ^b	Cy-K(alloc)LVfFAE (10d)	8.3/15	$[\text{M}+\text{H}]^+$ 918.5084	918.5090

HPLC: ^aAgilent 1260 Infinity II prime LC system, Eclipse Plus C18 Agilent column (4.6 × 250mm, 5 μm).

^bThermo Scientific system, Poroshell 120 EC C18 Agilent column (4.6 × 100mm, 4 μm). t_R = HPLC retention time.

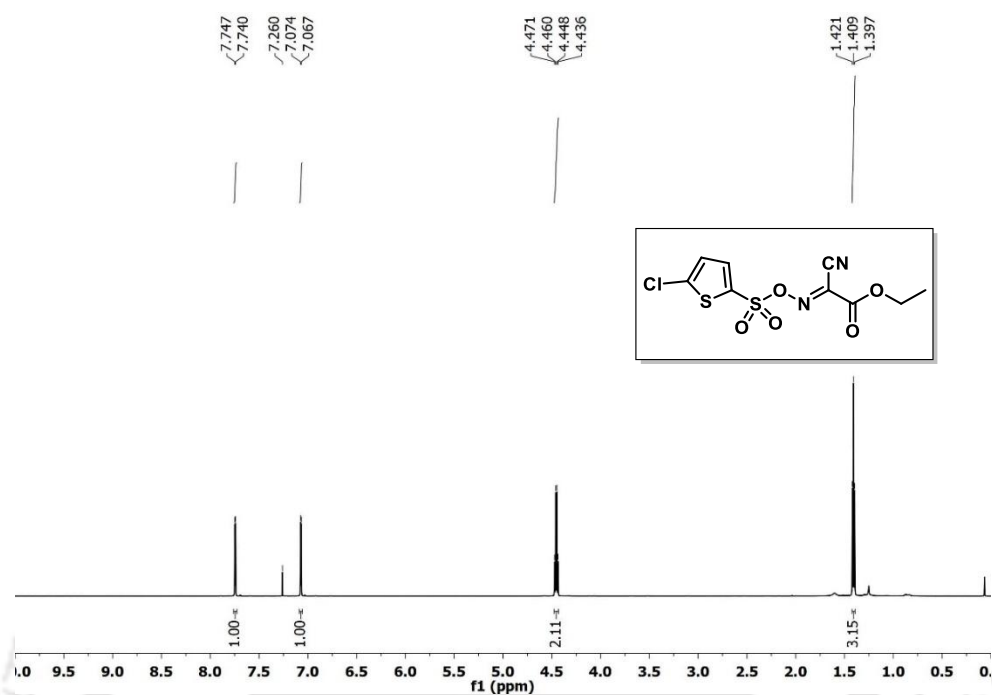
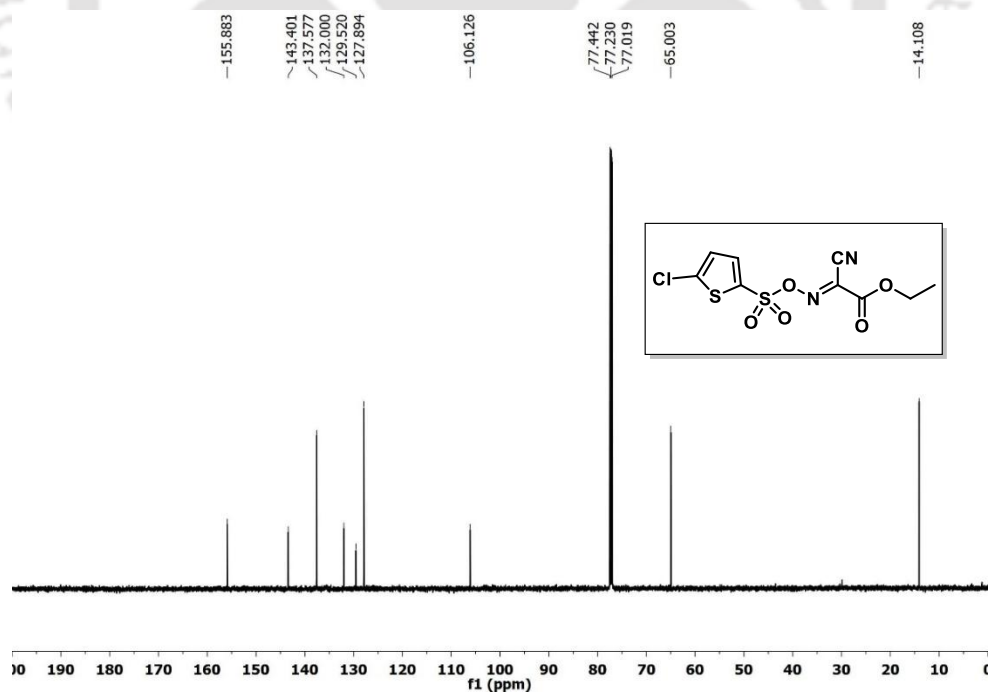
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2.17. Selected spectra

Figure 2.5. ¹H NMR spectrum of coupling reagent (o-CTSOXY, **1B**)Figure 2.6. ¹³C NMR spectrum of coupling reagent (o-CTSOXY, **1B**)

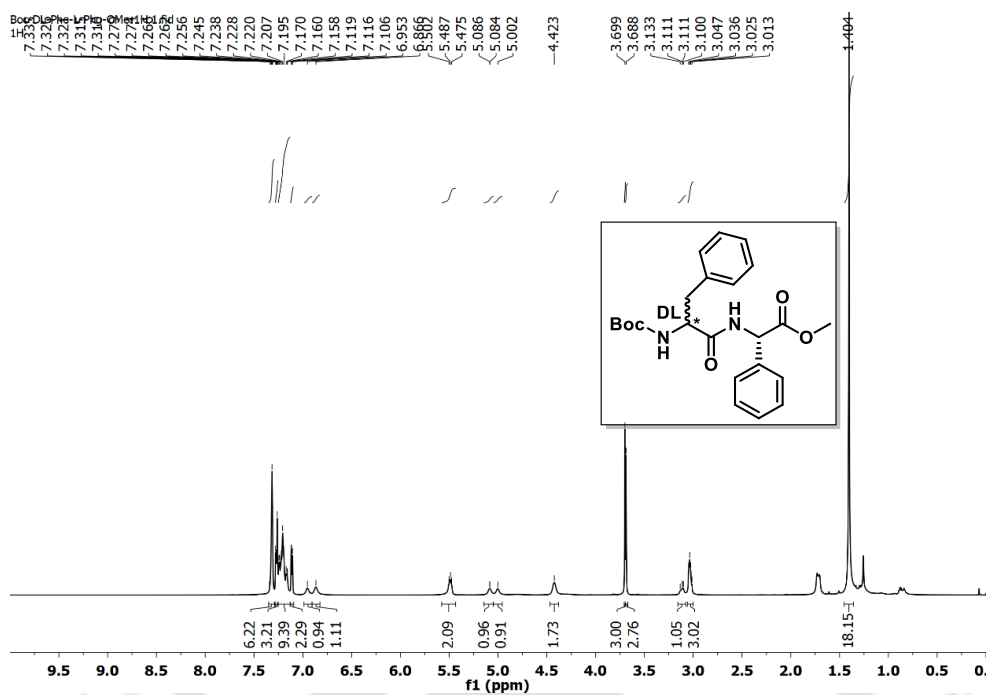


Figure 2.7. ¹H NMR spectrum of peptide **Boc-DL-Phe-L-Phg-OMe (9r)**

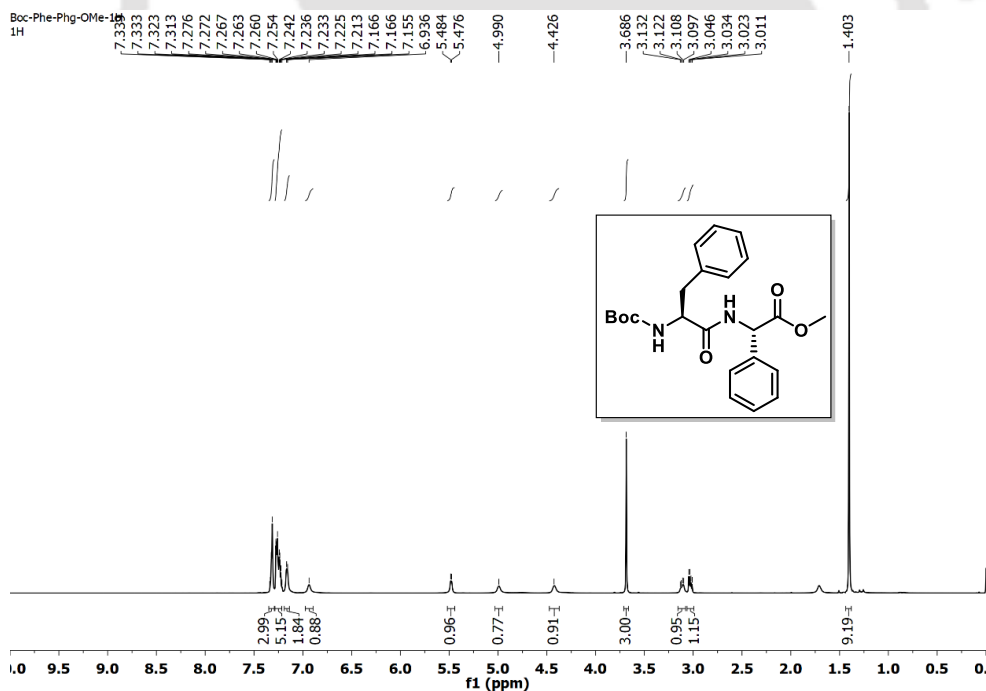
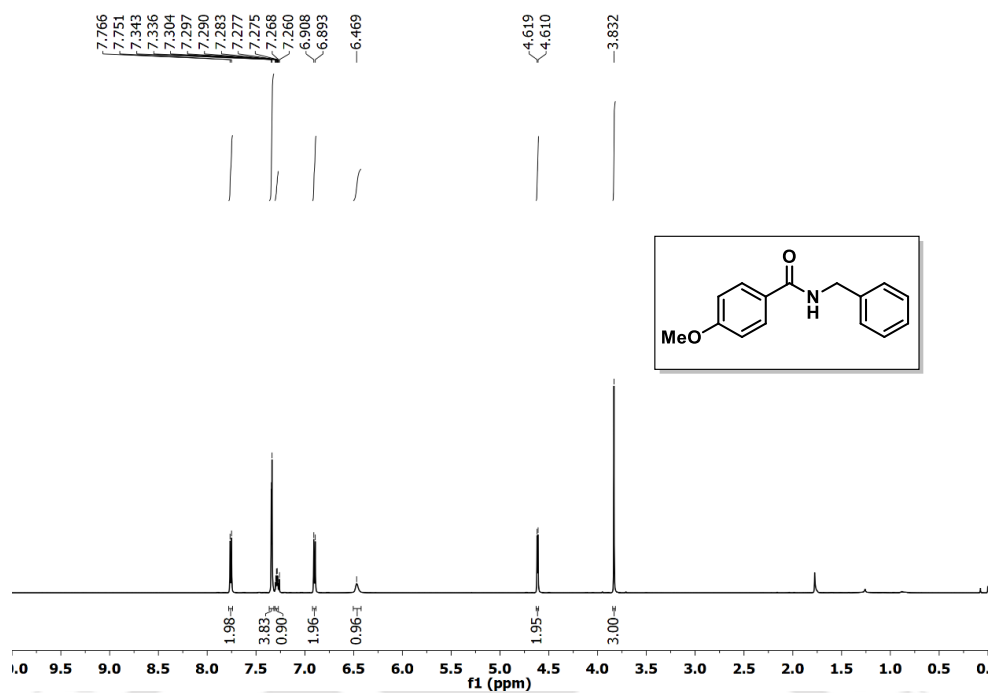
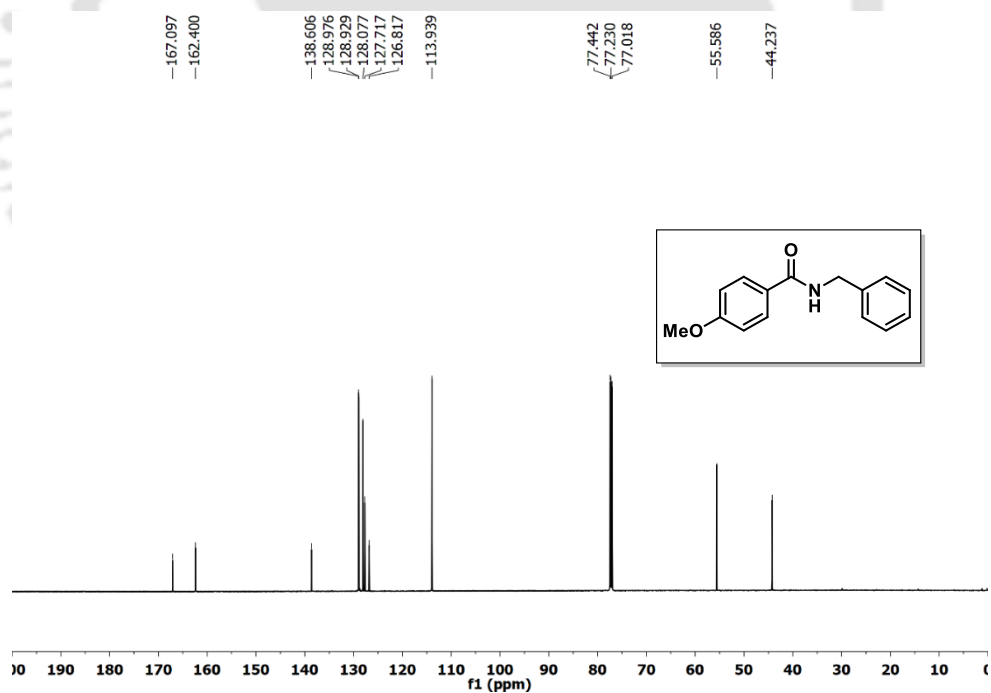
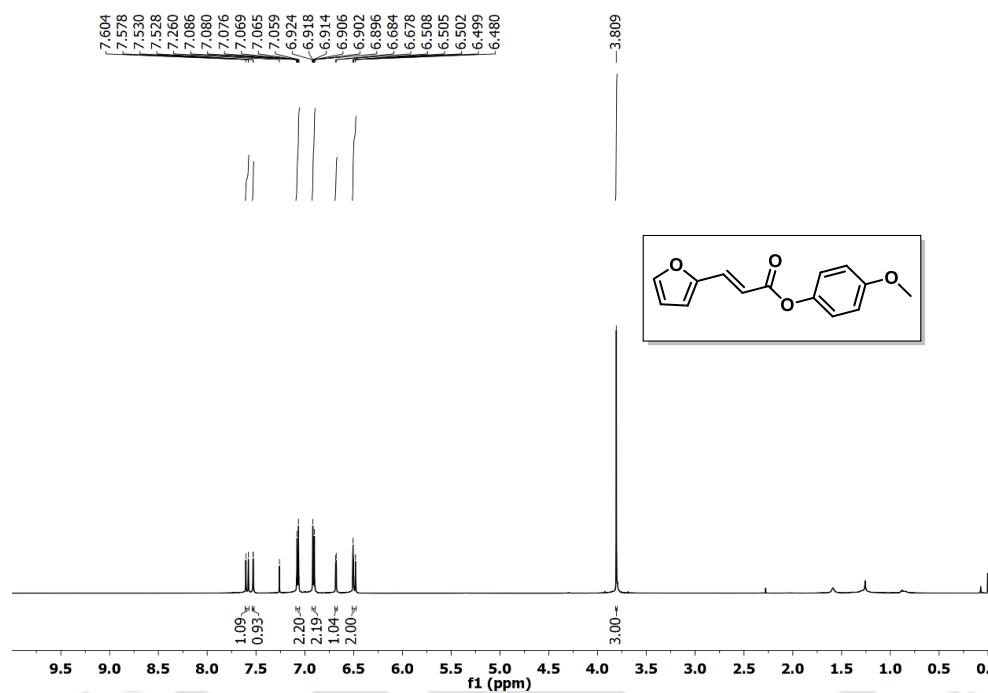
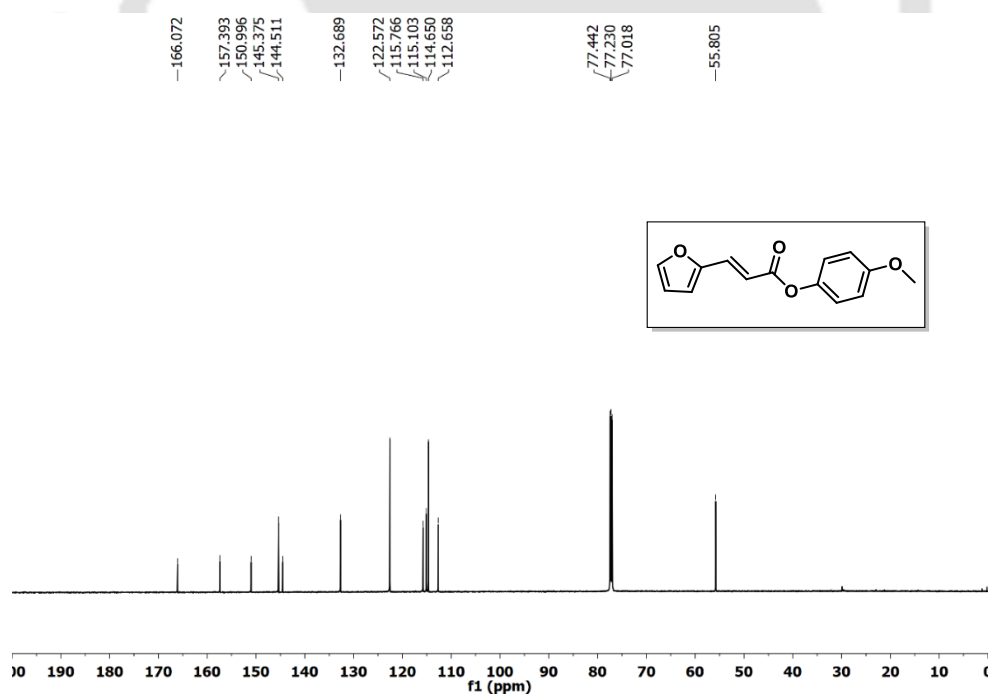
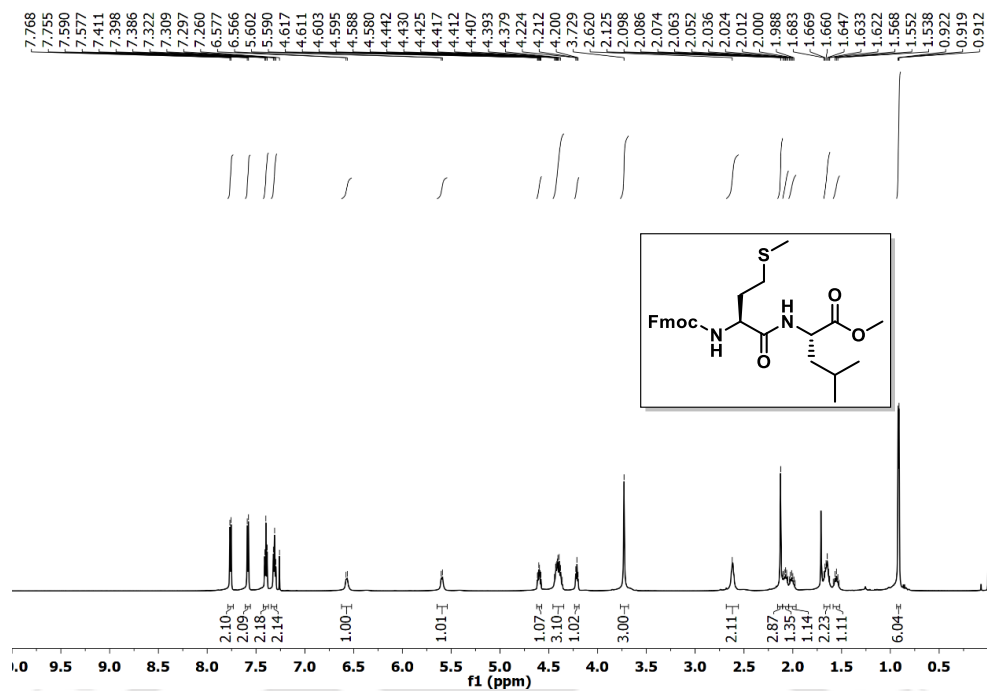
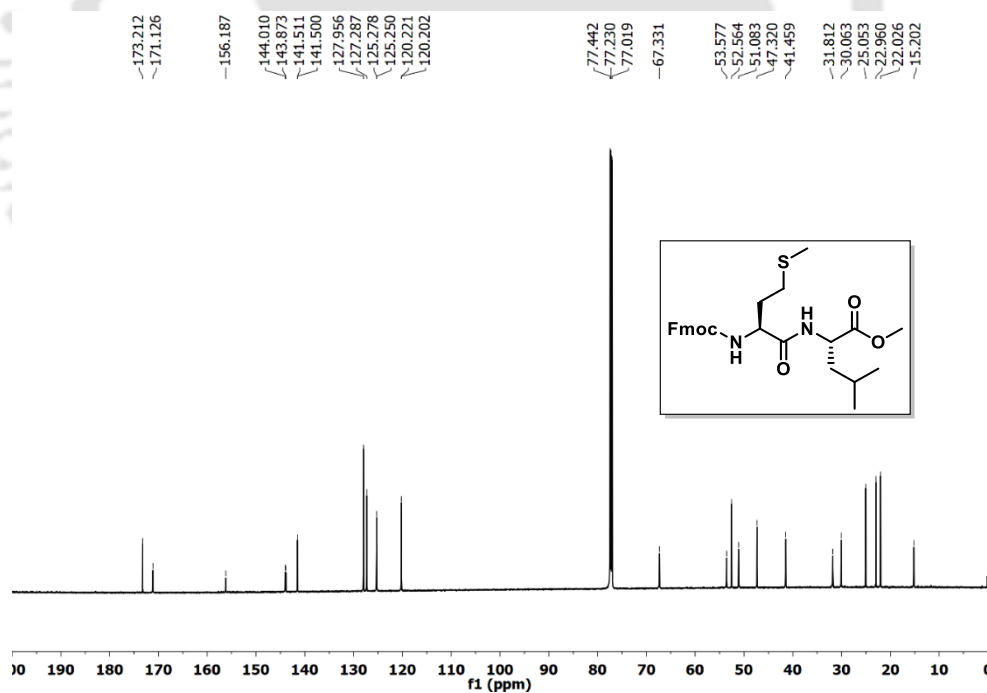


Figure 2.8. ¹H NMR spectrum of peptide **Boc-L-Phe-L-Phg-OMe (9f)**

Figure 2.9. ¹H NMR spectrum of amide **3d**Figure 2.10. ¹³C NMR spectrum of amide **3d**

Figure 2.11. ¹H NMR spectrum of ester 6jFigure 2.12. ¹³C NMR spectrum of ester 6j

Figure 2.13. ¹H NMR spectrum of peptide 9bFigure 2.14. ¹³C NMR spectrum of peptide 9b

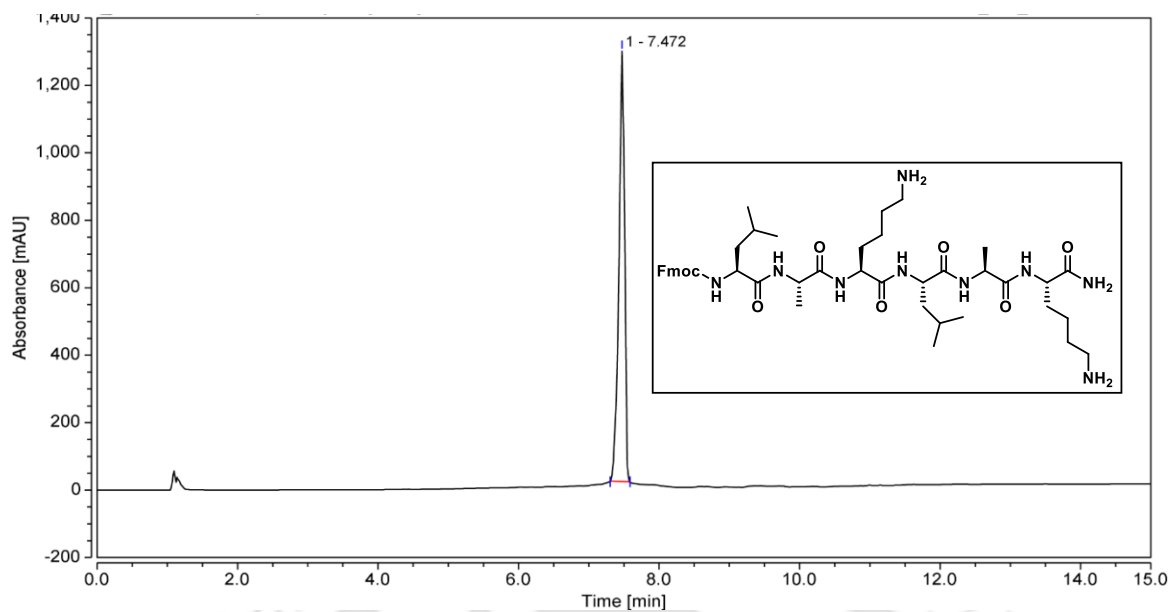


Figure 2.15. HPLC profile of peptide 10a

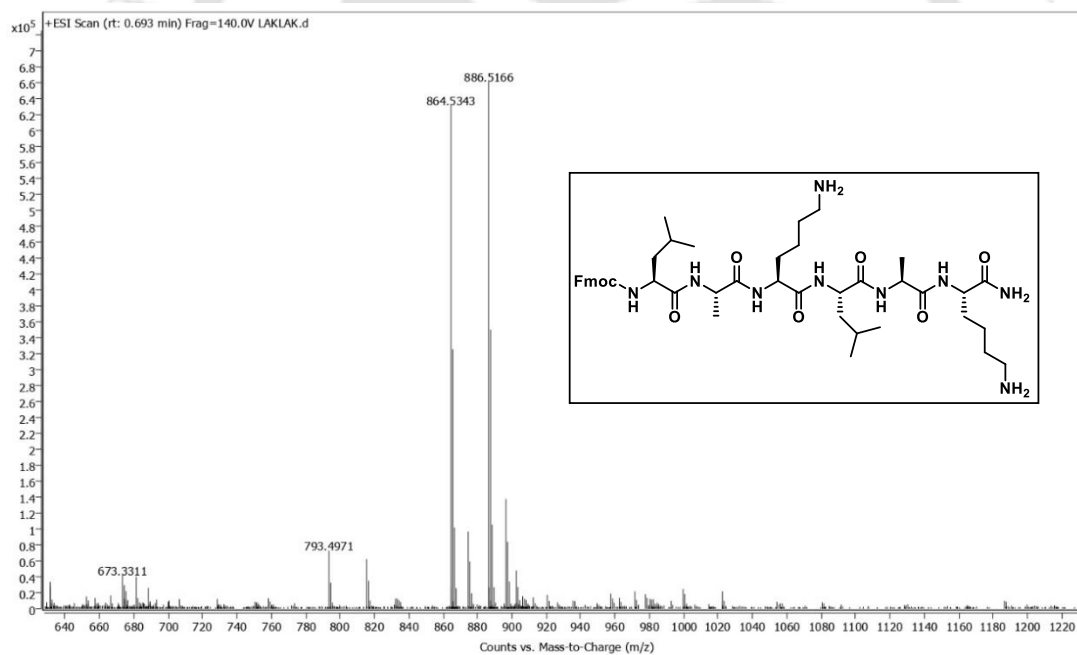


Figure 2.16. HRMS spectrum of peptide 10a

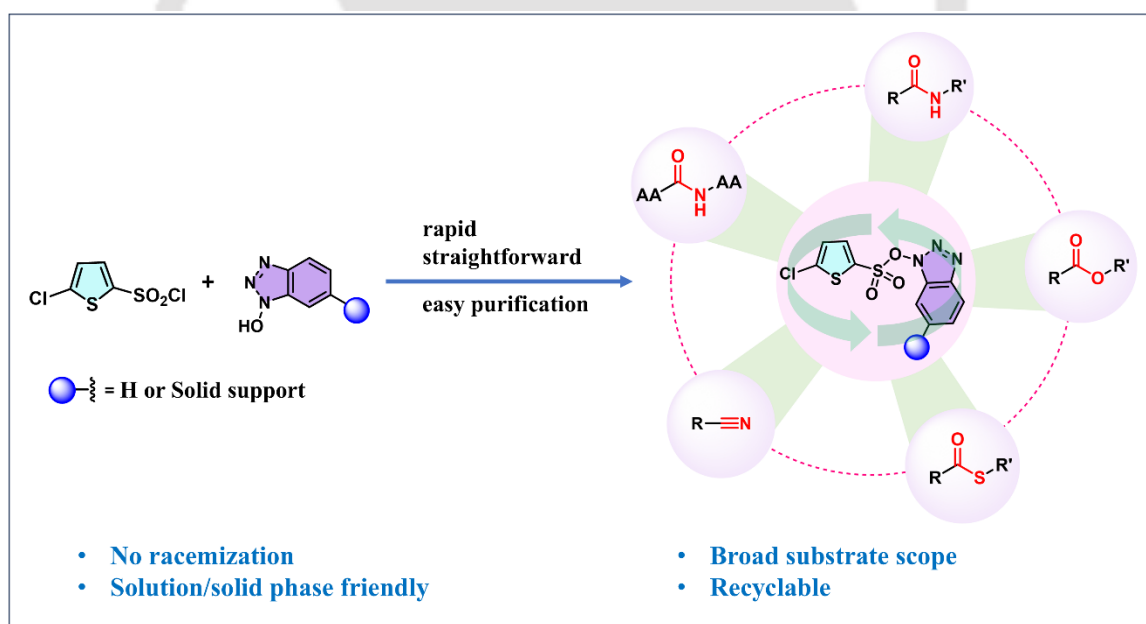
2.18. Crystallographic data

Table 2.12. Crystallographic data of *o*-CTSOXY (1B), Bz-Oxyrna (V) and amide 3f

Parameters	<i>o</i> -CTSOXY (1B)	Bz-Oxyrna (V)	amide 3f
Formula	C ₉ H ₇ Cl N ₂ O ₅ S ₂	C ₁₂ H ₁₀ N ₂ O ₄	C ₁₄ H ₁₂ Cl N O
Fw	322.74	246.22	245.70
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	<i>P</i> 21/ <i>c</i>	<i>P</i> 21/ <i>n</i>	<i>P</i> 21/ <i>n</i>
<i>a</i> /Å	7.9312(19)	11.8080(10)	5.734(3)
<i>b</i> /Å	7.1906(17)	8.0711(7)	26.752(11)
<i>c</i> /Å	24.649(6)	13.2548(11)	8.165(3)
α /°	90.00	90.00	90.00
β /°	95.382(7)	100.736(2)	91.464(17)
γ /°	90.00	90.00	90.00
<i>V</i> /Å ³	1399.6(6)	1241.12(18)	1252.0(9)
<i>Z</i>	4	4	4
<i>D_c</i> /g cm ⁻³	1.532	1.318	1.303
μ Mo K α /mm ⁻¹	0.586	0.101	0.287
<i>F</i> 000	656	512	512
<i>T</i> /K	302(2)	296(2)	296(2)
θ max.	26.26	26.33	24.25
Total no. of reflections	29816	26547	14806
Independent reflections	2455	2174	2200
Observed reflections	2104	1567	1527
Parameters refined	173	164	154
<i>R</i> ₁ , <i>I</i> > 2 σ (<i>I</i>)	0.0411	0.0671	0.0473
<i>wR</i> ₂ , <i>I</i> > 2 σ (<i>I</i>)	0.1526	0.1898	0.1302
GOF (<i>F</i> ²)	1.344	1.153	1.068
CCDC No.	2296553	2296554	2296548

Chapter 3

Development of Heterocyclic Recyclable Coupling Reagents and Applications



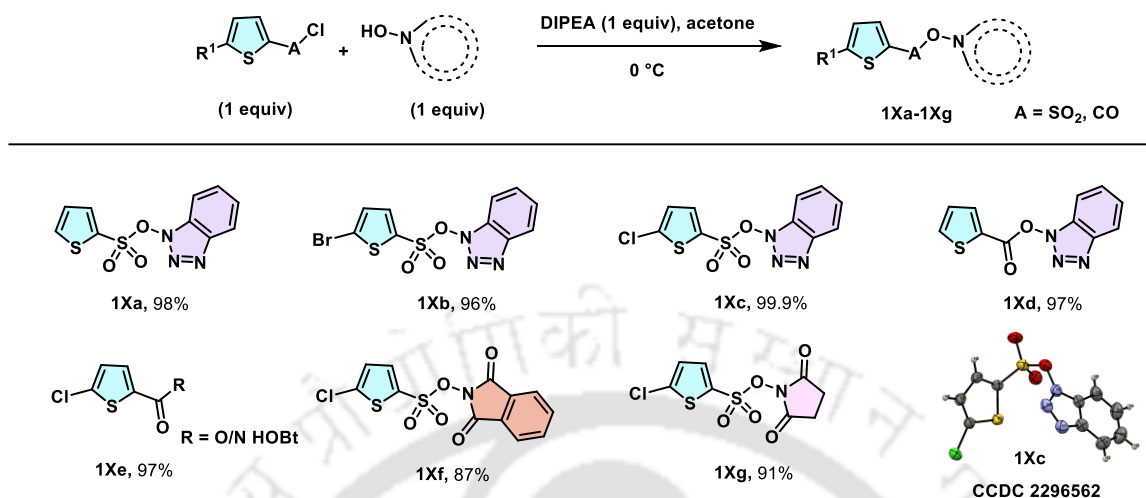
3.1. Background

In the previous chapter, we discussed the straightforward and time-efficient synthesis of *o*-CTSOXY and demonstrated its application in producing racemization-free amides, esters, peptides, and other compounds. While the reagent demonstrated efficiency in synthesizing the mentioned compounds, product yields were slightly compromised in certain cases. This chapter introduces novel heterocyclic coupling reagents to efficiently synthesize racemization-free amides, esters, thioesters, peptides, and nitriles.

Although several benzotriazole-based coupling reagents have been developed,¹ many produce toxic byproducts, pose guanidylation² concerns, have explosive properties, and fail in complex peptide coupling. They often involve harsh synthetic processes, are incompatible with green solvents, and are not recyclable. In contrast, our protocols provide straightforward synthesis, avoid the generation of toxic byproducts, eliminate guanidylation issues, and function effectively in cost-effective green solvents. Additionally, we performed solvent and byproduct recovery, as well as the recycling of coupling reagents, representing a significant step toward reducing environmental impact.

3.2. Design, synthesis, and efficiency of coupling reagents

In recent decades, the demand for intricate peptide structures, especially for biomedical research and large-scale production of peptide-based drugs, has spurred the search for efficient and cost-effective coupling reagents and synthetic methods. As a result, the quest for versatile, recyclable, cost-effective coupling reagents has become a significant challenge. To address this, we envisioned synthesizing a thiophene ring containing HOBt, N-hydroxyphthalimide, or N-hydroxysuccinimide-based new-generation recyclable coupling reagents. We synthesized a series of new reagents by reacting thiophene sulfonyl or carbonyl chlorides with HOBt, N-hydroxyphthalimide, or N-hydroxysuccinimide in the presence of DIPEA and acetone with excellent yields (Table 3.1). The coupling efficiency of these reagents was assessed via amidation. Using benzoic acid and benzylamine as starting materials, we observed varying yields of amide **3a** with different reagents in DCM. Notably, reagents **1Xf** and **1Xg** did not participate in the reaction, while reagent **1Xc** yielded the highest **3a** (83%). Thus, reagent **1Xc** emerged as the optimal choice (Table 3.2).

Table 3.1. Synthesis of designed reagents^a

^aReaction conditions: Thiophene derivative (1 mmol), N-heterocycle (1 mmol), DIPEA (1 mmol), acetone (2 mL), 5–30 min (**1Xa–1Xe**), 12 h (**1Xf**), 6 h (**1Xg**).

Table 3.2. Amidation with different reagents

Entry ^a	Reagent	Yield ^b (%)
1	1Xa	73
2	1Xb	76
3	1Xc	83
4	1Xd	64
5	1Xe	69
6	1Xf	---
7	1Xg	---

^aReaction conditions: **1a** (0.5 mmol), **2a** (0.5 mmol), **1Xa–1Xg** (0.5 mmol), DIPEA (0.6 mmol), DCM (1 mL), rt, 12 h (time after adding amine). ^bIsolated yields.

We further tested the synthesis of reagent **1Xc** in alternative solvents (EtOAc, DCM, ACN). Remarkably, all cases yielded over 99% within just 5 minutes. Additionally, we successfully performed a gram-scale synthesis: starting with 10g of 5-chlorothiophene-2-

sulfonyl chloride, we obtained an impressive 14.53 g (99.9%) of **1Xc**. We also tested the stability of reagent **1Xc** at room temperature and under refrigeration (0–4 °C). No deterioration was observed for up to one month at room temperature and one year under refrigeration.

3.3. DFT study

Analyzing molecular orbitals aids in understanding the chemical reactivity and kinetic stability of reagents. HOBt exhibits a desmotropic nature and can form both N-substituted and O-substituted products. The HOMO–LUMO gap for both forms was calculated using DFT with Gaussian 09W software and the B3LYP/6-31G(d, P) method.³ The DFT study revealed that the O-substituted **1Xc** is more stable than the N-substituted **1Xc(N)** due to a larger HOMO–LUMO gap (Figure 3.1). The crystallographic structure further validated the formation of the more stable **1Xc** (O-isomer).

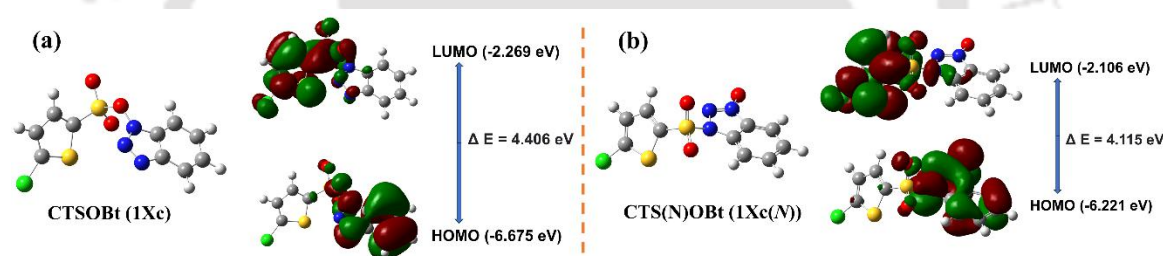
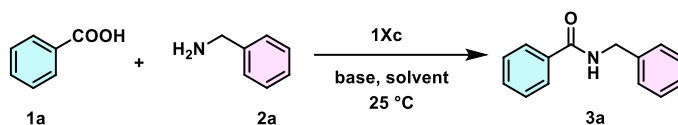


Figure 3.1. (a, b) Gas phase optimized structure and HOMO-LUMO energies of **1Xc** and **1Xc(N)**.

3.4. Reaction optimization

The exceptional coupling efficiency of **1Xc** could establish it as a universal platform for amidation and esterification. As previously discussed, reagent **1Xc** achieved an impressive 83% yield of **3a** in DCM. The synthesis of amide **3a** was optimized using various other solvents such as THF, EtOAc, ACN, DMSO, DMF, CHCl_3 , and acetone, along with other bases like Et_3N and DBU (Table 3.3). Different yields of amide **3a** were obtained with different solvents and bases. The highest yield (**3a**, 98%) was obtained using acetone as the solvent and DIPEA as the base within 30 minutes (entry 8, Table 3.3), which were considered the optimized conditions.

Table 3.3. Optimization of amidation reaction

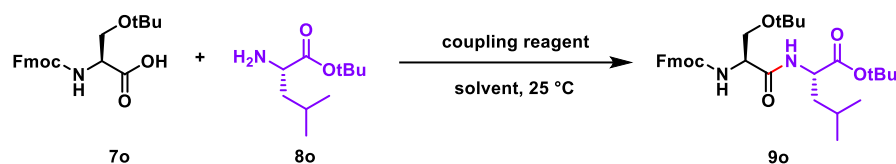


Entry ^a	Solvent	Base	Time ^b	Yield ^c (%)
1	DCM	DIPEA	12 h	83
2	THF	DIPEA	2 h	64
3	EtOAc	DIPEA	1.3 h	91
4	ACN	DIPEA	35 min	88
5	DMSO	DIPEA	3 h	50
6	DMF	DIPEA	15 min	90
7	CHCl ₃	DIPEA	1.4 h	89
8	Acetone	DIPEA	30 min	98
9	Acetone	Et ₃ N	45 min	85
10	Acetone	DBU	50 min	79

^aReaction conditions: **1a** (0.5 mmol), **2a** (0.5 mmol), **1Xc** (0.5 mmol), base (0.6 mmol), solvent (1 mL), 25 °C. ^bTime after addition of amine. ^cIsolated yields.

3.5. Racemization and yield comparison during dipeptide synthesis

Racemization is a significant challenge with many coupling reagents. Loss of chiral integrity during drug discovery can result in severe toxic effects. After optimizing the reaction conditions, we compared the racemization suppression capacity and peptide yield production using our reagent (**1Xc**) with other coupling reagents. According to the literature, Fmoc-L-Ser(*t*Bu)-OH is highly prone to racemization. We synthesized the dipeptide Fmoc-L-Ser(*t*Bu)-L-Leu-O*t*Bu and, upon comparison with reported values,⁴ found that our coupling reagent **1Xc** demonstrated superior performance. In Table 3.4, conventional coupling reagents (entries 1–4) showed significant racemization. DEPBT had excellent chirality retention but low efficiency. On the other hand, MYMsA and MYTsA showed no epimerization/racemization but required longer reaction times. In contrast, **1Xc** produced an excellent yield of the desired dipeptide under optimized conditions (entry 8, Table 3.4) without detectable racemization at 25 °C and in significantly less time (Figure 3.9–3.11).

Table 3.4. Racemization and yield comparison in dipeptide synthesis

Entry	Coupling reagent	Additive	Time	Yield (%) ^c	dr ^d
1 ^a	HBTU	DIPEA	10 min	90	82:18
2 ^a	HATU	DIPEA	10 min	70	87:13
3 ^a	PyBop	DIPEA	10 min	91	88:12
4 ^a	DCC		10 min	98	91:9
5 ^a	DEPBT	DIPEA	20 min	61	99:1
6 ^a	MYMsA		22 h	99	100:0
7 ^a	MYTsA		22 h	94	100:0
8 ^b	1Xc	DIPEA	25 min	> 99	100:0

^aLiterature reported values.⁴ ^bSynthesis of **9o** using **1Xc** under optimized conditions. ^cIsolated yield.

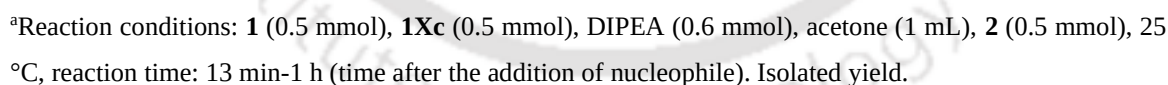
^dDetection using chiral HPLC.

3.6. Substrate scope

3.6.1. Scope of amides

After successfully testing racemization, we explored amidation under optimized conditions. Remarkably, all reactions yielded excellent results within a very short time (Table 3.5). Benzoic acid with electron-donating (p-OMe) and electron-withdrawing (p-NO₂) groups smoothly participated, producing the corresponding amides (**3b** and **3c**) with excellent yields. Electron-deficient aromatic amines also resulted in the corresponding amides **3d** and **3e** with yields of 96% and 97%. A long-chain hydrophobic acid and sterically hindered amine actively participated, affording the desired amides (**3f**, **3g**) with satisfactory yields (89% and 91%). Moderately long-chain hydrophobic and cyclic aliphatic amines also offered the desired amides **3h** and **3i** with 94% and 97% yields. Base-sensitive, sterically rigid Fmoc-amino acids reacted smoothly, producing the desired amides (**3j–3l**) with outstanding yields (98–99%). Methyl ester-protected amino acids also participated readily, yielding amides **3m** and **3n**. Heterocyclic aromatic acid reacted well

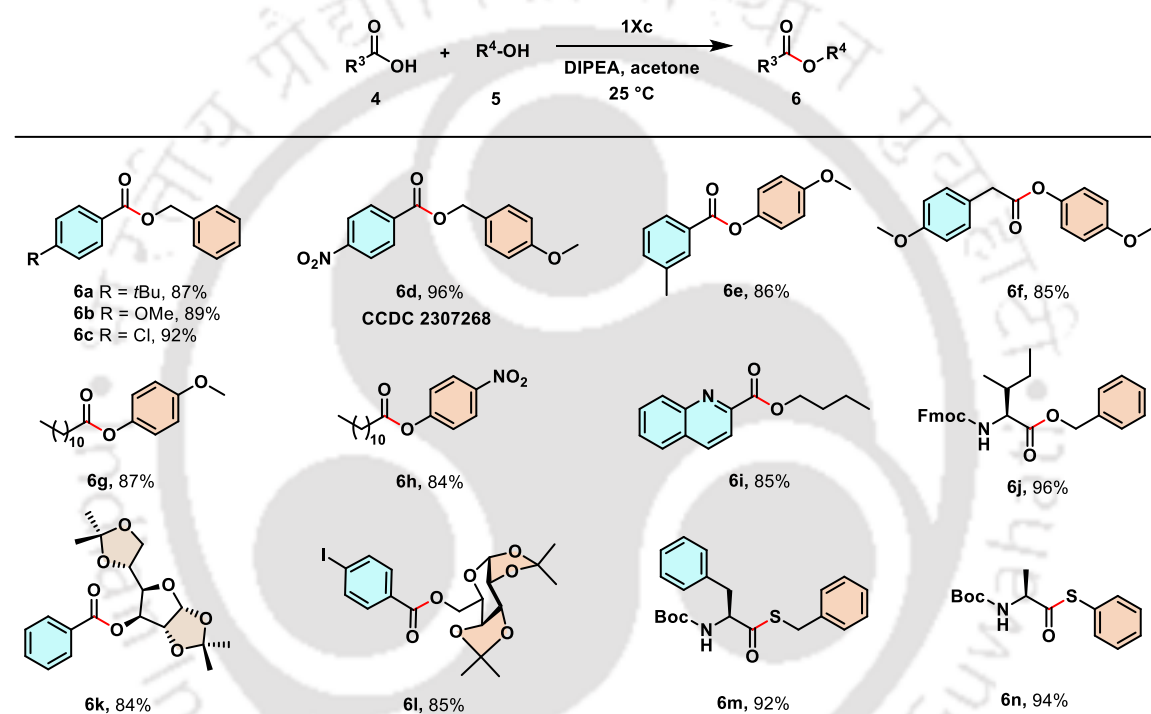
Table 3.5. Scope of amides^a



We further explored the generality of the process by synthesizing various esters under optimized conditions (Table 3.6). Benzoic acids with electron-donating (p-*t*Bu, p-OMe) and electron-withdrawing (p-Cl, p-NO₂) groups yielded the desired esters **6a–6d** with good to excellent yields (87–96%). Electron-deficient aromatic alcohols also participated readily, producing esters **6e–6h** with satisfactory yields (84–87%). Heterocyclic aromatic acid reacted with a moderately long chain alcohol under optimized conditions, forming

ester **6i** (85%). Fmoc-amino acid also reacted effectively (**6j**). Sterically crowded di-acetonide-protected glucose 3-OH acceptor (secondary alcohol) and galactose 6-OH acceptor also met the reaction conditions, producing esters **6k** and **6l** with good yields. Electron-deficient nucleophiles, such as benzylic and aromatic thiols, precipitated readily under the optimized conditions, yielding the desired thioesters (**6m**, **6n**) with excellent yields (92%, 94%).

Table 3.6. Scope of esters and thioesters^a



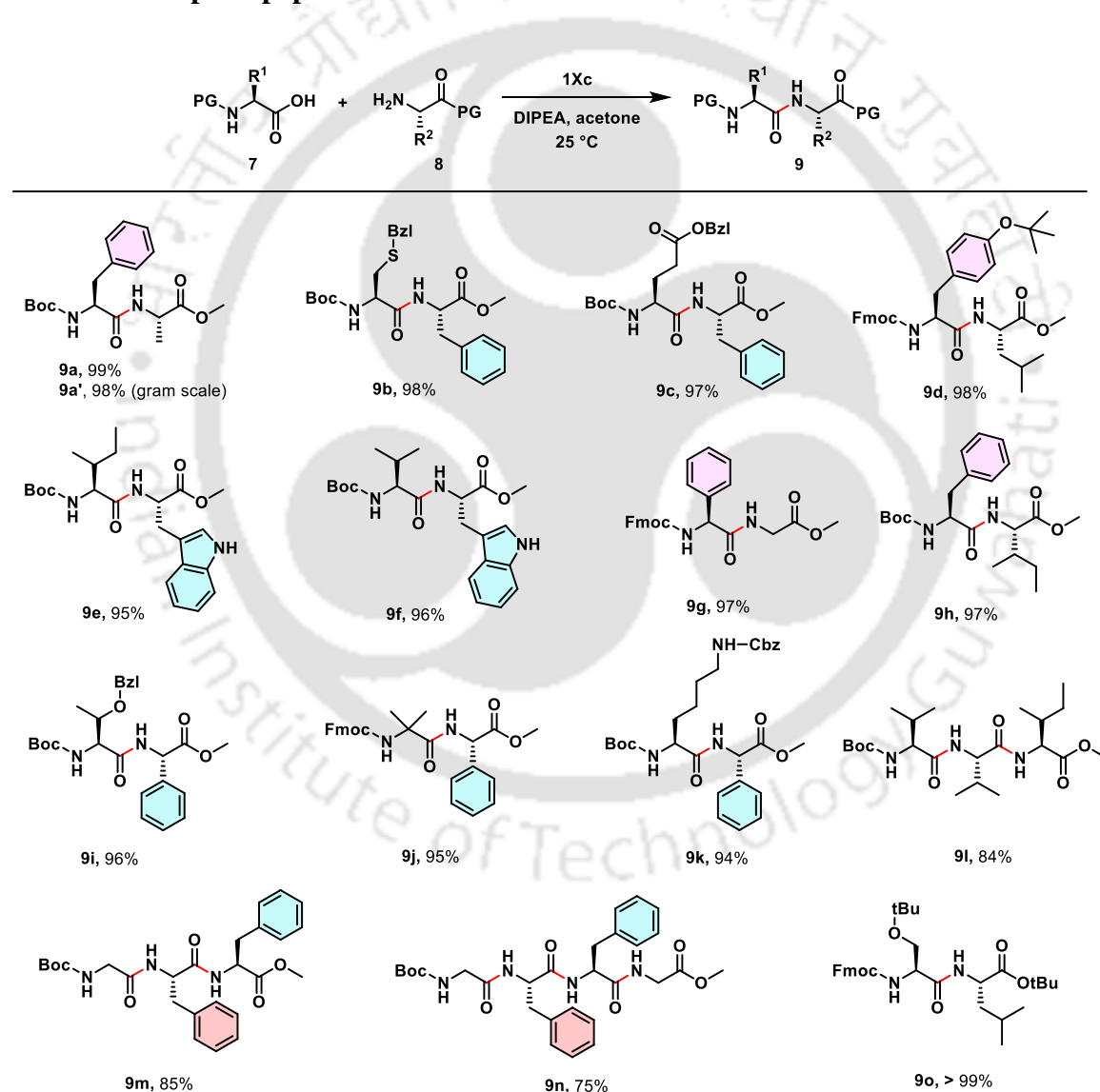
^aReaction conditions: **4** (0.5 mmol), **1Xc** (0.5 mmol), DIPEA (0.6 mmol), acetone (1 mL), **5** (0.5 mmol), 25 °C, reaction time: 2-8 h (time after the addition of nucleophile). Isolated yield.

3.6.3. Scope of peptides

The protocol's applicability was further explored by synthesizing challenging peptides (Table 3.7). Various protecting groups (Boc, Fmoc, Bzl, *t*Bu, Cbz) bearing amino acids were tested successfully. N-protected amino acids with Bzl or *t*Bu side chain protection produced the desired dipeptides (**9a–9d**) with excellent yields (97–99%). Sterically demanding Ile, Val, and Phg also participated readily under optimized conditions, affording peptides (**9e–9g**) with outstanding yields (95–97%). In case of **9e** and **9f**, the Trp indole

NH did not affect reactivity. Installing sterically rigid amino acids at the C-terminal offered peptides **9h**, **9i**, and **9k** with 97%, 96%, and 95% yields, respectively. Placing sterically rigid amino acids at both terminals of the sequence resulted in peptide **9j** with a 95% yield. Next, our focus shifted to synthesizing tri and tetra-peptides. The challenging tripeptide VVI (**9l**) was synthesized with a good yield (84%). Tripeptide GFF (**9m**, 85%) and tetrapeptide GFFG (**9n**, 75%) were also efficiently synthesized, demonstrating the protocol's effectiveness for synthesizing moderately long-chain peptides.

Table 3.7. Scope of peptides^a

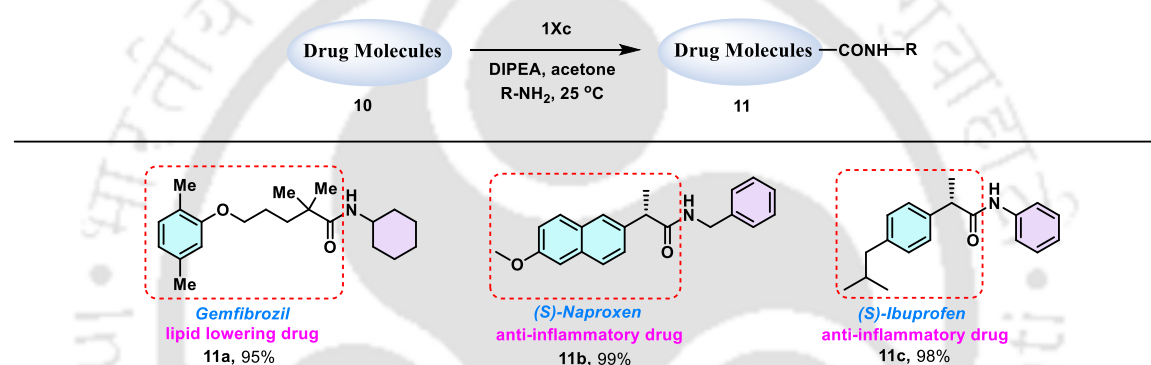


^aReaction conditions: **7** (0.5 mmol), **1Xc** (0.5 mmol), DIPEA (0.6 mmol), acetone (1 mL), **8** (0.5 mmol), 25 °C, reaction time: 30 min-3 h (time after the addition of nucleophile). Isolated yield.

3.6.4. Late-stage modification of drugs

Following our successes in amidation, esterification, and peptide synthesis, we found the protocol's suitability for late-stage drug modifications. Under optimized conditions, we modified several drugs through amidation with excellent yields (Table 3.8). Gemfibrozil, naproxen, and ibuprofen-bearing carboxylic acids participated smoothly, yielding modified drugs (**11a–11c**) with 95–99% yields. The use of reagent **1Xc** in drug modification demonstrated its practical utility.

Table 3.8. Late-stage modifications of drugs^a



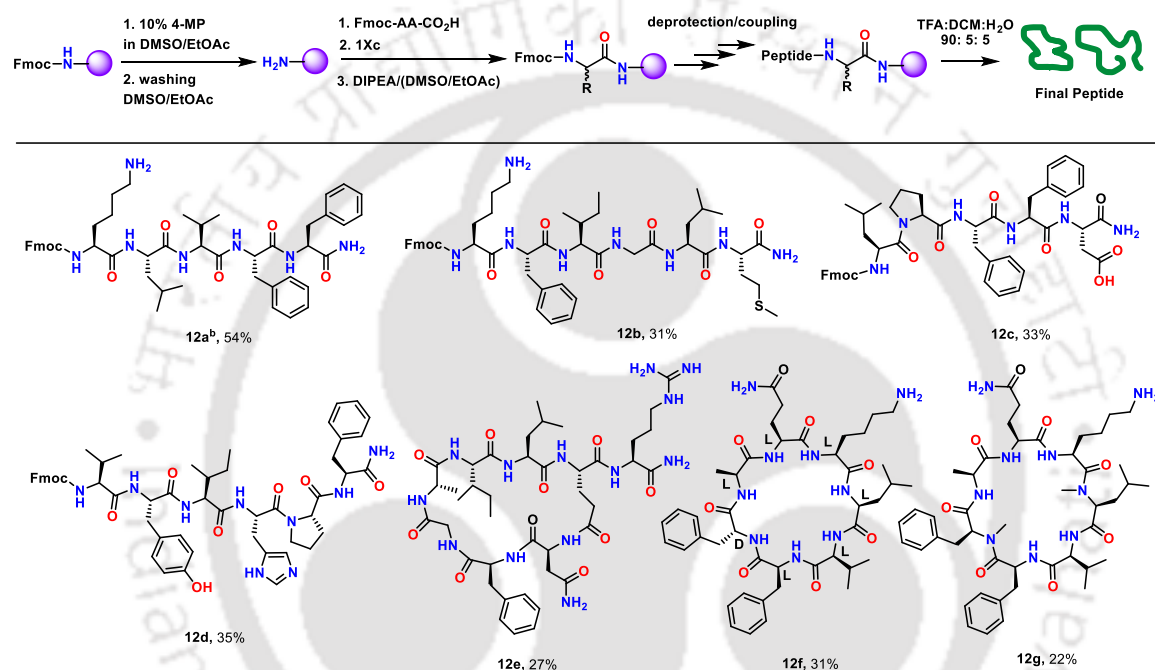
^aReaction conditions: **10** (0.5 mmol), **1Xc** (0.5 mmol), DIPEA (0.6 mmol), RNH₂ (0.5 mmol), acetone (1 mL), 25 °C, reaction time: 30 min–1 h (time after the addition of nucleophile). Isolated yield.

3.7. Solid phase peptide synthesis

Solid-phase peptide synthesis (SPPS) is essential for creating large peptide sequences. The Fmoc/tBu strategy⁵ is predominant; DMF, commonly used in SPPS, is toxic⁶. Seeking safer solvents, we tested acetone, achieving a 41% yield for Fmoc-KLVFF-NH₂ (**12a**), a key segment of the Aβ₄₂ peptide linked to Alzheimer's disease.⁷ Using a DMSO/EtOAc (1:9) mixture⁸ improved the yield to 53%, and DMSO/acetone (1:19) slightly increased it to 54%. Although 5% DMSO/acetone provided a better yield, resin swelling was poor, so DMSO/EtOAc (1:9) was chosen as the optimal solvent system for SPPS. We then synthesized additional peptides, including eldoisin⁹-related peptide (**12b**) with a 31% yield and Soto peptide LPFFD¹⁰ (**12c**) with a 33% yield. The hexapeptide VYIHPF (**12d**), part of tissue hormone Angiotensin II¹¹, was synthesized with a 35% yield. The protocol

also showed efficacy in cyclic peptide synthesis. Cy-NFGAILER-NH₂ (**12e**) was prepared using side chain to tail cyclization with a 27% yield. The hexapeptide NFGAIL originating from human islet amyloid polypeptide, is an amyloidogenic fragment.¹² Cy-KLVFFAE (**12f**), a head-to-tail cyclic peptide, inhibits A β ₁₋₄₀ aggregation.¹³ Cy-KL(N-Me)VFF(N-Me)AE (**12g**) a most challenging sequence was synthesized with a 22% yield (Table 3.9).

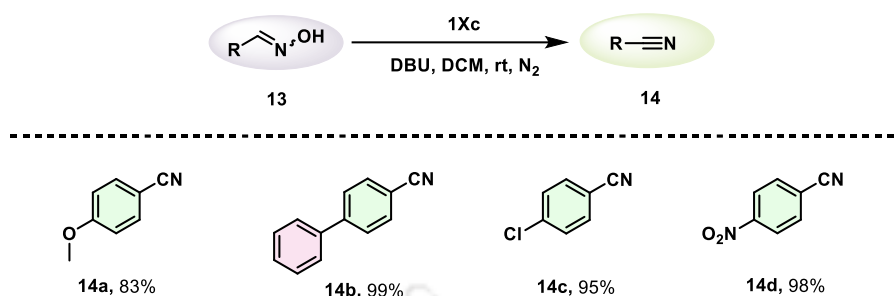
Table 3.9. Scope of solid phase peptides^a



^aReaction conditions: Rink amide MBHA resin (0.14 mmol, loading 0.7 mmol/g), amino acids (2 equiv), **1Xc** (2.5 equiv), DIPEA (5 equiv), solvent: DMSO/EtOAc (1:9), Fmoc deprotection: 10% 4-methylpiperidine (4-MP) in DMSO/EtOAc (1:9), coupling time: 3 h. ^bYield in DMSO/acetone (1:19). AA = amino acid.

3.8. Synthesis of nitriles

The reagent **1Xc** not only facilitates the synthesis of amides, esters, and peptides but also effectively synthesizes nitriles from aldoximes. Using literature-reported conditions,¹⁴ electron-donating (p-OMe, p-Ph) and electron-withdrawing (p-Cl, p-NO₂) groups containing aromatic aldoximes offered nitriles (**16a–16d**) with excellent yields (83–99%) (Table 3.10). This demonstrates the versatility and efficiency of reagent **1Xc** in various organic transformations.

Table 3.10. Scope of nitriles^a

^aReaction conditions: **13** (0.4 mmol), **1Xc** (0.4 mmol), DBU (0.8 mmol), DCM (1 mL), rt, N₂-atm, 2 h.

3.9. Solid-supported coupling reagent

Solid-supported coupling reagents enhance product purity, allowing for purification via simple washing/filtration. This method enables easy recovery of the solid support. To date, very few polymeric coupling reagents have been reported.¹⁵ Here, we developed a polymer-bound coupling reagent (**1Xp**) (section 3.13.8) for synthesizing amides, esters, thioesters, and peptides. IR and EDX analyses confirmed the synthesis of **1Xp**. In IR analysis, the disappearance of the 3421 cm⁻¹ peak (O-H stretching) and the appearance of 1398 and 1190 cm⁻¹ peaks (SO₃-ester), indicated successful synthesis of **1Xp**. In EDX, the presence of S and Cl atoms, along with C, N, and O atoms, confirmed the synthesis of **1Xp** (Figure 3.2).

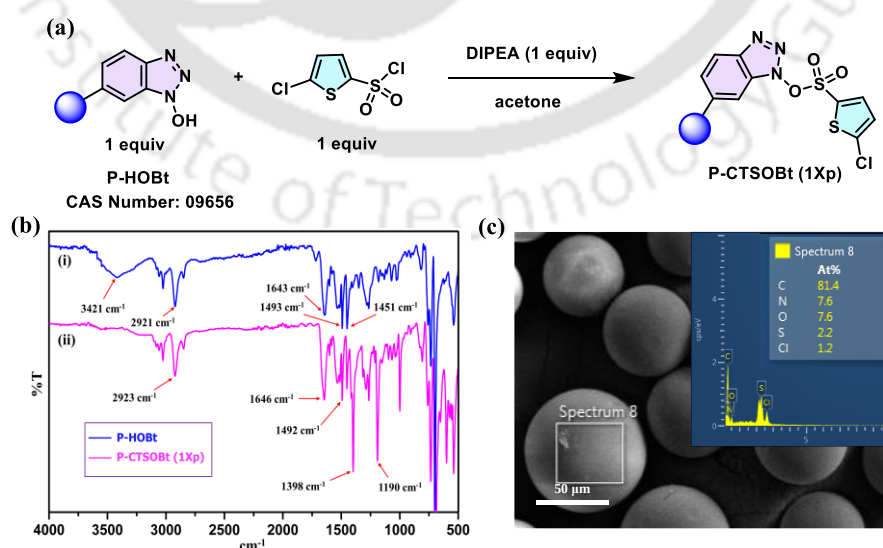
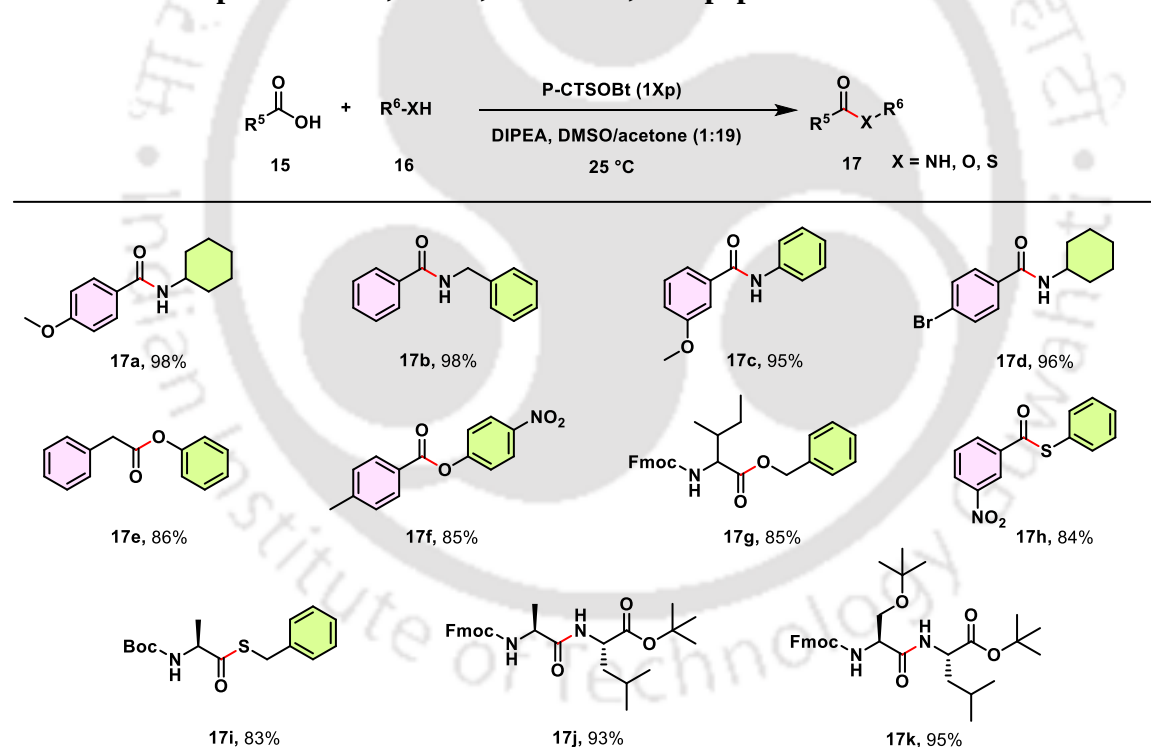


Figure 3.2. (a) Synthetic scheme of **1Xp**; (b) IR-spectra of (i) P-HOBt, (ii) **1Xp**, (c) EDX of **1Xp**.

3.10. Scope of amides, esters, thioesters, and peptides using 1Xp

The reactivity of **1Xp** was tested by synthesizing N-cyclohexyl-4-methoxybenzamide (**17a**) in acetone in the presence of DIPEA, yielding 90%. Screening other solvents like DMSO/EtOAc (1:9) and DMSO/acetone (1:19) resulted in 92% and 98% yields of **17a**, respectively, making DMSO/acetone (1:19) the optimized solvent. Various amides, esters, and thioesters (**17a–17i**) were synthesized with good to excellent yields (83–98%). Peptides (**17j–17k**) were also synthesized with high yields (93–95%) (Table 3.11). Products were collected by filtration and washes. Some of the esters and thioesters required column chromatography for purification. After the reaction, the solid-supported HOBt was easily recovered by simple washing with solvent and reused without altering its reactivity.

Table 3.11. Scope of amides, esters, thioesters, and peptides^a



^aReaction conditions: HOBt polymer resin: 0.2 mmol (loading: 1 mmol/g) **15** (0.2 mmol), **16** (0.2 mmol), DIPEA (0.24 mmol), solvent (1 mL), 25 °C, 1–6 h (time after the addition of nucleophile). Isolated yield.

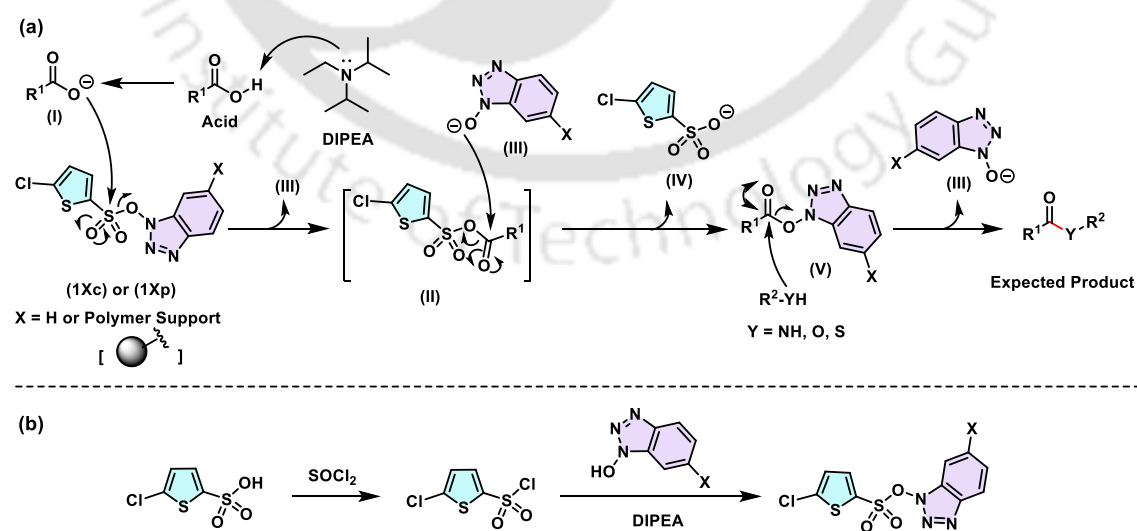
Solvents and byproducts were efficiently recovered using the protocol discussed in the previous chapter 2 (section 2.9). After synthesizing **1Xc** (using 10 g 5-chlorothiophene-2-sulfonyl chloride), 97% (97 mL out of 100 mL) acetone was recovered. For amide **3c** (using

1 g acid), 87% (13 mL out of 15 mL) acetone and 96% 5-chlorothiophene-2-sulfonic acid (SA)/HOBT byproduct mixture were recovered. In the case of **1Xp** (using 0.2 mmol P-HOBT resin), 90% (18 mL out of 20 mL) acetone was recaptured.

3.11. Plausible mechanism and recyclability of the coupling reagents

We proposed a mechanistic pathway (Scheme 3.1a) for synthesizing amides, esters, thioesters, and peptides using CTSOBt (**1Xc**) or P-CTSOBt (**1Xp**). DIPEA generates the carboxylate nucleophile (I), which adds to the sulfonyl center of **1Xc** or **1Xp**, forming intermediate (II) and removing OBt[−] (III). OBt[−] (III) then attacks intermediate (II), forming active intermediate (V) and releasing thiophene sulfonate (IV). Next, nucleophilic addition to intermediate (V) offers the desired product. Intermediate (V), derived from 4-methoxybenzoic acid and **1Xc**, was characterized using SC-XRD, NMR, and HRMS.

The coupling reagent (**1Xc**) was successfully regenerated from byproducts (SA+HOBT). The mixture of 5-chlorothiophene-2-sulfonic acid (SA) and HOBT was treated with SOCl₂ at 70 °C in toluene with catalytic DMF, forming sulfonyl chloride of SA in 30 minutes. After cooling, the reaction was neutralized with DIPEA and stirred, yielding **1Xc** within 5–10 minutes. In the case of polymeric coupling reagent, recovered P-HOBT was treated with 5-chlorothiophene-2-sulfonyl chloride alongside DIPEA offered **1Xp** (Scheme 3.1b).



Scheme 3.1. (a) Plausible mechanism for synthesizing amides, esters and peptides. (b) Recyclability of coupling reagent (**1Xc** or **1Xp**).

3.12. Conclusion

In conclusion, we have successfully introduced thiophene-ring containing HOBt and polymeric HOBt-based coupling reagents (**1Xc** and **1Xp**) for synthesizing racemization-free amides, esters, thioesters, and peptides. Additionally, reagent **1Xc** facilitates the synthesis of nitriles from aldoximes. DFT studies revealed the successful formation of an O-substituted coupling reagent (**1Xc**) rather than an N-substituted one, which was confirmed by SC-XRD. The isolation and characterization of the intermediate support our mechanistic proposal. Several key advantages of these methodologies include efficient synthesis of coupling reagents under mild conditions, easy purification, high bench stability, effectiveness in both solution and solid phase, use of green solvents, and prevention of side reactions (e.g., guanidylation). We also successfully demonstrated the easy recovery of solvents and byproducts. Additionally, recycling of coupling reagents from the recovered byproducts significantly contributes to waste reduction. These advantages make the reagents highly valuable for both academic research and industrial applications.

3.13. Experimental section

3.13.1. Materials and instrumentations

As described in chapter 8

3.13.2. General procedure for the synthesis of coupling reagent

DIPEA (1 mmol) and HOBt, N-hydroxyphthalimide, or N-hydroxysuccinimide (1 mmol) were mixed with acetone (2 mL) and kept stirring at 0 °C. Substituted thiophene-2-sulfonyl or carbonyl chloride (1 mmol) was added dropwise. The reaction was monitored by TLC. After completion, acetone was evaporated, and EtOAc was poured. The mixture was washed with 10% citric acid and 10% NaHCO₃ solution. The organic portion was dried over anhydrous Na₂SO₄, filtered, and then evaporated to yield the pure reagent.

3.13.3. General procedure for the synthesis of amides, esters, and thioesters

Acid (0.5 mmol) and DIPEA (0.6 mmol) were mixed in acetone (1 mL) at room temperature. CTSOBt (**1Xc**) (0.5 mmol) was introduced and stirred for 15–20 minutes. The desired nucleophile (amine, alcohol, or thiol) was then added. TLC was performed to track the reaction progress. After completion, acetone was evaporated, and EtOAc was poured. The mixture was sequentially washed twice with 10% citric acid and 10% NaHCO₃ solution, respectively. The organic part was dried over Na₂SO₄, filtered, and concentrated to obtain the crude product, which was purified using column chromatography.

3.13.4. General procedure for the synthesis of dipeptides and higher analogs

CTSOBt (**1Xc**) (0.5 mmol) was added to a mixture of N-protected amino acid (0.5 mmol) and DIPEA (0.6 mmol) in acetone (1 mL) at room temperature. After pre-activation (for 15–20 min), a neutral solution of methyl ester of a second amino acid (0.5 mmol) was added. The reaction progress was tracked by TLC. Upon completion, acetone was evaporated, and EtOAc was poured. The organic portion was washed twice with 10% citric acid and 10% NaHCO₃ solution, respectively. It was then dried over anhydrous Na₂SO₄, filtered, and concentrated to obtain the crude dipeptide. The crude product was purified using column chromatography.

For synthesizing higher analogs, the Boc-group of the dipeptide was deprotected using TFA/DCM (1:1) and evaporated with a rotavapor. The deprotected peptide was washed with diethyl ether and neutralized with DIPEA in acetone. Simultaneously, another N-protected amino acid (0.5 mmol), **1Xc** (0.5 mmol), and DIPEA (0.6 mmol) in acetone (1 mL) were stirred for 15–20 minutes. The neutralized Boc-deprotected dipeptide was added to this mixture to obtain the desired tripeptide. The synthesis of the tetrapeptide followed the same repeated process. All tri- and tetrapeptides were purified by washing and then performing column chromatography.

3.13.5. General procedure for solid phase peptide synthesis

As described in chapter 2, section 2.14.6. Here, we used coupling reagent CTSOBt (**1Xc**) instead of coupling reagent *o*-CTSOXY (**1B**).

3.13.6. Procedure for synthesizing stapled peptide Cy-NFGAILER-NH₂ (12e)

Amino acids were installed in the sequence using CTSOBt (**1Xc**). The first amino acid, Fmoc-Arg(Pbf)-OH (2 equiv), was successfully coupled with resin using CTSOBt (**1Xc**) (2.5 equiv) in a DMSO/EtOAc solvent (1:9). Coupling steps continued until the desired peptide, Fmoc-NFGAILER-resin, was obtained. Fmoc-deprotection of the last amino acid was performed using 10% 4-MP in DMSO/EtOAc (1:9). The *t*Bu-group of the second amino acid was deprotected using FeCl₃ (5 equiv) in DCM. The second amino acid was then coupled with the last amino acid using CTSOBt (**1Xc**) (2 equiv) and DIPEA (3 equiv) in DMSO/EtOAc (1:9). After successful side-chain to tail coupling, the stapled peptide Cy-NFGAILER-NH₂ was detached from the resin using a TFA:DCM:H₂O (90:5:5) cocktail, precipitated in cold diethyl ether, and purified using reversed-phase HPLC.

3.13.7. Procedure for synthesizing cyclic peptide Cy-KLVfAE (12f) and Cy-KL(N-Me)VFF(N-Me)AE (12g)

The synthesis of each cyclic peptide started with the installation of the Fmoc-Glu-OMe amino acid. The coupling steps were continued until we got the sequence Fmoc-KLVfAE-OMe-resin and Cy-KL(N-Me)VFF(N-Me)AE-OMe-resin. LiOH in THF/H₂O was used to cleave the methyl ester of the first amino acid, and then last amino acid Fmoc was deprotected using 10% 4-MP in DMSO/EtOAc (1:9). Head-to-tail cyclization was achieved with the help of CTSOBt (**1Xc**) (2 equiv), DIPEA (2.5 equiv) in DMSO/EtOAc for 12 hours. The final cleavage was done with TFA:DCM:H₂O (90:5:5) cocktail for 3 hours. It was then precipitated in cold diethyl ether and purified using reversed-phase HPLC.

3.13.8. Synthesis of polymer-supported coupling reagent [P-CTSOBt (**1Xp**)]

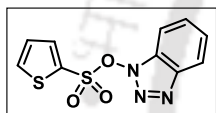
In a 2mL frit-fitted plastic syringe 200 mg polymer-bound HOBt resin (0.2 mmol, loading: 1 mmol/g) was taken. Next, 5-chlorothiophene-2-sulfonyl chloride (0.2 mmol) and DIPEA (0.2 mmol) in acetone was added to it. It was then rotated for 30 min. The reaction progress was monitored by TLC. After the complete consumption of the starting material, the resin was washed with acetone to get pure P-CTSOBt (**1Xp**).

3.13.9. Procedure for synthesizing amides, esters, thioesters, and peptides using 1Xp

Acid (0.2 mmol) and DIPEA (0.24 mmol) in DMSO/acetone (1:19) were added to the syringe containing **1Xp** (200 mg) and allowed to rotate for 30–40 min. After that, a nucleophile (0.2 mmol) such as amine, alcohol, or C-protected amino acid was added to the syringe to obtain the desired product. The reaction progress was monitored by TLC. After completion, the filtrate was taken. It was then evaporated, and EtOAc was added. Next, the organic part was washed twice with 10% citric acid and 10% NaHCO₃ solution, respectively. It was then dried using anhydrous Na₂SO₄ and filtered. The filtrate was then evaporated to obtain the pure product. In some cases, further purification was needed.

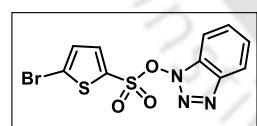
3.14. Characterization data

1H-benzo[d][1,2,3]triazol-1-yl thiophene-2-sulfonate (**1Xa**):



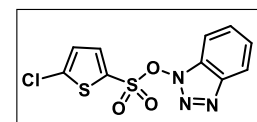
Yield: 98% (275 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 4.8 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 4.4 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 143.08, 139.01, 138.66, 130.40, 129.64, 128.87, 128.85, 125.60, 120.54, 109.55; HRMS: (ESI) *m/z* calcd for C₁₀H₈N₃O₃S₂, 282.0002 [M+H]⁺; found 282.0007.

1H-benzo[d][1,2,3]triazol-1-yl 5-bromothiophene-2-sulfonate (**1Xb**):

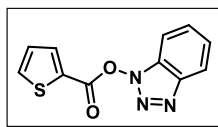


Yield: 96% (346 mg); faint brownish solid; ¹H NMR (600 MHz, CDCl₃) δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 4.1 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 4.1 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 143.10, 139.26, 131.94, 130.87, 129.80, 128.86, 127.55, 125.73, 120.62, 109.54; HRMS: (ESI) *m/z* calcd for C₁₀H₇BrN₃O₃S₂, 359.9107 [M+H]⁺; found 359.9123.

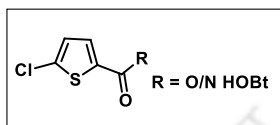
1H-benzo[d][1,2,3]triazol-1-yl 5-chlorothiophene-2-sulfonate (**1Xc**):



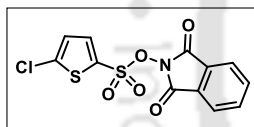
Yield: 99.9% (315.4 mg); faint yellowish solid; mp 85–87 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 4.2 Hz, 1H), 7.48–7.44 (m, 1H), 7.07 (d, *J* = 4.2 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 144.64, 143.14, 138.75, 129.81, 128.90, 128.38, 128.07, 125.74, 120.65, 109.57; HRMS: (ESI) *m/z* calcd for C₁₀H₇ClN₃O₃S₂, 315.9612 [M+H]⁺; found 315.9608.

1H-benzo[d][1,2,3]triazol-1-yl thiophene-2-carboxylate (1Xd):

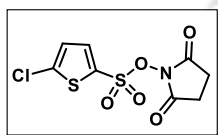
Yield: 97% (238 mg); white solid; $R_f = 0.5$ in 1:4 EtOAc/Hexane; ^1H NMR (600 MHz, CDCl_3) δ 8.19 (ddd, $J = 5.1, 3.9, 1.9$ Hz, 1H), 8.12 – 8.08 (m, 1H), 7.89 (ddd, $J = 6.2, 5.0, 1.3$ Hz, 1H), 7.59 – 7.54 (m, 1H), 7.50 (ddt, $J = 8.3, 2.1, 1.1$ Hz, 1H), 7.47 – 7.42 (m, 1H), 7.33 – 7.28 (m, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 158.36, 143.66, 137.49, 136.80, 136.78, 129.07, 129.01, 128.97, 125.13, 120.76, 108.59; HRMS: (ESI) m/z calcd for $\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S}$, 246.0332 $[\text{M}+\text{H}]^+$; found 246.0334.

1H-benzo[d][1,2,3]triazol-1-yl 5-chlorothiophene-2-carboxylate (1Xe):

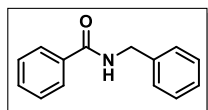
Yield: 97% (271 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.10 (d, $J = 8.4$ Hz, 1H), 7.99 (d, $J = 4.1$ Hz, 1H), 7.59 – 7.55 (m, 1H), 7.51 – 7.43 (m, 2H), 7.14 (d, $J = 4.1$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 157.35, 156.01, 143.62, 143.27, 142.42, 138.20, 137.31, 133.62, 133.53, 132.72, 130.51, 129.18, 128.88, 128.56, 127.97, 127.40, 125.20, 124.74, 120.82, 116.75, 115.87, 108.47; HRMS: (ESI) m/z calcd for $\text{C}_{11}\text{H}_7\text{ClN}_3\text{O}_2\text{S}$, 279.9943 $[\text{M}+\text{H}]^+$; found 279.9958.

1,3-dioxoisindolin-2-yl 5-chlorothiophene-2-sulfonate (1Xf):

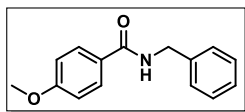
Yield: 87% (299 mg); faint raddish solid; ^1H NMR (600 MHz, CDCl_3) δ 7.90 (dd, $J = 5.5, 3.1$ Hz, 2H), 7.83 (dd, $J = 5.5, 3.1$ Hz, 2H), 7.73 (d, $J = 4.2$ Hz, 1H), 7.06 (d, $J = 4.2$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 161.43, 143.10, 137.50, 135.58, 130.31, 128.65, 127.90, 124.68; HRMS: (ESI) m/z calcd for $\text{C}_{12}\text{H}_6\text{ClNNaO}_5\text{S}_2$, 365.9269 $[\text{M}+\text{Na}]^+$; found 365.9272.

2,5-dioxopyrrolidin-1-yl 5-chlorothiophene-2-sulfonate (1Xg):

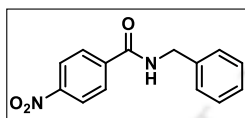
Yield: 91% (269 mg); brown solid; ^1H NMR (600 MHz, CDCl_3) δ 7.73 (d, $J = 4.2$ Hz, 1H), 7.05 (d, $J = 4.2$ Hz, 1H), 2.84 (s, 4H); ^{13}C NMR (151 MHz, CDCl_3) δ 168.43, 143.17, 137.52, 130.58, 127.87, 25.61; HRMS: (ESI) m/z calcd for $\text{C}_8\text{H}_6\text{ClNNaO}_5\text{S}_2$, 317.9269 $[\text{M}+\text{Na}]^+$; found 317.9268.

N-Benzylbenzamide (3a):

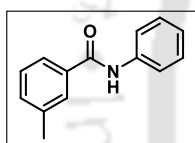
Yield: 98% (103 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, $J = 7.5$ Hz, 2H), 7.50 (t, $J = 7.4$ Hz, 1H), 7.42 (t, $J = 7.7$ Hz, 2H), 7.35 (d, $J = 4.3$ Hz, 4H), 7.32 – 7.28 (m, 1H), 6.48 (s, 1H), 4.65 (d, $J = 5.7$ Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.57, 138.37, 134.57, 131.76, 128.99, 128.80, 128.12, 127.83, 127.15, 44.35; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{14}\text{NO}$, 212.1070 $[\text{M}+\text{H}]^+$; found 212.1076.

N-benzyl-4-methoxybenzamide (3b):

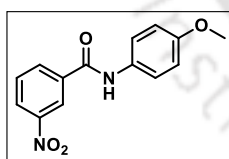
Yield: 97% (117 mg); colorless solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 8.9$ Hz, 2H), 7.34 (d, $J = 4.4$ Hz, 4H), 7.28 (ddd, $J = 8.7, 4.7, 3.9$ Hz, 1H), 6.90 (d, $J = 8.9$ Hz, 2H), 6.49 (s, 1H), 4.61 (d, $J = 5.7$ Hz, 2H), 3.83 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.08, 162.38, 138.61, 128.97, 128.91, 128.06, 127.70, 126.82, 113.92, 55.58, 44.23; HRMS: (ESI) m/z calcd for $\text{C}_{15}\text{H}_{16}\text{NO}_2$, 242.1176 $[\text{M}+\text{H}]^+$; found 242.1179.

N-benzyl-4-nitrobenzamide (3c):

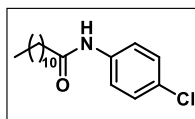
Yield: 97% (124 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.24 (d, $J = 8.8$ Hz, 2H), 7.93 (d, $J = 8.8$ Hz, 2H), 7.36 – 7.33 (m, 5H), 6.68 (s, 1H), 4.64 (d, $J = 5.7$ Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.56, 149.79, 140.09, 137.63, 129.11, 128.39, 128.15, 128.13, 124.01, 44.64; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_3$, 257.0921 $[\text{M}+\text{H}]^+$; found 257.0926.

3-methyl-N-phenylbenzamide (3d):

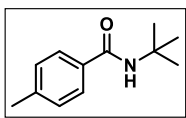
Yield: 96% (101 mg); faint orange solid; ^1H NMR (600 MHz, CDCl_3) δ 7.94 (s, 1H), 7.67 – 7.63 (m, 4H), 7.37 – 7.33 (m, 4H), 7.14 (t, $J = 7.4$ Hz, 1H), 2.40 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.21, 138.86, 138.20, 135.17, 132.75, 129.25, 128.81, 128.00, 124.68, 124.17, 120.41, 21.57; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{14}\text{NO}$, 212.1070 $[\text{M}+\text{H}]^+$; found 212.1072.

N-(4-methoxyphenyl)-3-nitrobenzamide (3e):

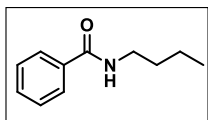
Yield: 97% (132 mg); faint greenish solid; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.46 (s, 1H), 8.78 (s, 1H), 8.41 (dd, $J = 13.1, 8.1$ Hz, 2H), 7.82 (t, $J = 7.9$ Hz, 1H), 7.69 (d, $J = 8.6$ Hz, 2H), 6.95 (d, $J = 8.6$ Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 162.83, 155.87, 147.74, 136.36, 134.05, 131.70, 130.13, 125.98, 122.30, 122.18, 113.81, 55.18; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_4$, 273.0870 $[\text{M}+\text{H}]^+$; found 273.0874.

N-(4-chlorophenyl)dodecanamide (3f):

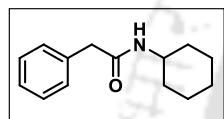
Yield: 89% (138 mg); faint pinkish solid; ^1H NMR (600 MHz, CDCl_3) δ 7.47 (d, $J = 8.8$ Hz, 2H), 7.29 (s, 1H), 7.26 (d, $J = 8.5$ Hz, 2H), 2.34 (t, $J = 7.6$ Hz, 2H), 1.72 – 1.65 (m, 2H), 1.34 – 1.24 (m, 16H), 0.87 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.74, 136.68, 129.25, 129.16, 121.16, 37.98, 32.11, 29.82, 29.81, 29.68, 29.58, 29.55, 29.46, 25.77, 22.90, 14.36; HRMS: (ESI) m/z calcd for $\text{C}_{18}\text{H}_{29}\text{ClNO}$, 310.1933 $[\text{M}+\text{H}]^+$; found 310.1931.

N-(tert-butyl)-4-methylbenzamide (3g):

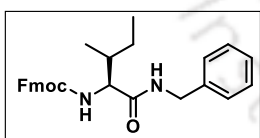
Yield: 91% (87 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.61 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.3 Hz, 2H), 5.92 (s, 1H), 2.37 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.04, 141.56, 133.24, 129.28, 126.87, 51.67, 29.09, 21.56; HRMS: (ESI) m/z calcd for $\text{C}_{12}\text{H}_{18}\text{NO}$, 192.1383 $[\text{M}+\text{H}]^+$; found 192.1382.

N-butylbenzamide (3h):

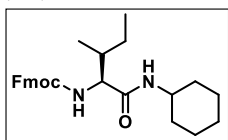
Yield: 94% (83 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.75 – 7.74 (m, 2H), 7.47 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.6 Hz, 2H), 6.28 (s, 1H), 3.44 (dd, J = 13.1, 7.1 Hz, 2H), 1.61 – 1.56 (m, 2H), 1.42 – 1.36 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.77, 135.04, 131.46, 128.69, 127.02, 39.99, 31.91, 20.34, 13.97; HRMS: (ESI) m/z calcd for $\text{C}_{11}\text{H}_{16}\text{NO}$, 178.1227 $[\text{M}+\text{H}]^+$; found 178.1233.

N-cyclohexyl-2-phenylacetamide (3i):

Yield: 97% (105 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.34 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 7.4 Hz, 1H), 7.24 (d, J = 7.2 Hz, 2H), 5.26 (s, 1H), 3.79 – 3.70 (m, 1H), 3.54 (s, 2H), 1.83 – 1.80 (m, 2H), 1.61 – 1.54 (m, 3H), 1.34 – 1.27 (m, 2H), 1.11 – 1.04 (m, 1H), 1.02 – 0.96 (m, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 170.23, 135.26, 129.56, 129.15, 127.45, 48.33, 44.16, 33.06, 25.60, 24.87; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{20}\text{NO}$, 218.1540 $[\text{M}+\text{H}]^+$; found 218.1551.

(9H-fluoren-9-yl)methyl((2S,3R)-1-(benzylamino)-3-methyl-1-oxopentan-2-yl)carbamate (3j):

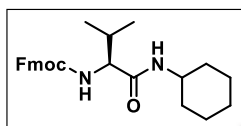
Yield: 98% (217 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.74 (d, J = 7.5 Hz, 2H), 7.54 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.29 – 7.21 (m, 7H), 6.46 (s, 1H), 5.50 (d, J = 8.6 Hz, 1H), 4.48 – 4.26 (m, 4H), 4.16 (t, J = 6.8 Hz, 1H), 4.03 (t, J = 7.7 Hz, 1H), 1.88 (s, 1H), 1.52 (s, 1H), 1.53 – 1.08 (m, 1H), 0.92 – 0.88 (dd, J = 12.6, 6.5 Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.43, 156.63, 143.99, 141.50, 138.02, 128.89, 127.92, 127.90, 127.74, 127.29, 125.23, 120.18, 67.18, 60.04, 47.36, 43.74, 37.56, 25.00, 15.75, 11.48; HRMS: (ESI) m/z calcd for $\text{C}_{28}\text{H}_{31}\text{N}_2\text{O}_3$, 443.2335 $[\text{M}+\text{H}]^+$; found 443.2358.

(9H-fluoren-9-yl)methyl((2S,3R)-1-(cyclohexylamino)-3-methyl-1-oxopentan-2-yl)carbamate (3k):

Yield: 99% (215 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.75 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.2 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 5.93 (d, J = 7.4 Hz, 1H), 5.58 (d, J = 8.6 Hz, 1H), 4.36 (dt, J =

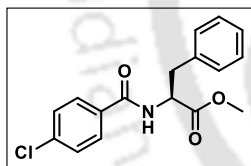
17.3, 10.3 Hz, 2H), 4.19 (t, $J = 7.0$ Hz, 1H), 3.93 (t, $J = 7.9$ Hz, 1H), 3.84 – 3.69 (m, 1H), 1.86 – 1.84 (m, 3H), 1.69 – 1.65 (m, 2H), 1.60 – 1.57 (m, 2H), 1.35 – 1.25 (m, 2H), 1.15 – 1.09 (m, 4H), 0.92 (d, $J = 6.7$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.32, 156.54, 144.05, 143.98, 141.49, 127.90, 127.26, 125.24, 120.17, 120.15, 67.22, 60.01, 48.51, 47.36, 37.78, 33.31, 33.09, 25.64, 25.17, 24.99, 24.97, 15.60, 11.55; HRMS: (ESI) m/z calcd for $\text{C}_{27}\text{H}_{35}\text{N}_2\text{O}_3$, 435.2648 $[\text{M}+\text{H}]^+$; found 435.2669.

(9H-fluoren-9-yl)methyl (S)-(1-(cyclohexylamino)-3-methyl-1-oxobutan-2-yl)carbamate (3l):



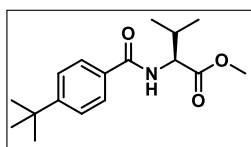
Yield: 99% (108 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.68 (d, $J = 7.5$ Hz, 2H), 7.51 (d, $J = 7.3$ Hz, 2H), 7.32 (t, $J = 7.4$ Hz, 2H), 7.23 (t, $J = 7.4$ Hz, 2H), 5.76 (d, $J = 7.1$ Hz, 1H), 5.45 (d, $J = 8.5$ Hz, 1H), 4.30 (dt, $J = 17.2, 10.3$ Hz, 2H), 4.13 (t, $J = 7.0$ Hz, 1H), 3.81 (t, $J = 7.6$ Hz, 1H), 3.72 – 3.65 (m, 1H), 2.04 – 1.97 (m, 1H), 1.80 – 1.78 (m, 2H), 1.62 – 1.59 (m, 2H), 1.53 – 1.50 (m, 1H), 1.31 – 1.21 (m, 2H), 1.09 – 1.00 (m, 3H), 0.87 (s, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.25, 156.62, 144.05, 144.00, 141.51, 127.92, 127.29, 125.25, 120.19, 120.17, 67.25, 60.87, 48.53, 47.38, 33.34, 33.13, 31.53, 25.65, 24.99, 24.97, 19.35, 18.33; HRMS: (ESI) m/z calcd for $\text{C}_{26}\text{H}_{33}\text{N}_2\text{O}_3$, 421.2486 $[\text{M}+\text{H}]^+$; found 421.2514.

Methyl (4-chlorobenzoyl)-L-phenylalaninate (3m):

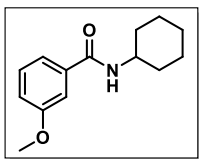


Yield: 95% (151 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.65 (d, $J = 8.4$ Hz, 2H), 7.39 (d, $J = 8.5$ Hz, 2H), 7.29 (t, $J = 7.2$ Hz, 2H), 7.27 – 7.24 (m, 1H), 7.12 (d, $J = 7.0$ Hz, 2H), 6.57 (d, $J = 7.1$ Hz, 1H), 5.07 (q, $J = 5.7$ Hz, 1H), 3.77 (s, 3H), 3.31 – 3.19 (m, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.17, 165.96, 138.26, 135.91, 132.41, 129.49, 129.08, 128.85, 128.64, 127.46, 53.75, 52.70, 38.01; HRMS: (ESI) m/z calcd for $\text{C}_{17}\text{H}_{17}\text{ClNO}_3$, 318.0892 $[\text{M}+\text{H}]^+$; found 318.0910.

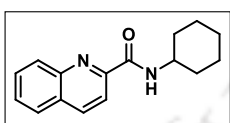
Methyl (4-(tert-butyl)benzoyl)-L-valinate (3n):



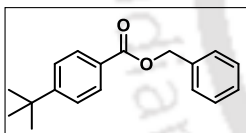
Yield: 94% (137 mg); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.74 (d, $J = 8.2$ Hz, 2H), 7.44 (d, $J = 8.3$ Hz, 2H), 6.68 (d, $J = 8.2$ Hz, 1H), 4.77 (dd, $J = 8.6, 5.0$ Hz, 1H), 3.74 (s, 3H), 2.25 (td, $J = 13.5, 6.8$ Hz, 1H), 1.31 (s, 9H), 0.976 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.86, 167.34, 155.36, 131.41, 127.07, 125.66, 57.51, 52.32, 35.06, 31.76, 31.28, 19.14, 18.11; HRMS: (ESI) m/z calcd for $\text{C}_{17}\text{H}_{26}\text{NO}_3$, 292.1908 $[\text{M}+\text{H}]^+$; found 292.1935.

N-cyclohexyl-3-methoxybenzamide (3o):

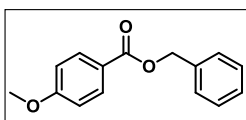
Yield: 98% (114 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.34 – 7.32 (m, 1H), 7.30 (t, $J = 7.9$ Hz, 1H), 7.25 (dt, $J = 7.6, 1.3$ Hz, 1H), 7.02 – 6.99 (m, 1H), 6.03 (d, $J = 7.3$ Hz, 1H), 3.99 – 3.91 (m, 1H), 3.83 (s, 3H), 2.04 – 1.98 (m, 2H), 1.74 (dt, $J = 13.9, 3.9$ Hz, 2H), 1.64 (dp, $J = 11.5, 3.8$ Hz, 1H), 1.45 – 1.37 (m, 2H), 1.22 (dtd, $J = 23.8, 12.0, 3.7$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.64, 159.96, 136.81, 129.64, 118.73, 117.53, 112.53, 55.61, 48.89, 33.37, 25.75, 25.09; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{20}\text{NO}_2$, 234.1489 $[\text{M}+\text{H}]^+$; found 234.1486.

N-cyclohexylquinoline-2-carboxamide (3p):

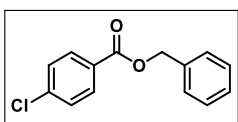
Yield: 96% (122 mg); colorless liquid; ^1H NMR (400 MHz, CDCl_3) δ 8.27 (dd, $J = 20.7, 8.5$ Hz, 2H), 8.17 (d, $J = 7.9$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 7.82 (d, $J = 8.1$ Hz, 1H), 7.71 (t, $J = 7.6$ Hz, 1H), 7.56 (t, $J = 7.5$ Hz, 1H), 4.04 – 3.96 (m, 1H), 2.04 (d, $J = 11.0$ Hz, 2H), 1.78 – 1.75 (m, 2H), 1.64 (d, $J = 13.2$ Hz, 1H), 1.47 – 1.30 (m, 4H), 1.26 – 1.17 (m, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 163.56, 150.23, 146.55, 137.47, 130.07, 129.75, 129.33, 127.84, 127.82, 118.98, 48.50, 33.22, 25.73, 25.06; HRMS: (ESI) m/z calcd for $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}$, 255.1492 $[\text{M}+\text{H}]^+$; found 255.1519.

Benzyl 4-(tert-butyl)benzoate (6a):

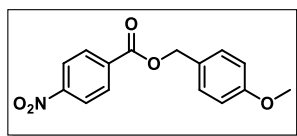
Yield: 87% (117 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.01 (d, $J = 8.2$ Hz, 2H), 7.45 – 7.43 (m, 4H), 7.38 (t, $J = 7.4$ Hz, 2H), 7.33 – 7.31 (m, 1H), 5.35 (s, 2H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.66, 156.91, 136.46, 129.79, 128.76, 128.34, 128.26, 127.55, 125.56, 66.64, 35.28, 31.31; HRMS: (ESI) m/z calcd for $\text{C}_{18}\text{H}_{20}\text{NaO}_2$, 291.1356 $[\text{M}+\text{Na}]^+$; found 291.1339.

Benzyl 4-methoxybenzoate (6b):

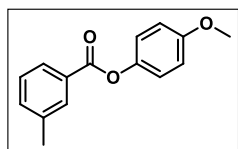
Yield: 89% (108 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.05 (d, $J = 8.9$ Hz, 2H), 7.45 (d, $J = 7.3$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.35 (t, $J = 7.3$ Hz, 1H), 6.92 (d, $J = 8.9$ Hz, 2H), 5.34 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.36, 163.55, 136.41, 131.90, 128.72, 128.31, 128.26, 122.61, 113.76, 66.56, 55.58; HRMS: (ESI) m/z calcd for $\text{C}_{15}\text{H}_{15}\text{O}_3$, 243.1016 $[\text{M}+\text{H}]^+$; found 243.1033.

Benzyl 4-chlorobenzoate¹⁶ (6c):

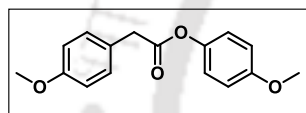
Yield: 92% (113 mg); colorless oil; ^1H NMR (600 MHz, CDCl_3) δ 8.02 (d, $J = 8.6$ Hz, 2H), 7.48 – 7.34 (m, 7H), 5.37 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.74, 139.68, 135.97, 131.28, 128.91, 128.83, 128.76, 128.56, 128.43, 67.13.

4-Methoxybenzyl 4-nitrobenzoate¹⁷ (6d):

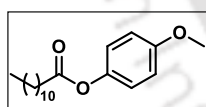
Yield: 96% (138 mg); white solid; ¹H NMR (400 MHz, CDCl₃) δ 8.26 (d, *J* = 8.8 Hz, 2H), 8.21 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.33 (s, 2H), 3.82 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 164.81, 160.16, 150.75, 135.87, 130.99, 130.58, 127.55, 123.71, 114.31, 67.74, 55.52.

4-Methoxyphenyl 3-methylbenzoate (6e):

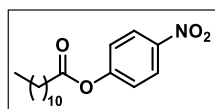
Yield: 86% (104 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 8.03 – 7.99 (m, 2H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 3.83 (s, 3H), 2.45 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 165.92, 157.48, 144.66, 138.57, 134.47, 130.83, 129.75, 128.63, 127.48, 122.66, 114.71, 55.80, 21.48; HRMS: (ESI) *m/z* calcd for C₁₅H₁₅O₃, 243.1016 [M+H]⁺; found 243.1039.

4-Methoxyphenyl 2-(4-methoxyphenyl)acetate (6f):

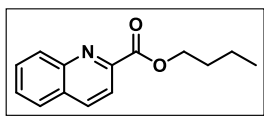
Yield: 85% (116 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.30 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 9.1 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 9.1 Hz, 2H), 3.79 (d, *J* = 18.0 Hz, 8H); ¹³C NMR (151 MHz, CDCl₃) δ 171.01, 158.94, 157.34, 144.34, 130.52, 125.70, 122.38, 114.50, 114.23, 55.71, 55.43, 40.61; HRMS: (ESI) *m/z* calcd for C₁₆H₁₇O₄, 273.1122 [M+H]⁺; found 273.1134.

4-Methoxyphenyl dodecanoate (6g):

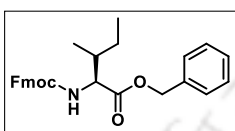
Yield: 87% (133 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 6.99 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 9.1 Hz, 2H), 3.79 (s, 3H), 2.53 (t, *J* = 7.5 Hz, 2H), 1.74 (p, *J* = 7.5 Hz, 2H), 1.40 (p, *J* = 6.9 Hz, 3H), 1.32 – 1.26 (m, 13H), 0.88 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 172.95, 157.36, 144.47, 122.52, 114.62, 55.78, 34.56, 32.12, 29.81, 29.67, 29.54, 29.46, 29.33, 25.19, 22.89, 14.32; HRMS: (ESI) *m/z* calcd for C₁₉H₃₁O₃, 307.2268 [M+H]⁺; found 307.2268.

4-Nitrophenyl dodecanoate (6h):

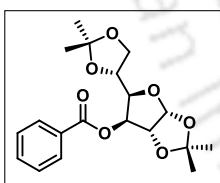
Yield: 84% (135 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 8.27 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.9 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 1.76 (p, *J* = 7.6 Hz, 2H), 1.41 (p, *J* = 7.6, 7.1 Hz, 2H), 1.36 – 1.52 (q, *J* = 8.2 Hz, 14H), 0.88 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.56, 155.74, 145.45, 125.39, 122.64, 34.54, 32.10, 29.79, 29.78, 29.63, 29.53, 29.42, 29.25, 24.95, 22.88, 14.31; HRMS: (ESI) *m/z* calcd for C₁₈H₂₈NO₄, 322.2013 [M+H]⁺; found 322.3057.

Butyl quinoline-2-carboxylate (6i):

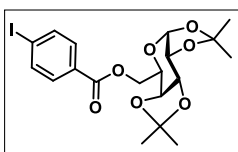
Yield: 85% (97 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.29 (dd, $J = 11.5, 8.7$ Hz, 2H), 8.16 (d, $J = 8.5$ Hz, 1H), 7.86 (d, $J = 8.2$ Hz, 1H), 7.77 (t, $J = 7.7$ Hz, 1H), 7.63 (t, $J = 7.5$ Hz, 1H), 4.48 (t, $J = 6.9$ Hz, 2H), 1.84 (dt, $J = 14.7, 7.0$ Hz, 2H), 1.51 – 1.45 (m, 2H), 0.98 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.58, 148.32, 147.74, 137.44, 130.92, 130.39, 129.41, 128.72, 127.67, 121.17, 66.26, 30.86, 19.34, 13.98; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{16}\text{NO}_2$, 230.1176 $[\text{M}+\text{H}]^+$; found 230.1209.

Benzyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alloisoleucinate (6j):

Yield: 96% (213 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.77 (d, $J = 7.5$ Hz, 2H), 7.60 (d, $J = 7.4$ Hz, 2H), 7.41 (t, $J = 7.3$ Hz, 2H), 7.37 – 7.30 (m, 7H), 5.34 (d, $J = 8.8$ Hz, 1H), 5.19 (dd, $J = 40.4, 12.2$ Hz, 2H), 4.40 (d, $J = 6.9$ Hz, 2H), 4.23 (t, $J = 6.9$ Hz, 1H), 1.92 (d, $J = 2.2$ Hz, 1H), 1.61 (s, 1H), 1.42 – 1.35 (m, 1H), 1.19 – 1.12 (m, 1H), 0.92 – 0.88 (m, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.16, 156.32, 144.14, 143.97, 141.51, 135.49, 128.81, 128.68, 128.59, 127.90, 127.27, 125.30, 120.19, 120.17, 67.25, 65.58, 58.61, 47.40, 38.30, 25.12, 15.72, 11.78; HRMS: (ESI) m/z calcd for $\text{C}_{28}\text{H}_{30}\text{NO}_4$, 444.2170 $[\text{M}+\text{H}]^+$; found 444.2178.

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)-4,4,6,6-tetramethylhexahydrofuro[3,4-b]furan-3-yl benzoate (6k):

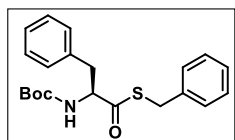
Yield: 84% (153 mg); colorless oil; ^1H NMR (600 MHz, CDCl_3) δ 8.03 (d, $J = 7.1$ Hz, 2H), 7.59 (t, $J = 7.4$ Hz, 1H), 7.46 (t, $J = 7.8$ Hz, 2H), 5.95 (d, $J = 3.6$ Hz, 1H), 5.50 (d, $J = 2.8$ Hz, 1H), 4.63 (d, $J = 3.6$ Hz, 1H), 4.39 – 4.32 (m, 2H), 4.14 – 4.07 (m, 2H), 1.56 (s, 3H), 1.42 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.42, 133.68, 129.92, 129.76, 128.75, 112.58, 109.61, 105.36, 83.61, 80.19, 76.85, 72.80, 67.47, 27.03, 26.95, 26.42, 25.42; HRMS: (ESI) m/z calcd for $\text{C}_{19}\text{H}_{24}\text{NaO}_7$, 387.1415 $[\text{M}+\text{Na}]^+$; found 387.1417.

((3aR,5R,5aS,8aS,8bR)-2,2,7,7-Tetramethyltetrahydro-5H-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl 4-iodobenzoate (6l):

Yield: 85% (208 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, $J = 8.5$ Hz, 2H), 7.75 (d, $J = 8.5$ Hz, 2H), 5.56 (d, $J = 4.9$ Hz, 1H), 4.65 (dd, $J = 7.9, 2.4$ Hz, 1H), 4.52 (dd, $J = 11.6, 4.6$ Hz, 1H), 4.41 (dd, $J = 11.5, 7.7$ Hz, 1H), 4.34 (dd, $J = 4.9, 2.5$ Hz, 1H), 4.31 (dd, $J = 7.9, 1.8$ Hz, 1H), 4.16 (ddd, $J = 6.9, 4.5, 1.6$ Hz, 1H), 1.50 (s, 3H), 1.47 (s, 3H), 1.35 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (151

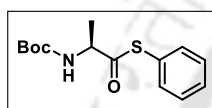
MHz, CDCl₃) δ 166.19, 137.94, 131.36, 129.77, 109.97, 109.04, 101.02, 96.54, 71.35, 70.97, 70.73, 66.34, 64.39, 26.23, 26.19, 25.18, 24.71; HRMS: (ESI) m/z calcd for C₁₉H₂₄IO₇, 491.0562 [M+H]⁺; found 491.0571.

S-Benzyl (S)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanethioate (6m):



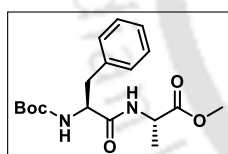
Yield: 92% (171 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.23 – 7.15 (m, 8H), 7.00 (d, J = 6.2 Hz, 2H), 4.79 (d, J = 8.9 Hz, 1H), 4.59 (q, J = 6.8 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.03 (qd, J = 14.0, 6.2 Hz, 2H), 1.33 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 200.66, 155.17, 137.36, 135.72, 129.60, 129.14, 128.81, 128.78, 127.51, 127.25, 80.58, 61.02, 38.56, 33.54, 28.48; HRMS: (ESI) m/z calcd for C₂₁H₂₅NNaO₃S, 394.1448 [M+Na]⁺; found 394.1450.

S-Phenyl (S)-2-((*tert*-butoxycarbonyl)amino)propanethioate (6n):



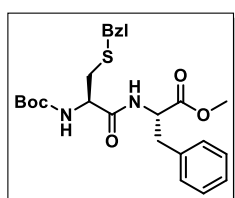
Yield: 94% (132 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.41 (s, 5H), 5.03 (s, 1H), 4.52 (p, J = 7.3 Hz, 1H), 1.49 (s, 9H), 1.44 (d, J = 7.3 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 200.23, 155.12, 134.87, 129.62, 129.41, 127.48, 80.62, 56.45, 28.57, 18.94; HRMS: (ESI) m/z calcd for C₁₄H₁₉NNaO₃S, 304.0978 [M+Na]⁺; found 304.0982.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-alaninate (9a):



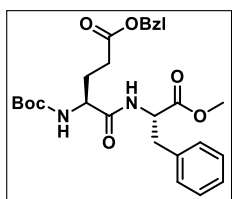
Yield: 99% (173 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.29 (t, J = 7.4 Hz, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.20 (d, J = 7.5 Hz, 2H), 6.49 (s, 1H), 5.03 (s, 1H), 4.52 (q, J = 7.7, 7.2 Hz, 1H), 4.37 (q, J = 7.4 Hz, 1H), 3.70 (s, 3H), 3.10 – 3.02 (m, 2H), 1.40 (s, 9H), 1.34 (d, J = 7.2 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 173.06, 170.91, 155.53, 136.65, 129.56, 128.82, 127.13, 80.43, 55.71, 52.69, 48.26, 38.48, 28.43, 18.57; HRMS: (ESI) m/z calcd for C₁₈H₂₇N₂O₅, 351.1915 [M+H]⁺; found 351.1913.

Methyl S-benzyl-N-(*tert*-butoxycarbonyl)-L-cysteinyl-L-phenylalaninate (9b):



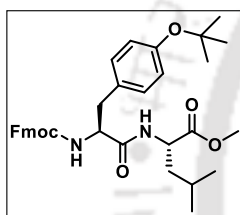
Yield: 98% (231 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.33 – 7.22 (m, 8H), 7.11 (d, J = 6.9 Hz, 2H), 6.77 (s, 1H), 5.20 (s, 1H), 4.86 – 4.80 (m, 1H), 4.21 (s, 1H), 3.70 (s, 5H), 3.17 – 3.06 (m, 2H), 2.75 (ddd, J = 51.8, 14.5, 6.4 Hz, 2H), 1.44 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 171.63, 170.48, 155.48, 138.02, 135.88, 129.49, 129.22, 128.81, 127.44, 127.38, 80.57, 53.84, 53.61, 52.56, 38.08, 36.66, 33.78, 28.46; HRMS: (ESI) m/z calcd for C₂₅H₃₃N₂O₅S, 473.2105 [M+H]⁺; found 473.2113.

Benzyl-(S)-4-((tert-butoxycarbonyl)amino)-5-(((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-5-oxopentanoate (9c):



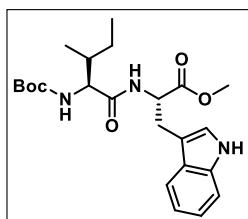
Yield: 97% (242 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.40 – 7.31 (m, 5H), 7.30 – 7.26 (m, 2H), 7.25 – 7.21 (m, 1H), 7.11 (d, $J = 7.3$ Hz, 2H), 6.80 – 6.65 (m, 1H), 5.23 (d, $J = 6.5$ Hz, 1H), 5.11 (s, 2H), 4.83 (q, $J = 6.3$ Hz, 1H), 4.17 (q, $J = 7.0, 6.4$ Hz, 1H), 3.70 (s, 3H), 3.10 (ddd, $J = 51.8, 14.0, 6.1$ Hz, 2H), 2.45 (ddt, $J = 23.8, 16.3, 7.4$ Hz, 2H), 2.10 (dq, $J = 13.7, 6.8$ Hz, 1H), 1.89 (dq, $J = 14.6, 7.4$ Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.37, 171.82, 171.35, 155.69, 135.84, 135.82, 129.41, 128.81, 128.78, 128.51, 128.48, 127.36, 80.31, 66.77, 53.73, 53.46, 52.58, 38.01, 30.54, 28.46, 28.13; HRMS: (ESI) m/z calcd for $\text{C}_{27}\text{H}_{35}\text{N}_2\text{O}_7$, 499.2439 $[\text{M}+\text{H}]^+$; found 499.2440.

Methyl((S)-3-(4-(tert-butoxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)-L-leucinate (9d):

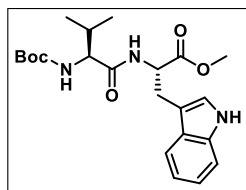


Yield: 98% (287 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.56 (d, $J = 7.4$ Hz, 2H), 7.40 (t, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.4$ Hz, 2H), 7.13 – 7.04 (m, 2H), 6.90 (d, $J = 8.1$ Hz, 2H), 6.14 (s, 1H), 5.38 (s, 1H), 4.54 (td, $J = 8.6, 5.4$ Hz, 1H), 4.42 (d, $J = 8.3$ Hz, 2H), 4.33 (d, $J = 10.6$ Hz, 1H), 4.20 (t, $J = 7.0$ Hz, 1H), 3.70 (s, 3H), 3.04 (ddd, $J = 69.7, 14.6, 6.6$ Hz, 2H), 1.55 (ddt, $J = 33.4, 13.2, 6.9$ Hz, 2H), 1.44 (dt, $J = 15.8, 8.1$ Hz, 1H), 1.32 (s, 9H), 0.87 (dd, $J = 12.6, 6.3$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.93, 170.76, 156.11, 154.68, 143.97, 143.88, 141.49, 131.17, 130.07, 127.95, 127.30, 125.26, 125.21, 124.49, 120.19, 78.61, 67.31, 56.27, 52.51, 51.08, 47.32, 41.67, 37.98, 29.02, 24.93, 22.86, 22.11; HRMS: (ESI) m/z calcd for $\text{C}_{35}\text{H}_{43}\text{N}_2\text{O}_6$, 587.3116 $[\text{M}+\text{H}]^+$; found 587.3119.

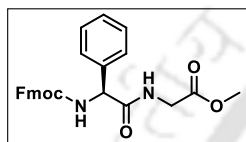
Methyl (tert-butoxycarbonyl)-L-alloisoleucyl-L-tryptophanate (9e):



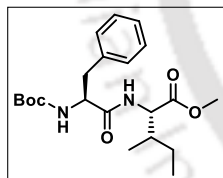
Yield: 95% (205 mg); faint yellowish solid; ^1H NMR (600 MHz, CDCl_3) δ 8.26 (s, 1H), 7.52 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.18 (t, $J = 7.5$ Hz, 1H), 7.12 (t, $J = 7.5$ Hz, 1H), 7.02 (s, 1H), 6.42 (d, $J = 7.9$ Hz, 1H), 5.07 (d, $J = 9.0$ Hz, 1H), 4.91 (dt, $J = 7.9, 5.5$ Hz, 1H), 3.96 (t, $J = 7.7$ Hz, 1H), 3.65 (s, 3H), 3.30 (qd, $J = 14.8, 5.5$ Hz, 2H), 1.82 – 1.72 (m, 2H), 1.43 (s, 9H), 1.03 (td, $J = 14.8, 14.3, 7.5$ Hz, 1H), 0.91 – 0.79 (m, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.23, 171.48, 155.91, 136.32, 127.67, 123.28, 122.43, 119.89, 118.68, 111.49, 109.86, 80.02, 59.39, 52.94, 52.53, 37.64, 28.51, 27.84, 24.80, 15.53, 11.61; HRMS: (ESI) m/z calcd for $\text{C}_{23}\text{H}_{34}\text{N}_3\text{O}_5$, 432.2493 $[\text{M}+\text{H}]^+$; found 432.2496.

Methyl (*tert*-butoxycarbonyl)-*L*-valyl-*L*-tryptophanate (9f):

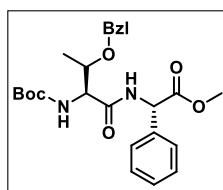
Yield: 96% (200 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.27 (s, 1H), 7.52 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.18 (t, $J = 7.5$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 7.01 (s, 1H), 6.43 (s, 1H), 5.08 (d, $J = 9.0$ Hz, 1H), 4.91 (dt, $J = 8.0, 5.6$ Hz, 1H), 3.93 (d, $J = 7.4$ Hz, 1H), 3.65 (s, 3H), 3.30 (qd, $J = 14.9, 5.5$ Hz, 2H), 2.11 – 1.99 (m, 1H), 1.43 (s, 9H), 0.90 (d, $J = 6.8$ Hz, 3H), 0.84 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.26, 171.54, 156.01, 136.33, 127.67, 123.26, 122.44, 119.89, 118.67, 111.51, 109.85, 80.01, 59.99, 52.97, 52.54, 31.28, 28.50, 27.84, 19.30, 17.79; HRMS: (ESI) m/z calcd for $\text{C}_{22}\text{H}_{32}\text{N}_3\text{O}_5$, 418.2337 $[\text{M}+\text{H}]^+$; found 418.2340.

Methyl (2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-phenylacetyl)glycinate (9g):

Yield: 97% (215 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.75 (d, $J = 7.5$ Hz, 2H), 7.57 (s, 2H), 7.38 (h, $J = 8.8, 7.2$ Hz, 7H), 7.31 – 7.27 (m, 2H), 6.28 (s, 1H), 6.13 (s, 1H), 5.29 (s, 1H), 4.37 (tt, $J = 17.7, 9.4$ Hz, 2H), 4.19 (t, $J = 7.4$ Hz, 1H), 4.08 (dd, $J = 18.6, 5.4$ Hz, 1H), 3.99 – 3.92 (m, 1H), 3.72 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 169.98, 155.88, 144.02, 143.94, 141.48, 137.79, 129.39, 128.92, 127.90, 127.60, 127.29, 125.30, 120.17, 67.37, 59.04, 52.69, 47.33, 41.65; HRMS: (ESI) m/z calcd for $\text{C}_{26}\text{H}_{25}\text{N}_2\text{O}_5$, 445.1758 $[\text{M}+\text{H}]^+$; found 445.1764.

Methyl (*tert*-butoxycarbonyl)-*L*-phenylalanyl-*L*-alloisoleucinate (9h):

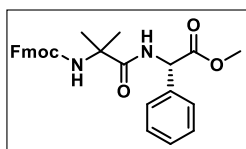
Yield: 97% (190 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.29 (t, $J = 7.5$ Hz, 2H), 7.25 – 7.19 (m, 3H), 6.40 (s, 1H), 5.04 (s, 1H), 4.49 (dd, $J = 8.4, 5.1$ Hz, 1H), 4.35 (q, $J = 7.4$ Hz, 1H), 3.68 (s, 3H), 3.12 – 3.02 (m, 2H), 1.85 – 1.78 (m, 1H), 1.41 (s, 9H), 1.35 (dtd, $J = 14.5, 7.5, 4.7$ Hz, 1H), 1.08 (ddq, $J = 14.3, 9.1, 7.3$ Hz, 1H), 0.87 (t, $J = 7.4$ Hz, 3H), 0.82 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.96, 171.12, 155.60, 136.77, 129.52, 128.84, 127.09, 80.44, 56.69, 55.93, 52.29, 38.16, 38.04, 28.44, 25.21, 15.47, 11.71; HRMS: (ESI) m/z calcd for $\text{C}_{21}\text{H}_{33}\text{N}_2\text{O}_5$, 393.2384 $[\text{M}+\text{H}]^+$; found 393.2382.

Methyl(S)-2-((2S,3S)-3-(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)butanamido)-2-phenylacetate (9i):

Yield: 96% (219 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.68 (d, $J = 7.1$ Hz, 1H), 7.33 – 7.25 (m, 9H), 7.21 (dd, $J = 6.7, 3.0$ Hz, 2H), 5.52 (t, $J = 7.4$ Hz, 1H), 4.57 (d, $J = 11.2$ Hz, 1H), 4.47 (d, $J = 11.2$ Hz, 1H), 4.34 (dd, $J = 7.0, 3.0$ Hz, 1H), 4.17 (qd, $J = 6.4, 2.9$ Hz, 1H), 3.70 (s, 3H), 1.46 (s, 9H), 1.26 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.12, 169.73, 155.98, 137.94, 136.19,

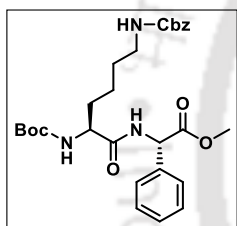
129.16, 128.72, 128.58, 127.96, 127.93, 127.39, 80.40, 74.98, 71.71, 57.61, 56.86, 52.98, 28.48, 15.32; HRMS: (ESI) m/z calcd for $C_{25}H_{33}N_2O_6$, 457.2334 $[M+H]^+$; found 457.2338.

Methyl (S)-2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanamido)-2-phenylacetate (9j):



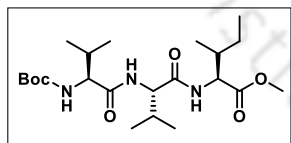
Yield: 95% (224 mg); colorless gum; 1H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, $J = 7.5$ Hz, 2H), 7.58 (t, $J = 7.7$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.37 – 7.25 (m, 8H), 5.57 – 5.38 (m, 2H), 4.39 (d, $J = 6.9$ Hz, 2H), 4.18 (s, 1H), 3.70 (s, 3H), 1.55 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.91, 171.38, 155.30, 144.00, 143.98, 141.47, 141.46, 136.51, 129.09, 128.66, 127.87, 127.29, 127.26, 127.24, 125.19, 125.16, 120.15, 66.88, 57.00, 52.98, 47.32, 25.60, 25.41; HRMS: (ESI) m/z calcd for $C_{28}H_{29}N_2O_5$, 473.2071 $[M+H]^+$; found 473.2077.

Methyl-(S)-2-((S)-6-(((benzyloxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-phenylacetate (9k):

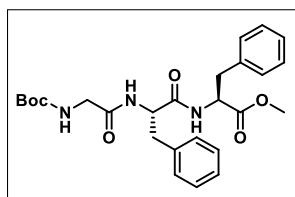


Yield: 94% (248 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 11H), 5.53 (d, $J = 7.1$ Hz, 1H), 5.10 (d, $J = 26.5$ Hz, 3H), 5.03 – 4.92 (m, 1H), 4.13 (s, 1H), 3.69 (s, 3H), 3.19 (t, $J = 6.7$ Hz, 2H), 1.92 – 1.77 (m, 2H), 1.51 (q, $J = 7.5, 6.9$ Hz, 2H), 1.41 (d, $J = 2.6$ Hz, 11H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.62, 171.29, 156.81, 155.97, 136.80, 136.30, 129.18, 128.80, 128.71, 128.33, 128.29, 127.48, 80.34, 66.85, 56.65, 54.28, 53.02, 40.54, 31.86, 29.59, 28.47, 22.52; HRMS: (ESI) m/z calcd for $C_{28}H_{38}N_3O_7$, 528.2705 $[M+H]^+$; found 528.2709.

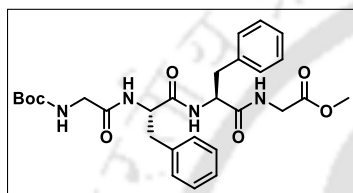
Methyl (tert-butoxycarbonyl)-L-valyl-L-valyl-L-alloisoleucinate (9l):



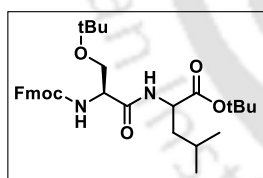
Yield: 84% (186 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 6.86 – 6.59 (m, 2H), 5.24 (d, $J = 25.4$ Hz, 1H), 4.56 (dd, $J = 8.6, 5.1$ Hz, 1H), 4.38 – 4.26 (m, 1H), 3.92 (t, $J = 7.8$ Hz, 1H), 3.72 (s, 3H), 2.16 – 2.06 (m, 2H), 1.93 – 1.83 (m, 2H), 1.42 (s, 9H), 1.21 – 1.11 (m, 1H), 0.93 (q, $J = 4.9, 4.1$ Hz, 10H), 0.90 – 0.86 (m, 8H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.29, 172.12, 171.14, 156.12, 80.06, 60.46, 58.84, 56.67, 52.27, 37.84, 31.04, 30.80, 28.50, 25.26, 19.48, 19.31, 18.37, 18.15, 15.62, 11.68; HRMS: (ESI) m/z calcd for $C_{22}H_{42}N_3O_6$, 444.3069 $[M+H]^+$; found 444.3074.

Methyl (*tert*-butoxycarbonyl)glycyl-*L*-phenylalanyl-*L*-phenylalaninate (9m):

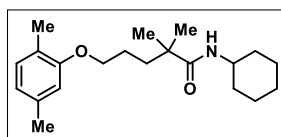
Yield: 85% (205 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.28 – 7.20 (m, 6H), 7.16 (d, $J = 7.3$ Hz, 2H), 7.01 (d, $J = 6.9$ Hz, 2H), 6.69 (d, $J = 7.5$ Hz, 1H), 6.43 (s, 1H), 5.10 (s, 1H), 4.75 (q, $J = 6.6$ Hz, 1H), 4.66 (q, $J = 7.1$ Hz, 1H), 3.75 (dd, $J = 17.0, 5.8$ Hz, 1H), 3.69 (d, $J = 5.0$ Hz, 1H), 3.67 (s, 3H), 3.10 – 2.94 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.53, 170.40, 169.57, 156.15, 136.39, 135.88, 129.52, 129.39, 128.86, 128.75, 127.29, 127.25, 80.57, 54.32, 53.58, 52.53, 44.41, 38.18, 38.02, 28.50; HRMS: (ESI) m/z calcd for $\text{C}_{26}\text{H}_{34}\text{N}_3\text{O}_6$, 484.2443 $[\text{M}+\text{H}]^+$; found 484.2447.

Methyl (*tert*-butoxycarbonyl)glycyl-*L*-phenylalanyl-*L*-phenylalanylglycinate (9n):

Yield: 75% (203 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.26 – 7.18 (m, 6H), 7.10 (d, $J = 6.7$ Hz, 4H), 6.78 (d, $J = 44.8$ Hz, 4H), 5.16 (s, 1H), 4.78 (q, $J = 7.3$ Hz, 1H), 4.59 (q, $J = 6.9$ Hz, 1H), 4.06 (dd, $J = 18.2, 5.7$ Hz, 1H), 3.83 (dd, $J = 18.0, 4.9$ Hz, 1H), 3.73 (s, 3H), 3.14 (dd, $J = 14.8, 6.1$ Hz, 1H), 2.97 (ddd, $J = 29.8, 13.8, 6.8$ Hz, 4H), 1.44 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.22, 170.79, 170.31, 169.93, 156.36, 136.72, 136.21, 129.50, 128.87, 128.61, 127.24, 126.97, 80.45, 54.66, 54.10, 52.55, 44.33, 41.23, 38.40, 38.23, 28.50; HRMS: (ESI) m/z calcd for $\text{C}_{28}\text{H}_{37}\text{N}_4\text{O}_7$, 541.2657 $[\text{M}+\text{H}]^+$; found 541.2663.

***Tert*-butyl *N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*O*-(*tert*-butyl)-*L*-serylleucinate (9o):**

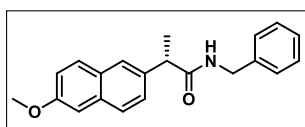
Yield: 99.9% (276 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.61 (d, $J = 7.0$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.32 (t, $J = 7.4$ Hz, 2H), 7.22 (d, $J = 6.2$ Hz, 1H), 5.78 (d, $J = 6.2$ Hz, 1H), 4.47 (q, $J = 7.5$ Hz, 1H), 4.39 (q, $J = 10.6, 8.3$ Hz, 2H), 4.24 (q, $J = 7.2, 6.8$ Hz, 2H), 3.83 (dd, $J = 8.6, 3.8$ Hz, 1H), 3.40 (t, $J = 8.4$ Hz, 1H), 1.68 – 1.61 (m, 2H), 1.52 (ddd, $J = 14.0, 8.8, 5.7$ Hz, 1H), 1.46 (s, 9H), 1.23 (s, 9H), 0.95 (d, $J = 6.4$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.84, 170.13, 156.26, 144.13, 143.98, 141.50, 127.91, 127.28, 125.35, 120.18, 81.92, 74.50, 67.31, 61.99, 54.46, 51.87, 47.35, 42.07, 28.19, 27.56, 25.08, 23.03, 22.35; HRMS (ESI) m/z : calcd for $\text{C}_{32}\text{H}_{44}\text{N}_2\text{NaO}_6$, 575.3092 $[\text{M}+\text{Na}]^+$; found 575.3104.

***N*-Cyclohexyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanamide (11a):**

Yield: 95% (157 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.00 (d, $J = 7.4$ Hz, 1H), 6.65 (d, $J = 7.4$ Hz, 1H), 6.60 (s, 1H), 5.47 (d, $J = 7.1$ Hz, 1H), 3.91 (t, $J = 6.0$ Hz, 2H), 3.77 (tdt, $J = 11.3, 7.9, 4.0$ Hz, 1H), 2.30 (s, 3H), 2.17 (s, 3H), 1.89 (dt, $J = 12.2, 4.1$ Hz, 2H), 1.75 – 1.65 (m, 6H), 1.61 (dt, $J = 12.8,$

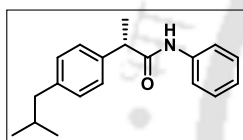
3.9 Hz, 1H), 1.36 (tdd, $J = 15.8, 12.2, 3.6$ Hz, 2H), 1.19 (s, 6H), 1.18 – 1.05 (m, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 176.57, 157.09, 136.71, 130.47, 123.70, 120.89, 112.24, 68.17, 48.19, 41.85, 37.93, 33.38, 25.76, 25.26, 25.08, 21.60, 16.05; HRMS: (ESI) m/z calcd for $\text{C}_{21}\text{H}_{34}\text{NO}_2$, 332.2585 $[\text{M}+\text{H}]^+$; found 332.2584.

(S)-N-Benzyl-2-(6-methoxynaphthalen-2-yl)propanamide (11b):



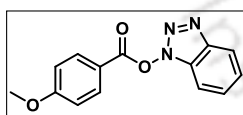
Yield: 99% (158 mg); faint yellowish solid; ^1H NMR (600 MHz, CDCl_3) δ 7.77 – 7.68 (m, 3H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.31 – 7.22 (m, 3H), 7.21 – 7.12 (m, 4H), 5.75 (s, 1H), 4.41 (qd, $J = 15.0, 5.7$ Hz, 2H), 3.94 (s, 3H), 3.76 (q, $J = 7.1$ Hz, 1H), 1.66 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 174.42, 157.93, 138.49, 136.54, 133.94, 129.40, 129.14, 128.79, 127.79, 127.69, 127.53, 126.48, 126.38, 119.39, 105.80, 55.53, 47.28, 43.77, 18.74; HRMS: (ESI) m/z calcd for $\text{C}_{21}\text{H}_{22}\text{NO}_2$, 320.1646 $[\text{M}+\text{H}]^+$; found 320.1645.

(S)-2-(4-Isobutylphenyl)-N-phenylpropanamide (11c):



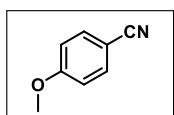
Yield: 98% (138 mg); faint yellowish solid; ^1H NMR (600 MHz, CDCl_3) δ 7.34 (d, $J = 8.0$ Hz, 2H), 7.22 – 7.16 (m, 4H), 7.08 (d, $J = 7.8$ Hz, 2H), 7.05 – 6.94 (m, 2H), 3.62 (q, $J = 7.1$ Hz, 1H), 2.40 (d, $J = 7.2$ Hz, 2H), 1.79 (dp, $J = 13.5, 6.7$ Hz, 1H), 1.52 (d, $J = 7.1$ Hz, 3H), 0.83 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.79, 141.33, 138.22, 138.08, 130.08, 129.10, 127.64, 124.36, 119.79, 47.96, 45.20, 30.39, 22.58, 18.69; HRMS: (ESI) m/z calcd for $\text{C}_{19}\text{H}_{24}\text{NO}$, 282.1853 $[\text{M}+\text{H}]^+$; found 282.1852.

Intermediate 4-methoxybenzoate-OBt (V):

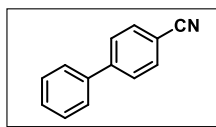


Yield: 99.9% (134 mg); faint brown solid, ^1H NMR (600 MHz, CDCl_3) δ 8.25 – 8.20 (m, 2H), 8.09 (dt, $J = 8.5, 0.9$ Hz, 1H), 7.54 (ddd, $J = 8.5, 6.9, 0.9$ Hz, 1H), 7.47 (dt, $J = 8.3, 1.0$ Hz, 1H), 7.43 (ddd, $J = 8.1, 6.8, 1.1$ Hz, 1H), 7.08 – 7.04 (m, 2H), 3.93 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.58, 162.58, 143.76, 133.29, 129.09, 128.87, 124.98, 120.69, 116.81, 114.78, 108.65, 55.93; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{12}\text{N}_3\text{O}_3$, 270.0874 $[\text{M}+\text{H}]^+$; found 270.0878.

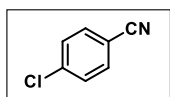
4-Methoxybenzonitrile¹⁸ (14a):



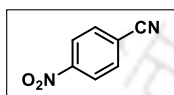
Yield: 83% (44 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.61 – 7.55 (m, 2H), 6.97 – 6.91 (m, 2H), 3.85 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 163.04, 134.18, 119.42, 114.94, 104.16, 55.73.

[1,1'-Biphenyl]-4-carbonitrile¹⁹ (14b):

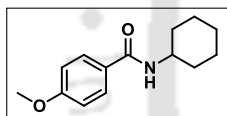
Yield: 99% (71 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.49 (t, *J* = 7.5 Hz, 2H), 7.43 (t, *J* = 7.3 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 145.86, 139.36, 132.80, 129.31, 128.86, 127.93, 127.42, 119.16, 111.08.

4-Chlorobenzonitrile²⁰ (14c):

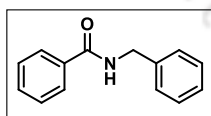
Yield: 95% (52 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.60 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 139.76, 133.59, 129.90, 118.19, 110.96.

4-Nitrobenzonitrile²¹ (14d):

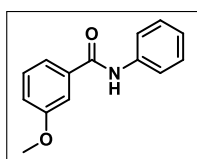
Yield: 98% (58 mg); ¹H NMR (600 MHz, CDCl₃) δ 8.36 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 150.22, 133.67, 124.48, 118.52, 116.99.

***N*-cyclohexyl-4-methoxybenzamide (17a):**

Yield: 98% (46 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.71 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.2 Hz, 2H), 5.92 (s, 1H), 3.99 – 3.91 (m, 1H), 3.83 (s, 3H), 2.01 (d, *J* = 11.1 Hz, 2H), 1.74 (dq, *J* = 13.8, 4.2 Hz, 2H), 1.64 (dt, *J* = 13.1, 4.0 Hz, 1H), 1.46 – 1.37 (m, 2H), 1.22 (qd, *J* = 11.6, 3.5 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 166.31, 162.18, 128.79, 127.60, 113.86, 55.59, 48.77, 33.52, 25.81, 25.14; HRMS: (ESI) *m/z* calcd for C₁₄H₂₀NO₂, 234.1489 [M+H]⁺; found 234.1495.

***N*-Benzylbenzamide (17b):**

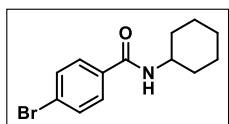
Yield: 98% (41 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.42 (td, *J* = 7.9, 3.1 Hz, 2H), 7.35 (t, *J* = 3.8 Hz, 4H), 7.30 (td, *J* = 6.3, 5.0, 2.6 Hz, 1H), 6.56 (d, *J* = 43.5 Hz, 1H), 4.63 (t, *J* = 5.5 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 167.58, 138.39, 134.55, 131.73, 128.96, 128.77, 128.09, 127.78, 127.17, 44.29; HRMS: (ESI) *m/z* calcd for C₁₄H₁₄NO, 212.1075 [M+H]⁺; found 212.1072.

3-Methoxy-*N*-phenylbenzamide (17c):

Yield: 95% (43 mg); faint radish solid; ¹H NMR (600 MHz, CDCl₃) δ 7.95 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.43 (s, 1H), 7.36 (td, *J* = 7.9, 3.2 Hz, 4H), 7.15 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 3.84 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 165.84, 160.15, 138.10, 136.66, 129.94, 129.28, 124.77, 120.41,

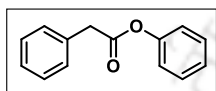
118.89, 118.22, 112.66, 55.66; HRMS: (ESI) m/z calcd for $C_{14}H_{14}NO_2$, 228.1020 $[M+H]^+$; found 228.1028.

4-bromo-N-cyclohexylbenzamide (17d):



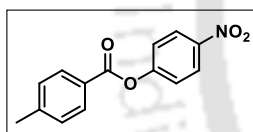
Yield: 96% (54 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 5.98 (s, 1H), 3.94 (tdt, J = 11.3, 8.0, 3.9 Hz, 1H), 2.01 (dt, J = 12.7, 3.9 Hz, 2H), 1.75 (dp, J = 11.9, 4.0 Hz, 2H), 1.65 (dt, J = 13.1, 3.9 Hz, 1H), 1.46 – 1.37 (m, 2H), 1.21 (ddt, J = 21.3, 13.0, 3.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.85, 134.09, 131.90, 128.69, 126.04, 49.06, 33.38, 25.73, 25.10.; HRMS: (ESI) m/z calcd for $C_{13}H_{17}BrNO$, 282.0489, 284.0468 $[M+H]^+$; found 282.0492, 282.0472.

Phenyl 2-phenylacetate (17e):



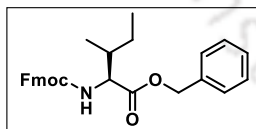
Yield: 86% (37 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.34 (m, 6H), 7.34 – 7.30 (m, 1H), 7.24 – 7.20 (m, 1H), 7.08 – 7.05 (m, 2H), 3.87 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.23, 150.93, 133.67, 129.59, 129.52, 128.94, 127.56, 126.07, 121.65, 41.65; HRMS: (ESI) m/z calcd for $C_{14}H_{13}O_2$, 213.0911 $[M+H]^+$; found 213.0918.

4-Nitrophenyl 4-methylbenzoate²² (17f):

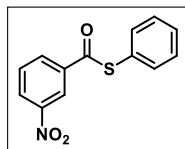


Yield: 85% (44 mg); faint yellowish solid; 1H NMR (600 MHz, $CDCl_3$) δ 8.32 (d, J = 8.8 Hz, 2H), 8.09 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 2.47 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.49, 156.05, 145.52, 145.49, 130.58, 129.72, 125.94, 125.45, 122.86, 22.04.

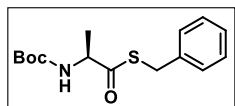
Benzyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alloisoleucinate (17g):



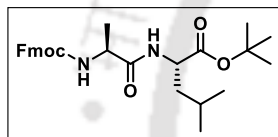
Yield: 85% (75 mg); faint pinkish solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, J = 7.4 Hz, 2H), 7.60 (d, J = 6.9 Hz, 2H), 7.41 (t, J = 7.1 Hz, 2H), 7.38 – 7.27 (m, 7H), 5.33 (t, J = 9.1 Hz, 1H), 5.26 – 5.11 (m, 2H), 4.40 (d, J = 6.8 Hz, 3H), 4.23 (t, J = 6.5 Hz, 1H), 1.92 (h, J = 5.3, 4.8 Hz, 1H), 1.39 (tq, J = 7.9, 4.0 Hz, 1H), 1.16 (dt, J = 14.6, 7.8 Hz, 1H), 0.90 (ddt, J = 17.8, 7.8, 2.9 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.16, 156.32, 144.16, 143.99, 141.52, 135.51, 128.82, 128.68, 128.60, 127.91, 127.28, 125.31, 120.20, 120.18, 67.26, 58.62, 47.43, 38.32, 29.92, 25.13, 15.73, 11.78; HRMS (ESI) m/z : calcd for $C_{28}H_{30}NO_4$, 444.2170 $[M+H]^+$; found 444.2195.

S-phenyl 3-nitrobenzothioate²³ (17h):

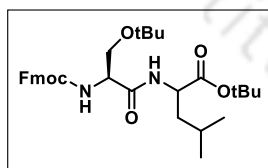
Yield: 84% (44 mg); yellowish solid; ¹H NMR (600 MHz, CDCl₃) δ 8.86 (s, 1H), 8.47 (d, *J* = 8.1 Hz, 1H), 8.33 (d, *J* = 7.7 Hz, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.56 – 7.46 (m, 5H); ¹³C NMR (151 MHz, CDCl₃) δ 188.60, 148.61, 138.16, 135.17, 133.13, 130.27, 129.72, 128.03, 126.30, 122.64.

S-Benzyl (S)-2-((tert-butoxycarbonyl)amino)propanethioate (17i):

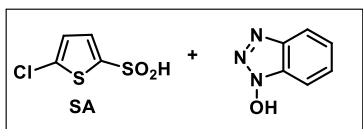
Yield: 83% (49 mg); faint pinkish solid; ¹H NMR (600 MHz, CDCl₃) δ 7.31 – 7.27 (m, 4H), 7.23 (ddd, *J* = 8.7, 5.5, 2.3 Hz, 1H), 4.96 (d, *J* = 7.3 Hz, 1H), 4.42 (p, *J* = 7.0 Hz, 1H), 4.15 – 4.07 (m, 2H), 1.45 (s, 9H), 1.38 (d, *J* = 7.2 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 201.63, 155.12, 137.31, 129.05, 128.82, 127.52, 80.52, 56.39, 33.41, 28.53, 18.98; HRMS (ESI) *m/z*: calcd for C₁₅H₂₁NNaO₃S, 318.1135 [M+Na]⁺; found 318.1149.

Tert-butyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanyl-L-leucinate (17j):

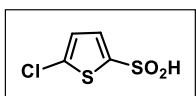
Yield: 93% (89 mg); liquid; ¹H NMR (600 MHz, CDCl₃) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.39 (d, *J* = 6.8 Hz, 1H), 5.51 (d, *J* = 6.6 Hz, 1H), 4.48 (td, *J* = 8.5, 5.2 Hz, 1H), 4.38 (d, *J* = 6.9 Hz, 2H), 4.30 (p, *J* = 7.1 Hz, 1H), 4.21 (t, *J* = 7.1 Hz, 1H), 1.66 – 1.59 (m, 2H), 1.45 (s, 9H), 1.41 (d, *J* = 7.1 Hz, 4H), 0.91 (dd, *J* = 6.3, 3.6 Hz, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 172.02, 171.99, 156.09, 144.05, 143.96, 141.49, 127.92, 127.28, 125.29, 120.18, 82.20, 67.32, 51.69, 50.58, 47.31, 41.90, 28.17, 25.11, 22.95, 22.26, 19.04; HRMS (ESI) *m/z*: calcd for C₂₈H₃₆N₂NaO₅, 503.2517 [M+Na]⁺; found 503.2522.

Tert-butyl-N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(tert-butyl)-L-seryl-L-leucinate (17k):

Yield: 95% (105 mg); ¹H NMR (600 MHz, CDCl₃) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.23 (d, *J* = 6.9 Hz, 1H), 5.78 (d, *J* = 6.3 Hz, 1H), 4.47 (q, *J* = 7.6 Hz, 1H), 4.44 – 4.36 (m, 2H), 4.24 (q, *J* = 7.3, 6.4 Hz, 2H), 3.83 (dd, *J* = 8.6, 3.8 Hz, 1H), 3.39 (t, *J* = 8.5 Hz, 1H), 1.68 – 1.60 (m, 2H), 1.55 – 1.50 (m, 1H), 1.46 (s, 9H), 1.23 (s, 9H), 0.95 (d, *J* = 6.4 Hz, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 171.83, 170.12, 156.25, 144.13, 143.97, 141.49, 127.91, 127.28, 125.36, 120.18, 81.91, 74.50, 67.30, 61.98, 54.43, 51.86, 47.34, 42.07, 28.19, 27.56, 25.08, 23.03, 22.35; HRMS (ESI) *m/z*: calcd for C₃₂H₄₄N₂NaO₆, 575.3092 [M+Na]⁺; found 575.3098.

Recovered 5-chlorothiophene-2-sulfonic acid (SA) + HOBt:

Yield: 96% (1.92g); white solid; ^1H NMR (600 MHz, CD_3OD) δ 7.87 (d, $J = 8.4$ Hz, 1H), 7.74 (d, $J = 8.4$ Hz, 1H), 7.55 (t, $J = 7.6$ Hz, 1H), 7.49 (t, $J = 7.7$ Hz, 1H), 7.21 (d, $J = 3.9$ Hz, 1H), 6.89 (d, $J = 3.8$ Hz, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 147.20, 142.76, 133.18, 129.73, 128.51, 128.33, 127.45, 127.22, 118.69, 111.60; HRMS: (ESI) m/z : calcd for SA ($\text{C}_4\text{H}_2\text{ClO}_3\text{S}_2$), 196.9139 $[\text{M}-\text{H}]^-$; found 196.9147; HOBt ($\text{C}_6\text{H}_4\text{N}_3\text{O}$), 134.0360 $[\text{M}-\text{H}]^-$; found 134.0363.

Recovered 5-chlorothiophene-2-sulfonic acid (SA)

Recovered yield: 97% (38 mg); white solid; ^1H NMR (600 MHz, CD_3OD) δ 7.22 (d, $J = 3.9$ Hz, 1H), 6.90 (d, $J = 3.9$ Hz, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 147.18, 133.19, 128.35, 127.23; HRMS (ESI) m/z : calcd for $\text{C}_4\text{H}_2\text{ClO}_3\text{S}$, 196.9139 $[\text{M}-\text{H}]^-$; found 196.9141.

Table 3.12. HPLC retention time and observed mass of solid phase synthesized peptides

Entry	Peptide sequence	t_R /Total run time (min)	Expected mass (m/z)	Observed mass (m/z)
1 ^a	Fmoc-KLVFF-NH ₂ (12a)	8.49/15	$[\text{M}+\text{H}]^+$ 874.4862	874.4875
2 ^b	Fmoc-KFIGLM-NH ₂ (12b)	8.86/15	$[\text{M}+\text{H}]^+$ 929.4954	929.4964
3 ^a	Fmoc-LPFFD-NH ₂ (12c)	10.04/15	$[\text{M}+\text{H}]^+$ 859.4026	859.4033
4 ^a	Fmoc-VYIHPF-NH ₂ (12d)	7.39/15	$[\text{M}+\text{H}]^+$ 996.4978	996.4985
5 ^a	Cy-NFGAILER-NH ₂ (12e)	8.61/15	$[\text{M}+\text{H}]^+$ 900.5051	900.5045
6 ^a	Cy-KLVFFAE (12f)	7.20/15	$[\text{M}+\text{H}]^+$ 834.4873	834.4879
7 ^a	Cy-KL(N-Me)VFF(N-Me)AE (12g)	8.78/15	$[\text{M}+\text{Na}]^+$ 884.5005	885.3850

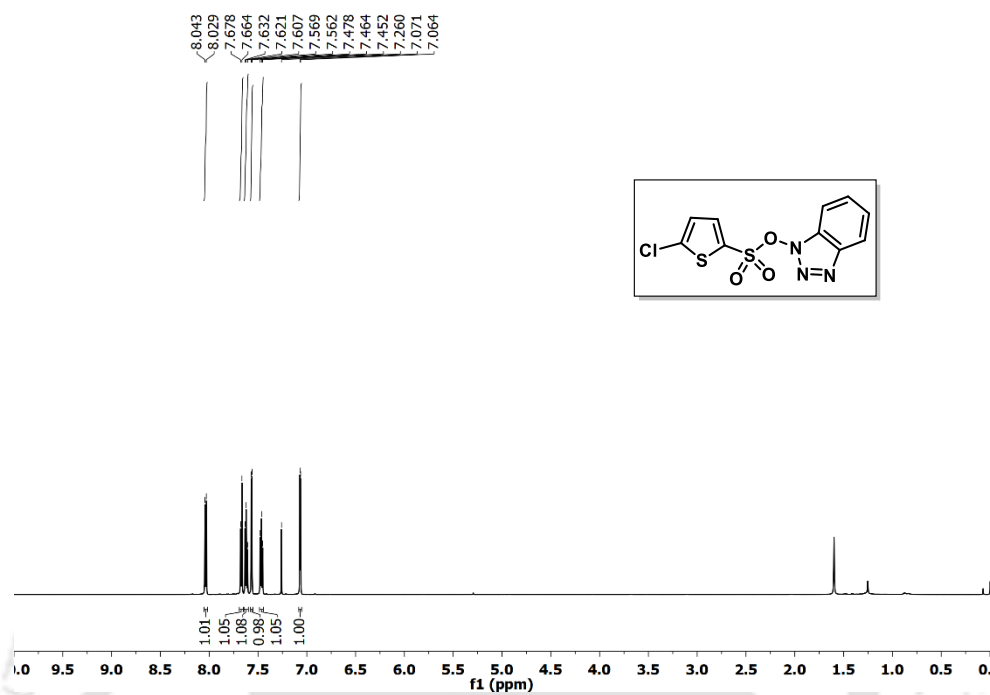
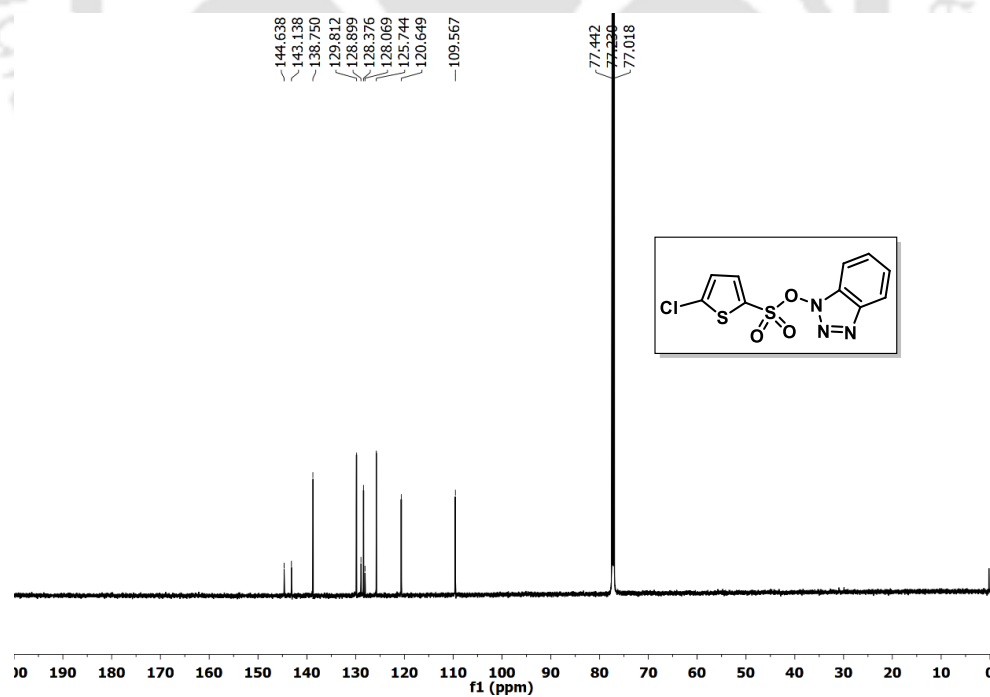
HPLC: ^aThermo Scientific system, Poroshell 120 EC C18 Agilent column ($4.6 \times 100\text{mm}$, $4 \mu\text{m}$). ^bAgilent 1260 Infinity II prime LC system, Eclipse Plus C18 Agilent column ($4.6 \times 250\text{mm}$, $5 \mu\text{m}$). t_R = HPLC retention time.

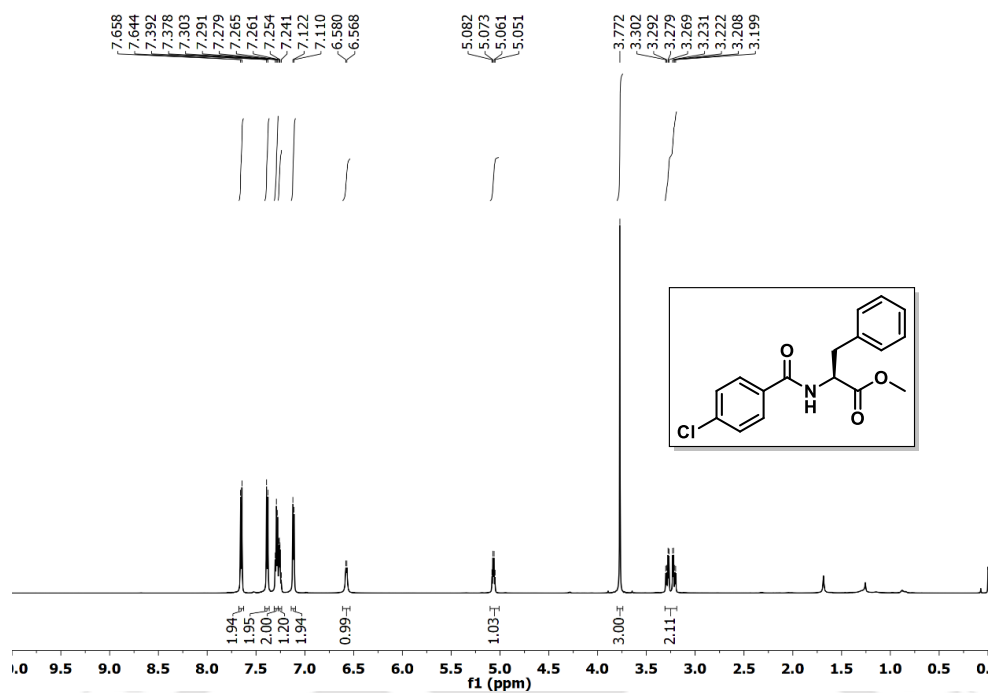
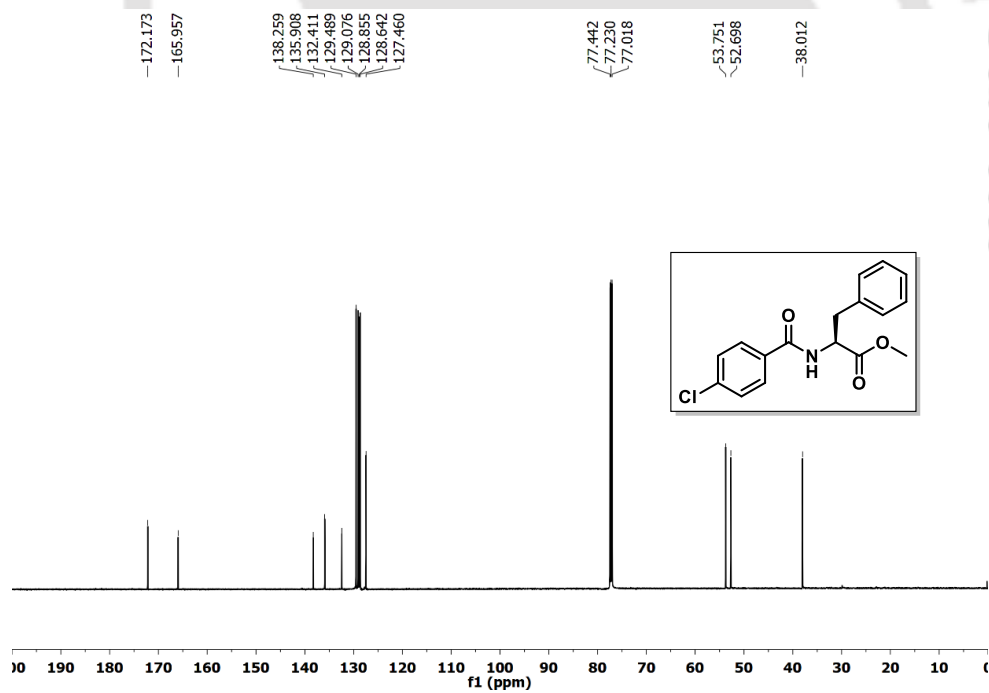
3.15. References

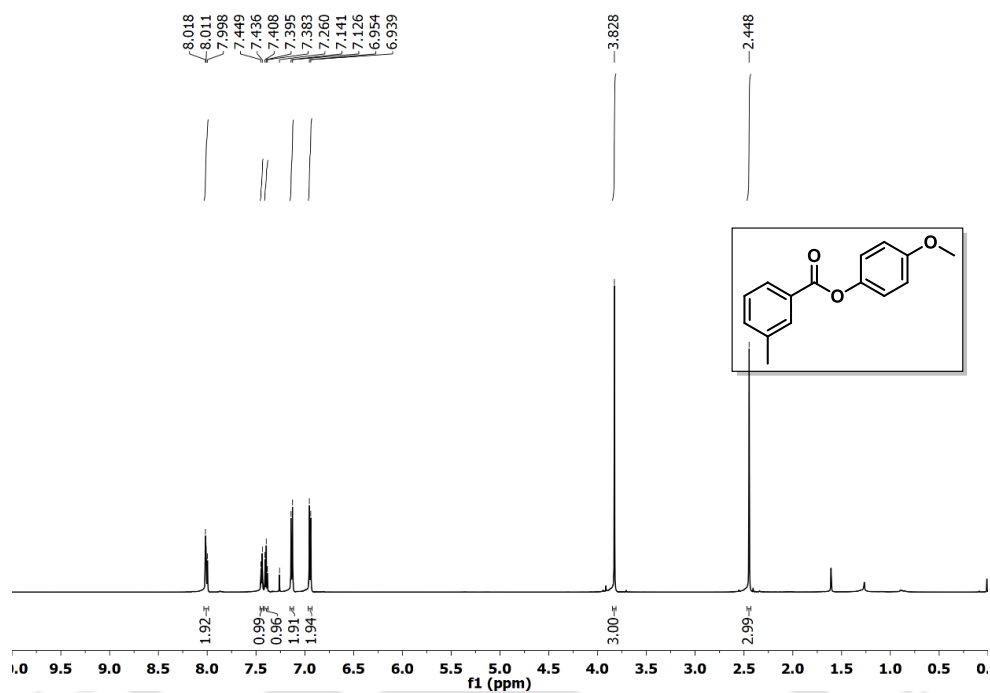
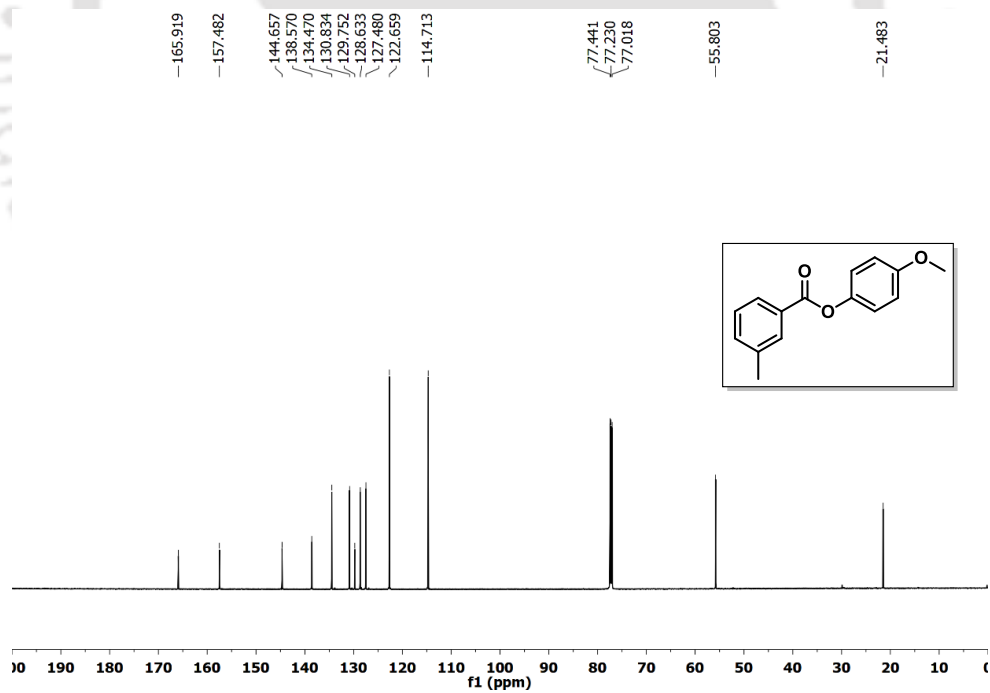
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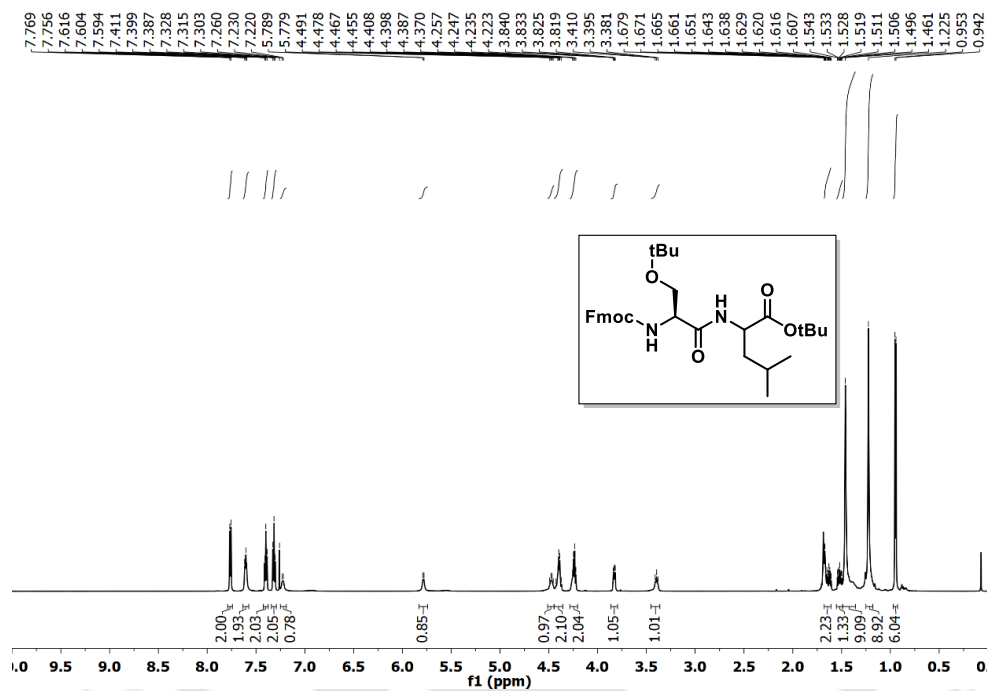
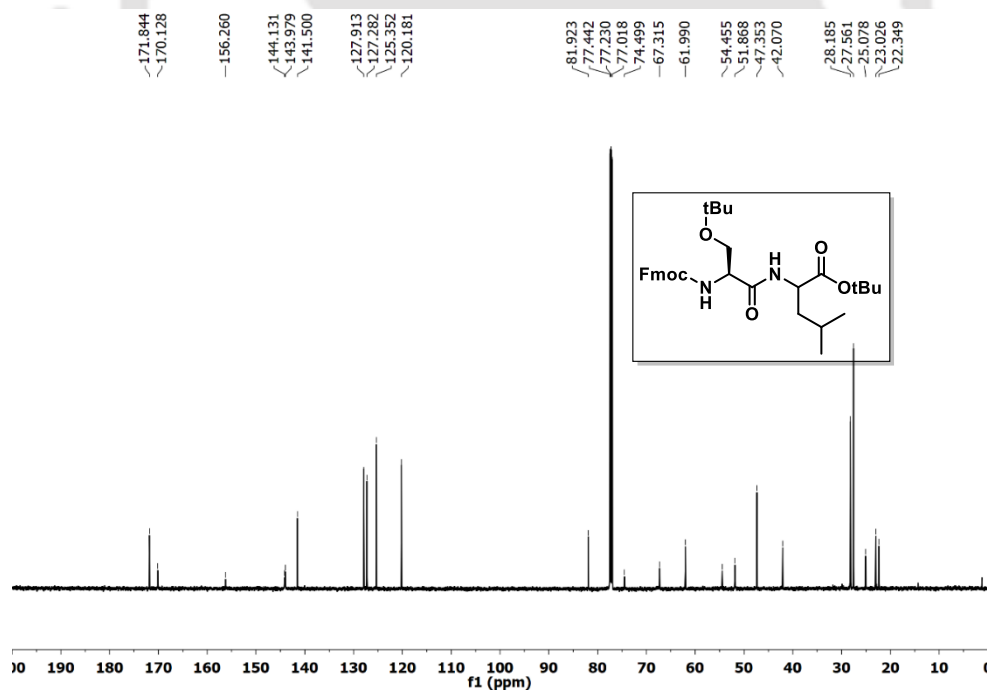
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3.16. Selected spectra

Figure 3.3. ¹H NMR spectrum of coupling reagent (CTSOBt, 1Xc)Figure 3.4. ¹³C NMR spectrum of coupling reagent (CTSOBt, 1Xc)

Figure 3.5. ^1H NMR spectrum of amide 3mFigure 3.6. ^{13}C NMR spectrum of amide 3m

Figure 3.7. ¹H NMR spectrum of ester 6eFigure 3.8. ¹³C NMR spectrum of ester 6e

Figure 3.9. ¹H NMR spectrum of peptide 9oFigure 3.10. ¹³C NMR spectrum of peptide 9o

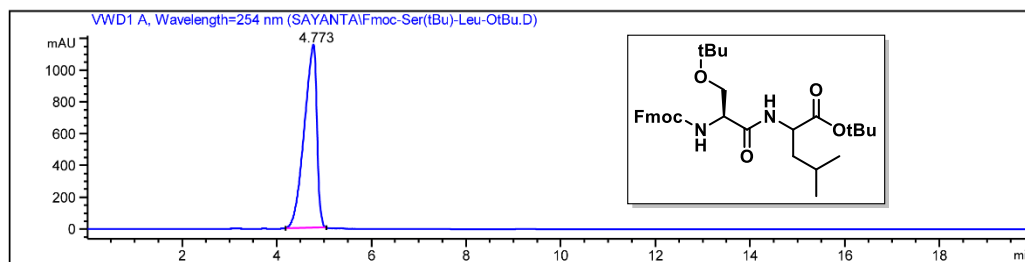


Figure 3.11. Chiral HPLC profile of peptide 9o

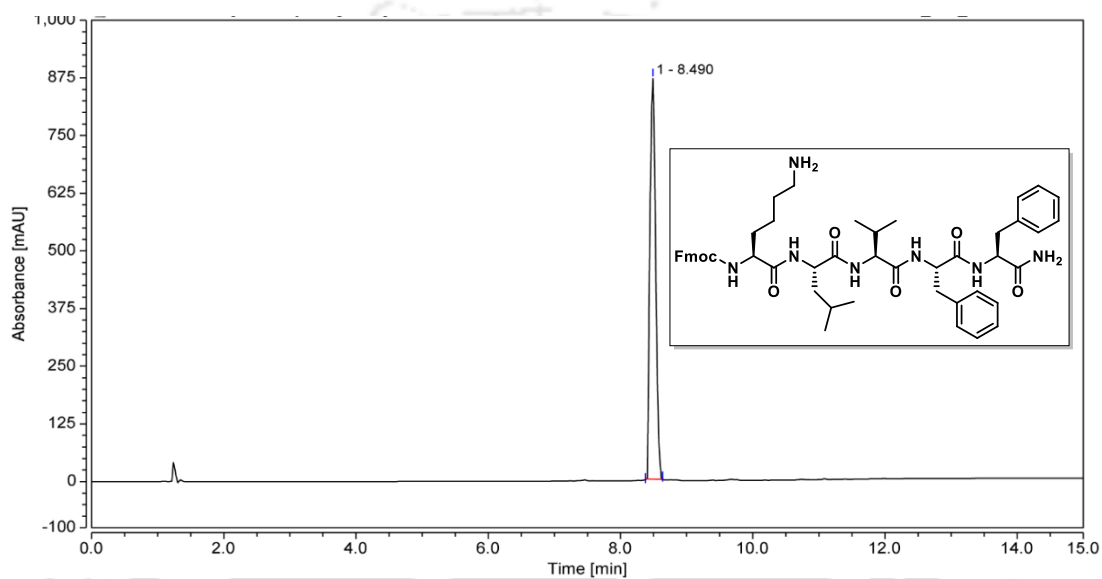


Figure 3.12. HPLC profile of peptide 12a

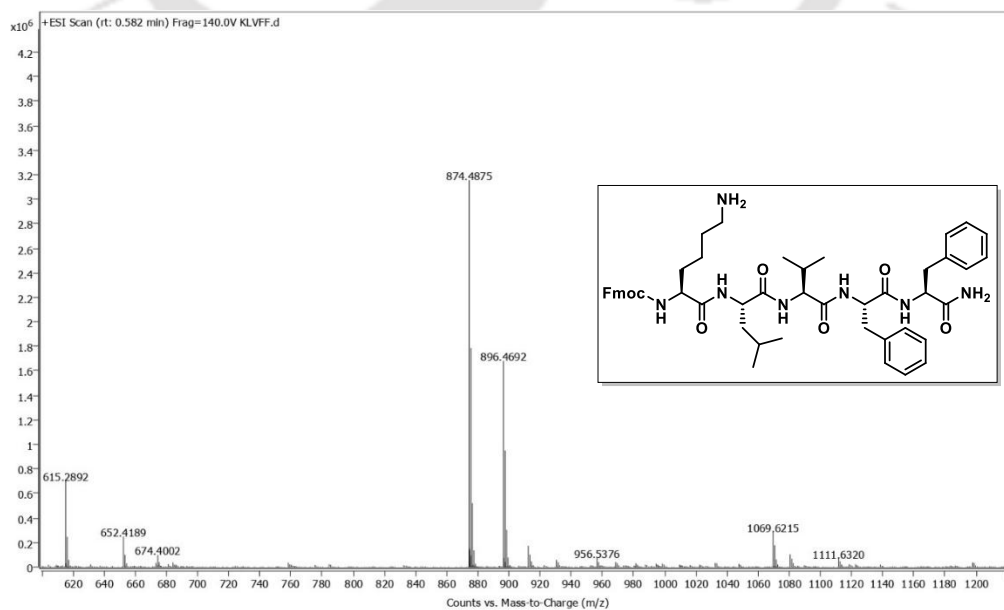
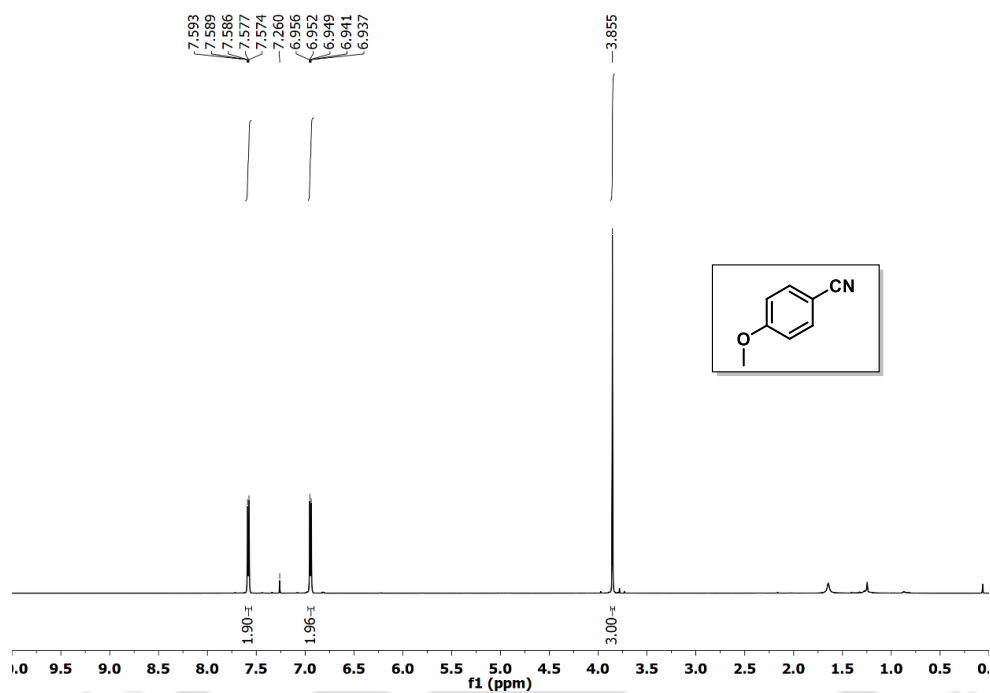
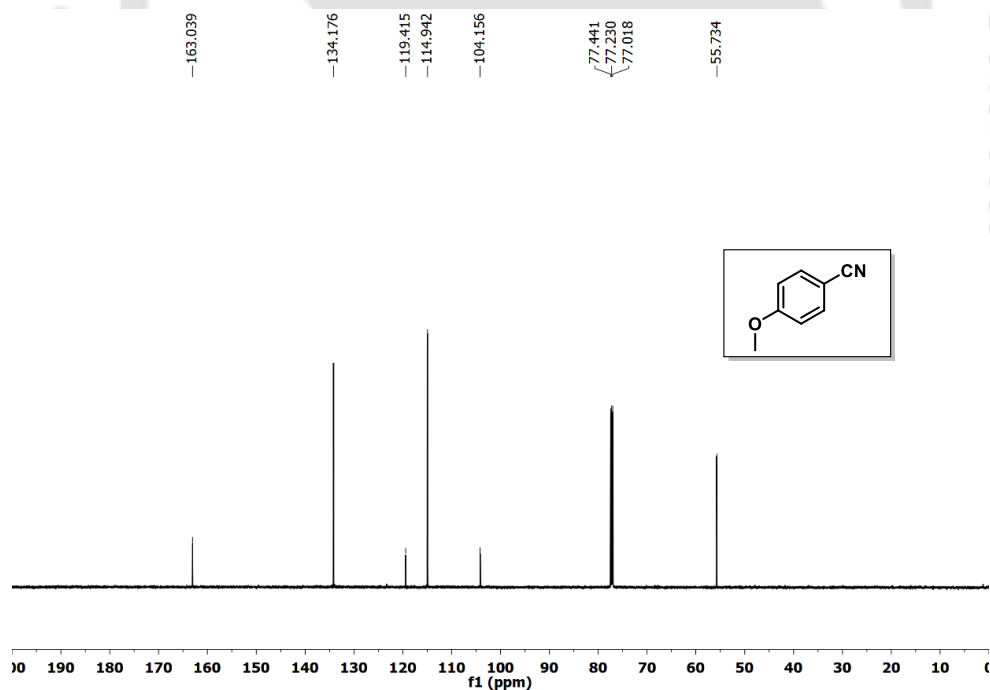


Figure 3.13. HRMS spectrum of peptide 12a

Figure 3.14. ¹H NMR spectrum of nitrile **14a**Figure 3.15. ¹³C NMR spectrum of nitrile **14a**

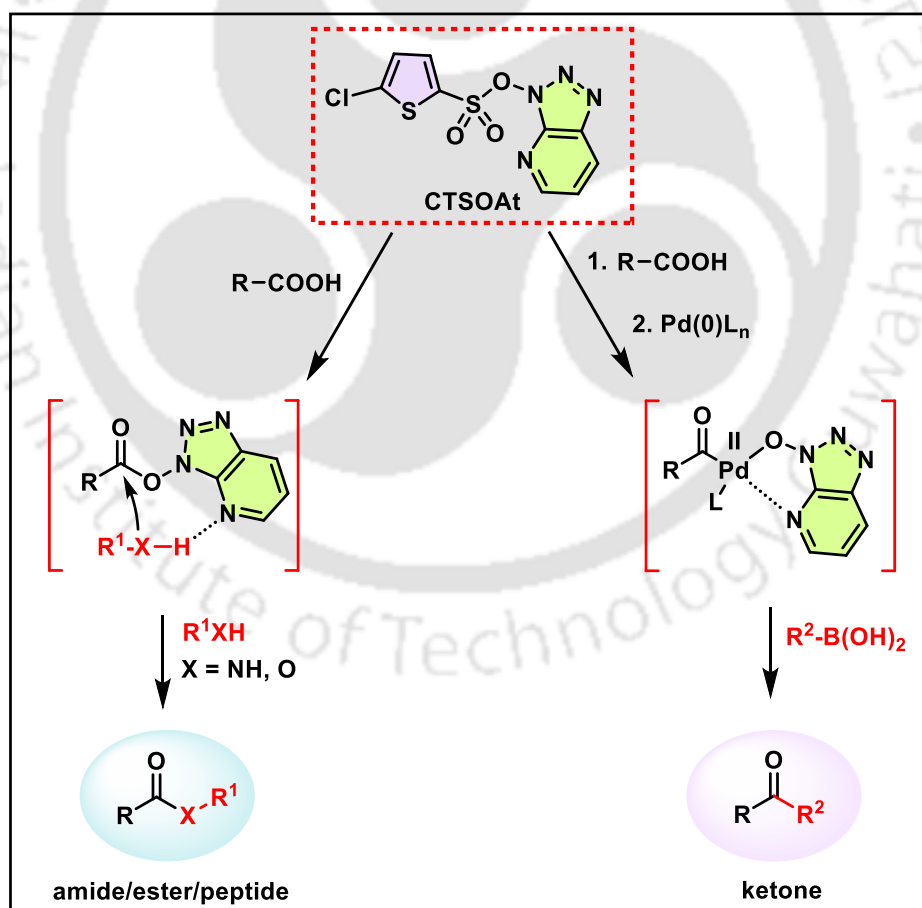
3.17. Crystallographic data

Table 3.13. Crystallographic data of CTSOBt (1Xc), 4-methoxybenzoate-OBt (V), and 6d

Parameters	CTSOBt (1Xc)	Intermediate (V)	6d
Formula	C10 H6 Cl N3 O3 S2	C14 H11 N3 O3	C15 H13 N O5
Fw	315.75	269.26	287.26
Crystal system	monoclinic	monoclinic	monoclinic
Space group	<i>P</i> 21/ <i>c</i>	<i>P</i> 21/ <i>c</i>	<i>P</i> 21/ <i>c</i>
<i>a</i> /Å	12.2804(6)	11.657(4)	4.348(3)
<i>b</i> /Å	7.1009(3)	9.460(3)	11.715(7)
<i>c</i> /Å	14.6740(7)	11.889(4)	27.266(16)
α /°	90	90	90
β /°	100.0050(10)	104.138(9)	93.875(16)
γ /°	90	90	90
<i>V</i> /Å ³	1260.14(10)	1271.4(8)	1385.5(14)
<i>Z</i>	4	4	4
<i>D_c</i> /g cm ⁻³	1.664	1.407	1.377
μ Mo K α /mm ⁻¹	0.640	0.102	0.105
F000	640	560	600
T/K	299(2)	298(2)	296(2)
θ max.	26.41	26.23	24.86
Total no. of reflections	33306	28027	19329
Independent reflections	2196	2204	2285
Observed reflections	2027	1942	1576
Parameters refined	172	182	191
<i>R</i> ₁ , <i>I</i> > 2 σ (<i>I</i>)	0.0272	0.0366	0.1015
<i>wR</i> ₂ , <i>I</i> > 2 σ (<i>I</i>)	0.0698	0.1089	0.2058
GOF (<i>F</i> ²)	1.073	0.732	1.244
CCDC No.	2296562	2305638	2307268

Chapter 4

A Novel Coupling Reagent Mediated Construction of C-N, C-O and C-C Bonds



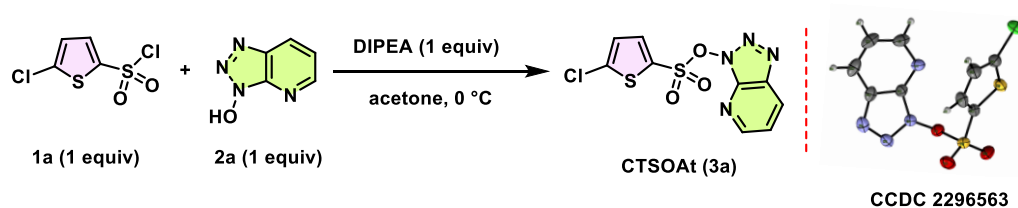
4.1. Background

In the previous chapters (2 and 3), we discussed the development of Oxyma and HOBT-based coupling reagents for efficient, racemization-free amide (C–N) and ester (C–O) bond formation. However, there is a research gap in synthesizing C–C bonds, which are crucial for ketone construction in organic synthesis. Traditional method, such as Friedel-Crafts acylation, faces challenges like poor yield, low regioselectivity, and the production of by-products.¹ The nucleophilic addition of organometallic compounds to carboxylic acid derivatives also encounters issues with chemoselectivity, functional group tolerance, and over-addition.² Establishing efficient methods for preparing ketones directly from carboxylic acids is important in synthetic chemistry. Organoboron compound-mediated cross-coupling processes are widely used for new C–C bond formation due to their non-toxicity and stability under thermal, air, and moisture conditions.³

In this chapter, we developed a HOAt-based coupling reagent 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl 5-chlorothiophene-2-sulfonate that efficiently activates carboxylic acids, enabling the construction of amide, ester, and ketone bonds. The nitrogen atom at the 7th position of the benzotriazole ring enhances reactivity and minimizes configurational loss, making it highly effective.⁴ Here, we demonstrated an organoboron compound-mediated cross-coupling process for efficiently constructing ketones from activated carboxylic acids. This versatile coupling reagent is highly useful for various organic transformations in the chemical laboratory.

4.2. Design and synthesis of coupling reagent

To date, a thiophene ring containing HOAt-based coupling reagent has not been established. Here, we reported a HOAt-based recyclable coupling reagent for the synthesis of amide, ester, and ketone bonds. The reaction of 5-chlorothiophene-2-sulfonyl chloride with HOAt in presence of DIPEA in acetone at room temperature yielded 99.9% of reagent CTSOAt (**3a**) (Scheme 4.1), characterized by NMR and HRMS. Screening with other solvents like DCM, EtOAc, and ACN also resulted in excellent yields (>99%). We also performed gram-scale production, where 10 g of 5-chlorothiophene-2-sulfonyl chloride yielded 99.9% of **3a**. The reagent showed stability in ambient conditions.



Scheme 4.1. Synthesis of CTSOAt (3a).

4.3. DFT study

The HOMO-LUMO energy calculation helps to determine molecular stability and reactivity. HOAt, like HOBt, exhibits desmotropic nature, allowing it to react from both the N- and O-nucleophilic sides, potentially forming either N-substituted or O-substituted products. Using the B3LYP/6-31G(d, P) basis set with Gaussian 09W software,⁵ the HOMO-LUMO gap for both O- and N-substituted forms was calculated. A higher HOMO-LUMO gap indicates a more stable molecule. The calculated HOMO-LUMO gap for **3a** was 5.005 eV, while for **3aN** it was 3.976 eV, indicating that the O-substituted form (**3a**) is more stable (Figure 4.1). The crystallographic structure also revealed the formation of the more stable **3a** (O-isomer).

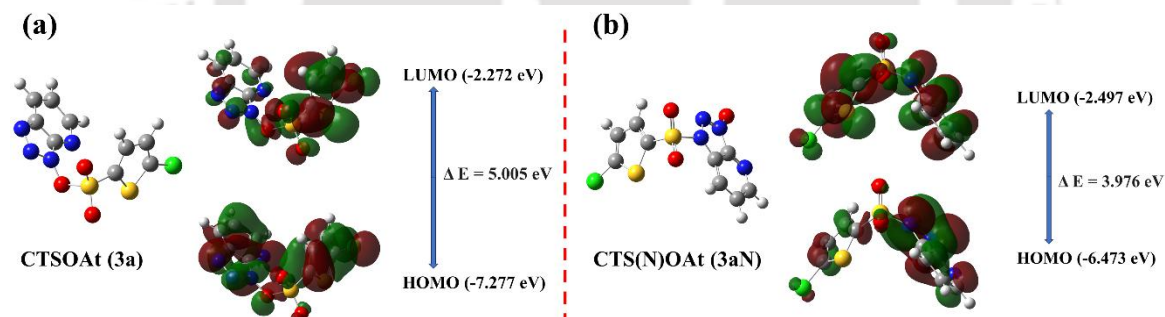


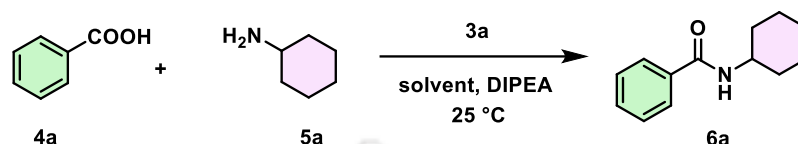
Figure 4.1. Gas phase optimized structures and $\Delta E_{\text{HOMO-LUMO}}$ of (a) CTSOAt (**3a**) and (b) CTS(N)OAt (**3aN**).

4.4. Reaction optimization

Next, we evaluated the efficiency of reagent **3a** by synthesizing amide **6a** from benzoic acid and benzylamine. Using DCM, the reaction yielded 96% of amide **6a**. We then tested the reaction in various solvents, including THF, EtOAc, ACN, DMF, acetone, and water. EtOAc, ACN, DMF, and acetone provided excellent yields of **6a**. However, EtOAc and

acetone required slightly more time to complete the reaction. ACN offered over 99% of amide **6a** within 15 minutes, making it the optimized solvent (Table 4.1).

Table 4.1. Reaction optimization



Entry ^a	Solvent	Time ^b	Yield ^c (%)
1	DCM	4 h	96
2	THF	12 h	39
3	EtOAc	5 h	98
4	ACN	15 min	>99
5	DMF	20 min	99
6	Acetone	3 h	99
7	H ₂ O	36 h	---

^aReaction conditions: **4a** (0.5 mmol), **3a** (0.5 mmol), DIPEA (0.6 mmol), solvent (1 mL), **5a** (0.5 mmol), 25 °C. ^bTime after the addition of amine. ^cIsolated yield.

4.5. Racemization test

After successfully optimizing the reaction conditions, we investigated the racemization suppression capacity of reagent **3a**. Chiral impurities complicate purification and reduce yield. Using optimized conditions, we synthesized dipeptides Boc-L-Phe-L-Ala-OMe and Boc-DL-Phe-L-Ala-OMe. Chiral HPLC of Boc-L-Phe-L-Ala-OMe showed one peak at 4.07 min, indicating one isomer. Peptide Boc-DL-Phe-L-Ala-OMe showed two peaks at 4.07 and 8.86 min, indicating two diastereomers (Figure 4.2). This confirmed the racemization-free synthetic process, also supported by NMR experiment. For Boc-L-Phe-L-Ala-OMe, only one peak was observed near δ values 6.4 ppm and 5.0 ppm in ¹H NMR (Figure 4.5). For Boc-DL-Phe-L-Ala-OMe, two peaks appeared at the same δ values (Figure 4.6), indicating one isomer for Boc-L-Phe-L-Ala-OMe and two isomers for peptide Boc-DL-Phe-L-Ala-OMe. In the ¹³C NMR of Boc-L-Phe-L-Ala-OMe, two peaks at 173–170 ppm, three peaks at 129–127 ppm, and four peaks at 55–38 ppm were observed. For Boc-DL-Phe-L-Ala-OMe, four peaks, five peaks, and eight peaks appeared at the same

δ values (characterization data, peptides **9c**, **9d**), confirming one isomer for Boc-L-Phe-L-Ala-OMe and two isomers for peptide Boc-DL-Phe-L-Ala-OMe.

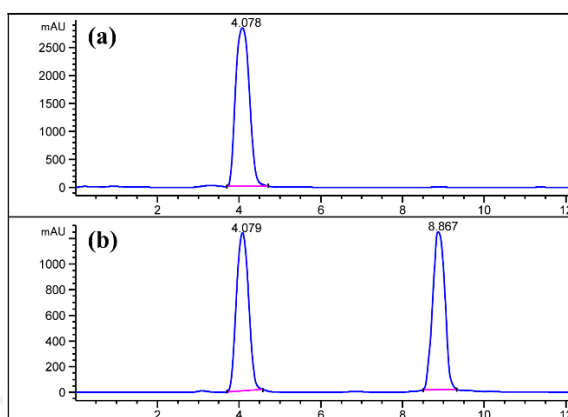


Figure 4.2. HPLC profile of (a) Boc-L-Phe-L-Ala-OMe, (b) Boc-DL-Phe-L-Ala-OMe. Agilent 1260 Infinity II prime LC system, CHIRALPAK[®] AS-H column (5 μ m, 4.6 $\times$ 250 mm), isocratic gradient of 20% isopropanol in hexane, run time 20 min.

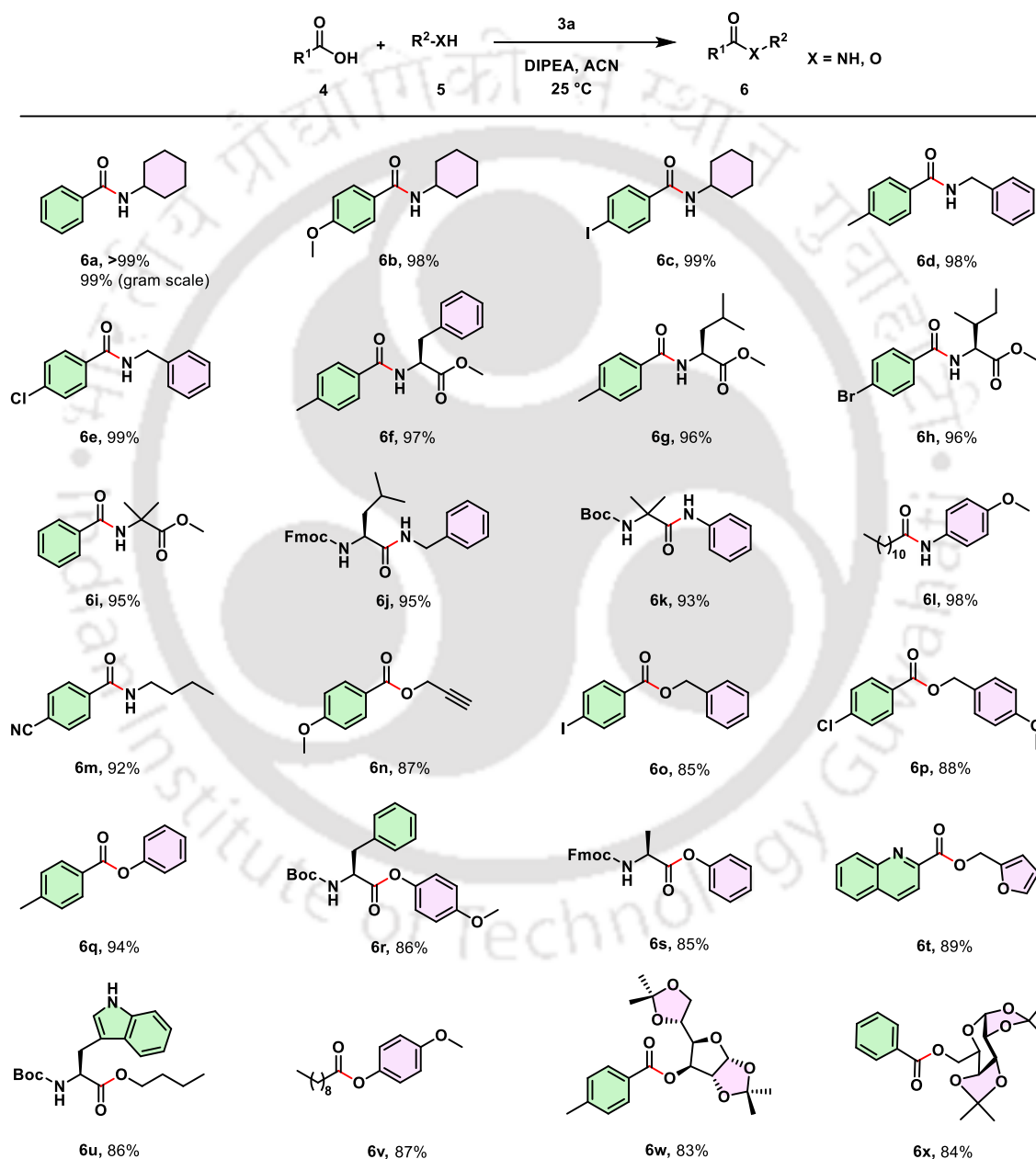
4.6. Substrate scope

4.6.1. Scope of amides and esters

Having achieved favorable results, we explored the scope of amides and esters using optimized conditions. Various acids, amines, and alcohols reacted to form amides and esters with excellent yields (Table 4.2). Aromatic acids bearing p-OMe and p-I groups produced amides **6b** and **6c** with excellent yields (98% and 99%). Benzylamine efficiently formed amides **6d** and **6e**. Methyl ester-protected amino acids yielded amides **6f** and **6g** with outstanding yields (97% and 96%). Sterically demanding Ile and Aib readily participated in the reaction, producing amides (**6h**, **6i**) with high yields (96%, 95%). Base-sensitive Fmoc amino acid produced amide **6j** (95%). Electron-deficient aromatic amines reacted with sterically hindered Boc-Aib-OH and long-chain aliphatic acid, yielding desired amides **6k** and **6l** (93%, 98%). Moderately long-chain hydrophobic amine also satisfied the reaction conditions, resulting in amide **6m**. We also synthesized esters with good to excellent yields. Aromatic acids with electron-donating and electron-withdrawing groups yielded esters **6n–6p** (85–88%). Poor nucleophilic aromatic alcohols reacted with aromatic acid, Boc, and Fmoc amino acids, offering the desired esters **6q–6s** with good to excellent yields (85–94%). Heterocyclic acid and amino acid satisfied the reaction

conditions and gave esters **6t** and **6u**. Long-chain aliphatic acid produced ester **6v** with a good yield (87%). Carbohydrate alcohols also formed esters **6w** and **6x** with satisfactory yields (83%, 84%). The successful synthesis of **6a** with a 99% yield using 1g of benzoic acid was achieved. We recovered 92% mixture of SA and HOAt from this.

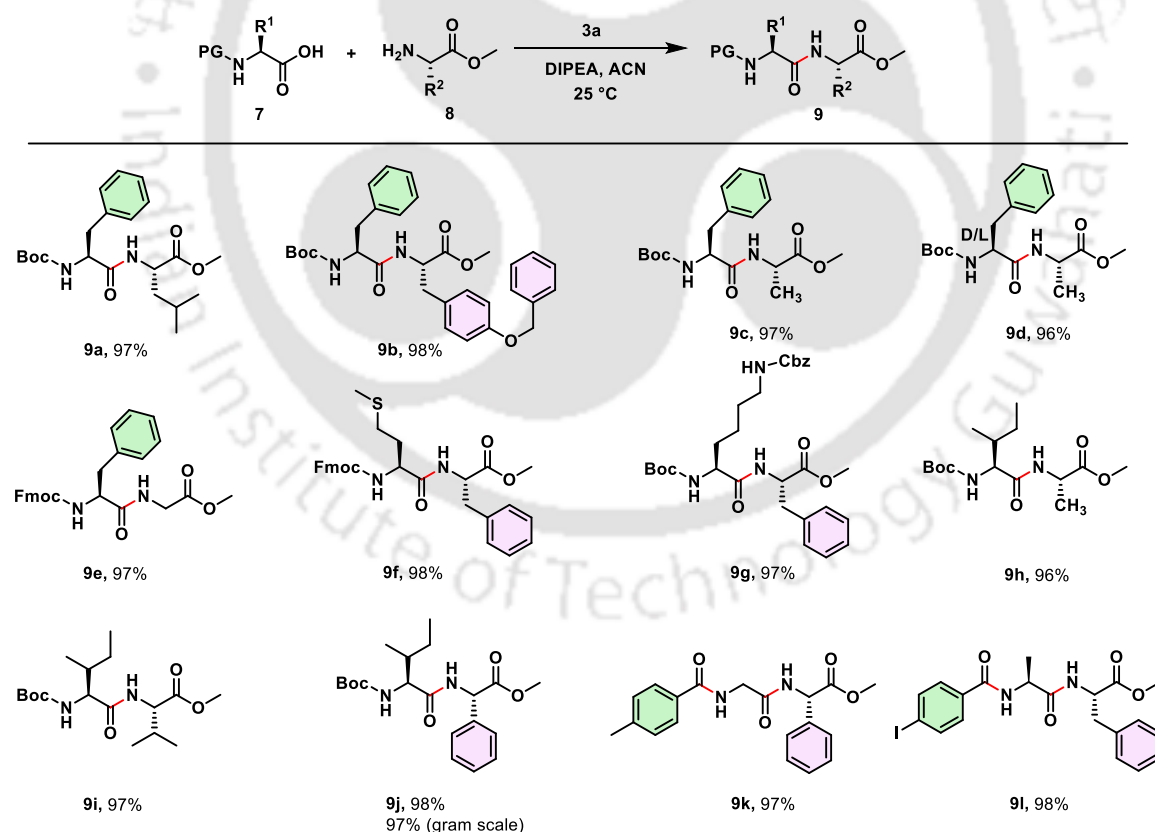
Table 4.2. Scope of amides and esters^a



^aReaction conditions: **4** (0.5 mmol), **3a** (0.5 mmol), DIPEA (0.6 mmol), ACN (1 mL), **5** (0.5 mmol), 25 °C, 15 min–30 min (amides); 5 h (esters) (time after adding nucleophile). Isolated yield.

4.6.2. Peptide synthesis in solution

After successfully synthesizing numerous amides and esters, we explored the reaction's generality by synthesizing various peptides under optimized conditions (Table 4.3). A wide range of protecting groups were well tolerated. Boc-Phe-OH yielded peptides (**9a–9c**) with 97–98% yields. The method was compatible with Fmoc-protected amino acids, yielding dipeptides (**9e, 9f**) efficiently. Cbz-protected aliphatic side chain and sterically hindered amino acids also yielded desired peptides (**9g, 9h**) with excellent yields (97%, and 96%). Installing sterically hindered amino acids on both sides of the peptide sequence (**9i, 9j**) was successful with satisfactory yields. We then synthesized pseudo tripeptides (**9k, 9l**) satisfactorily. Using 1g of Boc-Ile-OH resulted in a 97% yield for peptide **9j**, demonstrating the protocol's reliability and efficiency.

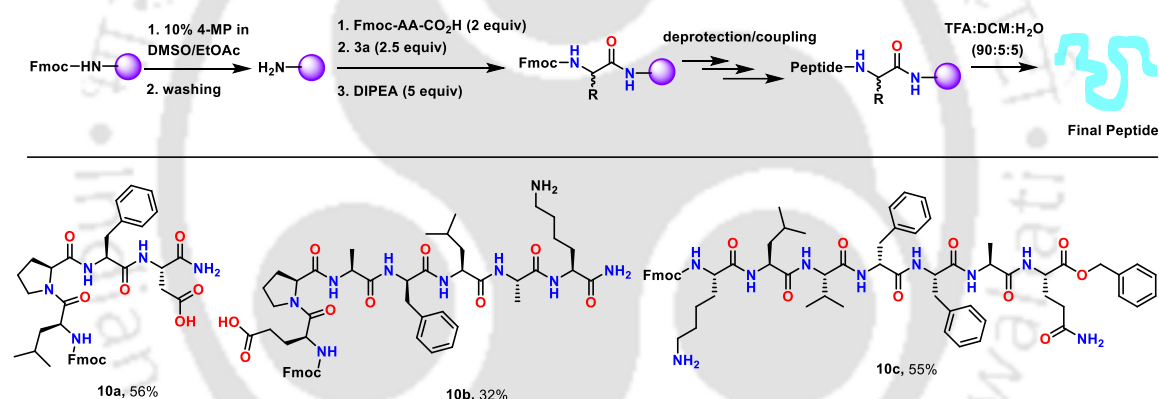
Table 4.3. Scope of peptides^a

^aReaction conditions: **7** (0.5 mmol), **3a** (0.5 mmol), DIPEA (0.6 mmol), ACN (1 mL), **8** (0.5 mmol), 25 °C, 30 min–2 h (time after adding nucleophile). Isolated yield.

4.6.3. Solid phase peptide synthesis

Synthesizing long-chain peptides is a challenging task. Commonly used solid-phase solvents like DMF, DCM, and NMP are hazardous. Recently, binary mixture solvents such as DMSO/ethyl acetate have shown comparable performance to DMF in terms of synthetic yield and purity.⁶ Using a 10% DMSO/ethyl acetate mixture, we achieved a satisfactory yield for peptide **10a** (56%). The peptide sequence was grown on Rink amide MBHA resin using the Fmoc/tBu solid-phase strategy.⁷ Additionally, we synthesized peptide Fmoc-EPAfLAKNH₂ (**10b**) with a satisfactory yield (32%). An analog peptide **10c** of KLVFFAE, a part of the A β peptide responsible for Alzheimer's disease⁸, was also synthesized with good yields (55%) (Table 4.4).

Table 4.4. Scope of solid phase peptides^a

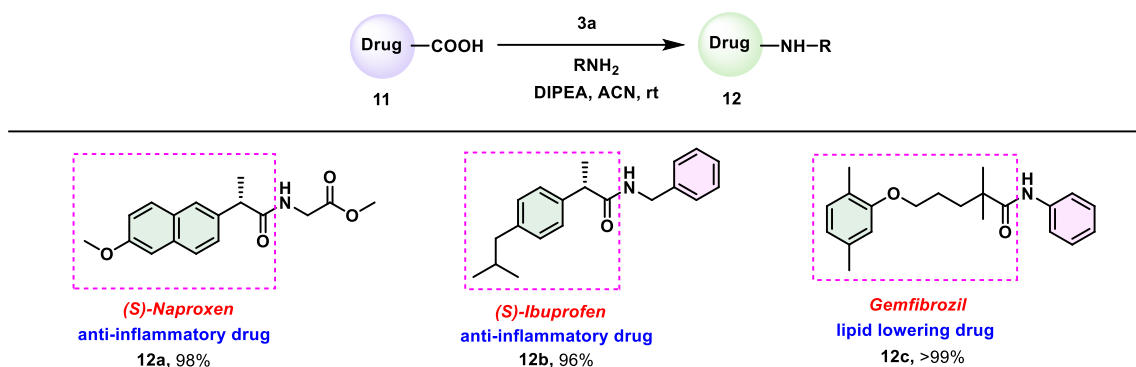


^aReaction conditions: Rink amide MBHA resin: 200 mg (0.14 mmol, loading: 0.7 mmol/g), amino acid (0.28 mmol), **3a** (0.35 mmol), DIPEA (0.7 mmol), solvent: DMSO/EtOAc (1:9), Fmoc deprotection: 10% 4-MP in DMSO/EtOAc (1:9), coupling time: 3 h, Isolated yields.

4.6.4. Late-stage modification of drugs

The reagent demonstrated high efficiency in modifying various drug molecules, including naproxen, ibuprofen, and gemfibrozil, through amidation. These drugs met the reaction conditions and offered the modified drugs (**12a–12c**) with excellent yields (96–99%) (Table 4.5). This successful drug modification highlights the practical applicability of reagent **3a**.

Table 4.5. Modification of some bioactive drugs^a

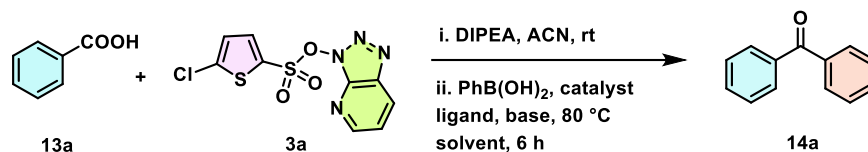


^aReaction conditions: **11** (0.5 mmol), **3a** (0.5 mmol), DIPEA (0.6 mmol), ACN (1 mL), RNH₂ (0.5 mmol), 25 °C, 30 min–1 h (time after adding nucleophile). Isolated yield.

4.7. Ketone synthesis via metal-catalyzed cross-coupling reaction

The reagent's efficiency was also demonstrated in ketone synthesis. It facilitated C-C bond formation via Suzuki–Miyaura coupling reactions with organoborane⁹. Reagent **3a** activates carboxylic acids, forming acyl-OAt intermediates that efficiently produce ketones through cross-coupling. Our goal of synthesizing ketones from activated carboxylic acids was successfully achieved. Initially, carboxylic acid was activated using reagent **3a** under optimized conditions. Then the activated carboxylic acid was treated with phenylboronic acid, 2 mol% Pd(OAc)₂, 4 mol% PCy₃, K₂CO₃, and dioxane at 80 °C, yielding 62% of ketone **14a** (entry 1, Table 4.6). We then moved on to optimize the reaction conditions by varying the catalysts, ligands, bases, and solvents. Altering the ligand from PCy₃ to DPPP resulted in a trace product. Using PPh₃ instead of PCy₃ did not improve the yield. Conducting the reaction without any ligand also failed to improve the yield. Next, we altered bases while keeping the rest of the reagents intact. Using KOAc as a base did not improve the yield. Interestingly, using Cs₂CO₃ as a base, we achieved an improved yield (86%) for ketone **14a** (entry 6, Table 4.6). Employing NaHCO₃ as a base yielded 60% of **14a**. Adding an organic base instead of an inorganic one resulted in only trace amounts of ketone (entries 8, 9, Table 4.6). We also examined the reaction in different solvents (e.g., ACN, toluene, DCE), but no improvement in yield was observed. Changing the metal catalyst (PdCl₂, Pd(PPh₃)₄, Pd₂(dba)₃, PdCl₂(PPh₃)₂, or Pd(COOCF₃)₂) did not improve product formation (entries 13–17, Table 4.6). When we used 4 mol% Pd(OAc)₂ and 8 mol% PCy₃, the reaction offered a 79% yield of ketone **14a**. Among all the conditions tested, entry 6 was deemed the optimal one.

Table 4.6. Optimization for ketone synthesis



Entry ^a	Catalyst	Ligand	Base	Solvent	Yield ^c %
1	Pd(OAc) ₂	PCy ₃	K ₂ CO ₃	dioxane	62
2	Pd(OAc) ₂	DPPP	K ₂ CO ₃	dioxane	trace
3	Pd(OAc) ₂	PPh ₃	K ₂ CO ₃	dioxane	23
4	Pd(OAc) ₂	----	K ₂ CO ₃	dioxane	29
5	Pd(OAc) ₂	PCy ₃	KOAc	dioxane	27
6	Pd(OAc) ₂	PCy ₃	Cs ₂ CO ₃	dioxane	86
7	Pd(OAc) ₂	PCy ₃	NaHCO ₃	dioxane	60
8	Pd(OAc) ₂	PCy ₃	DIPEA	dioxane	trace
9	Pd(OAc) ₂	PCy ₃	PS	dioxane	trace
10	Pd(OAc) ₂	PCy ₃	Cs ₂ CO ₃	ACN	---
11	Pd(OAc) ₂	PCy ₃	Cs ₂ CO ₃	toluene	62
12	Pd(OAc) ₂	PCy ₃	Cs ₂ CO ₃	DCE	64
13	PdCl ₂	PCy ₃	Cs ₂ CO ₃	dioxane	---
14	Pd(PPh ₃) ₄	PCy ₃	Cs ₂ CO ₃	dioxane	trace
15	Pd ₂ (dba) ₃	PCy ₃	Cs ₂ CO ₃	dioxane	55
16	PdCl ₂ (PPh ₃) ₂	PCy ₃	Cs ₂ CO ₃	dioxane	20
17	Pd(COOCF ₃) ₂	PCy ₃	Cs ₂ CO ₃	dioxane	50
18 ^b	Pd(OAc) ₂	PCy ₃	Cs ₂ CO ₃	dioxane	79

^aReaction conditions: step-i. **13a** (0.2 mmol), **3a** (0.2 mmol), DIPEA (0.2 mmol), ACN (1 mL); step-ii. PhB(OH)₂ (0.3 mmol), catalyst (2 mol%), ligand (4 mol%), base (0.4 mmol), solvent (1 mL), 80 °C;

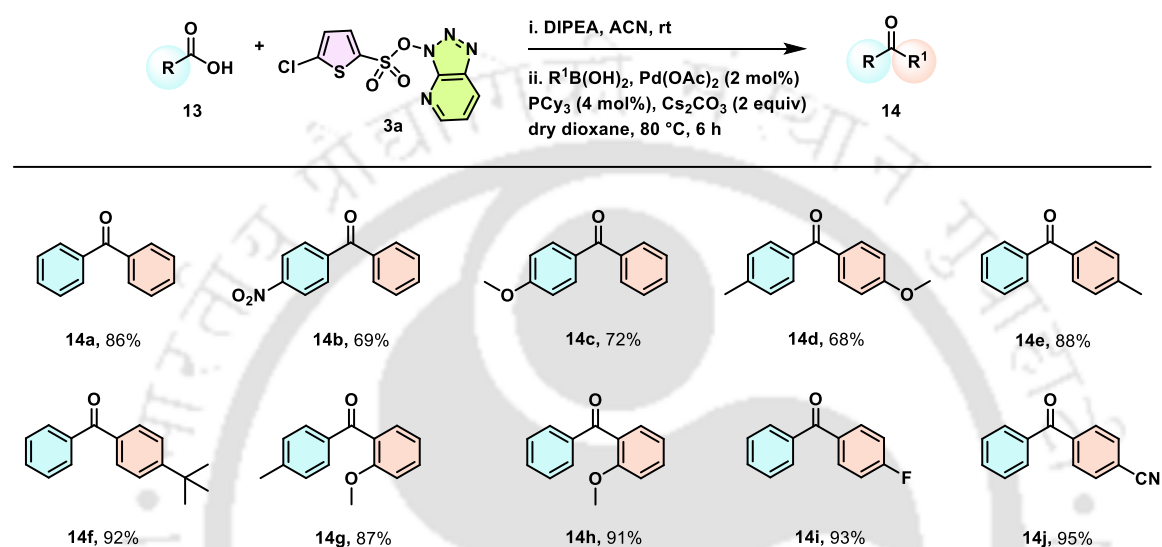
^bPd(OAc)₂ (4 mol%), PCy₃ (8 mol%); ^cisolated yield; PS = proton sponge¹⁰.

4.7.1. Scope of ketones

Utilizing the optimized conditions, we proceeded to synthesize a range of ketones. Both electron-withdrawing and electron-donating groups bearing aromatic acids and aryl boronic acids performed well under optimized conditions. Electron-withdrawing (p-NO₂) and donating (p-OMe) groups bearing benzoic acids successfully yielded ketones **14b** and **14c** with moderate to good yields (69%, 72%). Electron-donating groups (p-OMe, p-Me, p-tBu) containing aryl boronic acids also reacted smoothly, producing ketones **14d–14f**

with good to excellent yields (68–92%). Even ortho-methoxy substituted aryl boronic acids offered the desired ketones **14g** and **14h** with excellent yields (87%, 91%). Arylboronic acids with electron-withdrawing groups (p-F, p-CN) resulted in the desired ketones **14i** and **14j** with excellent yields (Table 4.7).

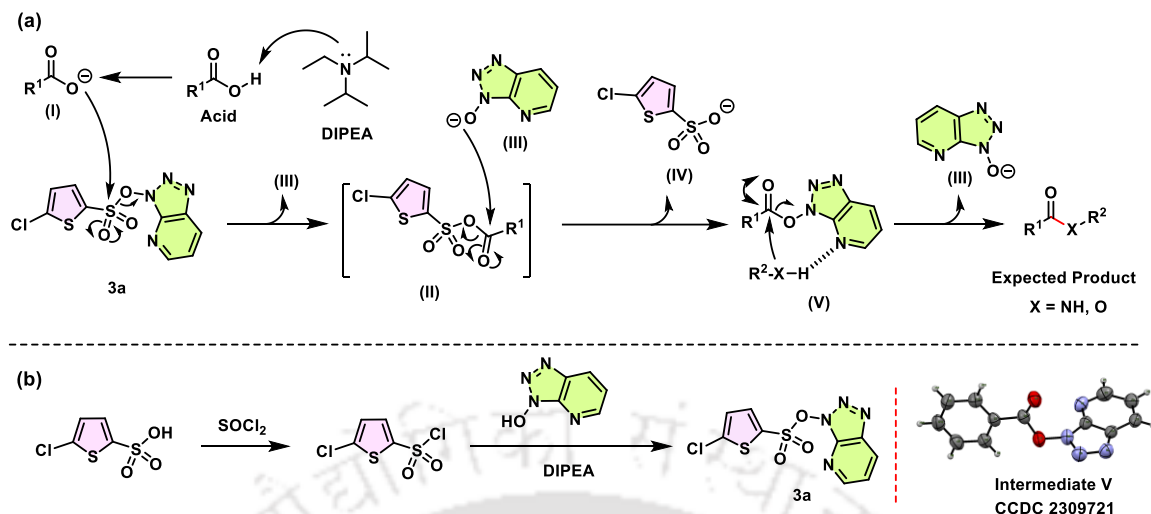
Table 4.7. Scope of ketones^a



^aReaction conditions: step-i. **13** (0.2 mmol), **3a** (0.2 mmol), DIPEA (0.2 mmol), ACN (1 mL); step-ii. $R^1B(OH)_2$ (0.3 mmol), $Pd(OAc)_2$ (2 mol%), PCy_3 (4 mol%), Cs_2CO_3 (0.4 mmol), dioxane (1 mL), 80 °C.

4.8. Plausible mechanism for amidation and esterification

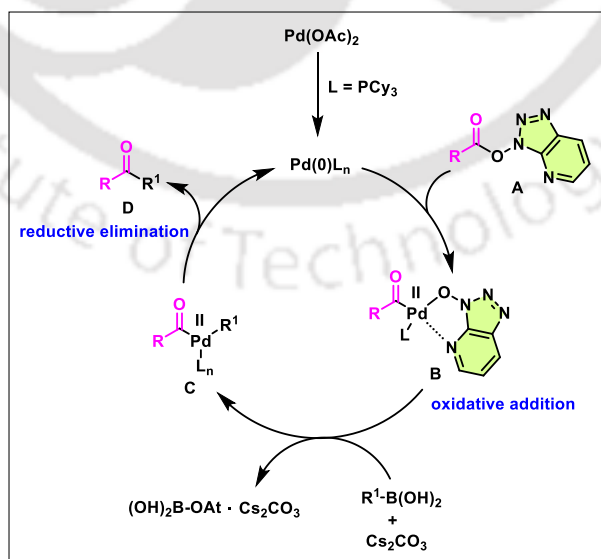
The plausible mechanistic pathway for amidation and esterification is shown in Scheme 4.2a. Initially, DIPEA generates carboxylate nucleophile (I), which attacks the sulfonyl center of **3a**, forming intermediate (II) and eliminating OAt^- (III). OAt^- (III) then attacks the carbonyl carbon of intermediate (II), creating active intermediate (V) and eliminating sulfonate ion (IV). Finally, nucleophiles attack the intermediate (V) to form the desired products. The 7th N atom of the HOAt ring enhances nucleophilicity of nucleophiles by NGP and leaving group character of OAt^- . The mechanism is supported by isolating and characterizing the reaction intermediate (V). The coupling reagent was recycled from recovered sulfonic acid and HOAt acquired from gram scale amidation. The sulfonic acid and HOAt mixture were treated with $SOCl_2$ in toluene with catalytic DMF at 70 °C. It was then cooled and treated with a base to reform reagent **3a** (Scheme 4.2b).



Scheme 4.2. Plausible mechanism for amidation and esterification and recyclability of the coupling reagent.

4.9. Plausible mechanism for ketone synthesis

We proposed a plausible mechanism for the Pd-catalysed Suzuki–Miyaura cross-coupling reaction¹¹ in Scheme 4.3. Initially, $\text{Pd}(\text{OAc})_2$ reacts with the ligand (PCy_3) to form an in-situ $\text{Pd}(0)$ complex. The oxidative addition of $\text{Pd}(0)$ into the ester C-O bond of **A** forms intermediate **B**. Base-assisted transmetalation between aryl boronic acid and intermediate **B** forms intermediate **C**. Finally, reductive elimination converts intermediate **C** into product **D**, regenerating $\text{Pd}(0)$.



Scheme 4.3. A plausible mechanism for Pd-catalysed C-C bond formation.

4.10. Conclusion

In conclusion, our synthetic protocol for the novel coupling reagent CTSOAt (**3a**) offers a mild, rapid, and environmentally friendly approach. This reagent readily engages with carboxylic acids, forming acyl-OAt intermediates that enable one-pot amidation or esterification while preserving stereochemistry. It excels in both solution and solid-phase peptide synthesis. The 7th N-atom of HOAt enhances reactivity and leads to high product yields without racemization. Furthermore, the reagent facilitates efficient ketone formation from carboxylic acids via Pd-catalyzed Suzuki–Miyaura cross-coupling under relatively mild conditions. Thus, the reagent is excellent for various organic transformations, which may make it attractive to synthetic chemists.

4.11. Experimental section

4.11.1. Materials and instrumentations

As described in chapter 8.

4.11.2. General procedure for the synthesis of coupling reagent

As described in chapter 3, section 3.13.2. Here, we used HOAt instead of HOBt.

4.11.3. General procedure for synthesizing amides and esters

As described in chapter 3, section 3.13.3. Here, we used coupling reagent CTSOAt (**3a**) and solvent acetonitrile instead of coupling reagent CTSOBt (**1Xc**) and solvent acetone.

4.11.4. General procedure for synthesizing dipeptides and higher analogs

As described in chapter 3, section 3.13.4. Here, we used coupling reagent CTSOAt (**3a**) and solvent acetonitrile instead of coupling reagent CTSOBt (**1Xc**) and solvent acetone.

4.11.5. General procedure for solid phase peptide synthesis

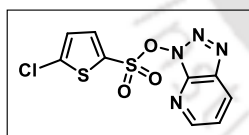
As described in chapter 2, section 2.14.6. Here, we used coupling reagent CTSOAt (**3a**) instead of coupling reagent *o*-CTSOXY (**1B**).

4.11.6. General procedure for the synthesis of ketones

In an oven-dried screw-capped glass vial, a mixture of carboxylic acid (0.2 mmol), CTSOAt (**3a**) (0.2 mmol), and DIPEA (0.2 mmol) in acetonitrile (1 mL) was taken and kept with stirring at room temperature. The complete intermediate formation was monitored by TLC. After the reaction, the solvent was evaporated, and EtOAc was pour. The organic part was washed with 10% citric acid and 10% NaHCO₃, then concentrated and dried. Next, the intermediate was transferred to another dry screw-capped glass vial. A mixture of aryl boronic acid (0.3 mmol), Pd(OAc)₂ (2 mol%), ligand (PCy₃, 4 mol%), and base (Cs₂CO₃, 0.4 mmol) in 1 mL dioxane was added to the glass vial and stirred at 80 °C. The reaction progress was tracked by TLC. After the reaction, it was passed through Celite, concentrated, and purified using column chromatography.

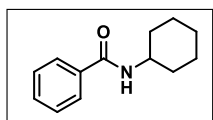
4.12. Characterization data

3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl 5-chlorothiophene-2-sulfonate (**3a**):

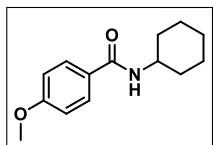


Yield: 99.9% (316.5 mg); faint yellowish solid; mp 119–121 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.77 (d, *J* = 4.4 Hz, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 4.1 Hz, 1H), 7.47 (dd, *J* = 8.3, 4.4 Hz, 1H), 7.08 (d, *J* = 4.1 Hz, 1H); ¹³C NMR (151 MHz, CDCl₃) δ 152.73, 144.50, 140.67, 138.66, 134.61, 129.78, 128.69, 128.30, 121.65; HRMS: (ESI) *m/z* calcd for C₉H₆ClN₄O₃S₂, 316.9565 [M+H]⁺; found 316.9564.

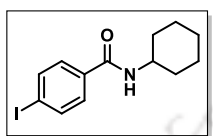
N-cyclohexylbenzamide (**6a**):



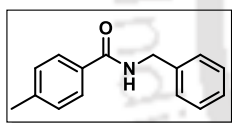
Yield: 99.4% (101 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.74 (d, *J* = 7.6 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 2H), 6.01 (s, 1H), 3.97 (tdt, *J* = 11.1, 8.0, 4.0 Hz, 1H), 2.03 (dd, *J* = 12.5, 4.2 Hz, 2H), 1.75 (dt, *J* = 13.6, 4.0 Hz, 2H), 1.65 (dq, *J* = 13.0, 4.0 Hz, 1H), 1.47 – 1.38 (m, 2H), 1.23 (dtd, *J* = 22.3, 11.7, 3.7 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 166.84, 135.33, 131.42, 128.70, 127.02, 48.88, 33.44, 25.78, 25.11; HRMS: (ESI) *m/z* calcd for C₁₃H₁₈NO, 204.1383 [M+H]⁺; found 204.1387.

N-cyclohexyl-4-methoxybenzamide (6b):

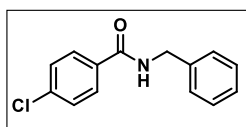
Yield: 98% (114 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.71 (d, J = 8.8 Hz, 2H), 6.90 (ddd, J = 8.7, 5.3, 2.6 Hz, 2H), 5.95 (s, 1H), 4.00 – 3.91 (m, 1H), 3.83 (dd, J = 4.7, 2.3 Hz, 3H), 2.01 (dt, J = 12.5, 4.0 Hz, 2H), 1.73 (dp, J = 11.6, 3.6 Hz, 2H), 1.64 (dq, J = 12.5, 3.8 Hz, 1H), 1.41 (tdq, J = 12.1, 8.1, 4.8 Hz, 2H), 1.21 (dtt, J = 18.1, 8.5, 4.1 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.31, 162.18, 128.79, 127.61, 113.85, 55.58, 48.78, 33.51, 25.81, 25.15; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{20}\text{NO}$, 234.1489 $[\text{M}+\text{H}]^+$; found 234.1488.

N-cyclohexyl-4-iodobenzamide (6c):

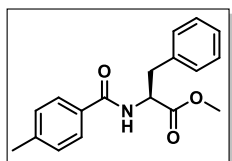
Yield: 99% (163 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 6.01 – 5.86 (m, 1H), 3.95 (tdt, J = 11.1, 8.0, 4.0 Hz, 1H), 2.01 (dt, J = 12.6, 4.0 Hz, 2H), 1.75 (dp, J = 12.0, 4.0 Hz, 2H), 1.65 (dt, J = 12.9, 4.0 Hz, 1H), 1.46 – 1.38 (m, 2H), 1.26 – 1.20 (m, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.00, 137.91, 134.72, 128.69, 98.24, 49.05, 33.41, 25.76, 25.11; HRMS: (ESI) m/z calcd for $\text{C}_{13}\text{H}_{17}\text{INO}$, 330.0349 $[\text{M}+\text{H}]^+$; found 330.0355.

N-benzyl-4-methylbenzamide (6d):

Yield: 98% (110 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.69 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 4.4 Hz, 4H), 7.32 – 7.27 (m, 1H), 7.22 (d, J = 7.8 Hz, 2H), 6.43 (s, 1H), 4.64 (dt, J = 5.7, 1.9 Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 167.48, 142.16, 138.53, 131.74, 129.44, 128.97, 128.11, 127.78, 127.16, 44.29, 21.64; HRMS: (ESI) m/z calcd for $\text{C}_{15}\text{H}_{16}\text{NO}$, 226.1226 $[\text{M}+\text{H}]^+$; found 226.1230.

N-benzyl-4-chlorobenzamide (6e):

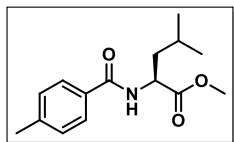
Yield: 99% (122 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.72 (d, J = 8.5 Hz, 2H), 7.40 – 7.28 (m, 7H), 6.56 (s, 1H), 4.61 (d, J = 5.7 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.52, 138.15, 137.97, 132.92, 129.01, 128.62, 128.10, 127.90, 44.40; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{13}\text{ClNO}$, 246.0680 $[\text{M}+\text{H}]^+$; found 246.0678.

Methyl (4-methylbenzoyl)-L-phenylalaninate (6f):

Yield: 97%, (142 mg); faint pink solid; ^1H NMR (600 MHz, CDCl_3) δ 7.62 (d, J = 8.1 Hz, 2H), 7.30 – 7.20 (m, 5H), 7.13 (d, J = 7.0 Hz, 2H), 6.55 (s, 1H), 5.12 – 5.04 (m, 1H), 3.76 (s, 3H), 3.32 – 3.19 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.32, 166.93, 142.46, 136.11, 131.25, 129.55,

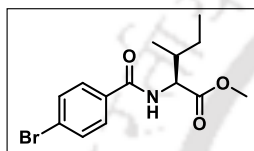
129.47, 128.80, 127.36, 127.21, 53.67, 52.58, 38.16, 21.66; HRMS: (ESI) m/z calcd for $C_{18}H_{20}NO_3$, 298.1438 $[M+H]^+$; found 298.1440.

Methyl (4-methylbenzoyl)leucinate (6g):



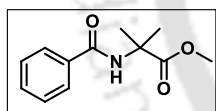
Yield: 96% (126 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.69 (d, J = 8.1 Hz, 2H), 7.22 (d, J = 7.8 Hz, 2H), 6.56 (s, 1H), 4.85 (td, J = 8.5, 4.8 Hz, 1H), 3.75 (s, 3H), 2.38 (s, 3H), 1.77 – 1.70 (m, 2H), 1.68 – 1.62 (m, 1H), 0.97 (dd, J = 11.0, 6.0 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.00, 167.23, 142.36, 131.28, 129.41, 127.26, 52.53, 51.27, 42.09, 25.18, 23.02, 22.27, 21.63; HRMS: (ESI) m/z calcd for $C_{15}H_{22}NO_3$, 264.1594 $[M+H]^+$; found 264.1595.

Methyl (4-bromobenzoyl)-L-alloisoleucinate (6h):



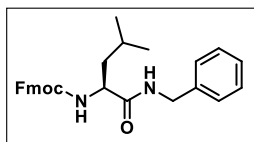
Yield: 96% (157 mg); colorless liquid; 1H NMR (600 MHz, $CDCl_3$) δ 7.66 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 6.65 (s, 1H), 4.83 – 4.74 (m, 1H), 3.77 (s, 3H), 2.04 – 1.96 (m, 1H), 1.55 – 1.47 (m, 1H), 1.29 – 1.21 (m, 2H), 1.00 – 0.93 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.75, 166.34, 133.15, 132.04, 128.87, 126.63, 57.05, 52.46, 38.44, 25.60, 15.68, 11.80; HRMS: (ESI) m/z calcd for $C_{14}H_{19}BrNO_3$, 328.0543 $[M+H]^+$; found 328.0541.

Methyl 2-benzamido-2-methylpropanoate (6i):

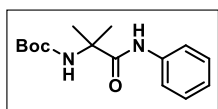


Yield: 95% (105 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.80 – 7.75 (m, 2H), 7.49 (dddd, J = 7.0, 5.3, 2.7, 1.5 Hz, 1H), 7.42 (tq, J = 7.0, 2.3 Hz, 2H), 6.81 (s, 1H), 3.78 (t, J = 2.1 Hz, 3H), 1.68 (t, J = 2.2 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.50, 166.78, 134.73, 131.75, 128.73, 127.14, 57.12, 52.98, 24.96; HRMS: (ESI) m/z calcd for $C_{12}H_{16}NO_3$, 222.1125 $[M+H]^+$; found 222.1126.

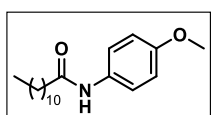
(9H-fluoren-9-yl)methyl (S)-(1-(benzylamino)-4-methyl-1-oxopentan-2-yl)carbamate (6j):



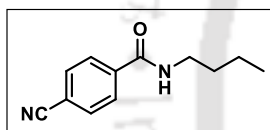
Yield: 95% (210 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, J = 7.3 Hz, 2H), 7.55 (d, J = 7.1 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.28 (dt, J = 12.1, 7.2 Hz, 4H), 7.23 (t, J = 8.2 Hz, 3H), 6.45 (s, 1H), 5.28 (s, 1H), 4.49 – 4.27 (m, 4H), 4.27 – 4.11 (m, 2H), 1.73 – 1.62 (m, 2H), 1.60 – 1.50 (m, 1H), 0.93 (t, J = 6.2 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.24, 156.51, 143.95, 141.53, 138.07, 128.90, 127.94, 127.82, 127.73, 127.30, 125.20, 120.20, 67.18, 53.80, 47.38, 43.76, 41.63, 24.92, 23.15, 22.21; HRMS: (ESI) m/z calcd for $C_{28}H_{31}N_2O_3$, 443.2329 $[M+H]^+$; found 443.2336.

Tert-butyl (2-methyl-1-oxo-1-(phenylamino)propan-2-yl)carbamate (6k):

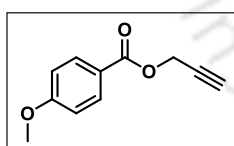
Yield: 93% (129 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.98 (s, 1H), 7.52 (d, $J = 7.9$ Hz, 2H), 7.31 (t, $J = 7.8$ Hz, 2H), 7.08 (t, $J = 7.4$ Hz, 1H), 4.99 (s, 1H), 1.56 (s, 6H), 1.44 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 172.88, 155.58, 138.47, 129.14, 124.19, 119.96, 81.09, 57.96, 28.45, 25.94; HRMS: (ESI) m/z calcd for $\text{C}_{15}\text{H}_{22}\text{N}_2\text{NaO}_3$, 301.1523 $[\text{M}+\text{Na}]^+$; found 301.1523.

N-(4-methoxyphenyl)dodecanamide (6l):

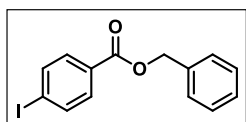
Yield: 98% (149 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.51 (d, $J = 35.0$ Hz, 1H), 7.40 (d, $J = 8.6$ Hz, 2H), 6.83 (ddt, $J = 9.0, 6.0, 3.4$ Hz, 2H), 3.81 – 3.75 (m, 3H), 2.31 (td, $J = 7.6, 3.0$ Hz, 2H), 1.74 – 1.66 (m, 2H), 1.27 (d, $J = 16.7$ Hz, 16H), 0.87 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.77, 156.50, 131.37, 122.04, 114.25, 55.64, 37.77, 32.10, 29.82, 29.80, 29.70, 29.65, 29.60, 29.53, 25.96, 22.87, 14.30; HRMS: (ESI) m/z calcd for $\text{C}_{19}\text{H}_{32}\text{NO}_2$, 306.2428 $[\text{M}+\text{H}]^+$; found 306.2428.

N-butyl-4-cyanobenzamide (6m):

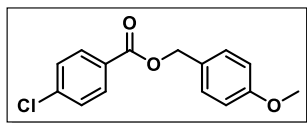
Yield: 92% (93 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.85 (d, $J = 8.2$ Hz, 2H), 7.70 (d, $J = 8.2$ Hz, 2H), 6.38 (s, 1H), 3.44 (q, $J = 6.9$ Hz, 2H), 1.59 (p, $J = 7.4$ Hz, 2H), 1.39 (h, $J = 7.4$ Hz, 2H), 0.94 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.95, 138.97, 132.57, 127.82, 118.22, 115.06, 40.27, 31.75, 20.31, 13.91; HRMS: (ESI) m/z calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{O}$, 203.1179 $[\text{M}+\text{H}]^+$; found 203.1177.

Prop-2-yn-1-yl 4-methoxybenzoate (6n):

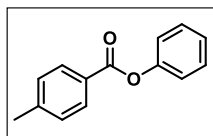
Yield: 87% (83 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 8.02 (d, $J = 8.9$ Hz, 2H), 6.92 (d, $J = 8.9$ Hz, 2H), 4.89 (d, $J = 2.4$ Hz, 2H), 3.85 (s, 3H), 2.50 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 165.71, 163.87, 132.08, 121.97, 113.89, 78.18, 74.99, 55.63, 52.35; HRMS: (ESI) m/z calcd for $\text{C}_{11}\text{H}_{11}\text{O}_3$, 191.0703 $[\text{M}+\text{H}]^+$; found 191.0707.

Benzyl 4-iodobenzoate (6o):

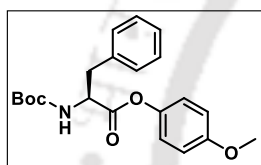
Yield: 85% (144 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.82 – 7.76 (m, 4H), 7.44 (d, $J = 7.4$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.35 (t, $J = 7.2$ Hz, 1H), 5.36 (s, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.15, 137.95, 135.98, 131.35, 129.83, 128.85, 128.58, 128.45, 101.06, 67.17; HRMS: (ESI) m/z calcd for $\text{C}_{14}\text{H}_{12}\text{IO}_2$, 338.9876 $[\text{M}+\text{H}]^+$; found 338.9882.

4-Methoxybenzyl 4-chlorobenzoate¹² (6p):

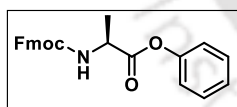
Yield: 88% (122 mg); colorless liquid; ¹H NMR (600 MHz, CDCl₃) δ 7.98 (d, *J* = 8.5 Hz, 2H), 7.39 (t, *J* = 8.6 Hz, 4H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.29 (s, 2H), 3.82 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 165.86, 159.97, 139.61, 131.28, 130.37, 128.94, 128.89, 128.11, 114.23, 67.02, 55.51.

Phenyl 4-methylbenzoate (6q):

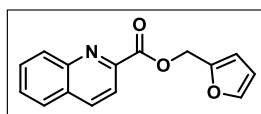
Yield: 94% (100 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 8.11 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.30 – 7.25 (m, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 2.46 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 165.44, 151.24, 144.59, 130.41, 129.65, 129.48, 127.04, 125.97, 121.96, 21.94; HRMS: (ESI) *m/z* calcd for C₁₄H₁₃O₂, 213.0910 [M+H]⁺; found 213.0925.

4-Methoxyphenyl (*tert*-butoxycarbonyl)-L-phenylalaninate (6r):

Yield: 86% (160 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.34 (t, *J* = 7.3 Hz, 2H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.24 (d, *J* = 7.1 Hz, 2H), 6.90 (d, *J* = 9.2 Hz, 2H), 6.86 (d, *J* = 9.1 Hz, 2H), 5.07 (d, *J* = 8.3 Hz, 1H), 4.80 (q, *J* = 6.9 Hz, 1H), 3.79 (s, 3H), 3.23 (d, *J* = 6.1 Hz, 2H), 1.44 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 171.13, 157.64, 155.35, 144.05, 136.02, 129.71, 128.90, 127.44, 122.26, 114.69, 80.36, 55.80, 54.80, 38.63, 28.52; HRMS: (ESI) *m/z* calcd for C₂₁H₂₅NNaO₅, 394.1625 [M+Na]⁺; found 394.1619.

Phenyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-alaninate (6s):

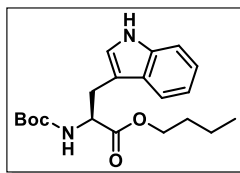
Yield: 85% (165 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (dd, *J* = 7.6, 3.3 Hz, 2H), 7.39 (q, *J* = 6.5, 5.7 Hz, 4H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.24 (m, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 5.39 (d, *J* = 7.9 Hz, 1H), 4.64 (p, *J* = 7.5 Hz, 1H), 4.48 – 4.37 (m, 2H), 4.24 (t, *J* = 7.1 Hz, 1H), 1.61 (d, *J* = 7.2 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.98, 155.91, 150.63, 144.07, 143.96, 141.55, 129.74, 127.95, 127.31, 126.41, 125.27, 121.47, 120.22, 67.32, 50.05, 47.38, 18.84; HRMS: (ESI) *m/z* calcd for C₂₄H₂₁NNaO₄, 410.1363 [M+Na]⁺; found 410.1353.

Furan-2-ylmethyl quinoline-2-carboxylate (6t):

Yield: 89% (113 mg); light brown solid; ¹H NMR (600 MHz, CDCl₃) δ 8.32 – 8.24 (m, 2H), 8.19 – 8.13 (m, 1H), 7.85 (ddd, *J* = 8.2, 3.8, 1.6 Hz, 1H), 7.76 (dddt, *J* = 8.4, 6.8, 3.0, 1.9 Hz, 1H), 7.63 (dddd, *J* = 9.9, 6.7, 3.1, 1.4 Hz, 1H), 7.44 (t, *J* = 1.9 Hz, 1H), 6.56 (t, *J* = 2.7 Hz, 1H), 6.38 (dq, *J* = 3.6, 1.9 Hz, 1H), 5.49 – 5.46 (m, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 165.19, 149.33, 147.95, 147.82, 143.57, 137.41,

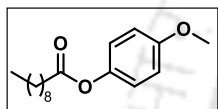
130.99, 130.42, 129.51, 128.83, 127.68, 121.32, 111.66, 110.84, 59.58; HRMS: (ESI) m/z calcd for $C_{15}H_{11}NNaO_3$, 276.0631 $[M+Na]^+$; found 276.0630.

Butyl (tert-butoxycarbonyl)-L-tryptophanate (6u):



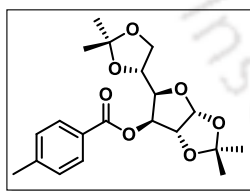
Yield: 86% (155 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.57 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.19 (t, $J = 7.6$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 6.99 (s, 1H), 5.09 (d, $J = 8.2$ Hz, 1H), 4.69 – 4.58 (m, 1H), 4.06 (tt, $J = 11.3, 5.4$ Hz, 2H), 3.34 – 3.22 (m, 2H), 1.53 (dt, $J = 15.0, 7.1$ Hz, 2H), 1.43 (s, 9H), 1.28 (dt, $J = 14.9, 7.4$ Hz, 2H), 0.88 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.64, 155.48, 136.33, 127.94, 122.90, 122.34, 119.74, 118.99, 111.33, 110.50, 79.97, 65.42, 54.48, 30.66, 28.53, 28.26, 19.19, 13.85; HRMS: (ESI) m/z calcd for $C_{20}H_{28}N_2NaO_4$, 383.1941 $[M+Na]^+$; found 383.1939.

4-methoxyphenyl decanoate (6v):



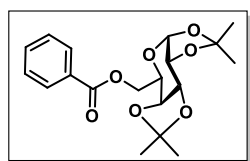
Yield: 87% (121 mg); colorless liquid; 1H NMR (600 MHz, $CDCl_3$) δ 6.99 (d, $J = 9.0$ Hz, 2H), 6.88 (d, $J = 9.0$ Hz, 2H), 3.79 (s, 3H), 2.53 (t, $J = 7.5$ Hz, 2H), 1.74 (p, $J = 7.5$ Hz, 2H), 1.40 (dt, $J = 15.1, 6.6$ Hz, 2H), 1.35 – 1.25 (m, 10H), 0.89 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.92, 157.37, 144.49, 122.51, 114.63, 55.78, 34.56, 32.07, 29.63, 29.46, 29.33, 25.19, 22.87, 14.30; HRMS: (ESI) m/z calcd for $C_{17}H_{27}O_3$, 279.1955 $[M+H]^+$; found 279.1955.

5-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-yl-4-methylbenzoate (6w):



Yield: 83% (157 mg); colorless liquid; 1H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, $J = 8.2$ Hz, 2H), 7.25 (d, $J = 8.1$ Hz, 2H), 5.94 (d, $J = 3.6$ Hz, 1H), 5.49 (d, $J = 2.6$ Hz, 1H), 4.62 (d, $J = 3.6$ Hz, 1H), 4.37 – 4.32 (m, 2H), 4.09 (q, $J = 8.6, 5.2$ Hz, 2H), 2.42 (s, 3H), 1.55 (s, 3H), 1.41 (s, 3H), 1.32 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.47, 144.50, 129.96, 129.46, 127.01, 112.55, 109.56, 105.36, 83.63, 80.21, 76.65, 72.83, 67.42, 27.02, 26.96, 26.42, 25.42, 21.91; HRMS: (ESI) m/z calcd for $C_{20}H_{26}NaO_7$, 401.1571 $[M+Na]^+$; found 401.1571.

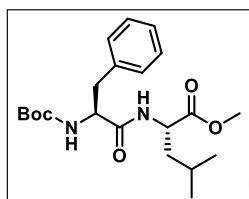
((3aR,5R,5aS,8aS,8bR)-2,2,7,7-tetramethyltetrahydro-5H-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl benzoate (6x):



Yield: 84% (153 mg); colorless liquid; 1H NMR (600 MHz, $CDCl_3$) δ 8.05 (d, $J = 7.2$ Hz, 2H), 7.55 (t, $J = 7.4$ Hz, 1H), 7.43 (t, $J = 7.7$ Hz, 2H), 5.57 (d, $J = 4.9$ Hz, 1H), 4.65 (dd, $J = 7.9, 2.5$ Hz, 1H), 4.53 (dd, $J = 11.5, 4.9$ Hz, 1H), 4.43 (dd, $J = 11.5, 7.6$ Hz, 1H), 4.36 – 4.32 (m, 2H), 4.19 (ddd, J

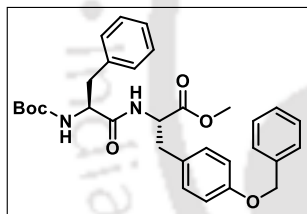
= 7.0, 4.8, 1.8 Hz, 1H), 1.52 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 166.67, 133.19, 130.31, 129.93, 128.56, 109.93, 109.03, 96.56, 71.38, 70.97, 70.78, 66.39, 64.10, 26.24, 26.20, 25.20, 24.72; HRMS: (ESI) m/z calcd for $\text{C}_{19}\text{H}_{25}\text{O}_7$, 365.1595 $[\text{M}+\text{H}]^+$; found 365.1612.

Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-leucinate (9a):



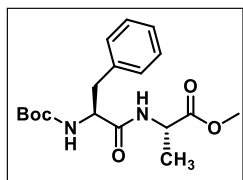
Yield: 97% (190 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.29 (t, J = 7.3 Hz, 2H), 7.24 (d, J = 6.8 Hz, 1H), 7.21 (d, J = 7.5 Hz, 2H), 6.24 (t, J = 9.6 Hz, 1H), 5.00 (s, 1H), 4.57 (td, J = 8.6, 4.8 Hz, 1H), 4.41 – 4.27 (m, 1H), 3.69 (s, 3H), 3.12 – 3.02 (m, 2H), 1.60 – 1.51 (m, 2H), 1.47 (ddd, J = 12.6, 9.1, 4.9 Hz, 1H), 1.41 (s, 9H), 0.90 (dd, J = 11.8, 6.2 Hz, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.03, 171.14, 155.61, 136.80, 129.60, 128.85, 127.14, 80.47, 55.91, 52.45, 50.97, 41.82, 38.25, 28.45, 24.87, 22.95, 22.10; HRMS: (ESI) m/z calcd for $\text{C}_{21}\text{H}_{33}\text{N}_2\text{O}_5$, 393.2389 $[\text{M}+\text{H}]^+$; found 393.2388.

Methyl (S)-3-(4-(benzyloxy)phenyl)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoate (9b):

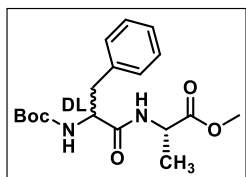


Yield: 98% (261 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.42 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.23 (t, J = 7.3 Hz, 1H), 7.19 (d, J = 7.1 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.25 (s, 1H), 5.02 (s, 2H), 4.95 (s, 1H), 4.74 (s, 1H), 4.33 (s, 1H), 3.67 (s, 3H), 3.07 – 2.94 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.62, 170.90, 158.13, 155.50, 137.14, 136.71, 130.47, 129.59, 128.88, 128.80, 128.20, 128.05, 127.71, 127.19, 115.10, 80.43, 70.19, 55.88, 53.61, 52.48, 38.49, 37.32, 28.46; HRMS: (ESI) m/z calcd for $\text{C}_{31}\text{H}_{37}\text{N}_2\text{O}_6$, 533.2652 $[\text{M}+\text{H}]^+$; found 533.2656.

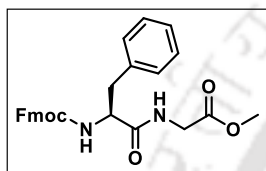
Methyl (tert-butoxycarbonyl)-L-phenylalanyl-D-alaninate (9c):



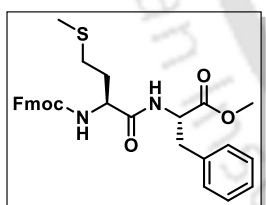
Yield: 97% (172 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.29 (t, J = 7.3 Hz, 2H), 7.24 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 7.1 Hz, 2H), 6.44 (d, J = 8.1 Hz, 1H), 5.01 (s, 1H), 4.51 (p, J = 7.2 Hz, 1H), 4.36 (s, 1H), 3.71 (s, 3H), 3.06 (td, J = 13.4, 12.5, 6.4 Hz, 2H), 1.40 (s, 9H), 1.34 (d, J = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.05, 170.90, 155.54, 136.70, 129.58, 128.84, 127.16, 80.43, 55.78, 52.66, 48.30, 38.51, 28.44, 18.60; HRMS: (ESI) m/z calcd for $\text{C}_{18}\text{H}_{26}\text{N}_2\text{NaO}_5$, 373.1734 $[\text{M}+\text{Na}]^+$; found 373.1741.

Methyl (tert-butoxycarbonyl)(D/L)phenylalanyl-L-alaninate (9d):

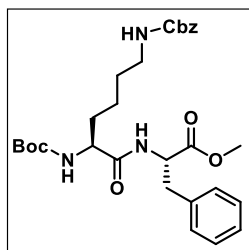
Yield: 96% (168 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.31 – 7.27 (m, 4H), 7.24 (d, J = 7.2 Hz, 2H), 7.20 (d, J = 7.5 Hz, 4H), 6.46 (d, J = 6.9 Hz, 1H), 6.30 (d, J = 7.2 Hz, 1H), 5.09 (s, 1H), 5.03 (s, 1H), 4.51 (q, J = 7.6 Hz, 2H), 4.43 – 4.28 (m, 2H), 3.71 (s, 6H), 3.05 (dq, J = 13.8, 8.9 Hz, 4H), 1.40 (s, 18H), 1.34 (d, J = 7.1 Hz, 3H), 1.24 (d, J = 7.7 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 173.21, 173.05, 170.92, 170.80, 155.57, 155.53, 136.84, 136.68, 129.58, 129.53, 128.87, 128.84, 127.16, 80.42, 80.39, 55.96, 55.77, 52.67, 52.65, 48.30, 48.09, 38.91, 38.51, 28.45, 18.58, 18.32; HRMS: (ESI) m/z calcd for $\text{C}_{18}\text{H}_{26}\text{N}_2\text{NaO}_5$, 373.1734 $[\text{M}+\text{Na}]^+$; found 373.1742.

Methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-phenylalanylglycinate (9e):

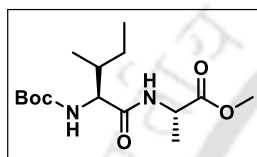
Yield: 97% (222 mg); white solid; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.50 (t, J = 5.9 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.70 – 7.59 (m, 3H), 7.40 (q, J = 7.0 Hz, 2H), 7.32 (d, J = 7.5 Hz, 3H), 7.26 (dt, J = 13.0, 7.5 Hz, 3H), 7.18 (t, J = 7.3 Hz, 1H), 4.29 (ddt, J = 10.6, 8.6, 3.8 Hz, 1H), 4.18 – 4.08 (m, 3H), 3.89 (qd, J = 17.4, 5.9 Hz, 2H), 3.64 (s, 3H), 3.04 (dd, J = 13.6, 3.2 Hz, 1H), 2.82 – 2.75 (m, 1H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 172.21, 170.26, 155.85, 143.73, 140.67, 138.21, 129.25, 128.08, 127.66, 127.08, 126.28, 125.40, 120.11, 65.69, 56.04, 51.76, 46.56, 40.66, 37.44; HRMS: (ESI) m/z calcd for $\text{C}_{27}\text{H}_{27}\text{N}_2\text{O}_5$, 459.1920 $[\text{M}+\text{H}]^+$; found 459.1920.

Methyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-methionyl-L-phenylalaninate (9f):

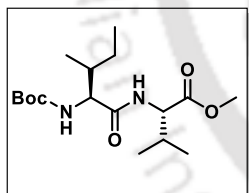
Yield: 98% (261 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.77 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 7.26 – 7.23 (m, 2H), 7.22 – 7.18 (m, 1H), 7.09 (d, J = 7.1 Hz, 2H), 6.61 (s, 1H), 5.51 (s, 1H), 4.86 (q, J = 6.2 Hz, 1H), 4.39 (dt, J = 32.1, 7.9 Hz, 3H), 4.21 (t, J = 6.9 Hz, 1H), 3.72 (s, 3H), 3.18 – 3.04 (m, 2H), 2.53 (hept, J = 6.9 Hz, 2H), 2.04 (d, J = 11.4 Hz, 4H), 1.94 (dq, J = 14.6, 7.4 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.77, 170.91, 156.12, 144.00, 143.92, 141.52, 135.68, 129.40, 128.89, 127.97, 127.47, 127.30, 125.30, 125.25, 120.23, 120.21, 67.31, 53.71, 53.42, 52.61, 47.34, 37.98, 31.76, 30.12, 15.22; HRMS: (ESI) m/z calcd for $\text{C}_{30}\text{H}_{33}\text{N}_2\text{O}_5\text{S}$, 533.2110 $[\text{M}+\text{H}]^+$; found 533.2111.

Methyl-*N*⁶-((benzyloxy)carbonyl)-*N*²-(*tert*-butoxycarbonyl)-*L*-lysyl-*L*-phenylalaninate (9g):

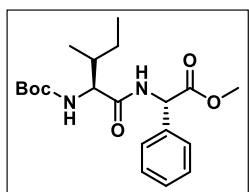
Yield: 97% (263 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.34 (d, *J* = 4.0 Hz, 4H), 7.33 – 7.26 (m, 3H), 7.23 (q, *J* = 7.3, 6.1 Hz, 1H), 7.10 (d, *J* = 7.4 Hz, 2H), 6.50 (s, 1H), 5.09 (d, *J* = 12.0 Hz, 3H), 4.95 (s, 1H), 4.84 (q, *J* = 6.4 Hz, 1H), 4.05 (s, 1H), 3.67 (s, 3H), 3.20 – 3.04 (m, 4H), 1.77 (s, 2H), 1.53 – 1.46 (m, 2H), 1.42 (s, 9H), 1.36 – 1.29 (m, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 171.98, 171.87, 156.77, 155.78, 136.79, 135.91, 129.43, 128.80, 128.69, 128.33, 128.28, 127.35, 80.28, 66.84, 54.46, 53.31, 52.54, 40.54, 38.06, 32.08, 29.56, 28.50, 22.46; HRMS: (ESI) *m/z* calcd for C₂₉H₄₀N₃O₇, 542.2866 [M+H]⁺; found 542.2864.

Methyl (*tert*-butoxycarbonyl)-*L*-alloisoleucyl-*L*-alaninate (9h):

Yield: 96% (152 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 6.45 (s, 1H), 5.06 (s, 1H), 4.58 (p, *J* = 7.3 Hz, 1H), 3.95 (d, *J* = 7.9 Hz, 1H), 3.74 (s, 3H), 1.91 – 1.82 (m, 1H), 1.50 (d, *J* = 17.6 Hz, 1H), 1.44 (s, 9H), 1.41 (d, *J* = 7.0 Hz, 3H), 1.14 (ddq, *J* = 14.3, 9.0, 7.3 Hz, 1H), 0.96 – 0.88 (m, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 173.35, 171.32, 155.98, 80.15, 59.37, 52.66, 48.20, 37.57, 28.51, 24.93, 18.55, 15.67, 11.65; HRMS: (ESI) *m/z* calcd for C₁₅H₂₈N₂NaO₅, 339.1896 [M+Na]⁺; found 339.1897.

Methyl (*tert*-butoxycarbonyl)-*L*-alloisoleucyl-*L*-valinate (9i):

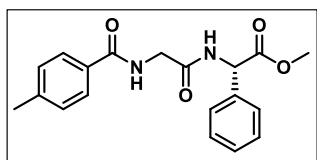
Yield: 97% (167 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 6.44 (s, 1H), 5.08 (s, 1H), 4.52 (dd, *J* = 8.7, 4.9 Hz, 1H), 3.93 (t, *J* = 8.0 Hz, 1H), 3.71 (s, 3H), 2.15 (ddd, *J* = 13.7, 9.5, 6.0 Hz, 1H), 1.88 – 1.82 (m, 1H), 1.52 (ddt, *J* = 18.8, 12.0, 7.0 Hz, 1H), 1.42 (s, 9H), 1.18 – 1.09 (m, 1H), 0.94 – 0.87 (m, 12H); ¹³C NMR (151 MHz, CDCl₃) δ 172.31, 171.85, 155.98, 80.06, 59.56, 57.24, 52.29, 37.10, 31.39, 28.48, 24.99, 19.10, 17.92, 15.68, 11.49; HRMS: (ESI) *m/z* calcd for C₁₇H₃₃N₂O₅, 345.4595 [M+H]⁺; found 345.4590.

Methyl-(*S*)-2-((2*S*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-methylpentanamido)-2-phenylacetate (9j):

Yield: 98% (185 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.37 – 7.29 (m, 5H), 6.92 (s, 1H), 5.53 (d, *J* = 6.8 Hz, 1H), 5.08 – 4.96 (m, 1H), 4.00 (t, *J* = 7.7 Hz, 1H), 3.72 (s, 3H), 1.87 (dt, *J* = 13.3, 10.2, 5.2 Hz, 1H), 1.53 – 1.46 (m, 1H), 1.42 (s, 9H), 1.18 – 1.11 (m, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.89 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.18, 171.17, 155.95, 136.38,

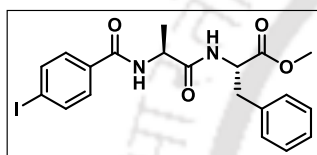
129.18, 128.79, 127.45, 80.19, 59.31, 56.69, 53.03, 37.41, 28.49, 24.98, 15.70, 11.61; HRMS: (ESI) m/z calcd for $C_{20}H_{31}N_2O_5$, 379.2233 $[M+H]^+$; found 379.2221.

Methyl (S)-2-(2-(4-methylbenzamido)acetamido)-2-phenylacetate (9k):



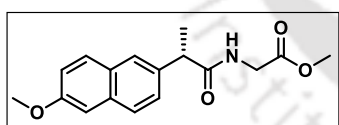
Yield: 97% (165 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.86 (d, J = 6.9 Hz, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.37 (dd, J = 6.5, 3.0 Hz, 2H), 7.31 (dq, J = 5.3, 3.5, 2.1 Hz, 4H), 7.18 (d, J = 8.0 Hz, 2H), 5.57 (d, J = 7.0 Hz, 1H), 4.31 – 4.15 (m, 2H), 3.70 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.18, 169.06, 167.92, 142.40, 136.14, 130.83, 129.36, 129.16, 128.78, 127.56, 127.39, 56.95, 52.97, 43.76, 21.62; HRMS: (ESI) m/z calcd for $C_{19}H_{21}N_2O_4$, 341.1496 $[M+H]^+$; found 341.1494.

Methyl (4-iodobenzoyl)-L-alanyl-L-phenylalaninate (9l):



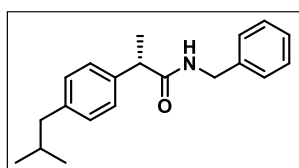
Yield: 98% (235 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.20 – 7.14 (m, 3H), 7.07 (dd, J = 7.3, 2.2 Hz, 2H), 6.83 (d, J = 7.4 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 4.89 – 4.83 (m, 1H), 4.67 (p, J = 7.0 Hz, 1H), 3.74 (s, 3H), 3.10 (ddd, J = 58.5, 13.9, 6.1 Hz, 2H), 1.44 (d, J = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.97, 171.83, 166.41, 137.99, 135.79, 133.31, 129.38, 128.91, 128.80, 127.37, 99.06, 53.56, 52.66, 49.28, 38.04, 18.55; HRMS: (ESI) m/z calcd for $C_{20}H_{22}IN_2O_4$, 481.0619 $[M+H]^+$; found 481.0626.

(S)-N-benzyl-2-(6-methoxynaphthalen-2-yl)propanamide (12a):



Yield: 98% (148 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.71 (dd, J = 19.4, 9.8 Hz, 3H), 7.39 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.9 Hz, 1H), 7.12 (s, 1H), 5.93 (s, 1H), 4.04 – 3.93 (m, 2H), 3.91 (s, 3H), 3.76 (q, J = 7.1 Hz, 1H), 3.69 (s, 3H), 1.61 (d, J = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.77, 170.54, 157.97, 136.18, 134.01, 129.45, 129.18, 127.78, 126.47, 119.38, 105.87, 55.53, 52.47, 47.00, 41.53, 18.60; HRMS: (ESI) m/z calcd for $C_{17}H_{20}NO_4$, 302.1387 $[M+H]^+$; found 302.1395.

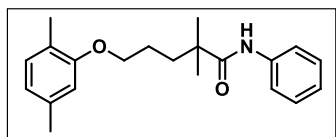
(S)-N-benzyl-2-(4-isobutylphenyl)propanamide (12b):



Yield: 96% (142 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 7.24 – 7.22 (m, 1H), 7.20 (dd, J = 8.2, 2.1 Hz, 2H), 7.15 – 7.08 (m, 4H), 5.63 (s, 1H), 4.39 (dd, J = 5.9, 2.9 Hz, 2H), 3.58 (q, J = 7.2 Hz, 1H), 2.45 (d, J = 7.1 Hz, 2H), 1.84 (hept, J = 6.8 Hz, 1H), 1.55 (d, J = 7.3 Hz, 3H), 0.89 (d, J = 6.6 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.59, 141.02,

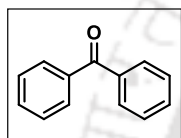
138.66, 138.64, 138.55, 129.88, 128.79, 127.58, 127.52, 47.02, 47.00, 45.20, 43.72, 30.38, 22.56, 18.63; HRMS: (ESI) m/z calcd for $C_{20}H_{26}NO$, 296.2009 $[M+H]^+$; found 296.2017.

5-(2,5-dimethylphenoxy)-2,2-dimethyl-N-phenylpentanamide (12c):



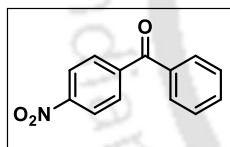
Yield: 99.6% (162 mg); faint brown solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, $J = 7.7$ Hz, 2H), 7.37 (d, $J = 8.7$ Hz, 1H), 7.32 (t, $J = 7.7$ Hz, 2H), 7.11 (t, $J = 7.3$ Hz, 1H), 7.00 (d, $J = 7.2$ Hz, 1H), 6.66 (d, $J = 7.1$ Hz, 1H), 6.61 (s, 1H), 3.95 (dd, $J = 6.1, 4.0$ Hz, 2H), 2.29 (s, 3H), 2.17 (s, 3H), 1.83 (d, $J = 3.2$ Hz, 4H), 1.34 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.92, 157.05, 138.06, 136.75, 130.52, 129.16, 124.52, 123.71, 121.03, 120.35, 112.34, 68.07, 43.01, 37.93, 25.84, 25.34, 21.58, 16.04; HRMS: (ESI) m/z calcd for $C_{21}H_{28}NO_2$, 326.2115 $[M+H]^+$; found 326.2115.

Benzophenone (14a):



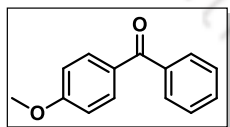
Yield: 86% (31 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) 7.81 (d, $J = 7.1$ Hz, 4H), 7.59 (t, $J = 7.4$ Hz, 2H), 7.49 (t, $J = 7.7$ Hz, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.99, 137.77, 132.63, 130.26, 128.47; HRMS (ESI) m/z : calcd for $C_{13}H_{10}O$, 183.0805 $[M+H]^+$; found 183.0805.

(4-nitrophenyl)(phenyl)methanone¹³ (14b):



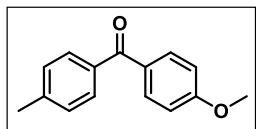
Yield: 69% (31 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 8.35 (d, $J = 8.7$ Hz, 2H), 7.94 (d, $J = 8.7$ Hz, 2H), 7.80 (d, $J = 7.1$ Hz, 2H), 7.66 (t, $J = 7.5$ Hz, 1H), 7.53 (t, $J = 7.8$ Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 195.06, 150.03, 143.09, 136.47, 133.71, 130.93, 130.32, 128.91, 123.77.

(4-methoxyphenyl)(phenyl)methanone (14c):

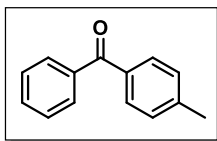


Yield: 72% (31 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, $J = 8.5$ Hz, 2H), 7.75 (d, $J = 7.7$ Hz, 2H), 7.56 (t, $J = 7.2$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 2H), 6.96 (d, $J = 8.5$ Hz, 2H), 3.88 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 195.78, 163.40, 138.44, 132.75, 132.08, 130.30, 129.91, 128.37, 113.73, 55.67; HRMS (ESI) m/z : calcd for $C_{14}H_{13}O_2$, 213.0911 $[M+H]^+$; found 213.0916.

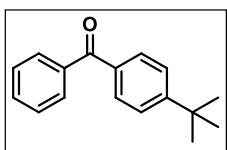
(4-methoxyphenyl)(p-tolyl)methanone (14d):



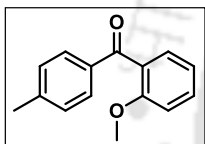
Yield: 68% (31 mg); white solid; 1H NMR (600 MHz, $CDCl_3$) δ 7.74 (d, $J = 8.5$ Hz, 2H), 7.60 (d, $J = 7.8$ Hz, 2H), 7.20 (d, $J = 7.8$ Hz, 2H), 6.88 (d, $J = 8.4$ Hz, 2H), 3.81 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 195.59, 163.22, 142.83, 135.68, 132.64, 130.65, 130.21, 129.07, 113.67, 55.68, 21.83; HRMS (ESI) m/z : calcd for $C_{15}H_{15}O_2$, 227.1067 $[M+H]^+$; found 227.1079.

phenyl(p-tolyl)methanone (14e):

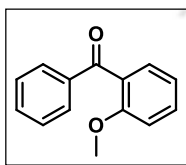
Yield: 88% (35 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, J = 7.0 Hz, 2H), 7.73 (d, J = 8.1 Hz, 2H), 7.58 (t, J = 7.4 Hz, 1H), 7.48 (t, J = 7.7 Hz, 2H), 7.28 (d, J = 7.9 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 196.75, 143.46, 138.13, 135.05, 132.37, 130.52, 130.14, 129.18, 128.41, 21.88; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{O}$, 197.0961 $[\text{M}+\text{H}]^+$; found 197.0968.

(4-(tert-butyl)phenyl)(phenyl)methanone (14f):

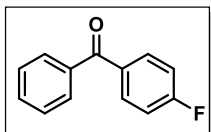
Yield: 92% (44 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.81 (d, J = 7.3 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.58 (t, J = 7.4 Hz, 1H), 7.51 – 7.45 (m, 4H), 1.37 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 196.71, 156.39, 138.12, 135.00, 132.39, 130.35, 130.19, 128.41, 125.46, 35.32, 31.35; HRMS (ESI) m/z : calcd for $\text{C}_{17}\text{H}_{19}\text{O}$, 239.1431 $[\text{M}+\text{H}]^+$; found 239.1430.

(2-methoxyphenyl)(p-tolyl)methanone (14g):

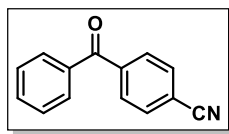
Yield: 87% (39 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.72 (d, J = 8.2 Hz, 2H), 7.46 (ddd, J = 8.4, 7.4, 1.8 Hz, 1H), 7.33 (dd, J = 7.5, 1.7 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.03 (td, J = 7.4, 1.0 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 3.73 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 196.36, 157.39, 144.04, 135.39, 131.80, 130.27, 129.60, 129.32, 129.17, 120.63, 111.59, 55.82, 21.93; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{15}\text{O}_2$, 227.1067 $[\text{M}+\text{H}]^+$; found 227.1073.

(2-methoxyphenyl)(phenyl)methanone (14h):

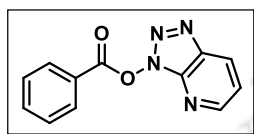
Yield: 91% (39 mg); colorless liquid; ^1H NMR (600 MHz, CDCl_3) δ 7.82 (d, J = 7.2 Hz, 2H), 7.55 (t, J = 7.4 Hz, 1H), 7.47 (ddd, J = 8.4, 7.5, 1.8 Hz, 1H), 7.43 (t, J = 7.7 Hz, 2H), 7.36 (dd, J = 7.5, 1.6 Hz, 1H), 7.04 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 3.73 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 196.70, 157.54, 137.98, 133.14, 132.08, 130.04, 129.79, 129.02, 128.42, 120.68, 111.62, 55.80; HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{13}\text{O}_2$, 213.0911 $[\text{M}+\text{H}]^+$; found 213.0917.

(4-fluorophenyl)(phenyl)methanone (14i):

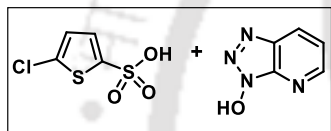
Yield: 93% (37 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.85 (dd, J = 8.7, 5.5 Hz, 2H), 7.77 (d, J = 7.7 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.49 (t, J = 7.7 Hz, 2H), 7.16 (t, J = 8.6 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 195.50, 166.43, 164.75, 137.69, 134.00, 132.91, 132.85, 132.69, 130.09, 130.00, 128.57, 115.74, 115.59; HRMS (ESI) m/z : calcd for $\text{C}_{13}\text{H}_{10}\text{FO}$, 201.0711 $[\text{M}+\text{H}]^+$; found 201.0718.

4-benzoylbenzonitrile (14j):

Yield: 95% (39 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.87 (d, J = 8.2 Hz, 2H), 7.81 – 7.76 (m, 4H), 7.64 (t, J = 7.4 Hz, 1H), 7.51 (t, J = 7.8 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ 195.25, 141.39, 136.48, 133.53, 132.36, 130.43, 130.26, 128.82, 118.21, 115.82; HRMS (ESI) m/z : calcd for $\text{C}_{15}\text{H}_{10}\text{NO}_2$, 208.0757 $[\text{M}+\text{H}]^+$; found 208.0762.

3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl benzoate (Bz-OAt):

Yield: 99% (119 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 8.73 (dd, J = 4.4, 1.3 Hz, 1H), 8.46 (dd, J = 8.4, 1.3 Hz, 1H), 8.34 – 8.26 (m, 2H), 7.79 – 7.74 (m, 1H), 7.60 (t, J = 7.9 Hz, 2H), 7.46 (dd, J = 8.4, 4.5 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 162.80, 152.01, 140.96, 135.71, 135.30, 131.11, 129.81, 129.35, 124.84, 121.13; HRMS (ESI) m/z : calcd for $\text{C}_{12}\text{H}_9\text{N}_4\text{O}_2$, 241.0721 $[\text{M}+\text{H}]^+$; found 241.0728.

Recovered 5-chlorothiophene-2-sulfonic acid (SA) + HOAt:

Yield: 93%; ^1H NMR (600 MHz, Methanol- d_4) δ 8.72 (dd, J = 4.5, 1.4 Hz, 1H), 8.42 (dd, J = 8.5, 1.4 Hz, 1H), 7.51 (dd, J = 8.4, 4.4 Hz, 1H), 7.21 (d, J = 3.9 Hz, 1H), 6.90 (d, J = 3.9 Hz, 1H); ^{13}C NMR (151 MHz, Methanol- d_4) δ 152.11, 147.22, 141.12, 136.47, 133.17, 129.98, 128.33, 127.24, 122.25; HRMS: (ESI) m/z calcd for SA ($\text{C}_4\text{H}_2\text{ClO}_3\text{S}_2$), 196.9139 $[\text{M}-\text{H}]^-$; found 196.9148; HOAt ($\text{C}_5\text{H}_3\text{N}_4\text{O}$), 135.0312 $[\text{M}-\text{H}]^-$; found 135.0314.

Table 4.8. HPLC retention time and observed mass of solid phase synthesized peptides

Entry	Peptide sequence	t_R /Total run time (min)	Expected mass (m/z)	Observed mass (m/z)
1 ^a	Fmoc-LPFD-NH ₂ (10a)	9.23/15	$[\text{M}+\text{Na}]^+$ 734.3160	734.3166
2 ^b	Fmoc-EPAfLAK-NH ₂ (10b)	6.83/10	$[\text{M}+\text{H}]^+$ 996.5190	996.8380
3 ^a	Fmoc-KLVfFAE(NH ₂)-OBn (10c)	9.74/15	$[\text{M}+\text{H}]^+$ 1164.6128	1164.6133

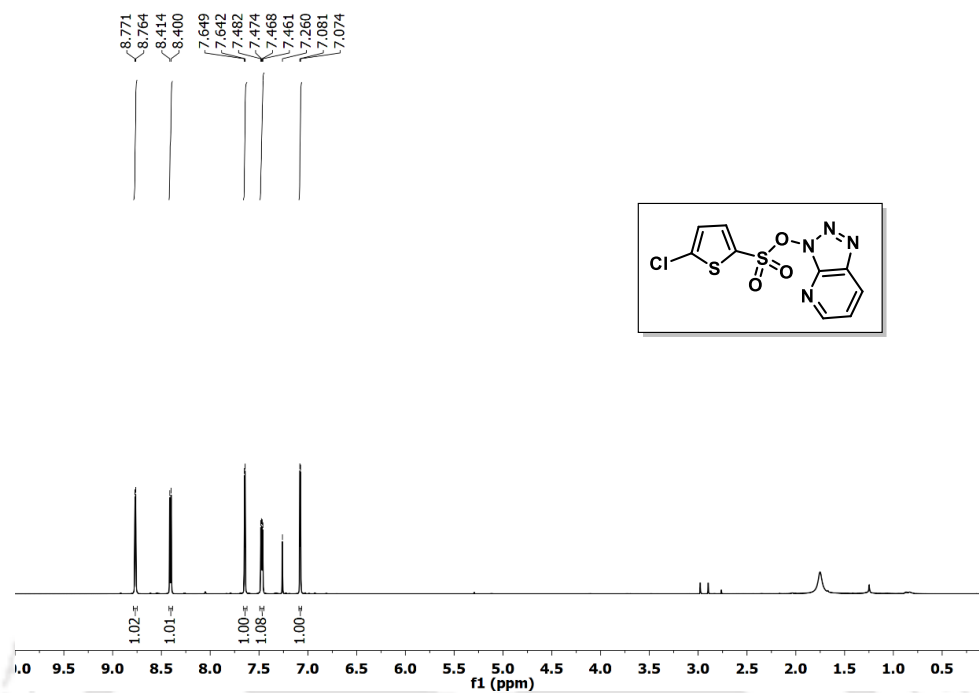
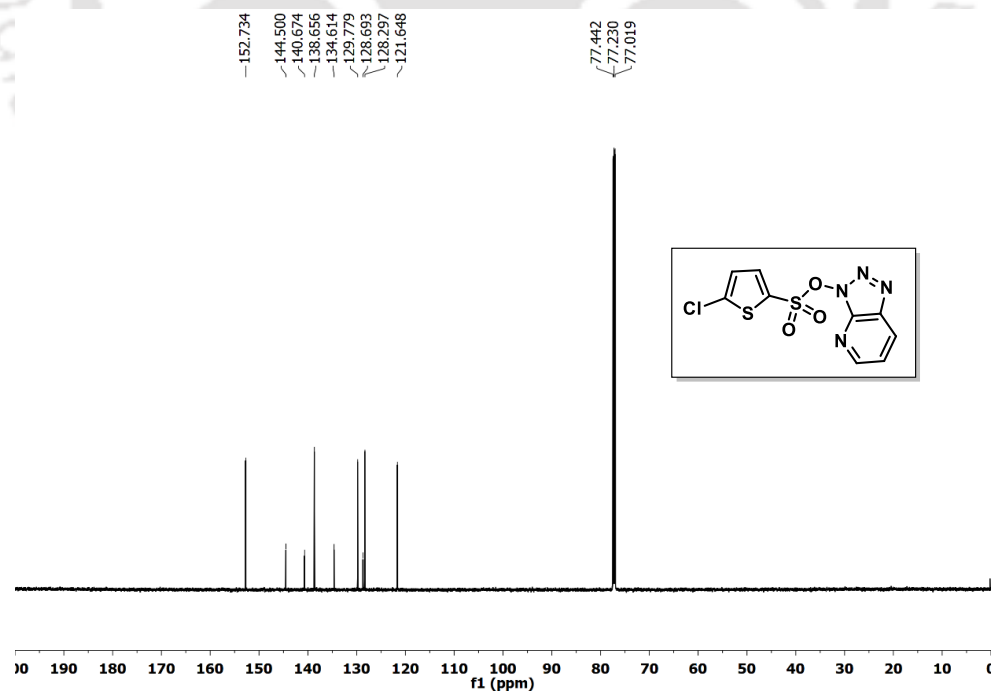
HPLC: ^aThermo Scientific system, ^bAgilent 1260 Infinity II prime LC system. Poroshell 120 EC C18

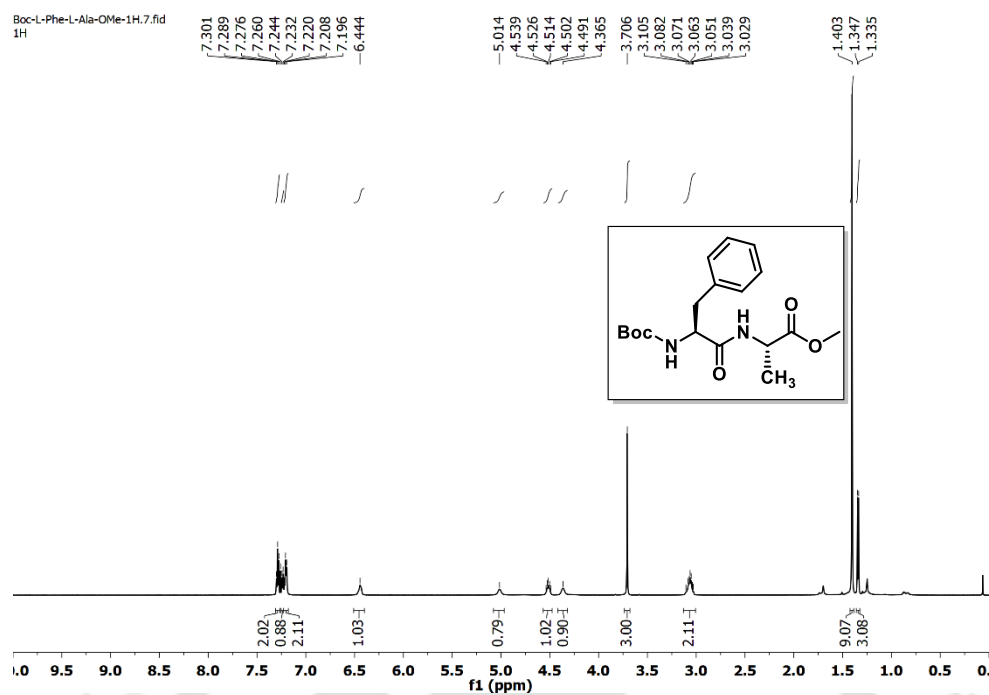
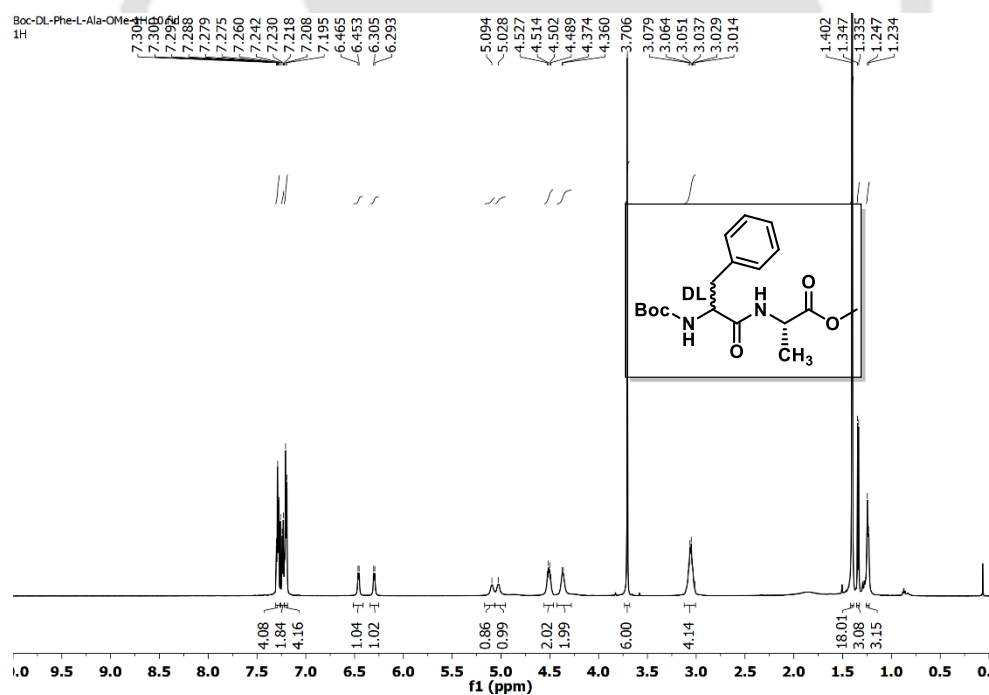
Agilent column (4.6 × 100mm, 4 μm). t_R = HPLC retention time.

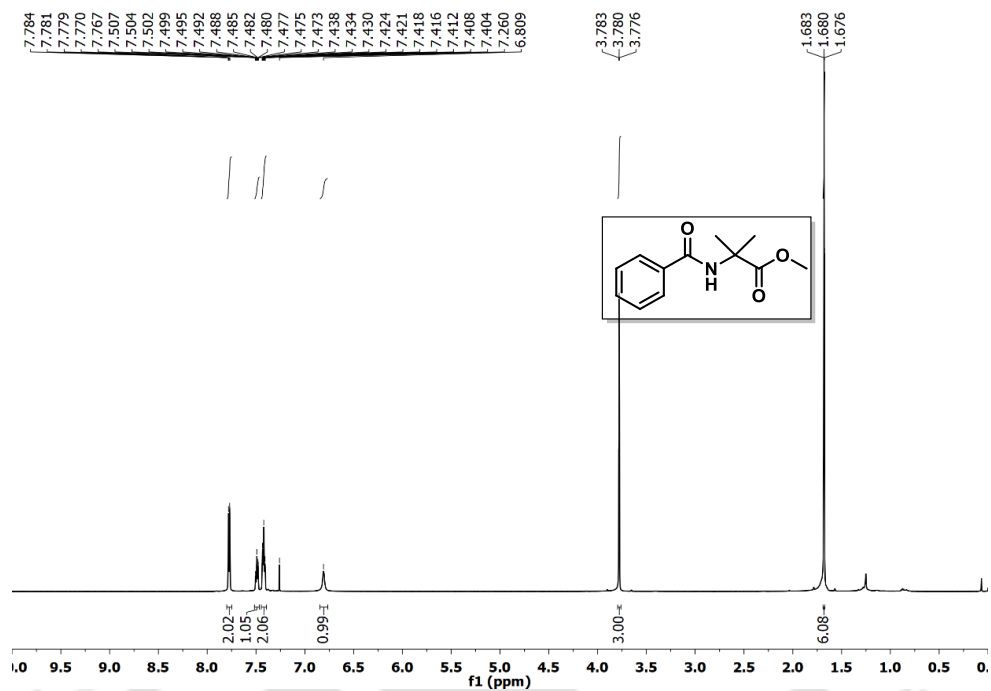
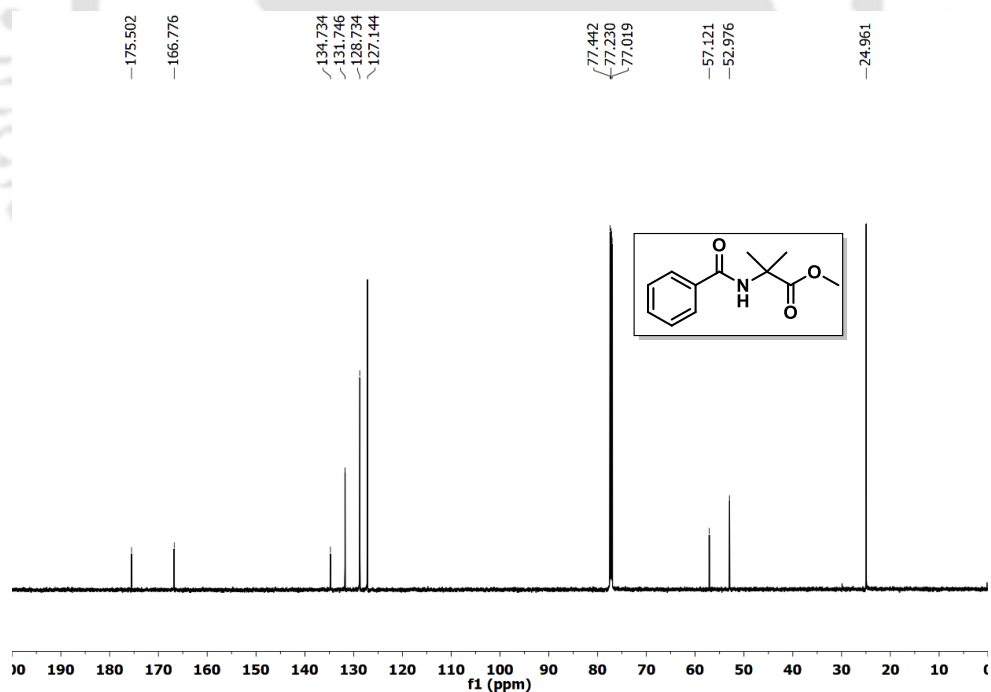
4.13. References

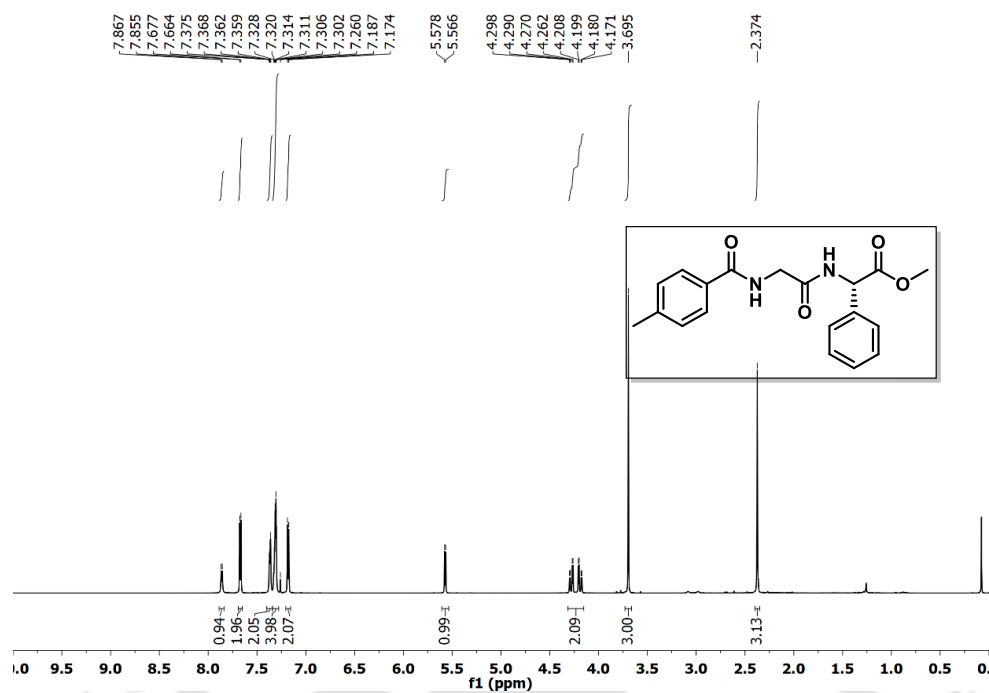
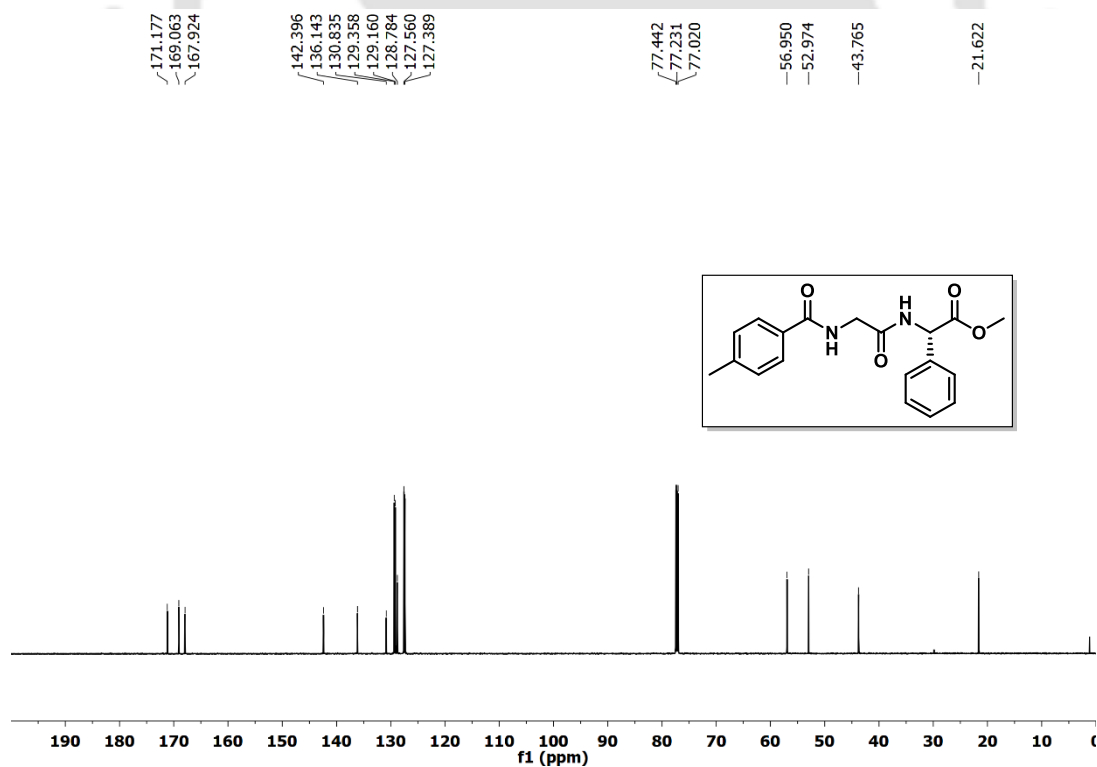
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4.14. Selected spectra

Figure 4.3. ¹H NMR spectrum of coupling reagent (CTSOAt, 3a)Figure 4.4. ¹³C NMR spectrum of coupling reagent (CTSOAt, 3a)

Figure 4.5. ^1H NMR spectrum of Boc-L-Phe-L-Ala-OMe (9c)Figure 4.6. ^1H NMR spectrum of Boc-DL-Phe-L-Ala-OMe (9d)

Figure 4.7. ¹H NMR spectrum of amide **6i**Figure 4.8. ¹³C NMR spectrum of amide **6i**

Figure 4.9. ¹H NMR spectrum of peptide 9kFigure 4.10. ¹³C NMR spectrum of peptide 9k

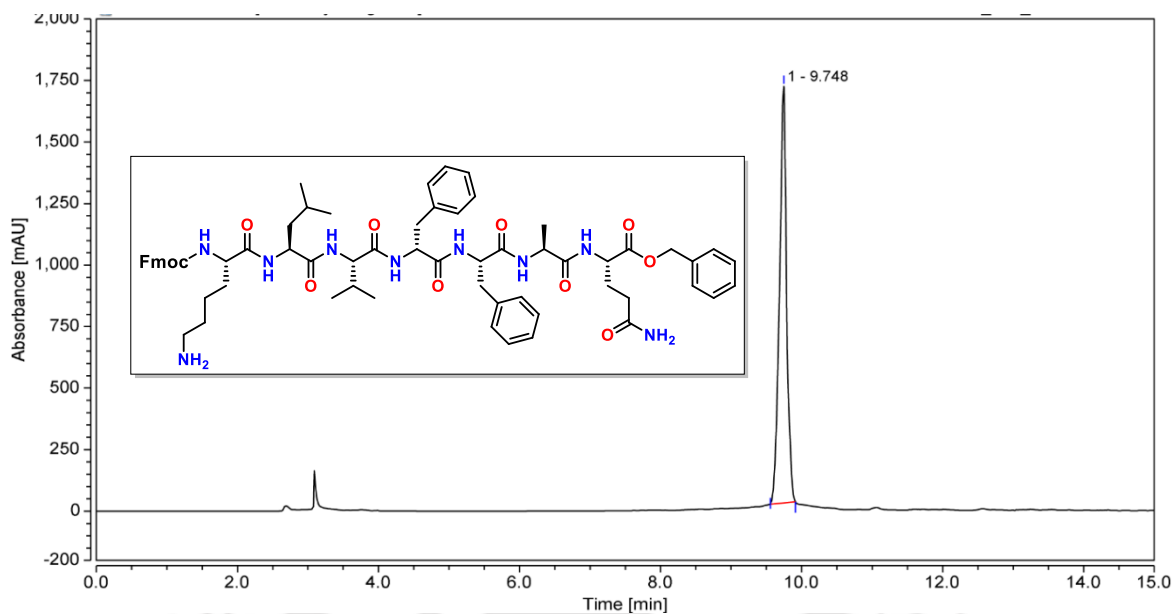


Figure 4.11. HPLC profile of peptide 10c

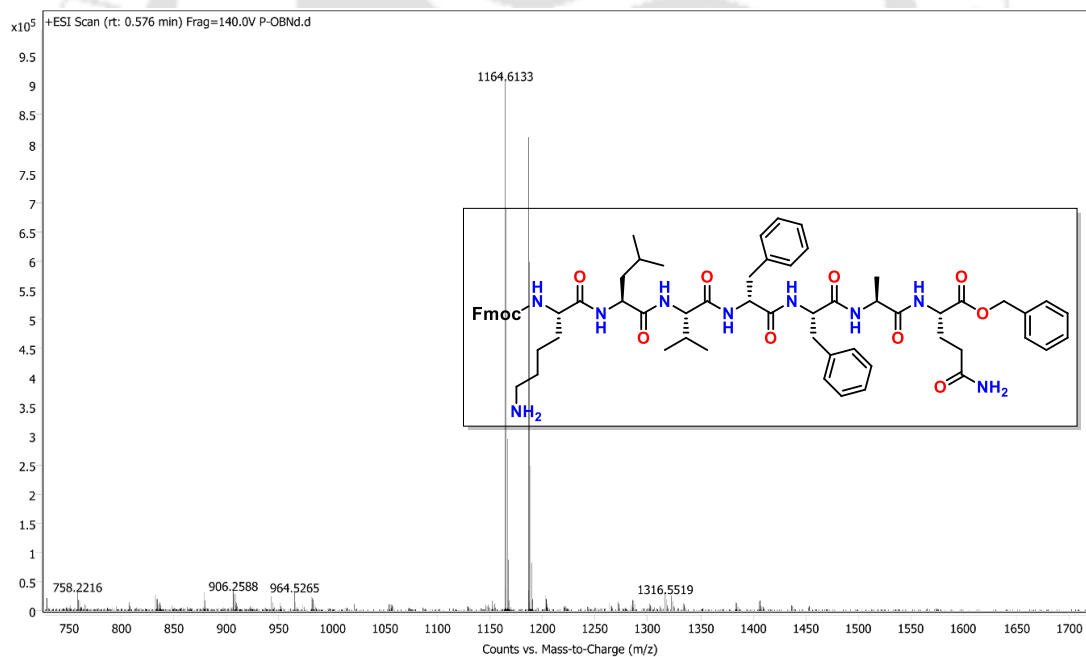
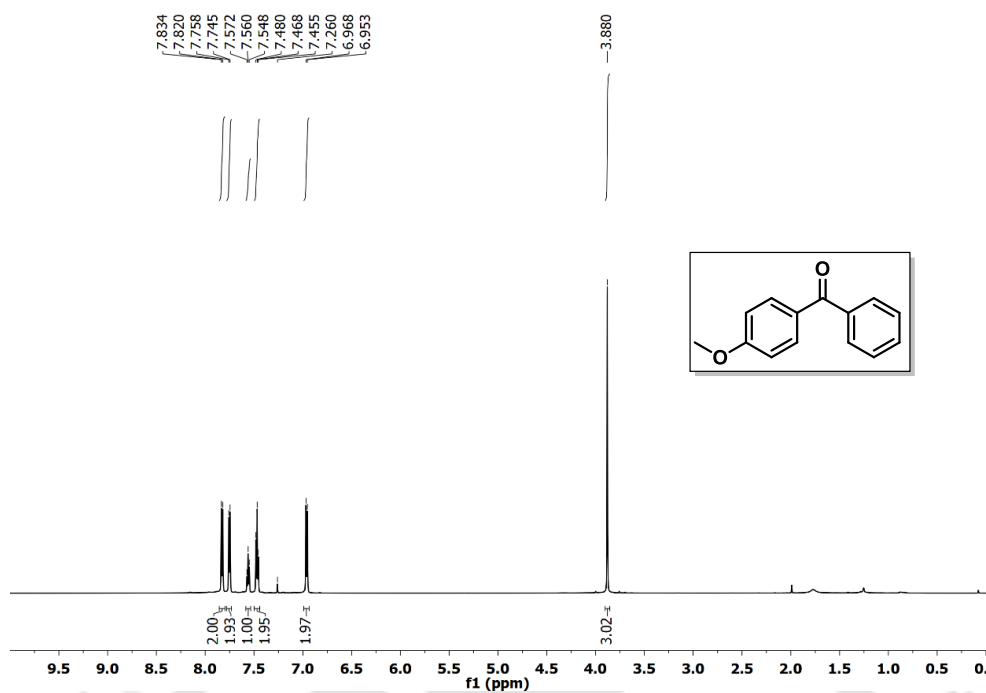
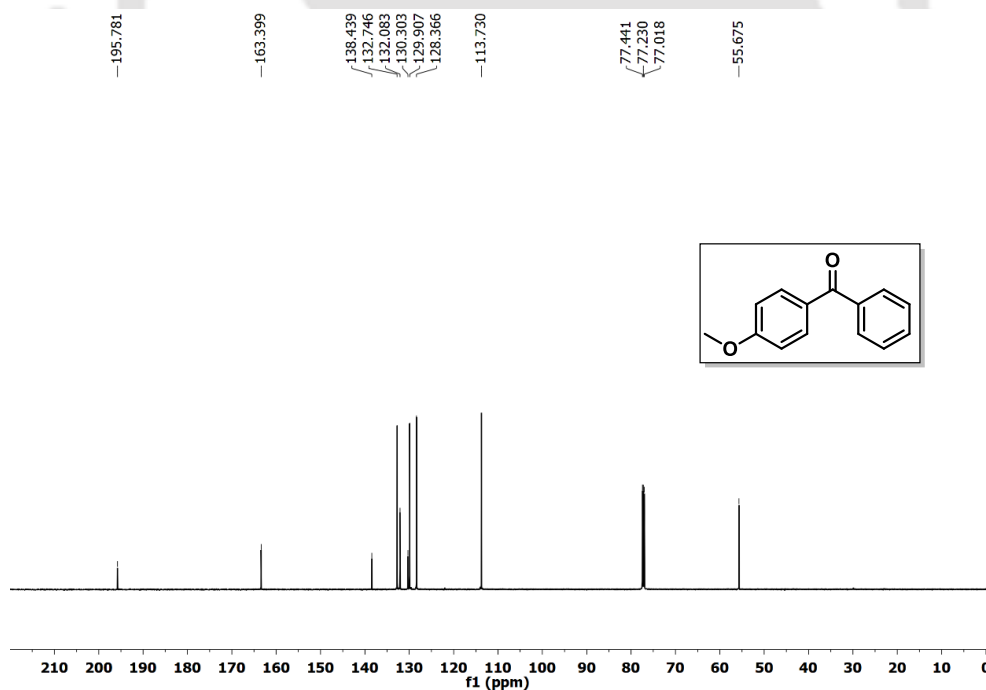


Figure 4.12. HRMS spectrum of peptide 10c

Figure 4.13. ¹H NMR spectrum of ketone **14c**Figure 4.14. ¹³C NMR spectrum of ketone **14c**

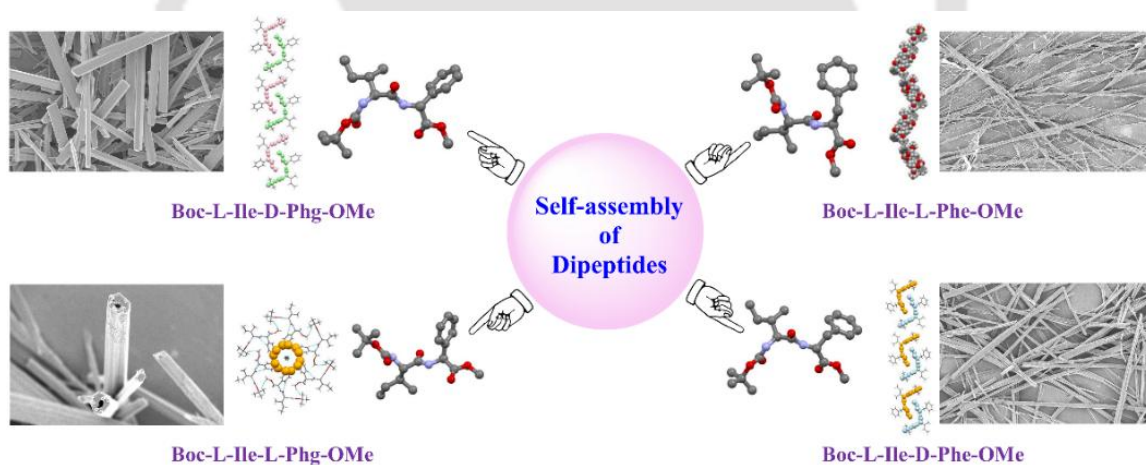
4.15. Crystallographic data

Table 4.9. Crystallographic data of CTSOAt (3a), Bz-OAt (V) and ketone 14j

Parameters	CTSOAt (3a)	Bz-OAt (V)	Ketone 14j
Formula	C ₉ H ₅ Cl N ₄ O ₃ S ₂	C ₁₂ H ₈ N ₄ O ₂	C ₁₄ H ₉ N O
Fw	316.74	240.22	207.22
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	P 21/c	C 2/c	P 21/n
a/Å	7.8618(17)	7.3009(7)	4.0327(4)
b/Å	25.170(5)	14.8494(13)	23.141(2)
c/Å	6.6676(14)	20.8531(19)	11.5231(12)
$\alpha/^\circ$	90.00	90.00	90
$\beta/^\circ$	110.425(5)	96.186(3)	92.475(2)
$\gamma/^\circ$	90.00	90.00	90
V/Å ³	1236.4(5)	2247.6(4)	1074.33(18)
Z	4	8	4
D _c /g cm ⁻³	1.702	1.420	1.281
μ Mo K α /mm ⁻¹	0.655	0.102	0.081
F000	640	992	432
T/K	297(2)	297(2)	298(2)
θ max.	26.32	26.26	25.58
Total no. of reflections	32005	28173	23652
Independent reflections	2166	2307	1888
Observed reflections	1962	1805	1567
Parameters refined	173	163	145
R ₁ , I > 2 σ (I)	0.0412	0.0562	0.0433
wR ₂ , I > 2 σ (I)	0.1245	0.1317	0.1259
GOF (F ²)	1.072	0.876	0.866
CCDC No.	2296563	2309721	2374954

Chapter 5

Influence of Amino Acids Side-Chain and Chirality on the Supramolecular Assemblies of Dipeptides



5.1. Background

In the previous chapters, we synthesized various amides, esters, peptides, and other compounds using novel coupling reagents. After successfully synthesizing a wide range of peptides, we obtained several peptide crystals. This prompted us to examine the self-assembly properties of these crystalline peptides, as peptide self-assembly serves as essential components for various material, device, and biomedical applications.¹⁻³ Through various non-covalent interactions, short peptides can self-assemble into diverse nanostructures such as nanofibrils, nanorods, hollow tubes, spheres, nanotapes, and nanovesicles.⁴⁻⁸ These nanostructures show tremendous potential in the fields of material science, nanoscience, and medicine.⁹

5.2. Design of peptides

Interestingly, we obtained two peptide crystals Boc-L-Ile-D-Phe-OMe and Boc-L-Ile-L-Phg-OMe, synthesized in chapter 2. To delve deeper into how side-chains and chirality affect dipeptide self-assembly composed of L/L and L/D amino acids, we designed two additional peptides: Boc-L-Ile-L-Phe-OMe and Boc-L-Ile-D-Phg-OMe and crystallized them. All the designed peptides contained L-Ile positioned at the N-terminal and L-Phe, D-Phe, L-Phg, or D-Phg (Phg: non-proteinogenic amino acid with a side-chain that has one methylene group less than Phe) at the C-terminal (Figure 5.1). D-amino acid-containing peptides are known to increase proteolytic stability.¹⁰ While there are several reports on L/L amino acids containing peptide self-assembly, the peptide self-assembly with alternating sequences of L/D amino acids is not well-explored. To address this research gap, we designed this project to investigate how side chains and chirality of L/L and L/D amino acids influence peptide self-assembly.

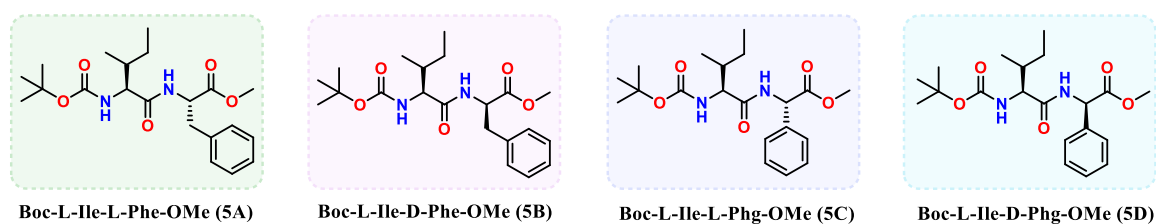


Figure 5.1. Chemical structure of peptides 5A, 5B, 5C, and 5D.

5.3. Synthesis of peptides

To obtain an adequate amount of peptides for studying self-assembly, we synthesized all the N- and C-protected dipeptides using the coupling reagent-based conventional solution-phase peptide synthesis method as described in section 5.9.2. The characterization of all synthesized peptides was performed using NMR (^1H and ^{13}C) spectroscopy and mass spectrometry.

5.4. Examination of dipeptide self-assembly via SC-XRD

We conducted SC-XRD studies of these peptides to understand their molecular arrangements and conformations. Colorless, well-ordered, block-shaped single crystals of the peptides (**5A**, **5B**, **5C**, and **5D**) were obtained through the gradual evaporation of the peptides containing acetonitrile-water at room temperature. Peptide **5A** formed crystal in orthorhombic ($P 21 21 21$) crystal system, and peptide **5C** crystallized in a hexagonal ($P 63$) crystal system. While both peptides **5B** and **5D** formed crystals in the monoclinic ($P 21$) crystal system. Each peptide was comprised of a single molecule in its own asymmetric unit. The torsion angles of the peptide backbone indicated that all peptides exhibited parallel β -sheet structure. Each subunit of peptide **5A** was linked to each other by intermolecular hydrogen bonds ($\text{N1-H1}\dots\text{O2}$ and $\text{N2-H2}\dots\text{O3}$), forming a parallel β -sheet structure (Figure 5.2b). These subunits subsequently self-assembled into single helix-like structure in higher-order packing along the a -axis. (Figure 5.2c).

Table 5.1. List of torsion angles (deg)

Peptide	$\phi 1$	$\psi 1$	$\phi 2$	$\psi 2$	$\omega 1$	$\omega 2$
5A	C5-N1-C6-C11 = -96.8(6)	N1-C6-C11- N2 = 99.6(5)	C11-N2-C12- C13 = -124.8(5)	N2-C12-C13- O5 = 32.5(6)	O1-C5-N1-C6 = 179.0(4)	C6-C11-N2- C12 = 179.0(4)
5B	C5-N1-C6-C11 = -123.5(4)	N1-C6-C11- N2 = 111.0(4)	C11-N2-C12- C13 = 150.9(4)	N2-C12-C13- O5 = 176.9(4)	O1-C5-N1-C6 = 178.3(4)	C6-C11-N2- C12 = -171.3(3)
5C	C5-N1-C6-C11 = -114.5(7)	N1-C6-C11- N2 = 122.7(7)	C11-N2-C12- C13 = -150.3(7)	N2-C12-C13- O5 = 169.1(6)	O1-C5-N1-C6 = -178.2(6)	C6-C11-N2- C12 = 178.2(6)
5D	C5-N1-C6-C11 = -118.9(5)	N1-C6-C11- N2 = 110.3(5)	C11-N2-C12- C13 = 148.2(5)	N2-C12-C13- O5 = -175.6(5)	O1-C5-N1-C6 = 176.2(5)	C6-C11-N2- C12 = -178.4(5)

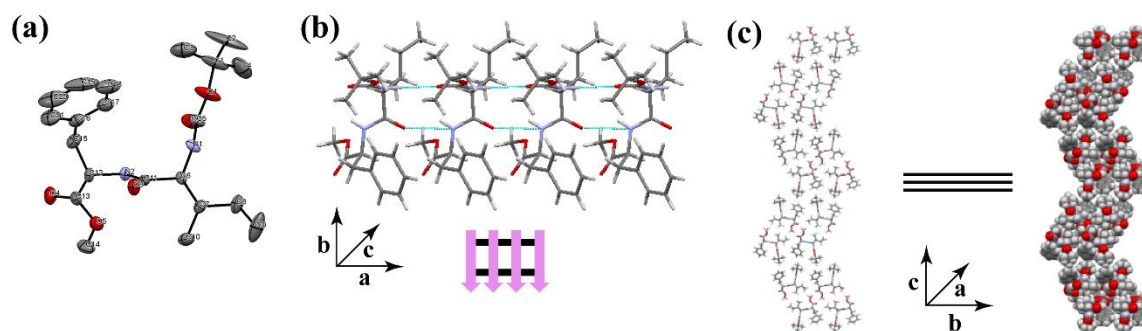


Figure 5.2. (a) ORTEP diagram, (b) parallel β -sheet structure, and (c) single helix-like arrangement of peptide **5A**.

Similarly, peptide **5B** formed parallel β -sheet structure via intermolecular hydrogen bonds (N1-H1...O2 and N2-H2...O3) in *a*-direction (Figure 5.3b). Further, peptide subunits self-organized into a discrete double-stranded helix-like structure along the *b*-axis in higher-order packing (Figure 5.3c). These strands gained stability from C-H...O and C-H... π (C=O) interactions (Figure 5.3c). Notably, substituting L-Phe with D-Phe at the C-terminus resulted in peptide **5B**, exhibiting different backbone orientations and different packing patterns.

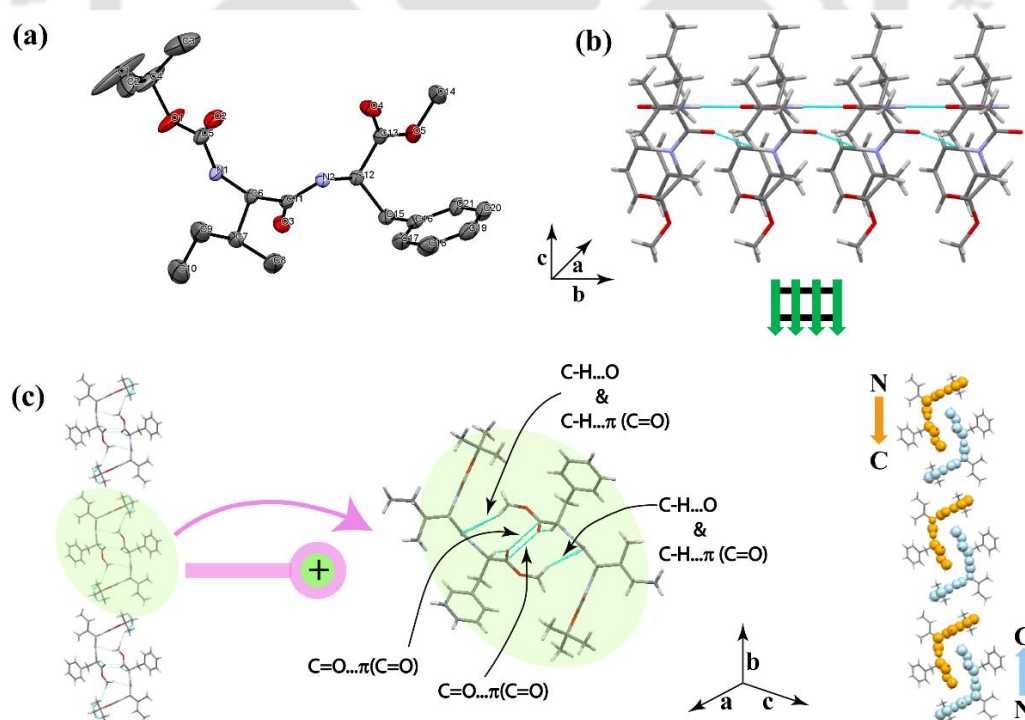


Figure 5.3. (a) ORTEP diagram, (b) parallel β -sheet structure, and (c) discrete double-stranded helix-like arrangement of peptide **5B**.

Conversely, peptide **5C** formed a parallel β -sheet structure (Figure 5.4b) via intermolecular hydrogen bonds (N1-H1...O2 and N2-H2...O3) in the *b*-direction. In higher-order packing, six peptide subunits self-associated, encircling a water molecule via van der Waals interactions among the phenyl rings of C-terminal L-Phg, creating a hexagonal hollow tube-like structure in the *c*-direction (Figure 5.4c). Notably, replacing L-Phe with an unnatural (non-coded) amino acid L-Phg at the C-terminal of peptide **5C** significantly altered its crystal packing arrangement compared to peptide **5A**.

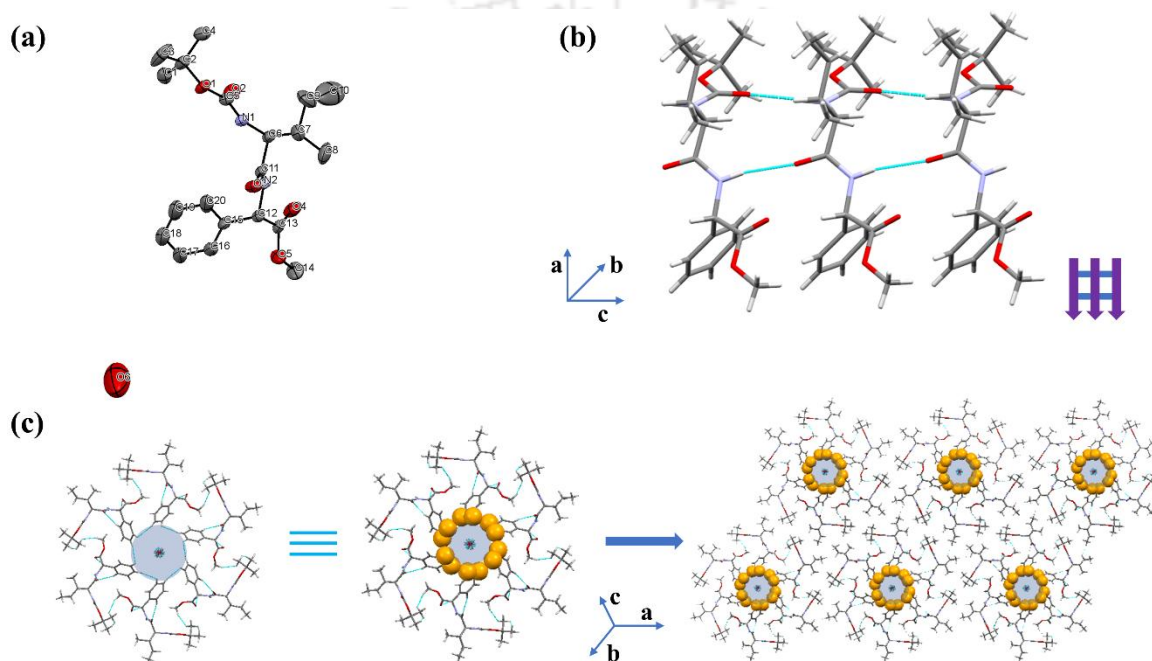


Figure 5.4. (a) ORTEP diagram, (b) parallel β -sheet structure, and (c) hexagonal hollow tube-like architecture of peptide **5C**.

Peptide **5D** formed a parallel β -sheet structure (Figure 5.5b), resembling peptide **5B**. The molecules self-organized into a discrete double-stranded helix-like structure in the *b*-direction (Figure 5.5c). These strands gained stability via C-H... π (C=O) interactions (Figure 5.5c).

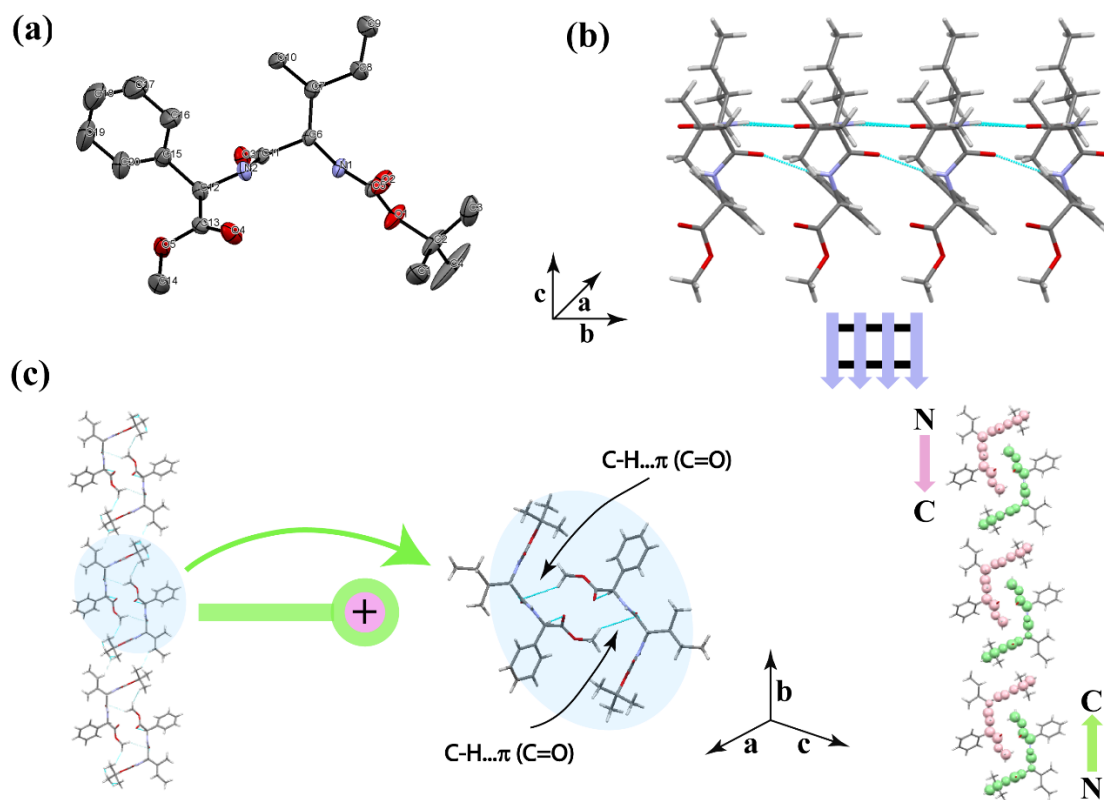


Figure 5.5. (a) ORTEP diagram, (b) parallel β -sheet structure, and (c) discrete double-stranded helix-like arrangement of peptide 5D.

5.5. Structural comparison of peptides

Subsequently, we analyzed and compared the conformations of these peptides' molecular structures. Despite **5C** being an analog of **5A**, differing only by one methylene unit in the side-chain at the C-terminal, their backbone orientation is distinct (Figures 5.6a and 5.6c). The N2-C12-C13 (τ) bond angles in peptides **5A** and **5C** were measured to be $110.7(4)^\circ$ and $108.5(6)^\circ$, respectively. In peptide **5A**, the phenyl ring is linked to the $\text{C}\alpha$ in Phe via a methylene group, resulting in no significant steric repulsion between the $\text{C}\alpha$ (C12) proton and the ortho protons of phenyl ring (Figure 5.6a). Consequently, the sp^3 carbon (C12) remains flexible, leading to a less elongated backbone. In contrast, the phenyl ring in Phg of peptide **5C** is directly linked to the $\text{C}\alpha$ (C12) (Figure 5.6c), causing steric repulsion between the $\text{C}\alpha$ (C12) proton and the phenyl ring's ortho protons, as well as between ortho protons of the phenyl ring and the NH of Phg (N2H). Consequently, the peptide **5C** backbone is more extended compared to that of peptide **5A**. Interestingly, the backbone orientation of peptides **5B** and **5D** appeared similar because of the inclusion of L/D amino

acids in their sequences (Figure 5.6b and 5.6d). This similarity is likely caused by substantial steric hindrance from the two side chains oriented on the same side.

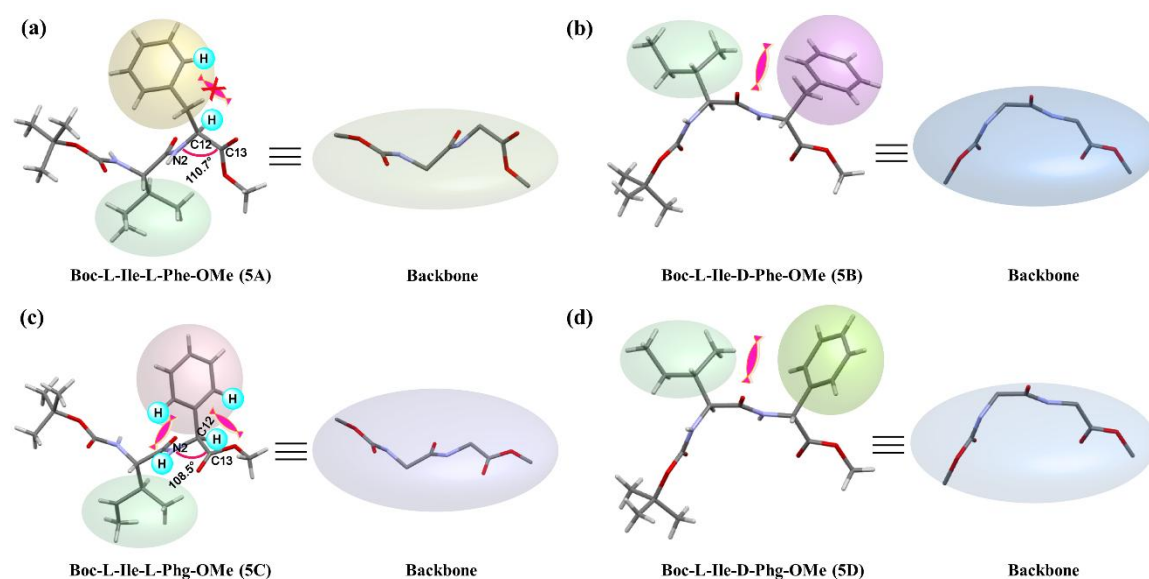


Figure 5.6. Comparative analysis of peptide molecular structures.

5.6. Conformational analysis using CD experiments

To analyze peptide conformations in solution, 1.5 mM peptide samples were incubated for 4 days at 37 °C in acetonitrile-water (1:1) mixture. After incubation, we diluted the solutions to 375 μ M to prevent high voltage issues. Finally, CD experiment was conducted. Peptide **5A** appeared with two positive bands at 217 nm and 195 nm (Figure 5.7, black curve), whereas peptide **5B** displayed negative bands at 218 nm and 196 nm (Figure 5.7, red curve). This is likely caused by the incorporation of reverse chiral (D) amino acid. Likewise, peptide **5C** exhibited positive bands at 218 nm and 198 nm (Figure 5.7, blue curve), while peptide **5D** displayed negative bands at 219 nm and 200 nm (Figure 5.7, magenta curve). Detailed analysis of the CD spectrum revealed that both peptides **5A** and **5C** adopted a combination of β -sheet and β -turn like structures in the solution. While they exhibited β -sheet in their crystalline state, the conformation might somewhat alter in solution.

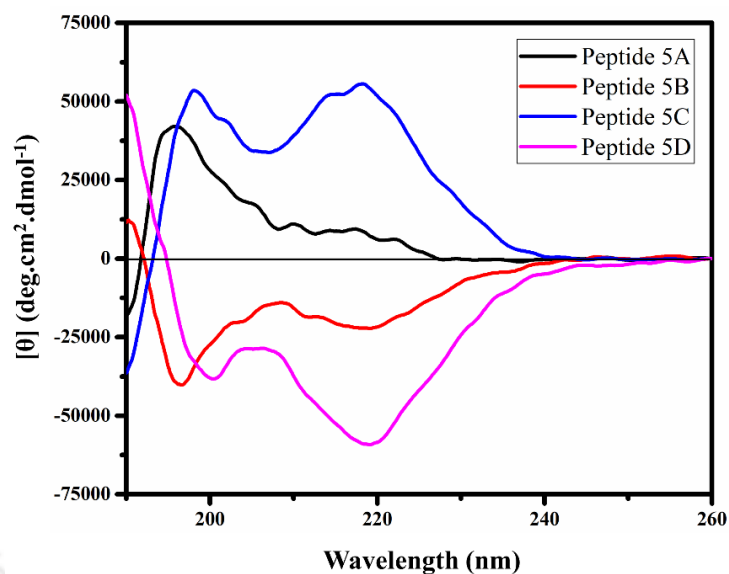


Figure 5.7. CD spectrum of 5A (black curve), 5B (red curve), 5C (blue curve), and 5D (magenta curve).

5.7. Morphology study

To investigate the morphology of self-assembling dipeptides in solution, we conducted the FESEM experiment. 1.5 mM each peptide sample was incubated for 4 days at 37 °C in acetonitrile-water (1:1) mixture. Subsequently, 4-day-old peptide samples were gently placed on aluminum foil-coated small glass pieces and dried in a desiccator. FESEM images of the dried samples were then captured.

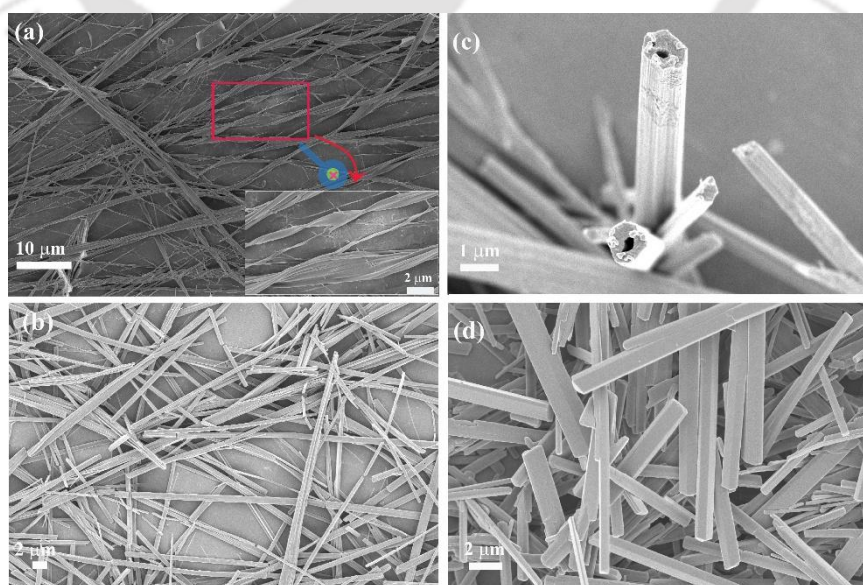


Figure 5.8. FESEM images peptides (a) 5A, (b) 5B, (c) 5C, and (d) 5D.

Interestingly, the images of peptides **5A** and **5C** showed distinct differences, while those of peptides **5B** and **5D** appeared nearly identical. Peptide **5A** showed a helical ribbon-like morphology, extending several micrometers in length (Figure 5.8a). In contrast, peptide **5C** exhibited a nanotube-like morphology with an inner diameter of approximately 261.9 nm and around 986.2 nm of outer diameter (Figure 5.8c). Conversely, peptides **5B** and **5D** were self-assembled into nanorod-like structures in the solution (Figure 5.8b, d).

5.8. Conclusion

In summary, we successfully investigated the supramolecular self-assembly and morphology of N-(Boc) and C-(OMe) protected L/L and L/D amino acids bearing dipeptides, Boc-L-Ile-L-Phe-OMe (**5A**), Boc-L-Ile-D-Phe-OMe (**5B**), Boc-L-Ile-L-Phg-OMe (**5C**), and Boc-L-Ile-D-Phg-OMe (**5D**). In crystalline state, all the peptides adopted parallel β -sheet structure via intermolecular hydrogen bonding. Interestingly, C-terminal L-Phe containing peptide **5A** self-assembled into a single helix-like structure, while C-terminal L-Phg containing peptide **5C** formed a hexagonal hollow tube-like structure encircling one water molecule in the crystalline state. On the other hand, L/D amino acids containing peptides **5B** and **5D** self-assembled into identical discontinuous double helix-like structures in their crystalline state. We also performed the FESEM experiment to investigate the morphology of the peptides in solution (50% ACN-H₂O). Peptide **5A** self-associated into a helical ribbon-like structure in solution, while peptide **5C** formed a nanotube-like structure. At the same time, peptides **5B** and **5D** are both self-associated into nanorod-like structures. There was no problem with the formation of the peptide's nanostructures due to their self-assembling nature. The nanostructures formed homogeneously without any issues. The self-assembly structures from SC-XRD and morphologies of peptides **5A** and **5C** differ because of their different backbone orientations, which are influenced by the backbone side-chains and their interactions (C-terminal Phe in peptide **5A** and Phg in peptide **5C**). This kind of comparative analysis could be valuable for designing peptidomimetics and developing novel materials.

5.9. Experimental section

5.9.1. Materials and instrumentations

As described in chapter 8

5.9.2. General procedure for the synthesis of dipeptides

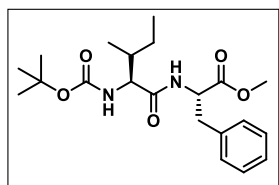
o-NoxylOXY (1 equiv) was mixed with Boc-protected amino acid (1 equiv) and DIPEA (1 equiv) in DCM. After pre-activating for a few minutes, a neutralized solution of C-protected (OMe) amino acid (1.2 equiv) was introduced. The depletion of the reactant was tracked using TLC. Post-reaction, some DCM was poured. The organic part was washed with 10% citric acid and saturated NaHCO₃ solution. The resulting organic part was dried using anhydrous Na₂SO₄, filtered, and concentrated using a rotavapor to obtain the crude. The crude peptides were purified using column chromatography. The characterization of all synthesized peptides was done by using NMR (¹H, ¹³C) spectroscopy and mass spectrometry.

5.9.3. Sample preparation

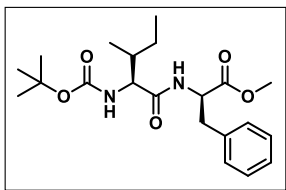
Peptide samples (1.5 mM, 1 mL each) were prepared in acetonitrile-water (1:1) mixture and incubated for 4 days at 37 °C. After aging for four days, these samples were subjected to CD and FESEM experiments.

5.10. Characterization data

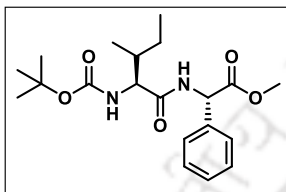
Boc-L-Ile-L-Phe-OMe (5A):



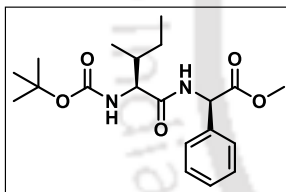
White solid, (452 mg, 89%), ¹H NMR (600 MHz, CDCl₃) δ 7.30-7.23 (m, 3H), 7.12-7.10 (d, *J* = 7.2 Hz, 2H), 6.35 (s, 1H), 5.02 (s, 1H), 4.89-4.87 (m, 1H), 3.95-3.92 (m, 1H), 3.71 (s, 3H), 3.14-3.09 (m, 2H), 1.83-1.75 (m, 2H), 1.44 (s, 9H), 1.09-1.06 (m, 1H), 0.89-0.88 (m, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 171.8, 171.4, 155.8, 135.8, 129.4, 128.8, 127.3, 80.0, 59.4, 53.2, 52.5, 38.1, 37.4, 28.5, 24.8, 15.6, 11.6. ESI-MS: calculated [M+H]⁺ 393.2391, found *m/z* 393.2580.

Boc-L-Ile-D-Phe-OMe (5B):

White solid, (444 mg, 87%), ^1H NMR (600 MHz, CDCl_3) δ 7.29-7.22 (m, 3H), 7.11-7.10 (d, $J = 7.2$ Hz, 2H), 6.44 (s, 1H), 4.97 (s, 1H), 4.91-4.87 (m, 1H), 4.02-4.00 (m, 1H), 3.71 (s, 3H), 3.15-3.06 (m, 2H), 1.86-1.71 (m, 2H), 1.43 (s, 9H), 1.01-0.99 (m, 1H), 0.86-0.82 (m, 6H); ^{13}C NMR (CDCl_3 , 151 MHz) δ 171.0, 171.4, 155.9, 135.9, 129.3, 128.8, 127.3, 80.1, 59.3, 53.1, 52.5, 38.1, 37.3, 28.4, 24.4, 15.6, 11.6; ESI-MS: calculated $[\text{M}+\text{H}]^+$ 393.2391, found m/z 393.2578.

Boc-L-Ile-L-Phg-OMe (5C):

White solid, (578 mg, 88%), ^1H NMR (600 MHz, CDCl_3) δ 7.35-7.33 (m, 5H); 6.96 (s, 1H); 5.54-5.53 (m, 1H); 5.04 (s, 1H); 4.01-3.99 (m, 1H); 3.72 (s, 3H); 1.78-1.73 (m, 2H); 1.42 (s, 9H); 1.17-1.12 (m, 1H); 0.91-0.88 (t, $J = 7.2$ Hz, $J = 6.6$ Hz, 3H); 0.95-0.94 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.2, 171.1, 155.9, 136.4, 129.1, 128.7, 127.4, 80.1, 59.3, 56.7, 52.9, 37.4, 28.4, 24.9, 15.7, 11.5; ESI-MS: calculated $[\text{M}+\text{H}]^+$ 379.2235, found m/z 379.2412.

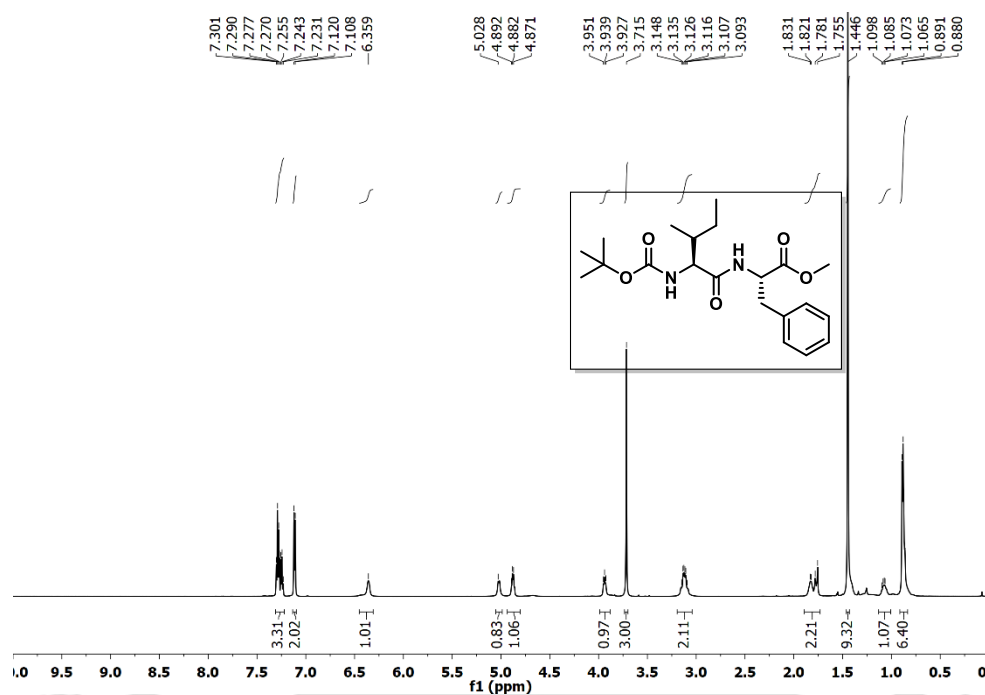
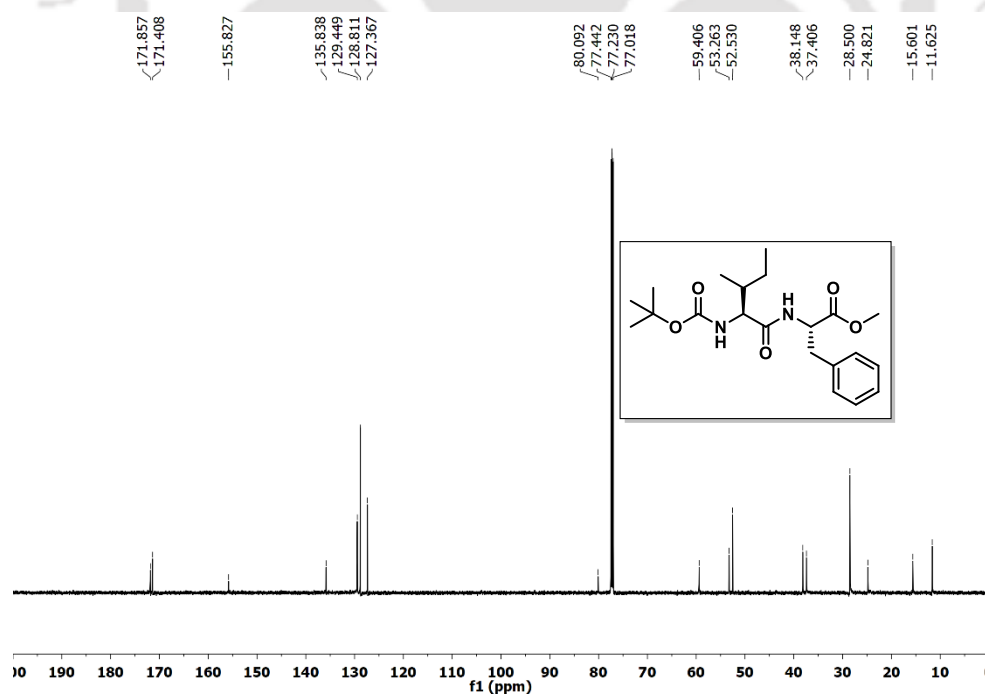
Boc-L-Ile-D-Phg-OMe (5D):

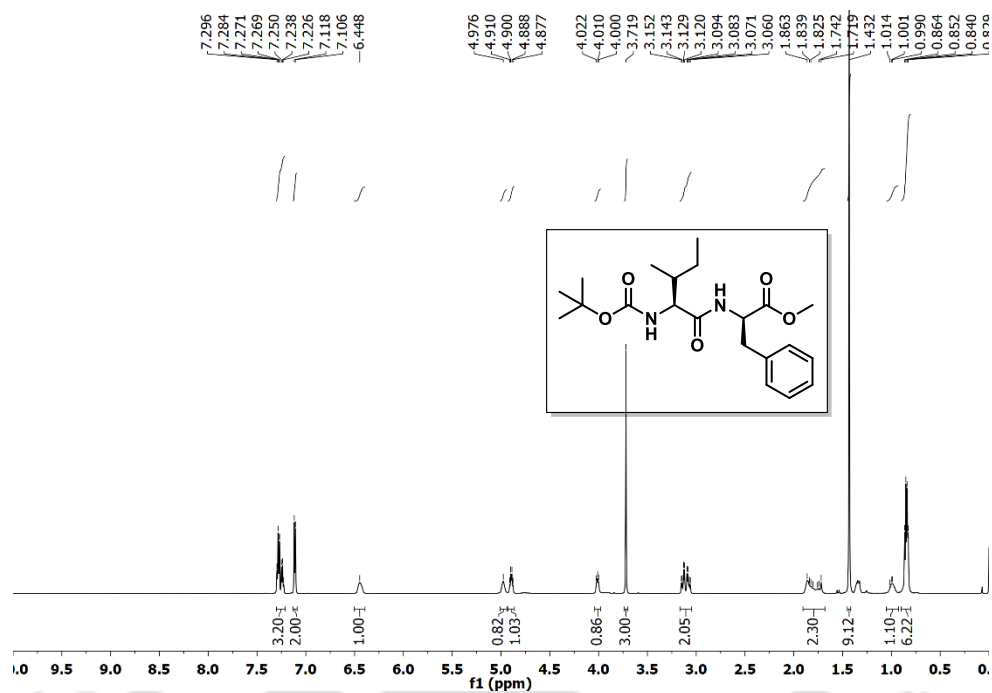
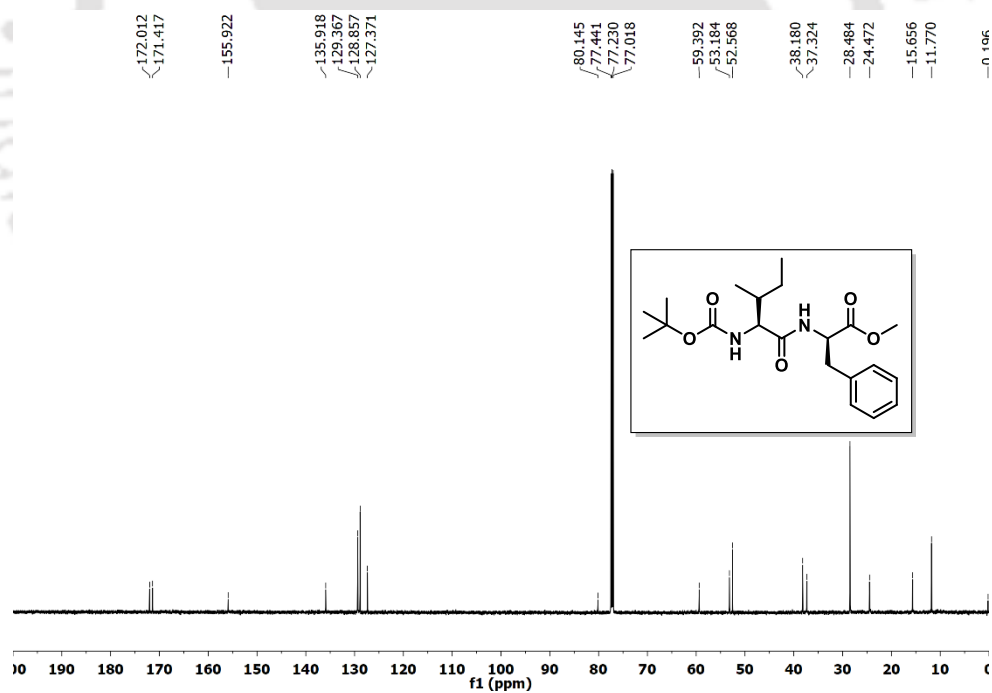
White solid, (570 mg, 87%), ^1H NMR 600 MHz, CDCl_3) δ 7.36-7.32 (m, 5H); 7.04 (s, 1H); 5.55-5.54 (m, 1H); 5.04 (s, 1H); 4.05 (s, 1H); 3.72 (s, 3H); 1.88-1.84 (m, 2H); 1.43 (s, 9H); 1.08-1.07 (m, 1H); 0.87 (brs, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.3, 171.2, 156.0, 136.4, 129.1, 128.7, 127.4, 80.2, 59.2, 56.6, 53.0, 37.4, 28.4, 24.7, 15.7, 11.6; ESI-MS: calculated $[\text{M}+\text{H}]^+$ 379.2235, found m/z 379.2411.

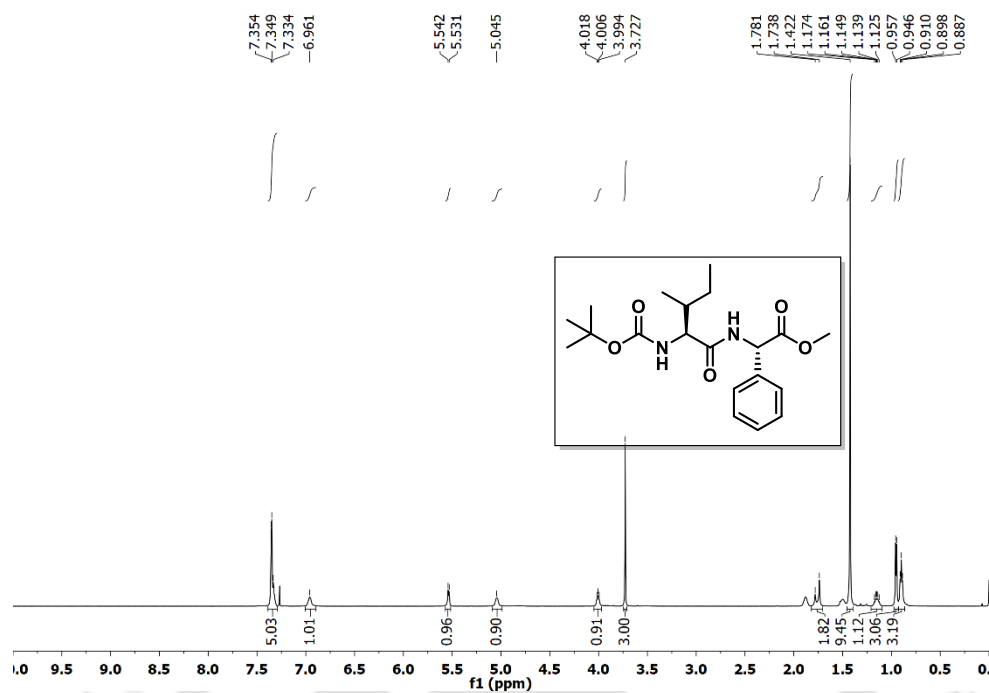
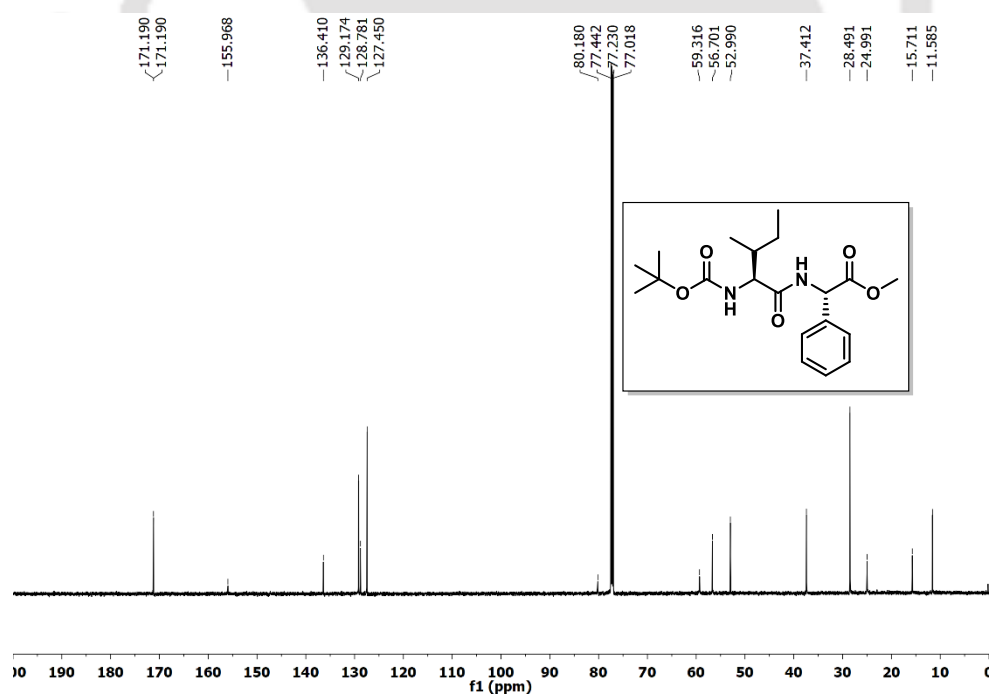
5.11. References

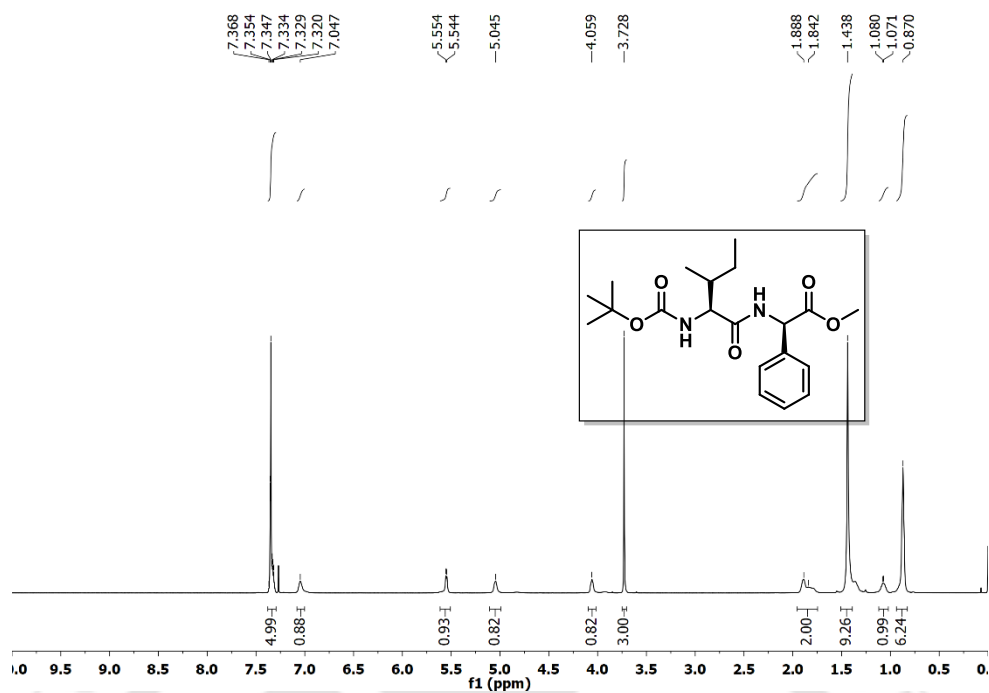
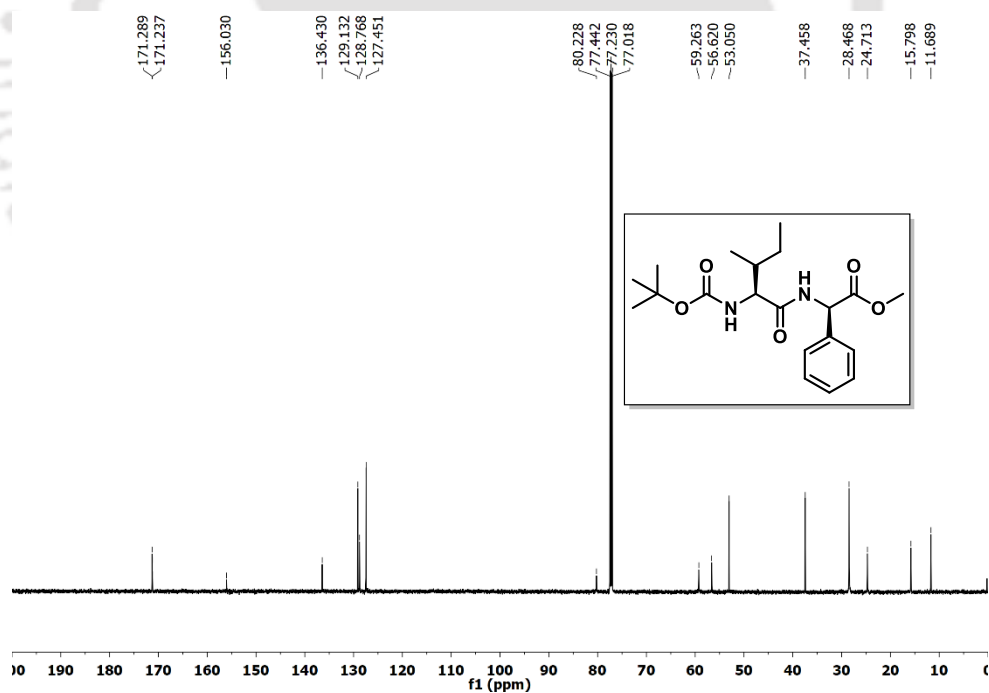
1. Chen, J.; Zou, X. Self-assemble peptide biomaterials and their biomedical applications. *Bioact. Mater.* **2019**, *4*, 120-131.
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4. Jiang, Q.; Liu, X.; Liang, G.; Sun, X. Self-assembly of peptide nanofibers for imaging applications. *Nanoscale* **2021**, *13*, 15142-15150.
5. Giri, R. S.; Pal, S.; Roy, S.; Dolai, G.; Manne, S. R.; Paul, S.; Mandal, B. Nanostructures from protected L/L and D/L amino acid containing dipeptides. *Pept. Sci.* **2021**, *113*, e24176.
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9. Acuña, S. M.; Veloso, M. C.; Toledo, P. G. Self-Assembly of Diphenylalanine-Based Nanostructures in Water and Electrolyte Solutions. *J. Nanomater.* **2018**, *2018*, 8140954.
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5.12. Selected spectra

Figure 5.9. ¹H NMR spectrum of peptide 5AFigure 5.10. ¹³C NMR spectrum of peptide 5A

Figure 5.11. ¹H NMR spectrum of peptide 5BFigure 5.12. ¹³C NMR spectrum of peptide 5B

Figure 5.13. ¹H NMR spectrum of peptide 5CFigure 5.14. ¹³C NMR spectrum of peptide 5C

Figure 5.15. ¹H NMR spectrum of peptide 5DFigure 5.16. ¹³C NMR spectrum of peptide 5D

5.13. Crystallographic data

Table 5.2. Hydrogen bonding distances (Å) and Bond angles (°) of peptide 5A, 5B, 5C and 5D

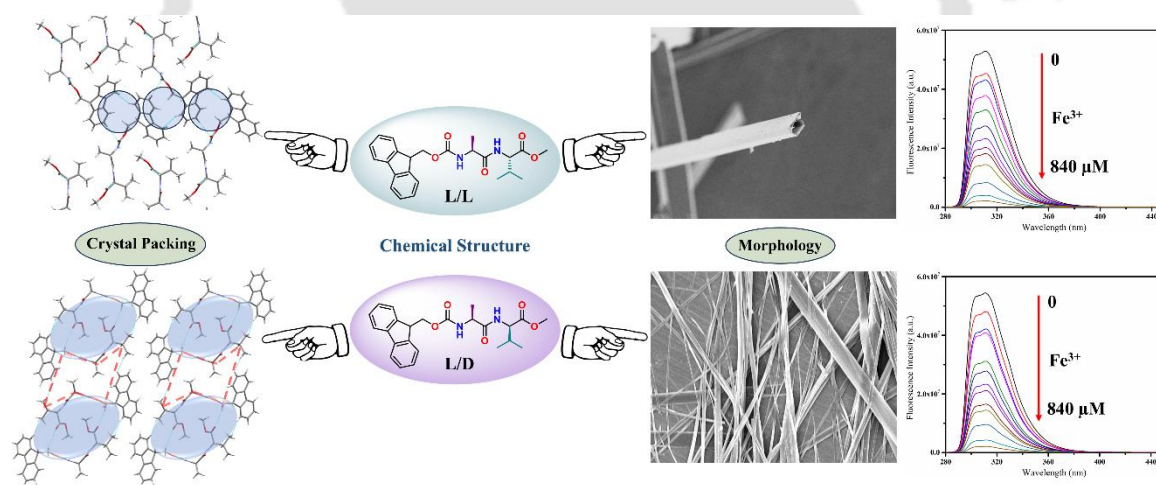
Peptide	D–H...A	$d(D\cdots H)/\text{\AA}$	$d(H\cdots A)/\text{\AA}$	$d(D\cdots A)/\text{\AA}$	$\angle D-H\cdots A/^\circ$
Peptide 5A	N1-H1...O2	0.86	2.15	2.992(5)	166
	N2-H2...O3	0.86	2.19	2.871(5)	135
Peptide 5B	N1-H1...O2	0.86	2.18	3.040(5)	177
	N2-H2N...O3	0.86	2.30	3.147(5)	167
	C6-H6...O3	0.98	2.57	3.480(5)	155
	C12-H12...O4	0.98	2.37	3.287(5)	156
Peptide 5C	N1-H1...O2	0.86	2.18	3.0261(2)	168
	N2-H2N...O3	0.86	2.37	3.1355(2)	148
Peptide 5D	N1-H1...O2	0.86	2.19	3.041(7)	171
	N2-H2N...O3	0.86	2.24	3.082(6)	165
	C12-H12...O4	0.98	2.46	3.440(8)	175

Table 5.3. Crystal parameters and refinement data of peptides 5A, 5B, 5C and 5D

Parameters	Peptide 5A	Peptide 5B	Peptide 5C	Peptide 5D
Formula	C ₂₁ H ₃₂ N ₂ O ₅	C ₂₁ H ₃₂ N ₂ O ₅	3(C ₂₀ H ₂₉ N ₂ O ₅), H ₂ O	C ₂₀ H ₃₀ N ₂ O ₅
Fw	392.49	392.49	1150.37	378.46
Crystal system	orthorhombic	monoclinic	hexagonal	monoclinic
Space group	<i>P</i> 21 21 21	<i>P</i> 21	<i>P</i> 63	<i>P</i> 21
a/Å	5.1231(3)	14.3440(12)	27.5075(16)	14.4958(18)
b/Å	11.1061(14)	5.0356(3)	27.5075(16)	5.0228(5)
c/Å	40.227(3)	17.1233(17)	5.1256(3)	16.5927(18)
α/°	90.00	90.00	90.00	90.00
β/°	90.00	113.079(10)	90.00	114.858(14)
γ/°	90.00	90.00	120.00	90.00
V/Å ³	2288.8(4)	1137.84(18)	3358.8(5)	1096.2(2)
Z	4	2	2	2
D _c /g cm ⁻³	1.139	1.146	1.138	1.147
μ Mo Kα/mm ⁻¹	0.081	0.081	0.082	0.082
F000	848	424	1238.0	408
T/K	293(2)	293(2)	293(2)	293(2)
θ max.	28.769	28.620	25.000	28.853
Total no. of reflections	6475	5081	6521	4770
Independent reflections	4593	4159	2784	3691
Observed reflections	2028	3034	1853	2190
Parameters refined	259	259	256	250
R ₁ , I > 2σ(I)	0.0665	0.0629	0.0696	0.0729
wR ₂ , I > 2σ(I)	0.1196	0.1439	0.2095	0.1580
GOF (F ²)	0.935	1.066	0.849	1.057
CCDC No.	1973966	1973967	1973968	1973969

Chapter 6

Chirality Controlled Self-Assembly of Dipeptides and Selective Sensing of Fe^{3+}



6.1. Background

In chapter 5, we discussed the influence of side chains and the chirality of amino acids on the self-assembly of Boc-dipeptides. While the self-assembly of small Fmoc-peptides containing L/L amino acids has been extensively explored, the self-assembly of small Fmoc-peptides with L/D amino acids is not well-explored. Additionally, peptides containing tyrosine, histidine, and tryptophan have been well-studied for metal ion sensing,^{1, 2} but peptides with hydrophobic amino acids have not been well-studied. Peptide-based metal ion sensors offer advantages such as low toxicity, high selectivity, and biocompatibility, rendering them valuable for environmental detection and biomedical analysis. Therefore, in this chapter, we aimed to investigate the impact of chirality on the self-assembly of Fmoc-dipeptides and peptide-based metal ion sensing.

6.2. Design of peptides

We obtained a Fmoc-dipeptide (Fmoc-L-Ala-L-Val-OMe) crystal, synthesized in chapter 2. To examine the impact of chirality, we synthesized another Fmoc dipeptide (Fmoc-L-Ala-D-Val-OMe), maintaining Fmoc-Ala-OH at N-terminal and altering the C-terminal amino acid from L-Val to D-Val (Figure 6.1). We then crystallized this peptide to investigate its self-assembly behavior in the crystalline state.

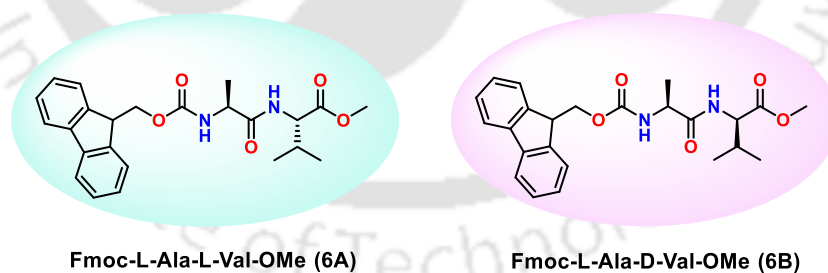


Figure 6.1. Chemical structure of dipeptides.

6.3. Synthesis of peptides

To conduct various studies related to self-assembly, we synthesized both dipeptides in adequate amounts using the conventional coupling reagent-based peptide synthesis method as described in chapter 5, section 5.9.2. The synthesized dipeptides were purified using column chromatography and characterized by NMR and mass spectrometry.

6.4. Investigation of the self-assembly of dipeptides using SC-XRD

The molecular conformation and arrangement of these peptides were investigated using SC-XRD experiments. Needle-shaped, colorless crystals of both peptides were obtained through the gradual evaporation of an acetonitrile-water solvent. Both peptides formed crystals in monoclinic ($P2_1$) crystal system, with a single molecule in the asymmetric unit. Peptide **6A** subunits were interconnected through intermolecular hydrogen bonding ($\text{N1-H1}\dots\text{O2} = 2.96 \text{ \AA}$ and $\text{N2-H2}\dots\text{O3} = 3.06 \text{ \AA}$), forming a parallel β -sheet structure. Between two subunits had a meridional distance of $4.90 \text{ \AA}$ (Figure 6.2b). In higher-order packing, a connection between two subunits occurred via the interaction between the urethane carbonyl oxygen of one subunit and the Fmoc-aromatic C-H group of the other subunit ($\text{C-H}\dots\text{O} = 2.68 \text{ \AA}$) in an antiparallel fashion. Subsequently, the Fmoc groups of the two subunits were also connected through an additional $\text{C-H}\dots\pi$ interaction ($2.78 \text{ \AA}$), resulting in a porous channel-like arrangement (Figure 6.2c).

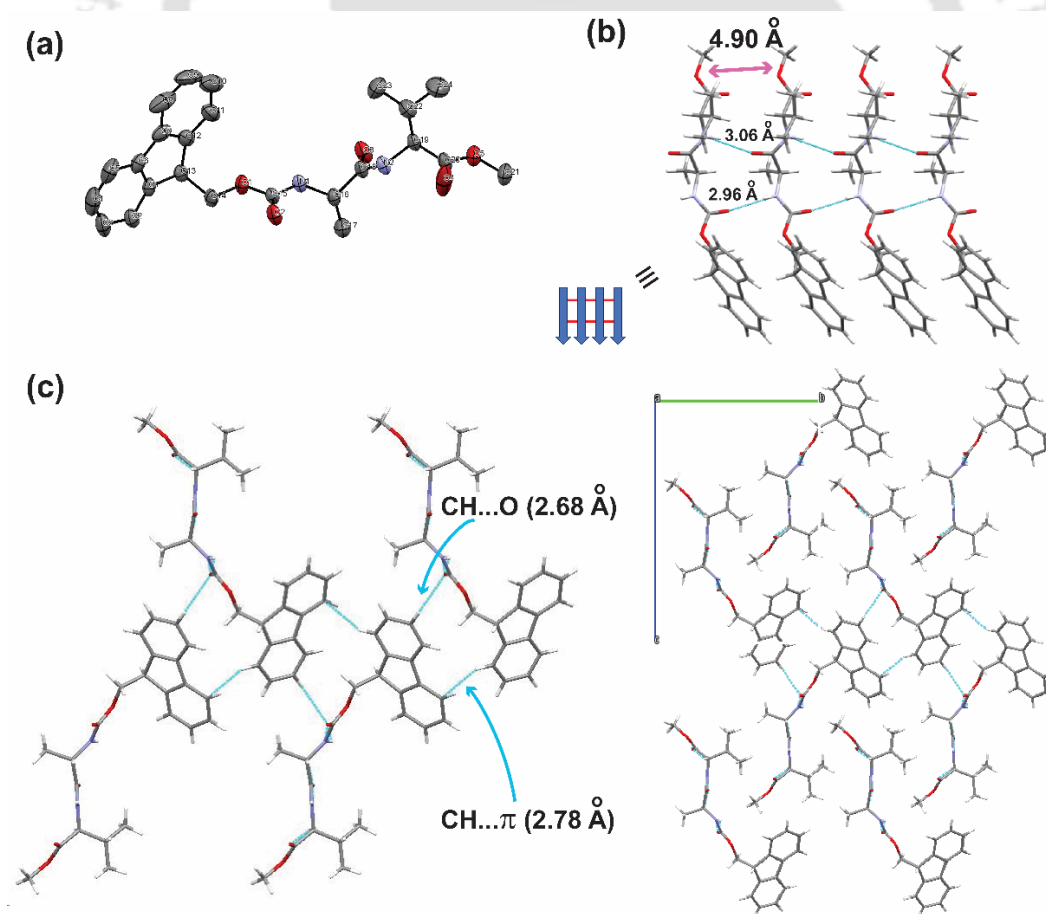


Figure 6.2. (a) ORTEP diagram, (b) β -sheet structure, and (c) higher-order packing structure of peptide **6A**.

Likewise, peptide **6B**, which contains D-Val, formed a parallel β -sheet structure facilitated by intermolecular hydrogen bonds ($\text{N1-H1}\dots\text{O2} = 2.86 \text{ \AA}$ and $\text{N2-H2}\dots\text{O3} = 2.90 \text{ \AA}$). The two subunits were separated by a meridional distance of $4.91 \text{ \AA}$ (Figure 6.3b). One subunit was linked with another in an antiparallel arrangement, forming a dimer via noncovalent interactions. The carbonyl oxygen of the methyl ester in one subunit was interlinked with the methylene H of the Fmoc group in another subunit ($\text{CH}\dots\text{O} = 2.55$ and $5.01 \text{ \AA}$), forming a dimer-like structure. Furthermore, an additional interaction was noted between the H of the Fmoc ring and the methyl H of the D-Val side chain ($\text{CH}\dots\text{H} = 2.35$ and $4.38 \text{ \AA}$, Figure 6.3c). Interestingly, a weak C-H...H interaction was detected between the C-H group of the D-Val side chain and the H of the Fmoc aromatic ring ($\text{CH}\dots\text{H} = 4.33$ and $2.30 \text{ \AA}$), which held the dimer units together (Figure 6.3c). Tables 6.1 and 6.3 provide detailed information on the backbone torsion angles and the crystallographic data, respectively.

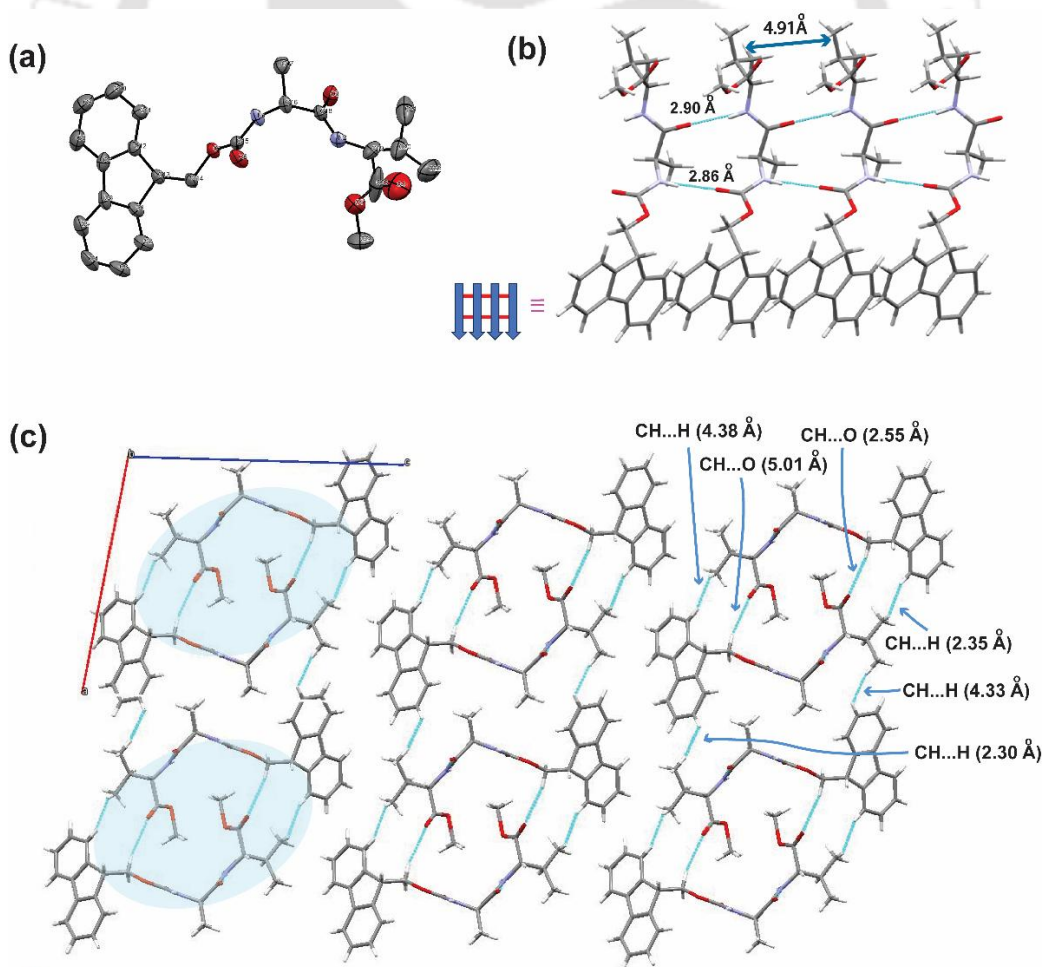


Figure 6.3. (a) ORTEP diagram, (b) β -sheet structure, and (c) higher-order packing structure of peptide **6B**.

Table 6.1. List of backbone Torsion angle (deg) of peptide 6A and peptide 6B

Peptides	$\phi 1$	$\psi 1$	$\phi 2$	$\psi 2$	$\omega 1$	$\omega 2$
Peptide 6A	C15-N1- C16-C18 = - 147.5(6)	N1-C16- C18-N2 = 129.2(6)	C18-N2- C19-C20 = - 132.5(7)	N2-C19- C20-O5 = 140.8(6)	O1-C15-N1- C16 = - 171.9(5)	C16-C18- N2-C19 = 174.2(6)
Peptide 6B	C15-N1- C16-C18= 113.3(6)	N1-C16- C18-N2= - 104.2(7)	C18-N2- C19-C23= - 105(1)	N2-C19- C23-O5= - 36(2)	O1-C15-N1- C16= - 179.3(5)	C16-C18- N2-C19= - 178.3(7)

6.5. Morphology study

To study the morphologies of self-assembling dipeptides, peptide samples aged for four days (1.5 mM in acetonitrile-water (1:1), at 37 °C) were drop-casted onto clean glass slides for optical microscopy, small aluminum-coated glass pieces for FESEM, and TEM grids (100 μ M prepared from 1.5 mM samples) and dried in a desiccator. We initially used optical microscopy to capture images of the dried peptides. Peptide **6A** exhibited a dense rod-like structure, while peptide **6B** formed fibrillar structure (Figure 6.4, 1a and 2a). FESEM analysis confirmed these findings, with peptide **6A** displaying hollow tube-like morphology and peptide **6B** showing fibrillar morphology (Figure 6.4, 1b and 2b). The FETEM experiment further validated these structures. The hollow tube structure of peptide **6A** was confirmed by a faint white portion at the edge of the tubes. The tube diameters were determined to be approximately 1.13 μ m to 1.41 μ m, with internal diameters of about 0.93 μ m to 1.09 μ m. For peptide **6B**, the fibrillar diameters ranged from approximately 40 nm to 140 nm (Figure 6.4, 1c and 2c).

To obtain topographical details, we conducted the AFM experiment. For AFM, 30 μ M peptide solutions (freshly prepared from 1.5 mM four-day aged samples) were drop cast onto small clean glass pieces, dried in a desiccator, and then performed the AFM experiment. Peptide **6A** exhibited tube-like structure having a diameter of $\sim$ 400 nm and 204 nm of height, similar to image observed in FESEM. Peptide **6B** showed fibrillar morphology having a diameter of $\sim$ 588 nm and lengths in the micrometer range. The morphological variation may be due to chiral differences, leading to different backbone orientations. 2D and 3D images and height-to-distance plots are presented in Figure 6.5.

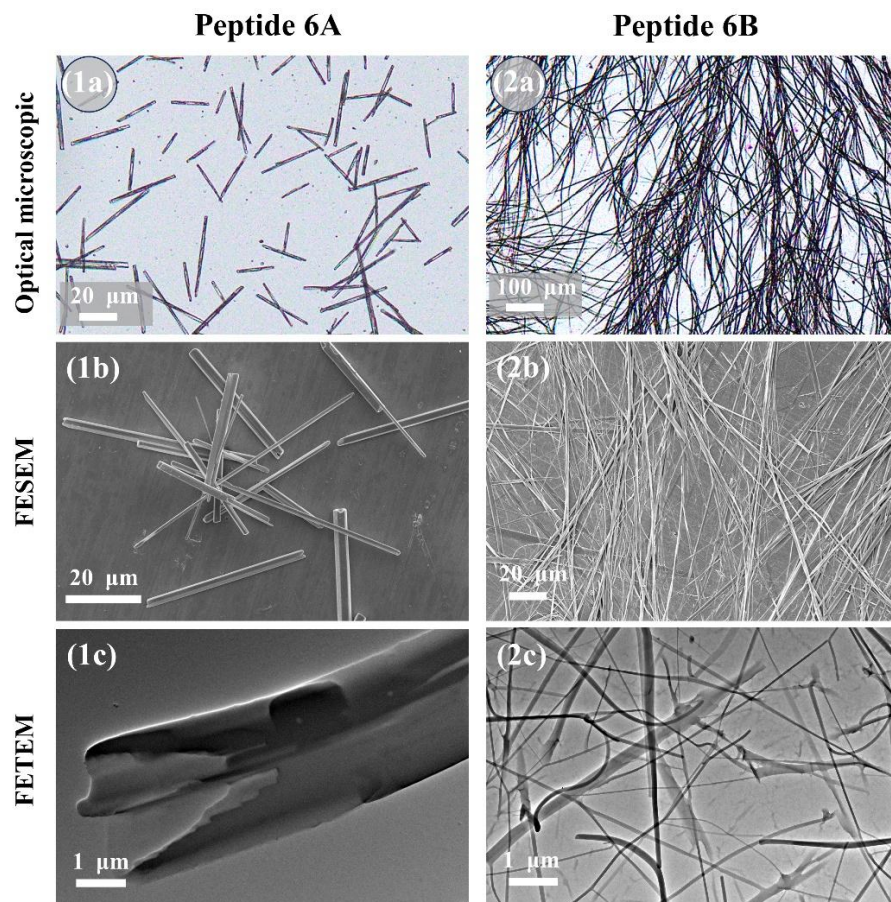


Figure 6.4. Optical microscopic (1a, 2a), FESEM (1b, 2b), and FETEM (1c, 2c) images of peptide **6A** and peptide **6B**, respectively.

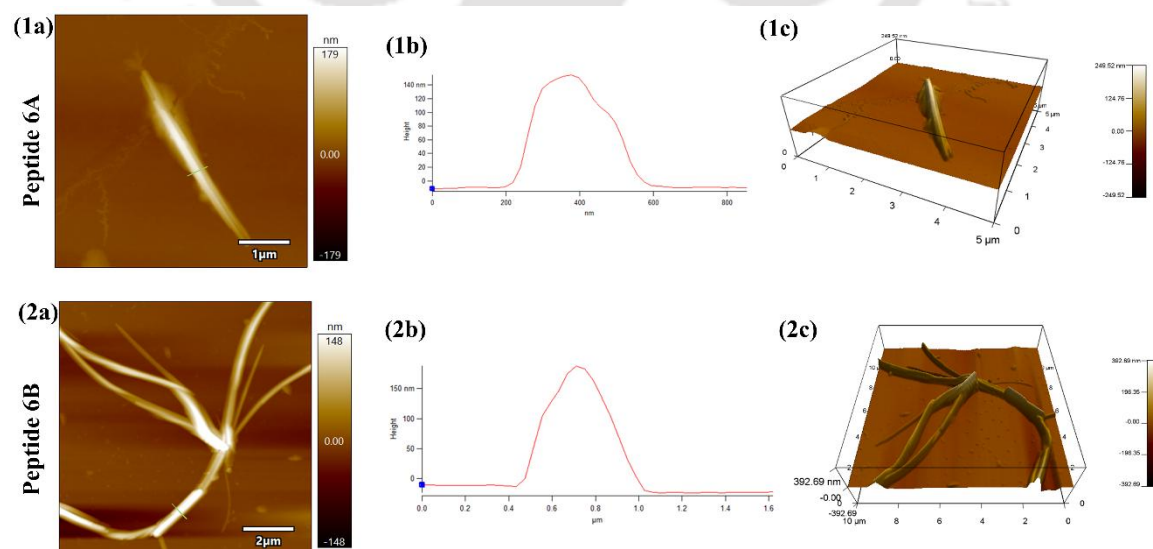


Figure 6.5. 2D AFM images (1a, 2a), height profile plots (1b, 2b), and 3D AFM images (1c, 2c) of peptides **6A** and **6B**, respectively.

6.6. Investigation on solvent-dependent self-assembly

After investigating the morphologies of both peptides in a mixed solvent, we examined their morphologies in pure acetonitrile, ethanol, methanol, and isopropanol. In pure acetonitrile, peptide **6A** exhibited a rod-like structure (Figure 6.6, 1a), while peptide **6B** displayed a wire-like structure (Figure 6.6, 2a). In the case of ethanol, peptide **6A** generated a rod-like structure (Figure 6.6, 1b), and peptide **6B** formed fibrous structure (Figure 6.6, 2b). Methanol led to hollow tubes for peptide **6A** (Figure 6.6, 1c) and fibrous structure for peptide **6B** (Figure 6.6, 2c). In isopropanol, peptide **6A** formed solid rods (Figure 6.6, 1d), and peptide **6B** exhibited fibrillar structure (Figure 6.6, 2d). These results indicate that peptide **6A** tends to form tube or rod-like structures, while peptide **6B** forms fibrillar, fibrous, or wire-like morphologies.

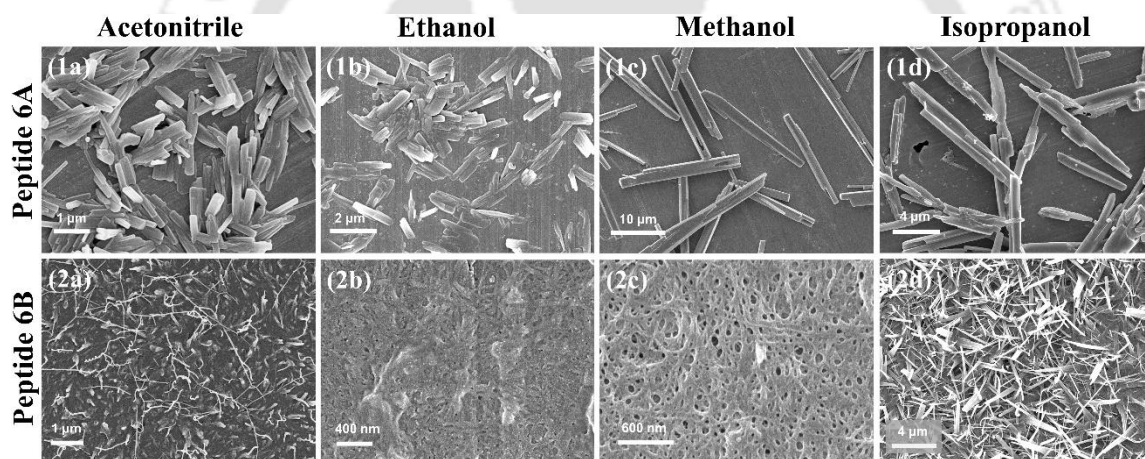


Figure 6.6. FESEM image of peptide **6A** and peptide **6B** in acetonitrile (1a, 2a), ethanol (1b, 2b), methanol (1c, 2c), and isopropanol (1d, 2d), respectively.

6.7. Secondary structure analysis using FT-IR

To analyze the secondary structure of peptides **6A** and **6B**, we used solid-state FT-IR. The amide I ($1600\text{--}1700\text{ cm}^{-1}$) and N-H ($3200\text{--}3500\text{ cm}^{-1}$) regions help determine the secondary structures of peptides.³ Peptide **6A** showed stretching frequencies at 3304 cm^{-1} and peptide **6B** at 3291 cm^{-1} , suggesting strongly hydrogen-bonded N-H groups. Urethane carbonyl stretching for both peptides (**6A** and **6B**) was noted at 1686 cm^{-1} and 1692 cm^{-1} , respectively, and ester carbonyl frequencies were noted at 1733 cm^{-1} and 1730 cm^{-1} ,

respectively. Both peptides (**6A** and **6B**) exhibited amide I bands at 1650 cm^{-1} and 1655 cm^{-1} , and amide II bands at 1520 cm^{-1} and 1538 cm^{-1} , respectively, suggesting β -sheet structure formation via intermolecular hydrogen bonding in the solid state (Figure 6.7a, c). We also conducted FT-IR analysis of freeze-dried, four-day aged peptide samples (1.5 mM) incubated in acetonitrile-water (1:1) at $37\text{ }^{\circ}\text{C}$. The aged peptides **6A** and **6B** showed amide I bands at 1652 cm^{-1} and 1653 cm^{-1} , amide II bands at 1522 cm^{-1} and 1535 cm^{-1} , urethane carbonyl stretching at 1687 cm^{-1} and 1690 cm^{-1} , ester carbonyl stretching at 1734 cm^{-1} and 1730 cm^{-1} , and N–H bands at 3305 cm^{-1} and 3293 cm^{-1} , respectively. These results suggested that peptides **6A** and **6B** self-assembled into β -sheet structures in solution via intermolecular hydrogen bonding, similar to the solid state (Figure 6.7b, d).

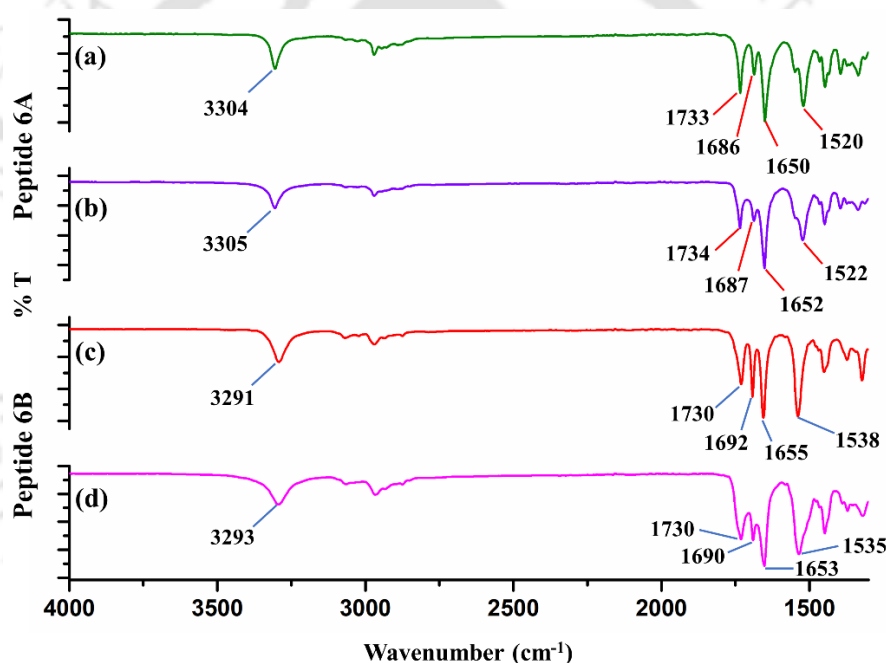


Figure 6.7. FTIR spectra of peptides **6A** and **6B** in the solid state (a, c) and incubated (b, d), respectively.

6.8. Thermal stability investigation using TGA

We conducted the TGA analysis to determine the incubated peptides' thermal stability. Typically, weight loss occurs in two stages: (i) $25\text{--}200\text{ }^{\circ}\text{C}$ attributed to escape of absorbed solvent or water molecules, and (ii) $200\text{--}500\text{ }^{\circ}\text{C}$ due to peptide bond breaking. Peptides **6A** and **6B** showed mass loss around $202\text{ }^{\circ}\text{C}$ (mp $155\text{--}157\text{ }^{\circ}\text{C}$) and $235\text{ }^{\circ}\text{C}$ (mp $170\text{--}172\text{ }^{\circ}\text{C}$), respectively, with no loss between $25\text{--}200\text{ }^{\circ}\text{C}$, indicating no absorbed solvent or water. The mass loss above $200\text{ }^{\circ}\text{C}$ suggested substantial thermal stability (Figure 6.8).

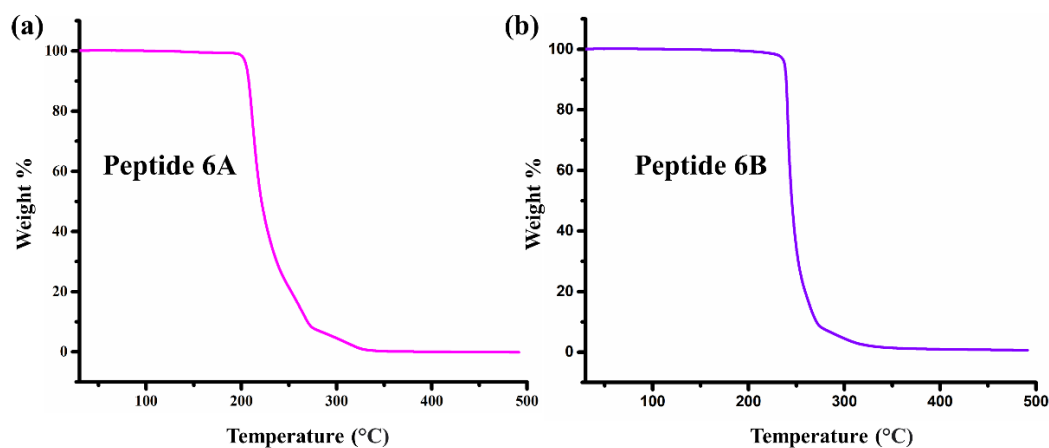


Figure 6.8. (a, b) TGA profile of peptides **6A** and **6B**, respectively.

6.9. Investigation of thermal stabilities of morphologies

We investigated the thermal stability of peptide morphologies using the FESEM experiment. Peptide samples were heated at 100 °C and 150 °C for 1 hour in a convection oven. At 100 °C, peptide **6A** maintained its hollow tube-like structure, and peptide **6B** preserved its fibrillar structure (Figure 6.9, 1a, 2a). At 150 °C, peptide **6A**'s surface was slightly deformed, while peptide **6B**'s morphology remained unchanged (Figure 6.9, 1b, 2b). This stability is likely due to H-bonding interactions in the crystalline state.

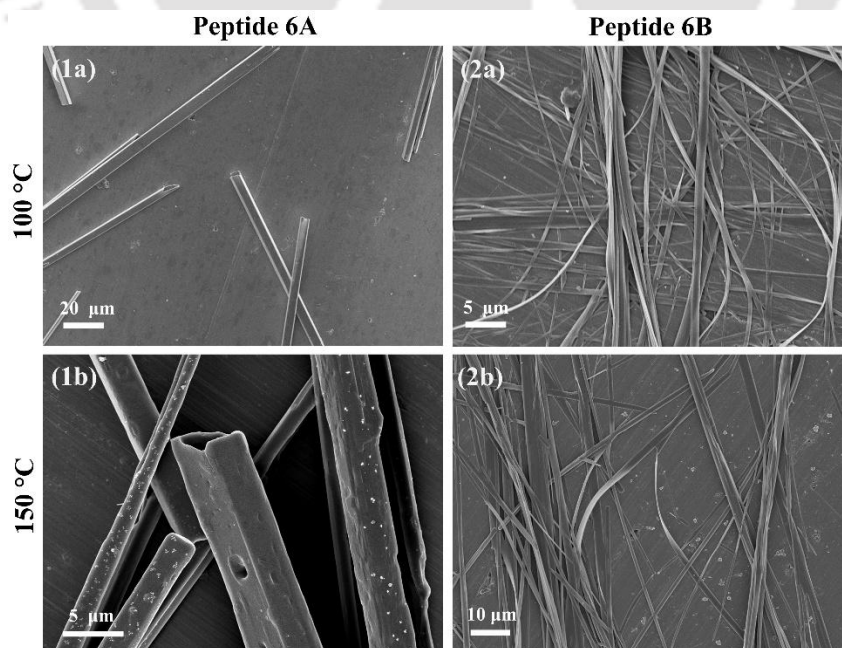


Figure 6.9. FESEM at 100 °C (1a, 2a) and 150 °C (1b, 2b) for peptides **6A** and **6B**, respectively.

6.10. Dye binding study

Bioactive dyes are commonly used to detect cellular structures.⁴ We examined the dye binding capacity of the peptides (**6A**, **6B**) in solution. To assess this, 1.5 mM dipeptide solutions in acetonitrile-water (1:1) were incubated with various dyes (Fluorescein (100 μM), Rhodamine B (10 μM), Thioflavin T (100 μM), and 5(6)-Carboxyfluorescein (100 μM)) for 4 days at 37 °C. The resulting solutions were then filtered and drop-casted (10 μL) onto clean glass slides and dried inside a desiccator. Fluorescence images showed distinct, intense green and red fluorescence from the tube (peptide **6A**) and fibrillar (peptide **6B**) morphologies, indicating high dye binding capacity (Figure 6.10).

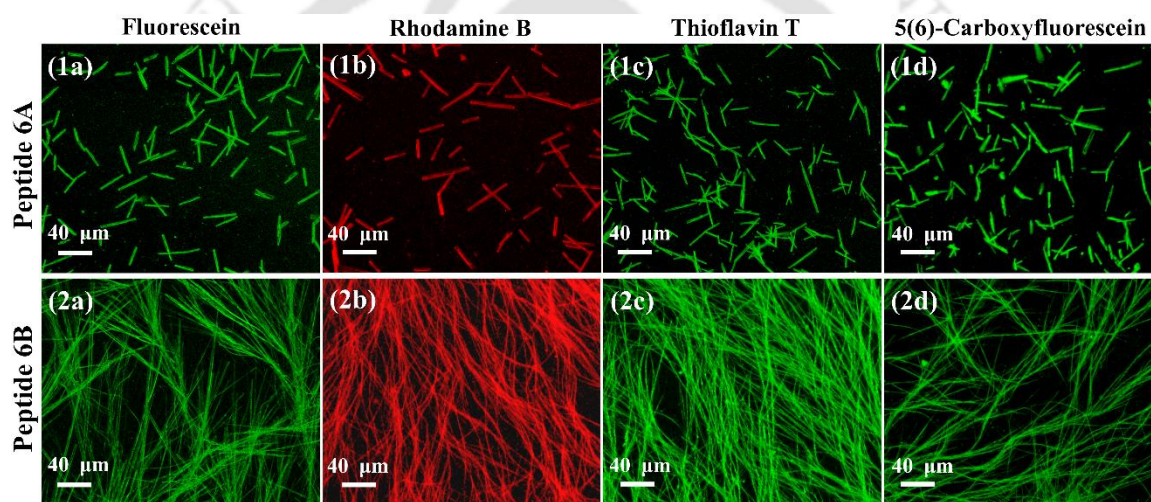


Figure 6.10. Fluorescence microscopic images of Fluorescein (1a, 2a), Rhodamine B (1b, 2b), Thioflavin T (1c, 2c), and 5(6)-Carboxyfluorescein (1d, 2d) stained peptide **6A** and peptide **6B**, respectively.

6.11. Investigation of self-assembly by PXRD

We performed the PXRD analysis of incubated freeze-dried peptide samples. 1.5 mM peptides were incubated in acetonitrile-water (1:1) at 37 °C for four days, then freeze-dried. The PXRD patterns of the freeze-dried peptides (2θ range 0 to 50°) were compared with those obtained from the CIF files of SC-XRD. The patterns from freeze-dried samples (peptides **6A** and **6B**) closely matched the CIF-derived patterns, indicating both peptides adopt a β -sheet structure in solution and crystalline states (Figure 6.11).

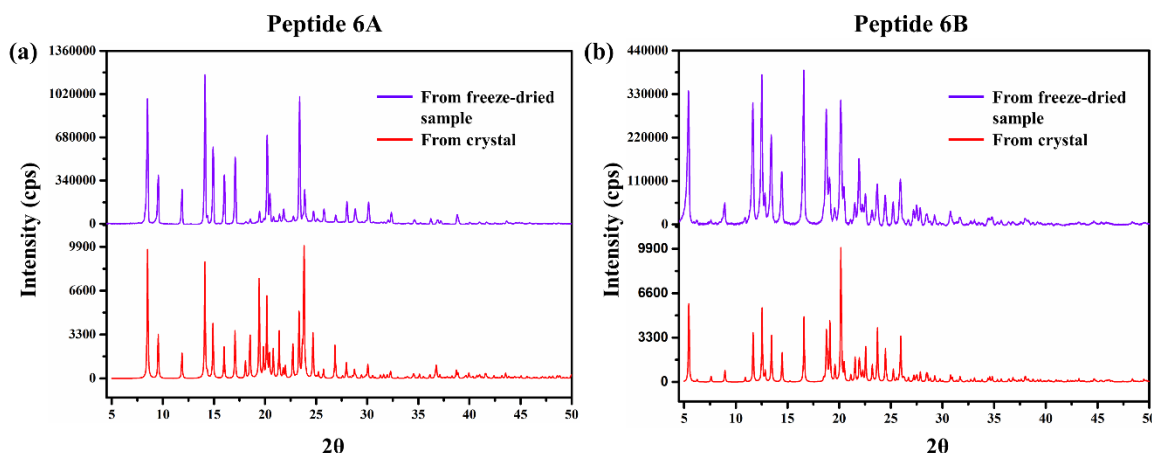


Figure 6.11. Comparison of freeze-dried PXRD with PXRD patterns obtained from SC-XRD of peptides (a) **6A** and (b) **6B**, respectively.

6.12. Metal ion sensing study

Metal ion sensing is crucial for biological processes and environmental monitoring.⁵ Various techniques like spectrometry, electrochemical, and colorimetric methods have been developed for heavy metal ion detection.⁶ Fluorescent probe-based methods are effective and widely used due to their simplicity, sensitivity and selectivity.⁷ Iron ions are essential for normal biological processes but harmful in excess levels in drinking water and the human body⁸, necessitating precise detection. Amino acids and peptides have excellent potential for developing metal ion sensors because of their good water solubility, binding affinity, biocompatibility, and low toxicity. We demonstrated Fmoc-dipeptide-based sensors for selective Fe^{3+} detection using fluorescence techniques.

6.12.1. UV-Vis and fluorescence spectroscopy

The absorbance spectra of dipeptides in acetonitrile-water (1:1) were measured at room temperature using a PerkinElmer Lambda 365+ spectrometer, showing absorption maxima at 265 nm (Figure 6.21). Fluorescence spectra were recorded in a 280 nm to 500 nm region with a HORIBA Scientific FluroMax-4 Spectrofluorometer, revealing prominent emission bands with dual peaks at 304 nm and 311 nm (Figure 6.16, 1a, 2a).

6.12.2. Screening different metal ions for sensing

We investigated the fluorescence response of Fmoc-dipeptides to various metal ions by recording their emission spectra. 1 mM stock solutions of different metal ions (Cu^{2+} , Zn^{2+} , Ca^{2+} , K^{+} , Na^{+} , Li^{+} , Ni^{2+} , Cd^{2+} , Co^{2+} , Al^{3+} , Fe^{3+} , Mg^{2+} , Pb^{2+} , Ag^{+} , Mn^{2+}) were prepared in acetonitrile-water (1:1). 50 μM peptides **6A** and **6B** were mixed with different metal ions solution (280 μM), and emission spectra were recorded. Most of the metal ions did not cause any change in fluorescence intensity, but a significant decrease in fluorescence intensity was noted with Fe^{3+} ion, with quenching efficiencies of 62.34% for peptide **6A** and 63.10% for peptide **6B** (Figure 6.12), suggesting that our dipeptides can serve as fluorescent probes for Fe^{3+} ions.

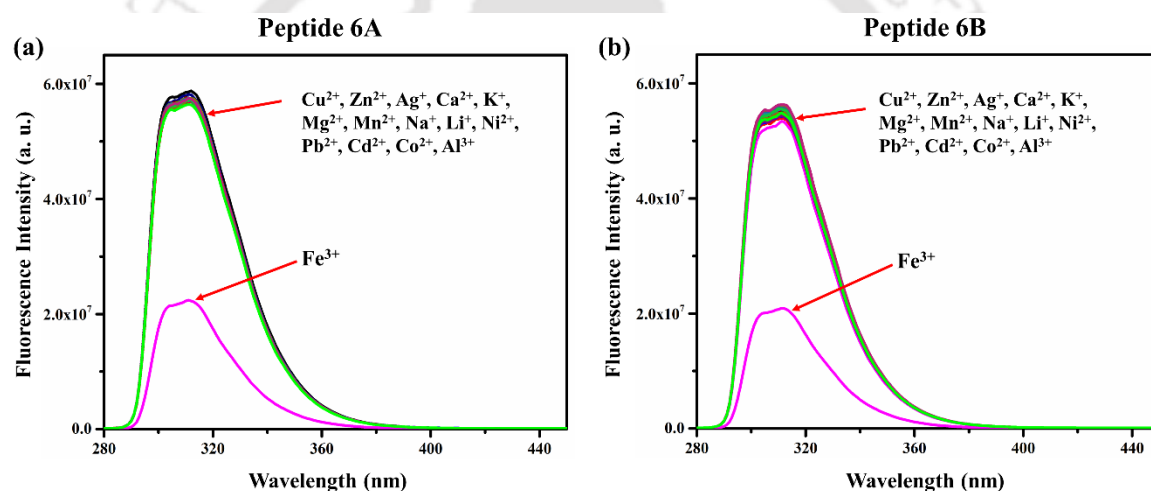


Figure 6.12. Emission spectra of (a) peptide **6A** (50 μM) and (b) peptide **6B** (50 μM) with various metal ions at a constant concentration of 280 μM .

6.12.3. Interfering experiments

We conducted competitive experiments to see the effect of different metal ions on Fe^{3+} detection using dipeptide probes. Competing metal ions (280 μM) were added to dipeptide (**6A**, **6B**) solutions (50 μM), followed by Fe^{3+} (280 μM). The addition of Fe^{3+} significantly reduced the emission intensity of the peptides (**6A** and **6B**), while the other metal ions did not induce any abrupt changes (Figure 6.13). The quenching effect when Fe^{3+} coexisted with different metal ions was almost similar to Fe^{3+} alone, indicating that the dipeptides' response to Fe^{3+} was unaffected by other metal ions.

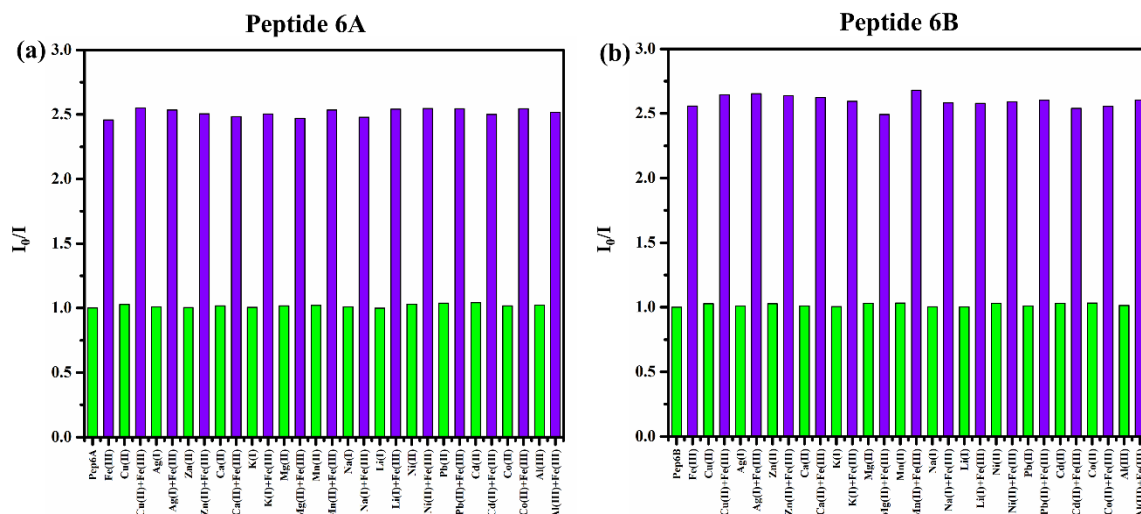


Figure 6.13. Fluorescence response of peptides **6A** and **6B** (50 μM) with various metal ions (280 μM), both with (violet) and without (green) Fe^{3+} (280 μM).

6.12.4. Screening anions for sensing

We investigated the interaction of peptides (**6A** and **6B**) (50 μM) with various anions (280 μM) using sodium salts of Cl^- , N_3^- , CO_3^{2-} , HCO_3^- , CH_3O^- , $\text{S}_2\text{O}_3^{2-}$, S^{2-} , F^- , SCN^- , NO_3^- , Br^- , and ClO_4^- . The emission spectra of the peptides remained unchanged in the presence of different anions (Figure 6.14), suggesting that anions do not substantially influence the fluorescence quenching process.

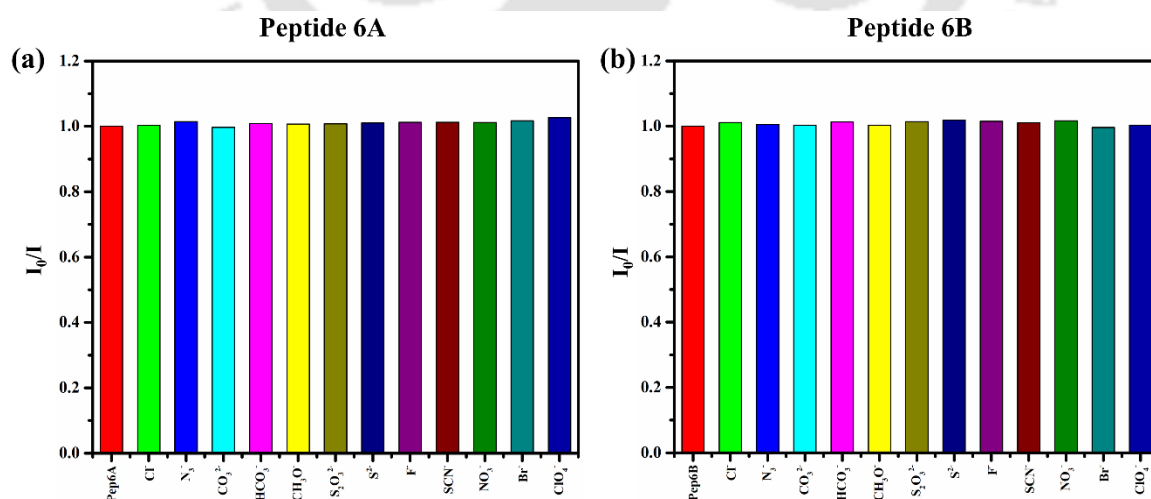


Figure 6.14. Fluorescence response (a, b) of peptides **6A** and **6B** (50 μM) in the presence of anions (280 μM), respectively.

6.12.5. Role of counter anion in fluorescence quenching

We tested the quenching efficiency using other salts of Fe^{3+} such as nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) and perchlorate ($\text{Fe}(\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$). Adding 280 μM of these salts to 50 μM peptides (**6A** and **6B**) showed results identical to FeCl_3 (Figure 6.15). The fluorescence quenching of the peptides was nearly the same for all Fe^{3+} salts tested, indicating that counter anions do not significantly influence Fe^{3+} sensing studies.

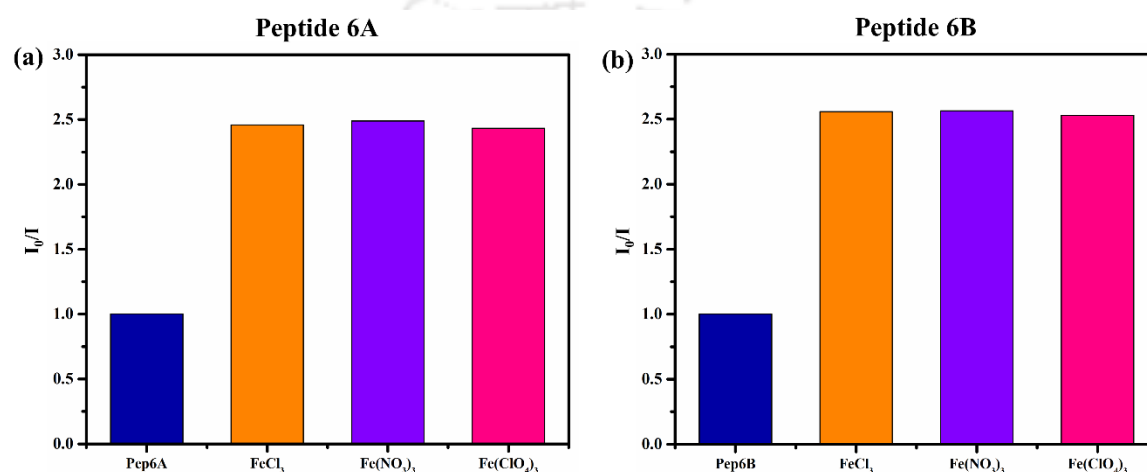


Figure 6.15. Fluorescence response (a, b) of peptides **6A** and **6B** (50 μM) with different Fe^{3+} salts (280 μM), respectively.

6.12.6. UV-Vis titration experiment for sensing of Fe^{3+}

We conducted UV-Vis titration experiments to test the probes' suitability for Fe^{3+} detection. Peptide solutions (50 μM) were titrated with varying Fe^{3+} concentrations (0–840 μM). Without Fe^{3+} , no peak appeared between 307–400 nm. Adding Fe^{3+} increased the absorbance in the 234–305 nm range, changing the solution color from colorless to yellowish, indicating Fe^{3+} addition. Additionally, the emergence of a new band in the 307–400 nm range signified the binding interaction of Fe^{3+} to peptides (**6A**, **6B**) (Figure 6.21).

6.12.7. Fluorescence titration experiment for sensing of Fe^{3+} and Stern-Volmer plot

The gradual decrease in fluorescence emission intensity of the peptides **6A** and **6B** (50 μM) was observed upon adding increasing concentrations of Fe^{3+} ion (0 μM to 840 μM).

The fluorescence intensity of peptides **6A** and **6B** was quenched by 96.05% and 96.25%, respectively, upon adding $840 \mu\text{M}$ Fe^{3+} (Figure 6.16, 1a, 2a). Titration experiments for both dipeptide-based probes showed almost identical results.

The quenching mechanism with metal ions was analyzed using the Stern-Volmer equation: $I_0/I = 1 + K_{\text{SV}}[Q]$, where (I_0) represents emission intensity without quencher, (I) is along with a quencher, K_{SV} denotes the Stern-Volmer constant, and $[Q]$ indicates the concentration of quencher.⁹ The plot of (I_0/I) vs. Fe^{3+} concentration showed a straight line, indicating a linear relation (Figure 6.16, 1b, 2b). The Stern-Volmer constants (K_{SV}) for dipeptides **6A** and **6B** were $5.35 \times 10^3 \text{ M}^{-1}$ and $7.41 \times 10^3 \text{ M}^{-1}$, respectively.

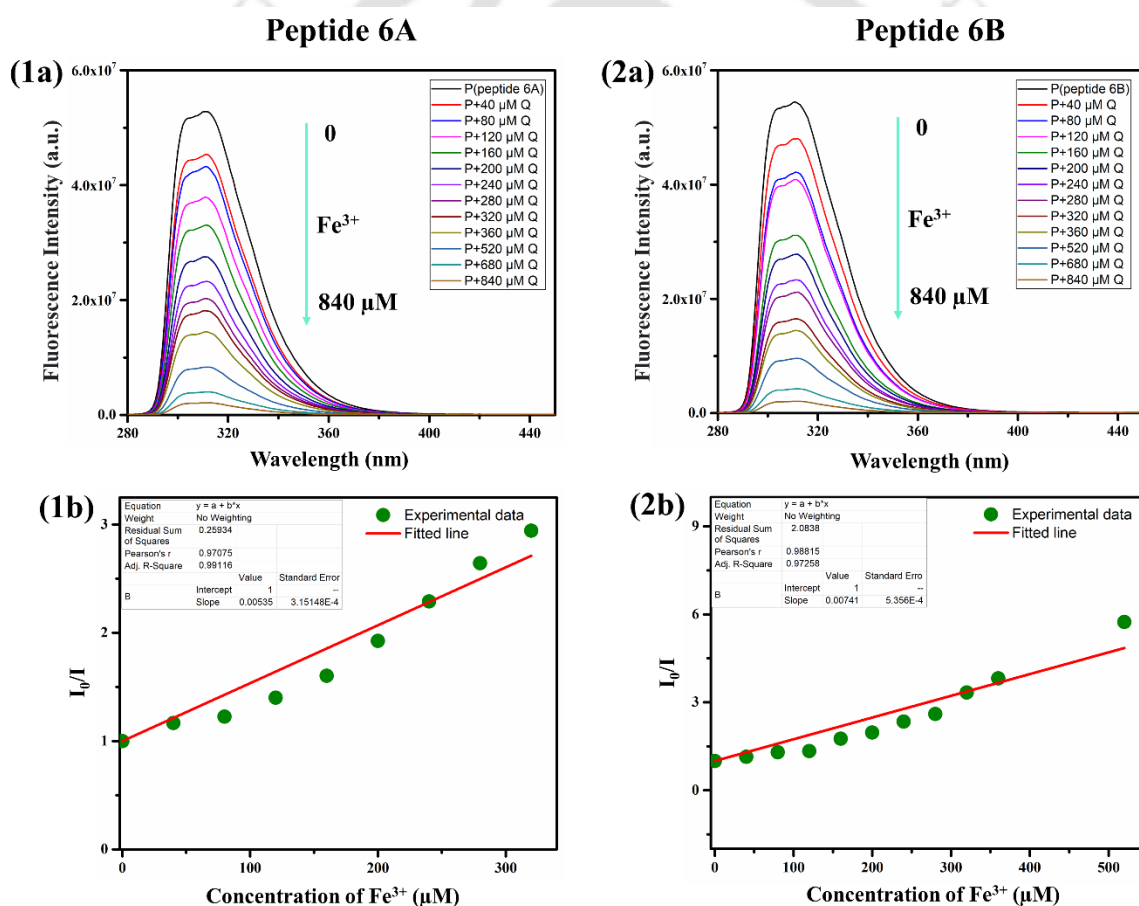


Figure 6.16. Emission spectra of (1a) peptide **6A** ($50 \mu\text{M}$) and (2a) peptide **6B** ($50 \mu\text{M}$) with varying concentrations of Fe^{3+} (0.0 – $840 \mu\text{M}$) in acetonitrile-water ($1:1$) mixture. Stern-Volmer plots for fluorescence quenching of (1b) peptide **6A** and (2b) peptide **6B** by Fe^{3+} ions.

6.12.8. Interaction and binding mode of Fe^{3+} to peptides

We examined the interaction and binding mechanism of Fe^{3+} using FT-IR and ^1H NMR experiments. FT-IR showed positive shifts in amide N-H bands and negative shifts in C=O bands, indicating Fe^{3+} coordination via carbonyl groups (Figure 6.22, 6.23). ^1H NMR titrations of dipeptides (**6A**, **6B**) with increasing Fe^{3+} concentrations showed downfield shifts in amide N-H peak due to deshielding of the N-H proton, suggesting the probable binding of Fe^{3+} to the carbonyl group (Figure 6.24, 6.25). These results implied that Fe^{3+} may interact with the carbonyl oxygen of amide/ester/carbamate units. Fe^{3+} , being a hard acid, prefers to bind with the hard oxygen of the carbonyl group. Its high positive charge and vacant d-orbitals facilitate effective interaction with the carbonyl oxygen, forming a coordination bond.

6.13. Conclusion

In conclusion, we illustrated the supramolecular self-assembly and morphology of N-(Fmoc) and C-(OMe) protected dipeptides, Fmoc-L-Ala-L-Val-OMe (**6A**) and Fmoc-L-Ala-D-Val-OMe (**6B**). Both peptides exhibited parallel β -sheet structures through intermolecular H bonding in their crystalline state. Peptide **6A** formed porous channel-like architecture, while peptide **6B** formed dimer. Morphological investigations using optical microscopy, FESEM, TEM, and AFM revealed distinct morphologies: peptide **6A** formed hollow tube-like structure, and peptide **6B** exhibited fibrillar structure in an acetonitrile-water (1:1) solvent mixture. The nanostructures formed homogeneously without any issues. The variation in morphology may be due to the altered chirality of Val, resulting in different backbone orientations. In various solvents, peptide **6A** formed rod or tube-like structures, while peptide **6B** formed fibrillar structures. Both peptide morphologies showed significant thermal stability upon dry heating and were effective in binding with bioactive dyes such as fluorescein, rhodamine B, thioflavin T, and 5(6)-carboxyfluorescein. The peptides also showed the ability to selectively sense Fe^{3+} ions through fluorescence techniques and may serve as fluorescent probes for Fe^{3+} ions. Such designs and discoveries significantly advance the creation of novel materials and therapeutics using small peptides.

6.14. Experimental section

6.14.1. Materials and instrumentations

As described in chapter 8

6.14.2. General procedure for synthesizing dipeptides

As described in chapter 5, in section 5.9.2.

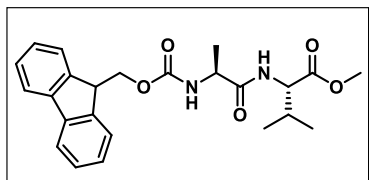
6.14.3. Sample preparation

Peptide samples (1.5 mM, 2 mL each) were prepared in a 50% acetonitrile-water solution and incubated at 37 °C for four days. Following incubation, various analyses were conducted using the samples, including optical microscopy, FESEM, FETEM, AFM, and thermal stability studies.

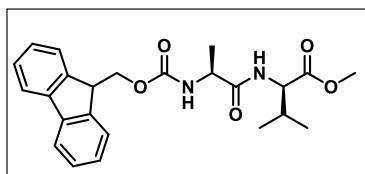
Stock solutions (1 mM) of various metal ions, including Cu²⁺, Zn²⁺, Ca²⁺, K⁺, Na⁺, Li⁺, Ni²⁺, Cd²⁺, Co²⁺, Al³⁺, Fe³⁺ (chloride salts); Mg²⁺ (sulfate salt); Pb²⁺ (nitrate salt); and Ag⁺, Mn²⁺ (acetic acid salts), were prepared in a 1:1 acetonitrile-water mixture. Additionally, 1.5 mM stock solutions of peptides were prepared by dissolving the compounds in 50% acetonitrile-water. Specific amounts of these metal solutions and peptides were then used for the sensing studies.

6.15. Characterization data

Boc-L-Ala-L-Val-OMe (6A):



Yield: 86% (363 mg); ¹H NMR (600 MHz, CDCl₃) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 6.57 (s, 1H), 5.47 (s, 1H), 4.54 (dd, *J* = 8.6, 4.8 Hz, 1H), 4.40 (d, *J* = 6.0 Hz, 2H), 4.33 (t, *J* = 7.6 Hz, 1H), 4.21 (t, *J* = 6.9 Hz, 1H), 3.73 (s, 3H), 2.18 (ddd, *J* = 18.7, 9.1, 4.6 Hz, 1H), 1.41 (d, *J* = 5.6 Hz, 3H), 0.92 (dd, *J* = 6.9, 2.5 Hz, 3H), 0.89 (dd, *J* = 6.8, 2.5 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 172.40, 172.38, 156.20, 143.99, 143.94, 141.49, 127.94, 127.28, 125.26, 120.19, 120.19, 67.35, 57.38, 52.41, 50.66, 47.28, 31.41, 19.12, 18.67, 17.89; HRMS (ESI) *m/z*: calcd for C₂₄H₂₉N₂O₅, 425.2071 [M+H]⁺; found 425.2084.

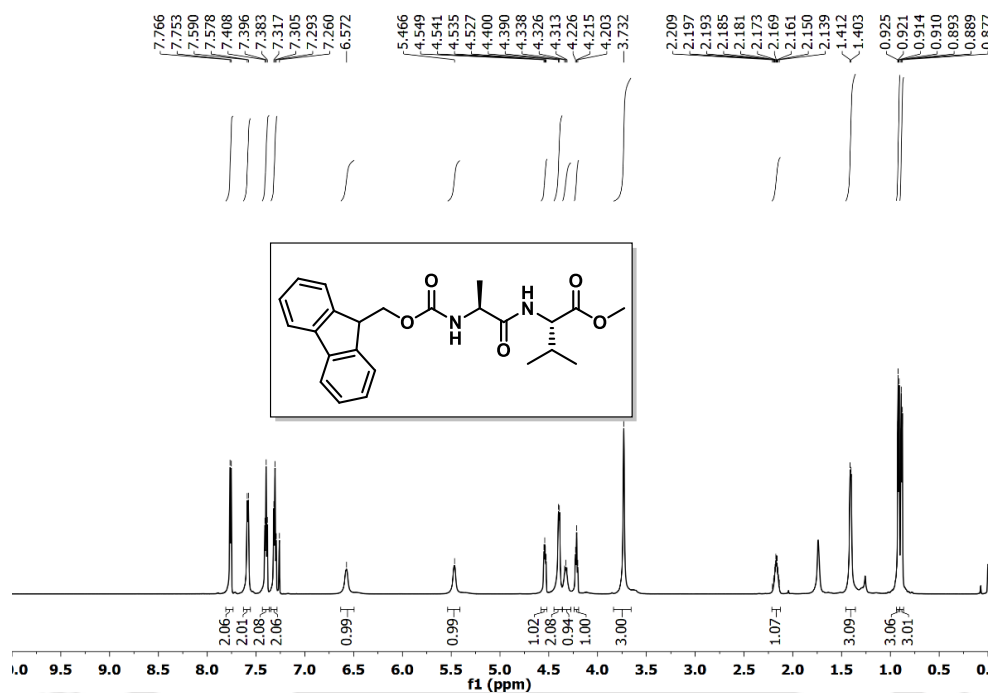
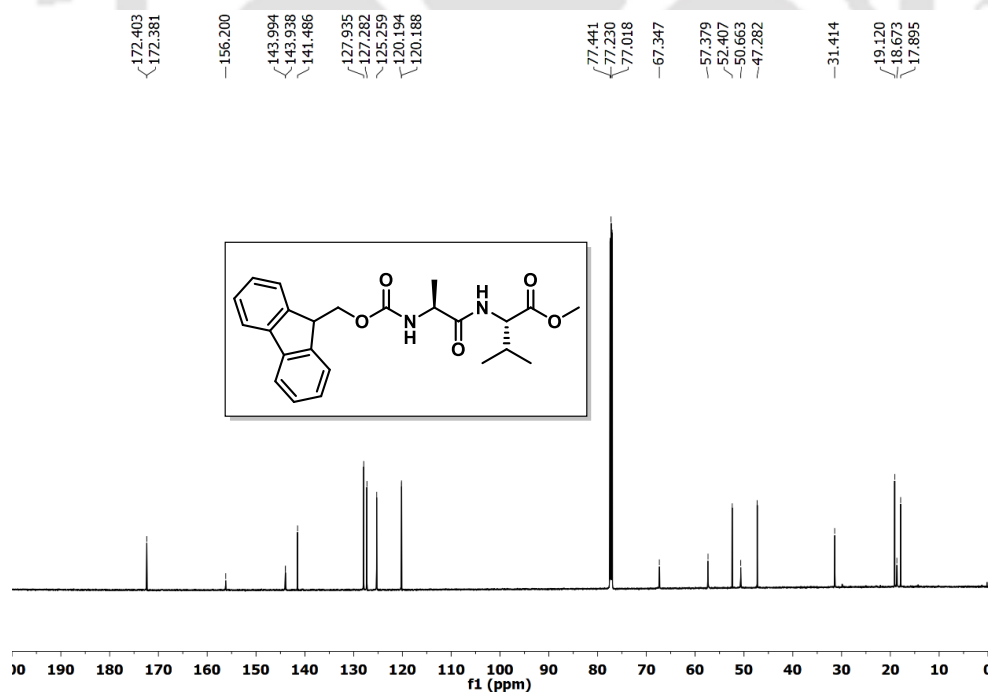
Boc-L-Ala-D-Val-OMe (6B):

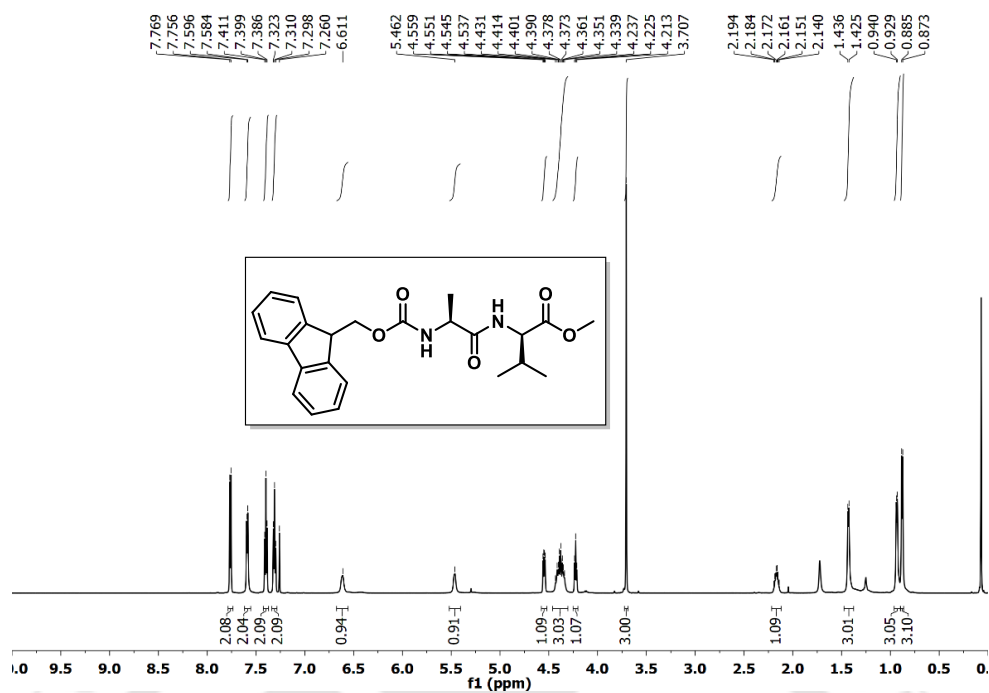
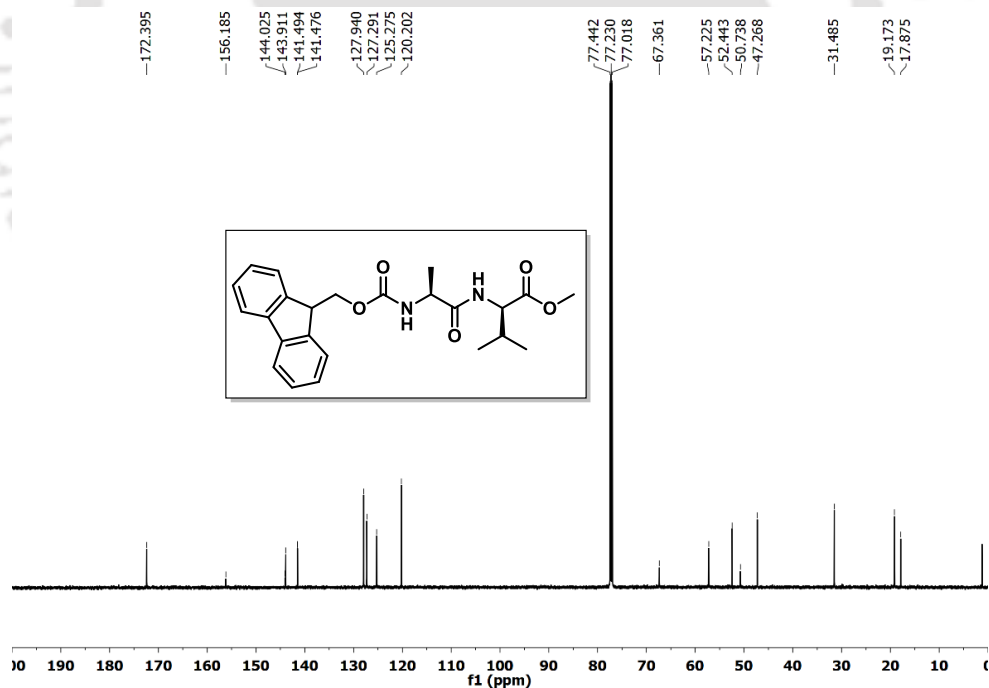
Yield: 85% (360 mg); white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 6.61 (s, 1H), 5.46 (s, 1H), 4.55 (dd, *J* = 8.8, 4.9 Hz, 1H), 4.38 (ddt, *J* = 20.2, 13.3, 7.3 Hz, 3H), 4.22 (t, *J* = 7.1 Hz, 1H), 3.71 (s, 3H), 2.17 (h, *J* = 6.5 Hz, 1H), 1.43 (d, *J* = 6.5 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 172.39, 156.19, 144.03, 143.91, 141.49, 141.48, 127.94, 127.29, 125.28, 120.20, 67.36, 57.22, 52.44, 50.74, 47.27, 31.49, 19.17, 17.87; HRMS (ESI) *m/z*: calcd for C₂₄H₂₉N₂O₅, 425.2071 [M+H]⁺; found 425.2058.

6.16. References

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6.17. Selected spectra

Figure 6.17. ^1H NMR spectrum of peptide 6AFigure 6.18. ^{13}C NMR spectrum of peptide 6A

Figure 6.19. ¹H NMR spectrum of peptide 6BFigure 6.20. ¹³C NMR spectrum of peptide 6B

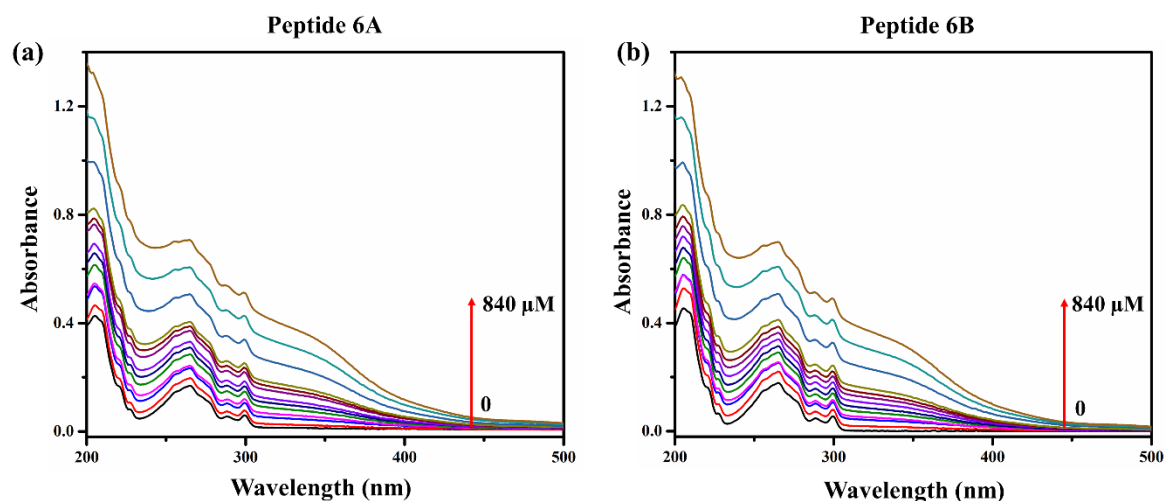


Figure 6.21. UV-Vis absorption spectra of peptides **6A** and **6B** (50 μM) with different concentrations of Fe^{3+} ions (0–840 μM).

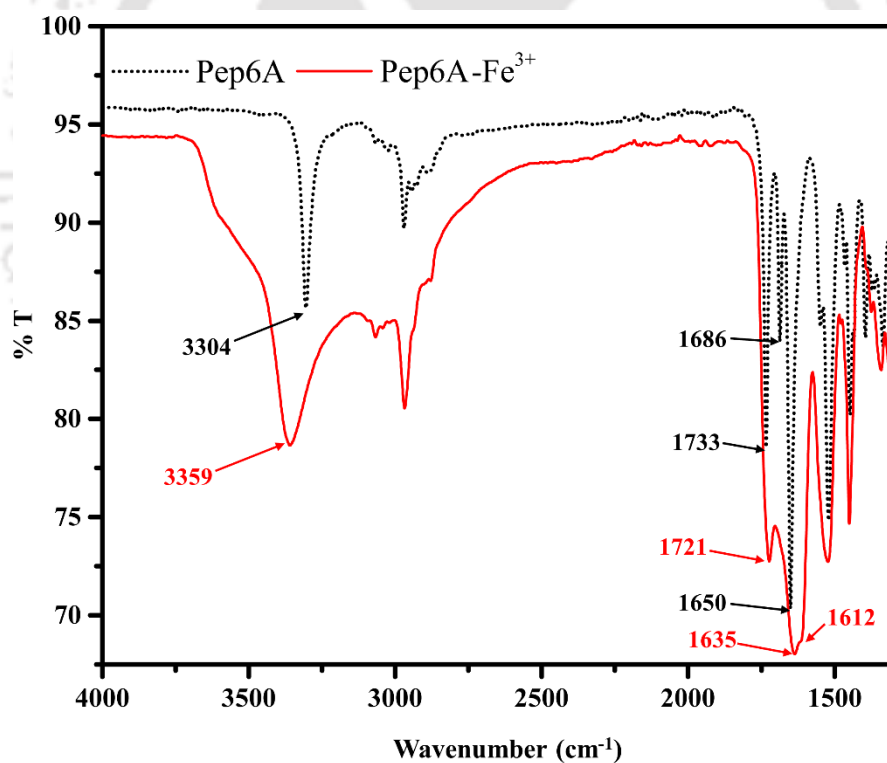


Figure 6.22. FT-IR spectra of peptide **6A** (12 mM) without (black dashed line) and with (red line) Fe^{3+} (18 mM).

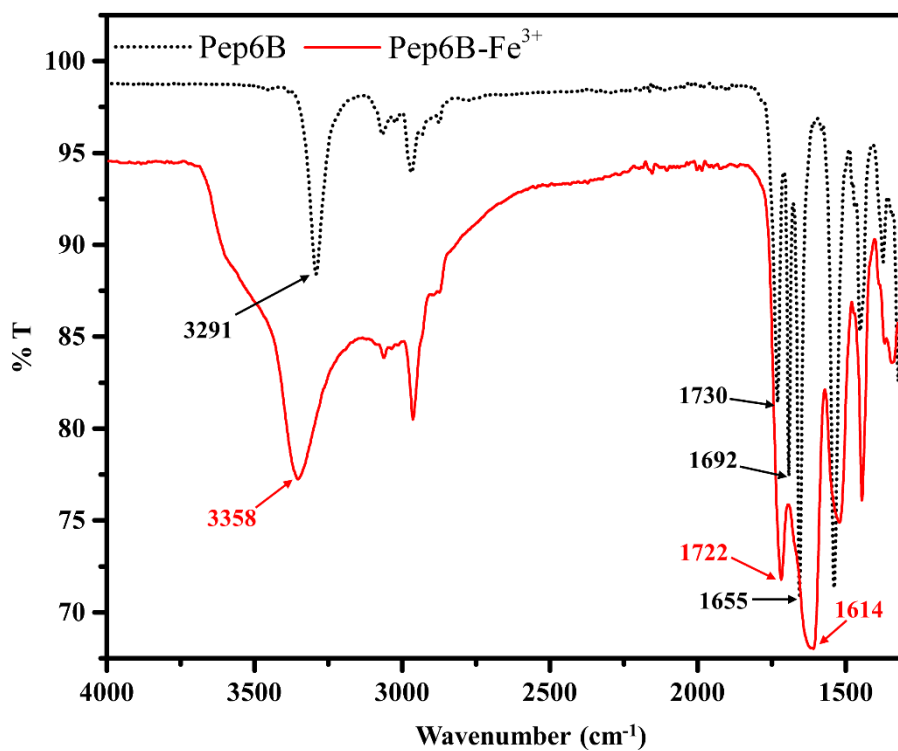


Figure 6.23. FT-IR spectra of peptide **6B** (12 mM) without (black dashed line) and with (red line) Fe^{3+} (18 mM).

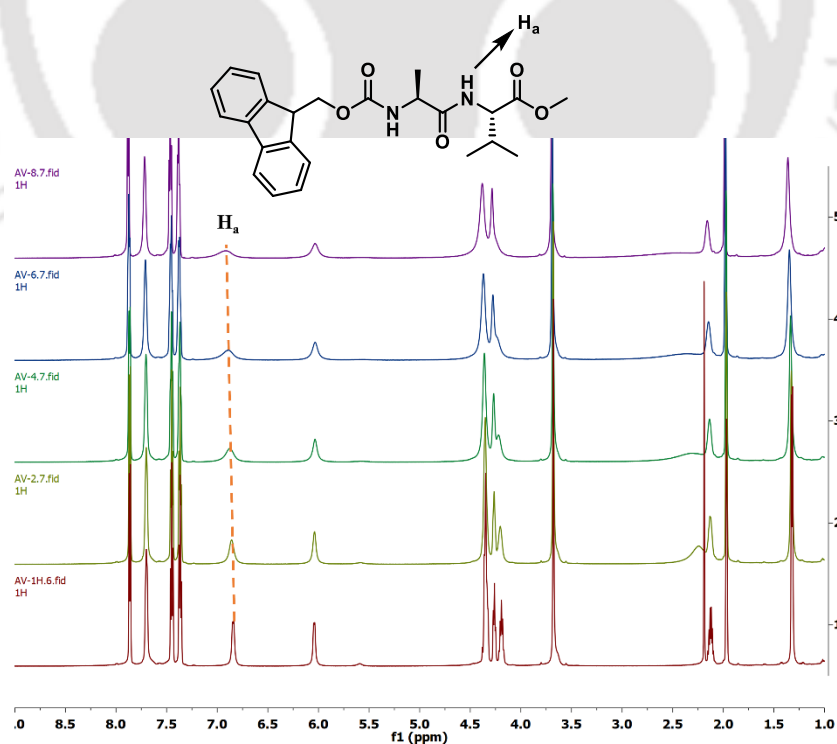


Figure 6.24. ^1H NMR titration of peptide **6A** (40 mM in CD_3CN) with incremental additions of Fe^{3+} solution (20 mM in CD_3CN , 2 μL each time).

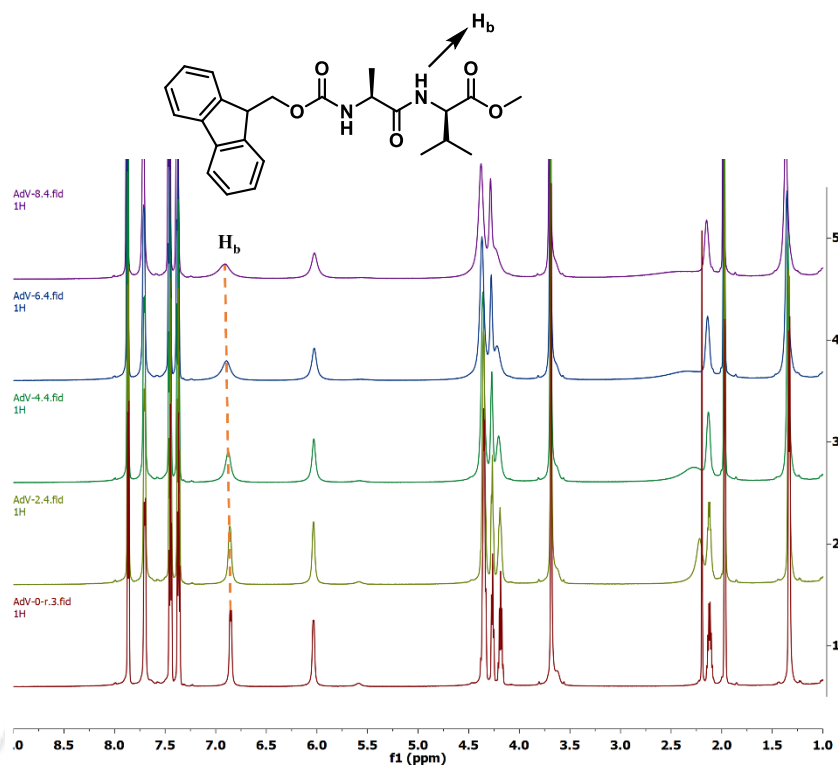


Figure 6.25. ^1H NMR titration of peptide **6B** (40 mM in CD_3CN) with incremental additions of Fe^{3+} solution (20 mM in CD_3CN , 2 μL each time).

6.18. Crystallographic data

Table 6.2. Hydrogen bonding distances ($\text{\AA}$) and Bond angles ($^\circ$) of peptides **6A**, **6B**

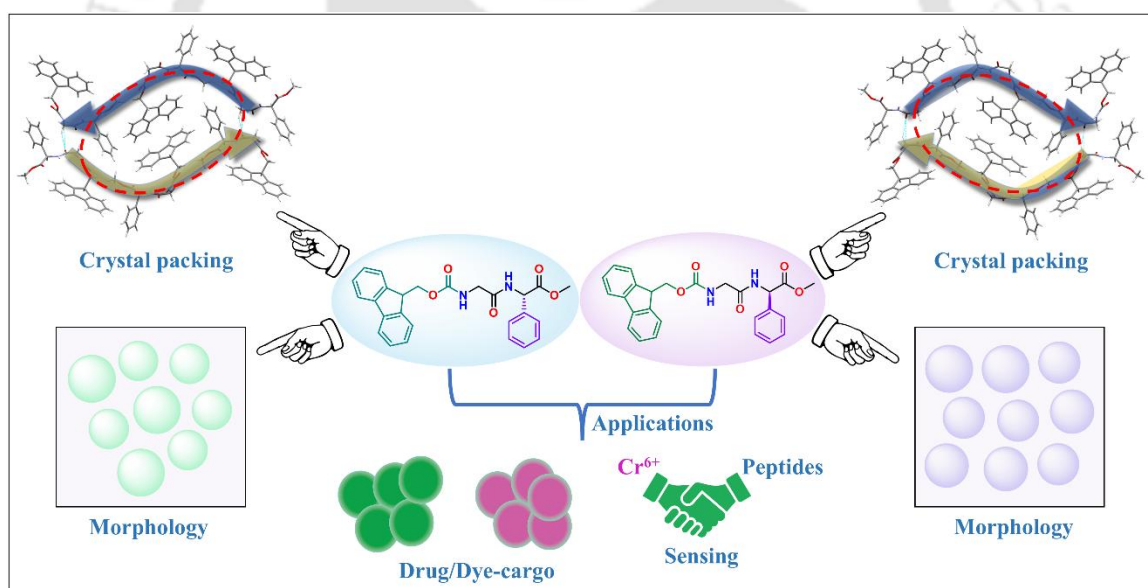
Peptides	D–H $\cdots$ A	$d(\text{D}\cdots\text{H})/\text{\AA}$	$d(\text{H}\cdots\text{A})/\text{\AA}$	$d(\text{D}\cdots\text{A})/\text{\AA}$	$\angle\text{D–H}\cdots\text{A}/^\circ$
Peptide 6A	N1–H1 $\cdots$ O2	0.86	2.22	2.964(7)	145
	N2–H2 $\cdots$ O3	0.86	2.23	3.067(7)	164
	C16–H16 $\cdots$ O3	0.98	2.35	3.274(9)	157'
	C19–H19 $\cdots$ O4	0.98	2.43	3.356(11)	157'
Peptide 6B	N1–H1 $\cdots$ O2	0.86	2.01	2.864(7)	173
	N2–H2 $\cdots$ O3	0.86	2.07	2.906(7)	164
	C14–H14B $\cdots$ O4	0.97	2.56	3.511(14)	168

Table 6.3. Hydrogen bonding distances (Å) and Bond angles (°) of peptides 6A, 6B

Parameters	Fmoc-L-Ala-L-Val-OMe (6A)	Fmoc-L-Ala-D-Val-OMe (6B)
Formula	C ₂₄ H ₂₈ N ₂ O ₅	C ₂₄ H ₂₈ N ₂ O ₅
Fw	424.48	424.48
Crystal system	Monoclinic	Monoclinic
Space group	<i>P</i> 21	<i>P</i> 21
<i>a</i> /Å	4.9063(13)	14.2952(12)
<i>b</i> /Å	12.542(3)	4.9152(4)
<i>c</i> /Å	18.529(5)	16.4095(14)
α /°	90.00	90.00
β /°	92.148(8)	99.331(2)
γ /°	90.00	90.00
<i>V</i> /Å ³	1139.4(5)	1137.74(16)
<i>Z</i>	2	2
<i>D_c</i> /g cm ⁻³	1.237	1.239
μ Mo K α /mm ⁻¹	0.087	0.087
<i>F</i> 000	452	452
<i>T</i> /K	297(2)	297(2)
θ max.	26.65	25.39
Total no. of reflections	23931	32659
Independent reflections	3963	3995
Observed reflections	2428	3169
Parameters refined	284	284
<i>R</i> ₁ , <i>I</i> > 2 σ (<i>I</i>)	0.0814	0.0891
<i>wR</i> ₂ , <i>I</i> > 2 σ (<i>I</i>)	0.1876	0.2401
GOF (<i>F</i> ²)	1.362	1.023
CCDC No.	2335186	2335187

Chapter 7

Self-Assembly of Enantiomeric Dipeptides and Applications



7.1. Background

Among the peptide crystals obtained in the previous chapters, one of the most intriguing was Fmoc-Gly-L-Phg-OMe, which contains a single chiral center. To further explore the influence of chirality on self-assembly, we designed and synthesized the enantiomeric dipeptide Fmoc-Gly-D-Phg-OMe by altering the chirality of L-Phg to D-Phg and then crystallized it. Our goal was to investigate the impact of chirality on the self-assembly of an enantiomeric Fmoc-dipeptide system.

In the previous two chapters, we discussed the influence of side chains and the chirality of amino acids on the self-assembly of diastereomeric dipeptides. The self-assembly of peptides containing natural amino acids has been extensively studied.¹ However, the self-assembly of enantiomeric dipeptides, especially those with one natural achiral and one unnatural chiral amino acid, has not been well explored. Here, we investigated the self-assembly behavior of one natural achiral and one unnatural chiral amino acid containing enantiomeric dipeptides to address this research gap.

7.2. Peptide design and synthesis

To address the aforementioned research gap, we synthesized our designed peptides Fmoc-Gly-L-Phg-OMe (**7A**) and Fmoc-Gly-D-Phg-OMe (**7B**) (Figure 7.1) using conventional solution-phase method for various self-assembly studies. The synthesized peptides were then characterized using NMR (^1H , ^{13}C) spectroscopy and mass spectrometry.

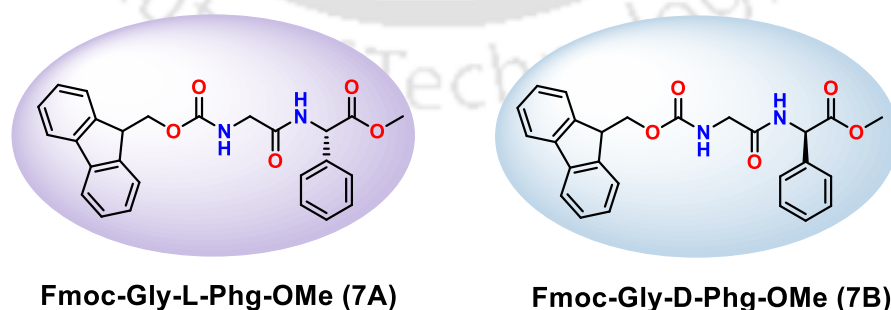


Figure 7.1. Chemical structure of peptides.

7.3. Investigation of morphologies using various microscopic techniques

To investigate the self-assembling properties of the dipeptides in solution, we initially conducted optical microscopic imaging. Both peptide samples (1.5 mM) were incubated for four days in a 1:1 acetonitrile-water mixture at 37 °C and then drop-casted onto clean glass slides, dried, and captured the images. Under bright-field optical microscopy, both peptides (**7A** and **7B**) displayed organized spherical morphologies (Figure 7.2, 1a, 2a).

Next, we investigated the self-assembly using advanced sophisticated techniques like FESEM, FETEM, and AFM. The incubated peptides were deposited onto silicon wafer pieces, dried, and captured the FESEM images. The FESEM images revealed densely packed, well-organized spherical structures for both peptides (**7A** and **7B**) (Figure 7.2, 1b, 2b).

To gain a more comprehensive understanding of the structural details, we performed the FETEM experiment. 100 μ M each peptide solution was prepared by diluting 1.5 mM incubated peptides with acetonitrile-water mixture (1:1). Next, each peptide sample was deposited on separate TEM grids and dried in a desiccator. FETEM images of the dried samples were captured. FETEM images revealed well-defined hollow vesicle structures of the dried peptide samples (**7A** and **7B**) (Figure 7.2, 1c, 2c). These vesicles exhibited internal diameters of 360-400 nm (peptide **7A**) and 320-370 nm (peptide **7B**), with corresponding external diameters of 490-600 nm (peptide **7A**) and 480-570 nm (peptide **7B**). The formation of these hollow vesicles was characterized by a distinct dark line at the periphery of each vesicle.

Next, we performed the Atomic Force Microscopy (AFM) experiment to investigate the topographical features of the nanostructures. AFM samples were prepared from 30 μ M peptide solutions derived from diluting 4-day-aged incubated peptides (1.5 mM). The AFM revealed vesicle morphologies of the peptides (**7A**, **7B**) similar to those observed in FESEM and FETEM. The diameters of the nanovesicles were approximately 690 nm for peptide **7A** and 665 nm for peptide **7B**. Despite different chirality, the peptides showed similar morphologies, possibly due to their identical backbone conformation. Figure 7.3 presents 2D images, height-to-distance profile plots, and 3D images of the vesicles.

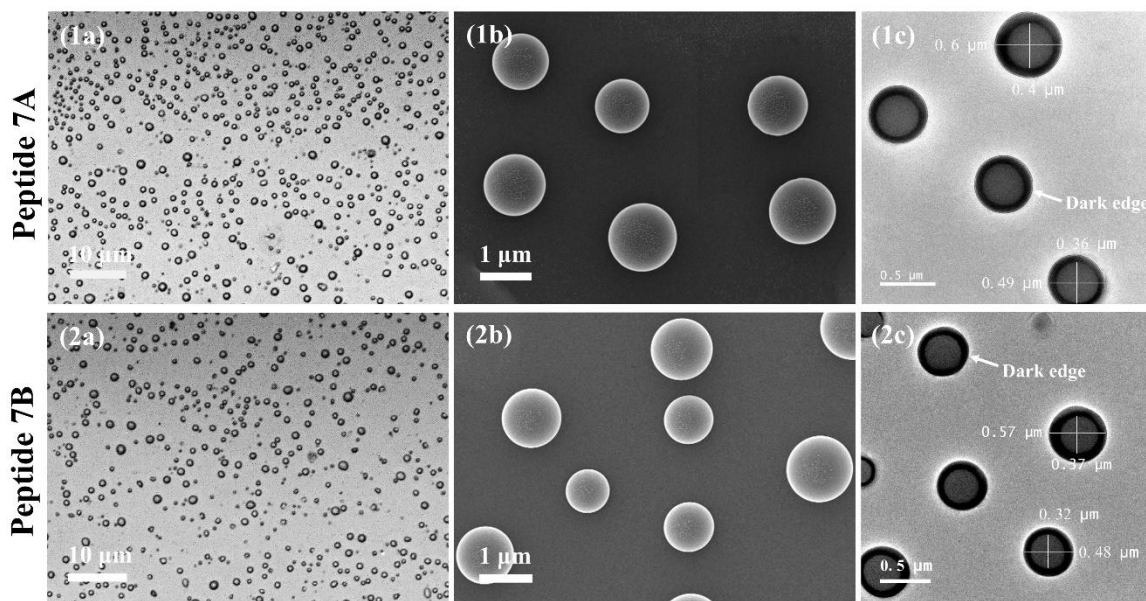


Figure 7.2. Optical microscopy (1a, 2a), FESEM (1b, 2b) revealed spherical structure, and FETEM (1c, 2c) revealed hollow vesicular structure of peptides 7A and 7B, respectively.

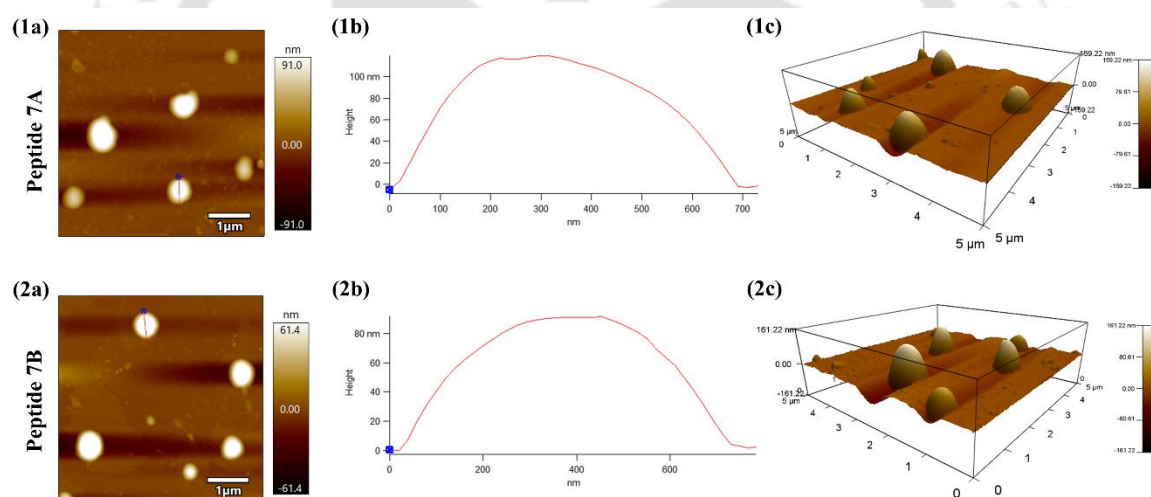


Figure 7.3. 2D images (1a, 2a), height profile plots (1b, 2b), and 3D images (1c, 2c) of peptides 7A and 7B, respectively.

7.4. Investigation of the self-assembly of dipeptides using SC-XRD

To elucidate the molecular arrangement and conformation, we employed single-crystal X-ray diffraction (SC-XRD). Gradual evaporation of sample containing acetonitrile-water mixture at room temperature offered colorless, needle-shaped crystals of peptides 7A and 7B. Both peptides formed crystals in the orthorhombic ($P 21 21 21$) crystal system with a

single molecule in the asymmetric unit. Peptide **7A** subunits formed a parallel β -sheet structure (Figure 7.4b) via intermolecular hydrogen bonding ($N1-H1...O3 = 3.00$ Å). Weak interactions were observed between the subunits (methylene H of Gly and urethane oxygen of subunits ($C-H...O = 2.57$ Å), and methylene H of the urethane group and carbonyl oxygen of the urethane group of subunits ($C-H...O = 2.26$ Å)), further stabilized the β -sheet. The peptide self-organized into a helical-like structure (Figure 7.4c) via head-to-tail interactions between the methyl H of the CH_3 group of the methyl ester of one subunit and the urethane carbonyl oxygen of another subunit ($C-H...O = 2.46$ Å). Six interconnected subunits created a void space (Figure 7.4d) through interactions between the methyl H of methyl ester CH_3 of one subunit and urethane carbonyl oxygen of the adjacent subunit ($CH...O = 2.46$ Å), and between methylene H of glycine of one subunit and the amide carbonyl oxygen of the neighboring subunit ($CH...O = 2.35$ Å).

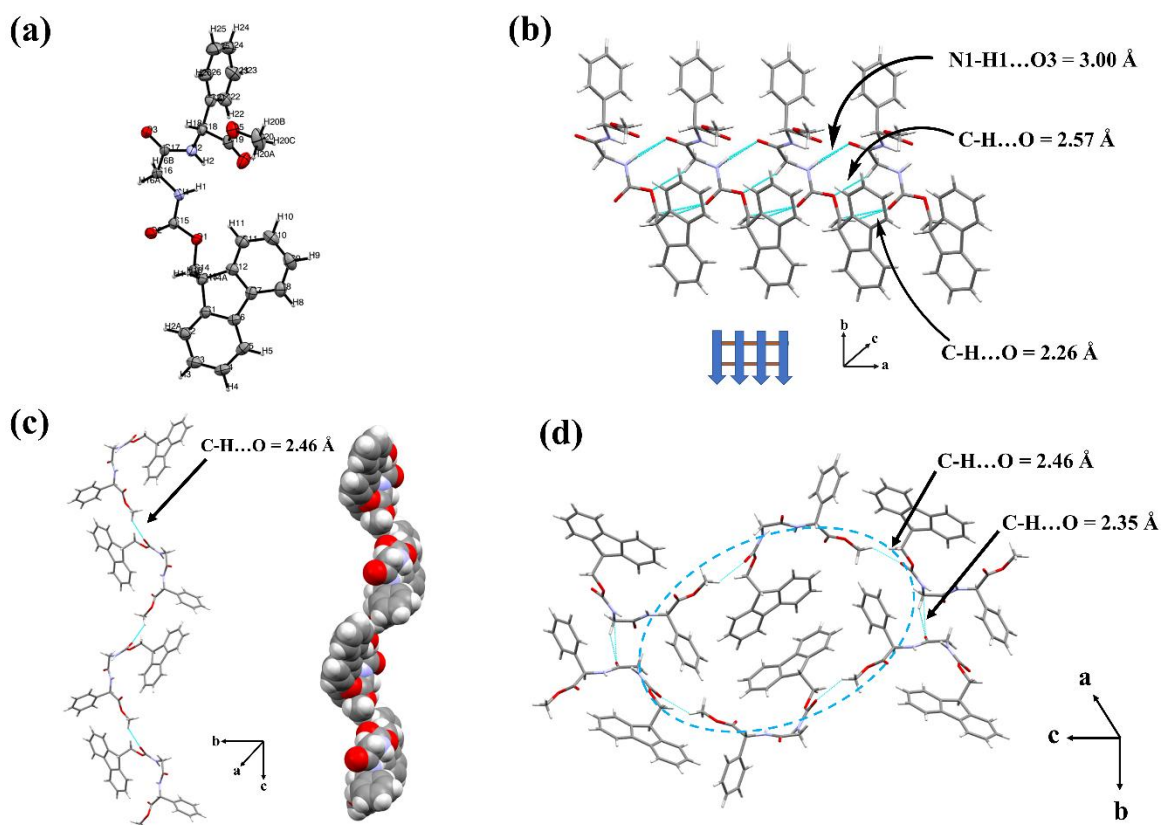


Figure 7.4. (a) ORTEP diagram, (b) parallel- β -sheet structure, and (c) and (d) higher-order packing of peptide **7A**.

Likewise, D-Phg-containing peptide **7B** also formed a parallel β -sheet structure (Figure 7.5b) via intermolecular hydrogen bonding between subunits ($\text{N1-H1}\cdots\text{O3} = 3.00 \text{ \AA}$). The parallel β -sheet structure was further stabilized by additional $\text{CH}\cdots\text{O}$ interactions between subunits (2.57 and $2.26 \text{ \AA}$). Subunits interconnected via head-to-tail interactions (methyl H of the methyl ester CH_3 of one subunit and the urethane carbonyl oxygen of another subunit, $\text{C-H}\cdots\text{O} = 2.40 \text{ \AA}$), similar to peptide **7A**, forming a helix-like architecture (Figure 7.5c). Six interconnected subunits formed a void space (Figure 7.5d) through interactions between the methyl H of the methyl ester CH_3 and urethane carbonyl oxygen of subunits ($\text{CH}\cdots\text{O} = 2.40 \text{ \AA}$), and between the methylene H of glycine and amide carbonyl oxygen ($\text{CH}\cdots\text{O} = 2.35 \text{ \AA}$) of subunits.

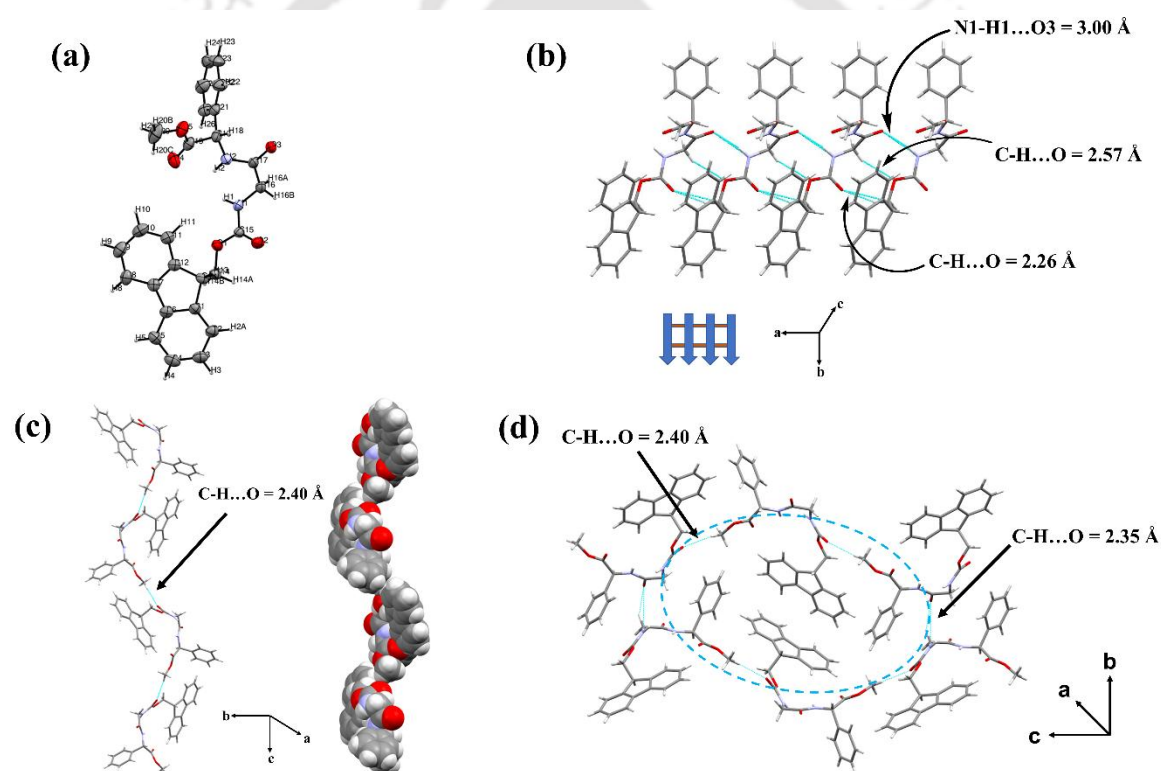


Figure 7.5. (a) ORTEP diagram, (b) parallel- β -sheet structure, and (c) and (d) higher-order packing of peptide **7B**.

Peptides **7A** and **7B**, each comprising one chiral amino acid (L-Phg or D-Phg) and one achiral amino acid, are structurally enantiomeric (Figure 7.6a). These enantiomeric peptides also displayed a mirror image relationship in their higher-order packing: peptide **7A** formed a right-handed helix-like, while peptide **7B** formed a left-handed helix-like

architecture (Figure 7.6b). Interestingly, in both peptides, two consecutive helical strands run anti-parallel without twisting into a double helix. These strands are stabilized by C-H $\cdots$ O interactions, creating void spaces (Figure 7.6c, 7.6d). These void spaces may facilitate the vesicle formation in solution, as observed by FESEM, FETEM, and AFM analysis.

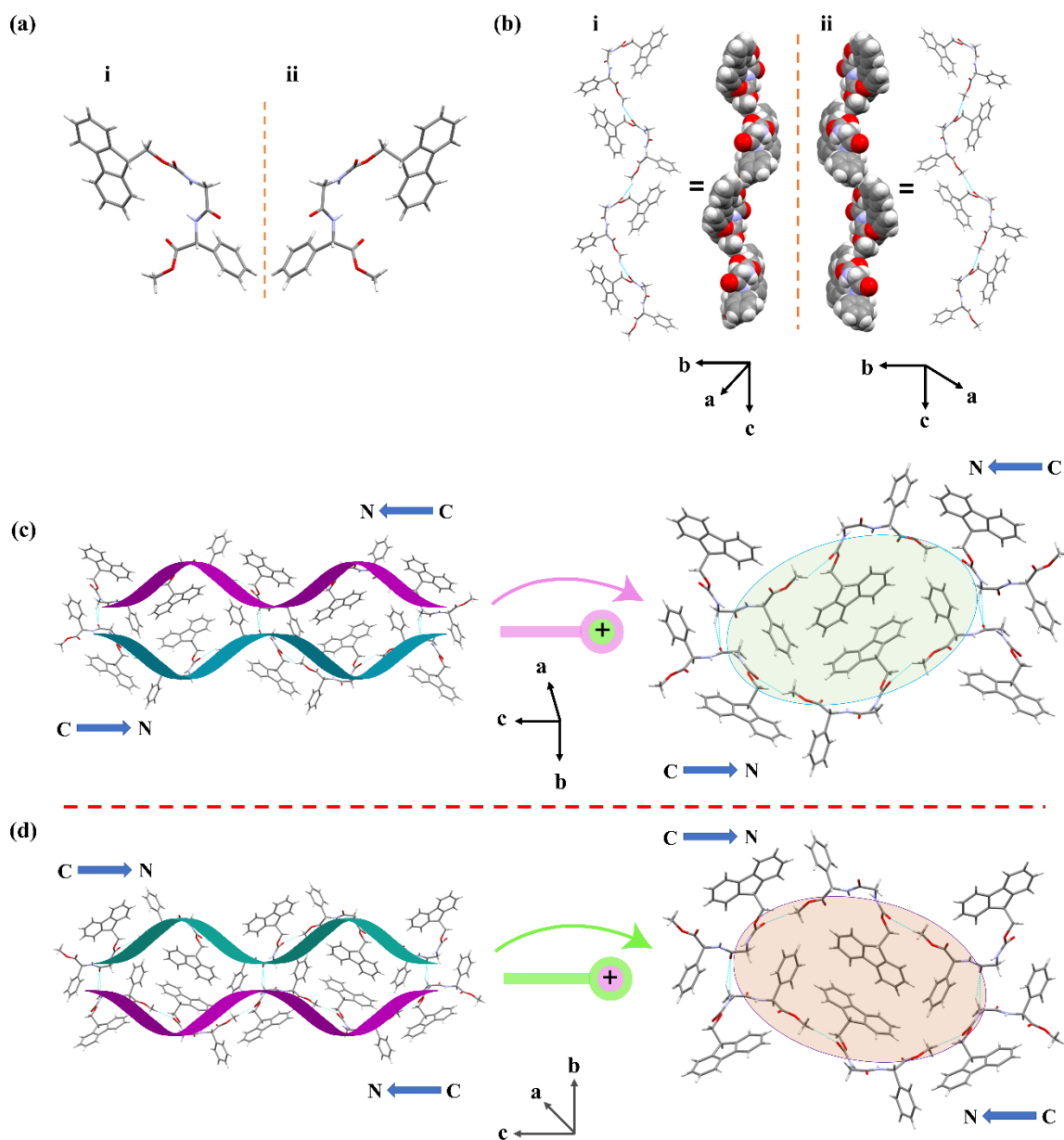


Figure 7.6. (ai, aii) Mirror images of the chemical structures of peptides **7A** and **7B**; (bi) right-handed helix-like, (bii) left-handed helix-like architecture, (c, d) void space created by the anti-parallel helix of peptides **7A**, and **7B**, respectively.

7.5. Investigation of the self-assembly of dipeptides using PXRD

Next, we conducted PXRD analysis of freeze-dried peptides **7A** and **7B**, incubated for four days at 37 °C in acetonitrile-water (1:1). The PXRD patterns (2θ range: 5–50°) were almost identical to the PXRD patterns obtained from the CIF of SC-XRD (Figure 7.7), indicating that peptides **7A** and **7B** adopted the β -sheet conformation in both solution and crystalline states.

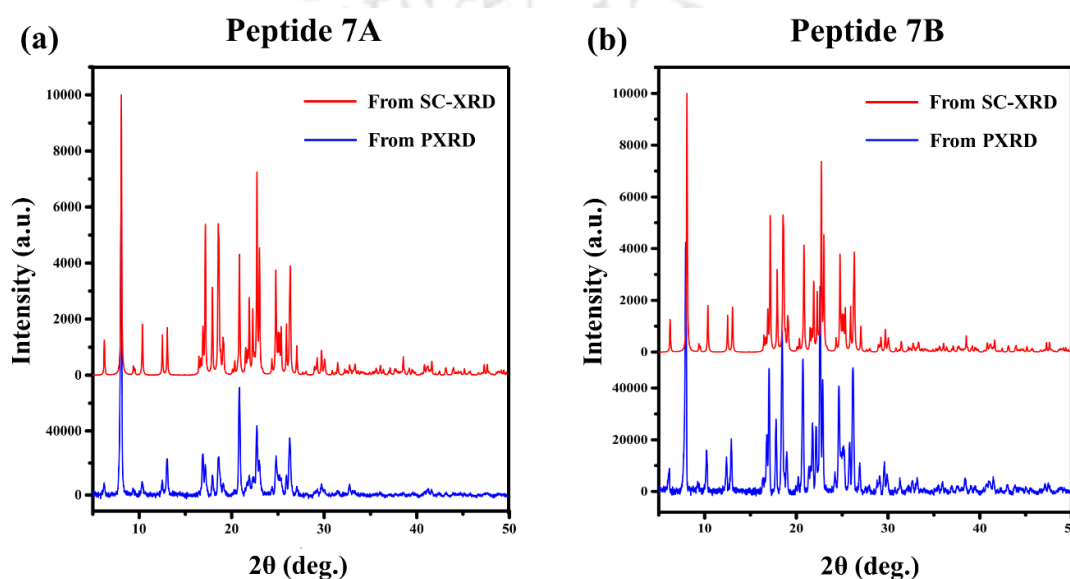


Figure 7.7. (a, b) PXRD patterns of peptides **7A** and **7B**, respectively. PXRD of freeze-dried incubated peptides (blue) identical to the patterns obtained from SC-XRD (red).

7.6. Investigation of the self-assembly of the peptides by FT-IR

We performed solid-state FT-IR spectroscopy to examine the secondary structure of peptides **7A** and **7B**. In FT-IR spectroscopy, the absorption spectra at 1600–1800 cm^{-1} correspond to the amide I band, 1470–1570 cm^{-1} to the amide II band, and 3200–3500 cm^{-1} to the N-H band.² Both peptides **7A** and **7B** exhibited characteristic bands at 3293 and 3291 cm^{-1} , respectively, indicating a strong intermolecular hydrogen bonding NH group. Additionally, bands at 3400 and 3399 cm^{-1} suggested that the NH group did not participate in intermolecular hydrogen bonding in both cases. Peptide **7A** exhibited amide I bands at 1727 and 1642 cm^{-1} and amide II bands at 1542 and 1517 cm^{-1} (Figure 7.8a). Peptide **7B** showed amide I bands at 1727 and 1643 cm^{-1} and amide II bands at 1545 and

1517 cm^{-1} (Figure 7.8c). The results suggested that both peptides adopted a β -sheet arrangement via intermolecular hydrogen bonding in the solid state.

We conducted the FT-IR experiment of freeze-dried incubated peptides (1.5 mM, at 37 $^{\circ}\text{C}$, in 1:1 acetonitrile-water for four days). For peptide **7A**, amide I bands were observed at 1728 and 1644 cm^{-1} , and amide II bands at 1545 and 1520 cm^{-1} (Figure 7.8b). For peptide **7B**, amide I bands appeared at 1727 and 1644 cm^{-1} , and amide II bands at 1542 and 1518 cm^{-1} (Figure 7.8d). N-H bands for peptide **7A** appeared at 3397 and 3291 cm^{-1} , and N-H bands for peptide **7B** appeared at 3399 and 3293 cm^{-1} . For both peptides, the bands at 3291 and 3293 cm^{-1} indicated intermolecular hydrogen-bonded N-H. These results suggested that both peptides formed a β -sheet structure in solution through self-assembly, similar to the solid state.

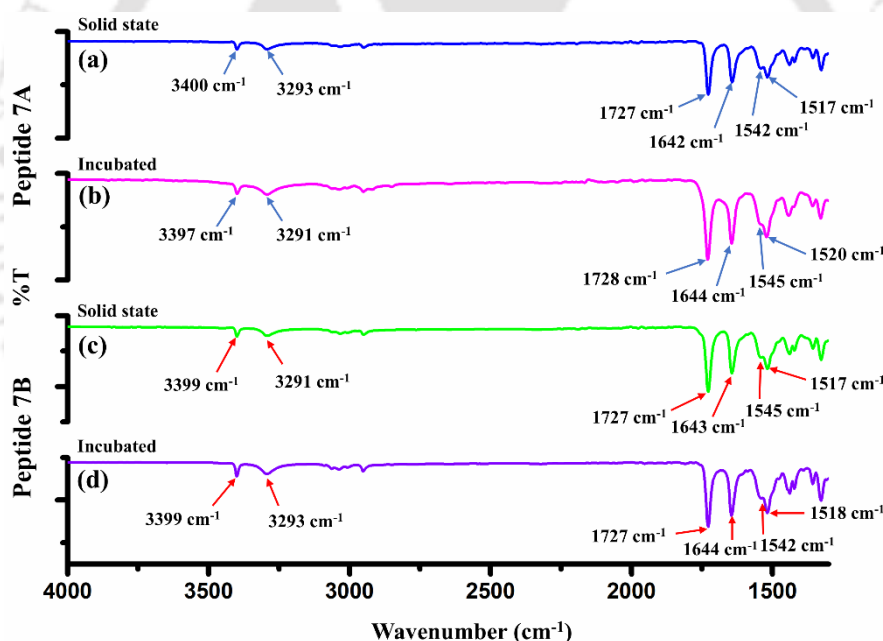


Figure 7.8. FT-IR spectra of peptides (**7A**, **7B**) in solid state (a, c) and freeze-dried (b, d), respectively.

7.7. Conformation analysis by CD experiment in solution

To analyze the conformations of peptides **7A** and **7B** in solution, we conducted Circular Dichroism (CD) spectroscopy. Peptide solutions were incubated for four days (1.5 mM) at 37 $^{\circ}\text{C}$ in 1:1 acetonitrile-water, then diluted to 375 μM to prevent high voltage. In the CD spectra, peptide **7A** showed positive bands at 217 nm and 199 nm, while peptide **7B**

exhibited negative bands at 217 nm and 198 nm, likely due to the presence of inverse chirality (D-Phg) (Figure 7.9). Thorough examination of the CD analysis revealed that peptide **7A** adopted a combination of β -turn and β -sheet structures in solution.

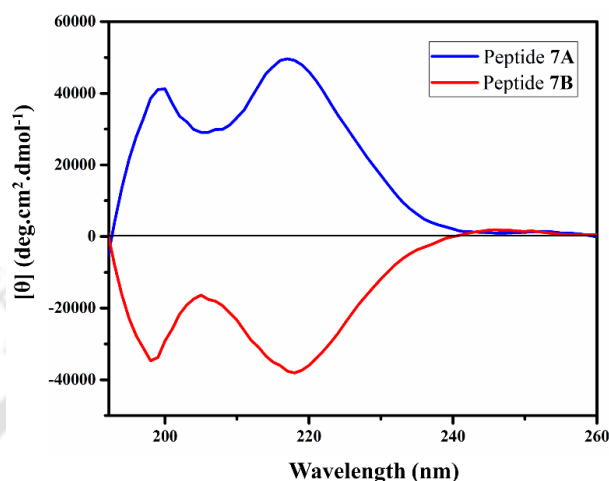


Figure 7.9. CD spectra of peptide **7A** (blue curve) and peptide **7B** (red curve), respectively.

7.8. DLS study

Next, we examined the dynamic light scattering (DLS) study to determine the size of vesicles formed in a 1:1 acetonitrile-water mixture. After four days of incubation, 1.5 mM peptide samples **7A** and **7B** formed nanovesicles with diameters of approximately 721 nm and 566 nm, respectively (Figure 7.10). These sizes nearly matched those obtained from TEM and AFM experiments, indicating well-defined nano vesicles.

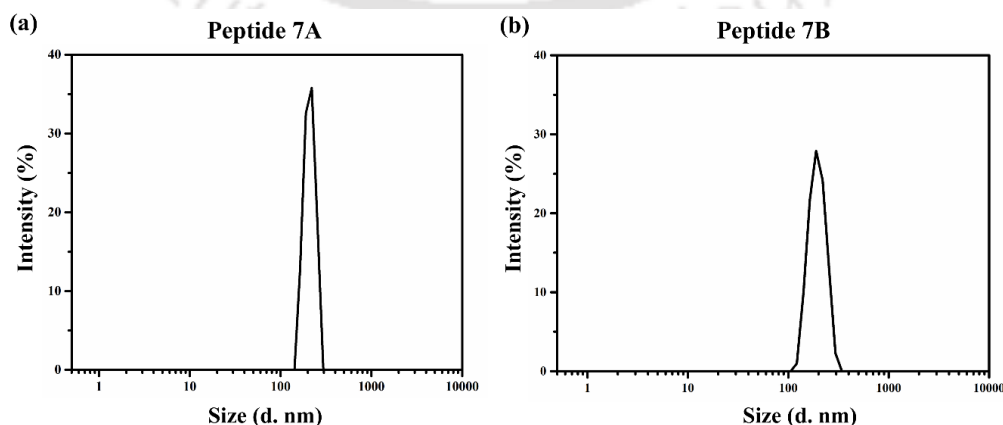


Figure 7.10. (a, b) DLS experiment and PDI values 0.984 and 0.818 of peptides **7A** and **7B**, respectively.

7.9. Investigation of thermal stabilities of peptide morphologies

We investigated the thermal stability of peptide nanostructures using the FESEM experiment. 1.5 mM four days incubated peptides (**7A**, **7B**) were drop-casted onto the silicon wafer pieces and heated in a convection oven at 100 °C and 150 °C for 1 hour. FESEM images showed that both peptides (**7A**, **7B**) maintained their morphologies at 100 °C and 150 °C (Figure 7.11). Such stability of the nanovesicles upon dry heating was likely due to their specific packing pattern and intermolecular hydrogen bonding in the crystalline state.

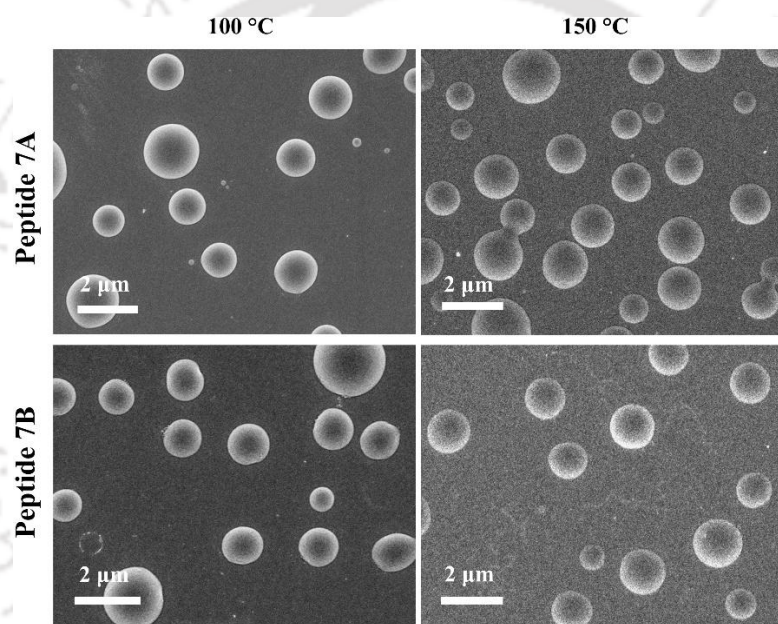


Figure 7.11. Thermal stability of the peptides (**7A**, **7B**) morphologies at 100 °C and 150 °C.

7.10. Investigation of thermal stability of peptides using TGA

To determine the thermal stability of the designed peptides, we conducted the TGA analysis of incubated peptides (1.5 mM in 1:1 acetonitrile-water, four days at 37 °C). In TGA, the weight loss observed at 25–200 °C is due to the evaporation of absorbed solvent/water molecules, and at 200–500 °C is due to the thermal degradation of peptide bonds. Peptides **7A** and **7B** showed weight loss near 196 °C and 173 °C, respectively, indicating the release of water/solvent molecules. Significant mass loss around 250 °C suggested substantial thermal stability of both peptides (Figure 7.12).

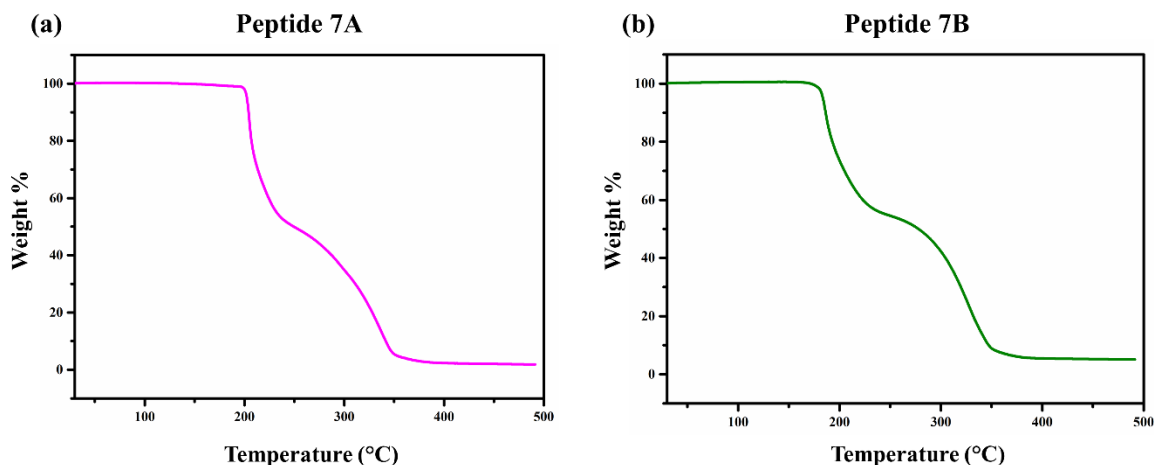


Figure 7.12. (a, b) TGA profile of peptides 7A and 7B, respectively.

7.11. DFT study

We performed the DFT study to optimize the peptide structures and correlate them with SC-XRD results. We calculated the HOMO–LUMO energies using the B3LYP/6-31G(d,p) basis set with Gaussian 09W software.³ The equal energy gap ($\Delta E = 5.018$ eV) between the HOMO and LUMO of peptides 7A and 7B suggested equal stability. The optimized structures of both peptides (7A, 7B) aligned with their crystalline conformations (Figure 7.13).

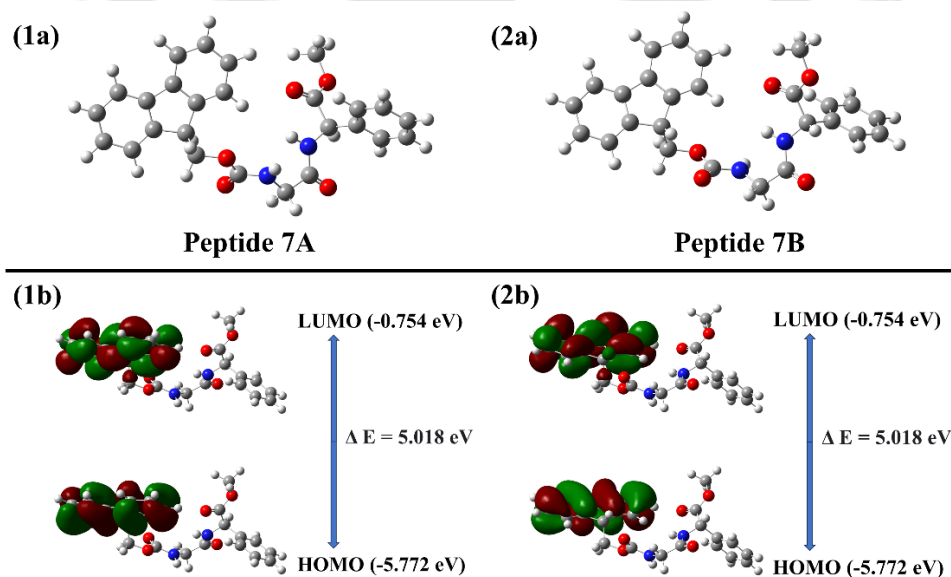


Figure 7.13. Optimized structures (1a, 2a), HOMO–LUMO (1b, 2b) gap of peptides (7A, 7B), respectively.

7.12. Drug and dye binding study

Dye-bound peptides are important tools for exploring protein structures and studying biological interactions.^{4, 5} Recent research has focused on binding drugs and dyes with bioactive peptides for various biomedical applications.⁶ Here, the binding capabilities were confirmed using curcumin (an anticancer drug) and Rhodamine B (a bioactive dye). Peptide solutions (1.5 mM) in 1:1 acetonitrile-water mixture were incubated with curcumin (50 μ M) and Rhodamine B (5 μ M) for four days at 37 °C. Subsequently, the samples were filtered, drop-casted onto glass slides, and dried, and then fluorescence images were captured. The bright green and red fluorescence from the peptide vesicles clearly demonstrated the successful binding of the drug and dye (Figure 7.14).

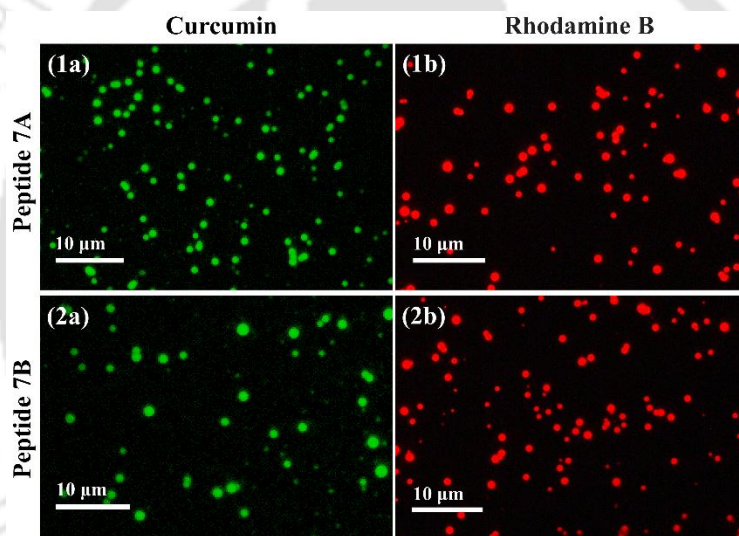


Figure 7.14. Fluorescence microscopic images of peptides (7A, 7B) strained with curcumin (1a, 2a) and rhodamine b (1b, 2b), respectively.

7.13. Metal ion sensing study

Supramolecular chemistry has advanced biosensor development for ion detection⁷, especially metal ions, due to their biological and environmental impact. Toxic heavy metals like Cr^{6+} are major pollutants, causing health issues.⁸ Traditional detection methods like atomic absorption spectrometry (AAS), electrochemical methods, and colorimetry are costly and complex.⁹ Fluorescence sensing methods offer a selective, sensitive, and cost-effective alternative. Although macrocyclic ligands have been

extensively studied, small peptide-based sensors for detecting heavy metal ions remain largely unexplored.

7.13.1. UV-Vis and fluorescence spectroscopy

This study highlights the selectivity and sensitivity of small peptide-based systems toward Cr^{6+} ions through fluorescence quenching. Initially, we recorded the UV-visible absorption spectra of peptides **7A** and **7B** in a 1:1 acetonitrile-water mixture at room temperature, finding an absorption maximum at 265 nm (Figure 7.22). Using this excitation wavelength, we recorded the emission spectra in a region of 280 nm to 500 nm, revealing prominent emission peaks at 306, 312, and 315 nm (Figure 7.16, 1a, 2a).

7.13.2. Screening of different metal ions for sensing

Next, we recorded the fluorescence spectra of the peptides (**7A** and **7B**) in the presence of various metal ions (Cd^{2+} , Na^+ , Co^{2+} , Al^{3+} , K^+ , Cr^{6+} , Ag^+ , Ni^{2+} , Ca^{2+} , Mn^{2+} , Mg^{2+} , Li^+ , Cu^{2+} , Cr^{3+}). Mixing 50 μM peptides (**7A**, **7B**) with 320 μM different metal ions showed no significant change in emission spectra, except for Cr^{6+} , which caused a sharp decrease in the emission intensity for both peptides (Figure 7.15). This suggested that the peptides can serve as fluorescent probes for Cr^{6+} ions. The quenching efficiency was 78.15% for peptide **7A** and 78.80% for peptide **7B**, highlighting their high selectivity for Cr^{6+} .

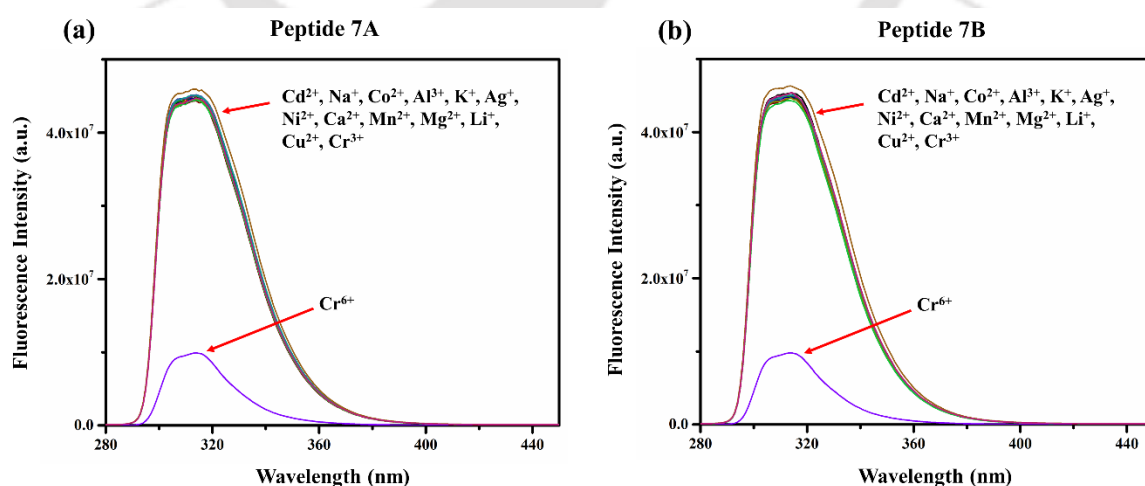


Figure 7.15. Emission spectra of (a) peptide **7A** (50 μM) and (b) peptide **7B** (50 μM) upon the presence of various metal ions of a constant concentration (320 μM).

7.13.3. Fluorescence titration experiment for sensing of Cr^{6+} and Stern-Volmer plot

To further explore the sensing capabilities of peptides **7A** and **7B** for Cr^{6+} ion, we recorded their fluorescence spectra with varying Cr^{6+} concentrations (0 to 840 μM) at room temperature. As the Cr^{6+} concentration increased in the 50 μM peptides (**7A**, **7B**), the fluorescence intensities gradually decreased (Figure 7.16, 1a, 2a). At 840 μM Cr^{6+} , the fluorescence of peptides **7A** and **7B** was quenched by 99.28% and 99.23%, respectively.

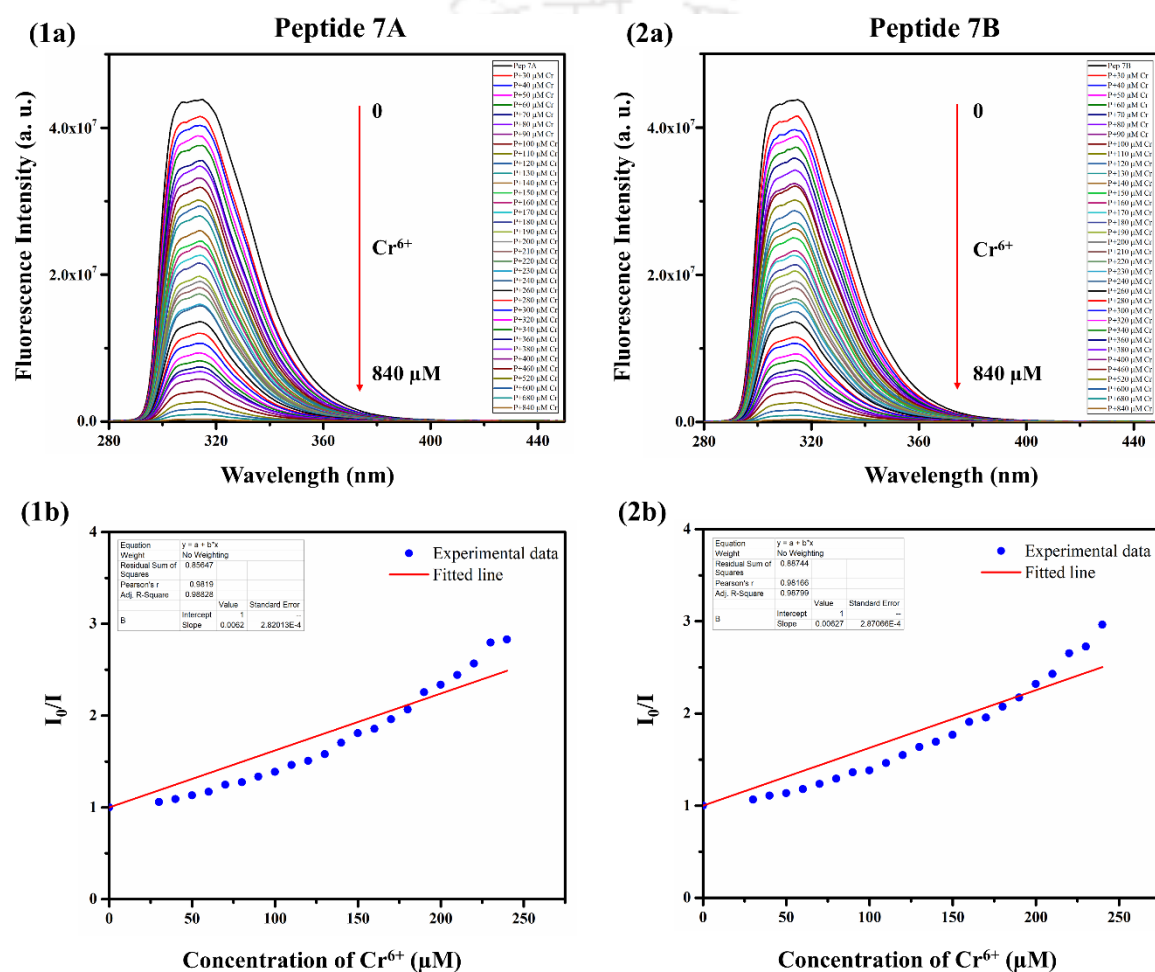


Figure 7.16. Fluorescence quenching of (1a) peptide **7A** and (1b) peptide **7B** at 50 μM concentration with varying Cr^{6+} concentrations (0–840 μM). Stern-Volmer plots for quenching of (1b) peptide **7A** and (2b) peptide **7B** by Cr^{6+} ion.

To analyze the quenching behavior of peptides **7A** and **7B** with varying Cr^{6+} concentrations, we used the Stern-Volmer equation: $I_0/I = 1 + K_{\text{sv}}[Q]$, where I_0 and I are the fluorescence intensities without and with Cr^{6+} , respectively, and K_{sv} is the Stern-

Volmer quenching constant. The plot of I_0/I against Cr^{6+} concentrations (Figure 7.16, 1b, 2b) showed a linear quenching trend for both peptides. The K_{SV} values for peptides **7A** and **7B** were calculated to be $6.2 \times 10^3 \text{ M}^{-1}$ and $6.27 \times 10^3 \text{ M}^{-1}$, respectively.

7.13.4. UV-Vis titration experiment for sensing of Cr^{6+}

The UV-Vis absorption behavior of peptides **7A** and **7B** with Cr^{6+} was recorded at room temperature. Peptide solutions ($50 \mu\text{M}$) were titrated with Cr^{6+} ($0\text{--}840 \mu\text{M}$). Without Cr^{6+} , Fmoc dipeptides showed bands at $234\text{--}305 \text{ nm}$ but none at $307\text{--}400 \text{ nm}$. Adding Cr^{6+} resulted in a new band at $307\text{--}400 \text{ nm}$, which increased with increasing Cr^{6+} concentrations (Figure 7.22), indicating favorable interactions with Cr^{6+} .

7.13.5. Screening anions for sensing

The interaction of peptides ($50 \mu\text{M}$) with various anions was studied using sodium salts ($320 \mu\text{M}$) of ions such as CH_3O^- , F^- , NO_3^- , HCO_3^- , SCN^- , NO_2^- , Br^- , CO_3^{2-} , Cl^- , $\text{S}_2\text{O}_3^{2-}$, N_3^- . No significant changes in the emission spectra of peptides **7A** and **7B** were observed, indicating that anions do not participate in the quenching process (Figure 7.17).

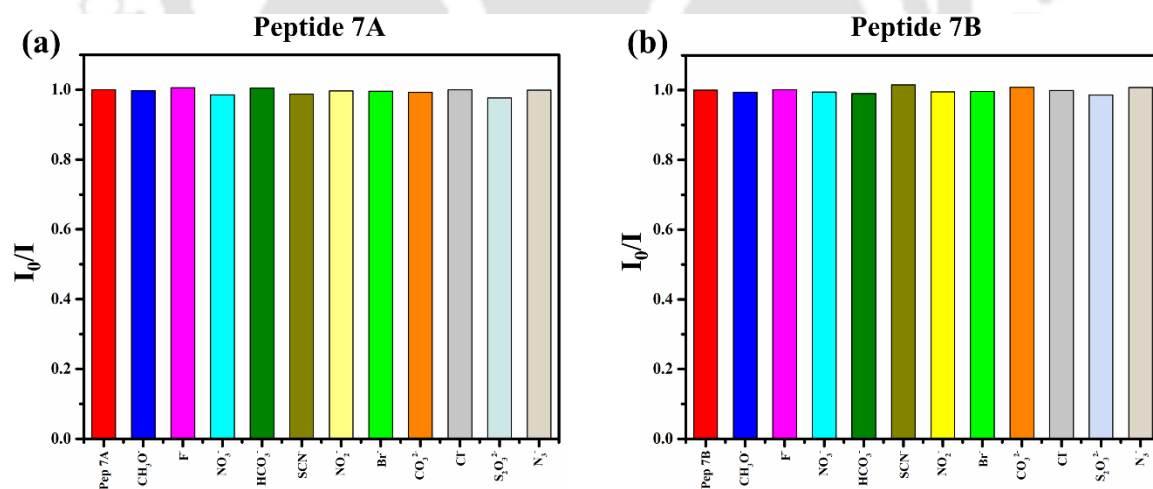


Figure 7.17. Fluorescence response (a, b) of peptides **7A** and **7B** ($50 \mu\text{M}$) with various anions ($320 \mu\text{M}$).

7.14. Conclusion

In our research, we explored the self-assembly of enantiomeric dipeptides: Fmoc-Gly-L-Phg-OMe (**7A**) and Fmoc-Gly-D-Phg-OMe (**7B**). We observed that both peptides formed

hollow vesicular structures in acetonitrile-water (1:1) mixture. The nanostructures formed homogeneously without any issues. Both peptides adopt a parallel β -sheet structure, creating void spaces within their higher-order crystal packing. These void spaces support the formation of hollow vesicles in solution. Interestingly, the enantiomeric dipeptides **7A** and **7B** formed right-handed and left-handed helices, respectively, through weak C–H...O interactions. Remarkably, both peptide nanostructures exhibited stability against dry heating and could bind with bioactive drugs and dyes. Additionally, they served as fluorescent probes for Cr^{6+} ions.

7.15. Experimental section

7.15.1. Materials and instrumentations

As described in chapter 8

7.15.2. General procedure for synthesizing dipeptides

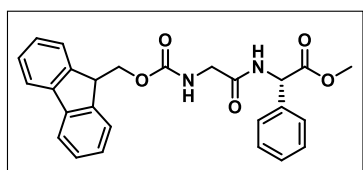
As described in chapter 5, in section 5.9.2.

7.15.3. Sample preparation

Peptide samples (1.5 mM) were prepared in 1:1 acetonitrile-water solution and incubated at 37 °C for four days. The incubated samples were used for various analysis. For the sensing study, stock solutions (2 mM) of various metal ions (Cd^{2+} , Na^+ , Co^{2+} , Al^{3+} , K^+ , Ni^{2+} , Ca^{2+} , Li^+ , Cu^{2+} from chloride salts; Mn^{2+} , Ag^+ from acetic acid salts; Mg^{2+} from sulfate salt; Cr^{3+} from nitrate salt; and Cr^{6+} from dichromate salt) and Na-salts of anions (1 mM) were prepared in 50% acetonitrile-water mixture. Additionally, 1 mM stock solutions of peptides **7A** and **7B** were prepared in 50% acetonitrile-water.

7.16. Characterization data

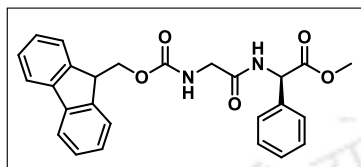
Fmoc-Gly-L-Phg-OMe (**7A**):



Yield: 93% (413 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.37 – 7.27 (m, 7H), 7.12 (s, 1H), 5.56 (dd, J =

16.7, 6.4 Hz, 2H), 4.38 (d, $J = 7.1$ Hz, 2H), 4.21 (t, $J = 7.3$ Hz, 1H), 4.03 – 3.87 (m, 2H), 3.71 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.27, 168.52, 156.77, 143.93, 141.48, 136.18, 129.25, 128.92, 127.94, 127.44, 127.29, 125.26, 120.19, 67.51, 56.67, 53.13, 47.24, 44.53; HRMS (ESI) m/z : calcd for $\text{C}_{26}\text{H}_{25}\text{N}_2\text{O}_5$, 445.1758 $[\text{M}+\text{H}]^+$; found 445.1758.

Fmoc-Gly-D-Phg-OMe (7B):

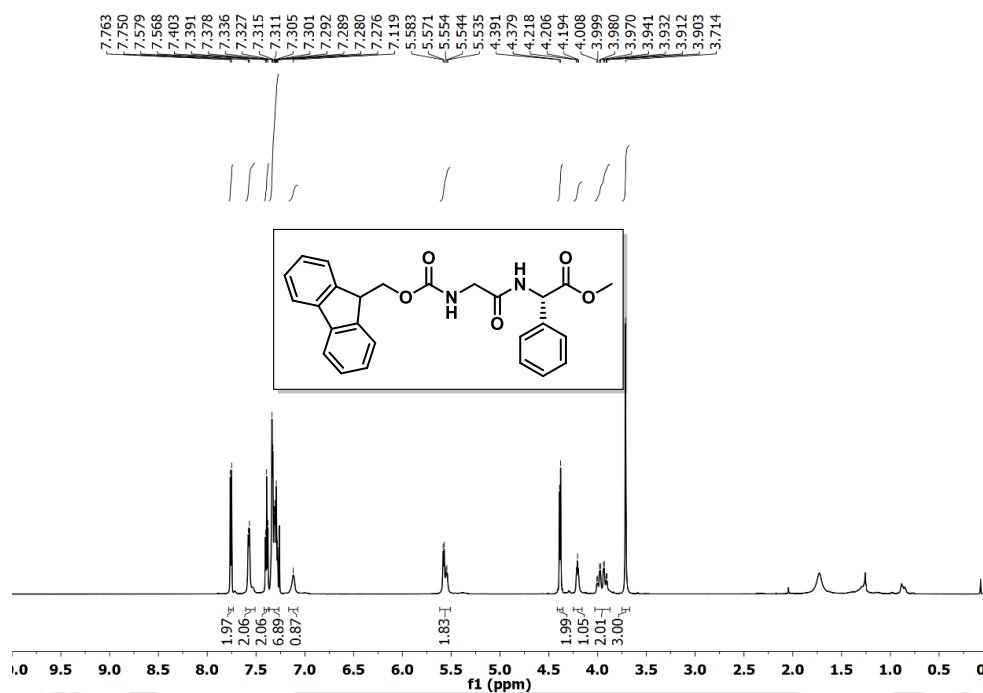
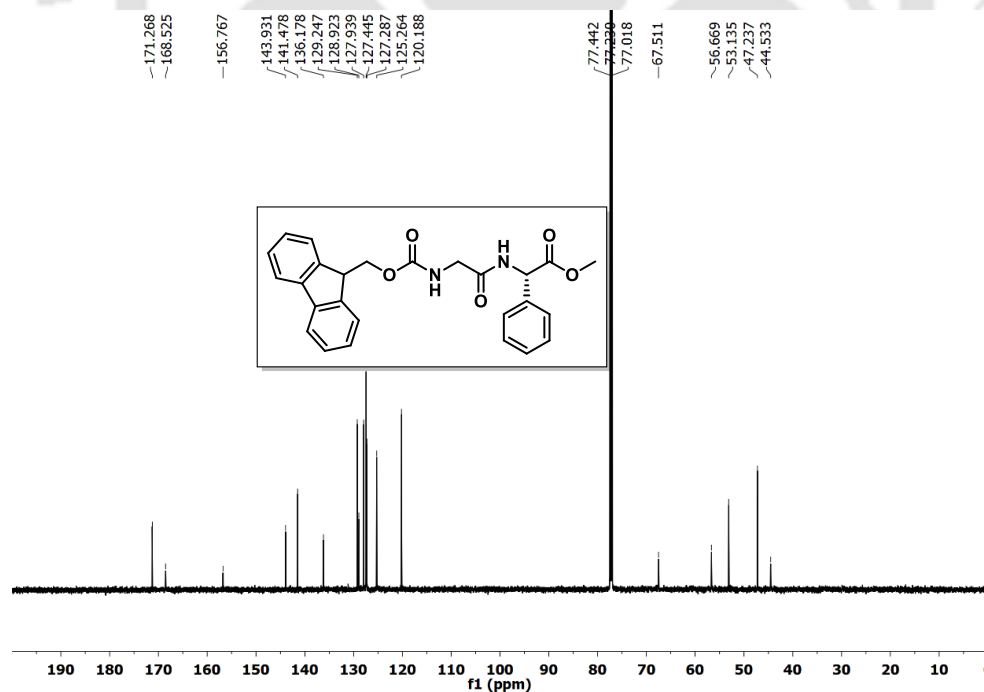


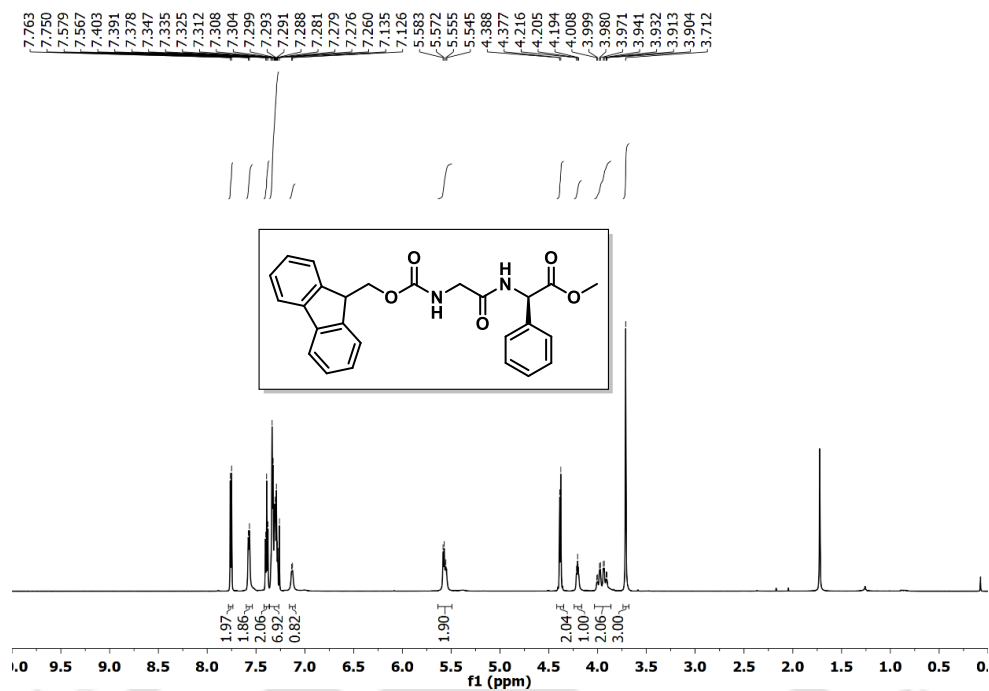
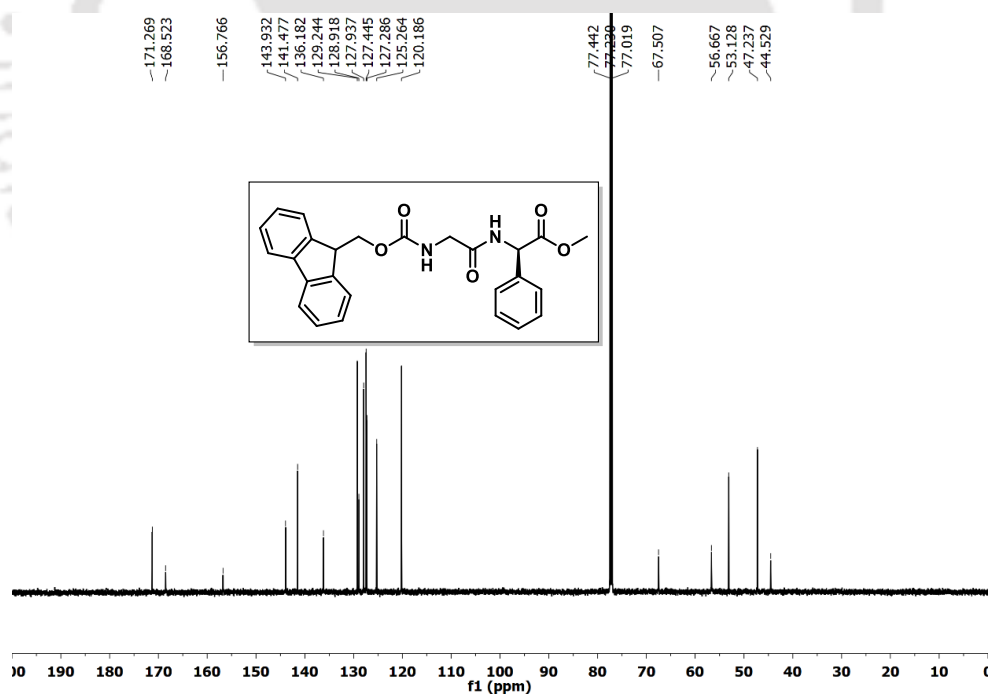
Yield: 92% (409 mg); white solid; ^1H NMR (600 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.57 (d, $J = 7.1$ Hz, 2H), 7.39 (t, $J = 7.4$ Hz, 2H), 7.36 – 7.27 (m, 7H), 7.16 – 7.10 (m, 1H), 5.64 – 5.49 (m, 2H), 4.38 (d, $J = 6.8$ Hz, 2H), 4.21 (t, $J = 6.6$ Hz, 1H), 4.03 – 3.86 (m, 2H), 3.71 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ 171.27, 168.52, 156.77, 143.93, 141.48, 136.18, 129.24, 128.92, 127.94, 127.44, 127.29, 125.26, 120.19, 67.51, 56.67, 53.13, 47.24, 44.53; HRMS (ESI) m/z : calcd for $\text{C}_{26}\text{H}_{25}\text{N}_2\text{O}_5$, 445.1758 $[\text{M}+\text{H}]^+$; found 445.1759.

7.17. References

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7.18. Selected spectra

Figure 7.18. ¹H NMR spectrum of peptide 7AFigure 7.19. ¹³C NMR spectrum of peptide 7A

Figure 7.20. ¹H NMR spectrum of peptide 7BFigure 7.21. ¹³C NMR spectrum of peptide 7B

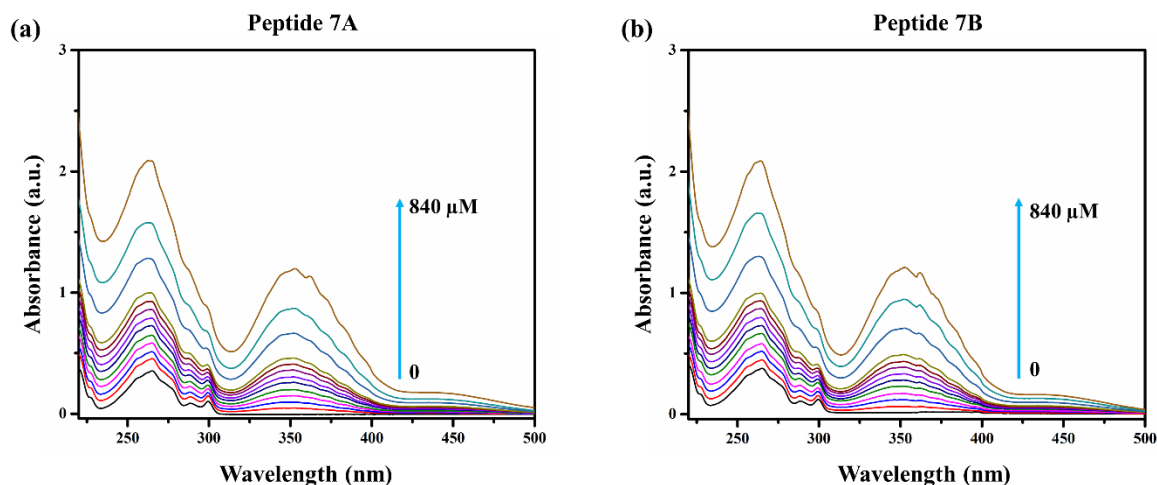


Figure 7.22. UV-Vis spectra of peptides 7A and 7B (50 μM) with increasing Cr^{6+} ions (0–840 μM).

7.19. Crystallographic data

Table 7.1. List of torsion angles (deg) of peptides 7A and 7B

Peptides	$\phi 1$	$\psi 1$	$\phi 2$	$\psi 2$	$\omega 1$	$\omega 2$
Peptide 7A	C15-N1-C16-C17 = 120.3(4)	N1-C16-C17-N2 = 7.4(5)	C17-N2-C18-C19 = 158.7(4)	N2-C18-C19-O5 = 158.2(4)	O1-C15-N1-C16 = 177.4(3)	C16-C17-N2-C18 = 178.2(4)
Peptide 7B	C15-N1-C16-C17 = 120.1(7)	N1-C16-C17-N2 = 7.6(9)	C17-N2-C18-C19 = 158.1(6)	N2-C18-C19-O5 = 158.1(6)	O1-C15-N1-C16 = 177.0(6)	C16-C17-N2-C18 = 178.2(6)

Table 7.2. Hydrogen bonding distances (Å) and Bond angles ($^\circ$) of peptides (7A, 7B)

Peptide	D-H $\cdots$ A	$d(\text{D}\cdots\text{H})/\text{\AA}$	$d(\text{H}\cdots\text{A})/\text{\AA}$	$d(\text{D}\cdots\text{A})/\text{\AA}$	$\angle \text{D-H}\cdots\text{A}/^\circ$
Peptide 7A	N1-H1 $\cdots$ O3	0.86	2.16	3.009(5)	170
	C14-H14A $\cdots$ O2	0.97	2.26	3.169(6)	155
	C16-H16A $\cdots$ O1	0.97	2.58	3.402(5)	143
	C16-H16B $\cdots$ O3	0.97	2.35	3.195(5)	145
	C20-H20B $\cdots$ O2	0.96	2.46	3.256(8)	140
Peptide 7B	N1-H1 $\cdots$ O3	0.86	2.15	3.006(8)	170
	C14-H14B $\cdots$ O2	0.97	2.27	3.168(9)	154
	C16-H16A $\cdots$ O3	0.97	2.35	3.198(9)	145
	C16-H16B $\cdots$ O1	0.97	2.58	3.404(9)	143
	C20-H20B $\cdots$ O2	0.96	2.40	3.264(11)	150

Table 7.3. Crystal parameters and refinement data of peptides 7A and 7B

Parameters	Peptide 7A	Peptide 7B
Formula	C ₂₆ H ₂₄ N ₂ O ₅	C ₂₆ H ₂₄ N ₂ O ₅
Fw	444.47	444.47
Crystal system	orthorhombic	orthorhombic
Space group	<i>P</i> 21 21 21	<i>P</i> 21 21 21
<i>a</i> /Å	5.5494(9)	5.5501(6)
<i>b</i> /Å	18.527(3)	18.537(2)
<i>c</i> /Å	21.867(4)	21.874(3)
α /°	90.00	90.00
β /°	90.00	90.00
γ /°	90.00	90.00
<i>V</i> /Å ³	2248.2(6)	2250.5(4)
<i>Z</i>	4	4
<i>D</i> _c /g cm ⁻³	1.313	1.312
μ Mo K α /mm ⁻¹	0.092	0.092
<i>F</i> ₀₀₀	936	936
<i>T</i> /K	296(2)	297(2)
θ max.	26.36	25.04
Total no. of reflections	57718	52357
Independent reflections	3967	3753
Observed reflections	2896	2598
Parameters refined	299	299
<i>R</i> ₁ , <i>I</i> > 2 σ (<i>I</i>)	0.0615	0.0940
<i>wR</i> ₂ , <i>I</i> > 2 σ (<i>I</i>)	0.1488	0.1999
GOF (<i>F</i> ²)	1.214	1.365
CCDC No.	2376387	2376388

Chapter 8

Materials and Instrumentations



8.1. Materials

All amino acids (protected and unprotected), Oxyma, 2-nitrobenzenesulfonyl chloride, and Rink amide MBHA resin (loading 0.7 mmol/g) were sourced from GL Biochem (Shanghai). 5-Chlorothiophene-2-sulfonyl chloride, HOBt, HOAt, DIPEA, and various aromatic and aliphatic acids, amines, and alcohols were purchased from Spectrochem (India), SRL (India), or Loba (India). Other thiophenesulfonyl or carbonyl chlorides, H-Leu-OtBu.HCl, and boronic acids were purchased from BLDpharm (India). Trifluoroacetic acid, citric acid, NaHCO₃, DMSO-*d*₆, ACN-*d*₃, DMF, DMSO, HPLC grade ACN, HPLC grade hexane, HPLC grade isopropanol, and normal solvent grade hexane, MeOH, CHCl₃, EtOAc and THF were procured from Merck (India). Fluorescein, rhodamine B, thioflavin T, 5(6)-carboxyfluorescein, curcumin, CDCl₃, and metal catalysts were sourced from Sigma Aldrich.

8.2. Instrumentations

8.2.1. Chromatography techniques

Reaction progress was tracked utilizing TLC on Silica gel 60 F₂₅₄ TLC plate with EtOAc/Hexane as the eluent. The crudes were purified over silica gel (60-120 mesh) column chromatography utilizing EtOAc/Hexane as the eluents.

8.2.2. High-performance liquid chromatography (HPLC)

The purification of solid-phase peptides was done using a Thermo Scientific Dionex S2 UltiMate 3000 Rapid Separation LC (RSLC) system connected with a C18(2) Phenomenex column. Analytical HPLC was employed to verify the purity of the peptides. An Agilent 1260 Infinity II Prime LC system connected with a CHIRALPAK® AS-H column (5 μm, 4.6×250 mm) was used for chiral HPLC. A binary solvent system of water and acetonitrile was used for the reverse phase, and hexane and isopropanol were used for the normal phase. Wavelengths of 214 nm and 254 nm were utilized for HPLC.

8.2.3. Solvent evaporation technique

All solvents were evaporated utilizing a Buchi rotary evaporator under vacuum. The HPLC-purified peptides were freeze-dried using a Labconco lyophilizer.

8.2.4. Mass spectrometry

Mass samples were prepared using ACN/H₂O solvent. High-resolution mass spectra (HRMS) of the synthesized compounds were obtained utilizing an electrospray ionization time-of-flight (ESI-TOF) mass spectrometer. MALDI-TOF mass spectra of the synthesized peptides were acquired utilizing a Bruker autoflex speed instrument.

8.2.5. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra of the synthesized compounds were recorded using CDCl₃, DMSO-*d*₆, and ACN-*d*₃ solvents on a 600 MHz or 400 MHz Bruker Ascend instrument. Calibration was performed using tetramethylsilane (Si(CH₃)₄: 0.00 ppm) or residual undeuterated solvent (CHCl₃: 7.260 ppm) for ¹H NMR and deuterated chloroform (CDCl₃: 77.230 ppm) for ¹³C NMR. For DMSO-*d*₆, the reference values were 2.50 ppm for ¹H NMR and 39.50 ppm for ¹³C NMR; and for ACN-*d*₃, 1.94 ppm for ¹H NMR and 118.26 ppm for ¹³C NMR. Coupling constants (*J*) were noted in Hz and chemical shifts in ppm (parts per million).

8.2.6. Single crystal X-ray diffraction (SC-XRD)

Single crystal XRD diffraction data was recorded on Bruker D8 QUEST system using MoK α X-ray radiation at 297K. The crystal structures of the molecules were obtained by solving the data using 'Bruker APEX2' and 'Bruker SAINT' software. The data refinement was done using the XShell 6.3.1 software.

8.2.7. Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of all compounds were obtained utilizing a PerkinElmer UATR Two FT-IR spectrometer, covering the wavelength range of 4000 to 400 cm⁻¹. This technique did not involve the use of KBr pellets. The final IR data plots were generated using OriginPro 15 software.

8.2.8. Melting point

The melting points of the compounds were determined using a Buchi MP apparatus.

8.2.9. Circular dichroism (CD)

The CD spectrum of the experimental peptides was obtained utilizing a JASCO J-1500 instrument within a wavelength range of 190 to 260 nm, with a path length and bandwidth of 1 mm. The reported data represent the average of three measurements. The CD plots of molar ellipticity versus wavelength were then generated. The formula mentioned below was applied to determine the mean residue molar ellipticity:

$$\theta \text{ (deg. cm}^2\text{.dmol}^{-1}\text{)} = \text{Ellipticity (mdeg). } 10^6 / \text{Pathlength (mm). [Protein] } (\mu\text{M}). N$$

8.2.10. Powder X-ray diffraction (PXRD)

The powder XRD data of the samples were obtained using a 9KW Powder X-Ray Diffraction System (Rigaku SmartLab).

8.2.11. Thermogravimetric analysis (TGA)

Peptide samples of 1.5 mM were incubated at 37 °C for 4 days, freeze-dried, and then subjected to thermogravimetric analysis. The analysis was conducted using a PerkinElmer TGA 4000 analyzer at a thermal rate of 10 °C/min under a nitrogen atmosphere.

8.2.12. Optical microscopic images

Optical bright field images of the samples were taken using an Olympus BX 51 microscope, employing various magnifications.

8.2.13. Field emission scanning electron microscopy (FESEM)

Peptide samples of 1.5 mM, incubated for four days, were drop-casted (10 μ L) onto aluminium foil-coated small glass pieces or silicon wafers and dried in a desiccator at room temperature. The FESEM images of the dried samples were captured utilizing SIGMA-300 (ZEISS) and Gemini-300 (ZEISS) instruments.

8.2.14. Field emission transmission electron microscopy (FETEM)

Freshly prepared 100 μ M peptide samples, obtained by diluting four days of incubated 1.5 mM peptides, were drop-casted onto TEM grids (carbon-coated copper grids). After floating for 1 minute, 10 μ L of a 2% uranyl acetate solution was introduced to each grid

and floated for another minute. Excess solvent was absorbed using tissue paper, and the grids were dried in a desiccator at room temperature. TEM images of the samples were captured using a JEOL 2100F instrument.

8.2.15. Atomic force microscopy (AFM)

Peptide solutions of 1.5 mM were incubated for four days and then diluted to a concentration of 30 μM . Then, 10 μL of each diluted sample was gently placed onto clean glass pieces and allowed to dry in a desiccator. Atomic force microscopy (AFM) images were then captured using the Oxford Cypher instrument.

8.2.16. Fluorescence imaging of dye and drug-binding peptides

Peptide solutions of 1.5 mM concentration were combined with various dyes and drugs of different concentrations (Fluorescein (100 μM), Thioflavin T (100 μM), Rhodamine B (10 μM or 5 μM), 5(6)-Carboxyfluorescein (100 μM), and Curcumin (50 μM)) and incubated at 37 $^{\circ}\text{C}$ for four days. Subsequently, 10 μL of each incubated sample was gently placed onto clean glass slides and dried in a desiccator at room temperature. Fluorescence microscopy images were then captured using an Olympus BX 51 fluorescence microscope.

8.2.17. Dynamic light scattering (DLS)

DLS experiments were performed using four days of incubated peptide samples (1.5 mM in 50% acetonitrile-water at 37 $^{\circ}\text{C}$) with the Nano-ZS90 Malvern instrument.

8.2.18. Density functional theory (DFT) study

The HOMO–LUMO gaps of the compounds were determined using density functional theory (DFT). These calculations were done with the B3LYP/6-31G(d,p) basis set, utilizing Gaussian 09W software.

8.2.19. UV-Vis and Fluorescence spectroscopy

PerkinElmer Lambda 365+ spectrometer was used to record the UV/Vis spectra of the compounds at room temperature. Fluorescence measurements were carried out using a HORIBA Scientific FluroMax-4 spectrofluorometer.

Conclusions and Future Directions

Conclusions

The works presented in this thesis focus on the development of novel coupling reagents and their applications. Additionally, the self-assembly of dipeptides and their applications are also described. The thesis overview is depicted in Scheme 1.

In chapter 1, we described the significance of the aforementioned compounds, their existing synthetic methods, and the associated drawbacks. We also discussed the self-assembly of small peptides and metal ion sensing.

In chapter 2, we developed Oxyma-based novel recyclable coupling reagent in a faster, eco-friendly manner. We demonstrated its applications in the racemization-free synthesis of amides, esters, thioesters, hydroxamates, and peptides using greener ways. The reagent showed equal effectiveness in both solution and solid-phase peptide synthesis.

In chapter 3, we developed heterocyclic novel recyclable coupling reagents and explored their applications in synthesizing amides, esters, thioesters, peptides, and nitriles with excellent yields in a sustainable manner. Interestingly, the reagent CTSOBt demonstrated equal effectiveness in both solution and solid-phase peptide synthesis.

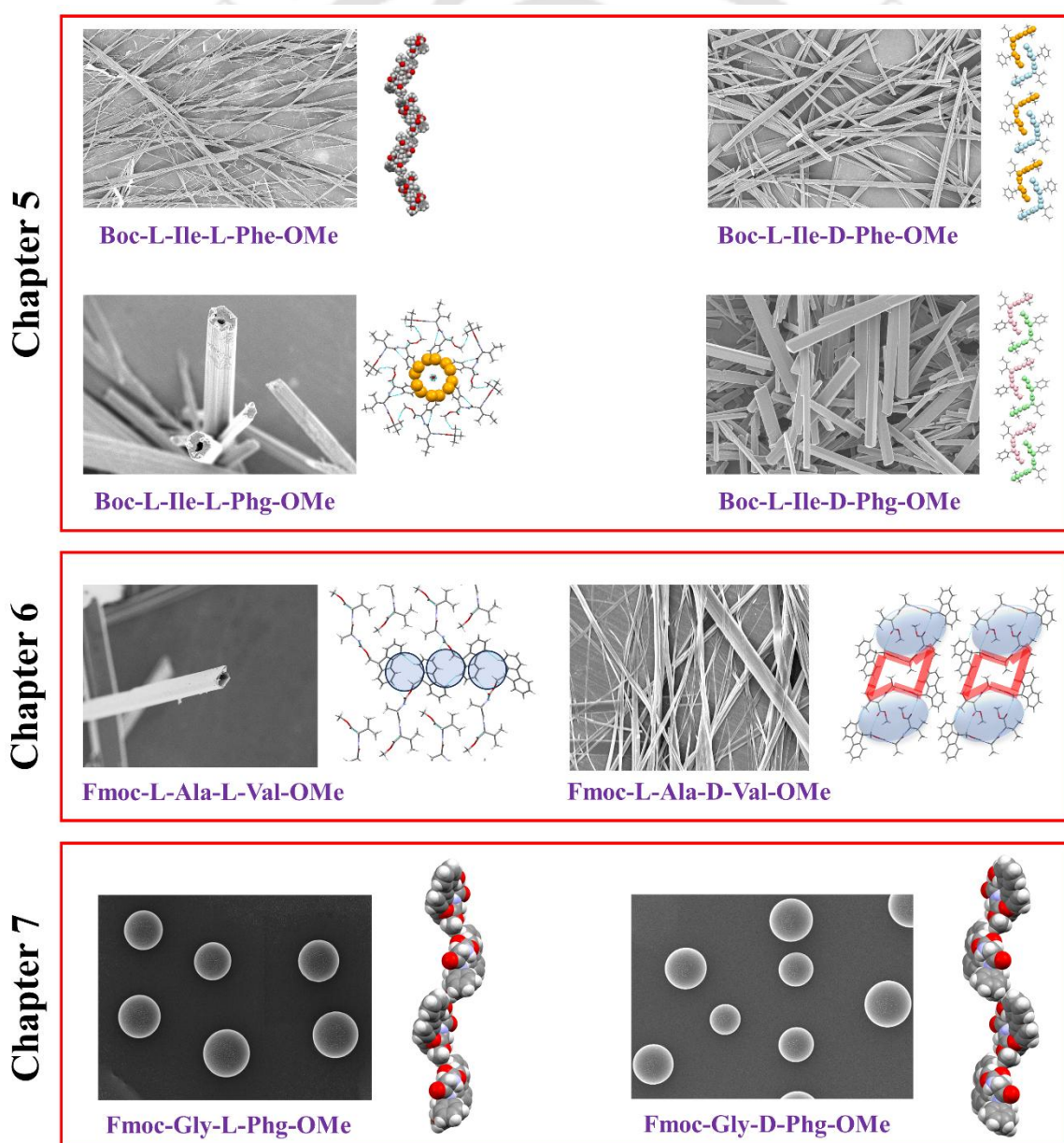
In chapter 4, we introduced a HOAt-based new recyclable coupling reagent for constructing C-N, C-O, and C-C bonds. Interestingly, this reagent helped in the direct synthesis of ketones from carboxylic acids using a Pd-catalyzed cross-coupling reaction.

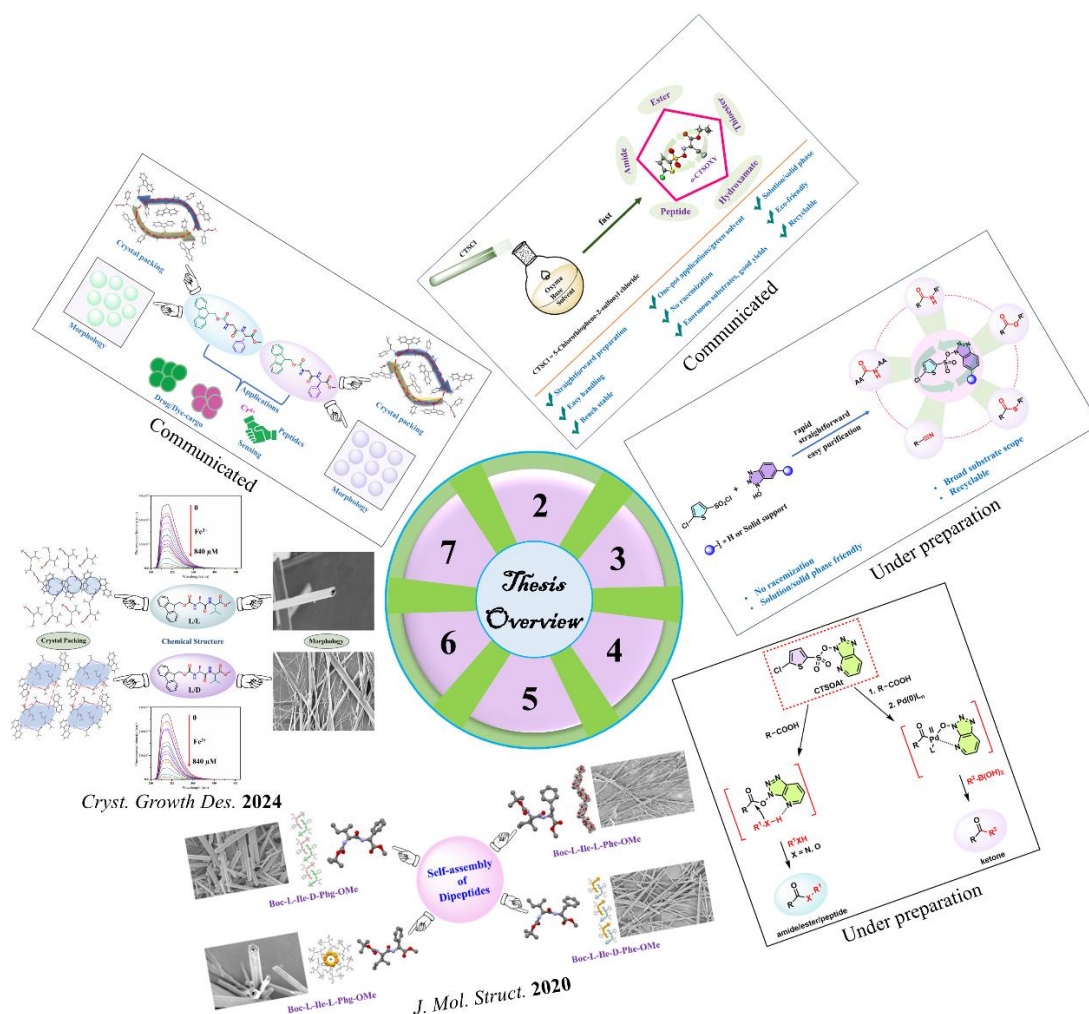
In chapter 5, we investigated the self-assembly of alternating L/L and L/D amino acid-containing dipeptides, focusing on the influence of side chains and the chirality of the amino acids. Significant changes in molecular arrangements and morphologies were observed upon altering the chirality and side chain in the sequence.

In chapter 6, we described the chirality controlled self-assembly of dipeptides and their applications in dye binding and selective sensing of Fe^{3+} . The alternating L/L and L/D amino acid-containing peptides created different morphologies. Both peptides demonstrated selectivity in sensing Fe^{3+} among various tested metal ions.

In chapter 7, we examined the self-assembly of enantiomeric dipeptides containing one chiral unnatural amino acid (L/D Phg) at the C-terminal of the sequence. Both peptides were self-assembled into nanovesicle structures in the solution. Interestingly, the formation of right-handed and left-handed helical structures was observed in crystalline state due to the alternation of chirality. In crystalline state, the formation of void spaces might be responsible for the vesicle formation in solution. Moreover, the peptides were effectively bound with bioactive drugs/dyes and exhibited the ability to sense Cr^{6+} ions.

The diagram below illustrates the chapter-wise chirality controlled self-assembly of peptides.





Scheme 1. Thesis overview

Future Directions

During my Ph.D., I developed several new, recyclable coupling reagents, such as *o*-CTSOXY, CTSOBt, P-CTSOBt, and CTSOAt. These reagents facilitate various organic transformations, including amidation, esterification, peptide and ketone synthesis. Additionally, these reagents can be used in synthesizing benzotriazoles, benzoxazoles, alcohols, acetals, and thioacetals. They can also be employed in rearrangement reactions to synthesize ureas, carbamates, thiocarbamates, and amides. The synthesis of aza-peptides and oligo- α -peptidyl ureas can also be done using these reagents.

Moreover, we explored the self-assembly of dipeptides based on chirality and side chains. Future research can be done on the self-assembly of small peptides influenced by various protecting groups, metal ions, and external factors. Higher-order peptide self-assembly and various applications, such as gas absorption and drug delivery, can be studied.

Research Outcome

Patents:

1. **Roy, S.;** Mandal, B. “A COUPLING REAGENT FOR AMIDATION, ESTERIFICATION AND PEPTIDE SYNTHESIS AND A METHOD OF PREPARATION THEREOF” as Indian patent application, application No. 202431026707 (complete application filled on the 30th March, 2024).
2. **Roy, S.;** Mandal, B. “NOVEL HETEROCYCLIC COUPLING REAGENTS, APPLICATIONS, AND METHODS THEREOF” as Indian patent application, application No. 202431027307 (complete application filled on the 2nd April, 2024).

Publications:

1. **Roy, S.;** Mandal, B. Ethyl (*E*)-2-(((5-chlorothiophen-2-yl)sulfonyl)oxy)imino)-2-cyanoacetate (*o*-CTSOXY) for Sustainable Organic Transformations (Communicated).
2. **Roy, S.;** Mandal, B. Sustainable Organic Transformations Using Novel Heterocyclic Recyclable Coupling Reagents (Under preparation).
3. **Roy, S.;** Mandal, B. CTSOAt Mediated Construction of C-N, C-O and C-C Bonds (Under preparation).
4. **Roy, S.;** Giri, R. S.; Dolai, G.; Shill, S.; Mandal, B. Vesicular Self-Assembly of Enantiomeric Helical Dipeptides and Toxic Cr⁶⁺ Sensing (Communicated).
5. Mondal, S.; **Roy, S.;** Sikder, A.; Border, A.; Paul, A.; Mandal, B. Intermolecular Ipso Aromatic Nucleophilic Substitution of Electron-Deficient Aryl Sulfonyl Chlorides. *ChemistrySelect* **2024**, e202403634.
6. **Roy, S.;** Giri, R. S.; Sarkar, A.; Mondal, S.; Mandal, B. Influence of Chirality on the Self-Assembly of Dipeptides and Sensing of Fe³⁺. *Cryst. Growth Des.* **2024**, 24, 5061-5077.
7. Dolai, G.; Shill, S.; **Roy, S.;** Mandal, B. Atomic Insight on Inhibition of Fibrillization of Dipeptides by Replacement of Phenylalanine with Tryptophan. *Langmuir* **2023**, 39, 9367-9383.

8. Dolai, G.; **Roy, S.**; Sen, S.; Giri, R. S.; Mandal, B. Crystal Structure of 1-(2,4,6-Trichlorobenzoyloxy) Benzotriazole (TCB-OBt): Observation of Uncommon Intermolecular Oxygen–Oxygen Interaction and Synthetic Application in Amidation. *New J. Chem.* **2021**, 45, 19804-19811.
9. Dolai, G.; Giri, R. S.; **Roy, S.**; Mandal, B. Crystal Structure and Supramolecular Arrangement of Heterochiral Tripeptides. *Pept. Sci.* **2021**, e24229.
10. **Roy, S.**; Giri, R. S.; Dolai, G.; Mandal, B. Role of Side-Chain and Chirality of the Amino Acids on the Supramolecular Assemblies of Dipeptides. *J. Mol. Struct.* **2020**, 1221, 128877.
11. Giri, R. S.; Pal, S.; **Roy, S.**; Dolai, G.; Manne, S. R.; Paul, S.; Mandal, B. Nanostructures from Protected L/L and D/L Amino Acid Containing Dipeptides. *Pept. Sci.* **2020**, e24176.
12. Giri, R. S.; **Roy, S.**; Dolai, G.; Manne, S. R.; Mandal, B. FeCl₃-Mediated Boc Deprotection: Mild Facile Boc-Chemistry in Solution and on Resin. *ChemistrySelect* **2020**, 5, 2050-2056.

Conferences:

Poster presentation:

1. **Roy, S.**; Giri, R. S.; Mandal, B. “Supramolecular Nanostructures of Alternating L/L and D/L Amino Acid Containing Dipeptides.” **Research and Industrial Conclave 2023**, 14-16th May 2023, IIT Guwahati, India (Poster).
2. **Roy, S.**; Mandal, B. “The Effect of Side Chain and Chirality of Amino Acids on Dipeptides Self-Assembly.” **Frontiers in Chemical Sciences - 2022**, 2-4th December 2022, Department of Chemistry, IIT Guwahati, India (P-123).
3. **Roy, S.**; Mandal, B. “Investigation of the Role of Chirality on Supramolecular Self Assemblies of Dipeptides.” **North-East Research Conclave (NERC) and Assam Biotech Conclave (ABC) 2022**, 20-22th May 2022, IIT Guwahati, Science Technology and Climate Change Dept. & Dept. of Education, Govt. of Assam, India.
4. **Roy, S.**; Giri, R. S.; Dolai, G.; Mandal, B. “Investigation of Supramolecular Self-Assembly of Two Alternating L/L and L/D Dipeptides.” **28th CRSI National**

Symposium in Chemistry (CRSI-NSC-28), 25-27th March 2022, Department of Chemistry, IIT Guwahati, India, Page 264 (P-185).

5. **Roy, S.**; Giri, R. S.; Dolai, G.; Mandal, B. “Supramolecular Assemblies of Boc-Protected Dipeptides and its Structural Features in Crystalline State.” **20th National Conference on Surfactants, Emulsions and Biocolloids NATCOSEB-XX**, 9-11th December 2021, Department of Chemistry, IIT Guwahati, in association with Indian Society for Surface Science and Technology, Kolkata, India, Page 74 (P-35).

Oral presentation:

Roy, S.; Mandal, B. “The Effect of Side Chain and Chirality of Amino Acids on Dipeptides Self-Assembly.” International Conference on Emerging Trends in Chemistry (ICETC-2023), 16-17th March 2023, Assam Don Bosco University, India (OP-05).

Workshops:

1. Workshop on “**Nuclear Magnetic Resonance: Technique and its Application.**” 23rd-24th August 2021, organized by North East Centre for Biological Sciences and Healthcare Engineering (NECBH), Indian Institute of Technology Guwahati, Assam in Association with BRUKER.
2. Workshop on “**Scanning Electron Microscopy: Technique and its Applications.**” 29th-30th July 2021, organized by North East Centre for Biological Sciences and Healthcare Engineering (NECBH), Indian Institute of Technology Guwahati, Assam in Association with ZEISS.
3. The silver jubilee **NMRS** workshop held during October 29-31, 2018, at Bose Institute, Darjeeling Campus, West Bengal, India.
4. Workshop on procedures and applications of **XRD, XRF, and Single Crystal XRD**, 27th July to 1st August, 2018, organized by Sophisticated Analytical Instrument Facility (SAIF), Guwahati, sponsored by Dept, of Science and Technology (DST), govt. of India.

Sayanta Roy

Personal Information:

Date of Birth: 15th January 1994

Father's Name: Dulal Ch. Roy

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Bataskuri, Dakshin Dinajpur, West Bengal-733124, India.

Academic Qualifications

01/2018–Present

Ph.D. in Chemistry

Indian Institute of Technology Guwahati, Assam-781039, India.

Thesis: **Development of Novel Recyclable Coupling Reagents and Investigation on Chirality Controlled Self-Assembly of Dipeptides**

Thesis advisor: Prof. Bhubaneswar Mandal

07/2014–07/2016

M.Sc. in Chemistry

Indian Institute of Technology Madras, Chennai-600036, India.

Thesis: **Palladium Catalyzed N-H-Insertion, Oxidative Aromatization and Cross-Coupling of 6-diazocyclohexenones and anilines: Efforts towards the Synthesis of Phenoxazines**

Thesis advisor: Prof. P. Anbarasan

07/2011–05/2014

B.Sc. in Chemistry Honours

with Physics and Mathematics

St. Paul's Cathedral Mission College, Calcutta University, West Bengal-700009, India.

Achievements and Awards

- Qualified “Joint Admission Test for M.Sc. (JAM 2014)” examination, India.
- Qualified State Eligibility Test (UGC, New Delhi) June-2018 in Chemical Science, India.
- Qualified Graduate Aptitude Test (GATE) 2016, 2017, 2019, 2020, 2021, 2022 in Chemistry, organized by the Ministry of Human Resource Development, Government of India.
- Received West Bengal Merit Cum Means Scholarship.

Research Experience

- During my Ph.D., I developed new coupling reagents for diverse organic transformations and gained expertise in synthesizing peptides both in solution and solid phase.
- I also worked on the formation of C-C bonds using metal-catalyzed cross-coupling reactions. I am skilled in synthesizing glycosyl acceptors and donors.
- Additionally, I investigated the self-assembly properties of various small peptides and explored their applications.
- Furthermore, I worked on sensing heavy toxic metal ions using peptides as probes.
- I have expertise in various instrumental techniques, e.g., HPLC, NMR, SC-XRD, CD, FT-IR, TGA, DLS, UV-Vis, Fluorescence, Optical-polarizable microscope, *etc.*

Industrial Experience

- I worked on a one-year industrial project at IIT Guwahati under the NEW GENERATION INNOVATION AND ENTREPRENEURSHIP DEVELOPMENT CENTRE.

Academic and Professional Skills

- **Laboratory and Instrumentation:** I have extensive experience in Synthetic Organic Chemistry, particularly in amidation, esterification, and peptide synthesis (both in solution and solid phases). I am proficient in constructing C-N and C-C bonds through cross-coupling processes. Additionally, I have expertise in the crystallization of small molecules and peptides. I have hands-on experience with various instrumental techniques, including NMR Spectroscopy, Single-Crystal X-ray Diffraction (SC-XRD), HPLC, FT-IR Spectroscopy, TGA, Circular Dichroism (CD), DLS, UV-Vis Spectroscopy, Fluorescence Spectroscopy, and Optical Polarizable and Fluorescence Microscopy. I am

also familiar with other techniques such as Powder-XRD, Mass Spectrometry (ESI, MALDI), FESEM, FETEM, AFM, EDX, and XPS.

- **Software:** MS Office, ChemDraw, MestreNova, MassHunter, Origin, Gaussian, Adobe Illustrator, Photoshop, APEX4, SHELXL, WINGX, Mercury, CrystalExplorer, Mendeley, EndNote, and Topspin.
- **Instrumental Experience:** Five years of experience as an authorized operator for the **Nuclear Magnetic Resonance** (NMR, 600 MHz Bruker Ascend) instrument at the **Central Instrument Facility (CIF), IIT Guwahati**.
- **Language:** Bengali, English, and Hindi.

Research Interests

- Peptide Chemistry
- Supramolecular Chemistry
- Chemical Biology
- Medicinal Chemistry
- Synthetic Organic Chemistry