Development of Greener Coupling Reagents and Methodologies for the Syntheses of Esters, Amides and Peptides

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Submitted by

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Dedicated

to

Lord Sai Baba & My Parents

INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

Department of Chemistry

STATEMENT

I do hereby declare that the matter embodied in this thesis entitled “**Development of Greener Coupling Reagents and Methodologies for the Syntheses of Esters, Amides and Peptides**” is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology Guwahati, India under the guidance of Dr. Bhubaneswar Mandal.

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

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CERTIFICATE

This is to certify that Miss. **Jyoti Chandra** has been working under my supervision since July 2013 as a regular registered Ph. D. student. I am forwarding her thesis entitled "**Development of Greener Coupling Reagents and Methodologies for the Syntheses of Esters, Amides and Peptides**" being submitted for the Ph. D. (Chemistry) Degree of this Institute. I certify that she has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in her thesis and this work has not been submitted elsewhere for a degree.

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Thesis Supervisor

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Jyoti Chandra

LIST OF ABBREVIATIONS

Boc	*tert*-butyloxycarbonyl
BOP	Benzotriazol-1-yloxy-tris(dimethylamino) phosphoniumhexafluorophosphate
Bz	Benzoyl
Bzl	Benzyl
Cbz	Benzyloxycarbonyl
CDI	*N*,*N*-carbonyldiimidazole
$CHCl_3$	Chloroform
CH_3CN	Acetonitrile
DCM	Dichloromethane
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide
DIPEA	Diisopropylethyl amine
DMAP	*N*,*N*-dimethylpyridin-4-amine
DMF	*N*,*N*-dimethylformamide
ESI MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
Et_3N	Triethylamine
Fmoc	9-Fluorenylmethoxycarbonyl
FT-IR	Fourier transformation infra red spectroscopy
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene)-*N*-methylmethanaminiumhexafluorophosphate *N*-oxide
HBTU	*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]*N*-methylmethanaminiumhexafluorophosphate *N*-oxide
HDMA	1-((dimethylamino)-(morpholino)methylene)-1*H*-[1,2,3]triazolo[4,5-*b*]pyridiniumhexafluorophosphate-3-oxide
HDMB	1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazoliumhexafluorophosphate-3-oxide
HOBt	1-hydroxy benzotriazole
HPLC	High pressure liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
LRMS	Low resonance masss spectrometry
mL	milli liter
MW	microwave
NCL	Native chemical ligation
o-NosylOXY	Ethyl 2-cyano-2-(2-nitrophenylsulfonyloxyimino)
NMI	*N*-methylimidazole

NMP	*N*-methyl-2- pyrrolidone
NMR	Nuclear magnetic resonance
Oxyma	Ethyl 2-cyano-2-(hydroxyimino)acetate
PTSA	*Para*-toulenesulphonic acid
PyBOP	Benzotriazole-1-yl-oxy-tris-pyrolidine-phosphonium hexafluorophosphate
RP	Reverse phase
SPPS	Solid phase peptide synthesis
TCBOXY	(*E*)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

Amino Acids		
Name	**3-letter code**	**1-letter code**
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

SYNOPSIS

The contents of this thesis have been divided into six chapters. Chapter 1 contains the introduction. Chapter 2 contains the detailed studies on the halogen-free synthesis of sulphonates of alcohol, Oxyma-*O*-sulphonates, and oxime-*O*-sulphonates under microwave irradiation. Chapter 3 contains our works on the greener approach towards the synthesis of amides and esters using the catalytic amount of *o*-NosylOXY. Chapter 4 contains the racemization free synthesis of peptides and polypeptides using the sub-stoichiometric amount of *o*-NosylOXY. The research works on the synthesis of 1,2,4-oxadiazoles by using the catalytic amount of *o*-NosylOXY is incorporated in the Chapter 5. Our efforts on the development of a new generation of an efficient coupling reagent, TCBOXY, for the synthesis of esters, thioesters, amides, and peptides are incorporated in the Chapter 6. The chapter-wise summaries of the mentioned research works are described below.

Chapter 1: Introduction

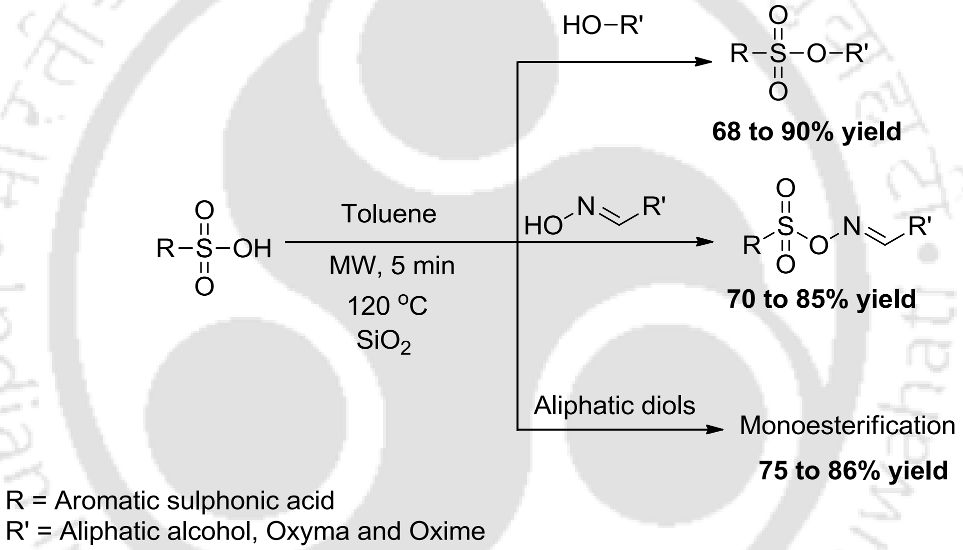
Sulphonate esters have interesting biological and pharmacological properties. Many methods are reported for the synthesis of sulphonate esters which involve various drawbacks such as the use of halogenated, expensive and less available reagents, vigorous reaction conditions and long reaction time. Halogenated reagents generate the excess amount of HCl which makes their industrial use difficult.

Peptides and proteins play essential roles in modern biology. Peptides are the polymers of amino acids and polypeptides are the longer peptides. For the synthesis of these biological macromolecules, amino acids are combined in the presence of coupling reagents, such as carbodiimides, phosphonium, and uronium/aminium salts, acyl halides, acyl azides, anhydrides, activated esters, and so forth. All of these activators are only effective to afford the fast reaction rates but not on the suppression of the undesired racemization during coupling reactions. For example, when DCC is used as the coupling reagent, the formation of an amide or peptide bond formation is associated with two kinds of annoying by-products, i.e., *N*, *N*'-dicyclohexylurea and the racemized peptide. In case of the acyl azides, many side reactions are possible. For example, acyl azide rearranges to acyl nitrene which undergoes Wolff rearrangement that leads to the formation of the isocyanate, which in turn reacts with the amino acid and gives urea as the side product along with the racemized product. Similarly, in case of BOP, hexamethylphosphoramide (HMPA) and HOBt are obtained as by-products which are highly toxic and carcinogenic. On the other hand, acyl halides and Yamaguchi reagent, TCBCl, have limited scope because of the danger of hydrolysis, racemization problem, cleavage of the protecting groups of amino acids and the generation of side reactions. TCB-DMAP is a modified version of the Yamaguchi reagent which is to overcome these problems. It decreased the drawbacks but a still significant limitation due to racemization persists. Because of the racemization problem, product yield gets reduced and purification becomes difficult. Also, the synthesis of the coupling reagents require hazardous chemicals, excess reagents for coupling reaction and have difficulties in recycling, thus generate a lot of chemical wastes and by-products. The cost of the coupling reagents is also high, and they have limited substrate scope.

Therefore, the invention of environment-friendly and cost-effective methods for organic transformation reactions with the highest level of efficacy and racemization suppression capability is highly desirable, but challenging. Hence, we wanted to develop newer strategies for peptide synthesis and different types of organic transformations to overcome the problems as mentioned earlier.

Chapter 2: Halogen Free Synthesis of Sulphonates of Alcohol, Oxyma-*O*-sulphonates, and Oxime-*O*-sulphonates Under Microwave Irradiation

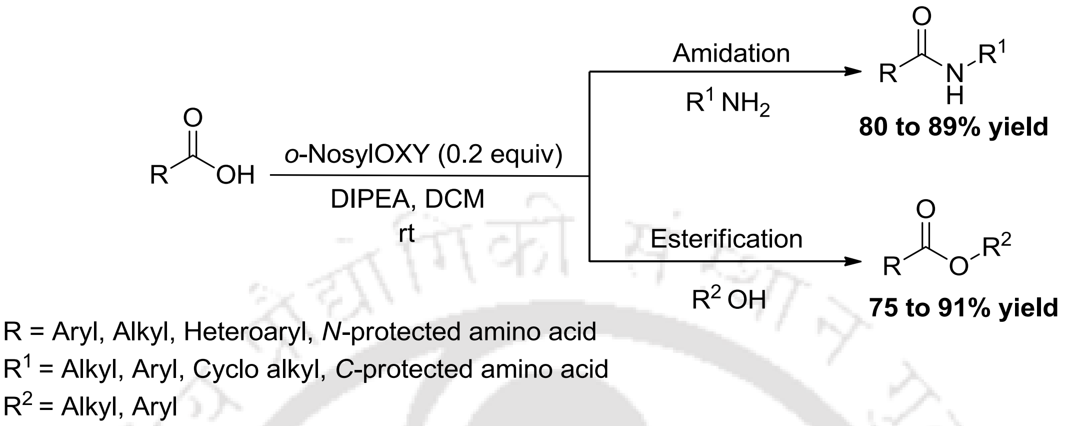
Sulphonate esters have drawn significant attention recently because of their attractive biological and pharmacological properties. The derivatives of sulphonate ester also exhibit moderate anti-proliferative effect against human cancer cell line SW756 and strong growth inhibition for mosquito Culexpipiens fourth instar larvae. Many methods are reported for the synthesis of sulphonate esters which involve various drawbacks such as use of halogenated, expensive and less available reagents, vigorous reaction conditions and long reaction time. Halogenated reagents generate excess amount of HCl which makes it difficult for industrial use. To overcome these drawbacks, we have developed a new halogen-free, industrially benign and environment-friendly microwave-assisted method for the synthesis of sulphonate esters by direct condensation of substituted benzene sulphonic acids and various primary and secondary alcohols or oximes, especially Oxyma. Reactions were completed within 5 min at 120 °C under microwave irradiation. Better yield was obtained by using inexpensive chromatographic silica gel as an additive. This method was also examined for the regioselective sulfonylation of diols, i.e., diols got monosulphonylated selectively with one equivalent of the benzene sulphonic acid and also higher selectivity towards the primary alcohol group than the secondary alcohol group. We could also recover the silica gel very quickly and reused for the next batches. This atom economic approach is green as the only generated by-product is water, and it represents a substantial improvement over previously described methods for the purpose.



Scheme 1. Synthesis of sulphonate esters

Chapter 3: Synthesis of Amides and Esters Using Catalytic Amount of *o*-NosylOXY

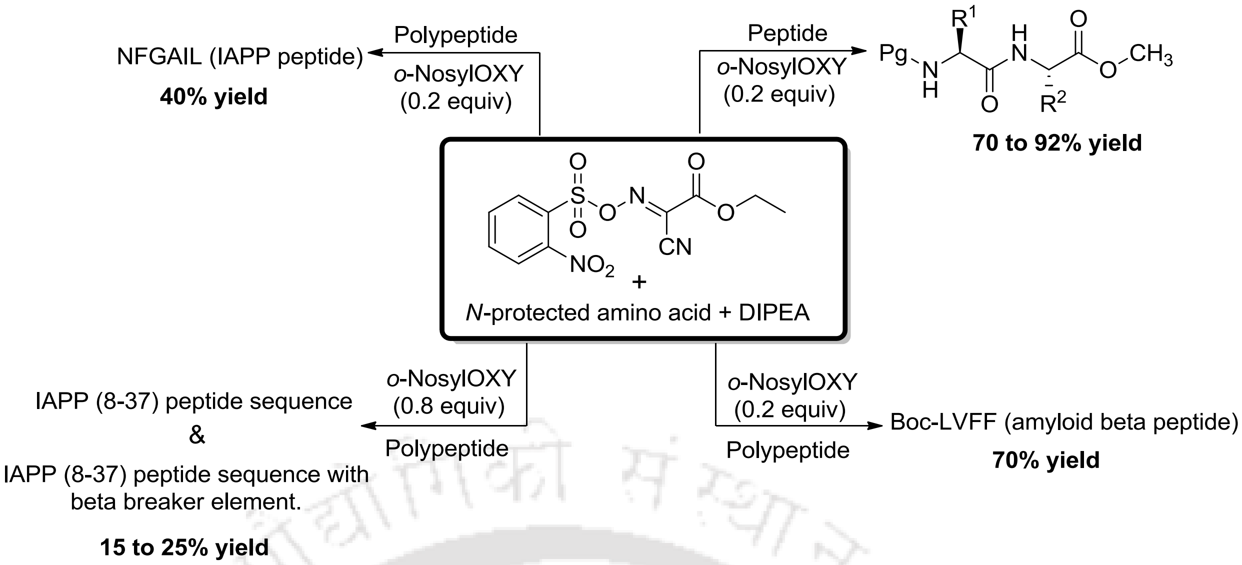
Amide and ester bond forming reactions are of tremendous significance in synthetic chemistry as these bonds are found in many natural products, bio-macromolecules, medicinally active compounds and drug molecules. In the past, methodological research has focused on this synthesis by different coupling reagents, metals, organo-catalyst, etc., but these methods have not been reached to satisfactory levels. In response to this acute demand, we have developed enantioselective syntheses of amides and esters using catalytic amount of *ortho*-NosylOXY. This method allows minimal waste production that makes it environment-friendly. Therefore, this concept is crucial in meeting one of the critical challenges of Green Chemistry.



Scheme 2. Synthesis of amides and esters using the catalytic amount of *ortho*-NosylOXY.

Chapter 4: Racemization Free Synthesis of Peptides and Polypeptides Using Sub-stoichiometric Amount of *o*-NosylOXY.

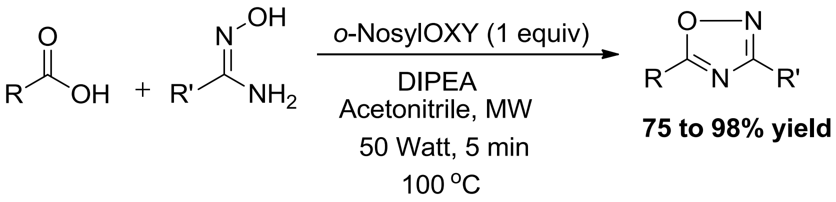
Peptides are an important class of biological molecules that are made up of amino acids. A long chain of the peptide is known as polypeptide that is important for biological functions in the body. There are several methods in literature for the synthesis of peptides as well as polypeptides, but those methods suffer from some drawbacks. Therefore, we developed an eco-friendly method for the synthesis of peptides and polypeptides using the sub-stoichiometric amount of *o*-NosylOXY. This method is compatible with the conventional *N*-protecting groups, such as Fmoc, Cbz, and Bz in solution phase and suppress the racemization during peptide synthesis. We demonstrated the utility of this protocol by synthesizing two biologically significant polypeptides. The first one is the fragment of Amylin peptide, which is known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes. The second one is the fragment of the amyloid β peptide (hydrophobic sequence with substantial steric hindrance responsible for Alzheimer’s disease). Thus, the use of the sub-stoichiometric amount of *o*-NosylOXY for solid phase peptide synthesis is demonstrated for the first time. This reduces the chemical waste generation that leads to the peptide synthesis in an environment-friendly manner.



Scheme 3. Synthesis of peptides and polypeptides using sub-stoichiometric amount of *o*-NosylOXY.

Chapter 5: Synthesis of 1,2,4-Oxadiazoles by Using Catalytic Amount of *o*-NosylOXY

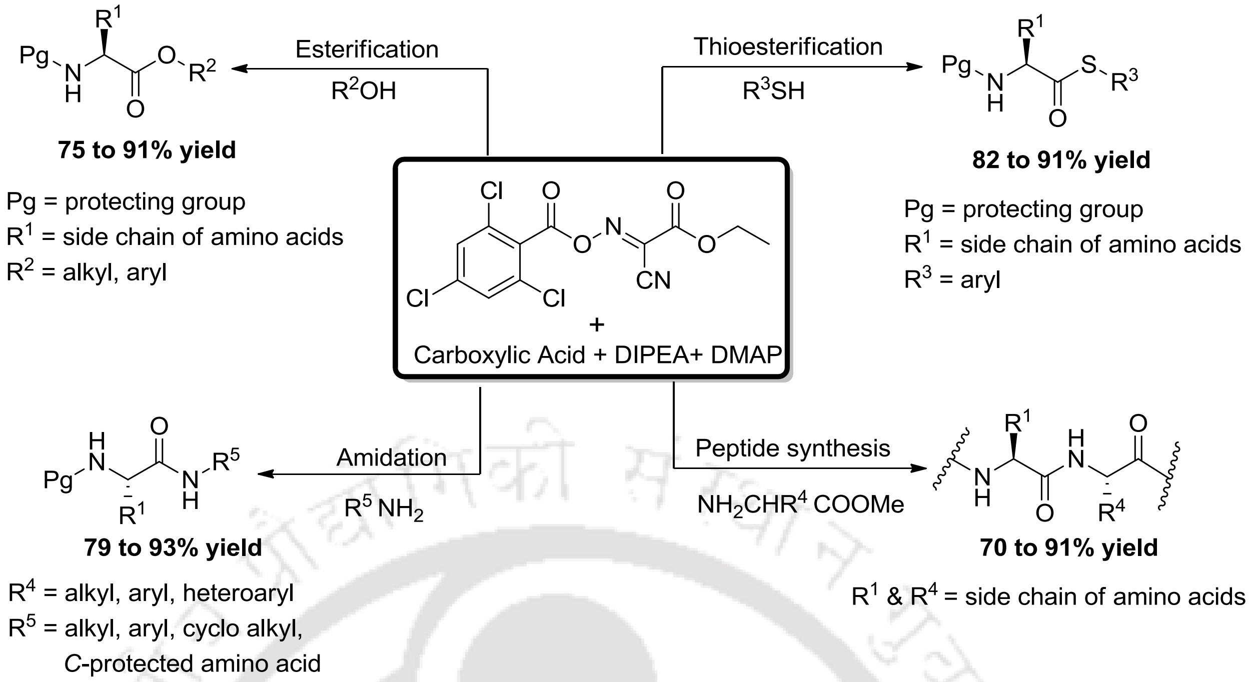
1,2,4-oxadiazoles are a class of heterocyclic compounds containing nitrogen and oxygen atoms which are found in many biologically active molecules and possess a variety of CNS (central nervous system) related activities. These heterocyclic compounds are also mostly used in medicines for the treatment of different type of fungal and bacterial infections along with the treatment of gastric ulcer, cancer, *etc*. In literature; there are many synthetic methods reported for the synthesis of 1,2,4-oxadiazoles by using coupling reagents with more than one equivalent. However, there is no report on coupling reagents which are used in catalytic amount for the synthesis of 1,2,4-oxadiazoles. In this chapter, we have discussed the effect of microwave irradiation on the reaction of carboxylic acids with different amidoximes for the synthesis of 1,2,4-oxadiazoles in the presence of the *ortho*-NosylOXY. All the substrates reacted smoothly and reaction completed within 5 min at 100 °C.



Scheme 4. Synthesis of 1,2,4-oxadiazoles using *ortho*-NosylOXY

Chapter 6: Development of a New Recyclable Coupling Reagent, (*E*)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY) for Synthesis of Esters, Thioesters, Amides and Peptides

To date, several coupling reagents have been developed. For example, Yamaguchi and coworkers have reported a coupling reagent, i.e., 2,4,6-trichlorobenzoyl chloride for the synthesis of carboxylic esters and lactones. Later, Shiina and coworkers developed a reagent similar to the Yamaguchi reagent, i.e., 2-methyl-6-nitrobenzoic anhydride for effective esterification process. In 2014, one more modified Yamaguchi reagent, i.e., 2,4,6-trichlorobenzoyl-4-dimethylaminopyridinium chloride (TCB-DMAP) was reported by Yoshinori and coworkers for esterification process. These mentioned coupling reagents suffer from drawbacks such as (a) not employed for amide and peptide synthesis (b) won’t suppress the formation of any undesired side product (c) not suppressed the racemization during the coupling reaction. Therefore, we developed a new reagent, **TCBOXY** for coupling reactions without causing detectable racemization. This reagent was successfully applied for the synthesis of esters, thioesters, amides, and peptides in good yield with broad substrate scope.



Scheme 5. Synthesis of esters, thioesters, amides, and peptides using TCBOXY.

Conclusion

In thesis work, we have developed a new halogen free method for the synthesis of Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (*ortho*-NosylOXY) as coupling reagent. And we have shown application of *ortho*-NosylOXY for the synthesis of amides, esters, peptides and polypeptides using the sub-stoichiometric amount of *o*-NosylOXY. In continuation to these syntheses, we discussed the synthesis of 1,2,4-oxadiazole using sub-stoichiometric amount of *o*-NosylOXY. Later, we developed another new recyclable Oxyma based coupling reagent, (*E*)-ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY, **II**), which was successfully applied for the synthesis of amides, peptides, esters, and thioesters in good yield with broad substrate scope.

TABLE OF CONTENTS

Chapter 1: Introduction

1.1. Background 1

1.2. Green Synthesis 1

1.3. Some biologically important class of compounds 2

1.3.1. Importance of sulphonate esters 2

1.3.2. Importance of amides 4

1.3.3. Importance of esters 5

1.3.4. Importance of peptides 6

1.3.5. Importance of 1,2,4-oxadiazole 8

1.4. Existing methods for the synthesis of relevant classes of compound 10

1.4.1. Existing methods for the synthesis of sulphonate esters 10

1.4.1.1. Synthesis of sulphonate esters of alcohol 10

1.4.1.2. Synthesis of sulphonate esters of Oxyma 11

1.4.1.3. Synthesis of sulphonate esters of oxime 12

1.4.2. Existing methods for the synthesis of amides, esters, and peptides 12

1.4.2.1. Solution phase peptide synthesis 12

1.4.2.1.1. Synthesis of amides, esters, and peptides via acyl halides 14

1.4.2.1.2. Synthesis of amides, esters, and peptides via acyl azides 14

1.4.2.1.3. Synthesis of amides, esters, and peptides via acyl anhydrides 15

1.4.2.1.4. Synthesis of amides, esters, and peptides via activated esters 17

1.4.2.1.5. Synthesis of amides, esters, and peptides via coupling reagents 17

1.4.2.1.6. Synthesis of peptides via native chemical ligation 19

1.4.2.2. Solid phase peptide synthesis 20

1.4.2.2.1. Fmoc based SPPS 21

1.4.2.2.2. Boc based SPPS 21

1.4.3. Existing methods for the synthesis of 1,2,4-oxadiazole 22

1.5. Drawbacks of existing methods 24

1.6. Objectives of thesis 26

1.7. References 26

Chapter 2: Halogen Free Synthesis of Sulphonates of Alcohol, Oxyma-*O*-sulphonates, and Oxime-*O*-sulphonates under Microwave Irradiation

2.1. A new halogen and metal free microwave assisted environment friendly method for the synthesis of sulphonate esters 39

2.1.1. Reaction optimization and substrate scope for formation of 40 sulphonate esters

2.2. Plausible mechanism 51
2.3. Conclusions 54
2.4. Experimental section 54
2.4.1. General consideration 54
2.4.2. General procedure for the direct synthesis of sulphonate ester from sulphonic acids 55
2.4.3. General procedure for the synthesis of sulphonate ester from sulphonic acids using silica gel 56
2.4.4. General procedure for the gram scale synthesis of sulphonate esters from sulphonic acids using silica gel 56
2.5. Characterization data 57
2.6. References 66
2.7. Selected spectra and chromatograms 69
2.7.1. ^{1}H NMR and ^{13}C NMR of selected compounds 69
2.7.2. GCMS analysis of intermediates 91

Chapter 3: Synthesis of Amides and Esters using Catalytic Amount of *o*-NosylOXY

3.1. Amide synthesis using catalytic amount of *o*-NosylOXY 94
3.2. Ester synthesis using catalytic amount of *o*-NosylOXY 97
3.3. Racemization study 98
3.4. Plausible mechanism 99
3.5. Conclusion 102
3.6. Experimental Section 103
3.6.1. Materials and methods 103
3.6.2. General procedure for the synthesis of amide 103
3.6.3. General procedure for the synthesis of ester 103
3.7. Characterization data 104
3.8. References 112
3.9. Selected spectra and chromatograms 114
3.9.1. ^{1}H NMR and ^{13}C NMR of amides and esters 114
3.9.2. HPLC Data for racemization test 123
3.9.3. Time dependent GCMS studies for Intermediates 125
3.9.4. $FeCl_3$ test for the evolution of water as the by-product 130

Chapter 4: Racemization Free Synthesis of Peptides and Polypeptide using Sub-stoichiometric Amount of *ortho*-NosylOXY

4.1. Reaction optimization and substrate scope for peptides and polypeptides synthesis using sub-stoichiometric amount of *o*-NosylOXY 132

4.2. Racemization suppression efficiency of **I** for peptide synthesis 136

4.3. Synthesis of polypeptides using sub-stiochiometric amount of *o*-NosylOXY 138

4.4. Plausible mechanism 140

4.5. Conclusion 140

4.6. Experimental Section 141

4.6.1. Materials and methods 141

4.6.2. General procedure for the synthesis of dipeptide 141

4.6.3. Solution Phase Synthesis of Boc-LVFF-OMe 141

4.6.4. Solid Phase Synthesis of NFGAIL-NH_2 142

4.6.5. Solid Phase Synthesis of IAPP (8-37) peptide and IAPP (8-37) peptide with one β-breaker element 143

4.7. Characterization data 143

4.8. Selected spectra and chromatograms 152

4.8.1. NMR (^{1}H and ^{13}C) and Mass spectra of peptides 152

4.8.2. HPLC Data for racemization test 160

4.8.3. HPLC chromatogram and mass spectra of Boc-LVFF-OMe via solution phase synthesis 162

4.8.4. HPLC chromatogram and mass spectra of NFGAIL-NH_2 via solid phase synthesis 163

4.8.5. HPLC chromatogram and mass spectra of ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-NH_2 via solid phase synthesis 164

4.8.6. HPLC chromatogram and mass spectra of ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTYG-NH_2 via solid phase synthesis 165

Chapter 5: Synthesis of 1,2,4-Oxadiazoles by Using the Catalytic Amount of *o*-NosylOXY

5.1. Reaction optimization and substrate scope for the synthesis of 1,2,4-oxadiazoles 168

5.2. Plausible Mechanism 179

5.3. Conclusion 181

5.4. Experimental Section 181

5.4.1. Materials and methods 181

5.4.2. General procedure for the synthesis of 1, 2, 4-Oxadiazoles from carboxylic acids (one-pot protocol) 182

5.5. Characterization Data 182

5.6. References 188

5.7. Selected spectra and chromatograms 189

5.7.1. ^{1}H NMR and ^{13}C NMR of selected compounds 189

Chapter 6: Development of a New Recyclable Coupling Reagent, (*E*)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY) for Synthesis of Esters, Thioesters, Amides and Peptides

6.1. TCBOXY as a new coupling reagent for racemization free amidation and peptide synthesis 207

6.1.1. Reaction optimization and substrate scope for esters, amides and peptides synthesis using TCBOXY 209

6.2. Racemization study 217

6.2.1. Racemization suppression efficiency of **II** for esters synthesis 217

6.2.2. Racemization suppression efficiency of **II** for peptide synthesis 218

6.3. Plausible mechanism 221

6.4. Recyclability of TCBOXY 222

6.5. Conclusion 224

6.6. Experimental section 225

6.6.1. Materials and methods 225

6.6.2. Procedure for synthesis of coupling reagent, (*E*)-ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino) acetate (TCBOXY, **II**) 225

6.6.3. General procedure for the synthesis of esters, thioesters, and amides 225

6.6.4. General procedure for the synthesis of peptides 226

6.6.5. Solution Phase Synthesis of Boc-VVIA-OMe 226

6.6.6. Solid Phase Synthesis of H-PGVGVPGVGV-NH_2 and H-DNASFVEDLG-NH_2 227

6.6.7. Procedure to identify the (*E*)-ethyl 9-cyano-1-(9H-fluoren-9-yl)-5-methyl-3,6-dioxo-2,7-dioxa-4,8-diazadec-8-en-10-oate 228

6.7. Characterization data 228

6.8. References 248

6.9. Selected spectra and chromatograms 251

6.9.1. NMR (^{1}H and ^{13}C), Mass spectra, and HPLC chromatogram of TCBOXY 251
6.9.2. NMR (^{1}H and ^{13}C) and Mass spectra of esters and thioesters 253
6.9.3. NMR (^{1}H and ^{13}C) and Mass spectra of amides and peptides 261
6.9.4. NMR (^{1}H and ^{13}C) spectra of Intermediate, VI 273
6.9.5. Data for racemization test 274
6.9.6. HPLC Chromatogram of dipeptides 282
6.9.7. HPLC Chromatogram and mass spectra of Boc-VVIA-OMe 284
6.9.8. HPLC chromatogram and mass spectra of DNASFVEDLG-NH_2 peptide 285
6.9.9. HPLC chromatogram and mass spectra of PGVGVPGVGV-NH_2 peptide 286
6.9.10. HPLC chromatogram and mass spectra of fragments of gramicidin via solid phase synthesis 287

Conclusions and Future Directions 291
Research Outcome 293
Curriculum Vitae 297

Chapter 1: Introduction

1.1. Background

The thesis describes the development of green methodologies for the synthesis of sulphonate esters, amides, peptides, esters, thioesters as well as 1,2,4-oxadiazole. These scaffolds are found in most of the pharmaceutical and biologically active molecules. This introductory chapter briefly focuses on the importance of above-mentioned compounds, their existing synthetic methods and drawbacks associated with the existing methods.

1.2. Green Synthesis

Green synthesis is an eco-friendly and cost-effective way of synthesis which is carried out under facile conditions in presence of non-toxic reagents. Cost-effectiveness, minimization of chemical waste generation, implementation of sustainable processes, enhancement in the yield of desired products and reduction in reaction time are the major drivers for the development of green synthesis. Coupling reactions such as synthesis of sulphonate esters, amides, esters, peptides, and 1,2,4-oxadiazoles gaining much more importance in academia and industry. Many synthetic methods have been extensively used for the synthesis of coupling reactions and various organic transformations via coupling reagents, but these coupling reagents are hazardous to the environment. Because, use of toxic chemicals for the synthesis of coupling reagents and use of excess reagents for coupling reactions, it generates a lot of chemical wastes. At present, the development of green approaches for the synthesis of coupling reactions and various organic transformations under ambient conditions using non-toxic reagents is highly desirable due to the increase in awareness about growing environmental concerns,. Therefore, we have focused on greener approach for the synthesis of the sulphonate esters, amides, esters, peptides, and 1,2,4-oxadiazole. We herein described the importance of these compounds.

1.3. Some biologically important class of compounds

1.3.1. Importance of sulphonate esters

Sulphonate esters are a class of sulphonic acid derivatives that have drawn much attention because of their interesting biological and pharmacological properties.[1] Sulphonate ester derivatives of alcohol showed a moderate anti-proliferative effect against human cancer cell line SW756 and strong growth inhibition for mosquito Culexpipiens 4th instar larvae.[2] For example, benzene sulphonate ester of pentafluorophenol (**1**, Figure 1.3.1.1) are found to be stable intermediates for sulphonamide formation,[3] which are extremely important antibacterial agents[4] and valuable structural motifs in medicinal chemistry.[5-6] Phenylsulphonate ester of paeonol (**2**) played an important role of a lead compound in anti-HBV therapy[7] which inhibit hepatitis B virus (HBV) gene expression and viral DNA replication.

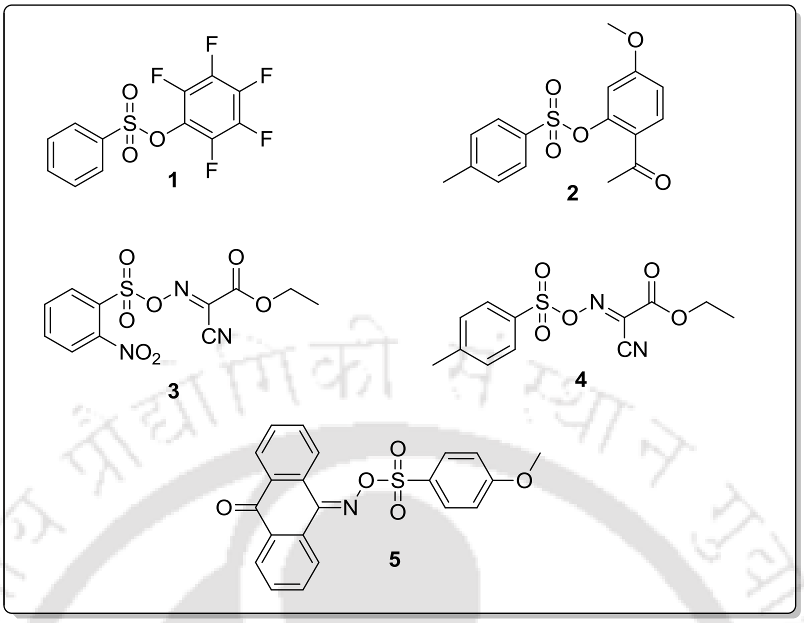


Figure 1.3.1.1. *Biologically active sulphonate esters*

The sulphonate esters of ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) are also well known for sulphonamide synthesis.[8] Suphonamides are often used in medicinal chemistry, e.g. antibacterial agents.[4] These esters are also known as coupling reagents in peptide chemistry for both solution and solid phase synthesis.[9] For example, Oxyma (**3** & **4**)[10,11] ester of substituted benzene sulphonic acids are the major class of peptide coupling reagents used to prevent the racemization during peptide synthesis which subsequently increased the yield of products.

Sulphonate esters of oxime and their derivatives are also valuable products or intermediates in the synthesis of medicinal compounds[12,13] and bioactive agents[14] For example, Anthracenone-based oxime ester (**5**) displayed strong antiproliferative activity against K562 leukemia cells and proved to be strong inhibitors of tubulin polymerization.[12] These microtubules are made up of two closely related globular polypeptides i.e. α- and β-tubulin which play an important role in anticancer therapeutic agents and in the formation and function of the spindle apparatus.[15]

1.3.2. Importance of amides

Amides are widespread in many compounds of biological and pharmaceutical importance[16]. The amide bonds are one of the most important parts of organic synthesis mainly present in proteins that act as enzymes, hormones, etc., which are essential to sustain life and are subunits of many pharmaceutical molecules, polymers, and natural products. For examples, carnosine[17] is a dipeptide (Figure 1.3.2.1) made up of amino acids beta-alanine and histidine. It is found in muscle and brain tissues in high concentration, can increase the Hayflick limit in human fibroblast as well as reduce the telomere shortening rate. It supports healthy cell function by inhibiting glycation and carbonylation. Carnosine acts as an anti-glycating agent, means it reduces the rate of formation of advanced glycation end-product (AGEs) that helps in the development of degenerative diseases such as diabetes, atherosclerosis, chronic renal failure and Alzheimer's disease. Similarly, Atorvastatin[18] is the top-selling marketed drug under trade name Lipitor which is a member of the medication class known as statins and used for inhibition of production of cholesterol in the body. It is also used for the treatment of dyslipidemia disease. This drug prevents strokes through anti-inflammatory activity and prevents cardiovascular diseases. Lidocaine[19] is the member of anti-arrhythmic drug class and it is used for the treatment of ventricular arrhythmias. It blocks sodium channels and decreases the rate of contractions of the heart. Lisinopril[20] is a peptide drug of angiotensin-converting enzyme inhibitor class and used for the treatment of high blood pressure, heart attacks, and heart failure. It also prevents kidney and eye complication problems in people suffering from diabetes. Valsartan[21] is an angiotensin II receptor with trade name Diovan which is used to treat high blood pressure, congestive heart attack and to decrease the death of peoples suffering from left ventricular dysfunction after having a heart attack. Likewise, Imatinib[22] is a drug sold under the brand name Gleevec. It is a small molecule tyrosine-kinase inhibitor which used to treat cancer such as chronic myelogenous leukemia and gastrointestinal stromal tumors.

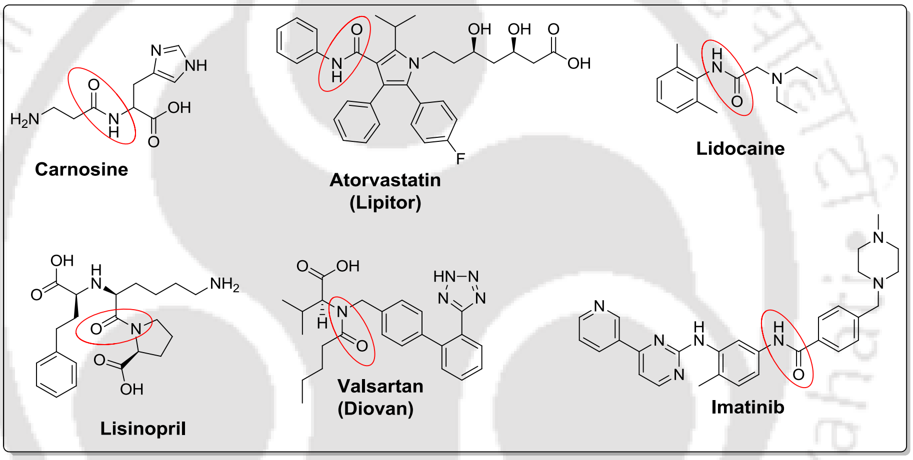


Figure 1.3.2.1. *Biologically active amides*

1.3.3. Importance of esters

An ester is an important class of molecules, derived from an organic (or inorganic) acid and alcohol. Ester linkages are present in numerous classes of molecule including natural products, biomacromolecules, medicinally active compounds and drug molecules. For examples, Phorbol ester[23] is tetracyclic diterpenoid (Figure 1.3.3.1) found in a variety of plants. It plays a major biological role in human cells to promote tumors. Menthyl isovalerate[24] is the methyl ester of isovaleric acid and its trade name is Validol. It is a mild sedative drug which has a direct effect on the central nervous system and improves the blood flow which lowers the blood pressure. Aspirin[25] is an ester of salicylic acid which is a drug, plays a central role in many biological functions such as pain killer, fever, and inflammatory diseases. It also prevents the cardiovascular diseases. Amylocaine[26] is a drug which is sold in the market with trade name Stovaine. It is an injection for relieving pain during surgery.

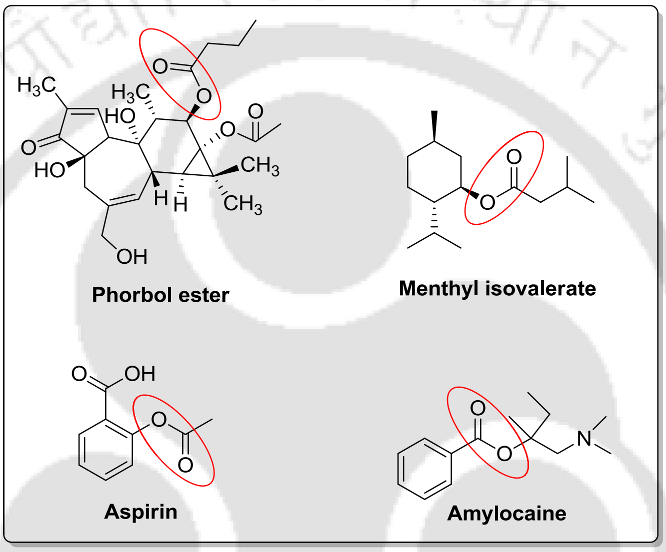


Figure 1.3.3.1. *Biologically active esters*

1.3.4. Importance of peptides

Peptides are important class of biological molecules that are made up of amino acids linked together by amide (peptide) bonds with the removal of water molecules. The formation of peptide bond is an example of condensation reaction which is a common class of reaction in living cells. Peptides are present in cell and tissue and play important roles in various biological processes. A long chain peptide is known as polypeptide or protein. Proteins are the most important class of biomolecules that acts as enzymes, hormones, and transporter to carry all biological function in the body. Peptide based drugs are recently very popular due to their effective, selective and benign nature. They are extensively used for curing numerous human diseases. For example, Lupron[27] is the peptide-based medicine (Figure 1.3.4.1) used for the treatment of prostate cancer, breast cancer, uterine fibroids etc. This drug is given by injection into muscles. Similarly, Goserelin[28] is a gonadotropin-releasing hormone superagonist (GnRH agonist). It is a decapeptide drug which is used to suppress the production of the sex hormones such as testosterone and estrogen.

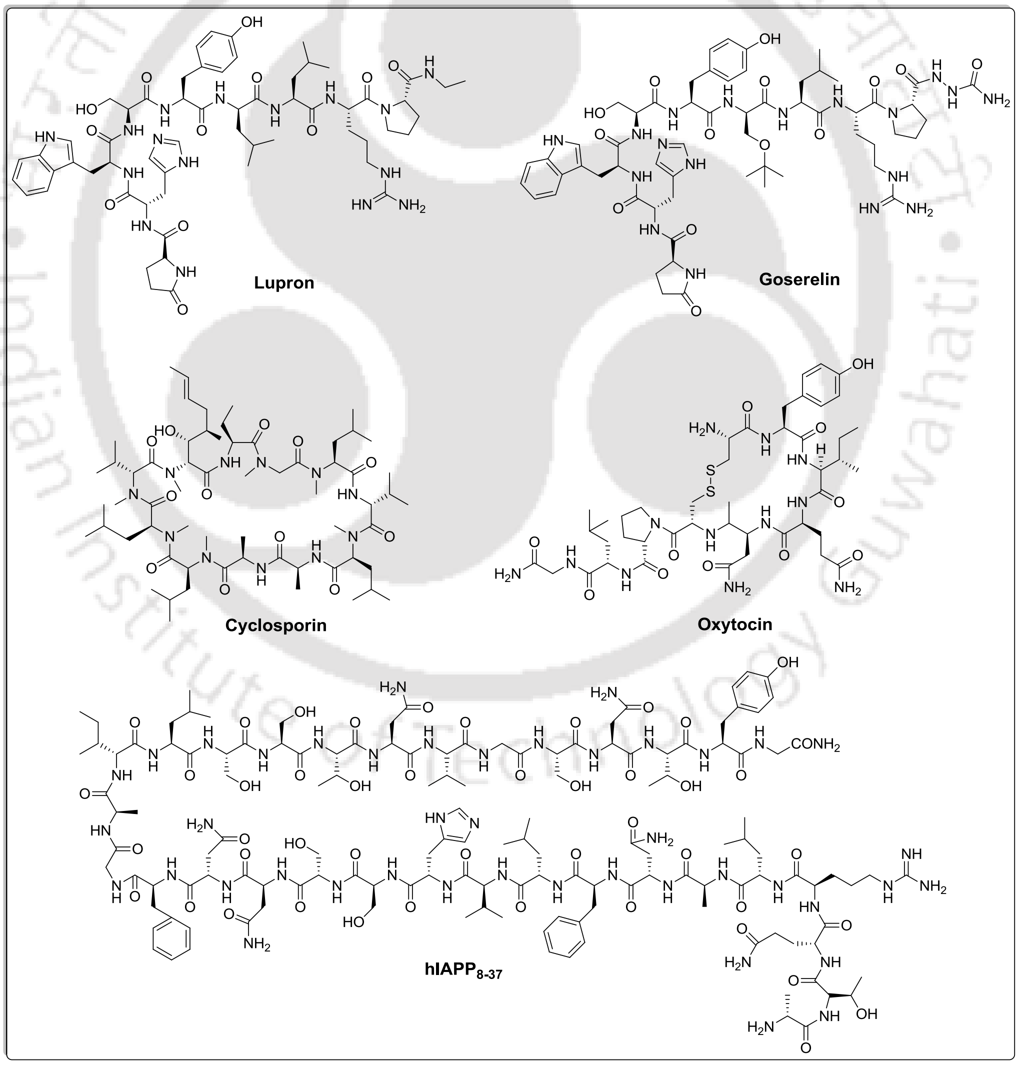


Figure 1.3.4.1. *Biologically active peptides*

It is used in the treatment of breast cancer as well as prostate cancer. Cyclosporin[29] is an immunosuppressant drug used to prevent organ rejection in people who have received liver, kidney or heart transplant. This drug is used along with other medicine to allow new organ in the body to function properly. Oxytocin[30] is a natural hormone that stimulates the uterus to contract. This medicine is used to induce labor during childbirth and control bleeding after childbirth. It also activates the breast to letdown milk within second after an infant begins to suckle. hIAPP$_{8\text{-}37}$ (human islet amyloid polypeptide)[31] is fragment of Amylin peptide which is known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes. Amylin is stored in insulin secretory granule and is thus released in response to insulin secretion. It plays an important role in controlling food intake, gastric emptying and glucose homeostasis.

1.3.5. Importance of 1,2,4-oxadiazole

1,2,4-oxadiazoles and their derivatives are an important class of heterocyclic compounds. They are mainly of five-membered rings containing nitrogen and oxygen atoms which have important medicinal and biological properties. It has a variety of CNS (central nervous system) related activities.[32] They are used as the drug for the treatment of different types of fungal and bacterial infections. For examples, Keto-1,2,4-oxadiazoles[33] (Figure 1.3.5.1, **1**) is a novel compound used to explore the P2 binding pocket of human mast cell tryptase and has high selectivity over human trypsin and other serine proteases. 3,5-diaryl-1,2,4-oxadiazole[34] (**2**) is a IL-8 antagonist have the activity in a binding assay as well as functional assay of IL-8 that induces elastase from neutrophils. IL-8 receptor antagonism acts as anti-inflammatory agents. DGAT-1[35] (**3**) is a member of 1,2,4-oxadiazole derivative acts as an enzyme which catalyzes the synthesis of mRNA in rodent models and its inhibitor used for the treatment of obesity and types II diabetes. 5- dichloromethyl-3-(*p*-sulfo namidophenyl)-[1,2,4]- oxadiazole[36] (**4**) is derivative of 1,2,4-oxadiazole is a drug acts as anti-tuberculosis agent. Similarly, 3-pyrazinyl-[1,2,4]-oxadiazole[37] derivatives (**5**) is tuberculostatic drugs which show higher activity against strains.

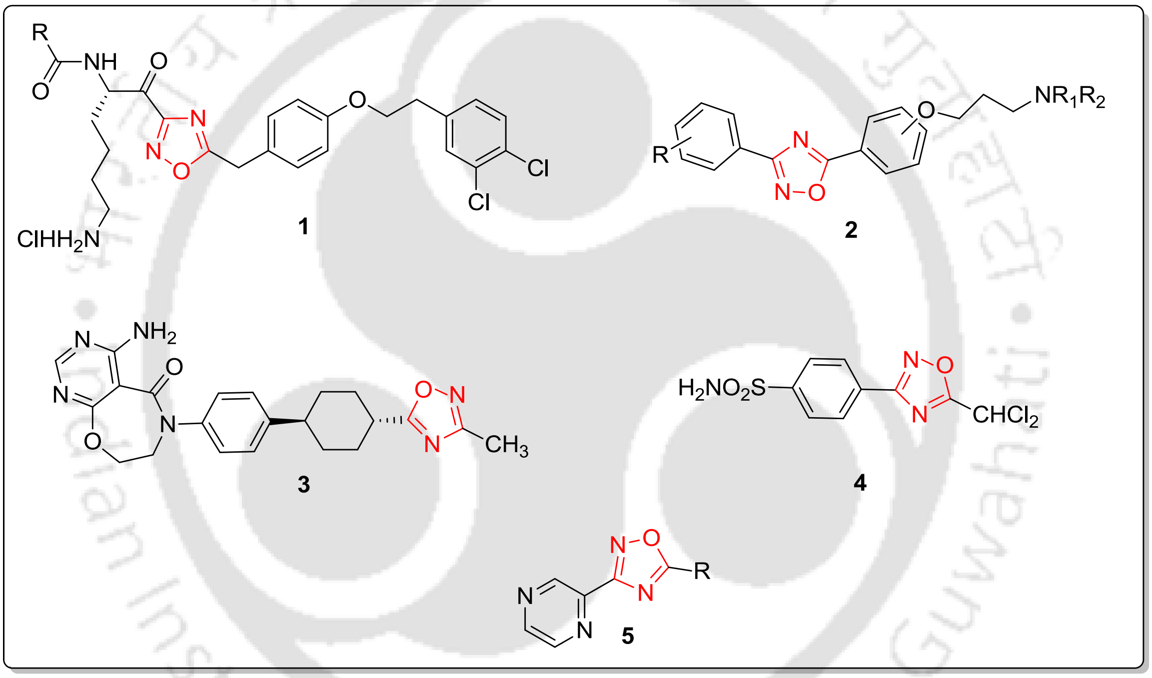


Figure 1.3.5.1. *Biologically active 1,2,4-oxadiazole*

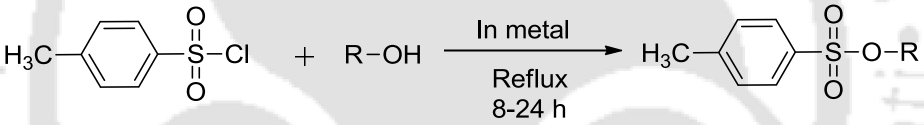
1.4. Existing methods for the synthesis of relevant classes of compound

1.4.1. Existing methods for the synthesis of sulphonate esters

Generally, sulphonate esters are synthesized by the reaction between sulphonyl chloride and alcohol or Oxyma or oxime. Many methods are reported in the literature for the synthesis of sulphonate esters using halogen based reagents or metal catalyst.

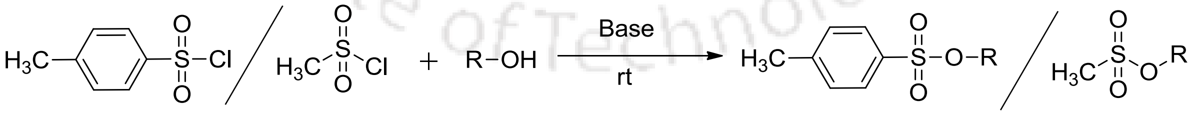
1.4.1.1. Synthesis of sulphonate esters of alcohol

Yan *et al*. described the synthesis of a wide range of sulphonate esters of alcohol from sulphonyl chlorides. In this method, alcohol was treated with *p*-toluenesulphonyl chloride in presence of indium metal for 8-24 h under reflux condition (Scheme 1.4.1.1.1).[38]



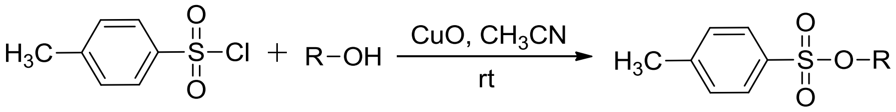
Scheme 1.4.1.1.1. *Synthesis of sulphonate ester of alcohol using metal*

Lie *et al*. illustrated a method for the synthesis of sulphonate ester via *p*-toluenesulphonyl chloride or mesityl chloride in presence of a base at room temperature (Scheme 1.4.1.1.2).[39]



Scheme 1.4.1.1.2. *Synthesis of sulphonate ester of an alcohol using mesityl chloride*

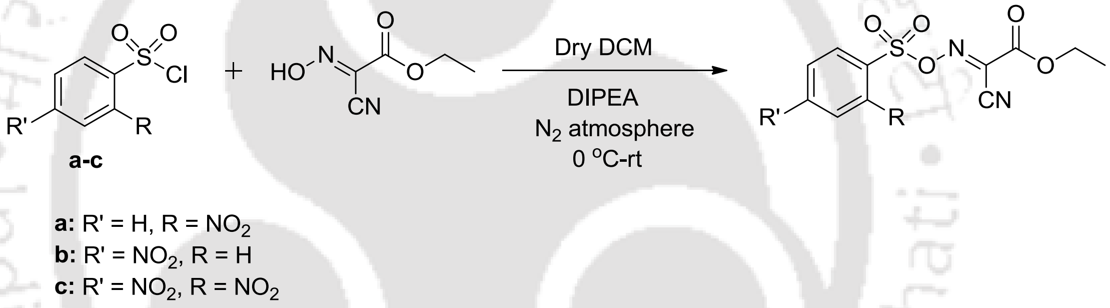
Meshram *et al.* synthesized sulphonate ester of alcohol from *p*-toluenesulphonyl chloride in presence of copper oxide at room temperature (Scheme 1.4.1.1.3).[40]



Scheme 1.4.1.1.3. *Synthesis of sulphonate ester of alcohol using copper oxide*

1.4.1.2. Synthesis of sulphonates ester of Oxyma

Similarly, sulphonate esters of Oxyma were also synthesized by using sulphonyl chloride. Mandal and co-workers[8] has prepared the sulphonate esters of Oxyma directly from sulphonyl chloride in presence of DIPEA as a base under nitrogen condition (Scheme 1.4.1.2.1).

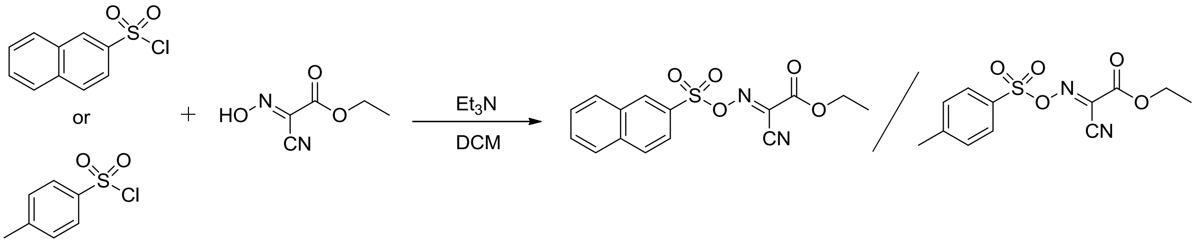


a-c

a: R' = H, R = NO_2
b: R' = NO_2, R = H
c: R' = NO_2, R = NO_2

Scheme 1.4.1.2.1. *Synthesis of sulphonate ester of Oxyma using sulphonyl chloride*

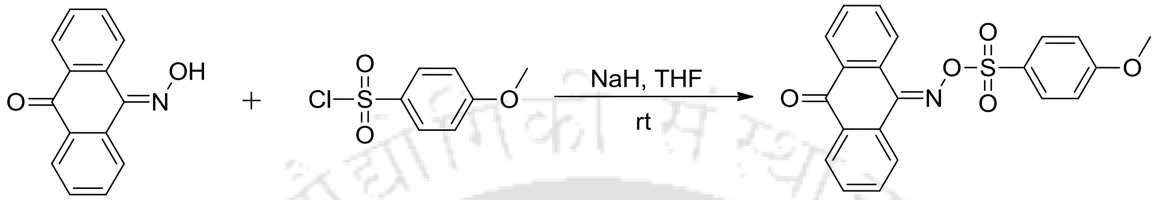
Khattab[11] synthesized sulphonate ester of Oxyma via reaction of 2-naphthalenesulphonyl chloride or *p*-tosyl chloride with Oxyma in presence of triethylamine in dry DCM under a nitrogen atmosphere at 0 ºC (Scheme 1.4.1.2.2).



Scheme 1.4.1.2.2. *Synthesis of sulphonate ester of Oxyma using tosyl chloride*

1.4.1.3. Synthesis of sulphonate esters of oxime

Surkau[41] *et al.* illustrated the synthesis of sulphonate ester of oxime from sulphonyl chloride in presence of sodium hydride at room temperature (Scheme 1.4.1.3.1).



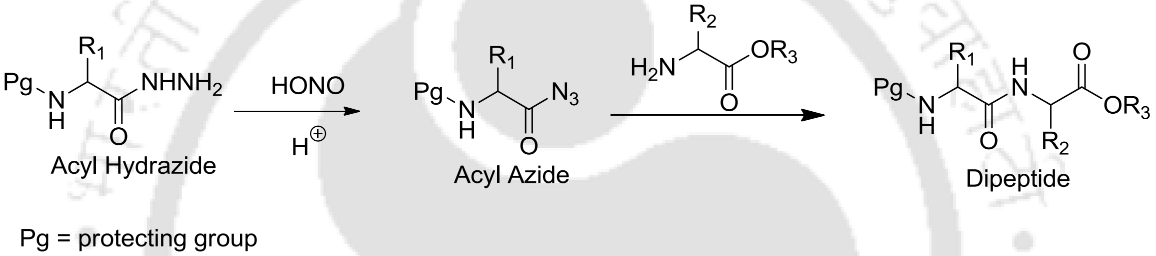
Scheme 1.4.1.3.1. *Synthesis of sulphonate ester of oxime using sulphonyl chloride*

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

Amide or ester or peptide bond formation takes place via activated intermediates of carboxylic acid using a suitable coupling reagent. Then, further addition of nucleophiles to the activated intermediate, e.g. amine or alcohol or *C*-protected amino acid, leads to the final product. The reaction of an activated intermediate of carboxylic acid with amine or alcohol or *C*-protected amino acid is known as coupling reaction and activators are coupling reagents.[42] In past two decades, a great effort has been devoted towards the development of efficient methods for the synthesis of amides, esters, and peptides from carboxylic acids using various coupling reagents. There are mainly two methods for peptide synthesis: a) ***Solution phase peptide synthesis*** b) ***Solid phase peptide synthesis***

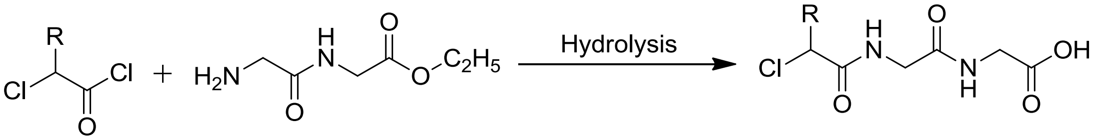
1.4.2.1. ***Solution phase peptide synthesis*****:** In this synthesis, the formation of peptide bond takes place in homogeneous solution between *N*-protected amino acid and *C*-protected amino acid without any solid support. After deprotection of *N*-terminal, the resulting dipeptide was coupled with another *N*-protected amino acid and this process continues till the last amoni acid of the sequence.

In the early 20th century, Curtius and Fisher independently began the first chemical synthesis of peptides. Acid azide method was developed by Curtius[43] for the synthesis of peptides. This method involves hydrazinolysis of alkyl esters and conversion of acyl hydrazide to acyl azide in presence of nitrous acid. Further, acyl azide reacts with the next amino acid in basic medium to form dipeptide (Scheme 1.4.2.1a).



Scheme 1.4.2.1a. *Curtius approach of peptide synthesis*

In 1901, Emil Fischer published an article which reports the synthesis of the first dipeptide, glycylglycine which considered as the beginning of peptide chemistry. Formation of polypeptide by acyl chloride method was developed by Fisher. In this method, the formation of dipeptide involves treatment of α-chloro acyl chloride with *C*-protected amino acid followed by hydrolysis of *C*-protected amino acid (Scheme 1.4.2.1b).[44]

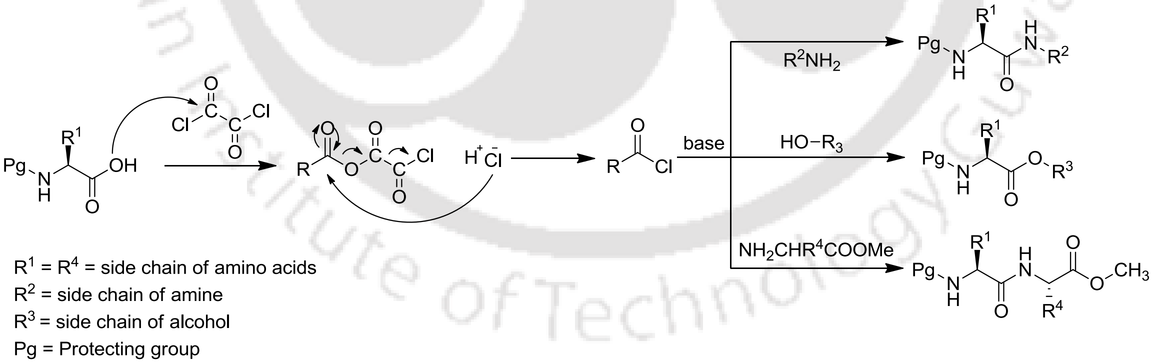


Scheme 1.4.2.1b. *Fisher approach of peptide synthesis*

There are different methods to activate the carboxylic acid to form activated intermediates such as acyl halides, acyl azides, anhydrides, activated esters etc.

1.4.2.1.1. Synthesis of amides, esters, and peptides via acyl halides

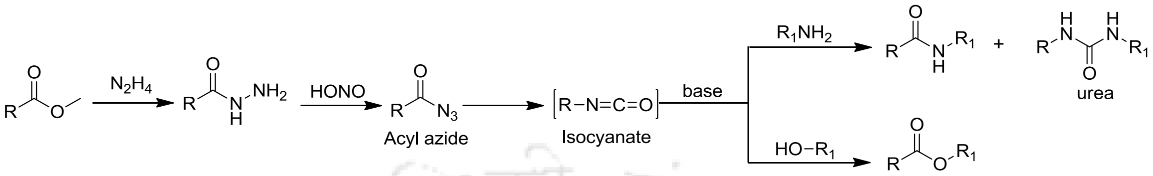
Acyl halide is one of the easiest methods to activate a carboxyl group of an amino acid for peptide bond formation. In this method, the carboxylic acid is activated using acyl halide reagents such as thionyl chloride,[45] oxalyl chloride,[46] phosphorus trichloride,[47] and phosphorus pentachloride[48] followed by a nucleophilic addition to the activated acid to form amides, esters, and peptides. The mechanism of acyl chloride formation from carboxylic acid using oxalyl chloride is illustrated in scheme 1.4.2.1.1.1. However, acyl chlorides have limited application in peptide coupling because of the danger of hydrolysis, and cleavage of protecting groups and other side reactions.



Scheme 1.4.2.1.1.1. *A mechanism for acyl chloride formation using oxalyl chloride*

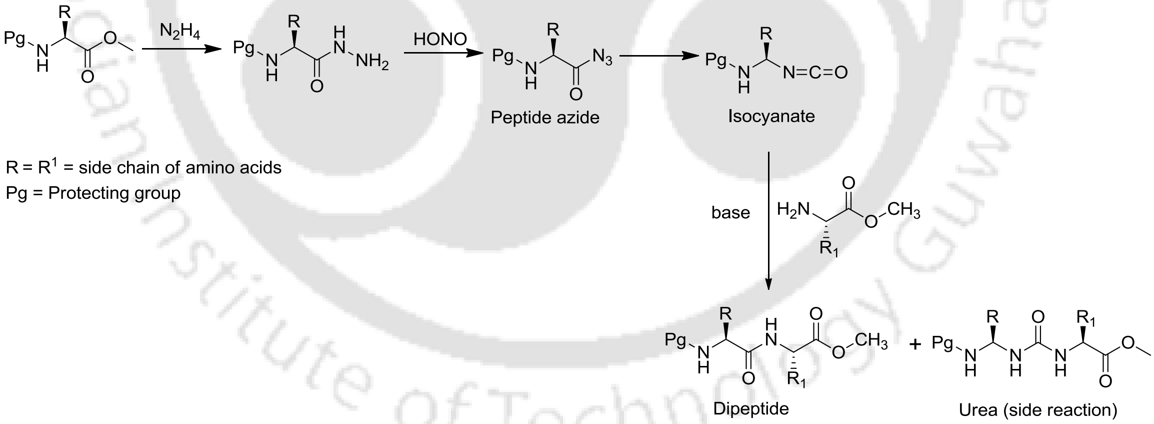
1.4.2.1.2. Synthesis of amides, esters, and peptides via acyl azides

The acyl azide method is the first method developed by Curtius[43] for peptide coupling. In this method, azides can be prepared directly from esters via two steps synthesis. The ester group first gets hydrolyzed with hydrazine to generate the acyl hydrazide which then undergoes a nitrosation reaction with nitrous acid to form the final acyl azide.



Scheme 1.4.2.1.2.1. *A mechanism for acyl azide formation and synthesis of amide and ester using Curtius rearrangement.*

Peptide azides are not stable and very reactive towards the Curtius rearrangement at high temperature. But this process has the following drawbacks: (a) formation of peptide bond takes several days if the reaction is carried out at low temperatures (b) Urea is generated as the side product and (c) This azide does not suppress the racemization.



Scheme 1.4.2.1.2.2. *A mechanism for peptide azide formation and synthesis of dipeptide using Curtius rearrangement.*

1.4.2.1.3. Synthesis of amides, esters, and peptides via acyl anhydrides

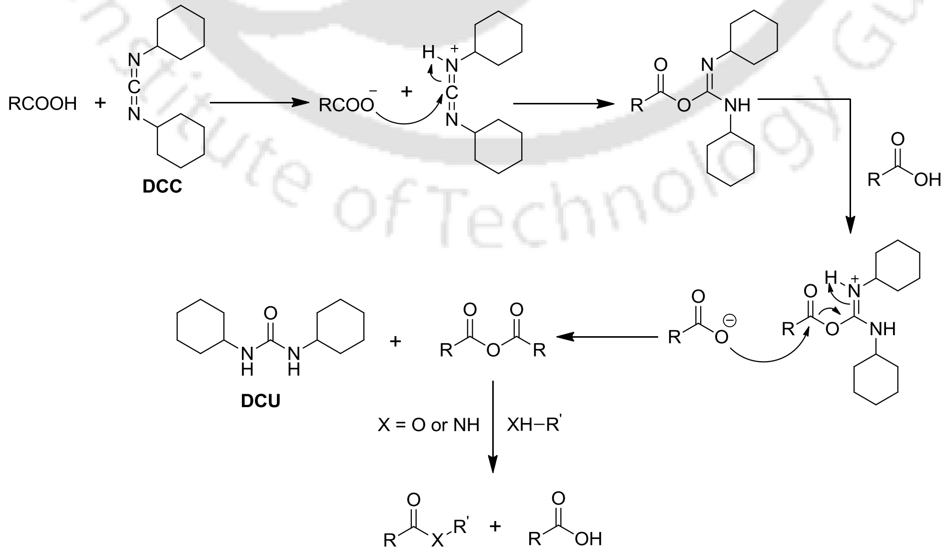
The strategy for the synthesis of amides, esters, and peptides ranges from the use of simple symmetric anhydrides to mixed anhydrides. The process of coupling of individual amino acids can be accomplished through employment of the carbodiimide,[49] 1-ethoxycarbonyl-1,2-dihydroquinoline (EDDQ),[50] cyclic anhydrides (*N*-carboxy anhydrides or Leuch's anhydrides),[51] urethane-protected *N*-carboxy anhydrides (UNCAs).[52]

When two molecules of carboxylic acid react in presence of one equivalent of dicyclohexyl carbodiimide (DCC) under reflux conditions then the formation of symmetric anhydrides takes place. This anhydride is then reacted with a vast range of nucleophiles such as alcohols, amines, and thiols. This coupling method is also compatible with peptide formation. The main limitations of this method are:

(a) Only half of the carboxylic acid is coupled and other half of acid is remained unreacted.

(b) Dicyclohexylurea (DCU) is formed as a byproduct along with symmetrical anhydride and

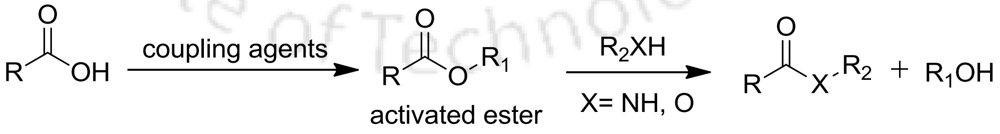
(c) Racemization complication



Scheme 1.4.2.1.3.1. *Synthesis of amide and ester using DCC*

1.4.2.1.4. Synthesis of amides, esters, and peptides via activated esters

Active esters are mixed anhydrides of carboxylic acid and a phenolic or hydroxamic acid which are formed in presence of coupling reagents. There are numerous type of active esters are available, such as HOBt ester,[53] HOAt ester,[54] *p*-nitrophenolic ester[55] pentafluorophenolic ester,[56] *N*-hydroxysuccinimide (HOSu),[57] *N*-hydroxy-5-norbornene-endo-2,3-dicarboxyimide (HONB) ester,[58] and 2,4,5-trichlorophenolic ester.[59] It was observed that methyl, ethyl and benzyl esters cannot consider as activated esters, although these esters are used as protecting group in peptide synthesis. However, the substituted phenyl esters work better than alkyl esters because of easier hydrolysis of phenyl esters with a variety of nucleophiles, like amines or alcohols. The esters synthesized from substituted hydroxamic acids are highly activated. These active esters generated fewer side reactions and racemization during the coupling reactions. The presence of activated esters helps in amidation in a shorter time and under milder conditions with good yields. The general reaction of the activated ester has shown in scheme 1.4.2.1.4.1.



Scheme 1.4.2.1.4.1. *Synthesis of amide and ester through activated ester*

1.4.2.1.5. Synthesis of amides, esters, and peptides via coupling reagents

The most desirable coupling reaction is the direct condensation of the carboxylic acid with amines and alcohols to produce amides, peptides, and esters with the loss of water molecules. In last two decades, a great effort has been devoted towards the development of efficient methods for the synthesis of amides, peptides, and esters using various coupling reagents including carbodiimides, phosphonium, and uronium/ aminium salts.[60-63] The amides and esters are also synthesized by activated esters such as acid halides, acid anhydrides or acid azides.[64-70] The other main strategy for amide bond formation involves the use of boron reagent that are used in stoichiometric amounts under reflux condition.[71-72] The use of catalytic boron derivatives[73], other homogeneous[74] or heterogeneous[75] catalysts and/or enzymes[76] has emerged as powerful alternatives to the traditional amidation process. Recently, Ishihara *et al*. presented the first catalytic method for a direct amide formation from free carboxylic acids and amines as the reaction partners.[77] These reactions have been neglected for many years principally because of the long reaction times and high temperatures needed.[78] Currently, microwave irradiation has also proven to be effective for the esterification reaction of carboxylic acids with alcohols[79] which reduced the reaction times but it required high temperature. Clark and co-workers[80] recently used a heterogeneous silica-based catalyst for amidation reactions, which demonstrated a better scope but with longer reaction times and need high temperatures to activate the catalyst.

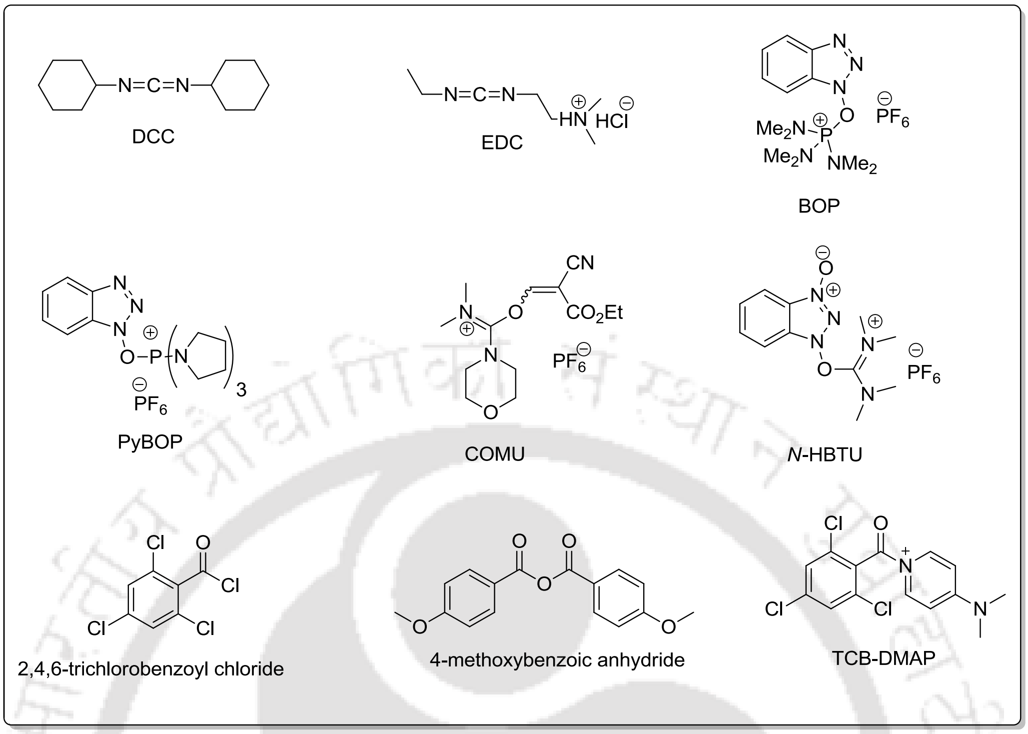
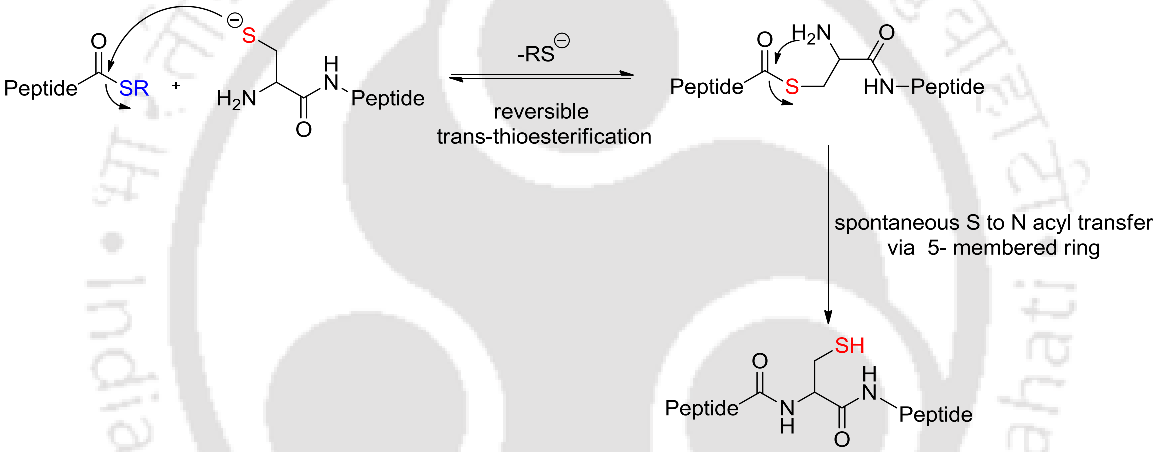


Figure 1.4.2.1.5.1. *Different types of coupling reagents*

On the other hand, Yamaguchi and coworkers have reported 2,4,6-trichlorobenzoyl chloride[81] as a coupling reagent for the synthesis of carboxylic esters and lactones. Later, Shiina and co-workers also developed a similar reagent to the Yamaguchi reagent for effective esterification process.[82] In 2014, Yoshinori and coworkers reported 2,4,6-trichlorobenzoyl-4-dimethylamino pyridinium chloride (TCB-DMAP)[83] as a coupling reagent for esterification process. These mentioned coupling reagents are capable to afford the reactions, but not effective in the suppression of racemization and undesired side product.

1.4.2.1.6. Synthesis of peptides via Native Chemical Ligation

NCL is non-enzymatic reactions that can join two unprotected peptide fragments to generate a ligated polypeptide or protein with a native peptide bond. The selective formation of an amide bond in the presence of unprotected functional groups is a challenging problem in peptide chemistry. In 1994, Kent and co-workers first introduced the native chemical ligation (NCL).[84] NCL is chemoselective reaction involves reversible trans-thioesterification step between thioester of one peptide and cysteine group of the second peptide to generate a ligated thioester intermediate which undergoes spontaneous S- to N- acyl transfer of thiol group via favorable five-membered ring (Scheme 1.4.2.1.6.1).

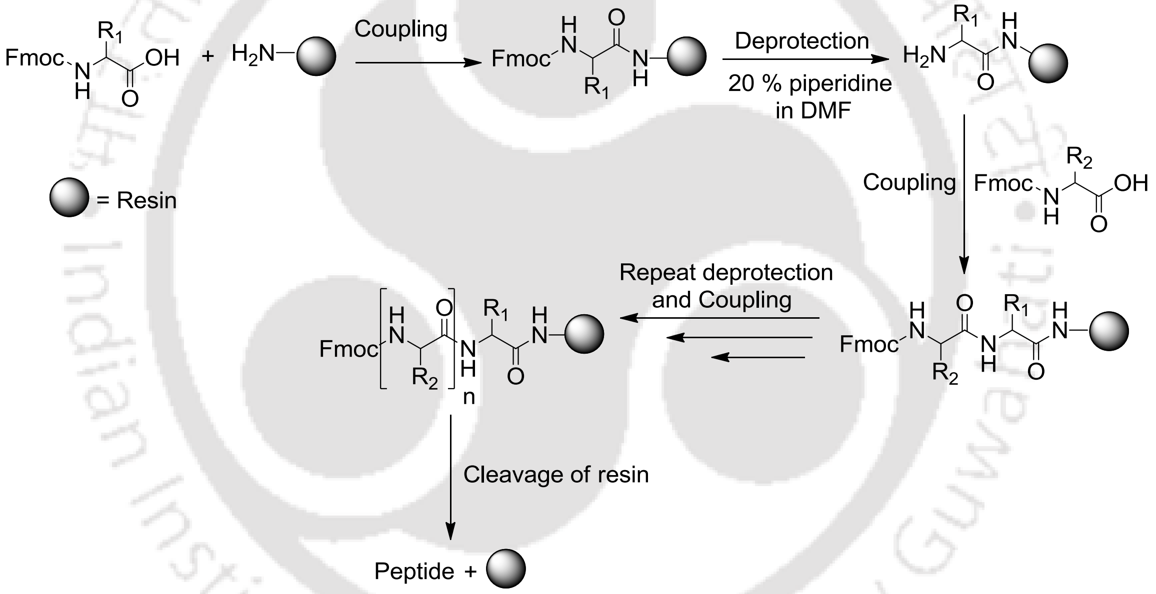


Scheme 1.4.2.1.6.1. *Native chemical ligation with thioester*

1.4.2.2. ***Solid phase peptide synthesis:*** In 1963, Prof. Robert Bruce Merrifield developed solid phase peptide synthesis (SPPS) for peptide synthesis.[85] The solid support is small and polymeric resin beads onto which peptide chains can be built. The peptide is covalently attached to the resin during the synthesis. In the end, crude peptide was cleaved from the solid support and can be purified using reversed phase HPLC. Mainly two strategies of SPPS method: Fmoc and Boc based SPPS.

1.4.2.2.1. Fmoc based SPPS

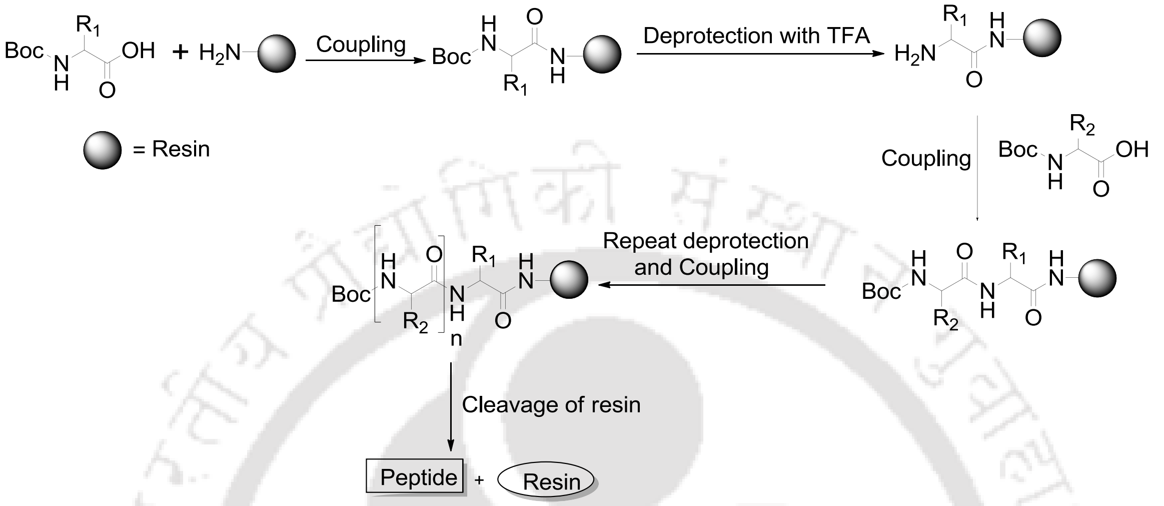
In this synthesis, first Fmoc-protected amino acid is loaded to a resin bead via linker using coupling reagents. Then, Fmoc-deprotection was carried by 10% of piperidine in DMF followed by coupling of second Fmoc-protected amino acid with first amino acid which is attached to a resin bead. This sequence is repeated until all desired amino acids are loaded. The last step involves the cleavage of the final peptide from resin bead under acidic conditions using TFA (Scheme 1.4.2.2.1.1).



Scheme 1.4.2.2.1.1. *Fmoc based SPPS*

1.4.2.2.2. Boc based SPPS

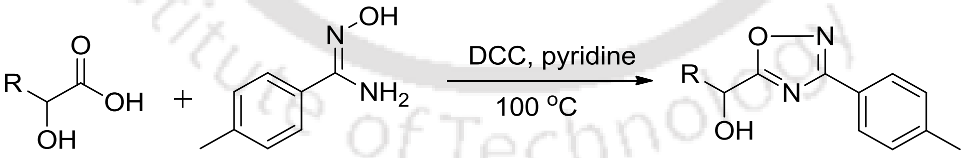
In this method, Boc protected amino acid is first attached to the resin linker. Deprotection of Boc from the resin is then performed using TFA. Next, Boc protected amino acid is then coupled with first amino acid via pre-activation with coupling reagents. After synthesis of the desired peptide, the peptide is deprotected from the resin by HF treatment (Scheme 1.4.2.2.2.1).



Scheme 1.4.2.2.2.1. *Boc based SPPS*

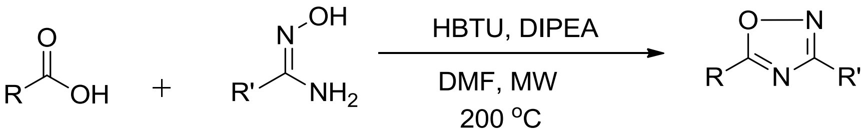
1.4.3. Existing methods for the synthesis of 1,2,4-oxadiazole

In literature many synthetic methods for the synthesis of 1,2,4-oxadiazoles are available. In 1996, Liang and Feng synthesized 1,2,4-Oxadiazole by DCC coupling reagent usually involved cyclization in refluxing pyridine condition[86] (Scheme 1.4.3.1).



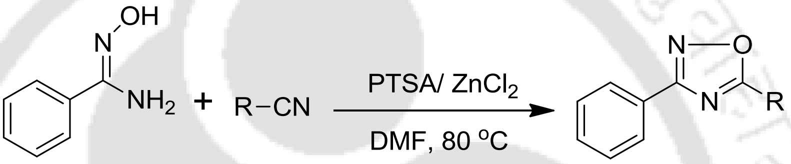
Scheme 1.4.3.1. *Synthesis of 1, 2, 4-Oxadiazoles by DCC*

In 2003, Marc[87] *et al*. reported the development of the microwave-assisted synthesis of 1,2,4-oxadiazole. The synthetic procedure involved the reaction between acid and amidoxime in presence of HBTU coupling reagent under MW irradiation (scheme 1.4.3.2).



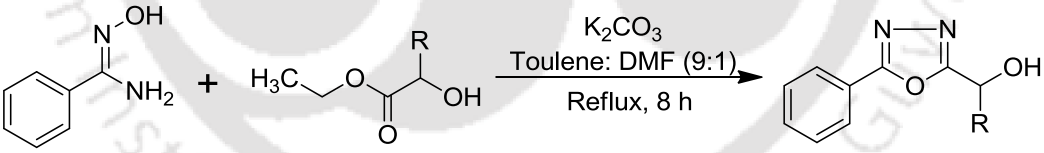
Scheme 1.4.3.2. *Synthesis of 1, 2, 4-Oxadiazoles by HBTU*

In 2009, Augustin[88] *et al.* illustrated the synthesis of 1,2,4-oxadiazole from organic nitrile in the presence of *p*-toluene sulphonic acid and zinc chloride in reflux condition (Scheme 1.4.3.3).



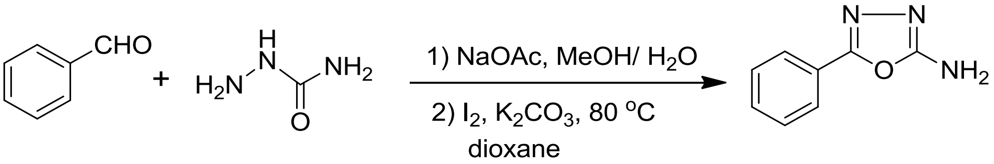
Scheme 1.4.3.3. *Synthesis of 1, 2, 4-Oxadiazoles by PTSA*

In 2013, Neves[89] *et al.* developed 3-aryl-5-hydroxyalkyl-1,2,4-Oxadiazoles in the presence of potassium carbonate under reflux condition (Scheme 1.4.3.4).



Scheme 1.4.3.4. *Synthesis of 3-aryl-5-hydroxyalkyl-1, 2, 4-Oxadiazoles*

In 2015, Pengfei[90] *et al.* reported the synthesis of 2-Amino-substituted 1,3,4-oxadiazoles via condensation of semicarbazide and the corresponding aldehydes followed by I_2 mediated oxidative C-O bond formation (Scheme 1.4.3.5).



Scheme 1.4.3.5. *Synthesis of 2- amino-1, 3, 4-Oxadiazole*

1.5. Drawbacks of existing methods

Sulphonate esters have interesting biological and pharmacological properties. Many methods are reported for the synthesis of sulphonate esters which involve various drawbacks such as use of halogenated, expensive and less available reagents, vigorous reaction conditions and long reaction time.[91-92] Halogenated reagents generate excess amount of HCl which make difficulty for industrial use. Peptides and proteins play important roles in modern biology. Peptides are the polymer of amino acids and polypeptides are the polymer of peptides. For the synthesis of these biological compounds, amino acids are combined together in the presence of coupling reagents, such as carbodiimides, phosphonium, and uronium/aminium salts, acyl halides, acyl azides, anhydrides, activated esters etc. All of these coupling reagents are only effective to afford the fast reaction rates for the formation of peptide bond but not on the suppression of the undesired racemization during coupling reactions. For examples, in DCC coupling reagent, the formation of an amide or peptide bond is associated with precipitation of two by-products i.e. *N*,*N*'-dicyclohexylurea and urea and racemization in presence of strong bases. In case of acyl azides, many side reactions are possible where acyl azide rearranges to acyl nitrene which undergoes Wolff rearrangement, leads to the formation of isocyanate that reacts with amino acid and gives urea as side product along with the racemized product. Similarly, in case of BOP, hexamethylphosphoramide (HMPA) and HOBt are obtained as by-products which are highly toxic and carcinogenic in nature. On the other hand, Acyl halides and Yamaguchi reagent, TCBCl have limited scope because of a danger of hydrolysis, racemization problem, cleavage of protecting groups of amino acid and generation of side reactions. TCB-DMAP is modified version of Yamaguchi reagent which was used to overcome this problem, it decreased their drawbacks but still have some major limitation i.e. racemization problems. Because of racemization problem, reduced product yield and purification becomes difficult. Also, the synthesis of coupling reagents require hazardous chemicals, excess reagents for coupling reaction and have difficulties in recycling, thus generate a lot of chemical wastes and by-products. The cost of the coupling reagents is also high and they have limited scope of substrates. Microwave technique has been developed to overcome these drawbacks. It has been successfully applied to reduce the reaction time for amidation and esterification but unfortunately, this reaction requires high temperature and also restricted to aliphatic carboxylic acids and primary aliphatic amines. Next, a heterogeneous silica-based catalyst is introduced for amidation reactions, which demonstrated a better scope but requires longer reaction times and very high temperatures to activate the catalyst.

Therefore, invention of environment-friendly methods, cost-effective and recyclable coupling reagents for peptide synthesis and organic transformation reactions with the highest level of efficacy and suppression of racemization is highly desirable and challenging. Hence, we fixed some specific objectives to develop newer strategies for peptide synthesis and different types of organic transformations to overcome the above-mentioned problems.

1.6. Objectives of thesis

The aim of the thesis work is to design and synthesize a cost-effective coupling reagent that would be easy to synthesize in the laboratory as well as its byproducts would be recyclable, so that waste generation could be reduced and meet one of the key challenges of Green Chemistry.

Based on these challenges, we have proposed the following objectives.

1. Synthesis of sulphonate esters of alcohol, Oxyma as well as oxime directly from sulphonic acid under microwave irradiation.
2. Synthesis of amides, esters and peptides in solution phase as well as in solid phase using a stoichiometric amount of Ethyl-2-cyano-2-(2-nitrophenylsulfonyloxyimino)acetate (*o*-NosylOXY).
3. Synthesis of 2,4,6-oxadiazole using a stoichiometric amount of *o*-NosylOXY.
4. Development of an Oxyma based new coupling reagent, (*E*)-ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate **(TCBOXY)** and its application for the racemization free synthesis of amides, esters, thioesters & peptides.

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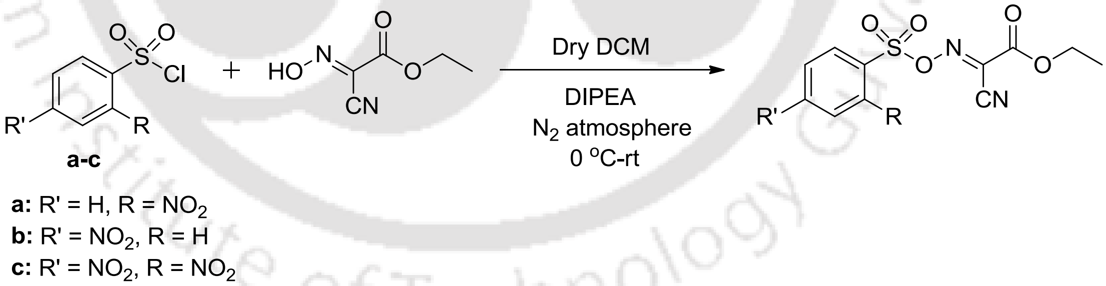
Chapter 2: Halogen Free Synthesis of Sulphonates of Alcohol, Oxyma-*O*-sulphonates, and Oxime-*O*-sulphonates under Microwave Irradiation

Previously, our lab members developed the sulphonate esters of Oxyma (*o*-NosylOXY) which is a good coupling reagent for coupling reactions, This coupling reagent require halogenated reagents for its synthesis (Scheme 2.1).[1] In this chapter, we have developed a new halogen-free, industrially benign and environment-friendly microwave-assisted method for the synthesis of sulphonate esters by direct condensation of substituted benzene sulphonic acids and alcohols or oximes, especially Oxyma.



Scheme 2.1. *Synthesis of sulphonate ester of Oxyma*

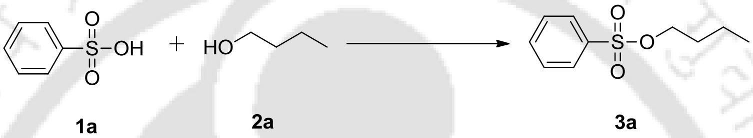
2.1. A new halogen and metal free microwave assisted environment friendly method for the synthesis of sulphonate esters

Sulphonic esters are classes of sulphonic acid derivatives that have drawn much attention for their interesting biological and pharmacological properties.[2] For example, sulphonate ester of alcohol i.e. Paeonol-phenylsulphonyl derivatives inhibit hepatitis B virus (HBV) gene expression and viral DNA replication which uses as a lead compound in anti-HBV therapy.[3] Sulphonate esters of oxime and their derivatives are also known as an important class of molecules which shows various pharmacological activities. For example, anthracenone based oxime ester shows strong antiproliferative activity against K562 leukemia cells and known as a strong inhibitor of tubulin polymerization.[4] The sulphonate esters of ethyl 2-cyano-2-(hydroxyimino) acetate (Oxyma)[5] are well-known reagents for peptide bond formation reaction for both solution and solid phase synthesis. These sulphonate esters are also found to be stable intermediates for the formation of sulphonamides, which are extremely important in medicinal chemistry.[6-9] Numerous methods are reported in the literature for the synthesis of sulphonate esters using halogen based reagents. These methods have major drawbacks, such as the use of toxic, expensive and less available reagents, vigorous reaction conditions, long reaction time, and generation of an excess amount of HCl which makes difficulty for industrial use. Herein, we reported a new halogen-free, industrially benign and environment-friendly microwave-assisted method for the synthesis of sulphonate esters by direct condensation of substituted benzene sulphonic acids and various primary and secondary alcohols or oximes, especially Oxyma.

2.1.1. Reaction optimization and substrate scope for formation of sulphonate esters

Prior to microwave heating, we first examined the reaction in reflux condition. For optimization of the reaction condition, synthesis of benzenesulphonate ester **(3a)** of butyl alcohol **(2a)** was used as a model reaction. The reaction was refluxed in toluene and progressed only with 40% yield (Table 2.1.1.1, entry **1**). Even after using a Dean-Stark trap, the yield of the product did not improve much (45%, entry **2**). Then we proceeded the reaction towards microwave irradiation and we observed that the yield was improved significantly (80%, entry **3**). The reaction in solvent-free condition under microwave irradiation although did not proceed, where the starting materials were recovered back (entry **4**).

Table 2.1.1.1. Preliminary optimization of the sulphonate ester formation reaction.

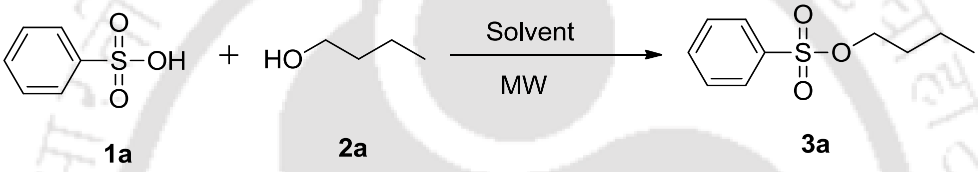


Entry	Conditions	Time (h)	% Yield[d]
1	reflux[a]	24	40
2	reflux with Dean-Stark trap[b]	24	45
3	Microwave irradiation[c]	0.5	80
4	Microwave irradiation neat[c]	0.5	0

[a] *The reaction was performed under reflux at 130 °C.* [b] *The reaction was performed with the Dean-Stark trap at 160 °C.* [c] *The sulphonic acid and alcohol were heated under microwave irradiation at 120 °C. All the reactions were performed with toluene except entry 4.* [d] *Isolated yield.*

Next, we screened a variety of solvents including, CH_3CN, H_2O, Toluene, Dioxane, *O*-Xylene and DMF for performing the reaction between benzene sulphonic acid and butyl alcohol under microwave irradiation (closed vessel, Table 2.1.1.2). We found that in acetonitrile and dioxane, the yield of the product was only 60% and 30%, respectively (entries **1** and **2**). There was no reaction in water, *o*-Xylene and DMF (entries **3**, **4** and **5**, respectively), starting materials were recovered back. A better yield of the product was obtained in toluene (entry **6**). Thus, toluene was accepted as the best among the screened solvents for this reaction.

Table 2.1.1.2. Optimization of the microwave-assisted reaction[a]



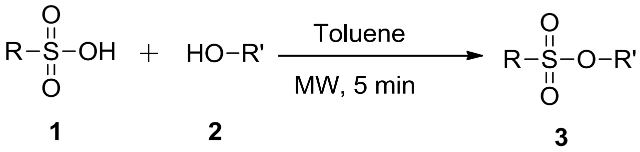
Entry	Solvents	Temp (°C)	MW energy (Watt)	Time (min)	Yield[b] (%)
1	CH_3CN	100	150	30	60
2	Dioxane	120	150	30	30
3	H_2O	120	150	45	0
4	*o*-Xylene	150	150	30	0
5	DMF	150	150	30	0
6	Toluene	120	150	30	80
7	Toluene	100	150	30	50
8	Toluene	110	150	30	70
9	Toluene	130	150	30	80
10	Toluene	120	150	25	80
11	Toluene	120	150	20	80

12	Toluene	120	150	15	80
13	Toluene	120	150	10	80
14	Toluene	120	150	5	80
15	Toluene	120	140	5	80
16	Toluene	120	120	5	80
17	Toluene	120	100	5	80
18	Toluene	120	80	5	80
19	**Toluene**	**120**	**60**	**5**	**80**
20	Toluene	120	40	5	77

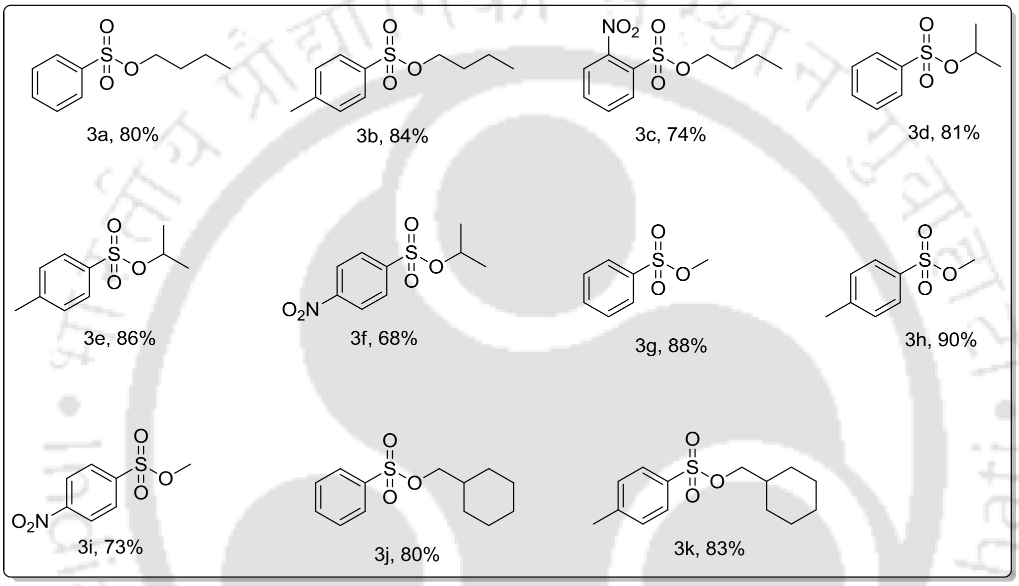
[a] *The reaction was performed with benzene sulphonic acid (1 mmol), butyl alcohol (1 mmol) were treated under microwave irradiation.* [b] *Isolated yield.*

Next, we screened the effect of temperature in toluene and the reaction time kept constant i.e. 30 min. We observed that the yield was only 50% at 100 °C (entry **7**); it increased with the elevation of temperature to 120 °C (entry **6** & **8**). A further increment of the yield of the reaction was not noticed even if the temperature was increased further (entry **9**). Therefore, we kept 120 °C as standard temperature for the further trials. Next, we optimized the reaction time and we observed no difference in yield even after reducing the duration of reaction time from 30 min to 5 min (entries **9-14**). Therefore, 5 min was accepted to be the standard duration. Thereafter, we varied the energy (Watt) of microwave irradiation and we observed that there is no difference in yield by lowering the energy of microwave irradiation from 150 watt to 60 Watt (entries **14-19**). However, below 60 Watt, the yield of product was lowered (entry **20**). Hence, 60 Watt was acquired as the optimum energy needed for the reaction.

Table 2.1.1.3. Esterification of sulphonic acids with alcohols.[a,b]



R = Aromatic sulphonic acid
R' = Aliphatic alcohol



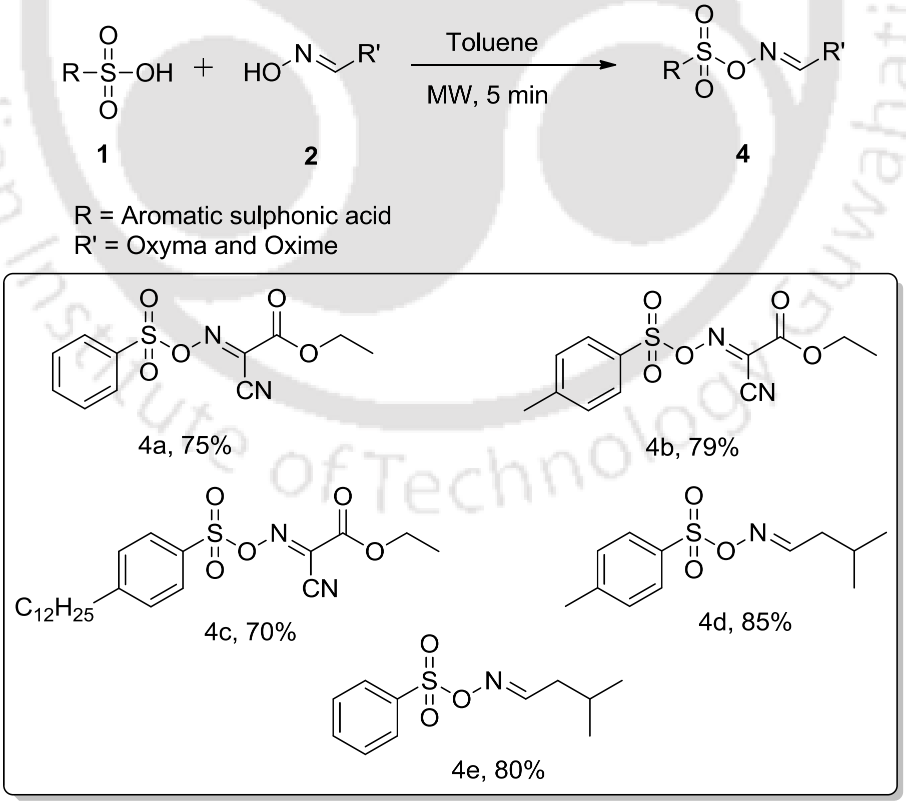
[a] *The reaction was performed with sulphonic acid (1 mmol), alcohol (1 mmol) and toluene (3 ml) under microwave irradiation (upper limit of MW was fixed at 60 Watt) for 5 min at 120 °C.* [b] *Isolated yield.*

With these promising results, we explored the scope of this method by using a wide range of substituted benzene sulphonic acid with the variety of aliphatic alcohols. All the reactions proceeded efficiently and the desired sulphonic esters were obtained in good yields with high purity (as determined by NMR spectroscopy).

Aromatic sulphonic acids having electron donating substituents reacted very well to give the corresponding sulphonate esters (Table 2.1.1.3, entries **3b**, **3e**, and **3h**) while sulphonic acids with electron withdrawing substituents, such as nitro group, did not react completely which in turn generated relatively low yield (entries **3c**, **3f**, and **3i**).

The scope of this method was further explored for the synthesis of sulphonate esters of oximes, especially, Oxyma (Table 2.1.1.4). All of these substrates reacted smoothly under the optimized conditions to provide the desired products. Synthesis of sulphonate ester of Oxyma worked very well with electron donating substituents (entries **4a**, **4b**, and **4c**), whereas sulphonic acids with electron withdrawing substituents showed somewhat lower reactivity with relatively low yield. In case of isovaleraldoxime also, we found benzene sulphonic acids with electron donating substituents worked well (entries **4d** and **4e**) but no product formed with electron withdrawing substituents.

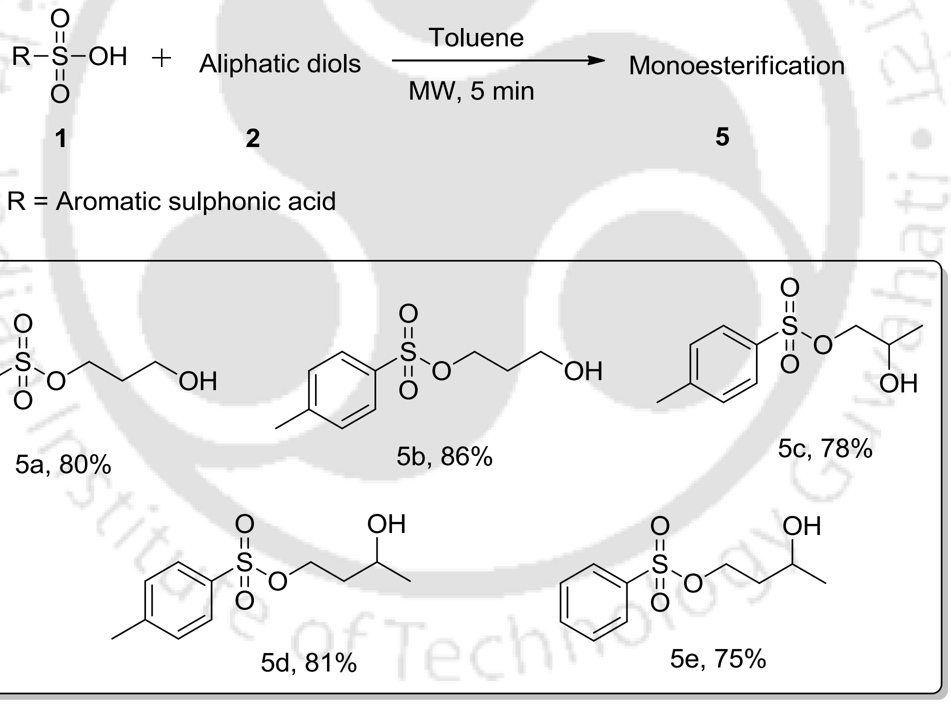
Table 2.1.1.4. Reaction of Oxyma and oxime with different sulphonic acids.[a,b]



[a]*The reaction conditions are same as mentioned in Table 2.1.1.3.* [b]*Isolated yield*

Next, we investigated the regioselectivity of the reaction with diols. Diols got monosulphonylated selectively with one equivalent of the benzene sulphonic acid (Table 2.1.1.5, entries **5a** and **5b**). In presence of a secondary alcohol, sulphonylation occurred selectively at the primary hydroxyl group (entries **5c**-**5e**). No trace of the disulphonylated product was observed as confirmed by NMR and ESI-MS spectra of the crude product (Figure S28-S33). On the other hand, the earlier method[10] using TsCl and pyridine offered a mixture of sulphonylation at the primary hydroxyl group as well as at the secondary hydroxyl group in 7:1 ratio, making the purification difficult.

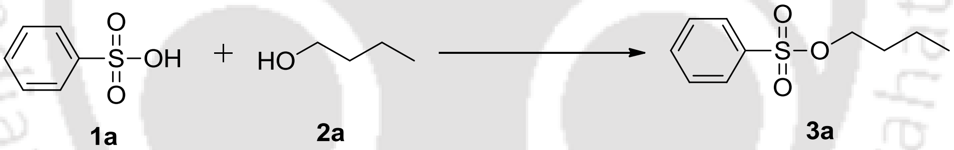
Table 2.1.1.5. Regioselectivity of diols.[a,b]



[a]The *reaction was performed with sulphonic acid (1 mmol), diol (1 mmol) and toluene (3 ml) under microwave irradiation (upper limit of MW was fixed at 60 Watt) for 5 min at 120 °C.* [b]*Isolated yield.*

All of these substrates reacted smoothly with electron donating substituents on the benzene sulphonic acid under the optimized conditions but either the yield was low or the reaction did not work for substrates containing electron withdrawing groups. Hence, we tried different additives (drying agents) to enhance the yield of the reaction and expected to get some products for the substrates containing electron withdrawing groups. For that, we took the same model reaction and tried the reaction with dry silica gel under reflux condition but the yield did not improve (Table 2.1.1.6, entry **1**). Then we tried the reaction under microwave irradiation with molecular sieves (3Å pore size) as an additive, we observed that the yield did not improve much (entry **2**). But, the combination of dry silica gel and MW together improved the yield significantly (entry **3**). However, reducing the amount of silica to 30 mg, the yield of the product decreased (entry **4**). Therefore, we kept the combination of dry silica gel (60 mg) and MW as the standard condition for other experiments.

Table 2.1.1.6. Preliminary optimization of the sulphonate ester formation reaction with silica.[a,b]

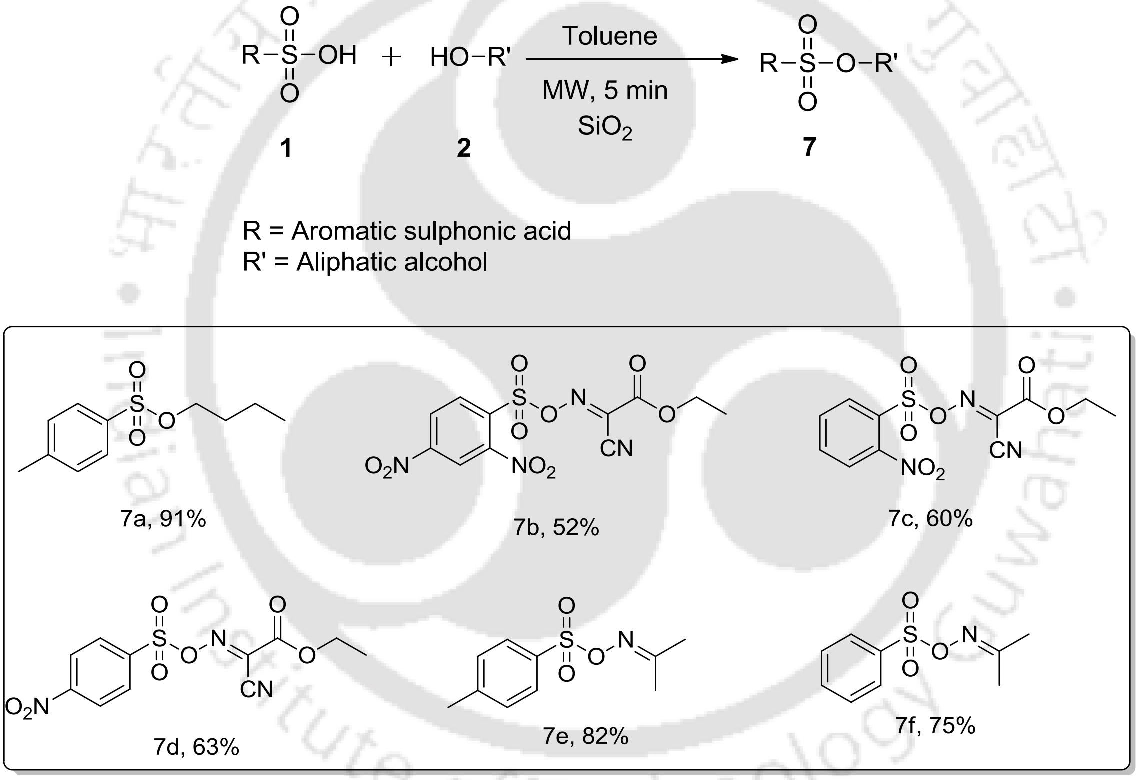


Entry	Conditions[a]	Weight of drying agent (mg)	Time (min)	% Yield[b]
1	Reflux with silica	60	1440	60
2	Microwave irradiation with molecular sieve (3Å)	100	5	84
3	Microwave irradiation with silica	60	5	91
4	Microwave irradiation with silica	30	5	66

[a] *The benzene sulphonic acid (1 mmol) and butyl alcohol (1 mmol) were heated under microwave irradiation at 120 ºC. All the reactions were performed in toluene.* [b] *Isolated yield.*

With the optimized conditions, we explored the reaction with substituted benzene sulphonic acids and (Table 2.1.1.7) alcohol (entry **7a**), Oxyma (entries **7b**-**7d**), and acetoxime (entries **7e**-**7f**) in presence of silica gel. These reactions proceeded efficiently in spite of the presence of the electron withdrawing substituents, which were unreactive in absence of silica. Yields of the desired sulphonate esters also increased.

Table 2.1.1.7. Esterification of sulphonic acids using silica gel.[a,b]



[a]*The reactions were performed with sulphonic acid (1 mmol), alcohol (1 mmol), silica gel 60 (60 mg) and toluene (3 ml) under microwave irradiation (upper limit of MW was fixed at 60 Watt) for 5 min at 120 °C.* [b]*Isolated yield.*

After the reaction, we could recover the silica gel just by filtration. The recovered silica gel was dried in the hot air oven and cooled down under desiccator, and then reused up to 5 cycles for the same reaction with no decrement of the yield of the product (Figure 2.1.1.1).

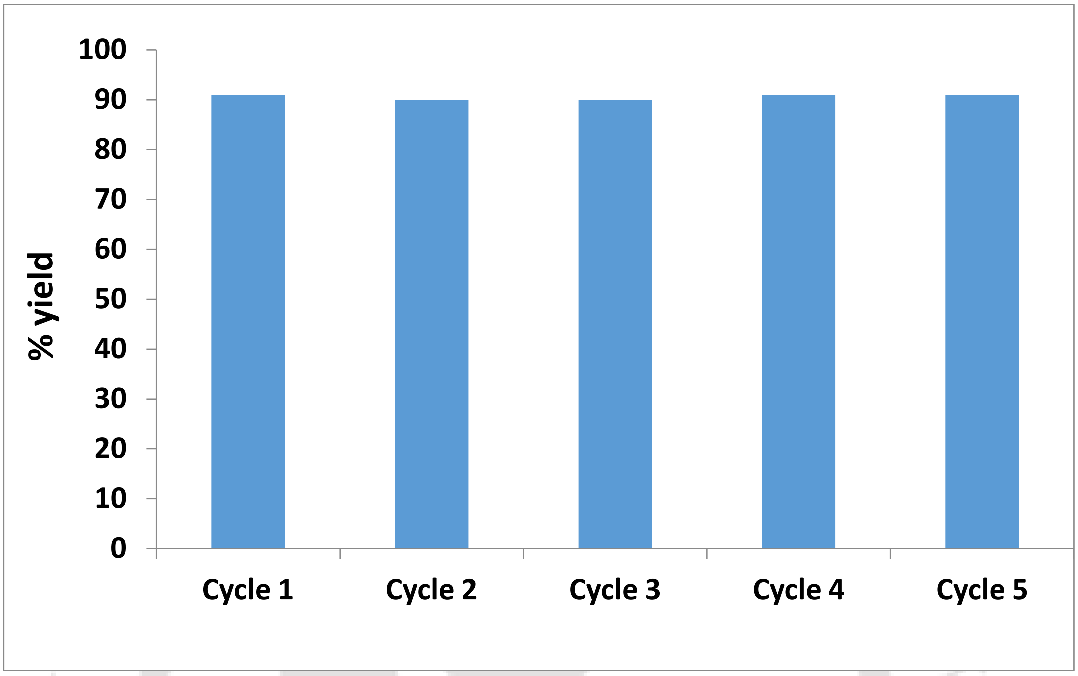


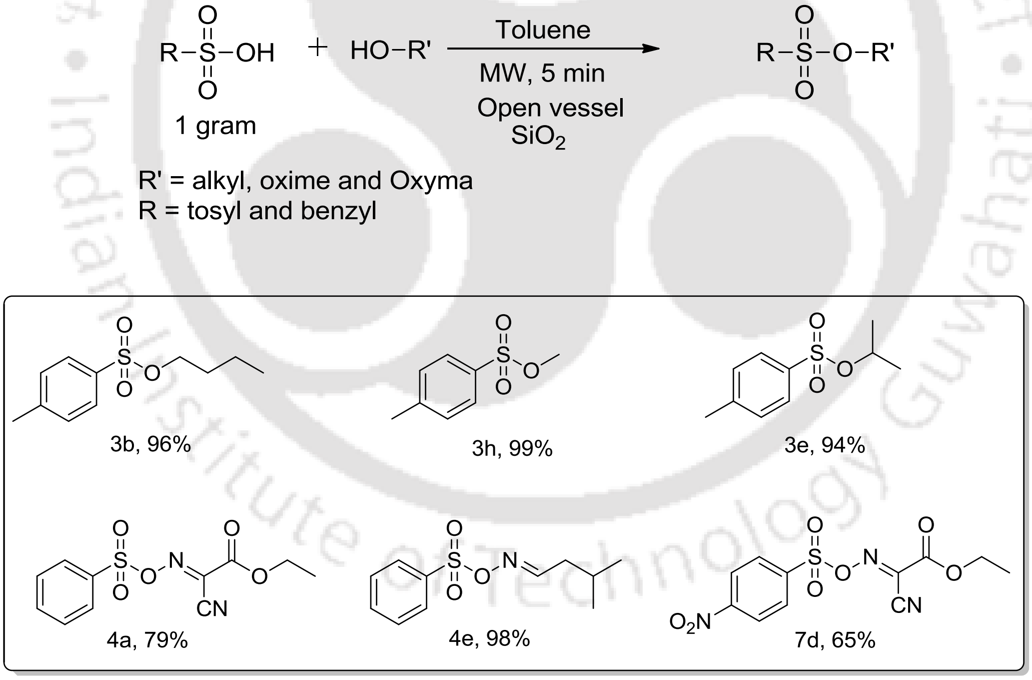
Figure 2.1.1.1. *Reusability of silica gel.*

After small-scale synthesis, we tried for the large-scale synthesis of sulphonate esters using the open vessel method. We observed that there is an improvement in yield of products. Previously, reaction with small-scale (1 mmol), we observed average yield but reaction with large-scale (1 g), excellent yield was obtained (Table 2.1.1.8). The reaction was clean and column chromatography was not required. Only filtration of silica gel and evaporation of the solvent generated pure products. In case of oximes, unreacted sulphonic acid and Oxyma was removed by washing with the bicarbonate solution.

However, the reaction with small-scale, the yield of the product **7d** was only 63% (Table 2.1.1.7). Even with large-scale protocol, the yield of **7d** did not improve much (~ 65%, Table 2.1.1.8). In this case, we could recover the rest of the starting materials by eluting with specific eluents and then recombined for next batch. But the reaction was not complete as before. However, the recovery and reuse were continued up to 5 cycles.

Overall 1.4 g of the product was obtained, whereas the weight of the unreacted Oxyma and 4-nitrobenzene sulphonic acid after the last cycle were 36 mg and 108 mg, respectively. Thus overall % of conversion of 4-nitrobenzene sulphonic acid was 89% and the overall percentage yield of the isolated product was 97% with respect to the mentioned conversion rate. More importantly, no side reactions were observed, therefore such cyclic operations were possible to perform. And the improvement of the conversion rate of an underactive substrate was possible in this way. Also, the above-mentioned procedure confirmed reversibility of the process.

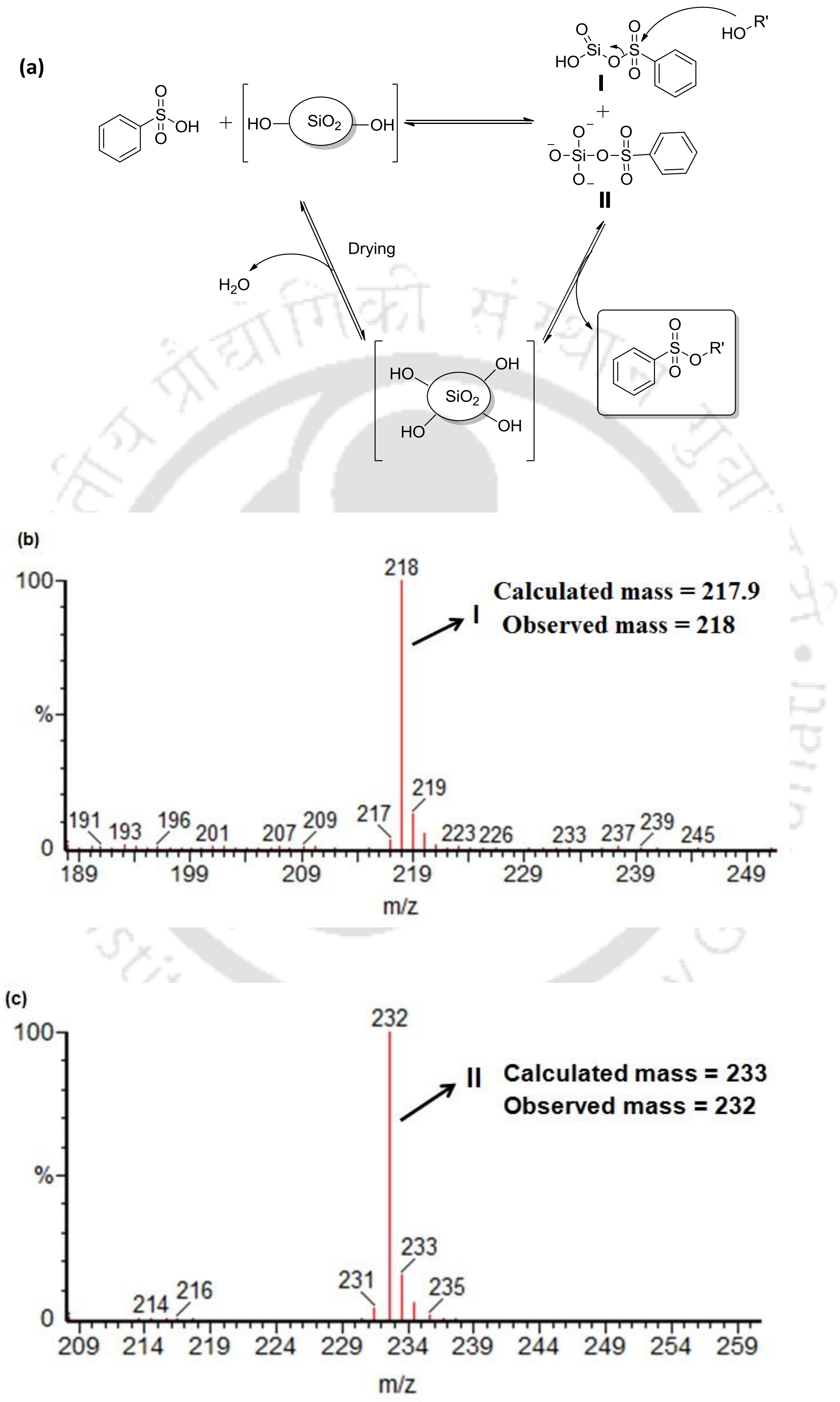
Table 2.1.1.8. Gram scale synthesis of sulphonate ester of alcohols, Oxyma and oxime.[a,b]



[a]*The gram scale reaction was performed with sulphonic acid (1 g, 1 equiv), alcohol (1 equiv), Oxyma (1 equiv) or oxime (1 equiv), silica gel 60 (1 g) and toluene (5 ml) under microwave irradiation (upper limit of MW was fixed at 60 Watt) for 5 min at 120 °C.* [b]*Isolated yield.*

2.2. Plausible mechanism

After exploring the scope of the substrate, we turned our attention to an elucidation of the mechanism of the reaction. Based on our experimental results, we have drawn a plausible mechanistic pathway (Figure 2.2.1**a**). At first, we thought silica gel is acting only as a dehydrating agent. But GCMS analysis of a reaction mixture containing silica gel and sulphonic acid, devoid of the nucleophile revealed two peaks of m/z values 218 and 232 (Figure 2.2.1**b** and 2.2.1**c,** & Figure S45a and S45d) that correspond to the depicted intermediates **I** and **II**, respectively. Therefore, we suggest that benzene sulphonic acid may react with the SiO_2 and other poly co-ordinated species present in the silica gel in the first step in a reversible manner and lead to the generation of the intermediate **I**, **II** or similar species. In the second step, **I** or **II** may react with the nucleophile to form the desired product. The FT-IR spectra (Figure 2.2.1**d**) of a premature reaction mixture (without nucleophile) indicate a broad signal at 3435 cm^{-1}. These vibrations are assigned to the stretching bands of Si-OH and of mutual linkage of water molecules with silanol groups. The vibration band at 1734 cm^{-1} indicates sulphonate ester bond (O=S-O-) with silica and the band at 1639 cm^{-1} indicates C=C bond of the benzene ring. The band at 1157 cm^{-1} is assigned to the Si-O-S bonding of benzene sulphonic acid over the surface of silica. The sharp bands at 1307 cm^{-1} and 1447 cm^{-1} indicate O=S=O stretching vibration bands, whereas the sharp bands at 576 cm^{-1} and 596 cm^{-1} are assigned to the O=S=O rocking bands.


(a)
SiO2
HO
OH
I
II
HO R'
Drying
H2O
(b)
218
Calculated mass = 217.9
Observed mass = 218
100
%
0
191
193
196
201
207
209
217
219
223
226
233
237
239
245
189
199
209
219
229
239
249
m/z
(c)
232
Calculated mass = 233
Observed mass = 232
214
216
231
233
235
209
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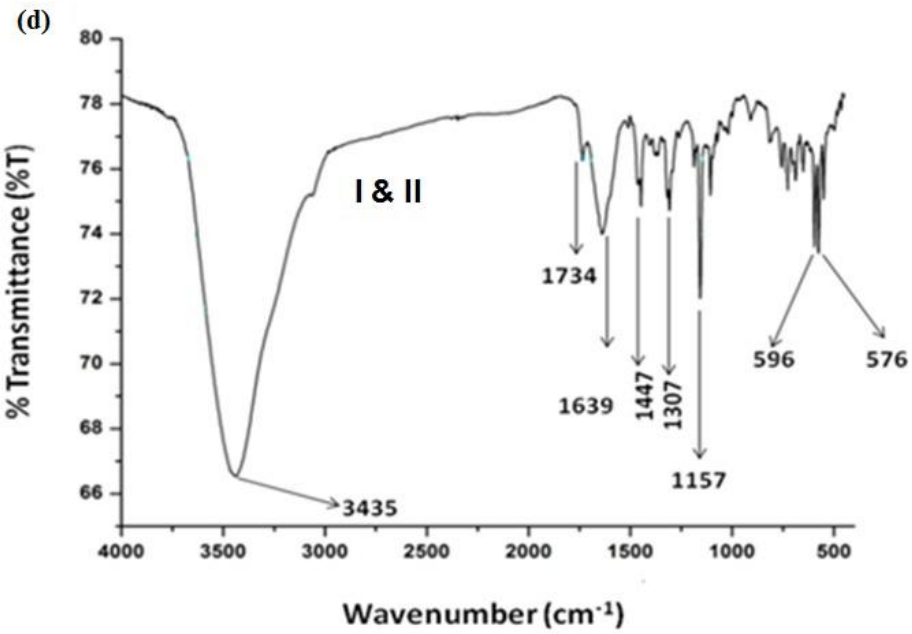
229
234
239
244
249
254
259
m/z



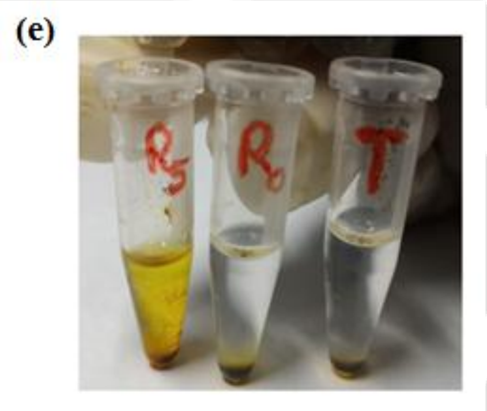


Figure 2.2.1*(a) A plausible mechanistic pathway. (b) GCMS spectra of intermediate* ***I****. (c) GCMS spectra of intermediate* ***II*** *(d) IR spectra of intermediate* ***I*** *(e) $FeCl_3$ test for the evolution of water as the by-product.*

Furthermore, the evolution of water was confirmed by adding anhydrous $FeCl_3$ to the reaction mixture (Figure 2.2.1**e**). $FeCl_3$ gives yellow or orange color in presence of water.[11] We took three sample vials, marked as R_5, T, and R_0; where R_5 indicates the reaction mixture after 5 min MW irradiation without silica gel (after the reaction), T indicates toluene solvent (reference), R_0 indicates the reaction mixture at 0 times (without

MW irradiation, reference). We added anhydrous $FeCl_3$ in each vial and observed that the solution in R_5 turns into yellow color immediately but the same in vials marked R_0 and T retains the black color of $FeCl_3$. The evolution of yellow color in R_5 confirms the evolution of water as the byproduct in the reaction mixture. Such condensation does not generate any by-product other than water. Therefore, our method is environment-friendly.

2.3. Conclusions

In summary, we have described a new, versatile and greener method for direct esterification of sulphonic acid with better yield using inexpensive chromatographic silica gel as additive under microwave irradiation without the use of any halogenating or activating reagents. This new halogen-free microwave technology-based method not only increased the yield but also reduced the reaction times from hours to minutes. This method was also examined for the regioselective sulphonylation of diols i.e., diols got monosulphonylated selectively with one equivalent of the benzene sulphonic acid and also higher selectivity towards the primary alcohol group than the secondary alcohol group. We could also recover the starting materials and silica gel very easily and reused for the next batches.

2.4. Experimental section

2.4.1. General consideration

All reagents were purchased from commercial sources. NMR spectra were recorded on 600 MHz and 400 MHz spectrometers using $CDCl_3$ as solvent and tetramethylsilane

(TMS) as an internal standard. Chemical shifts (δ) were reported in ppm and spin-spin coupling constants (J) were given in Hz. Abbreviations to denote the multiplicity of a particular signal are s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Reactions were monitored using thin layer chromatography with silica gel GF254. Purification of the reaction products was carried out by column chromatography using silica gel (60-120 mesh) using eluent EtOAc/Hexane. Solvents were removed under reduced pressure using a Buchi rotary evaporator. Melting points were determined using a dedicated melting point measuring apparatus and FT-IR spectra were recorded on an FT-IR spectrometer. CEM microwave reactor was used for microwave irradiation. X-Ray data were collected on a diffractometer equipped with a CCD area detector using Mo/Kα radiation. The structures were solved by the direct method using *SHELLX-97* (Göttingen, Germany) software. High-resolution mass spectra were recorded on a Micromass Q-TOF ESI-MS instrument (model HAB273) and Agilent 6500 Q-TOF LC/MS system; HPLC analysis was carried out with reverse phase column and CHIRAL PAK AS-H (5 μm, 2.1×150mm) column coupled to a UV detector. HPLC grade solvents were used for HPLC analysis.

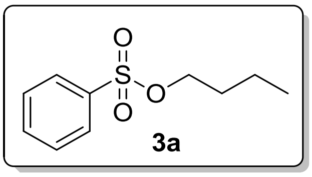
2.4.2. General procedure for the direct synthesis of sulphonate ester from sulphonic acids: Sulphonic acid (1 mmol), alcohol or Oxyma or oxime (1 mmol) were taken in microwave tube in Toluene (3 mL). The mixture was then placed in CEM microwave reactor and heated at 120 °C for 5 min. The progress of the reaction was monitored by TLC. After completion of the reaction, toluene was evaporated by rotavapor. The organic layer was diluted with ethyl acetate and washed with 5% $NaHCO_3$ (2×10 mL) and dried over anhydrous Na_2SO_4 and pure product was obtained, which was further characterized by usual spectroscopic techniques. Only in case of **4a**, **4b**, and **4c**, compounds were purified by column chromatography.

2.4.3. General procedure for the synthesis of sulphonate ester from sulphonic acids using silica gel: Sulphonic acid (1 mmol), alcohol or Oxyma or oxime (1 mmol) and silica gel 60 (230-400 mesh, 60 mg) were taken in microwave tube in Toluene (3 mL). The mixture was then placed in CEM microwave reactor and heated at 120 °C for 5 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered and SiO_2 was dried in a hot air oven for further use. The organic layer was diluted with ethyl acetate and was washed with 5% $NaHCO_3$ (2×10 mL) and dried over anhydrous Na_2SO_4 and pure product was obtained. But in case of **7b**, **7c**, and **7d**, compounds were purified by column chromatography.

2.4.4. General procedure for the gram scale synthesis of sulphonate esters from sulphonic acids using silica gel: Sulphonic acid (1 gram, 1 equiv), alcohol or Oxyma or oxime (1 equiv) and silica gel 60 (230-400 mesh, 1 gram) were taken in 50 ml RB flask in Toluene (5 mL). The mixture was then placed in CEM microwave cavity and heated at 120 °C for 5 min under open vessel condition using condenser and $CaCl_2$ guard tube. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered and SiO_2 was dried in a hot air oven for further use. The filtrate was evaporated and pure product was obtained, except for **4a** and **7d**.

2.5. Characterization data

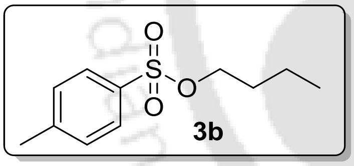
Butyl benzenesulfonate, 3a



3a

Sticky; (171 mg, 80%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.91-7.89 (d, J = 7.8 Hz, 2H), 7.66-7.63 (t, *J* = 7.2 Hz, 1H), 7.56-7.54 (t, *J* = 7.2 Hz, 2H), 4.05-4.03 (t, *J* = 6.6 Hz, 2H), 1.62-1.60 (t, *J* = 6.6 Hz, 2H), 1.34-1.31 (m, 2H), 0.85-0.83 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 136.3, 133.9, 129.4, 128.0, 70.8, 30.9, 18.7, 13.5; FT-IR (KBr, cm^{-1}): 2962, 1728, 1449, 1359, 1186, 1097, 939, 883, 756, 590; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{10}H_{15}O_3S$ 215.0742, found 215.0749.

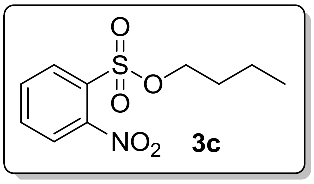
Butyl 4-methylbenzenesulfonate, 3b



3b

Sticky; (192 mg, 84%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.79-7.78 (d, *J* = 7.8 Hz, 2H), 7.35-7.33 (d, *J* = 7.8 Hz, 2H), 4.03-4.01 (t, *J* = 6.0 Hz, 2H), 2.45 (s, 3H), 1.63-1.61 (t, *J* = 6.6 Hz, 2H), 1.35-1.32 (m, 2H), 0.87-0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 144.8, 133.4, 130.0, 128.1, 70.6, 31.0, 21.8, 18.8, 13.6; FT-IR (KBr, cm^{-1}): 2962, 1728, 1455, 1359, 1180, 1097, 940, 884, 817, 664, 554; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{17}O_3S$ 229.0898, found 229.0903.

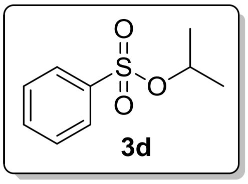
Butyl 2-nitrobenzenesulfonate, 3c



3c

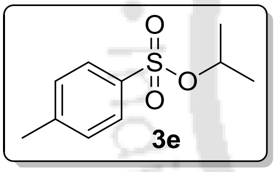
Sticky; (192 mg, 74%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.14-8.13 (d, *J* = 7.8 Hz, 1H), 7.81-7.74 (m, 3H), 4.30-4.28 (t, *J* = 6.6 Hz, 2H), 1.75-1.71 (m, 2H), 1.44-1.40 (m, 2H), 0.93-0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 144.9, 134.9, 132.3, 131.4, 130.0, 124.9, 72.6, 31.1, 18.8, 13.6; FT-IR (KBr, cm^{-1}): 2962, 1729, 1546, 1370, 1187, 936, 783, 740, 592.

Isopropyl benzenesulfonate, 3d


3d

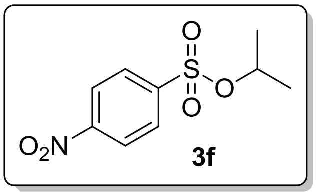
Sticky; (162 mg, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92-7.52 (m, 5H), 4.77-4.71 (m, 1H), 1.27-1.25 (d, *J* = 6.4 Hz 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 133.7, 129.3, 127.8, 60.6, 22.9; FT-IR (KBr, cm^{-1}): 2987, 1720, 1449, 1361, 1188, 1087, 918, 881, 781, 757, 690, 591; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_9H_{13}O_3S$ 201.0585, found 201.0589.

Isopropyl 4-methylbenzenesulfonate, 3e


3e

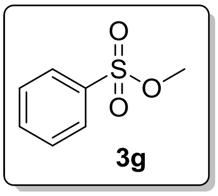
Sticky; (184 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.77 (d, *J* = 8.4 Hz, 2H), 7.34-7.31 (d, *J* = 8.4 Hz, 2H), 4.74-4.68 (m, 1H), 2.43 (s, 3H), 1.26-1.25 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.6, 134.7, 129.9, 127.8, 60.6, 23.0, 21.8; FT-IR (KBr, cm^{-1}): 2986, 1720, 1599, 1465, 1361, 1177, 1087, 919, 880, 816, 767, 663, 577, 555, 503; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{10}H_{15}O_3S$ 215.0742, found 215.0753.

Isopropyl 4-nitrobenzenesulfonate, 3f


3f

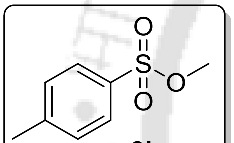
Solid; (167 mg, 68%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38-8.36 (d, *J* = 8.8 Hz, 2H), 7.90-7.88 (d, *J* = 8.4 Hz, 2H), 4.68-4.62 (m, 1H), 1.42-1.28 (dd, *J* = 6.4 Hz, *J* = 6.4 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.1, 150.1, 126.7, 124.4, 74.8, 24.0, 23.9; FT-IR (KBr, cm^{-1}): 2981, 1604, 1526, 1350, 1139, 1108, 923, 858, 737, 684, 564, 504; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_9H_{12}NO_5S$ 246.04, found 246.11.

Methyl benzenesulfonate, 3g


3g

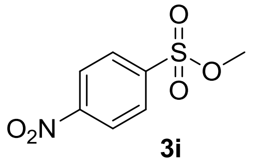
Sticky; (151 mg, 88%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.92-7.91 (d, *J* = 7.8 Hz, 2H), 7.68-7.66 (t, *J* = 7.2 Hz, 1H), 7.58-7.56 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 135.4, 134.1, 129.5, 128.2, 56.6; FT-IR (KBr, cm^{-1}): 2925, 1632, 1449, 1363, 1189, 1095, 990, 917, 789, 755, 710, 689, 588, 561; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_7H_9O_3S$ 173.0272, found 173.0263.

Methyl 4-methylbenzenesulfonate, 3h


3h

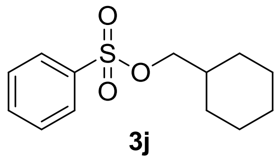
Sticky; (167 mg, 90%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.80-7.78 (d, *J* = 7.6 Hz, 2H), 7.36-7.34 (d, *J* = 8.0 Hz, 2H), 3.74 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 145.1, 132.4, 130.1, 128.3, 56.4, 21.9; FT-IR (KBr, cm^{-1}): 2922, 2848, 1728, 1597, 1525, 1456, 1361, 1177, 1096, 993, 817, 767, 686, 661, 556, 505; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_8H_{11}O_3S$ 187.0429, found 187.0427.

Methyl 4-nitrobenzenesulfonate, 3i


3i

Sticky; (158 mg, 73%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.43-8.41 (d, *J* = 8.8 Hz, 2H), 8.14-8.11 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 150.1, 149.0, 127.4, 123.8, 54.6; FT-IR (KBr, cm^{-1}): 2924, 1604, 1521, 1354, 1231, 1125, 1036, 743, 640, 550, 472; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_7H_8NO_5S$ 218.01, found 218.08.

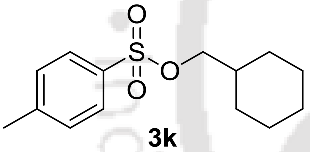
Cyclohexylmethyl benzenesulfonate, 3j



3j

Sticky; (203 mg, 80%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91-7.90 (d, *J* = 7.2 Hz, 2H), 7.66-7.64 (t, *J* = 7.8 Hz, 2H), 7.57-7.54 (t, *J* = 7.8 Hz, 1H), 3.44-3.43 (d, *J* = 6.6 Hz, 2H), 1.76-1.72 (m, 4H), 1.50-1.47 (m, 1H), 1.28-1.21 (m, 4H), 0.97-0.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.8, 130.5, 129.0, 127.5, 75.8, 40.7, 29.8, 26.8, 25.6; FT-IR (KBr, cm^{-1}): 2852, 1728, 1449, 1363, 1283, 1186, 1097, 973, 947, 829, 755, 689, 589; HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{13}H_{18}NaO_3S$ 277.0874, found 277.0877.

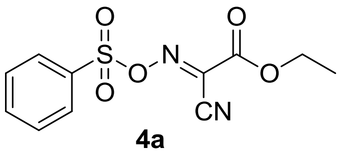
Cyclohexylmethyl-4-methylbenzenesulfonate, 3k



3k

Sticky; (222 mg, 83%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79-7.77 (d, *J* = 8.4 Hz, 2H), 7.35-7.33 (d, *J* = 8.4 Hz, 2H), 3.81-3.80 (d, *J* = 6.0 Hz, 2H), 2.45 (s, 3H), 1.69-1.67 (m, 3H), 1.46-141 (m, 1H), 1.29-1.25 (m, 4H), 0.92-0.84 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 133.4, 130.0, 128.1, 75.6, 37.4, 29.3, 25.6, 21.9; FT-IR (KBr, cm^{-1}): 2853, 1729, 1451, 1362, 1285, 1178, 1098, 974, 947, 893, 667, 580; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{21}O_3S$ 269.1211, found 269.1191.

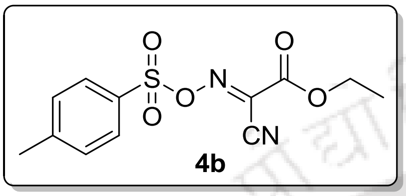
(*E*)-Ethyl-2-cyano-2-(((phenylsulfonyl)oxy)imino) acetate, 4a



4a

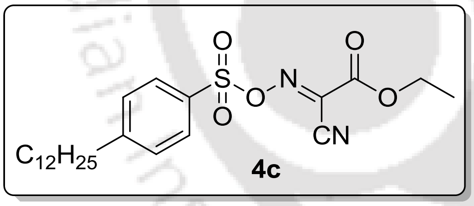
White crystalline solid; (212 mg, 75%); mp 96 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06-8.03 (d, *J* = 8.2 Hz, 2H), 7.79-7.75 (t, *J* = 7.2 Hz, 1H), 7.65-7.61 (t, *J* = 8 Hz, 2H), 4.44-4.38 (q, 2H), 1.39-1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 135.9, 133.4, 131.6, 129.8, 129.6, 106.2, 64.8, 14.1; FT-IR (KBr, cm^{-1}): 2977, 1755, 1591, 1450, 1405, 1294, 1198, 1002, 943, 824, 763, 668, 579; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{11}H_{11}N_2O_5S$ 283.0389, found 283.0388.

(*E*)-Ethyl-2-cyano-2-((tosyloxy)imino) acetate, 4b


4b

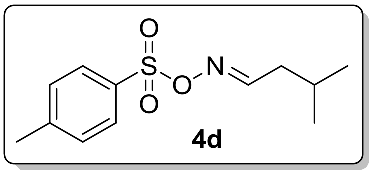
White solid; (234 mg, 79%); mp 70-71 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 8.4 Hz, 2H), 7.33-7.32 (d, *J* = 7.8 Hz, 2H), 4.09-4.06 (q, 2H), 2.42 (s, 3H), 1.28-1.25 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 147.5, 131.4, 130.4, 130.1, 129.6, 106.2, 64.7, 22.0, 14.0; FT-IR (KBr, cm^{-1}): 2925, 1758, 1688, 1594, 1393, 1293, 1199, 1014, 950, 844, 820, 679, 549; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{12}H_{13}N_2O_5S$ 297.05, found 297.28.

(*E*)-Ethyl-2-cyano-2-((((4-dodecylphenyl) sulfonyl)oxy) imino) acetate, 4c


4c

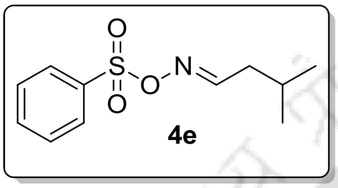
Sticky; (315 mg, 70%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06-8.03 (d, *J* = 8.4 Hz, 1H), 7.45-7.42 (t, *J* = 7.6 Hz, 1H), 4.44-4.37 (m, 5H), 4.14-4.09 (q, 2H), 1.45-1.37 (m, 15H), 1.29-1.24 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 132.9, 129.6, 128.3, 125.0, 108.1, 63.7, 29.8, 21.3, 14.6, 14.2; FT-IR (KBr, cm^{-1}): 2961, 2923, 2852, 1730, 1462,1261, 1095, 1023, 802.

(*E*)-3-Methylbutanal *O*-tosyl oxime, 4d


4d

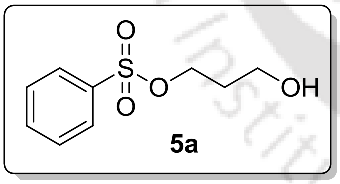
Oil; (217 mg, 85%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25-7.23 (t, *J* = 7.8 Hz, 2H), 7.17-7.15 (t, *J* = 7.2 Hz, 2H), 6.74-6.73 (t, *J* = 5.4 Hz, 1H), 2.34 (s, 3H), 2.29-2.27 (t, *J* = 6.0 Hz, 1H), 2.09-2.07 (t, *J* = 6.6 Hz, 1H), 1.86-1.79 (m, 1H), 0.96-0.93 (dd, *J* = 6.6 Hz, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.0, 138.0, 129.2, 128.4, 125.5, 38.3, 26.9, 22.6, 21.6; FT-IR (KBr, cm^{-1}): 2959, 1727, 1659, 1466, 1387, 1047, 926, 801, 694; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{12}H_{18}NO_3S$ 256.10, found 256.22.

(*E*)-3-Methylbutanal *O*-phenylsulfonyl oxime, 4e



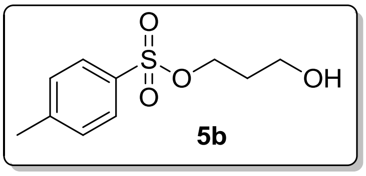
Oil; (193 mg, 80%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.37 (t, *J* = 6.6 Hz, 1H), 7.18-7.16 (t, *J* = 7.8 Hz, 1H), 7.10-7.07 (t, *J* = 7.8 Hz, 1H), 6.70-6.69 (t, *J* = 5.4 Hz, 1H), 2.24-2.22 (t, *J* = 6.6 Hz, 1H), 2.04-2.02 (t, *J* = 6.6 Hz, 1H), 1.82-1.72 (m, 1H), 0.91-0.88 (dd, *J* = 6.6 Hz, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.5, 137.6, 128.9, 128.1, 125.3, 38.0, 26.7, 22.1; FT-IR (KBr, cm^{-1}): 2960, 1709, 1660, 1467, 1387, 1048, 803, 694, 604.

3-Hydroxypropyl benzenesulfonate, 5a



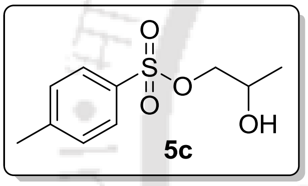
Sticky; (173 mg, 80%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91-7.90 (d, *J* = 8.4 Hz, 2H), 7.67-7.64 (t, *J* = 7.2 Hz, 1H), 7.57-7.54 (t, *J* = 7.8 Hz, 2H), 4.20-4.18 (t, *J* = 6.0 Hz, 2H), 3.70-3.69 (d, *J* = 4.8 Hz, 2H), 1.91-1.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ; 136.0, 134.1, 129.5, 128.0, 67.9, 58.3, 31.8; FT-IR (KBr, cm^{-1}): 2885, 1448,1359, 1187, 1097, 934, 836, 755, 689, 590; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_9H_{13}O_4S$ 217.0535, found 217.0535.

3-Hydroxypropyl 4-methylbenzenesulfonate, 5b


5b

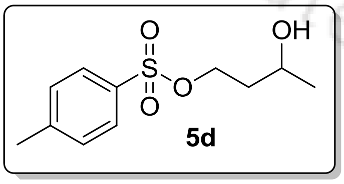
Sticky; (198 mg, 86%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.77 (d, *J* = 7.8 Hz, 2H), 7.35-7.33 (d, *J* = 7.8 Hz, 2H), 4.17-4.15 (t, *J* = 6.0 Hz, 2H), 3.70-3.68 (m, 2H), 2.44 (s, 3H), 1.89-1.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.1, 133.0, 130.1, 128.0, 68.0, 58.1, 31.8, 21.8; FT-IR (KBr, cm^{-1}): 2926, 1598, 1356, 1175, 1097, 933, 816, 665, 555; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{10}H_{15}O_4S$ 231.0691, found 231.0691.

2-Hydroxypropyl 4-methylbenzenesulfonate, 5c


5c

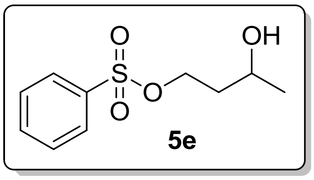
Sticky; (179 mg, 78%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81-7.79 (d, *J* = 8.4 Hz, 2H), 7.37-7.35 (d, *J* = 8.4Hz, 2H), 4.06-4.02 (m, 1H), 4.01-3.98 (m, 1H), 3.86-3.83 (m, 1H), 2.45 (s, 3H), 1.17-1.15 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.3, 132.8, 130.2, 128.2, 65.9, 21.9, 18.7; FT-IR (KBr, cm^{-1}): 2924, 1598, 1357, 1177, 1097, 981, 815, 667, 556; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{10}H_{15}O_4S$ 231.0691, found 231.0691.

3-Hydroxybutyl 4-methylbenzenesulfonate, 5d


5d

Sticky; (198 mg, 81%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80-7.78 (d, *J* = 8.4 Hz, 2H), 7.35-7.34 (d, *J* = 7.8 Hz, 2H), 4.25-4.21 (m, 1H), 4.12-4.09 (m, 1H), 3.95-3.92 (m, 1H), 2.45 (s, 3H), 1.72-1.66 (m, 2H), 1.19-1.18 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.1, 133.0, 130.1, 128.1, 68.0, 64.3, 38.0, 23.8, 21.9; FT-IR (KBr, cm^{-1}): 2926, 1356, 1176, 1097, 947, 816, 665, 555; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{11}H_{17}O_4S$ 245.0848, found 245.0802.

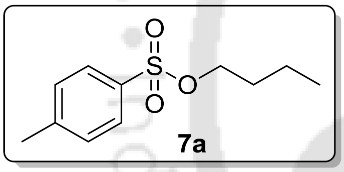
3-Hydroxybutyl benzenesulfonate, 5e



5e

Sticky; (173 mg, 75%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.93-7.92 (d, *J* = 7.8 Hz, 2H), 7.68-7.65 (t, *J* = 7.8 Hz, 1H), 7.58-7.55 (t, *J* = 7.8 Hz, 2H), 4.29-4.25 (m, 1H), 4.16-4.13 (m, 1H), 3.96-3.93 (m, 1H), 1.72-1.67 (m, 2H), 1.20-1.19 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 135.9, 134.0, 129.5, 128.1, 68.3, 64.3, 38.0, 23.8; FT-IR (KBr, cm^{-1}): 2924, 1448, 1358, 1187, 1096, 947, 755, 689, 589; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{10}H_{15}O_4S$ 231.0691, found 231.0641.

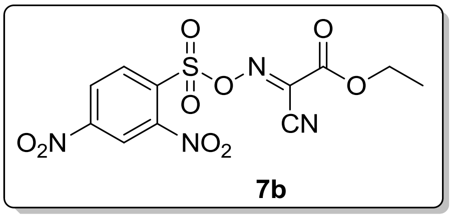
Butyl 4-methylbenzenesulfonate, 7a



7a

Sticky; (207 mg, 91%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.79-7.78 (d, *J* = 7.8 Hz, 2H), 7.35-7.33 (d, *J* = 7.8 Hz, 2H), 4.03-4.01 (t, *J* = 6.0 Hz, 2H), 2.45 (s, 3H), 1.63-1.61 (t, *J* = 6.6 Hz, 2H), 1.35-1.32 (m, 2H), 0.87-0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 144.8, 133.4, 130.0, 128.1, 70.6, 31.0, 21.8, 18.8, 13.6; FT-IR (KBr, cm^{-1}): 2962, 1728, 1455, 1359, 1180, 1097, 940, 884, 817, 664, 554; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{17}O_3S$ 229.0898, found 229.0903.

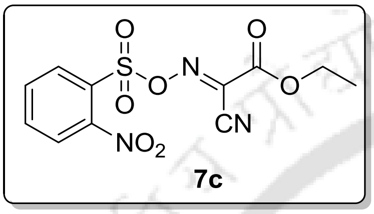
(*E*)-Ethyl-2-cyano-2-((((2,4-dinitrophenyl) sulfonyl)oxy) imino) acetate, 7b



7b

Yellow solid; (193 mg, 52%); mp 109 ºC; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.96-8.95 (d, *J* = 6.4 Hz, 1H), 8.76-8.75 (d, *J* = 2.8 Hz, 1H), 8.57-8.56 (d, *J* = 2.8 Hz, 1H), 4.55-4.50 (q, 2H), 1.49-1.47 (t, *J* = 3.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.2, 154.3, 144.0, 133.5, 129.7, 127.6, 122.4, 118.8, 106.7, 65.1, 14.1; FT-IR (KBr, cm^{-1}): 2925, 1752, 1603, 1536, 1472, 1347, 1222, 1067, 986, 832, 742; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_9N_4O_9S$ 373.00, found 373.30.

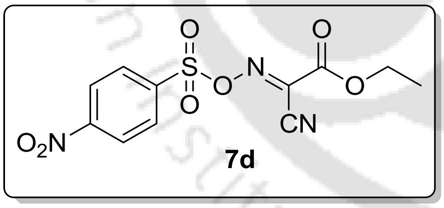
(*E*)-Ethyl-2-cyano-2-((((2-nitrophenyl) sulfonyl)oxy) imino) acetate, 7c



7c

White crystalline solid; (196 mg, 60%); mp 113 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27-8.25 (d, *J* = 8.0 Hz, 1H), 7.96-7.86 (m, 3H), 4.46-4.41 (q, 2H), 1.40-1.37 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 148.9, 137.1, 133.5, 132.8, 126.6, 125.6, 106.0, 65.1, 14.0; FT-IR (KBr, cm^{-1}): 2920, 2850, 1748, 1544, 1406, 1202, 1139, 928, 839, 742; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{10}N_3O_7S$ 328.0239, found 328.0242.

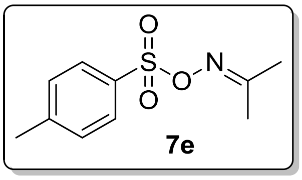
(*E*)-Ethyl-2-cyano-2-((((4-nitrophenyl) sulfonyl)oxy) imino) acetate, 7d



7d

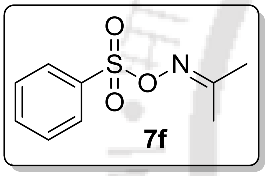
White crystalline solid; (206 mg, 63%); mp 110 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45-8.43 (d, *J* = 9.2 Hz, 2H), 8.26-8.23 (d, *J* = 8.8 Hz, 2H), 4.43-4.38 (q, 2H), 1.38-1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 151.9, 138.8, 132.7, 131.2, 124.9, 106.9, 65.1, 14.0; FT-IR (KBr, cm^{-1}): 2992, 1768, 1533, 1400, 1291, 1197, 933, 822, 742; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{10}N_3O_7S$ 328.0239, found 328.0242.

Propan-2-one *O*-tosyl oxime, 7e



Oil; (186 mg, 82%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.64-7.62 (d, *J* = 8.4 Hz, 2H), 7.13-7.12 (d, *J* = 8.4 Hz, 2H), 2.18 (s, 3H), 1.73 (s, 3H), 1.67 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 164.7, 144.5, 130.1, 129.2, 128.1, 21.0, 20.8, 16.1; FT-IR (KBr, cm^{-1}): 2926, 1643, 1446, 1364, 1190, 1038, 867, 778, 669; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{10}H_{14}NO_3S$ 228.06, found 228.10.

Propan-2-one *O*-phenylsulfonyl oxime, 7f



Oil; (160 mg, 75%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.83-7.82 (d, *J* = 7.8 Hz, 2H), 7.51-7.49 (t, *J* = 7.2 Hz, 1H), 7.42-7.39 (t, *J* = 7.8 Hz, 2H), 1.80 (s, 3H), 1.74 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 165.1, 135.6, 133.7, 129.7, 128.8, 128.3, 126.5, 21.1, 16.5; FT-IR (KBr, cm^{-1}): 2926, 1637, 1446, 1364, 1192, 1018, 997, 881, 795, 693.

2.6. References

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2.7. Selected spectra and chromatograms

2.7.1. ^{1}H NMR and ^{13}C NMR of selected compounds

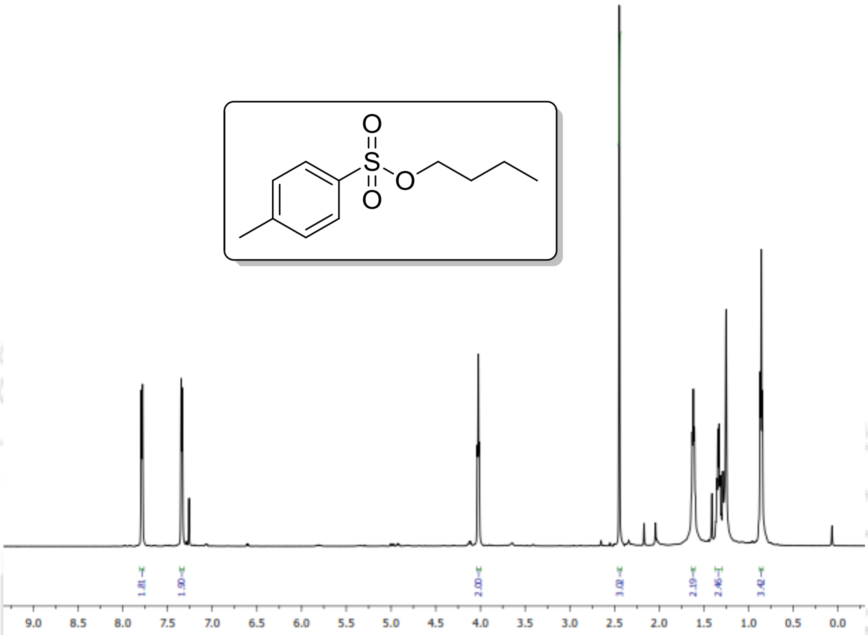


Figure S1. ^{1}H NMR spectra of compound 3b

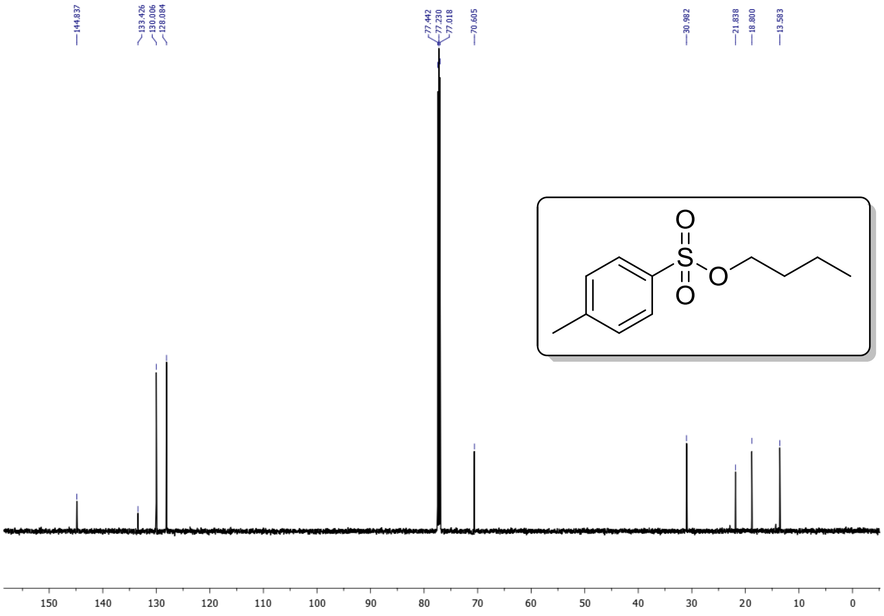


Figure S2. ^{13}C NMR spectra of compound 3b

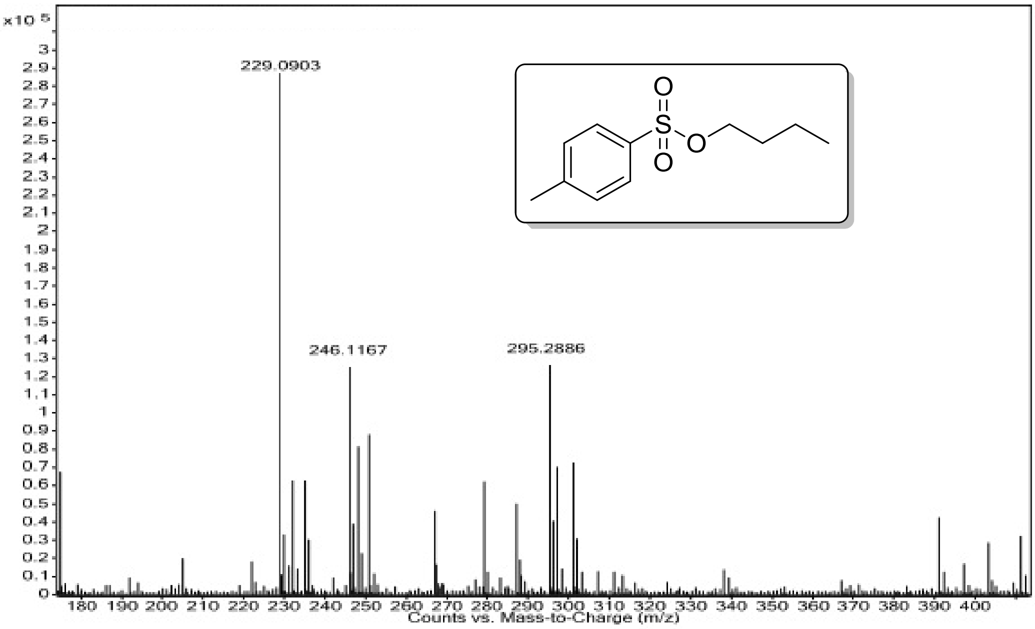


Figure S3. Mass spectra of compound 3b

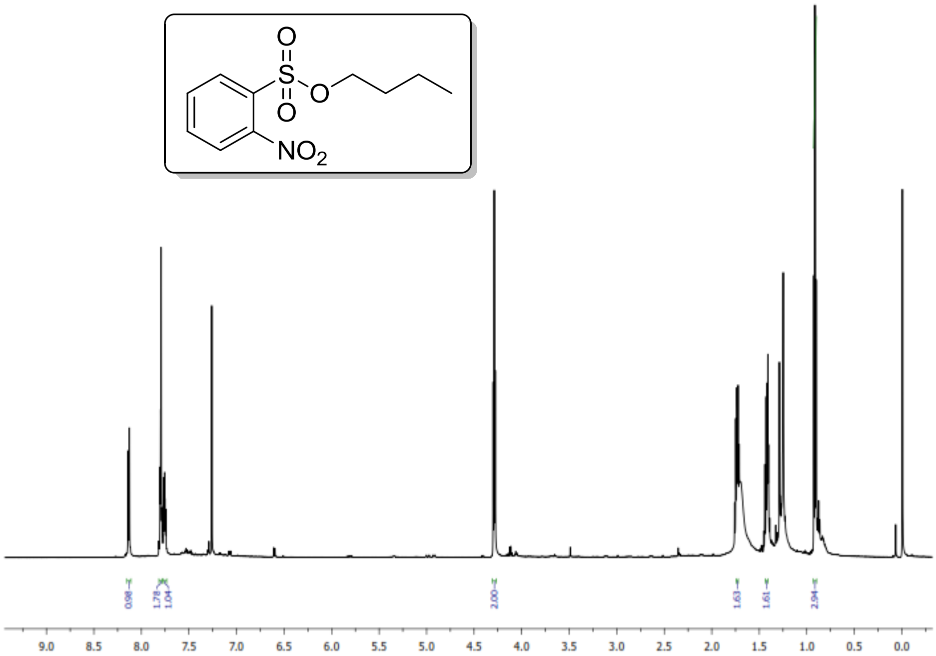


Figure S4. ^{1}H NMR spectra of compound **3c**

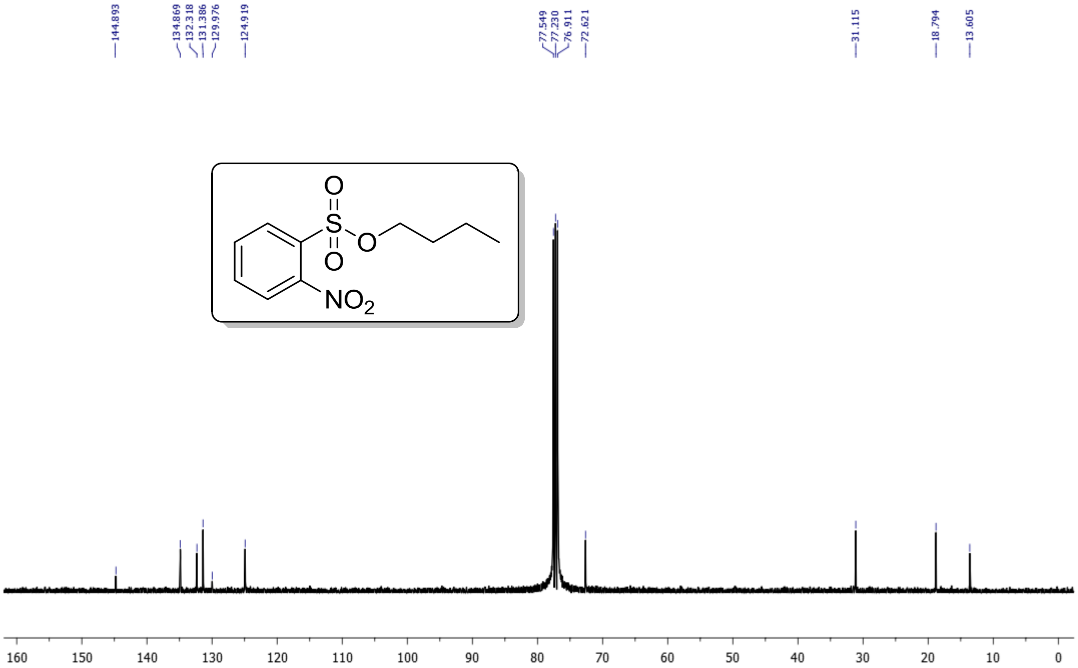


Figure S5. ^{13}C NMR spectra of compound **3c**

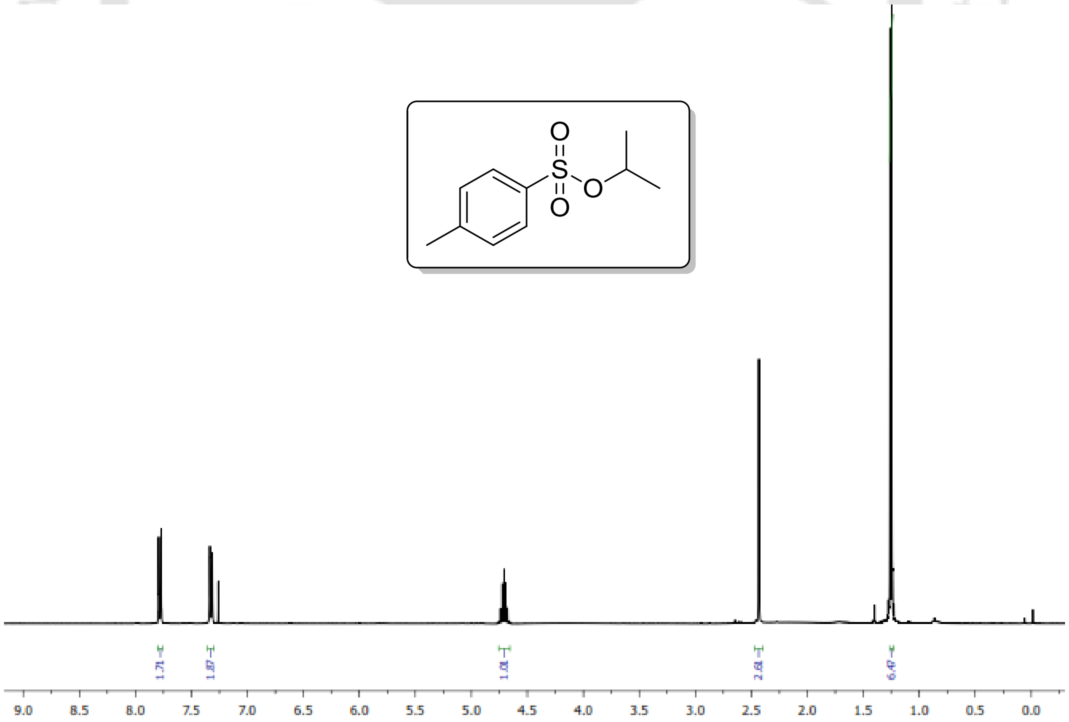


Figure S6. ^{1}H NMR spectra of compound **3e**

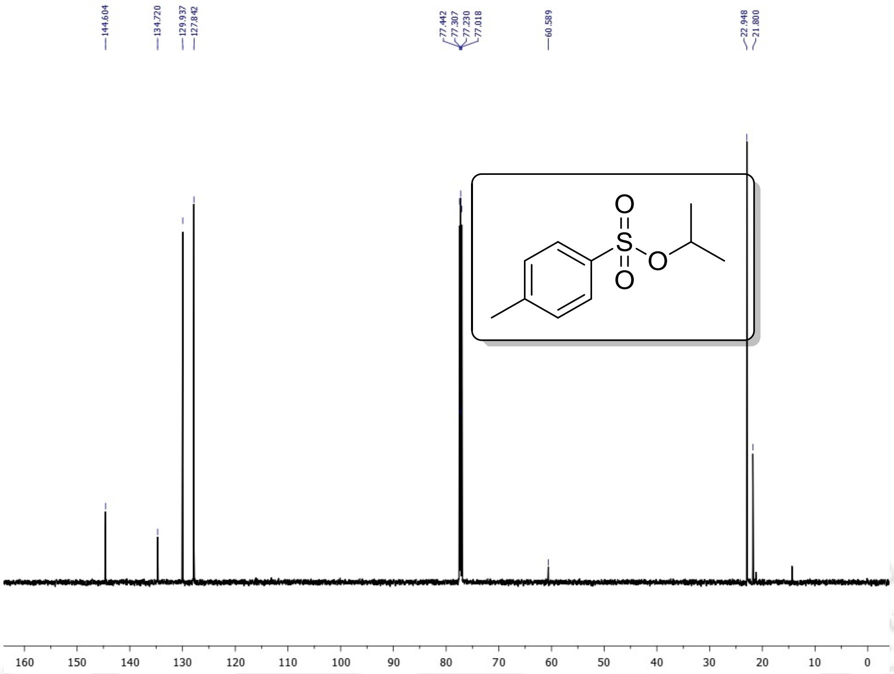


Figure S7. ^{13}C NMR spectra of compound **3e**

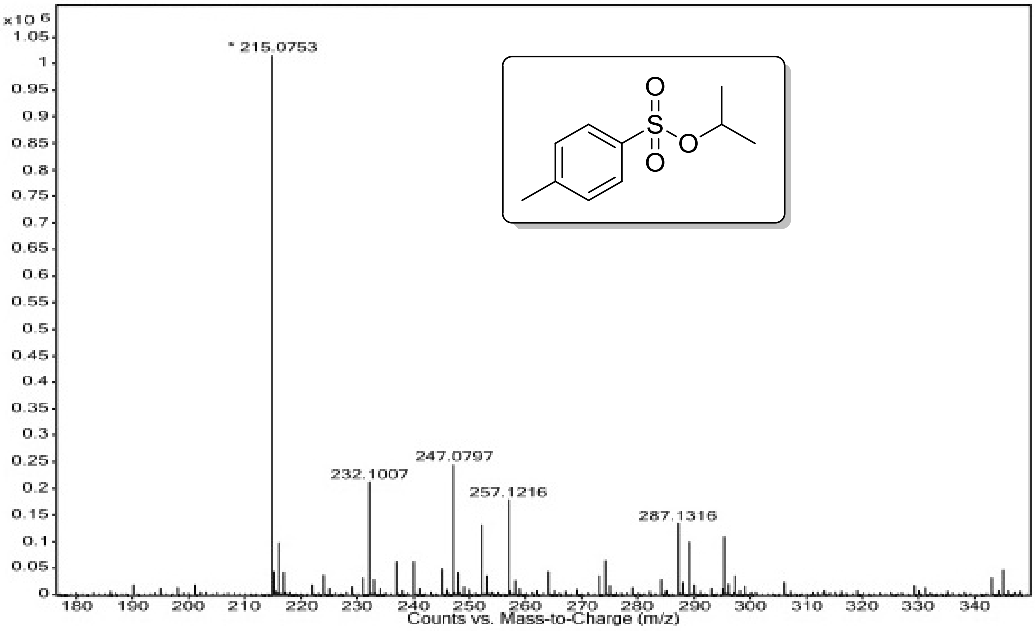


Figure S8. Mass pectra of compound **3e**

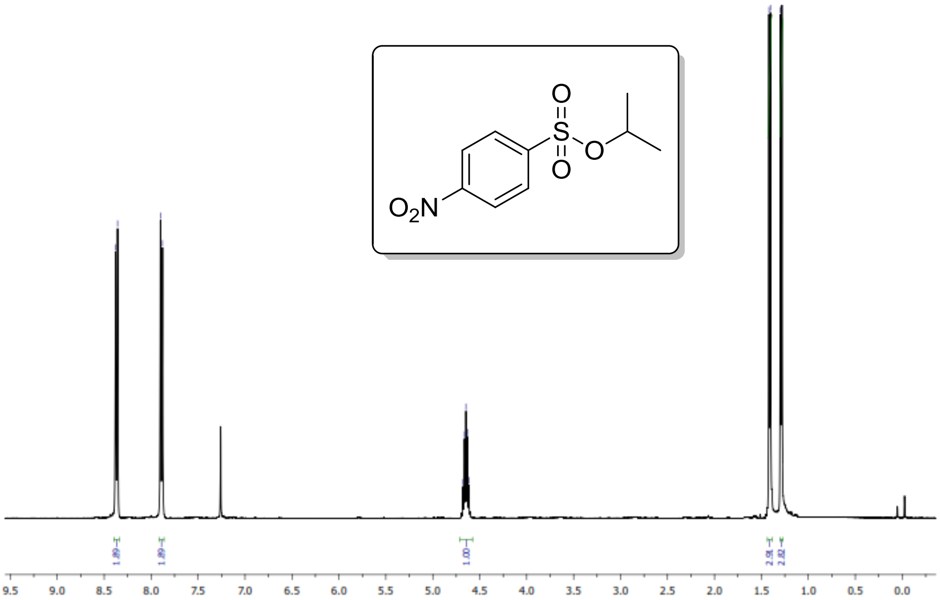


Figure S9. ^{1}H NMR spectra of compound **3f**

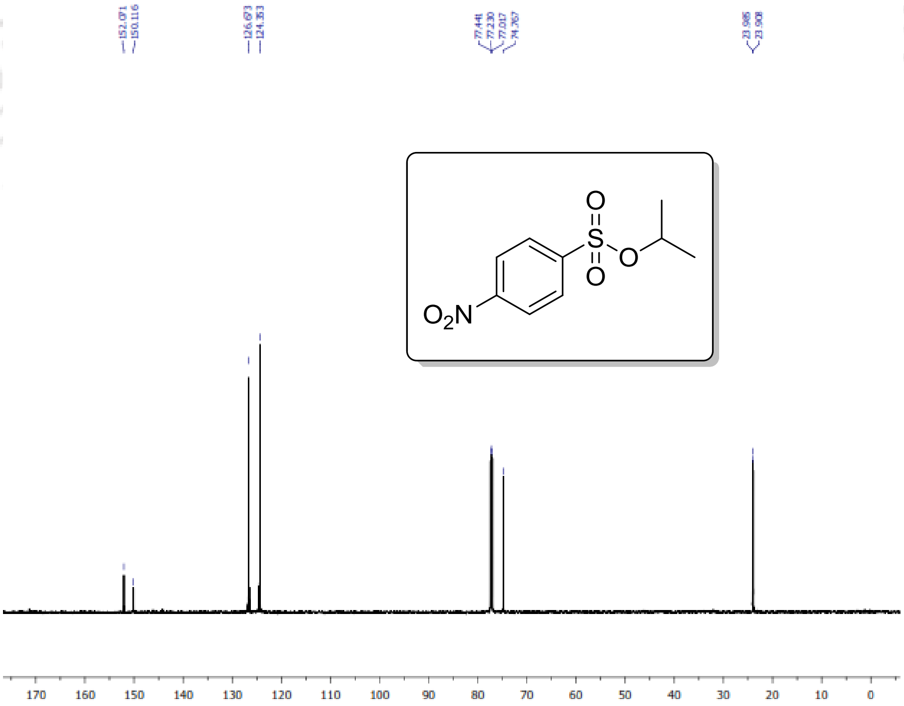


Figure S10. ^{13}C NMR spectra of compound **3f**

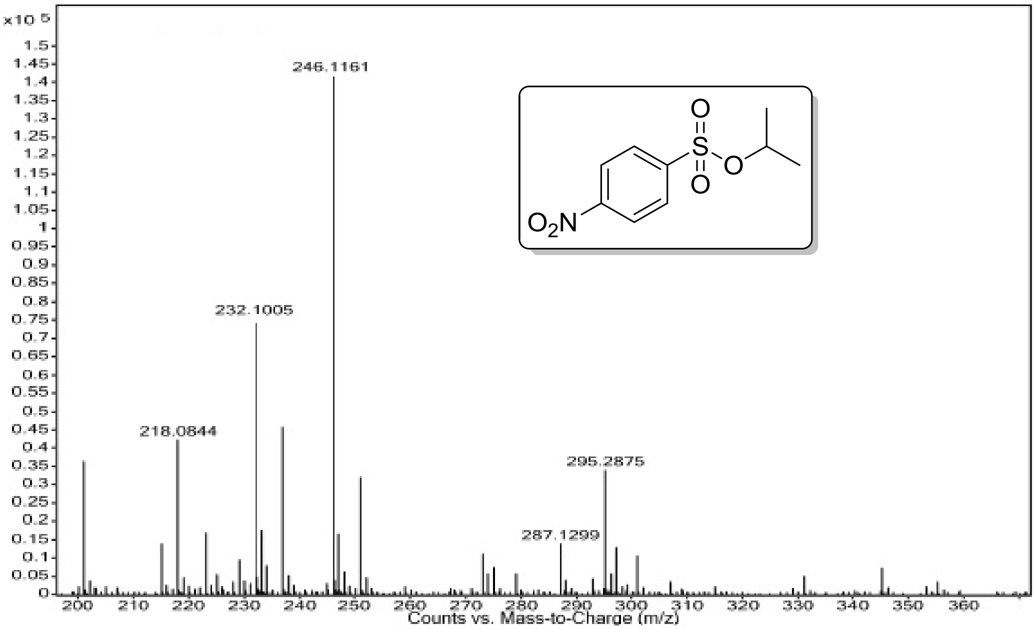


Figure S11. Mass spectra of compound **3f**

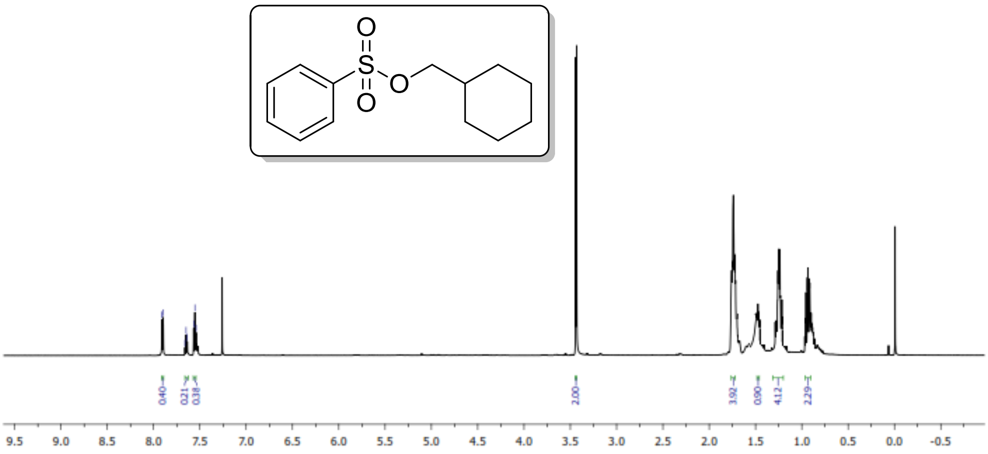


Figure S12. ^{1}H NMR spectra of compound **3j**

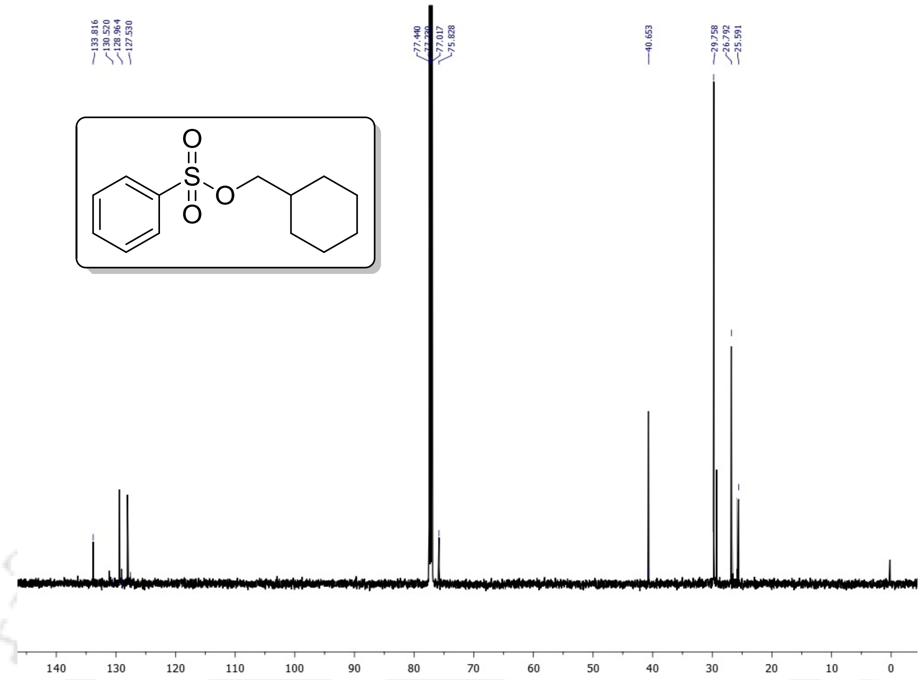


Figure S13. ^{13}C NMR spectra of compound **3j**

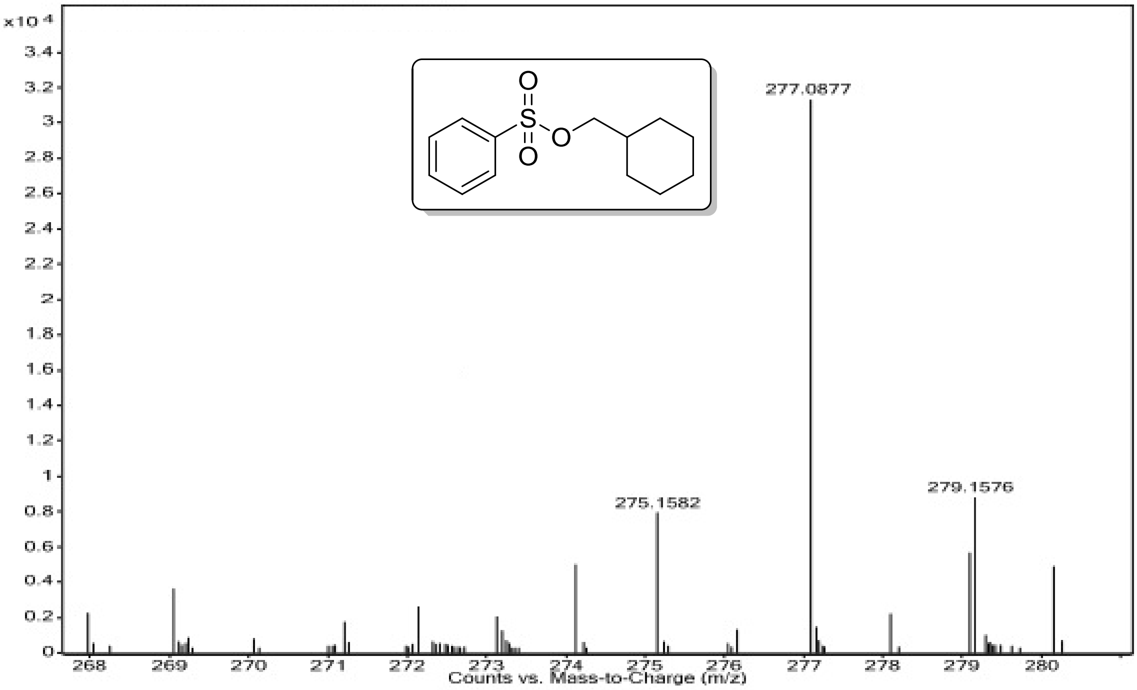


Figure S14. Mass spectra of compound **3j**

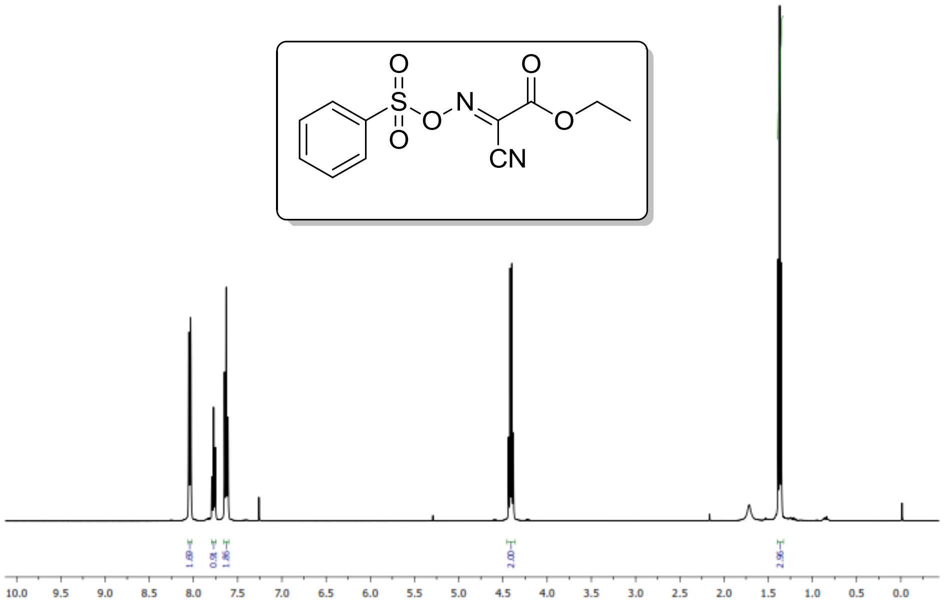


Figure S15. ^{1}H NMR spectra of compound **4a**

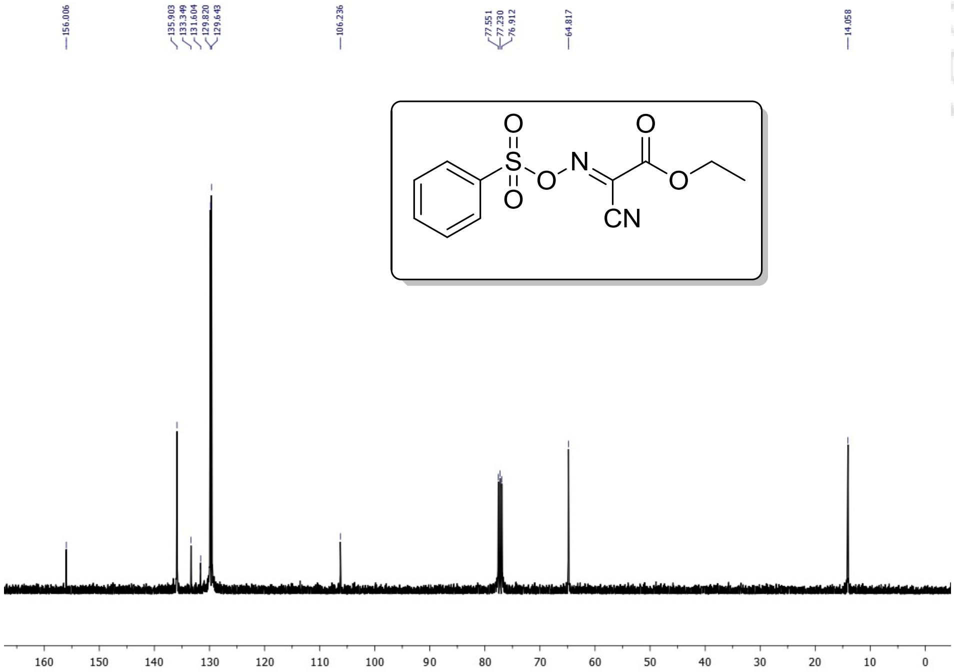


Figure S16. ^{13}C NMR spectra of compound **4a**

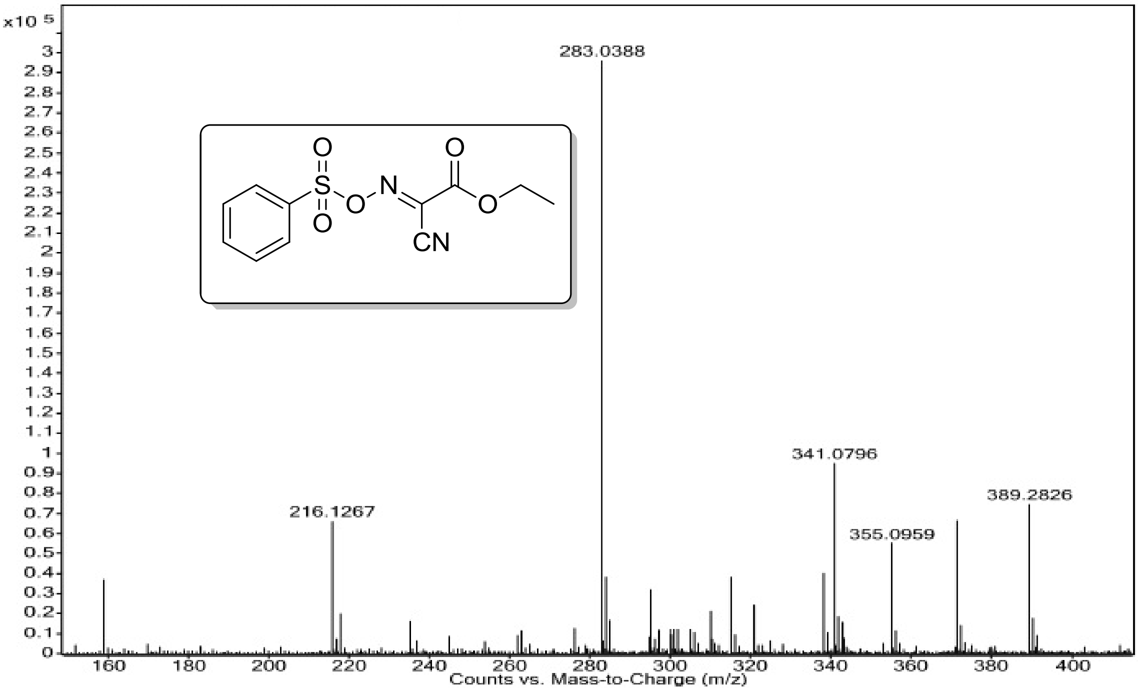


Figure S17. Mass spectra of compound **4a**

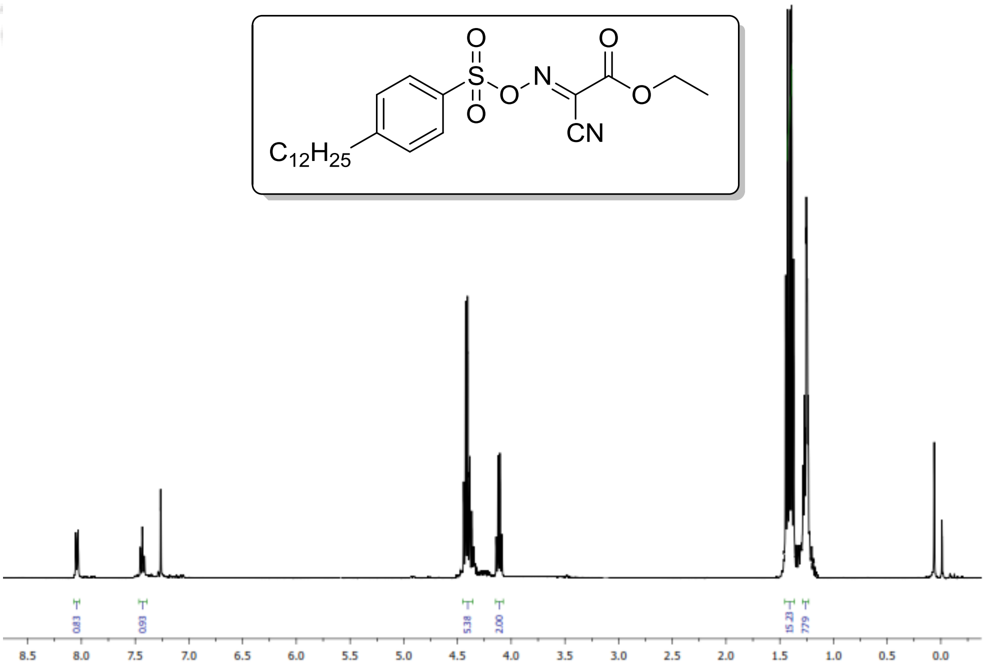


Figure S18. ^{1}H NMR spectra of compound **4c**

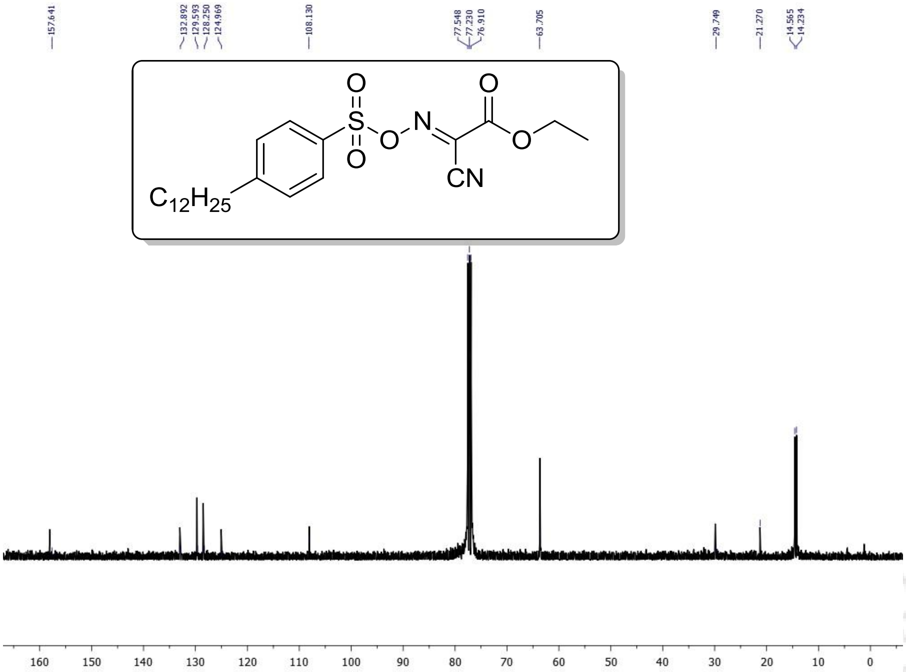


Figure S19. ^{13}C NMR spectra of compound **4c**

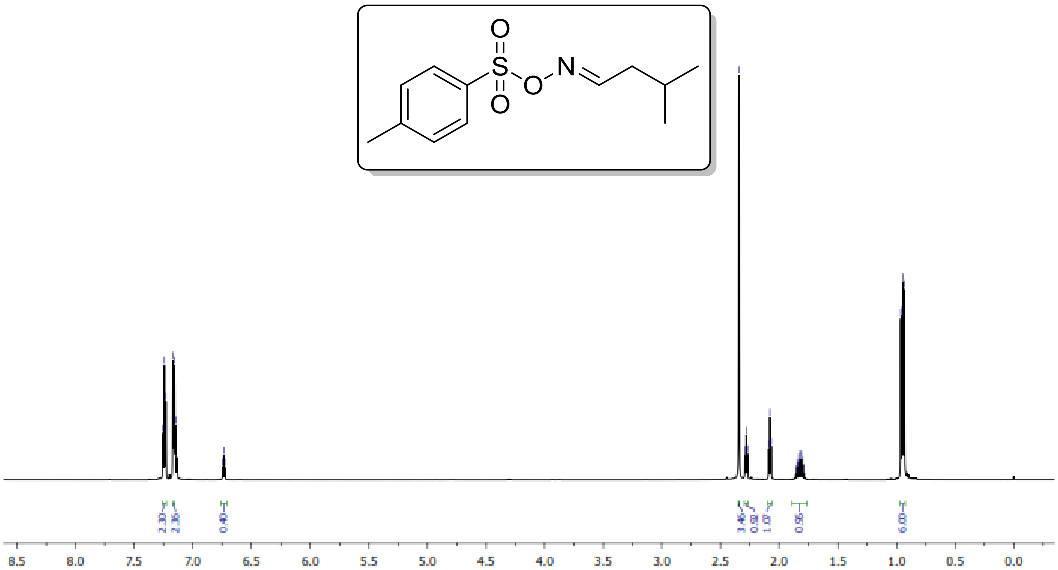


Figure S20. ^{1}H NMR spectra of compound **4d**

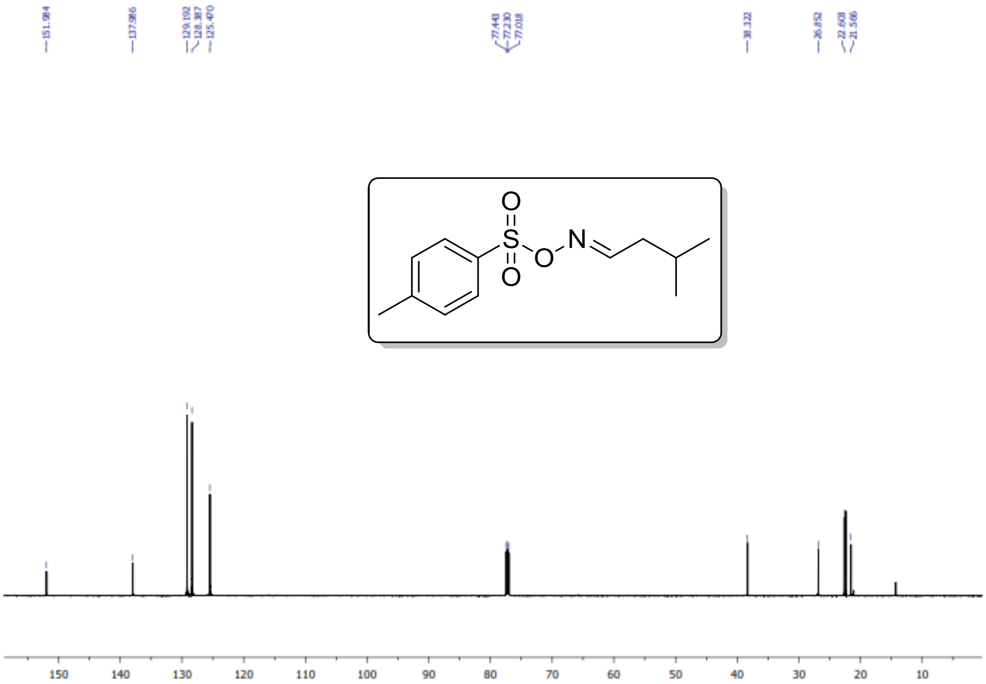


Figure S21. ^{13}C NMR spectra of compound **4d**

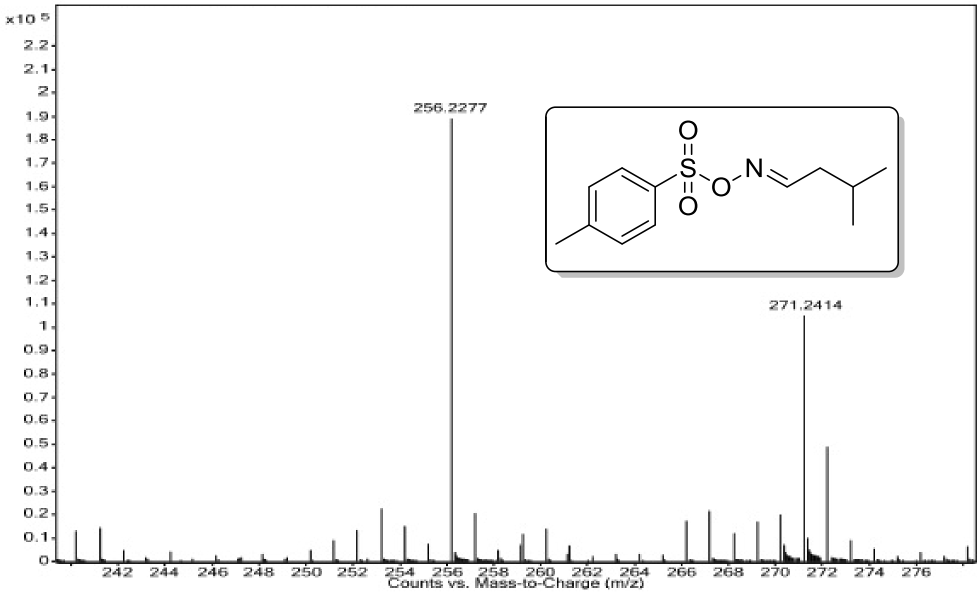


Figure S22. Mass spectra of compound **4d**

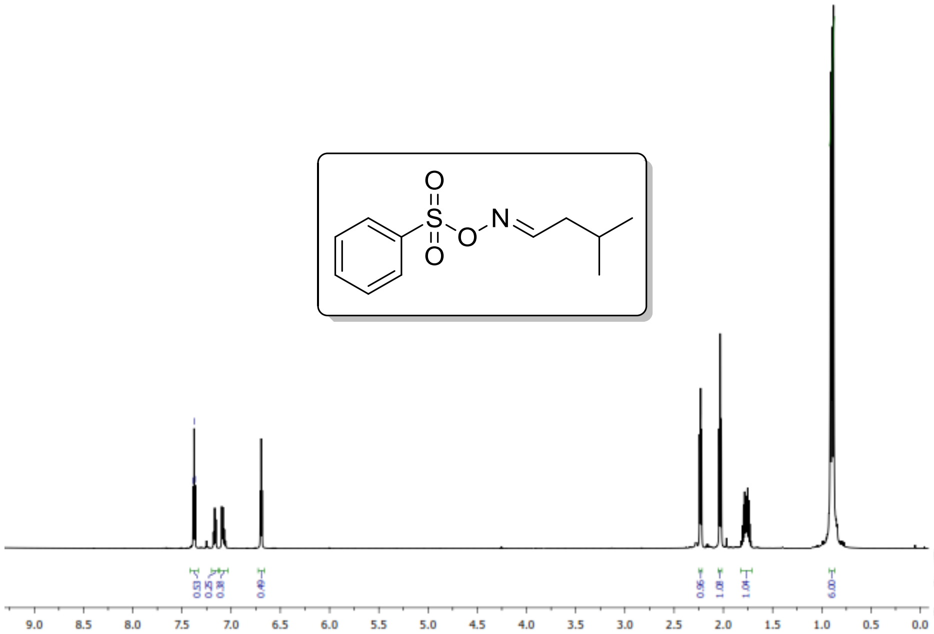


Figure S23. ^{1}H NMR spectra of compound **4e**

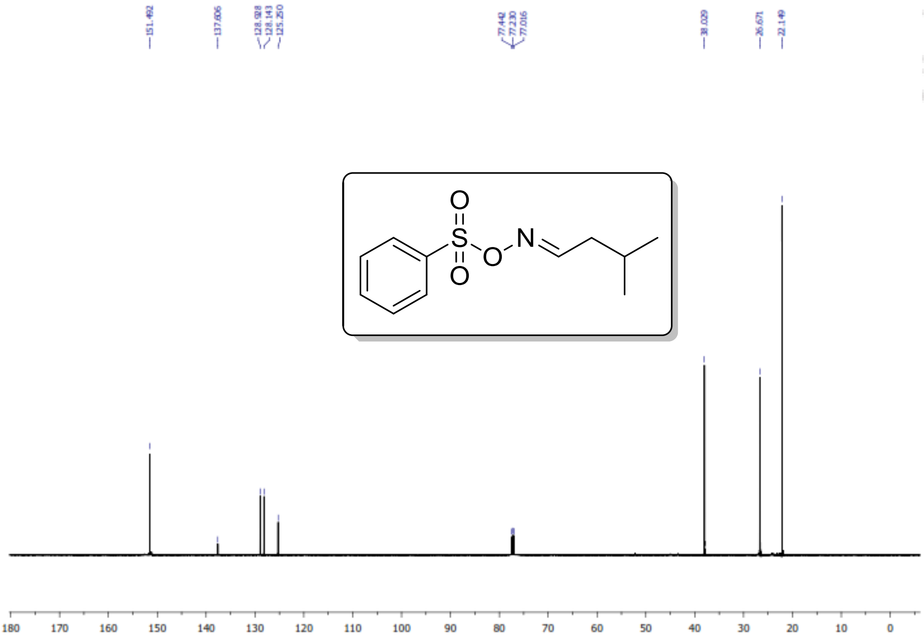


Figure S24. ^{13}CNMR spectra of compound **4e**

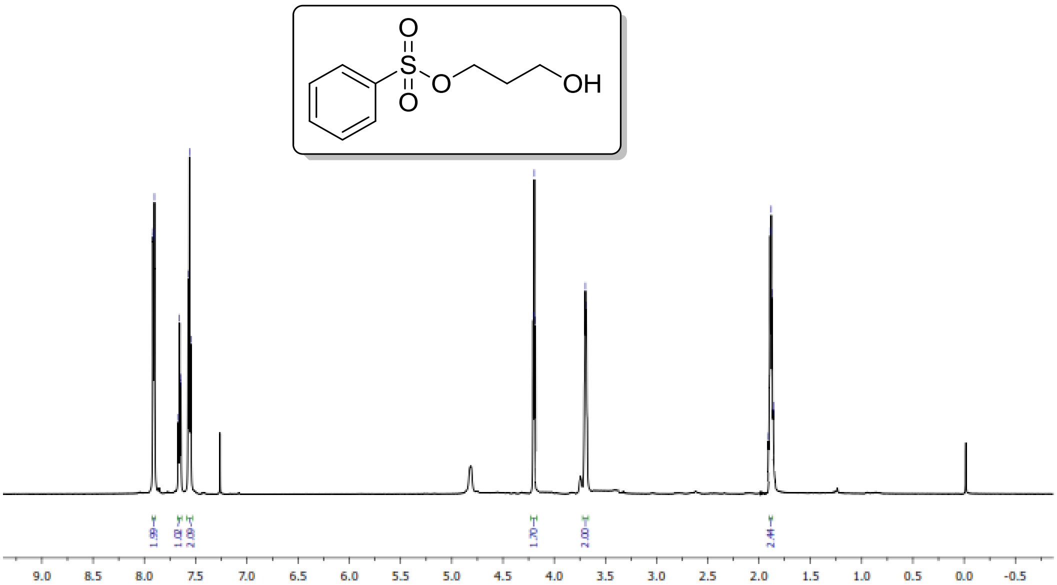


Figure S25. ^{1}H NMR spectra of compound **5a**

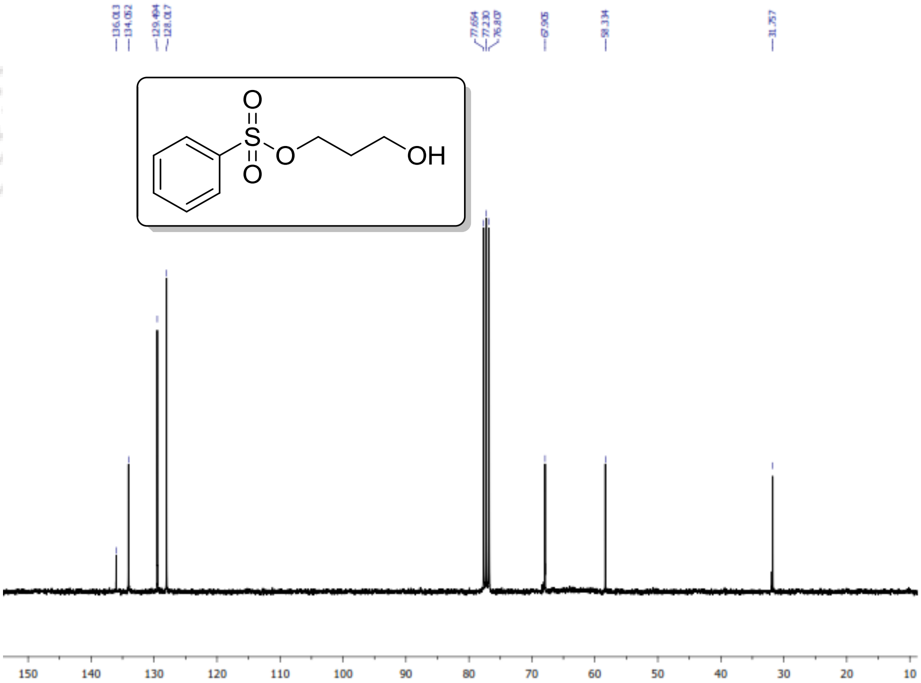


Figure S26. ^{13}C NMR spectra of compound **5a**

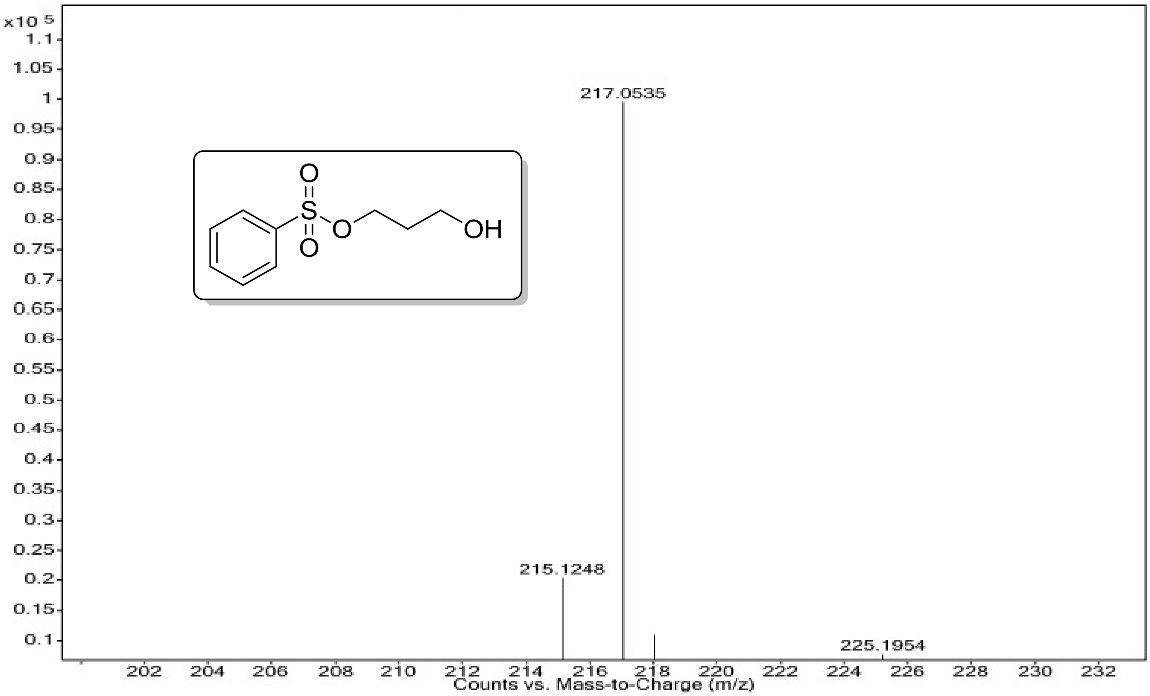


Figure S27. Mass spectra of compound **5a**

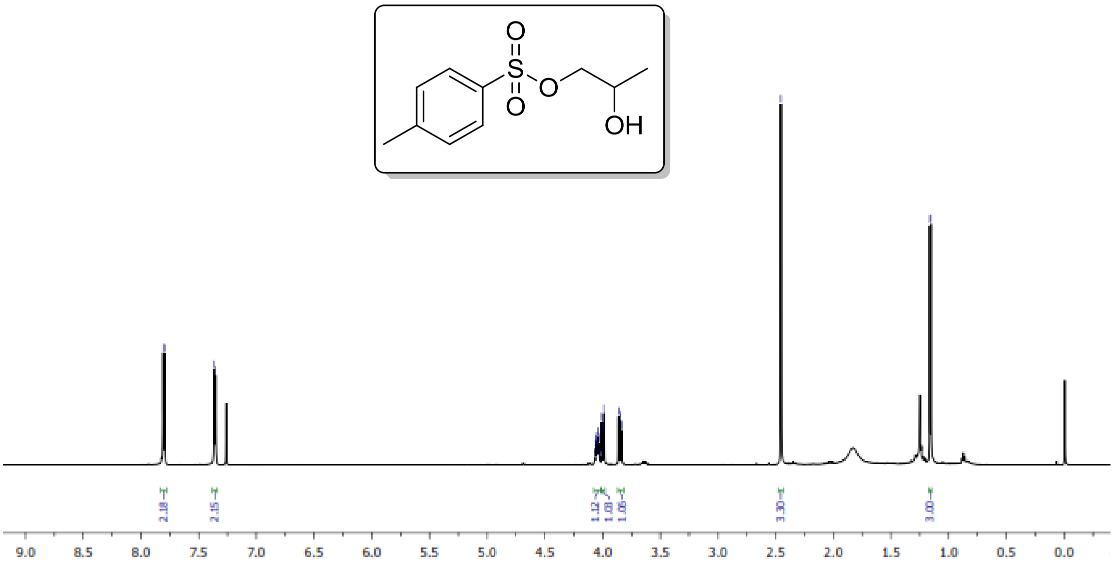


Figure S28. ^{1}H NMR spectra of compound **5c**

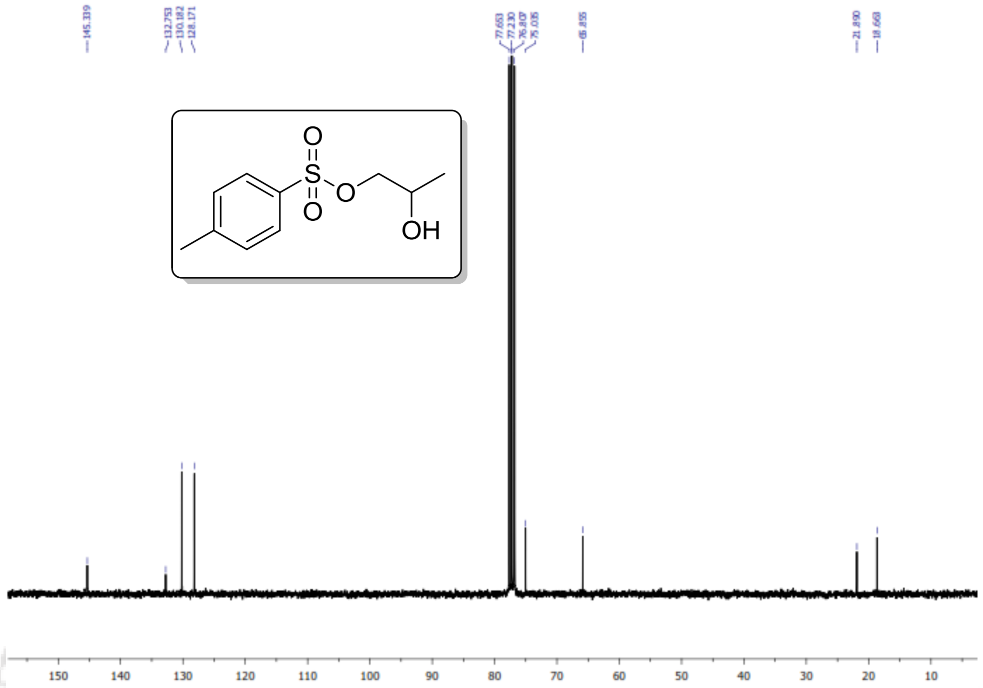


Figure S29. ^{13}C NMR spectra of compound **5c**

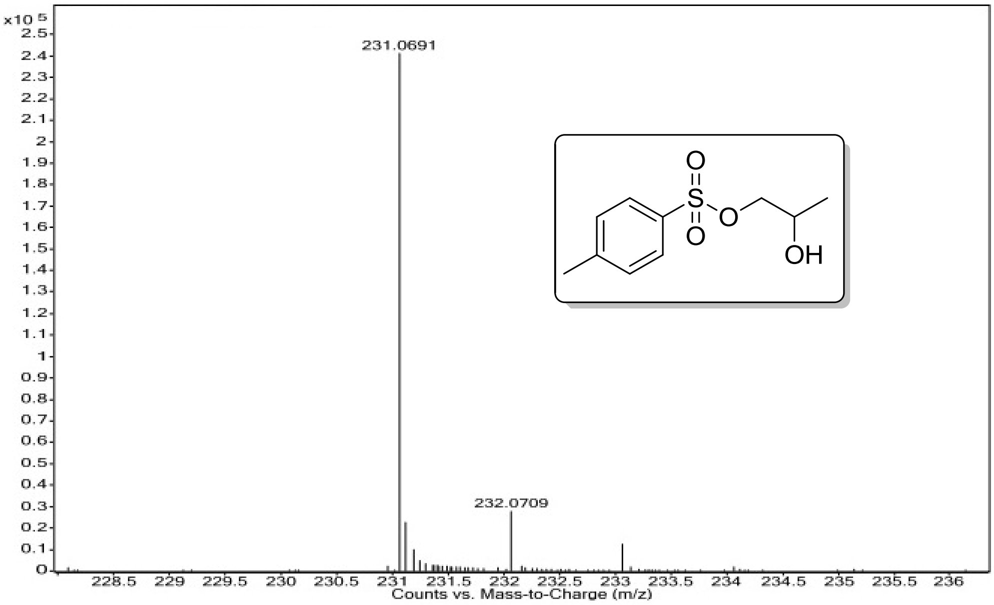


Figure S30. Mass spectra of compound **5c**

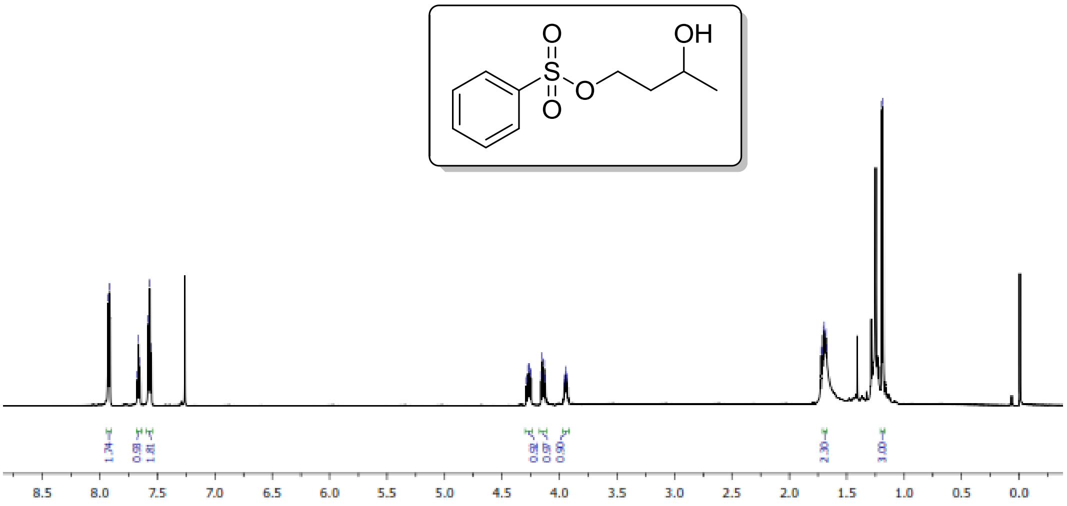


Figure S31. ^{1}H NMR spectra of compound **5e**

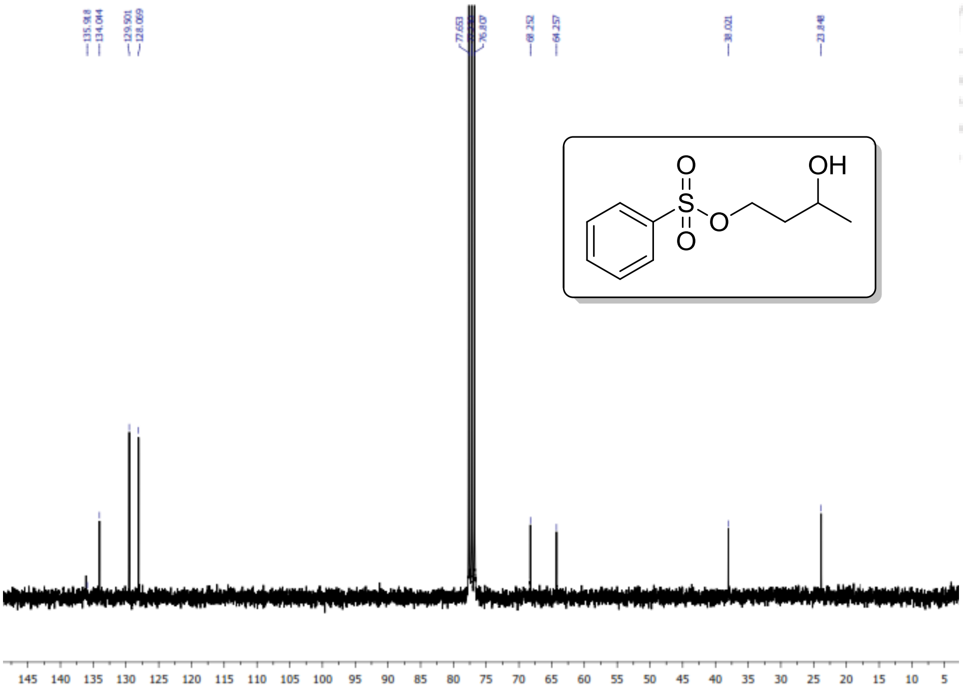


Figure S32. ^{13}C NMR spectra of compound **5e**

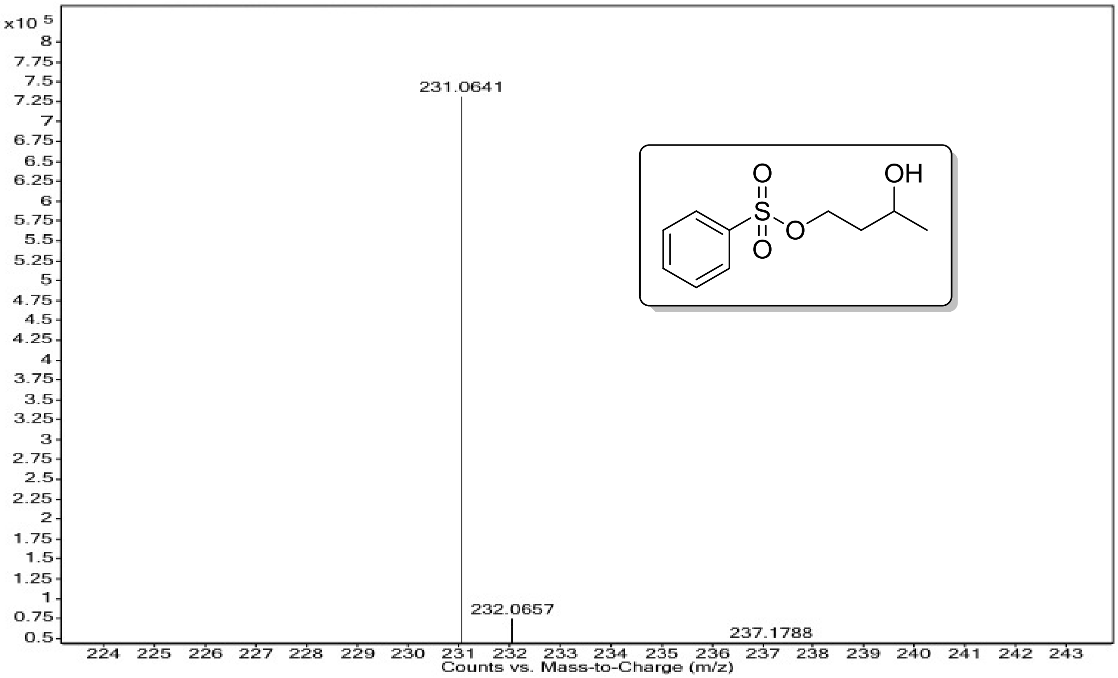


Figure S33. Mass spectra of compound **5e**

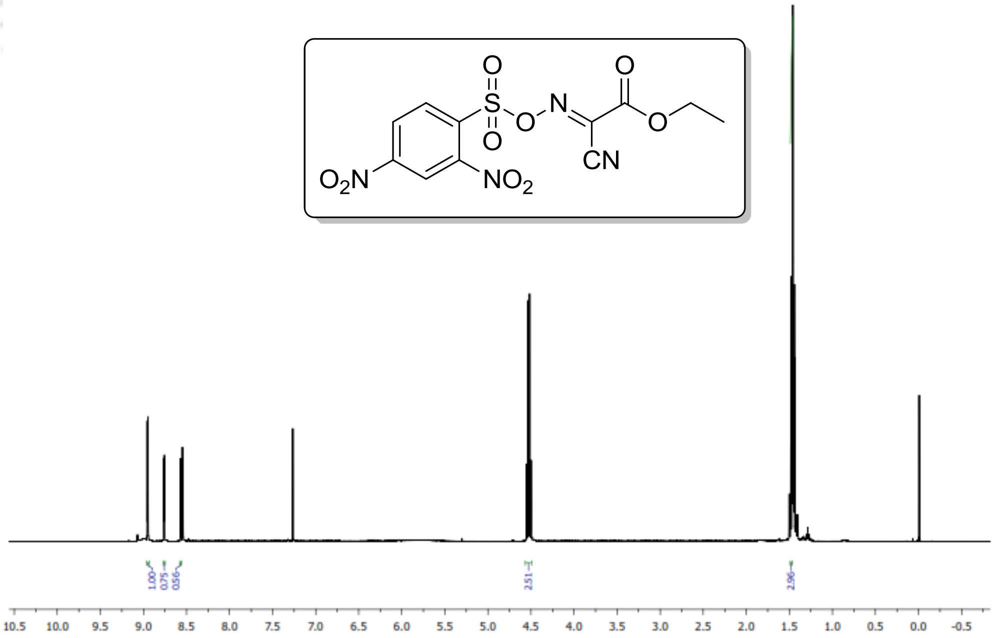


Figure S34. Mass spectra of compound **7b**

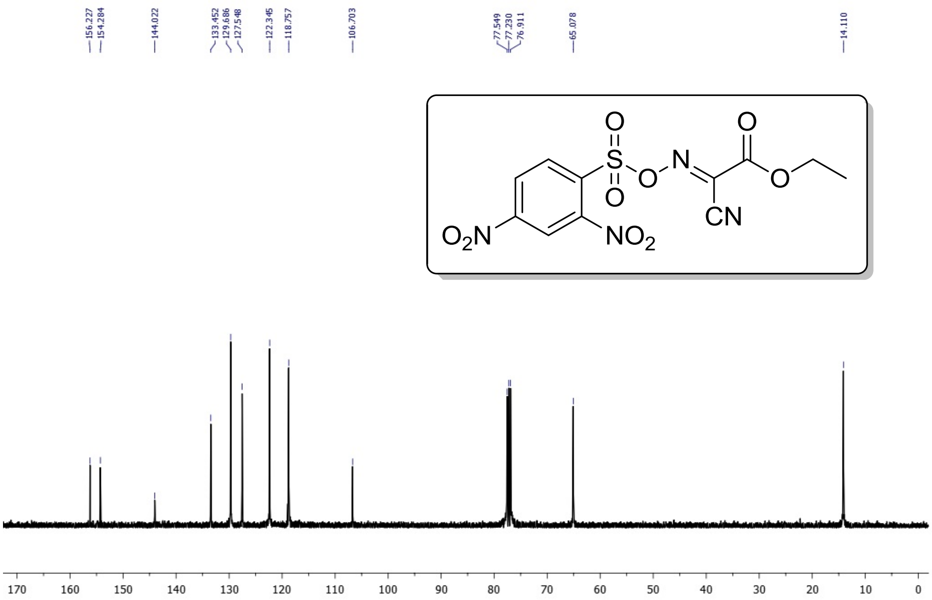


Figure S35. ^{13}C NMR spectra of compound **7b**

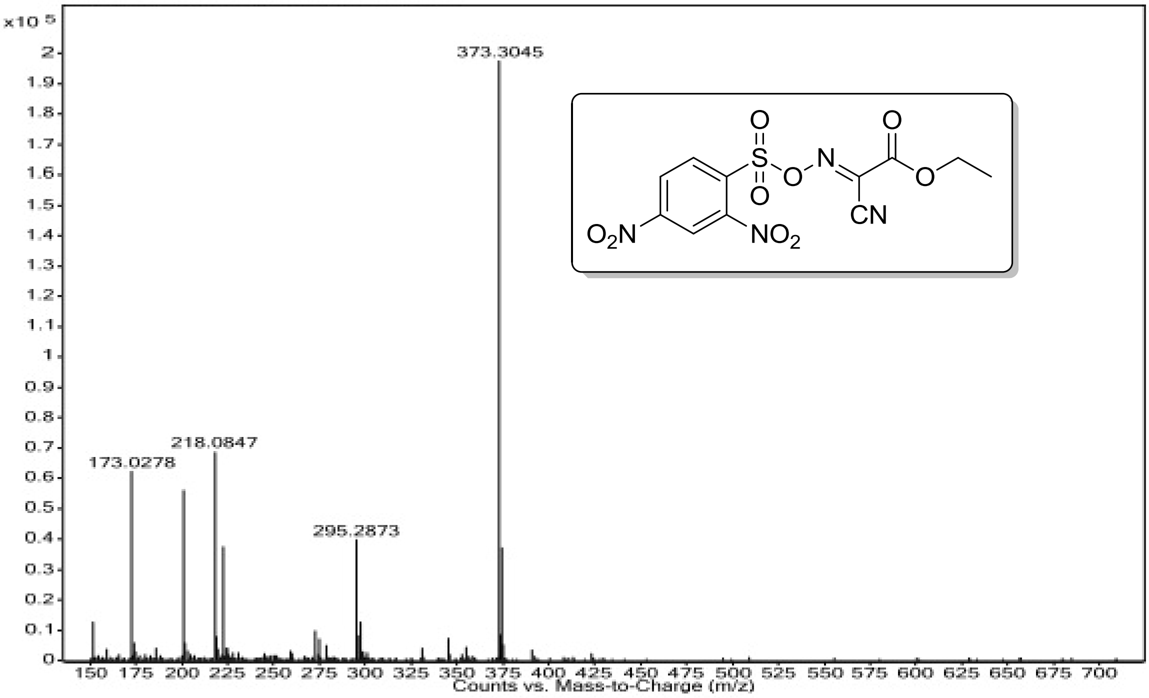


Figure S36. Mass spectra of compound **7b**

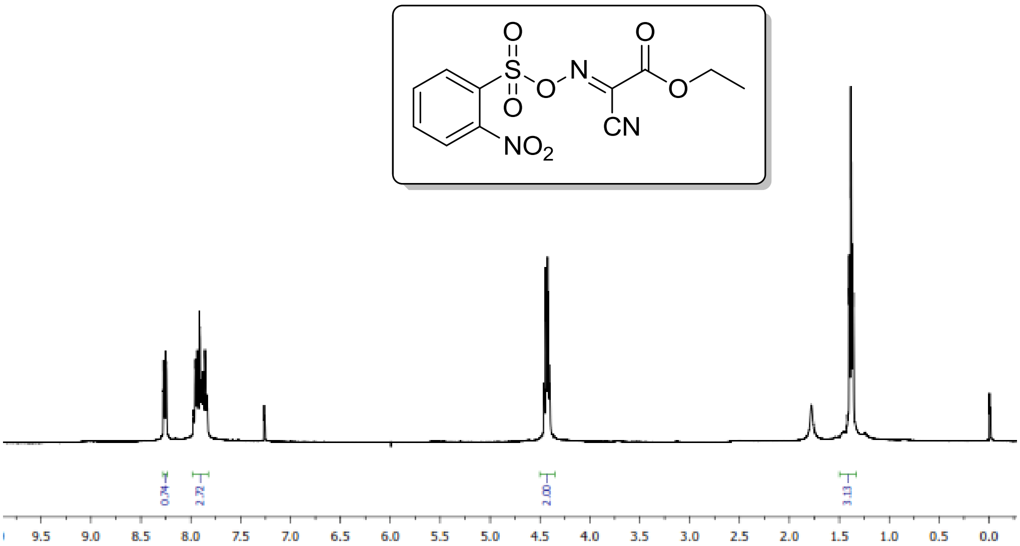


Figure S37. ^{1}H NMR spectra of compound **7c**

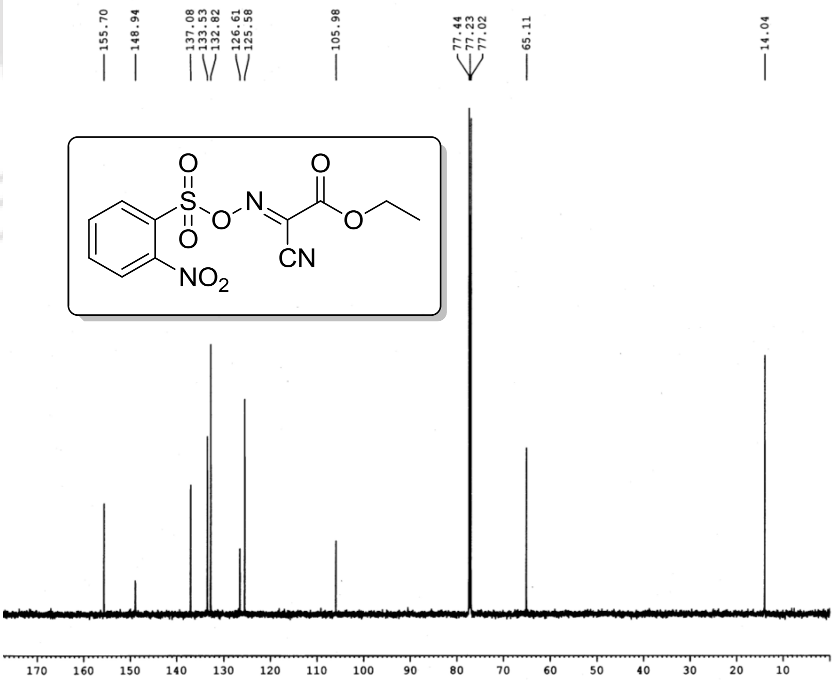


Figure S38. ^{13}C NMR spectra of compound **7c**

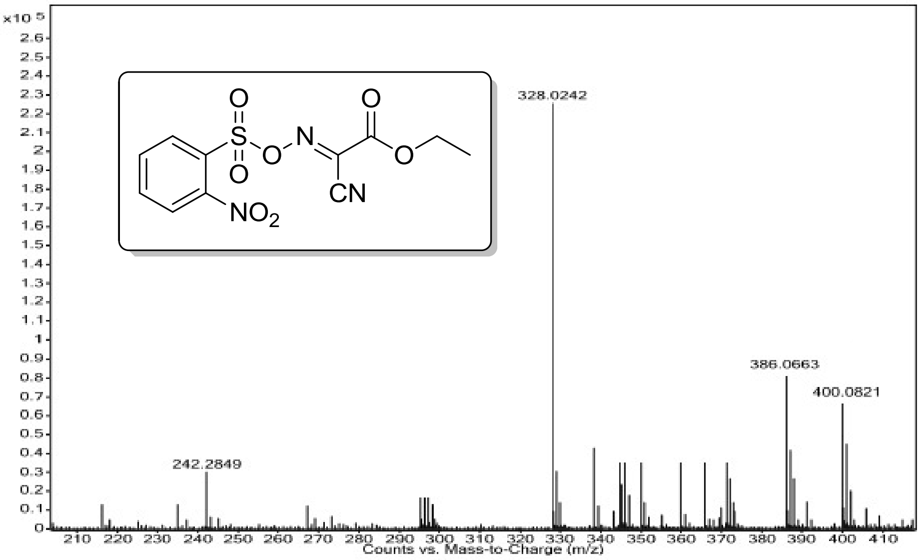


Figure S39. Mass spectra of compound **7c**

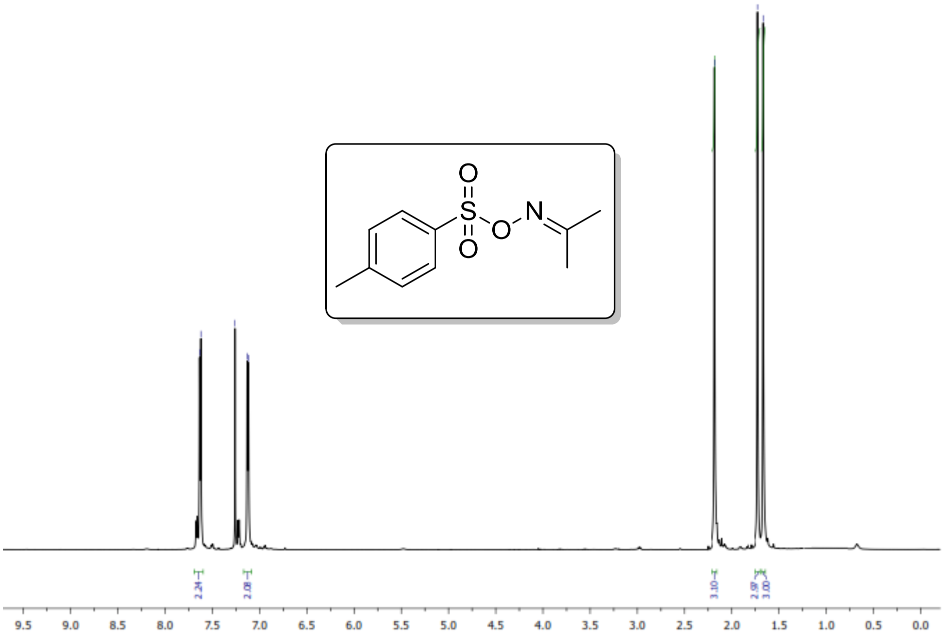


Figure S40. ^{1}H NMR spectra of compound **7e**

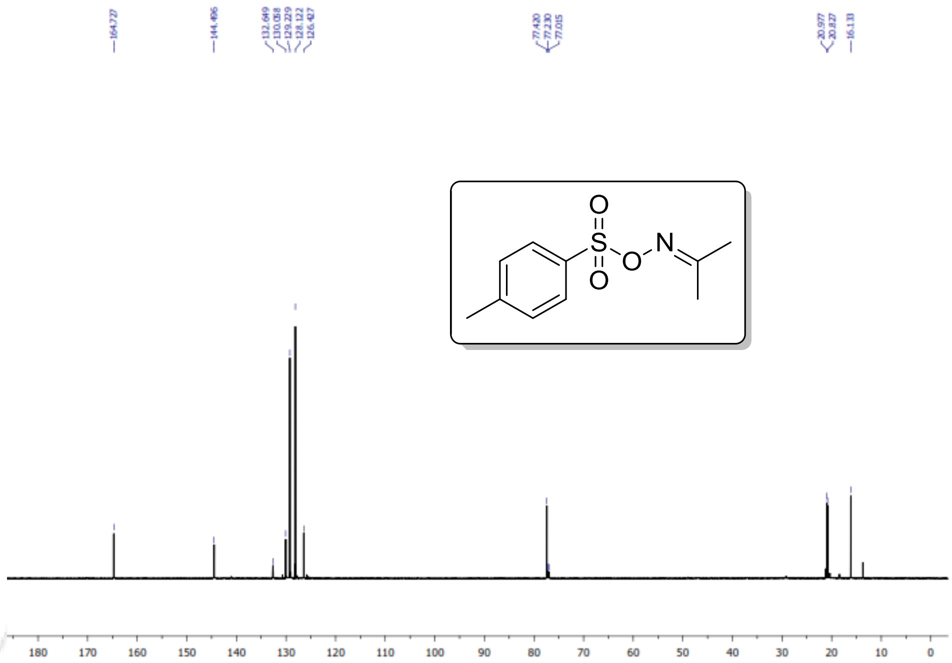


Figure S41. ^{13}C NMR spectra of compound **7e**

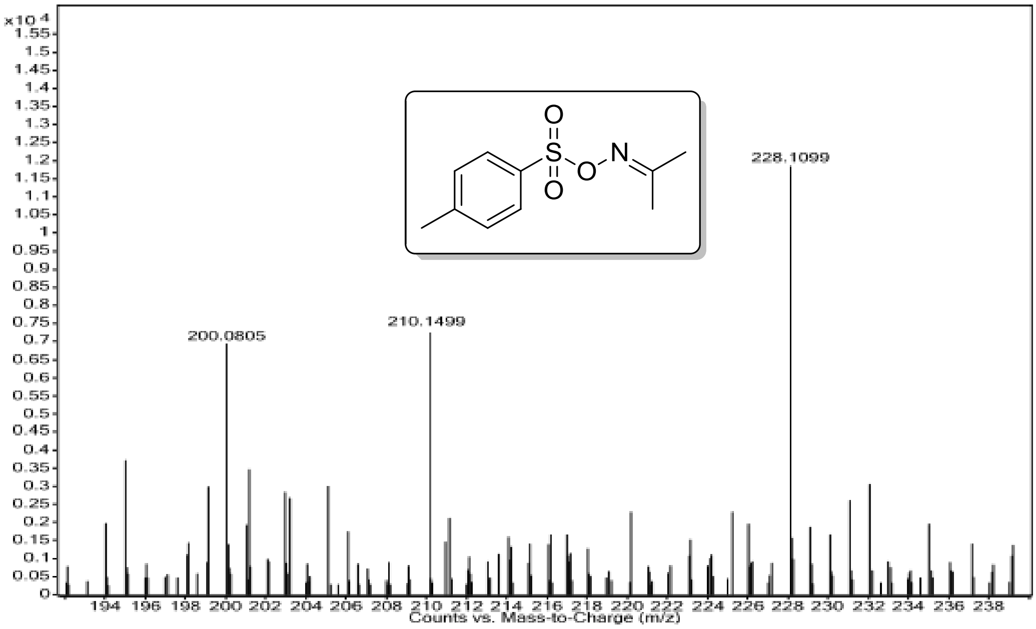


Figure S42. Mass spectra of compound **7e**

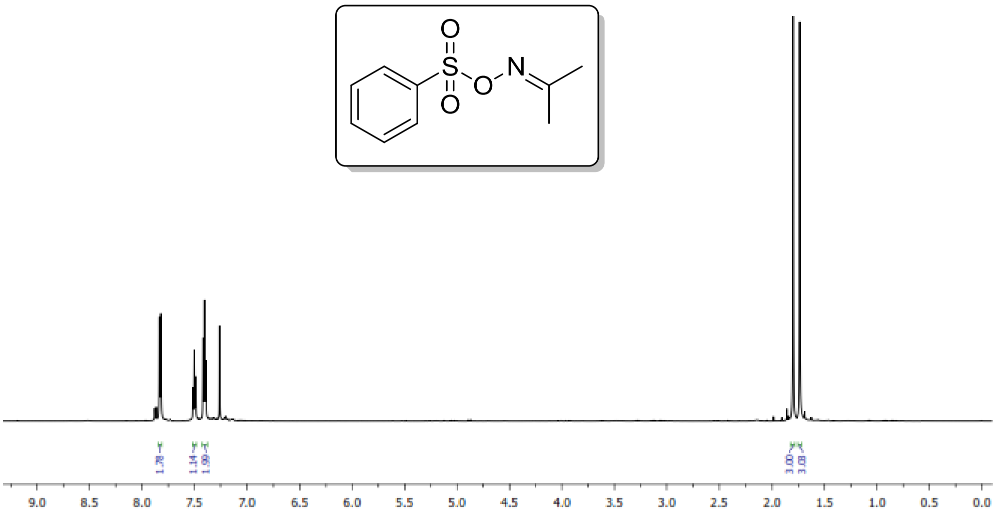


Figure S43. ^{1}H NMR spectra of compound **7f**

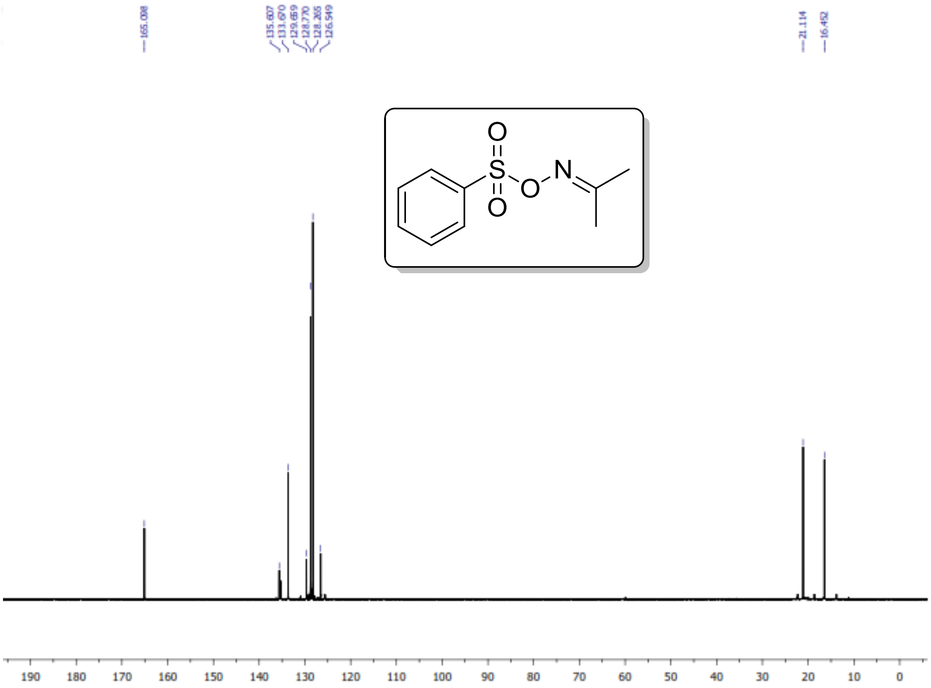
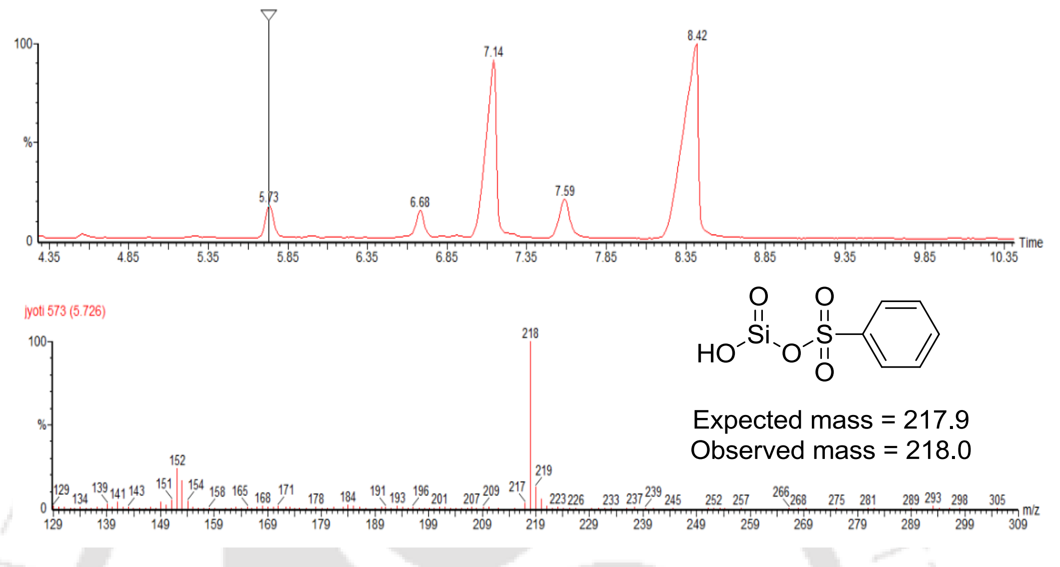


Figure S44. ^{13}C NMR spectra of compound **7f**

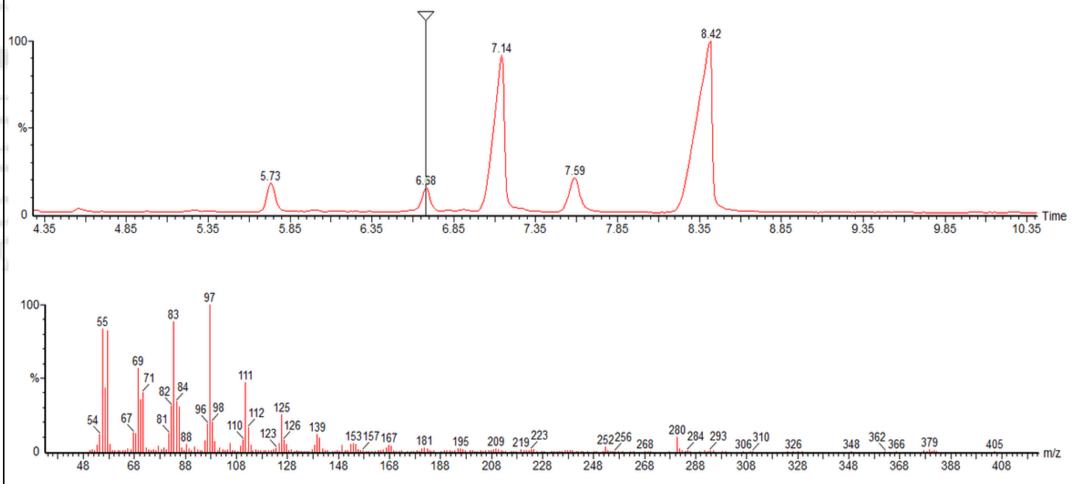
2.7.2. GCMS analysis of intermediates

(a)

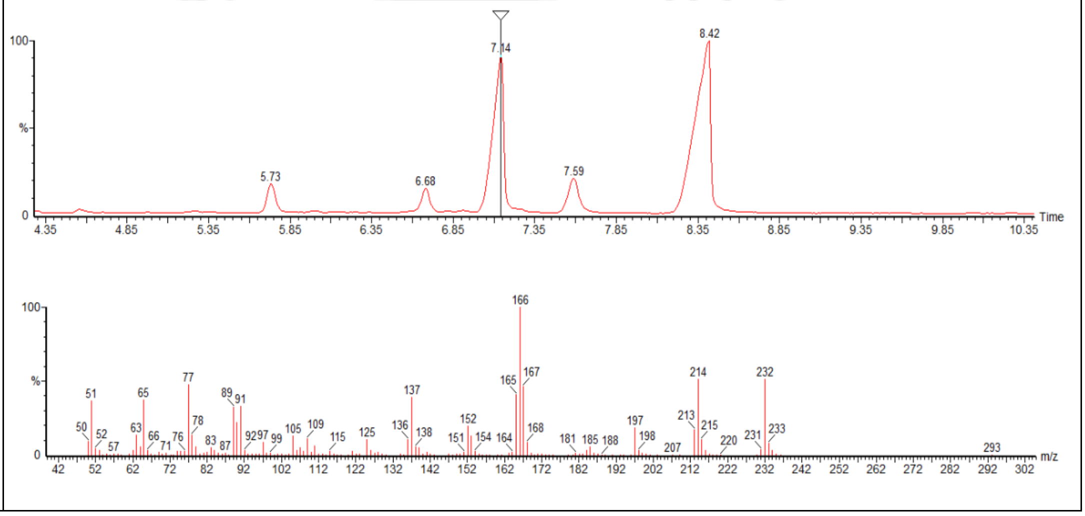


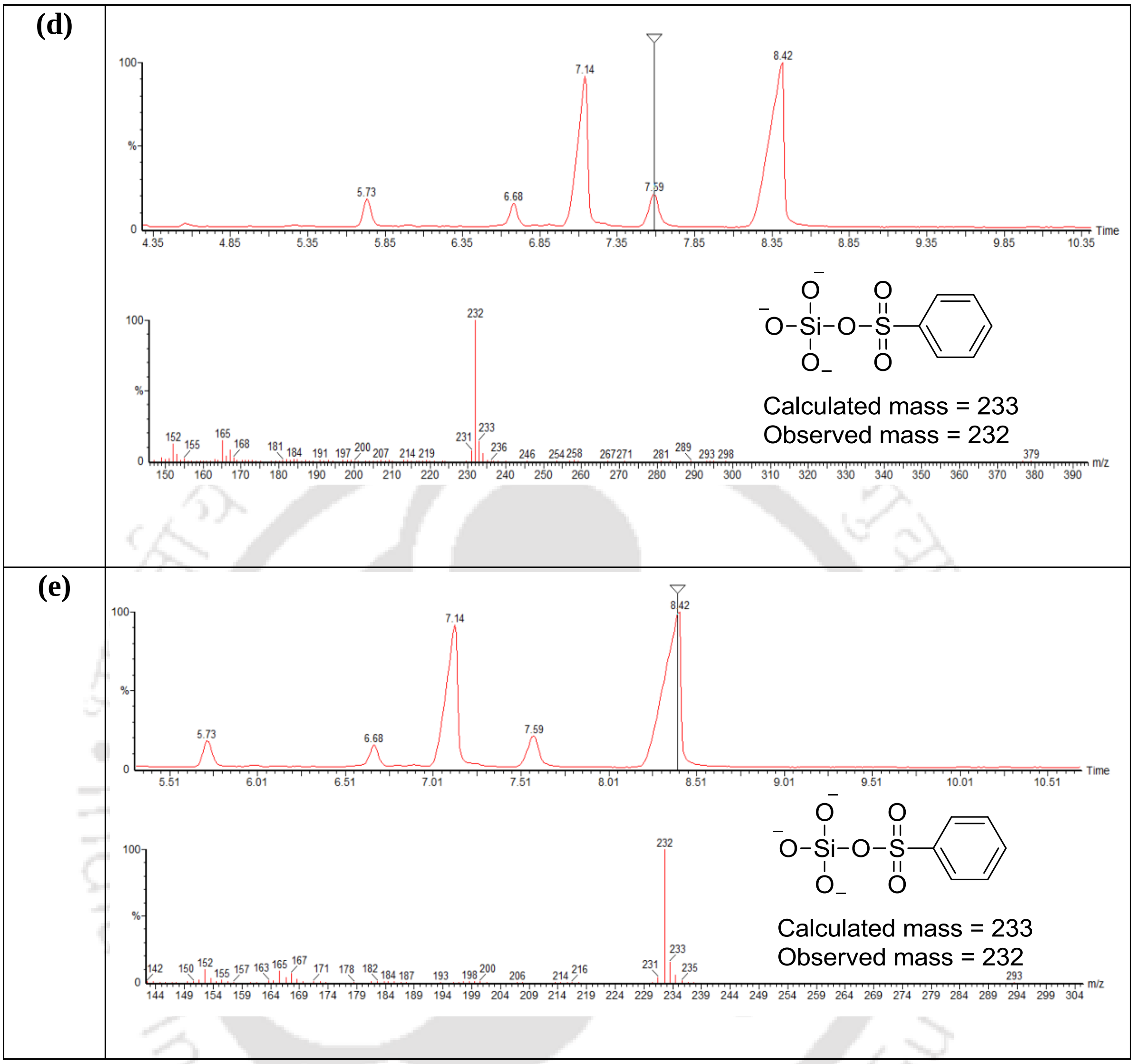
Expected mass = 217.9
Observed mass = 218.0

(b)



(c)





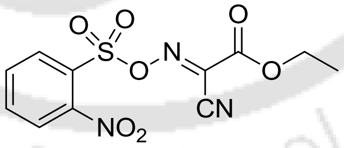
(d) Calculated mass = 233
Observed mass = 232

(e) Calculated mass = 233
Observed mass = 232

Figure S45. GCMS data of intermediates.

Chapter 3: Synthesis of Amides and Esters using Catalytic Amount of *o*-NosylOXY

In the previous chapter, we have documented a new halogen free method for the synthesis of Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate (*ortho*-NosylOXY, **I**, Fig. 3.1). Recently we have developed *ortho*-NosylOXY as a novel and efficient coupling reagent for coupling reactions and various organic transformations.[1] This coupling reagent is prepared by the reaction between Oxyma (ethyl 2-cyano-2-(hydroxyimino)acetate) and sulphonyl chloride in presence of DIPEA and dichloromethane as the solvent under nitrogen at room temperature. In this chapter, we have described a new approach for the synthesis of amides and esters by the catalytic amount of *o*-NosylOXY.



I

***Figure 3.1**. Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino)acetate, (ortho-NosylOXY, **I**)*

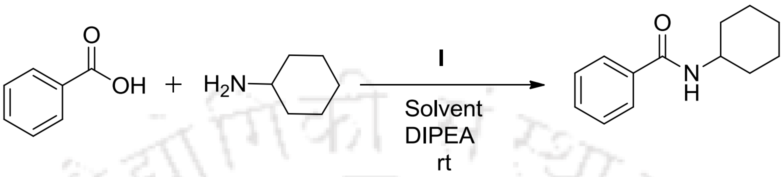
Amide bonds are mainly found in biological and pharmaceutical compounds (Chapter 1, section 1.3.2). Esters are also present in many natural products, bio-macromolecules, medicinally active compounds and drug molecules (Chapter 1, section 1.3.3). In the past, different methodological research has focused on these syntheses by different coupling reagents (Chapter 1, section 1.4.2). But these methods have not been reached impressive levels. All of the methodologies have significant drawbacks: (a) synthesis of the coupling reagents require hazardous chemicals, (b) syntheses of coupling reactions requires the excess amount of reagents, (c) generate lots of by-products that cannot be recycled easily, (d) cost of the coupling reagents are high, and (e) they have limited scope of substrates. Thus, they are neither environmental-friendly nor cost-effective. In response to this acute demand, we have developed synthesis of amides and esters using catalytic amount of *o*-NosylOXY.

3.1. Amide synthesis using catalytic amount of *o*-NosylOXY

o-NosylOXY is very stable at room temperature, can be stored for long period of time. We successfully achieved the conversion of the carboxylic acids to amides, peptides, esters, thioesters, hydroxamates and heterocyclic compounds with 1 equiv of *o*-NosylOXY.

We wanted to investigate the capability of this coupling reagent (**I**) for the synthesis of amides and esters in sub-stoichiometric amount. For optimization, we used benzoic acid (1 equiv) and *o*-NosylOXY (varied amount) in DCM solvent, then DIPEA (1.5 equiv) was added to the reaction mixture. After 3-5 min preactivation, cyclohexylamine (1.2 equiv) was added in above mixture and was kept in stirring condition at room temperature for 20 min. We found that the reaction worked very well with 84% yield using 0.2 equiv of **I** (Table 3.1.1, entry **5**). But when we used 0.1 equiv, we observed only 55% (Entries **4**). Therefore, 0.2 equiv of *o*-NosylOXY is kept as the standard amount for the coupling reaction. Next, for solvent optimization, we screened several solvents including THF, $CHCl_3$, DCM, DMF, EtOH, CH_3CN, MeOH and H_2O using 0.2 equiv of **I**. DCM was observed to be the best solvent with excellent yield (84%). But there was no reaction in EtOH, MeOH and water (Entry **9**, **11** and **12**, respectively).

Table 3.1.1. Optimization of reaction conditions.[a]



Entry	Solvents	I (equiv)	Yield[b] (%)
1	DCM	1.0	92
2	DCM	0.4	86
3	DCM	0.2	84
4	DCM	0.1	55
5	**DCM**	**0.2**	**84**
6	THF	0.2	70
7	$CHCl_3$	0.2	73
8	DMF	0.2	72
9	EtOH	0.2	0
10	CH_3CN	0.2	78
11	MeOH	0.2	0
12	H_2O	0.2	0

[a] *Reaction conditions: Acid (1 mmol), Reagent **I** (varied amount), DIPEA (1.5 mmol), Amine (1.2 mmol) at room temperature and the reaction time is 20 min.* [b] *Isolated yield.*

Further, we performed the same reaction with other coupling reagents such as DCC, EDC, HBTU, BenzylOXY, and TosylOXY with 0.2 equiv, we observed only 40% yield

(Table 3.1.2). Hence, only *o*-NosylOXY worked with catalytic amount and rest did not show any catalytic behaviour.

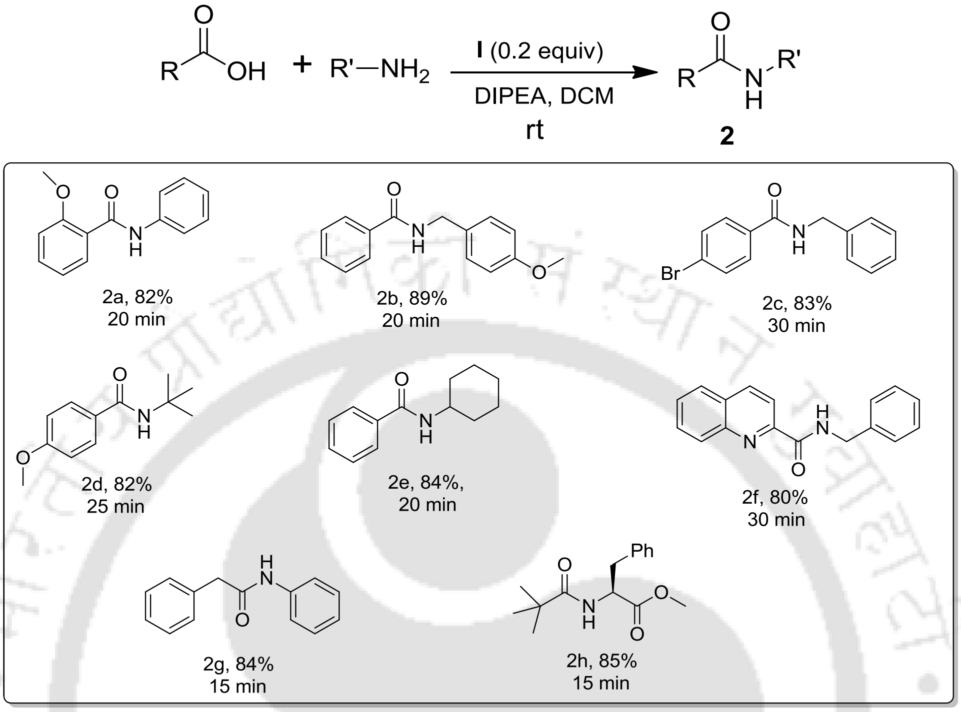
Table 3.1.2. Study of the catalytic behavior of various coupling reagents.[a]

Entry	**Coupling reagents**	**Time (h)**	**Yield[b] (%)**
1	DCC	24	15
2	EDC	36	17
3	HBTU	0.5	40
4	BenzylOXY	36	31
5	TosylOXY	36	35
6	***o*-NosylOXY**	0.5	**84**

[a] *Reaction conditions: Acid (1 mmol), Coupling reagents (0.2 mmol), DIPEA (1.5 mmol), Amine (1.2 mmol) at room temperature and the reaction time is 20 min.* [b] *Isolated yield*

With that optimized reaction conditions in hand, we explored the applicability of this method for amidation using various carboxylic acids and amines by using **I** (Scheme 3.1.1). The reaction worked well with aromatic (Entries **2a-2e**), heterocyclic containing carboxylic acid (Entry **2f**) and aliphatic (Entries **2g** and **2h**) and aromatic amines (Entries **2a** and **2g**), aliphatic amines (Entries **2b-2f**) as well as *C*-protected amino acid (Entry **2h**) with good to excellent yields.

Scheme 3.1.1. Wide scope of the synthesis of amide by using **I**.[a,b]

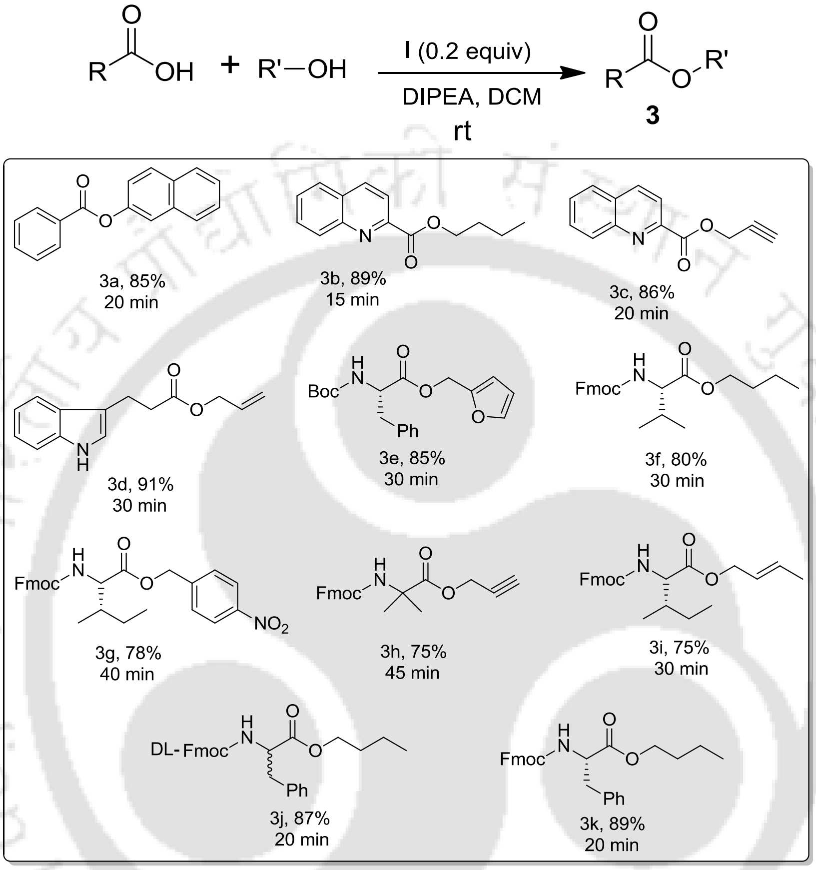


[a]*Reaction conditions: Acid (1 mmol), Reagent **I** (0.2 mmol), DIPEA (1.5 mmol), Amine (1.2 mmol) and DCM (2 ml) stirred at room temperature for 15-30 min.* [b]*Isolated yield*

3.2. Ester synthesis using catalytic amount of *o*-NosylOXY

After successful synthesis of amides using catalytic amount of *o*-NosylOXY, we further extended this protocol for esterification reactions. We did initial trials with benzoic acid and *β*-naphthol using 0.2 equiv of reagent **I**, obtained the desired product with excellent yield (85%, Scheme 3.2.1, entry **3a**). All of these substrates reacted smoothly under the optimized conditions to provide the desired products. The reactions worked well with aromatic carboxylic acid (Scheme 3.2.1, entry **3a**), heterocycle containing carboxylic acid (Entries **3b-3d**) as well as *N*-protected amino acid (Entries **3e-3k**) with excellent yield.

Scheme 3.2.1. Wide scope of the synthesis of ester by using **I**.[a,b]



*[a]Reaction conditions: Acid (1 mmol), Reagent **I** (0.2 mmol), DIPEA (1.5 mmol), Alcohol (1.2 mmol) and DCM (2 ml) stirred at room temperature for 15-45 min. [b]Isolated yield.*

3.3. Racemization study

For investigation of racemization potential, we synthesized Fmoc-DL-Phe-OBu and Fmoc-L-Phe-OBu esters using the optimized protocol and passed through a chiral column. In case of Fmoc-DL-Phe-OBu, two well-separated peaks observed which corresponding to the two enantiomers and in case of Fmoc-L-Phe-OBu, a single peak appeared which corresponds to the single enantiomer which was observed in HPLC chromatogram (Figure 3.3.1 and Figure S19-S23). This indicates no racemization occurred during the coupling reaction.

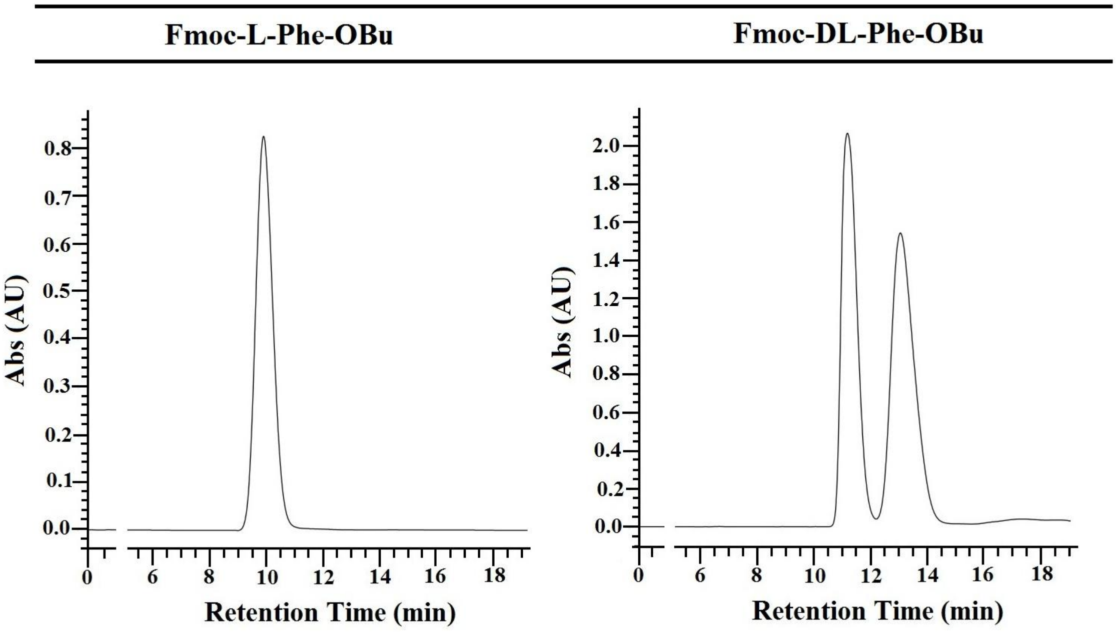


Figure 3.3.1. *HPLC Chromatogram of Fmoc-L-Phe-OBu (left panel) and Fmoc-DL-Phe-OBu (right panel)*

3.4. Plausible mechanism

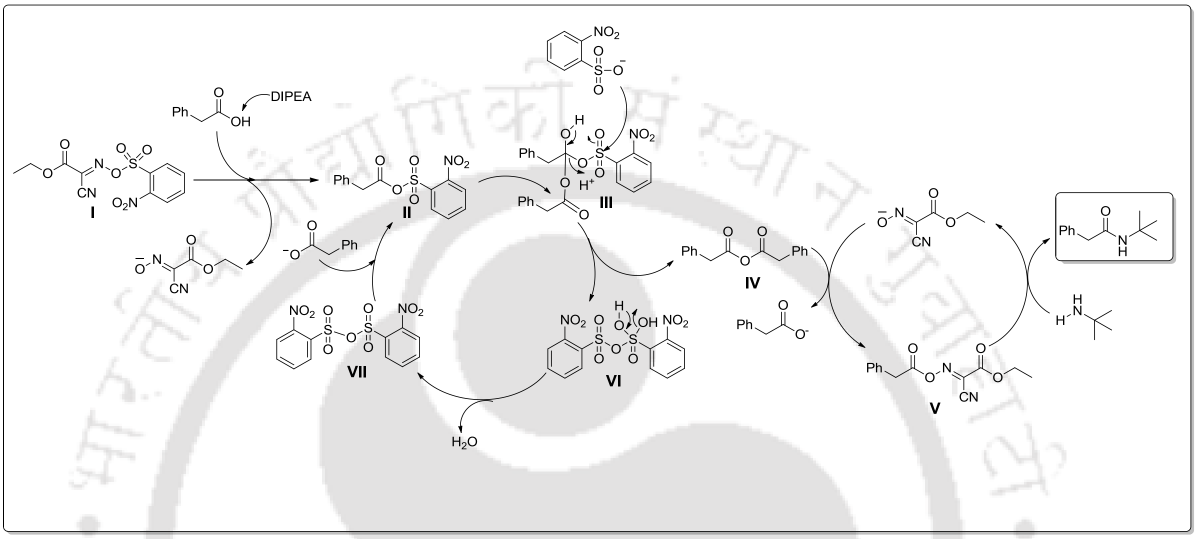
Having demonstrated the synthesis of amides and esters using catalytic amount of *o*-NosylOXY, we turned our attention to elucidate the mechanism of the catalytic effect.

Initially, we thought *o*-NosylOXY is working as a catalyst by in situ recombination of its by-products, 2-nitrobenzenesulphonic acid and Oxyma. To confirm, we performed two control reactions, one in presence and the other in absence of base, DIPEA, not using *o*-NosylOXY, but with its plausible precursors, 2-nitrobenzenesulphonic acid and Oxyma instead. We mixed 2-nitrobenzenesulphonic acid, Oxyma and the benzoic acid and stirred. After 15 min, *t*-butyl amine was added and kept the reaction for overnight; no reaction took place in both the cases. To be sure, we repeated the same reactions (in presence and in absence of base) with PTSA instead of 2-nitrobenzenesulphonic acid but reaction did not proceed, either. This means, in situ recombination of its byproducts is not happening in room temperature and it is not the cause of the catalytic effect of *o*-NosylOXY. However, such recombination is known to occur in high temperature or under microwave irradiation.[2]

Later, we monitored the reaction with phenylacetic acid (1 mmol), *o*-NosylOXY (0.2 mmol) in presence of DIPEA (1.5 mmol) by GCMS in a time dependent manner. Based on the results, we drew the most probable mechanism (Scheme 3.4.1), where the reagent **I** seemed to act as an initiator.

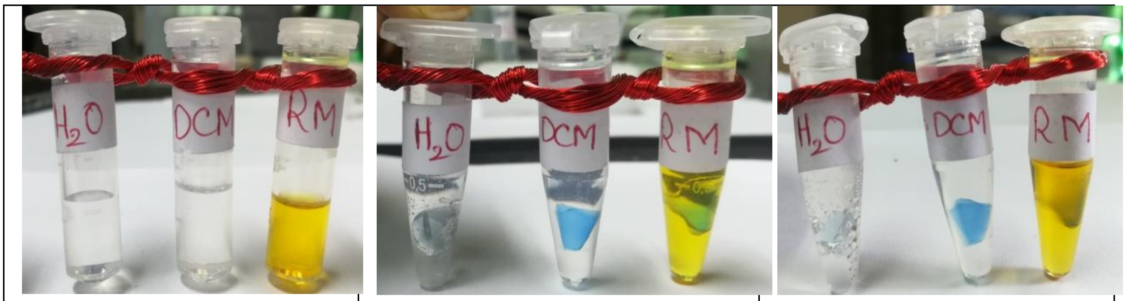
Most probably, at first the mixed anhydride **II** was evolved via the nucleophilic attack of the carboxylate anion on the reagent **I** with the expulsion of Oxyma anion. Next, another molecule of the carboxylate anion reacted with the intermediate **II** and formed the intermediate **III**. In the next step, most probably, the nucleophilic attack of 2-nitrobenzene sulphonate on **III** generated the symmetric anhydride **IV**. After that the intermediate **V** was generated by further attack of Oxyma anion to the intermediate **IV**. **V** subsequently was trapped with the nucleophilic amine to generate the desired amide. On the other hand, the intermediate **VI** was also formed by the reaction of the 2-nitrobenzene sulphonate on the intermediate **III** and **VI** was converted into **VII** with loss of a water molecule. This **VII** was activated by another carboxylate anion to produce **II** again. All the intermediates were identified by GCMS studies and furnished in Figure S24.

However, other kinds of attacks are also possible to explain the mechanism. For example, we noticed in one of the GCMS profile, presence of a peak corresponding to direct attack of amine to the intermediate **II**.



Scheme 3.4.1. *A plausible mechanistic pathway for the catalytic approach of o-NosylOXY.*

The evolution of water was confirmed by adding blue silica gel to the reaction mixture (Figure 3.4.1). Blue silica gel turns its colour to light blue gradually when it takes up moisture. For experiment, we took three sample vials of water, distilled DCM and the reaction mixture. After adding blue silica gel in each vial, colour of silica bead in water gradually turns into light blue and colour in reaction mixture gradually turns into green but in DCM vial, colour of silica bead remains as blue (Figure 3.4.1). When we kept this silica bead in solution overnight then we observed that color of blue silica gel turns its color into light blue in water containing vial and in reaction mixture vial. This experiment proved that water is generated as by-product in the reaction mixture as suggested by the drawn mechanistic pathway.



(a) Before adding blue silica gel

(b) 10 min after adding blue silica gel

(c) Kept overnight after adding blue silica gel

Figure 3.4.1. *Test for evolution of water*

Furthermore, the evolution of water was also confirmed by adding anhydrous $FeCl_3$ to the reaction mixture. $FeCl_3$ gives orange or reddish color in presence of water.[3] This experiment has shown in Figure S25. In this condensation, change of colour from orange to reddish confirmed that water is generated as by-product. All these experiments confirm that water comes out as the by-product as suggested by the drawn plausible mechanism. It also proves that our method is eco-friendly.

3.5. Conclusion

Here, we have reported the feasibility of catalytic behavior of *o*-NosylOXY as a coupling reagent for amides and esters synthesis. The developed method suppressed the racemization during the coupling reactions. *o*-NosylOXY is used in catalytic amount for coupling reaction, i.e. minimal waste production that leads to environment-friendly method. Therefore, this concept could be important for meeting one of the key challenges of Green Chemistry.

3.6. Experimental Section

3.6.1. Materials and methods

As described in chapter 2 section 2.4.1

3.6.2. General procedure for the synthesis of amide:

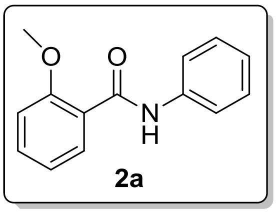
o-NosylOXY (0.2 equiv) was added to a solution of carboxylic acid (1 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 3-5 min for preactivation followed by the addition of amine (1.2 equiv). The reaction mixture was stirred at room temperature for 15-30 min. After completion of the reaction, the reaction mixture was diluted with 50 ml of ethyl acetate; the organic phase was washed with 5% citric acid (3×20 ml), 5% $NaHCO_3$ (3×20 ml) and dried by anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the product which was purified by column chromatography.

3.6.3. General procedure for the synthesis of ester:

o-NosylOXY (0.2 equiv) was added to a solution of carboxylic acid (1 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 3-5 min for preactivation followed by the addition of alcohol (1.2 equiv). The reaction mixture was stirred at room temperature for 15-45 min. After completion of the reaction, the reaction mixture was diluted with 50 ml of ethyl acetate; the organic phase was washed with 5% citric acid (3×20 ml), 5% $NaHCO_3$ (3×20 ml) and dried by anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the product which was purified by column chromatography.

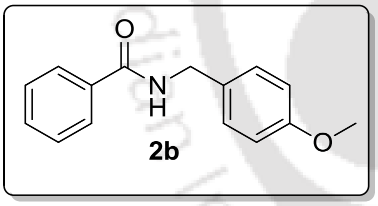
3.7. Characterization data

2-Methoxy-*N*-phenylbenzamide, 2a


2a

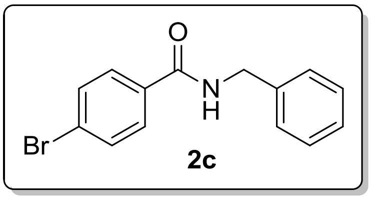
Oil; (186 mg, 82%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.29-8.28 (d, *J* = 7.8 Hz, 1H), 7.69-7.68 (t, *J* = 8.4 Hz, 2H), 7.50-7.47 (t, *J* = 7.8 Hz, 1H), 7.38-7.35 (t, *J* = 7.8 Hz, 2H), 7.14-7.11 (t, *J* = 7.8 Hz, 2H), 7.03-7.01 (d, *J* = 8.4 Hz, 1H), 4.04 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.4, 157.3, 138.5, 133.4, 132.6, 129.1, 124.3, 121.9, 121.8, 120.6, 111.7, 56.4; FT-IR (KBr, cm^{-1}): 3354, 2944, 1667, 1597, 1443, 1321, 1254, 1126, 1019, 754, 691, 510; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{14}H_{14}NO_2$ 228.1025, found 228.1036.

***N*-(4-Methoxybenzyl) benzamide, 2b**


2b

Solid; (214 mg, 89%); mp 99-101 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.78-7.77 (d, *J* = 7.8 Hz, 2H), 7.50-7.49 (t, *J* = 7.2 Hz, 1H), 7.42-7.40 (t, *J* = 7.8 Hz, 2H), 7.28-7.26 (t, *J* = 7.2 Hz, 2H) 6.88-6.87 (d, *J* = 7.2 Hz, 2H), 4.57-4.56 (d, *J* = 5.4 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 167.5, 159.0, 134.5, 131.5, 130.5, 129.2, 128.5, 127.1, 114.1, 55.3, 43.5; FT-IR (KBr, cm^{-1}): 3334, 2915, 1641, 1530, 1487, 1311, 1238, 1185, 1029, 719, 580; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{15}H_{16}NO_2$ 242.1181, found 242.1185.

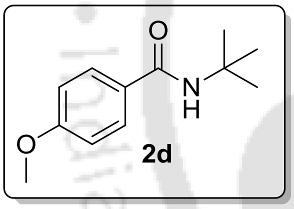
N-Benzyl-4-bromobenzamide, 2c



2c

Solid; (240 mg, 83%); mp 171-173 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.66-7.65 (d, *J* = 8.4 Hz, 2H), 7.57-7.55 (d, *J* = 8.4 Hz, 2H), 7.37-7.30 (m, 5H), 6.41 (br, 1H), 4.63-4.62 (d, *J* = 5.4 Hz, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 166.6, 138.1, 133.3, 132.0, 129.1, 128.8, 128.1, 127.9, 126.5, 44.4; FT-IR (KBr, cm^{-1}): 3314, 2918, 1640, 1549, 1483, 1321, 1256, 1010, 846, 731, 669; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{14}H_{13}BrNO$ 290.0181, found 290.0166.

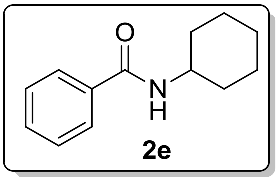
N-(*tert*-Butyl)-4-methoxybenzamide, 2d



2d

Solid; (170 mg, 82%); mp 106-108 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.70-7.68 (d, *J* = 8.4 Hz, 2H), 6.91-6.90 (d, *J* = 9.0 Hz, 2H), 3.84 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 166.7, 162.0, 132.3, 128.4, 113.8, 55.6, 51.7, 29.1; FT-IR (KBr, cm^{-1}): 3328, 2973, 1631, 1550, 1455, 1323, 1255, 1177, 1032, 841, 770; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{12}H_{18}NO_2$ 208.1338, found 208.1337.

N-Cyclohexylbenzamide, 2e

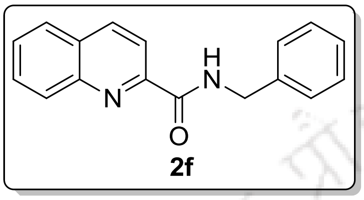


2e

Solid; (170 mg, 92%); mp 152-154 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.75-7.74 (d, *J* = 7.2 Hz, 2H), 7.49-7.46 (t, *J* = 7.2 Hz, 1H), 7.43-7.40 (t, *J* = 7.8 Hz, 2H), 4.00-3.94 (m, 1H), 2.04-2.01 (m, 2H), 1.76-1.73 (m, 3H), 1.66-1.64 (m, 1H), 1.45-1.38 (m, 2H), 1.26-1.16 (m, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 167.5, 134.8, 131.4, 128.5, 126.9, 48.9, 33.0, 32.9, 25.5, 25.0;

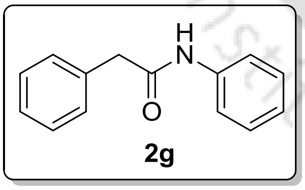
FT-IR (KBr, cm^{-1}): 3242, 2927, 2850, 1624, 1574, 1452, 1261, 1082, 700; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{18}NO$ 204.1388, found 204.1395.

_N_-Benzylquinoline-2-carboxamide, 2f


2f

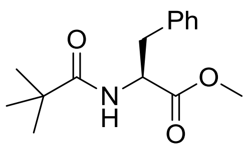
Solid; (210 mg, 80%); mp 125-127 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36-8.32 (q, 2H), 8.08-8.07 (d, *J* = 8.4 Hz, 1H), 7.89-7.88 (d, *J* = 8.4 Hz, 1H), 7.76-7.74 (t, *J* = 7.2 Hz, 1H), 7.63-7.61 (t, *J* = 7.2 Hz, 1H), 7.43-7.42 (d, *J* = 7.2 Hz, 2H), 7.39-7.36 (t, *J* = 7.2 Hz, 2H), 7.32-7.30 (t, *J* = 7.2 Hz, 1H), 4.76-4.75 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.6, 149.8, 146.6, 138.5, 137.7, 130.2, 129.8, 129.5, 128.9, 128.1, 128.0, 127.9, 127.6, 119.1, 43.7; FT-IR (KBr, cm^{-1}): 3329, 2931, 1657, 1525, 1498, 1365, 1208, 1161, 1080, 843, 702, 634; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{15}N_2O$ 263.1184, found 263.1183.

***N*, 2-Diphenylacetamide, 2g**


2g

Solid; (177 mg, 84%); mp 113-115 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.39 (t, *J* = 7.8 Hz, 4H), 7.35-7.33 (t, *J* = 7.8 Hz, 3H), 7.29-7.26 (m, 2H), 7.11- 7.07 (m, 1H), 3.74 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.4, 137.8, 134.6, 129.7, 129.4, 129.1, 127.9, 124.7, 120.1, 45.0; FT-IR (KBr, cm^{-1}): 3435, 2922, 1657, 1555, 1441, 1356, 1258, 1166, 1024, 755, 691, 504; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{14}NO$ 212.1075, found 212.1084.

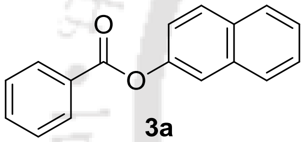
(*S*)-Methyl 3-phenyl-2-pivalamidopropanoate, 2h



2h

Solid; (223 mg, 85%); mp 88-90 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.25 (m, 3H), 7.09-7.07 (d, *J* = 7.2 Hz, 2H), 4.88-4.85 (m, 1H), 3.74 (s, 3H), 3.19-3.16 (m, 1H), 3.11-3.08 (m, 1H), 1.15 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.0, 172.4, 136.0, 129.4, 128.6, 127.2, 53.0, 52.4, 38.7, 37.8, 27.4; FT-IR (KBr, cm^{-1}): 2973, 1754, 1731, 1634, 1531, 1202, 1038, 754, 702; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{22}NO_3$ 264.1600, found 264.1608.

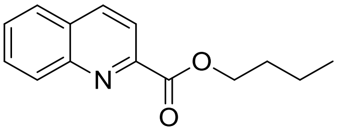
Naphthalen-2-yl benzoate, 3a



3a

Solid; (210 mg, 85%); mp 103-105 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27-8.26 (d, *J* = 7.2 Hz, 2H), 7.92-7.81 (m, 3H), 7.70-7.65 (m, 2H), 7.56-7.48 (m, 4H), 7.38-7.36 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.6, 148.8, 134.0, 133.9, 131.7, 130.4, 129.8, 129.7, 128.8, 128.0, 127.9, 126.8, 125.9, 121.4, 118.9; FT-IR (KBr, cm^{-1}): 3446, 2918, 1729, 1596, 1450, 1353, 1272, 1154, 1062, 955, 817, 708, 477; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{13}O_2$ 249.0916, found 249.0915.

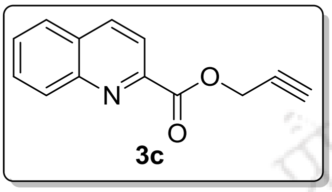
Butyl quinoline-2-carboxylate, 3b



3b

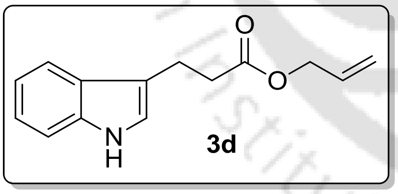
Oil; (204 mg, 89%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32-8.29 (m, 2H), 8.18-8.16 (d, *J* = 8.4 Hz, 1H), 7.88-7.87 (d, *J* = 7.8 Hz, 1H), 7.80-7.77 (t, *J* = 7.2 Hz, 1H), 7.66-7.63 (t, *J* = 7.2 Hz, 1H), 4.51-4.48 (t, *J* = 7.2 Hz, 2H), 1.88-1.83 (m, 2H), 1.52-1.48 (m, 2H), 1.01-0.98 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.6, 148.4, 147.8, 137.4, 131.0, 130.4, 129.5, 128.7, 127.7, 121.2, 66.3, 30.9, 19.4, 14.0; FT-IR (KBr, cm^{-1}): 3060, 2959, 1716, 1503, 1463, 1315, 1211, 1105, 845, 777, 625; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{16}NO_2$ 230.1181, found 230.1198.

Prop-2-yn-1-yl quinoline-2-carboxylate, 3c


3c

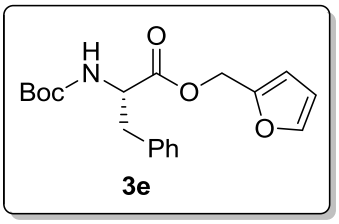
Solid; (181 mg, 86%); mp 111-113 ºC; ^1H NMR (600 MHz, $CDCl_3$) δ 8.31-8.30 (d, *J* = 7.8 Hz, 2H), 8.21-8.20 (d, *J* = 8.4 Hz, 1H), 7.88-7.87 (d, *J* = 7.8Hz, 1H), 7.80-7.78 (t, *J* = 7.2 Hz, 1H), 7.67-7.64 (t, *J* = 7.2 Hz, 1H), 5.08-5.07 (d, *J* = 2.4 Hz 2H), 2.56-2.55 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.8, 147.8, 147.4, 138.0, 131.0, 130.6, 129.6, 129.0, 127.7, 121.3, 77.5, 75.7, 53.6; FT-IR (KBr, cm^{-1}): 3193, 2931, 2127, 1714, 1445, 1310, 1209, 1134, 846, 782, 625; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{10}NO_2$ 212.0712, found 212.0720.

Allyl 3-(1H-indol-3-yl) propanoate, 3d


3d

Oil; (208 mg, 91%); ^1H NMR (600 MHz, $CDCl_3$) δ 8.21 (br s, 1H), 7.86-7.84 (d, *J* = 8.4 Hz, 1H), 7.60-7.59 (d, *J* = 7.8 Hz, 1H), 7.50 (s, 1H), 7.45-7.42 (t, *J* = 7.2 Hz, 1H), 7.38-7.35 (t, *J* = 7.2 Hz, 1H), 6.17-6.11 (m, 1H), 5.54-5.45 (m, 2H), 4.83-4.82 (d, *J* = 5.4 Hz, 2H), 3.38-3.35 (t, *J* = 7.8 Hz, 2H), 3.01-2.99 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 136.5, 132.4, 127.4, 122.3, 121.6, 119.5, 118.9, 118.4, 115.1, 111.3, 65.3, 35.1, 20.8; FT-IR (KBr, cm^{-1}): 3057, 2926, 1729, 1619, 1457, 1338, 1159, 742; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{16}NO_2$ 230.1181, found 230.1184.

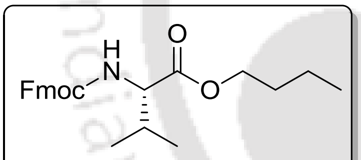
(*R*)-Furan-2-yl-methyl 2-((tert-butoxycarbonyl)amino)-3-phenylpropanoate, 3e



3e

Solid; (272 mg, 85%); mp 86-88 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.24-7.21 (t, *J* = 7.2 Hz, 3H), 7.03-7.02 (d, *J* = 6.6 Hz, 2H), 6.42-6.38 (m, 2H), 5.18-5.16 (d, *J* = 12.6 Hz, 1H), 5.05-5.03 (d, *J* = 12.6 Hz, 1H), 4.96-4.95 (d, *J* = 7.8 Hz,1H), 4.62-4.59 (m, 1H), 3.12-3.04 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.7, 155.3, 149.1, 143.6, 136.0, 129.5, 128.7, 127.1, 111.5, 110.8, 80.1, 58.9, 54.5, 38.3, 28.5; FT-IR (KBr, cm^{-1}): 3380, 2976, 1753, 1686, 1508, 1168, 1050, 739; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{19}H_{24}NO_5$ 346.1654, found 346.1211.

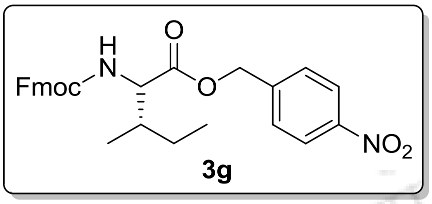
(*R*)-Butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoate, 3f



3f

Solid; (316 mg, 80%); mp 70-72 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.62-7.60 (m, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 4.41-4.39 (m, 2H), 4.32-4.30 (m, 1H), 4.25-4.23 (t, *J* = 7.2 Hz, 1H), 4.18-4.13 (m, 2H), 2.20-2.15 (m, 1H), 1.65-1.62 (m, 2H), 1.41-1.38 (m, 2H), 0.99-0.91 (m, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.4, 156.4, 143.9, 141.5, 127.9, 127.3, 125.3, 120.2, 67.2, 65.4, 59.2, 47.4, 31.6, 30.8, 19.3, 17.8, 13.9; FT-IR (KBr, cm^{-1}): 3416, 2966, 1725, 1514, 1448, 1306, 1224, 1088, 756; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{24}H_{30}NO_4$ 396.2175, found 396.2177.

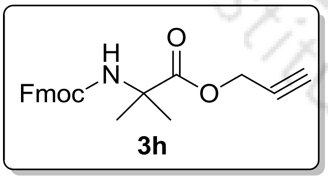
(*R*)-4-Nitrobenzyl-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoate, 3g



3g

Solid; (381 mg, 78%); mp 118-120 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21-8.19 (d, *J* = 8.4 Hz, 2H), 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.59-7.57 (d, *J* = 7.8 Hz, 2H), 7.50-7.49 (d, *J* = 8.4 Hz, 2H), 7.41-7.39 (m, 2H), 7.32-7.29 (t, *J* = 7.8 Hz, 2H), 5.25 (s, 2H), 4.48-4.45 (m, 1H), 4.42-4.41 (d, *J* = 7.2 Hz, 2H), 4.22-4.20 (t, *J* = 6.6 Hz, 1H), 1.71-1.64 (m, 2H), 1.25-1.23 (m, 1H), 0.96-0.95 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 156.2, 147.8, 143.8, 142.7, 141.4, 128.5, 127.9, 127.2, 125.1, 123.9, 120.2, 67.2, 65.6, 52.7, 47.2, 41.4, 24.9, 23.0, 21.8; FT-IR (KBr, cm^{-1}): 3438, 2961, 1740, 1687, 1526, 1347, 1205, 1104, 1032, 852, 740; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{29}N_2O_6$ 489.2026, found 489.2025.

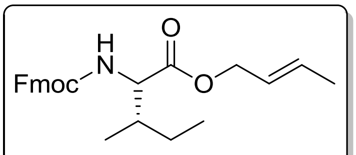
Prop-2-yn-1-yl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanoate, 3h



3h

Oil; (272 mg, 75%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.60-7.59 (d, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 5.37 (br, 1H), 4.73 (s, 2H), 4.38 (s, 2H), 4.24-4.22 (t, *J* = 7.2 Hz, 1H), 2.45 (s, 1H), 1.58 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.0, 155.1, 144.1, 141.5, 127.9, 127.2, 125.3, 120.2, 75.4, 66.8, 56.6, 53.2, 47.4, 25.2; FT-IR (KBr, cm^{-1}): 3066, 2988, 2128, 1720, 1524, 1450, 1388, 1282, 1144, 1089, 740, 621; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{22}H_{22}NO_4$ 364.1549, found 364.1552.

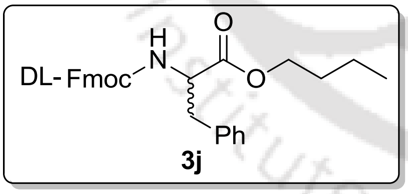
(2*R*,3*S*)-(*E*)-But-2-en-1-yl-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanoate, 3i



3i

Oil; (305 mg, 75%); ^1H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.61-7.60 (m, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 5.84-5.79 (m, 1H), 5.61-5.56 (m, 1H), 4.58-4.57 (d, *J* = 5.4 Hz, 2H), 4.40-4.39 (d, *J* = 7.2 Hz, 2H), 4.36-4.34 (m, 1H), 4.25-4.22 (t, *J* = 7.2 Hz, 1H), 1.91-1.89 (m, 1H), 1.73-1.72 (d, *J* = 6.6 Hz, 3H), 1.46-1.42 (m, 1H), 1.21-1.16 (m, 1H), 0.95-0.92 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.1, 156.3, 144.0, 141.5, 132.4, 127.9, 127.2, 125.3, 124.7, 120.2, 67.2, 66.1, 58.6, 47.4, 38.3, 25.2, 18.0, 15.7, 11.8; FT-IR (KBr, cm^{-1}): 3066, 2964, 1725, 1514, 1450, 1334, 1195, 1040, 740; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{30}NO_4$ 408.2175, found 408.2178.

DL-Butyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanoate, 3j

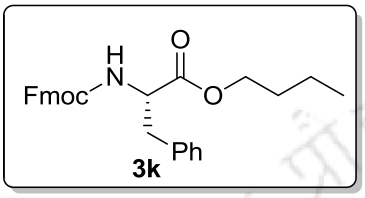


3j

Solid; (394 mg, 87%); mp 76-79 ºC; ^1H NMR (600 MHz, $CDCl_3$) δ 7.79-7.77 (d, *J* = 7.8 Hz, 2H), 7.60-7.57 (t, *J* = 7.8 Hz, 2H), 7.43-7.40 (t, *J* = 7.8 Hz, 2H), 7.34-7.27 (m, 4H), 7.14-7.12 (d, *J* = 7.2 Hz, 1H), 5.34 (br s, 1H), 4.70-4.67 (m, 1H), 4.47-4.44 (m, 1H), 4.37-4.34 (m, 1H), 4.24-4.21 (t, *J* = 7.2 Hz, 1H), 4.17-4.10 (m, 2H), 3.18-3.10 (m, 2H), 1.63-1.58 (m, 2H), 1.37-1.33 (m, 2H), 0.94-0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 155.7, 143.9, 141.5, 136.0, 129.5, 128.7, 127.9, 127.3, 127.2, 125.3, 125.2, 120.1, 67.1, 65.6, 55.0, 47.3, 38.5, 30.6, 19.2, 13.8; FT-IR (KBr, cm^{-1}):

2923, 1736, 1694, 1541, 1450, 1254, 1022, 736, 691.; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{30}NO_4$ 444.2175, found 444.2150.

(*S*)-Butyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanoate, 3k



3k

Solid; (394 mg, 89%); mp 76-79 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.77 (d, *J* = 7.8 Hz, 2H), 7.59-7.57 (t, *J* = 7.8 Hz, 2H), 7.42-7.40 (t, *J* = 7.2 Hz, 2H), 7.33-7.27 (m, 4H), 7.13-7.12 (d, *J* = 7.2 Hz, 1H), 5.33 (br s, 1H), 4.68-4.67 (d, *J* = 6.0 Hz, 1H), 4.46-4.43 (m, 1H), 4.36-4.33 (t, *J* = 6.6 Hz, 1H), 4.23-4.21 (t, *J* = 7.2 Hz, 1H), 4.17-4.09 (m, 2H), 3.17-3.09 (m, 2H), 1.62-1.58 (m, 2H), 1.36-1.33 (m, 2H), 0.94-0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 155.7, 143.9, 141.5, 136.0, 129.5, 128.7, 127.9, 127.3, 127.2, 125.3, 125.2, 120.2, 67.1, 65.6, 55.0, 47.3, 38.5, 30.6, 19.2, 13.8; FT-IR (KBr, cm^{-1}): 2923, 1736, 1694, 1541, 1450, 1254, 1022, 736, 691.; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{30}NO_4$ 444.2175, found 444.2145.

3.8. References

1. (a) Dharm Dev, Nani Babu Palakurthy, Kishore Thalluri, Jyoti Chandra and Bhubaneswar Mandal. Ethyl 2-cyano-2-(2-nitrobenzenesulfonyloxyimino)acetate (*o*-NosylOXY): A Recyclable coupling reagent for racemization free synthesis of peptide, amide, hydroxamate and ester. *J. Org. Chem.* **2014**, *45*, 5420-5431; (b) Kishore Thalluri, Srinivasa Rao Manne, Dharm Dev and Bhubaneswar Mandal. Ethyl 2-Cyano-2-(4-nitrophenylsulfonyloxyimino)acetate-Mediated lossen rearrangement: Single-pot racemization-free synthesis of hydroxamic acids and ureas from carboxylic acids. *J. Org. Chem.* **2014**, *79*, 3765-3775; (c) Dharm Dev,

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2. Jyoti Chandra,. Rohit Chaudhuri, Srinivasa Rao Manne, Sandip Mondal, Bhubaneswar Mandal. Direct synthesis of sulphonates of alcohol, Oxyma-*O*-sulphonates and Oxime-*O*-sulphonates under microwave irradiation, *ChemistrySelect.* **2017**, *2*, 8471–8477.

3. Saul Soloway and S. H. Wilen. Improved ferric chloride test for phenols. *Anal. Chem*. **1952**, *24*, 979-983.

3.9. Selected spectra and chromatograms

3.9.1. ^{1}H NMR and ^{13}C NMR of amides and esters

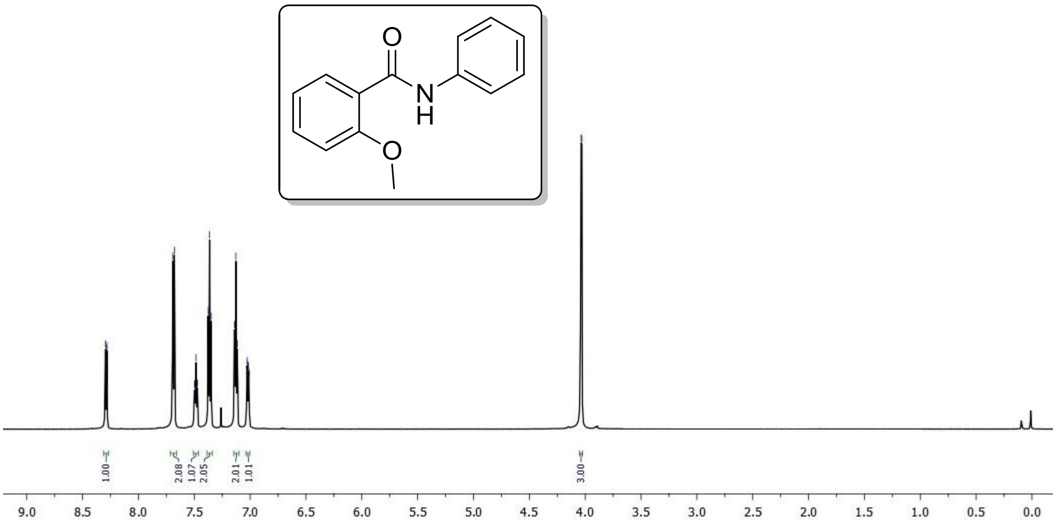


Figure S1. ^{1}H NMR spectra of compound **2a**

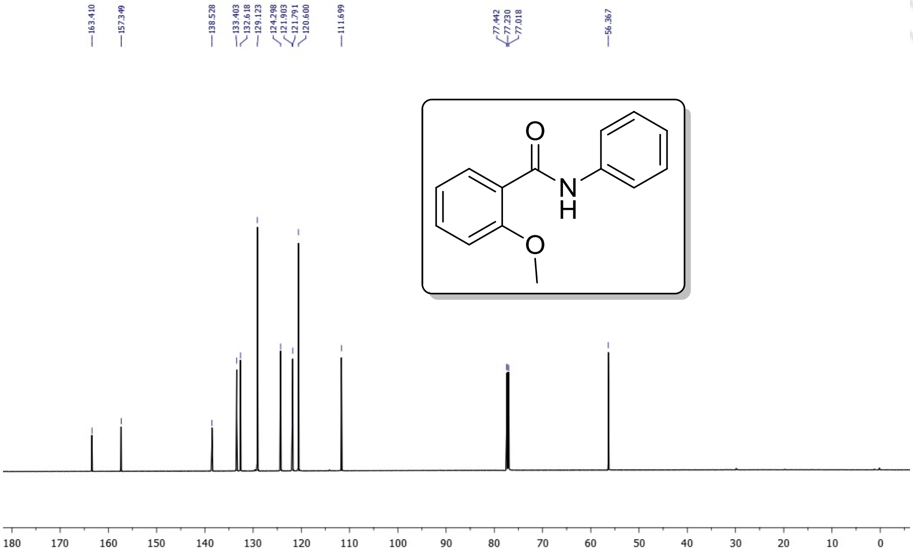


Figure S2. ^{13}C NMR spectra of compound **2a**

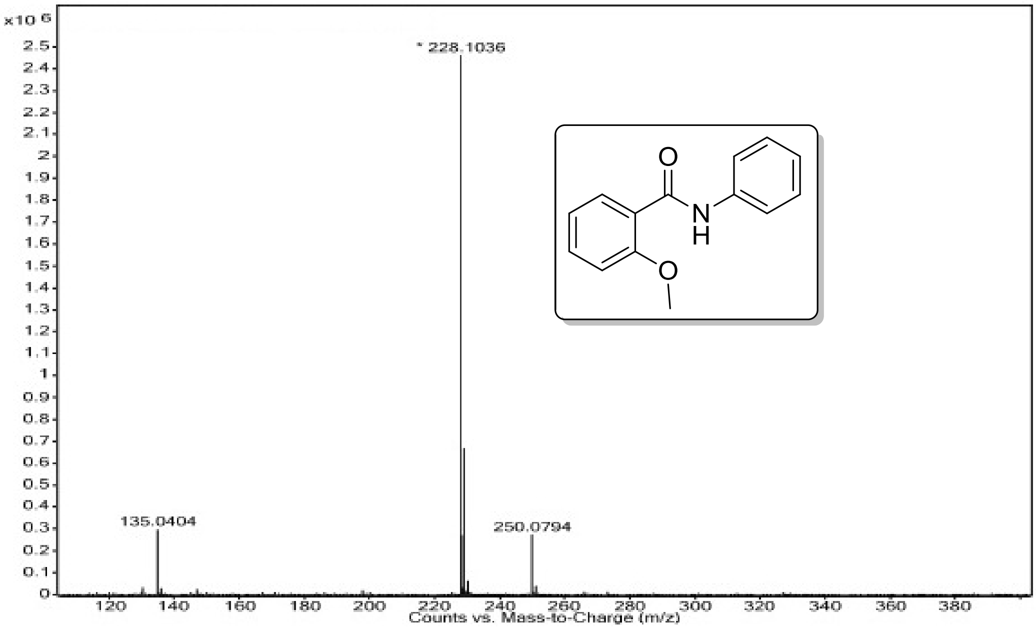


Figure S3. Mass spectra of compound **2a**

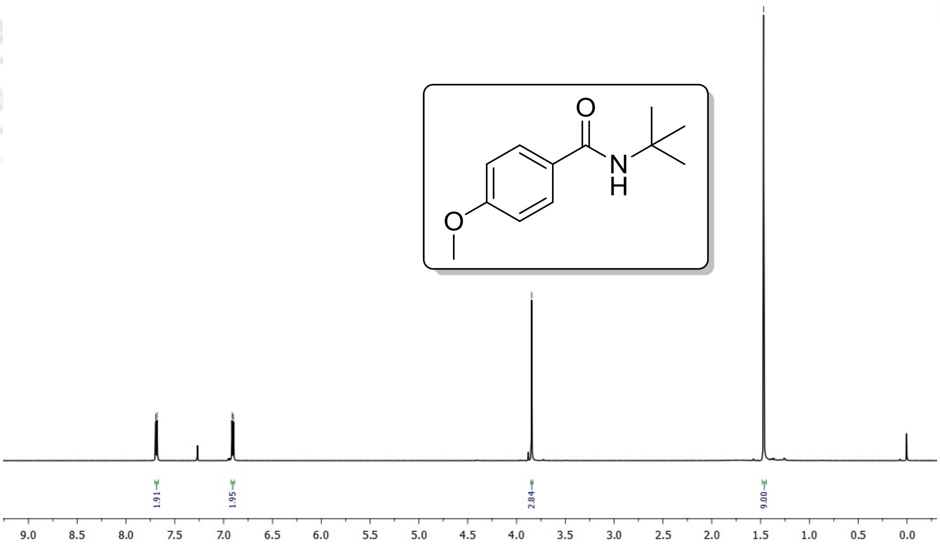


Figure S4. ^{1}H NMR spectra of compound **2d**

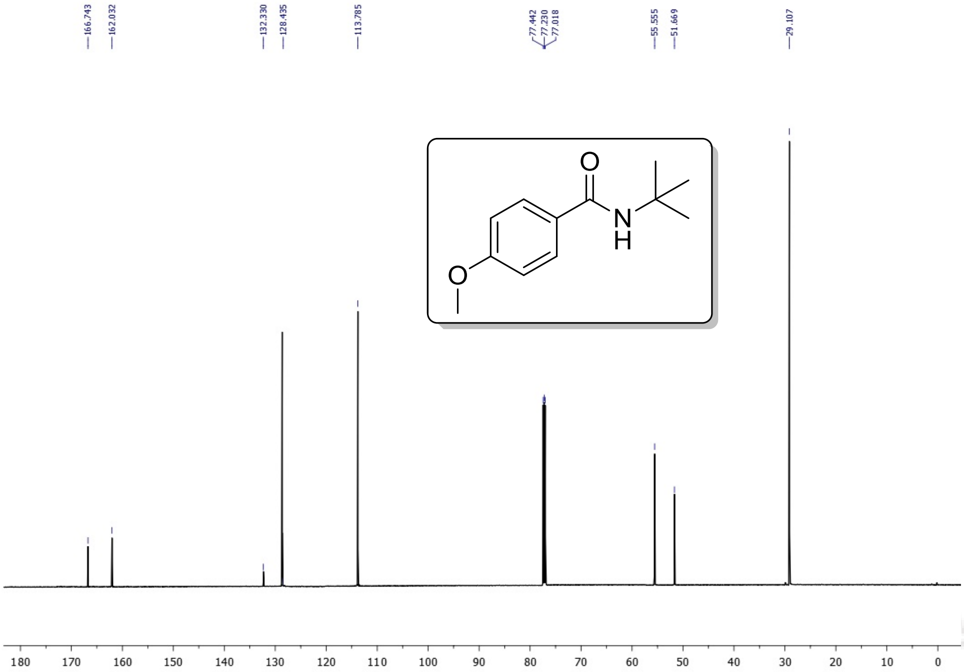


Figure S5. ^{13}C NMR spectra of compound **2d**

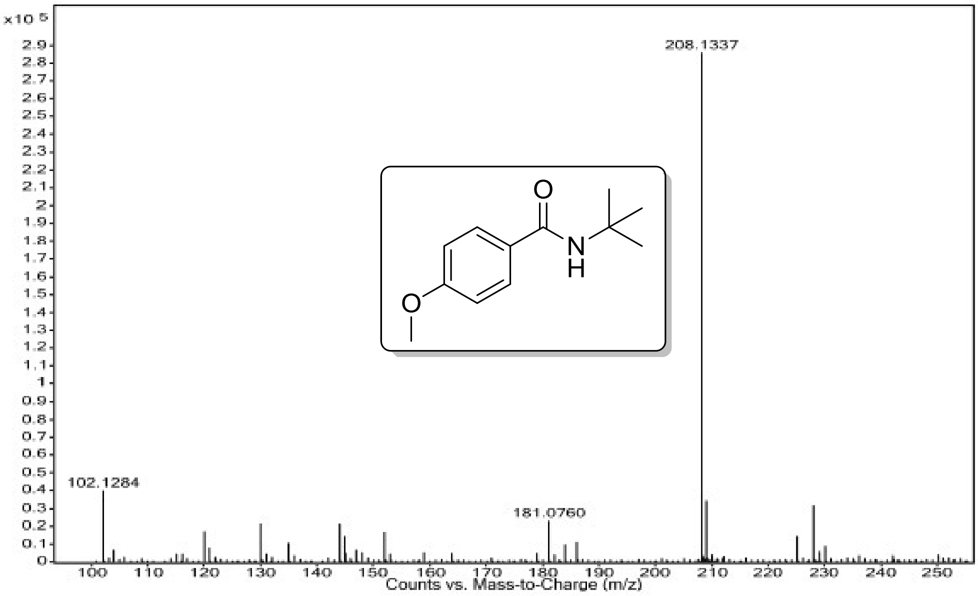


Figure S6. Mass spectra of compound **2d**

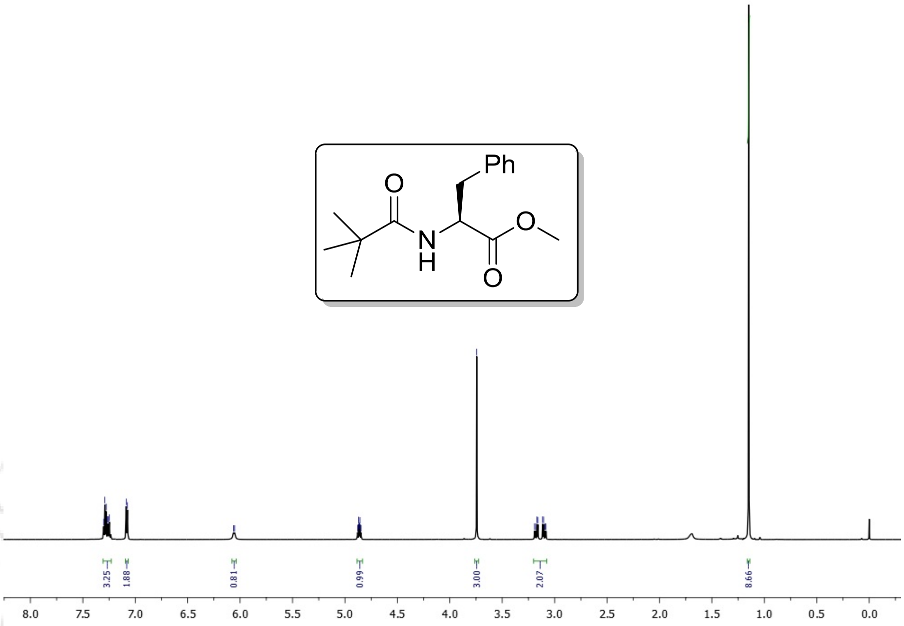


Figure S7. ^{1}H NMR spectra of compound **2h**

Figure S8. ^{13}C NMR spectra of compound **2h**

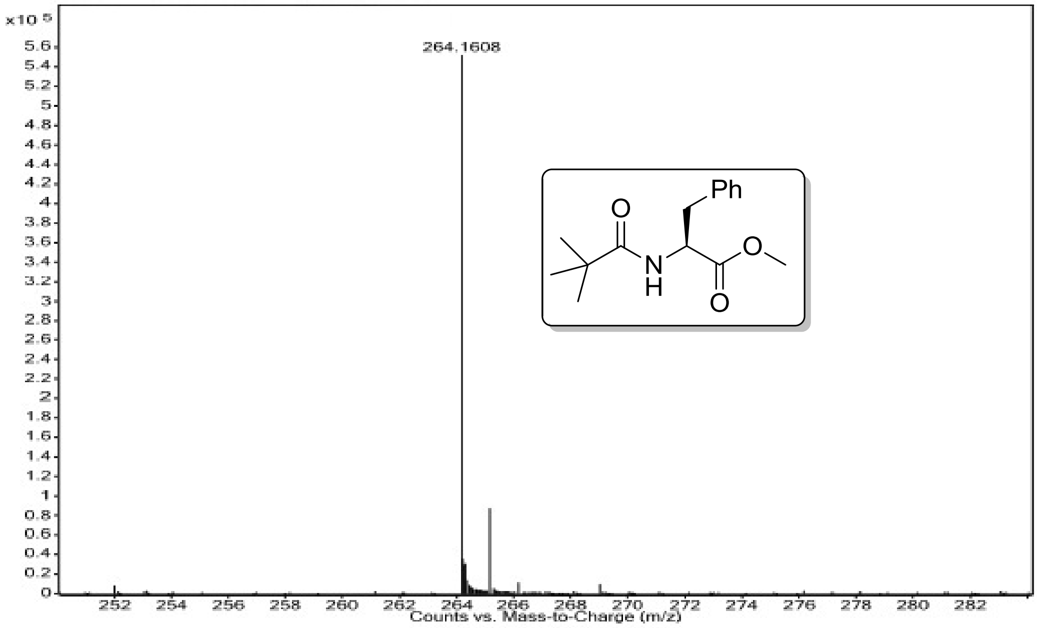


Figure S9. Mass spectra of compound **2h**

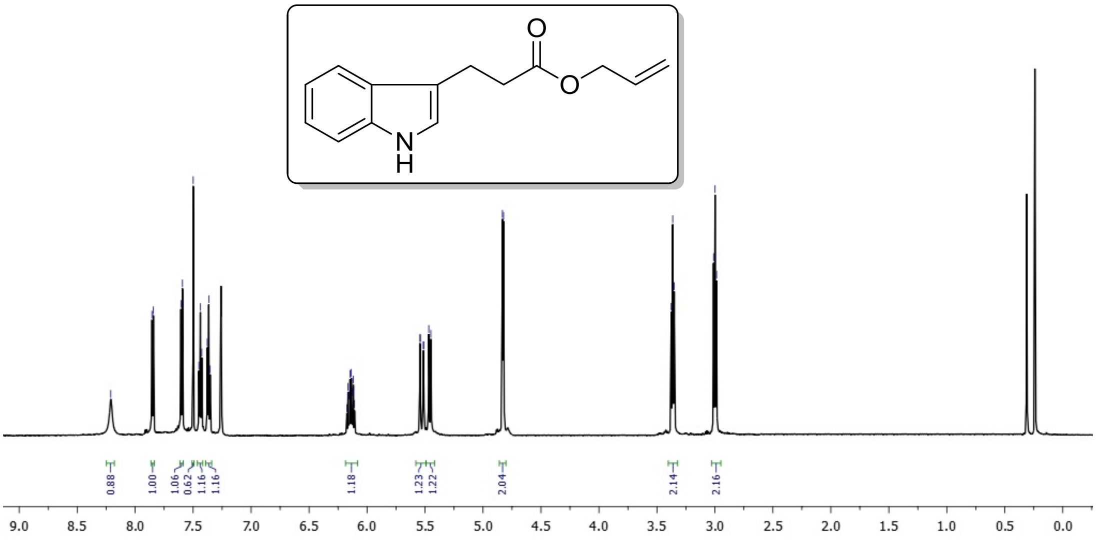


Figure S10. ^{1}H NMR spectra of compound **3d**

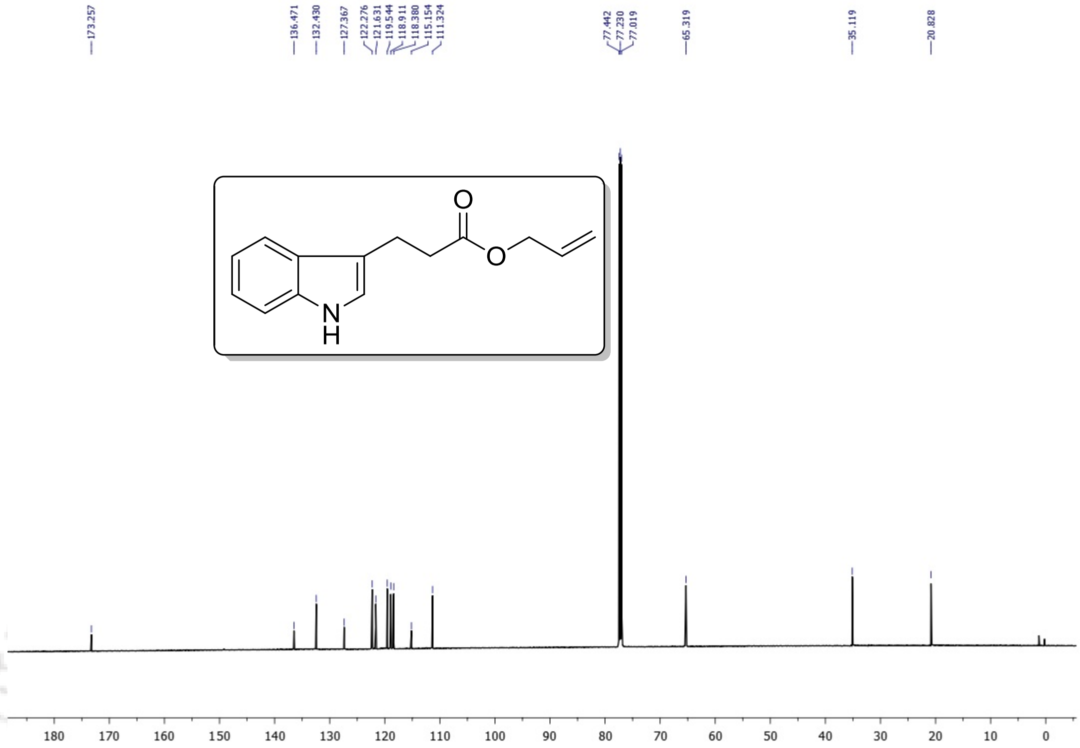


Figure S11. ^{13}C NMR spectra of compound **3d**

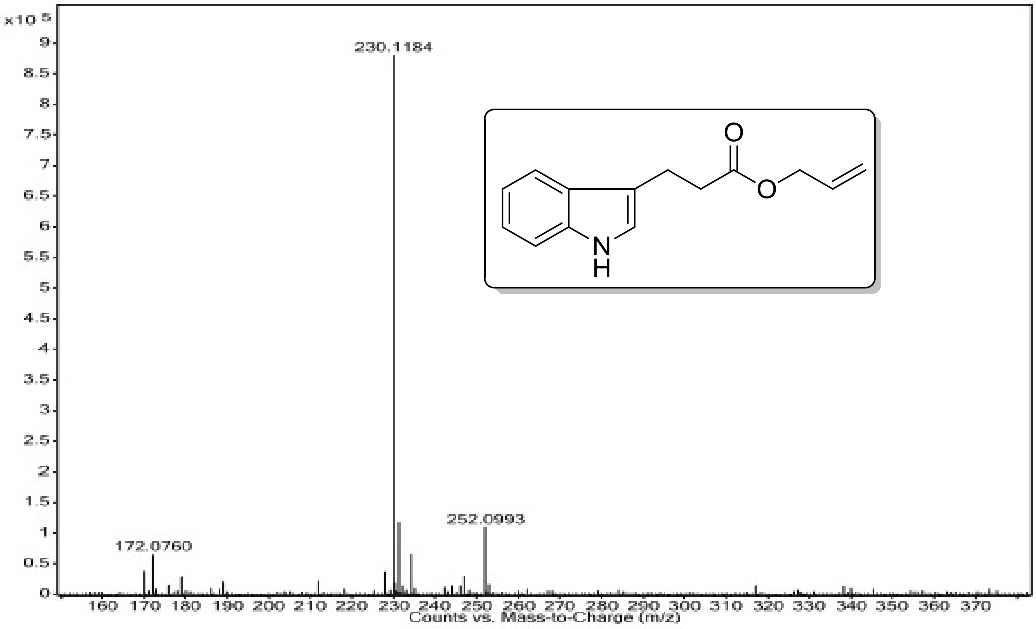


Figure S12. Mass spectra of compound **3d**

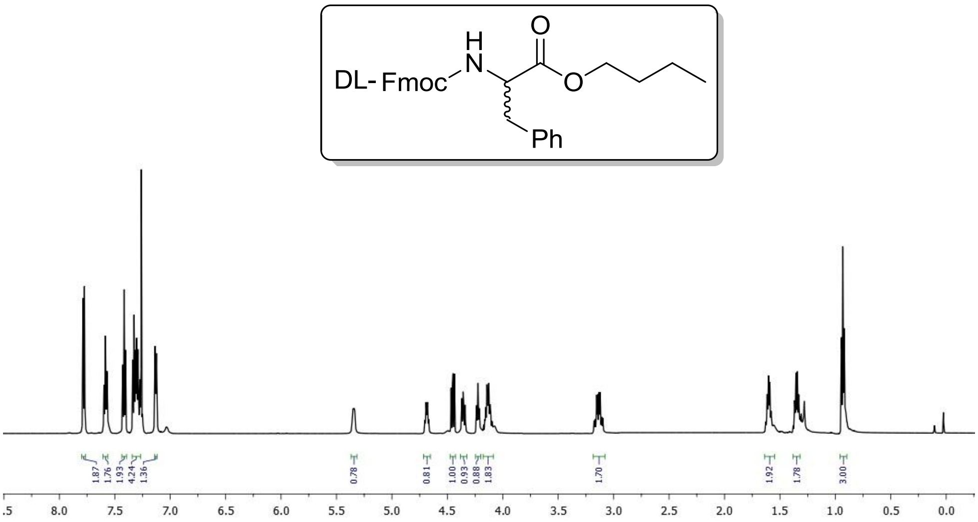


Figure S13. ^{1}H NMR spectra of compound **3j**

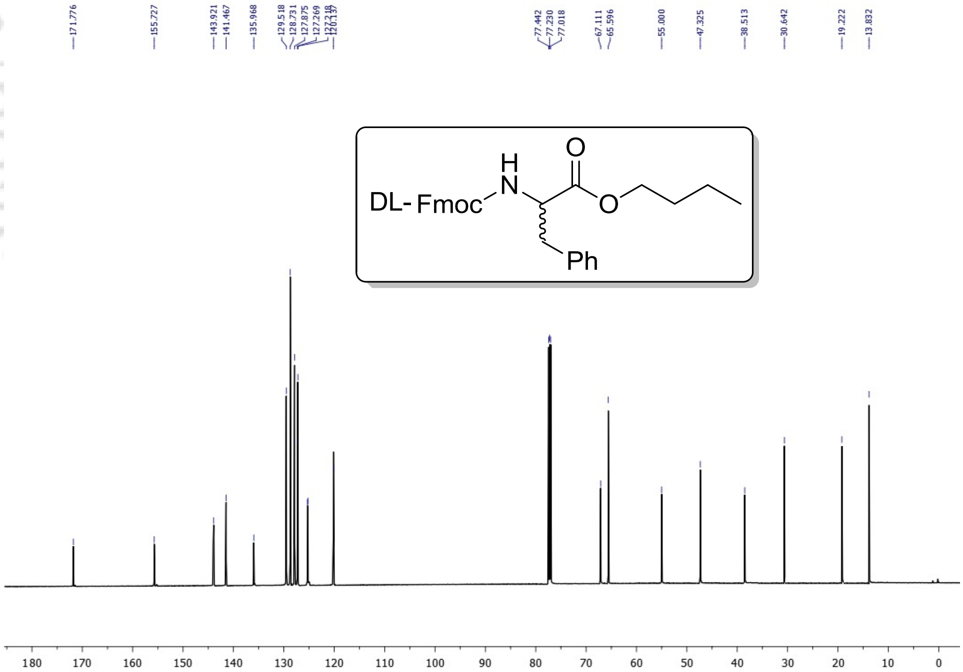


Figure S14. ^{13}C NMR spectra of compound **3j**

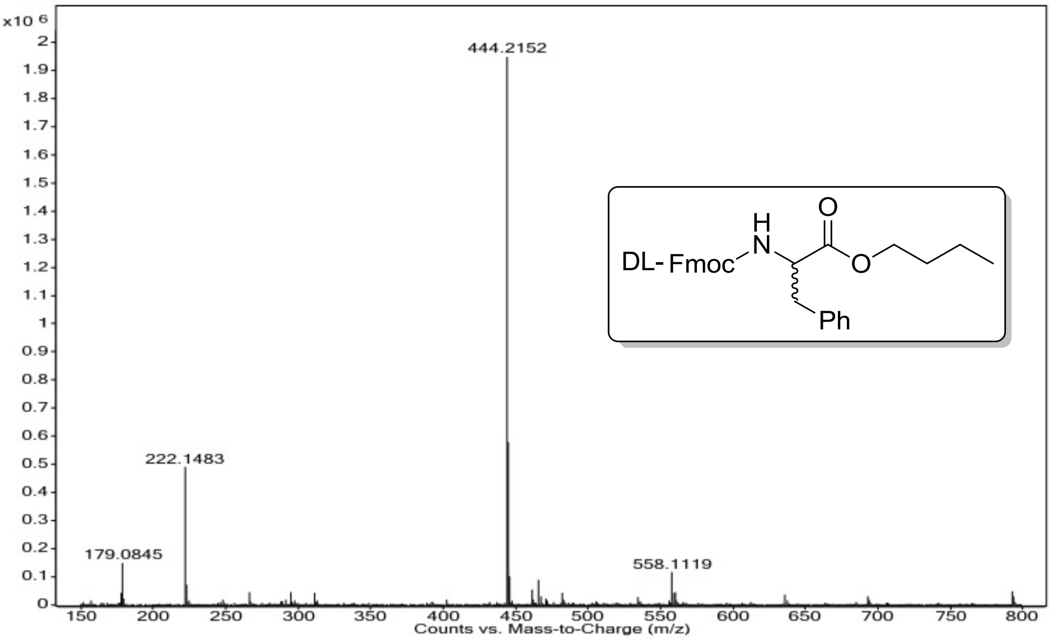


Figure S15. Mass spectra of compound **3j**

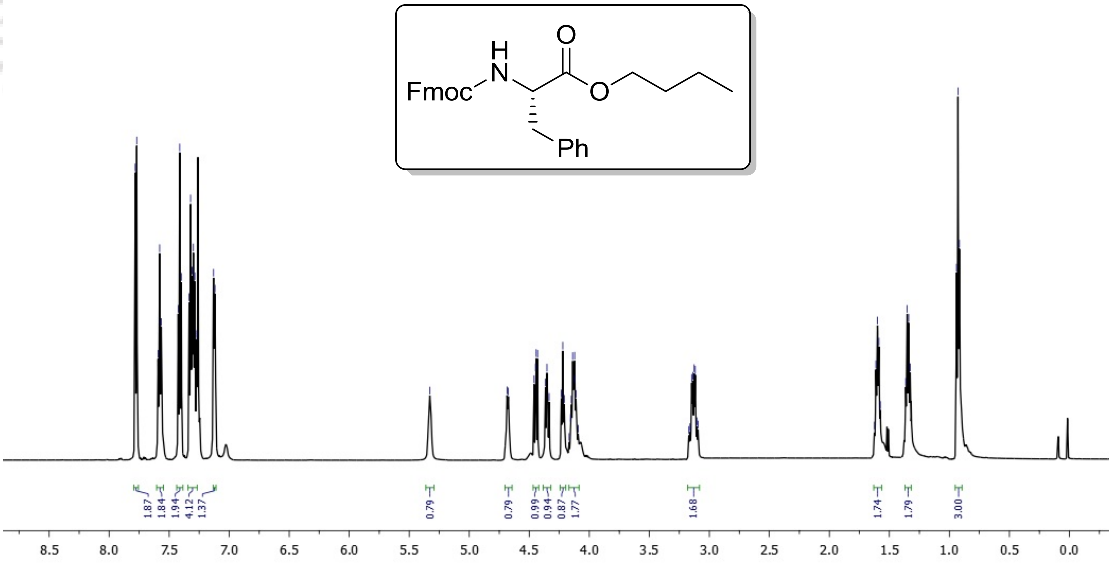


Figure S16. ^{1}H NMR spectra of compound **3k**

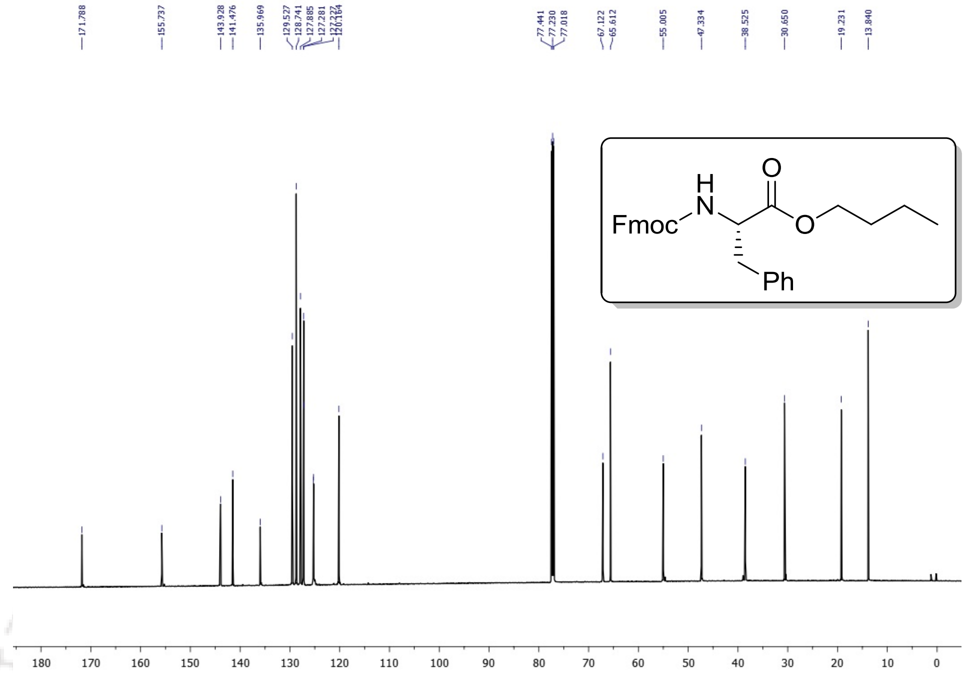


Figure S17. ^{13}C NMR spectra of compound **3k**

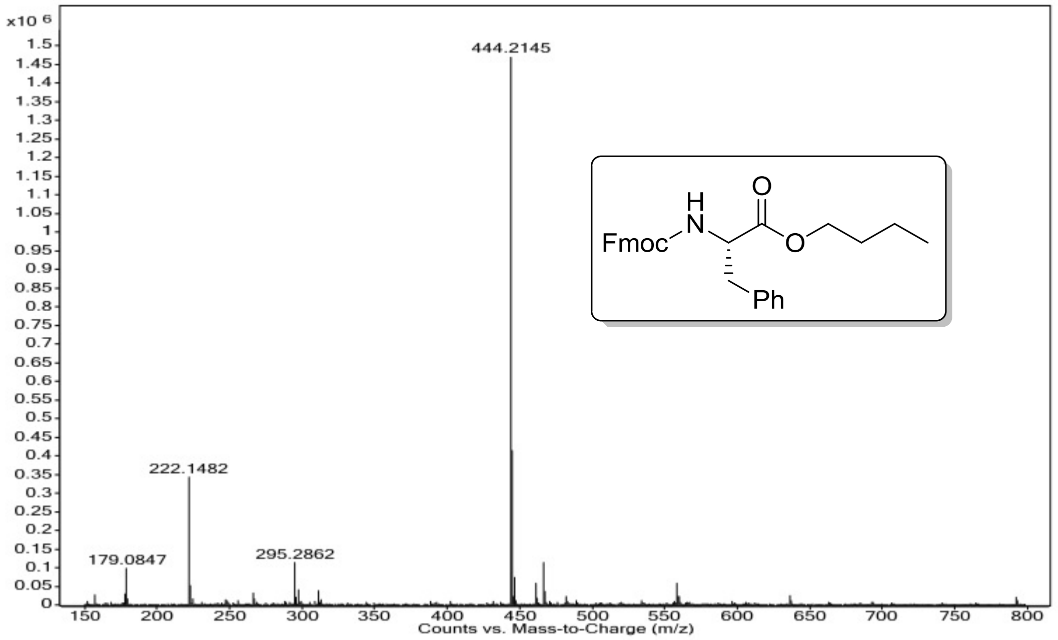


Figure S18. Mass spectra of compound **3k**

3.9.2. HPLC Data for racemization test

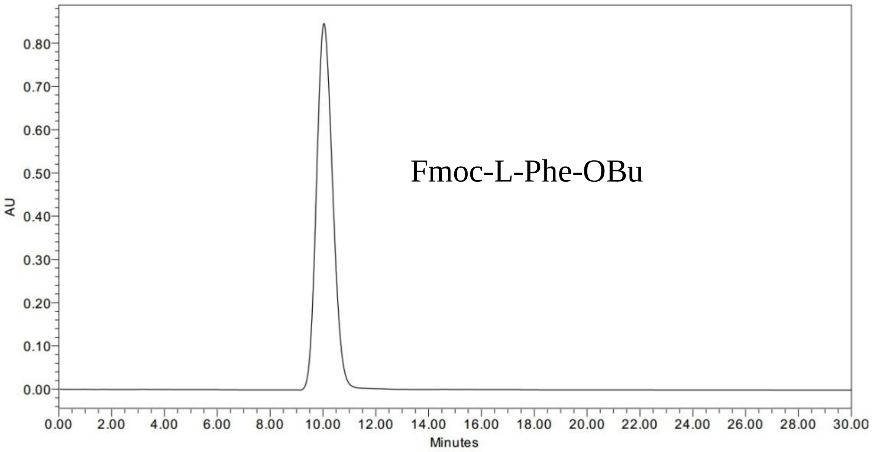


Figure S19. HPLC chromatogram of Fmoc-L-Phe-OBu ester run up to 30 min in CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Scheme 3.2.1, entry 3k).

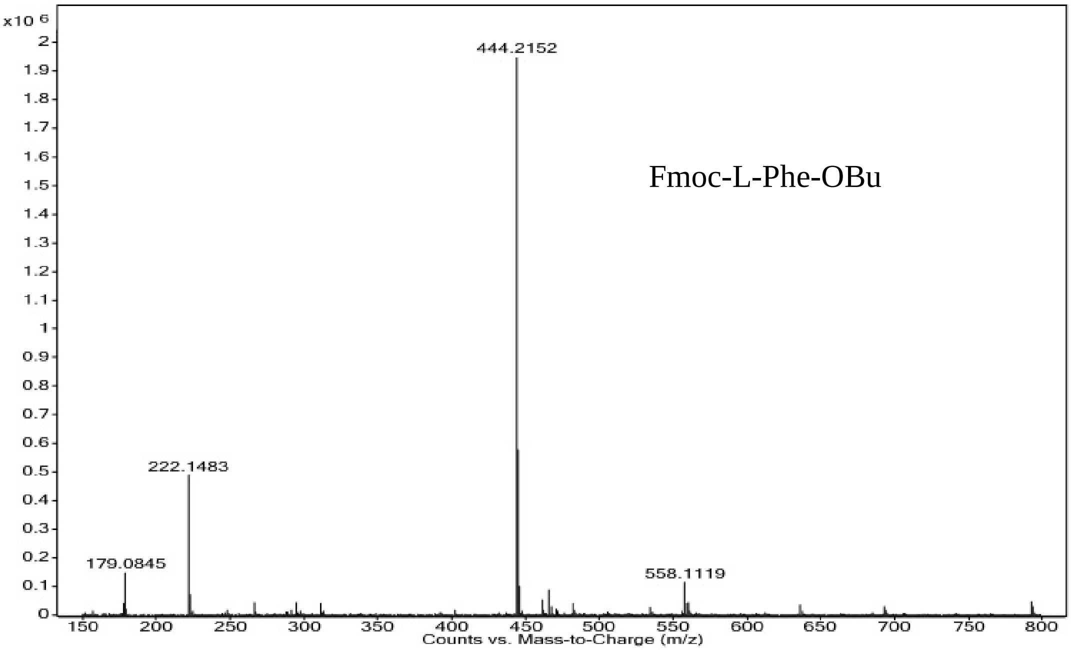


Figure S20. ESI-MS spectrum of Fmoc-L-Phe-OBu ester (Scheme 3.2.1, entry 3k).

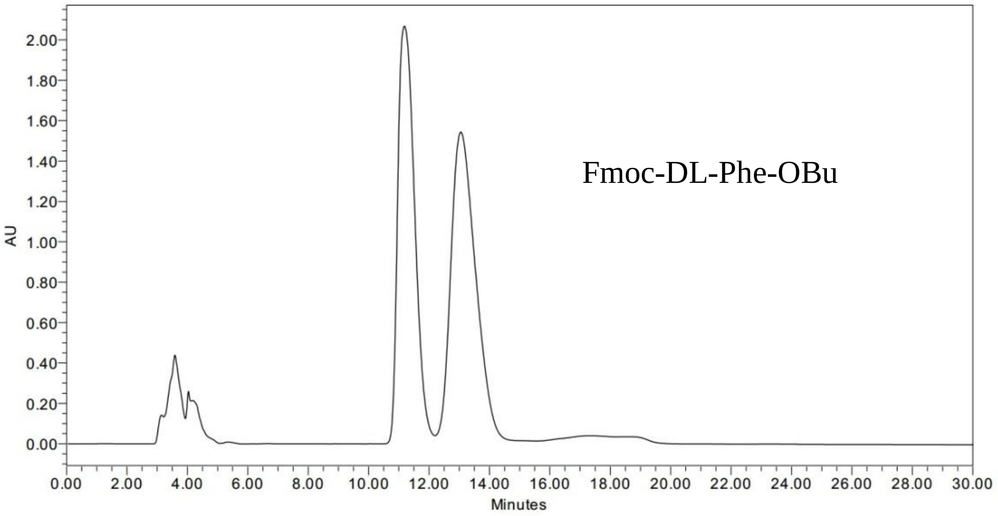


Figure S21. HPLC chromatogram of Fmoc-DL-Phe-OBu ester run up to 30 min in CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Scheme 3.2.1, entry 3j).

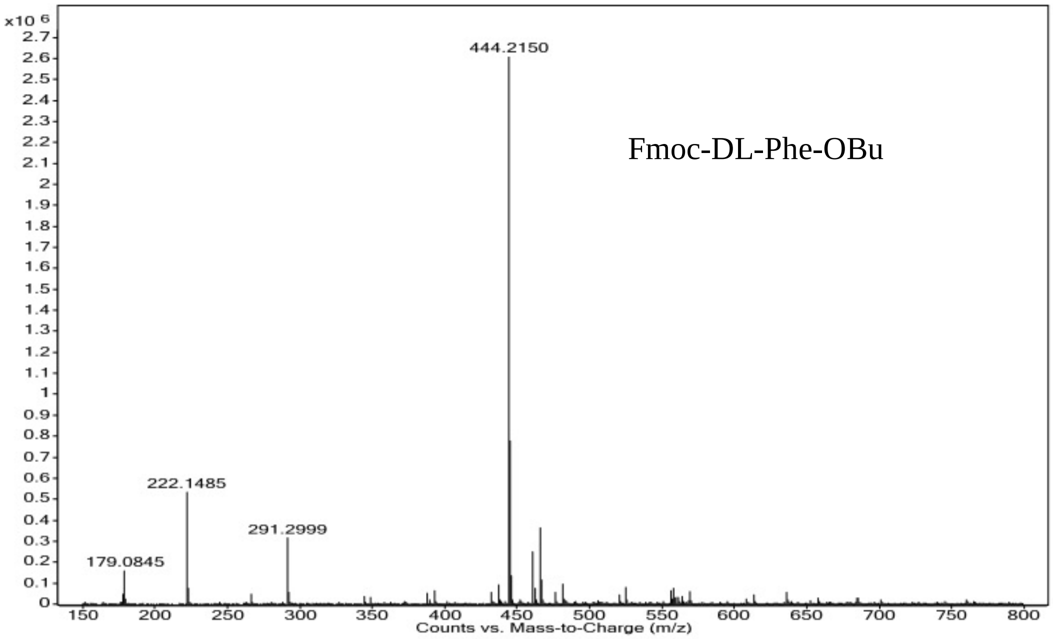


Figure S22. ESI-MS spectrum of Fmoc-DL-Phe-OBu ester (Scheme 3.2.1, entry 3j).

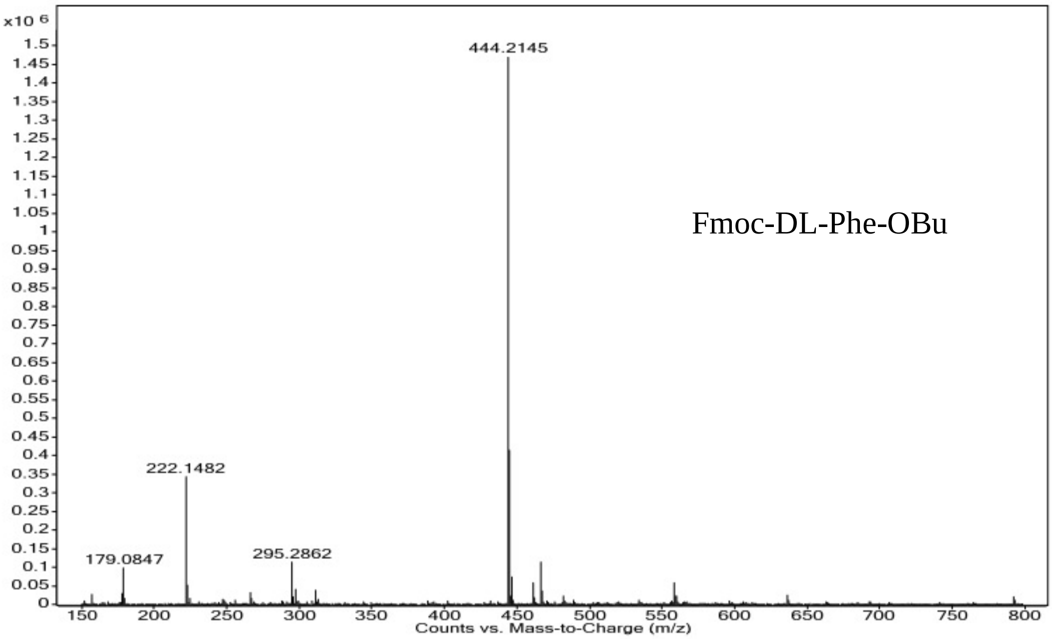
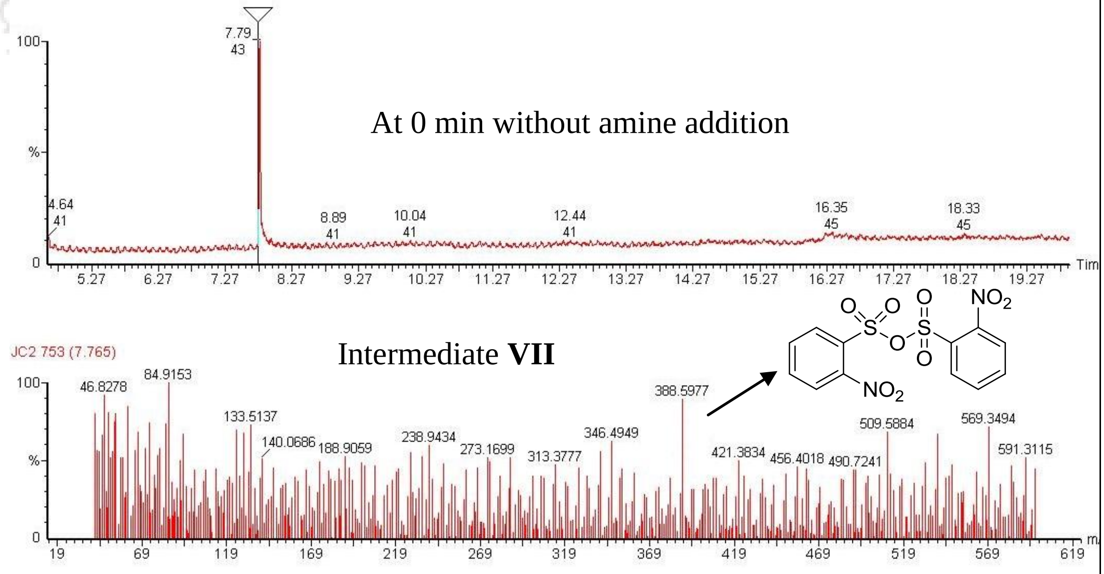


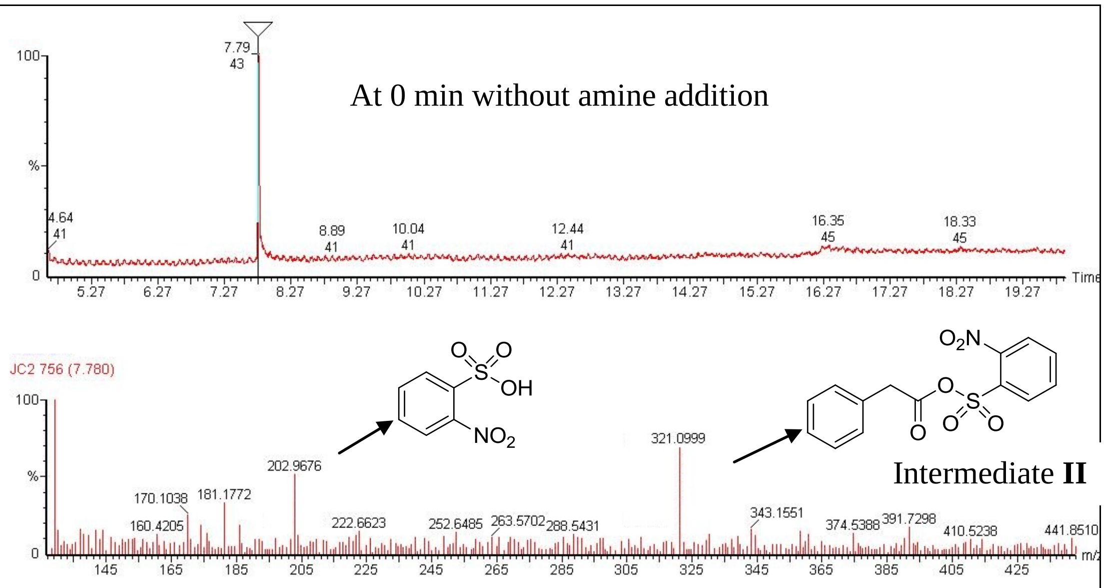
Figure S23. ESI-MS spectrum of Fmoc-DL-Phe-OBu ester (Scheme 3.2.1, entry 3j).

3.9.3. Time dependent GCMS studies for Intermediates

(1)



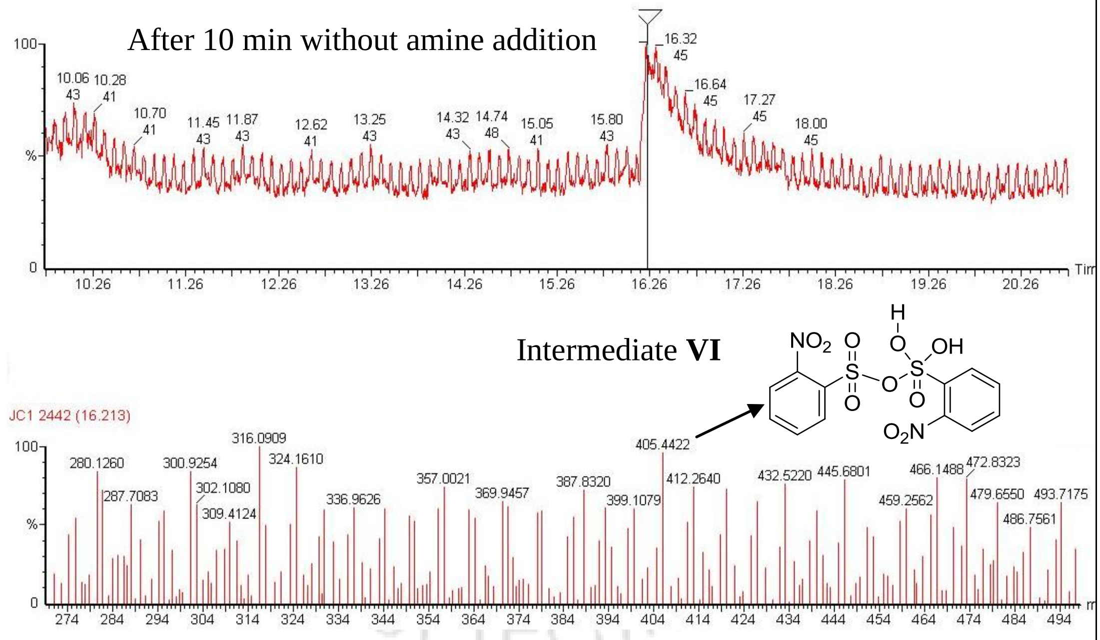
(2)



At 0 min without amine addition

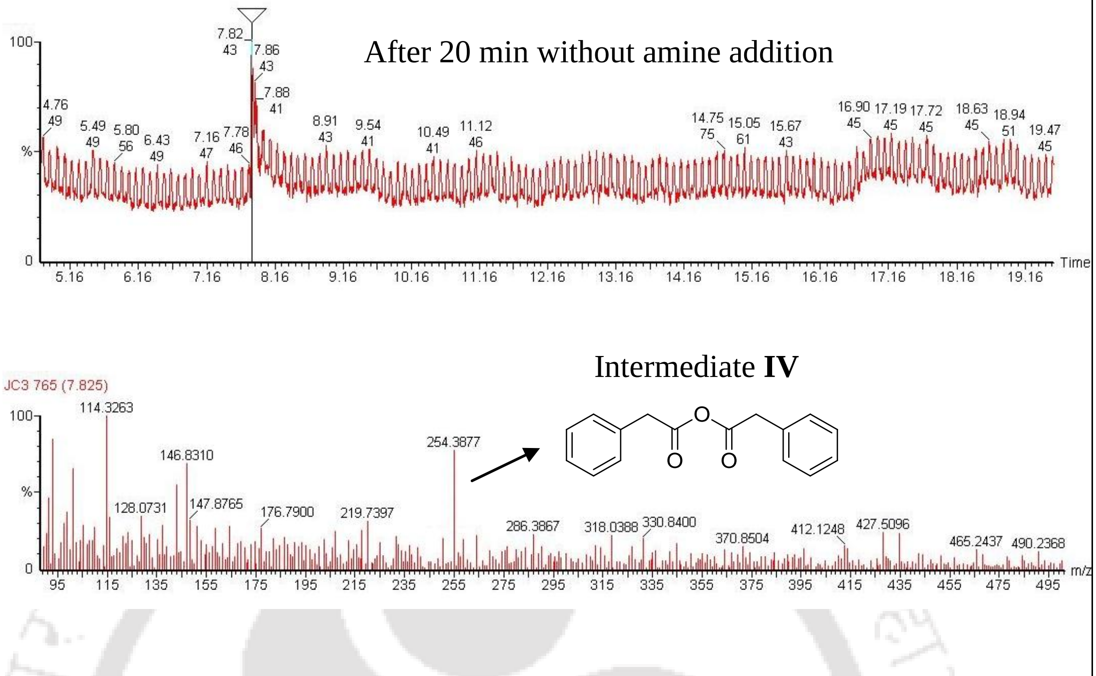
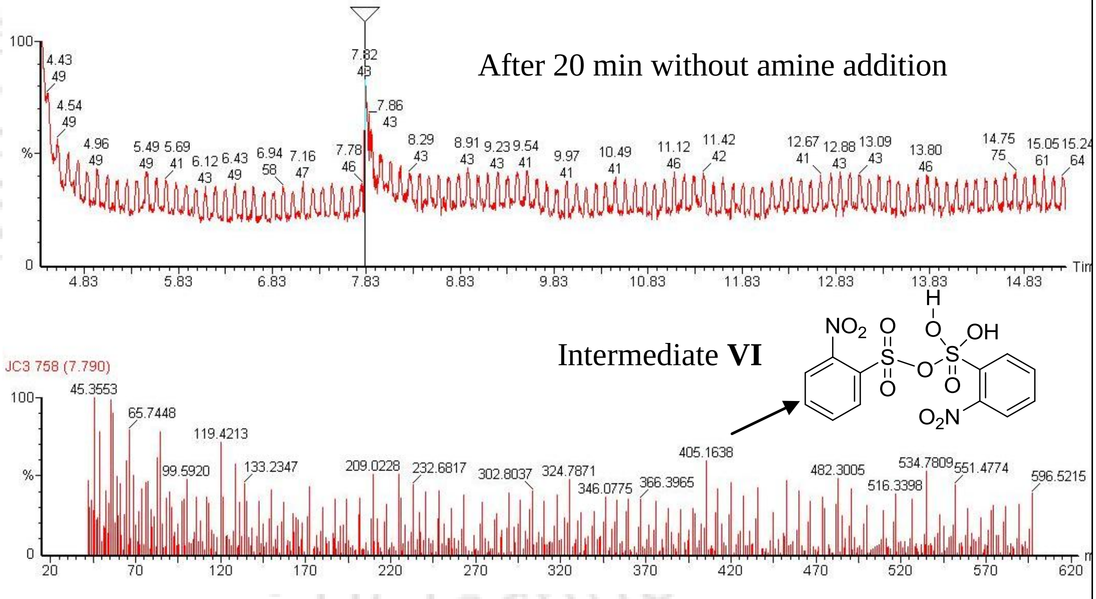
Intermediate **II**

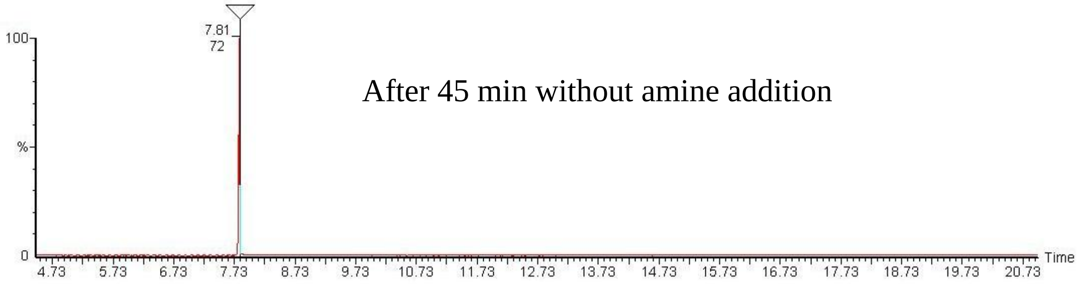
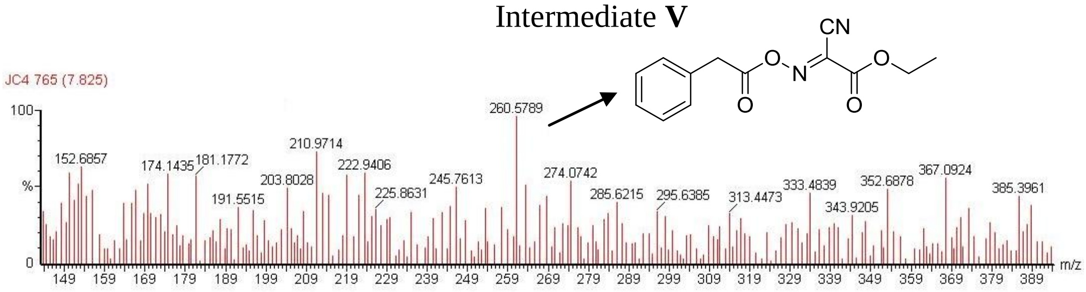
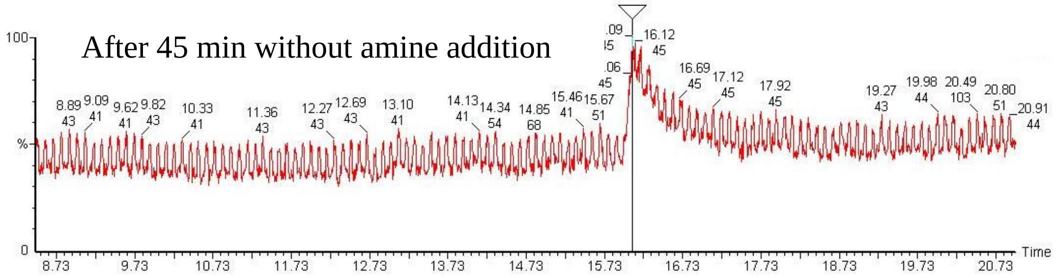
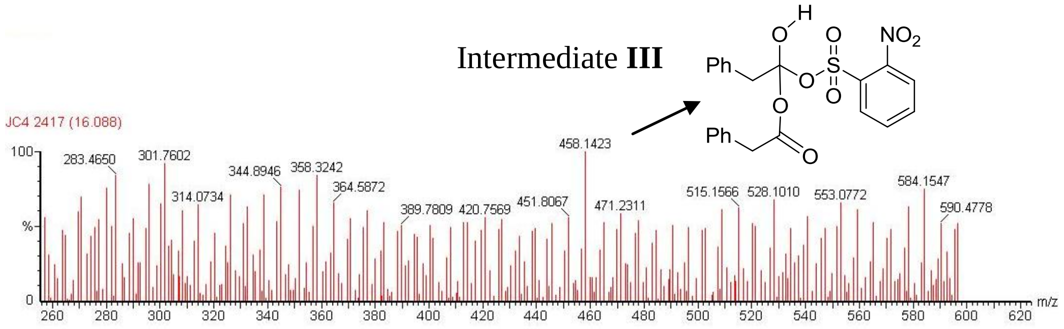
(3)

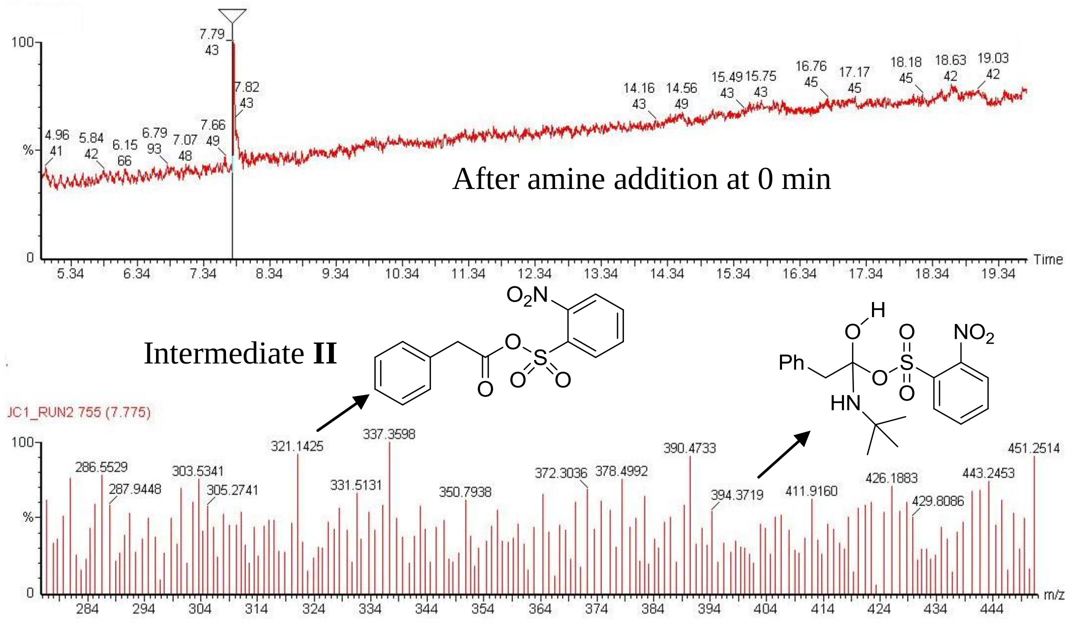
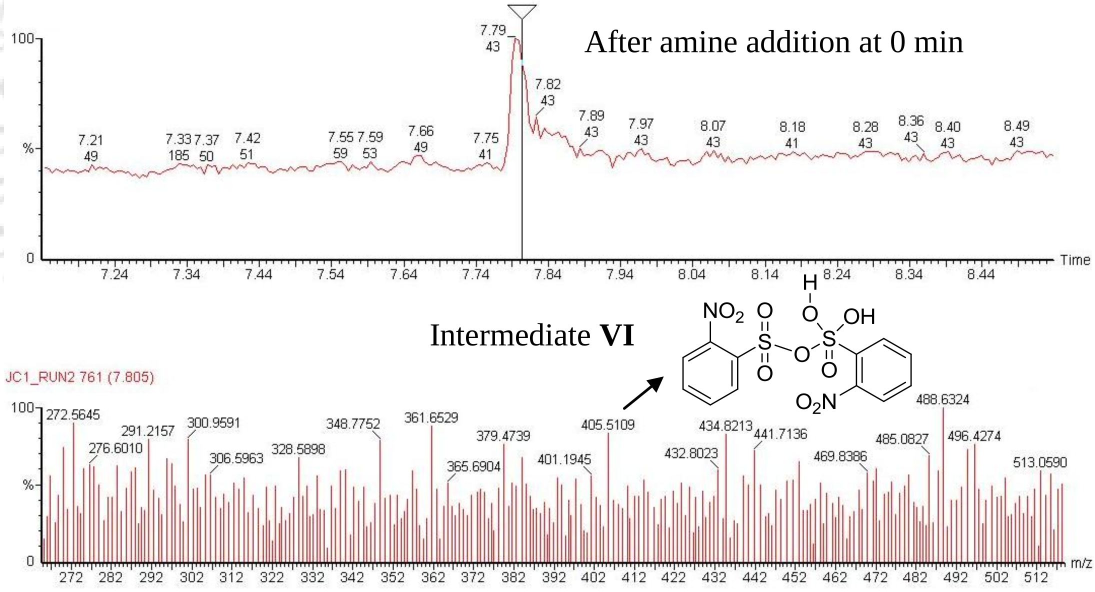


After 10 min without amine addition

Intermediate **VI**

(4)	 After 20 min without amine addition Intermediate **IV**
(5)	 After 20 min without amine addition Intermediate **VI**

(6)	 After 45 min without amine addition Intermediate **V** 
(7)	After 45 min without amine addition  Intermediate **III** 

(8)	 After amine addition at 0 min Intermediate **II**
(9)	 After amine addition at 0 min Intermediate **VI**

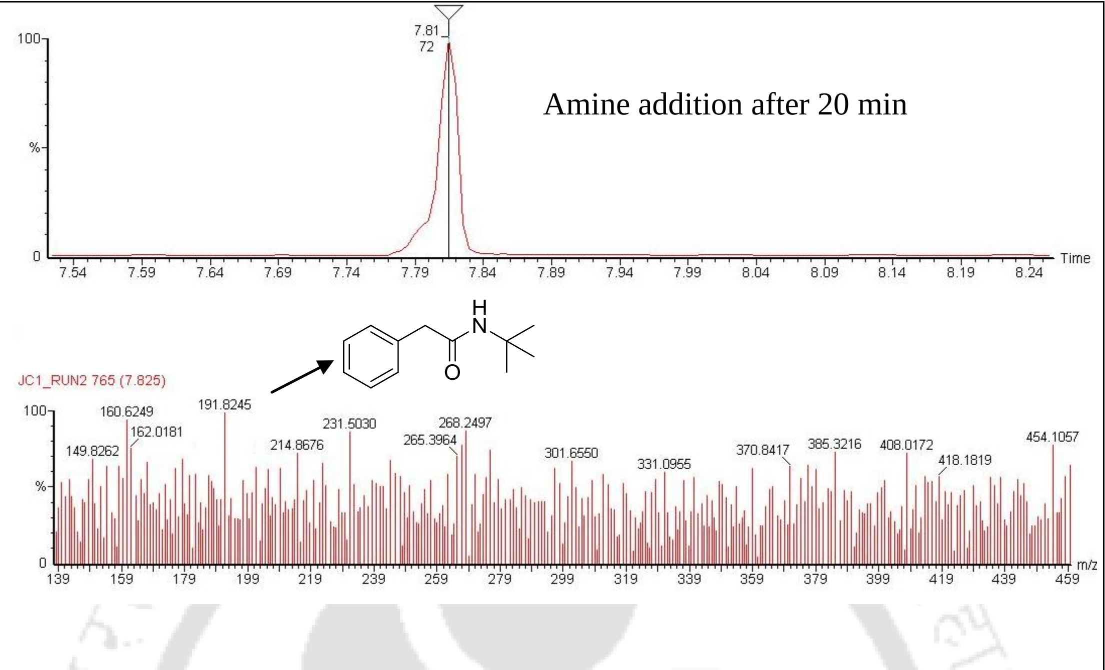
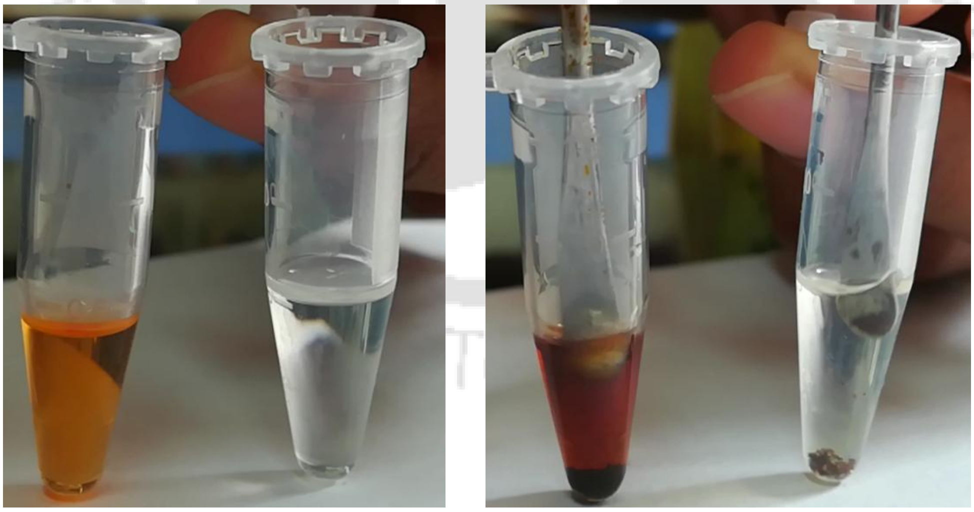
(10)	

Figure S24. GCMS data of intermediates

3.9.4. $FeCl_3$ test for the evolution of water as the by-product



Before adding $FeCl_3$ After adding $FeCl_3$

Figure S25. $FeCl_3$ test for evolution of water

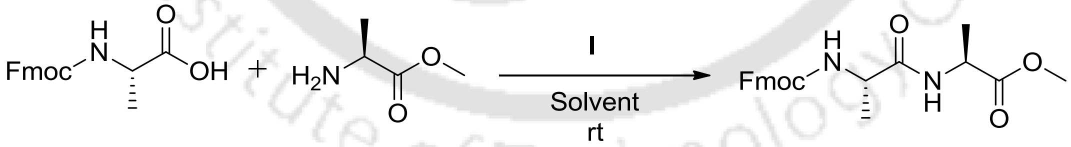
Chapter 4: Racemization Free Synthesis of Peptides and Polypeptides using Sub-Stoichiometric Amount of *ortho*-NosylOXY

In the previous chapter, we described the catalytic approach of *o*-NosylOXY for the racemization free synthesis of amides and esters. In this chapter, we extended the catalytic approach of *o*-NosylOXY for the racemization free synthesis of peptides and polypeptides. Peptides are an important class of biological molecules that are made up of amino acids and a long chain of the peptide is known as polypeptides that carry biological function in the body (Chapter 1, section 1.3.4). There are several methods in literature for the synthesis of peptides as well as polypeptides (Chapter 1, section 1.4.2.1 and 1.4.2.2), but those methods suffer from some drawbacks (Chapter 1, section 1.5). Most importantly, usually fivefold excess or tenfold excess of the coupling reagent is used in solid phase peptide synthesis. Therefore, we developed an eco-friendly method for the synthesis of peptides and polypeptides using the sub-stoichiometric amount of *o*-NosylOXY.

4.1. Reaction optimization and substrate scope for peptides and polypeptides synthesis using sub-stoichiometric amount of *o*-NosylOXY

For optimization, we took syntheisis of Fmoc-Ala-Ala-OMe dipeptide as a model reaction. We found that with decreased amount of **I** below 1 equiv also showed good coupling efficiency. With 0.2 equiv, the reaction worked well and generated 80% yield (Table 4.1.1, entry **5**). But as we decreased the amount of **I** less than 0.2 equiv, we observed only 53% & 30% with 0.1 & 0.05 equiv (Entries **6** and **7**), respectively. Therefore, we inferred that 0.2 equiv of *o*-NosylOXY is sufficient for the coupling reaction. Next, we screened several solvents such as EtOAc, DCM, CH_3CN, THF etc. using 0.2 equiv of **I.** We found that DCM afforded higher yield (Table 4.1.1). But there was no reaction in MeOH, EtOH, and water (Entries **8**, **12** and **13**). In this case, we observed only starting materials. Therefore, DCM and 0.2 equiv of **I** were kept as standard conditions for peptide synthesis.

Table 4.1.1. Optimization of reaction conditions.[a]



Entry	Solvents	I (equiv)	Yield[b] (%)
1	DCM	1.0	92
2	DCM	0.5	87
3	DCM	0.4	84
4	DCM	0.3	82

5	**DCM**	**0.2**	**80**
6	DCM	0.1	53
7	DCM	0.05	30
8	MeOH	0.2	0
9	THF	0.2	69
10	$CHCl_3$	0.2	75
11	CH_3CN	0.2	76
12	EtOH	0.2	0
13	H_2O	0.2	0
14	DMF	0.2	78

[a] *Reaction conditions: Fmoc-Ala-OH (1 mmol), o-NosylOXY (varied amount), DIPEA (1.5 mmol),* H_2N*-Ala-OMe (1.5 mmol) at room temperature and the reaction time is 30 min.* [b] *Isolated yield.*

Further, we performed the same model reaction with other coupling reagents such as DCC, EDC, HBTU, BenzylOXY, and TosylOXY with 0.2 equiv. We observed only up to 20% yield (Table 4.1.2). Hence, we concluded that only *o*-NosylOXY worked with catalytic amount and the rest did not show any catalytic behavior.

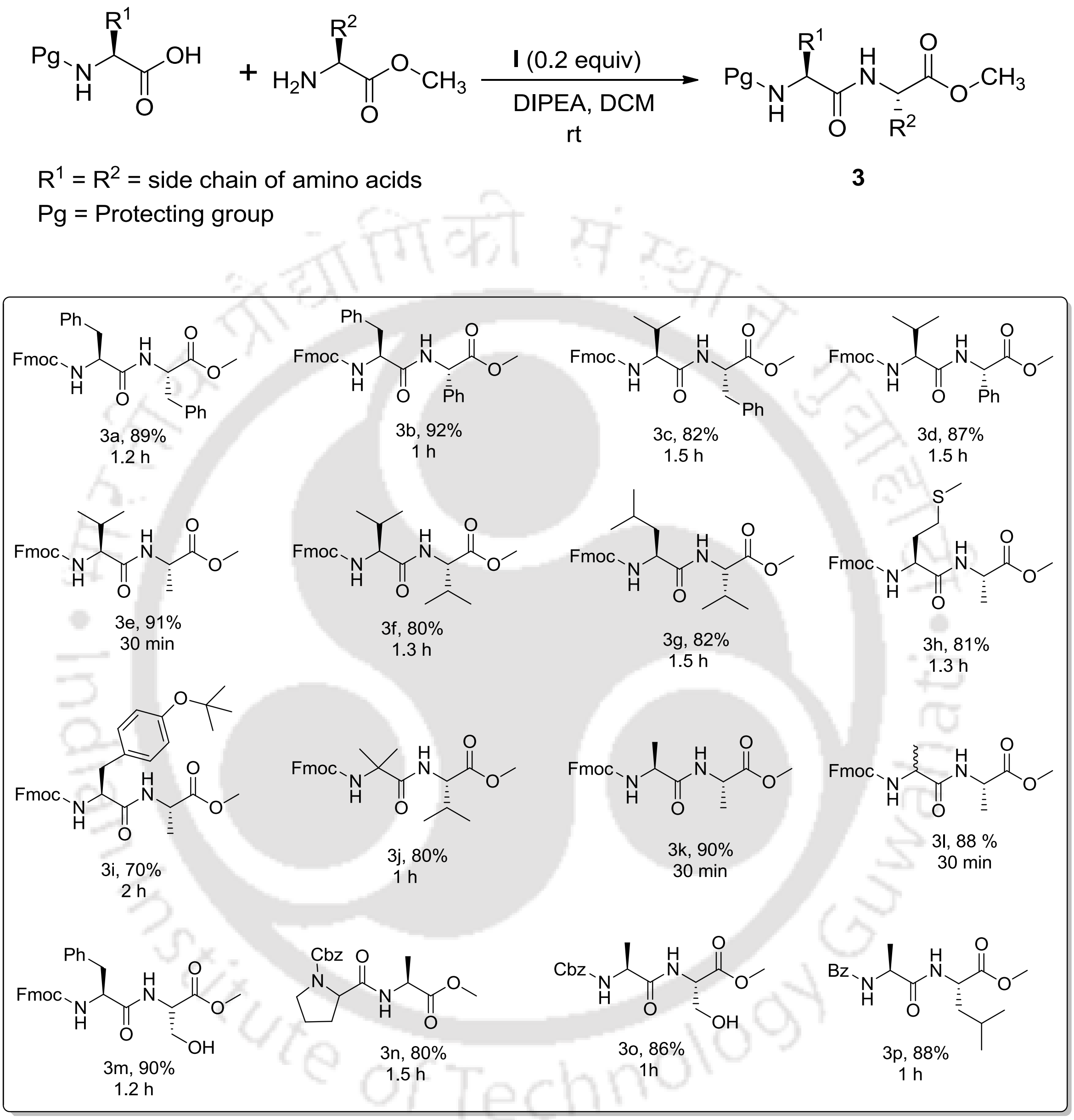
Table 4.1.2. Study of the catalytic behavior of various coupling reagents.[a]

Entry	Coupling reagents	Time (h)	Yield[b] (%)
1	DCC	24	12
2	EDC	36	14
3	HBTU	0.5	20
4	BenzylOXY	36	2
5	TosylOXY	36	5
6	***o*-NosylOXY**	0.5	**80**

[a]Reaction conditions: Fmoc-Ala-OH (1 mmol), Coupling reagents (0.2 mmol), DIPEA (1.5 mmol), H_2N-Ala-OMe (1.5 mmol) stirred at room temperature for 30 min. [b]Isolated yield

With that optimized reaction conditions in hand, we explored the applicability of this method for peptide synthesis from various *N*-protected amino acid with *C*-protected amino acid. The reaction worked well with the standard *N*-protecting groups of amino acids such as Fmoc (Scheme 4.1.1, entries **3a-3m**), Cbz (Entries **3n-3o**) and Bz (Entry **3p**) as well as with bulky side chain of amino acids with good yield.

Scheme 4.1.1. Wide scope for the synthesis of the peptide using **I**.[a,b]



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[a]*Reaction conditions: C-protected amino acid (1 mmol), Reagent* ***I*** *(0.2 mmol), DIPEA (1.5 mmol), N-protected amino acid (1.5 mmol) stirred at room temperature for 30-120 min.* [b]*Isolated yield.*

4.2. Racemization suppression efficiency of I for peptide synthesis

For racemization study, we synthesized Fmoc-DL-Ala-L-Ala-OMe and Fmoc-L-Ala-L-Ala-OMe dipeptides using the catalytic amount of **I** and compared their HPLC profiles. The appearance of the twin peak in the HPLC profile of Fmoc-DL-Ala-L-Ala-OMe corresponds to the two diastereomeric products (Figure 4.2.1, right panel) whereas the presence of a single peak of Fmoc-L-Ala-L-Ala-OMe indicates single stereoisomeric product (Figure 4.2.1, left panel). The ^{1}H and ^{13}C NMR spectra of these dipeptides were also compared (Figure 4.2.1 and Figure S4-S9). We found one singlet at δ = 3.75 ppm for the methoxy proton of **3k** and two singlets at δ = 3.75 and 3.73 ppm for the methoxy proton of **3l** in the ^{1}H NMR spectra. Similarly, in ^{13}C NMR spectra, we found two peaks at δ = 173.3, 172.0 ppm corresponding to the two carbonyls of the amide and the ester groups of **3k** indicating the presence of the single diastereomer and four peaks at δ = 173.4, 173.3, 173.0 and 172.9 ppm in ^{13}C NMR of **3l**, which indicate the presence of two diastereomers (Figure 4.2.1). We could not find any detectable racemization during the coupling reaction.

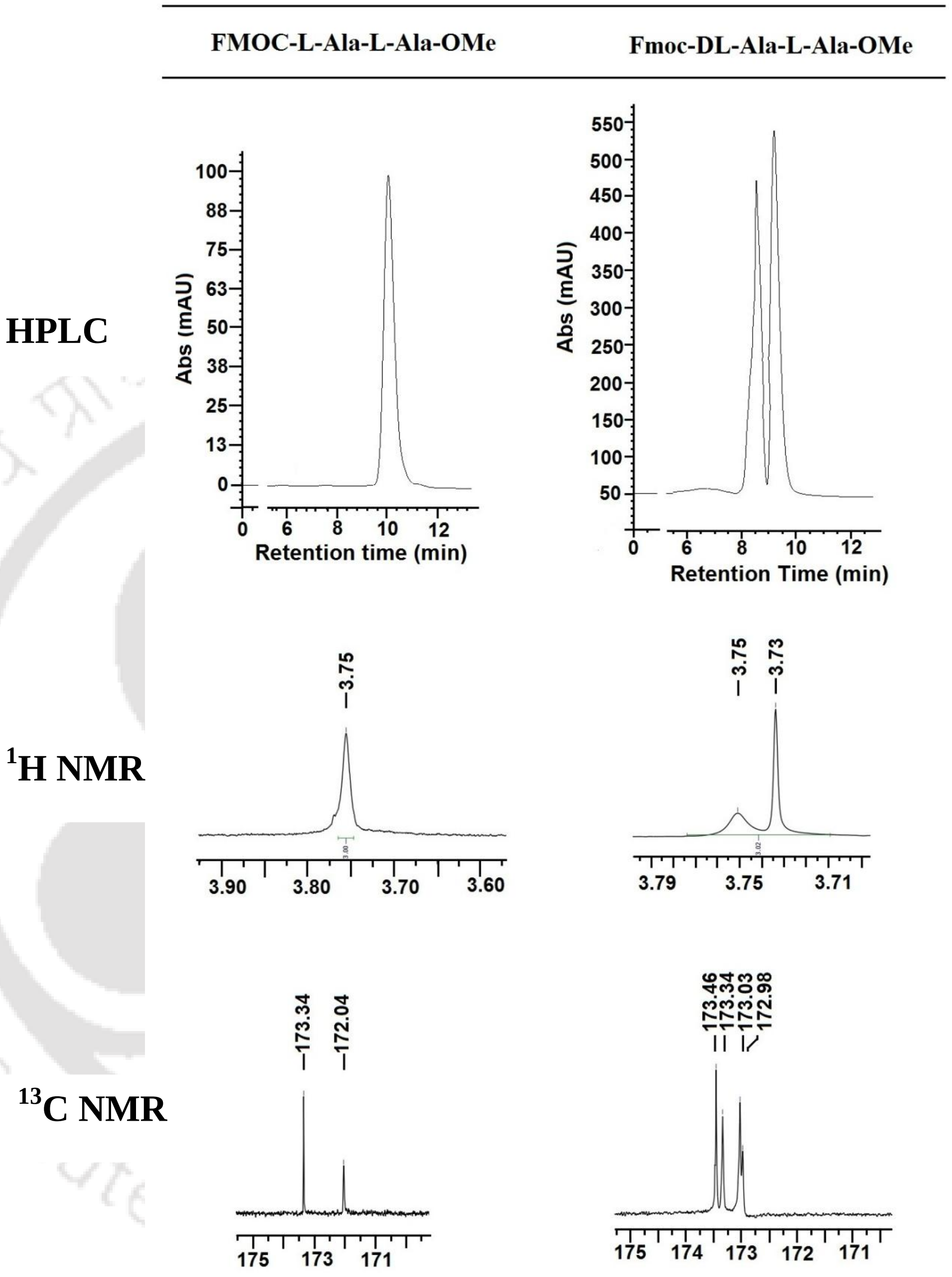


Figure 4.2.1. *Examination of racemization by comparison of the HPLC profiles, ^{1}H NMR and ^{13}C NMR of Fmoc-L-Ala-L-Ala-OMe (left panel) and Fmoc-DL-Ala-L-Ala-OMe (right panel)*

4.3. Synthesis of polypeptides using sub-stiochiometric amount of *o*-NosylOXY

After successful synthesis of peptides, we proceeded for the synthesis of polypeptides both in solution and on solid phase. First, we synthesized Boc-Leu-Val-Phe-Phe-OMe (Figure 4.3.1**a**) with 0.2 equiv of **I**, this is the fragment of the amyloid β peptide which is responsible for Alzheimer's disease. This fragment is highly hydrophobic in nature with huge steric hindrance and it is very difficult to synthesize. A Boc chemistry based stepwise coupling was performed in DCM following solution phase methodology with 70% yield. HPLC chromatogram of crude peptide was as good as purified peptide (Figure S20-S21). Further, we synthesized IAPP (22-27) peptide: Asn-Phe-Gly-Ala-Ile-Leu-NH_2 (Figure 4.3.2**b**), which is known to be the core sequence responsible for the initiation of aggregation of the amylin peptide that leads to type 2 diabetes. A Fmoc chemistry based stepwise coupling of amino acid was performed in DMF on rink amide MBHA resin following SPPS (solid phase peptide synthesis) protocol. In each step, 1.5 equiv of Fmoc amino acids, 0.2 equiv of **I** and 4 equiv of DIPEA were used and gently rotated. When we performed the reaction between Fmoc-Gly-OH and *C*-protected amino acid by using 0.2 equiv of **I**, the reaction did not work. But with 1 equiv of the coupling reagent, the reaction worked very well. We achieved this peptide with 40% yield corresponding to resin loading (Figure S22-23).

Next, we synthesized two more long chain peptides using SPPS methodology.

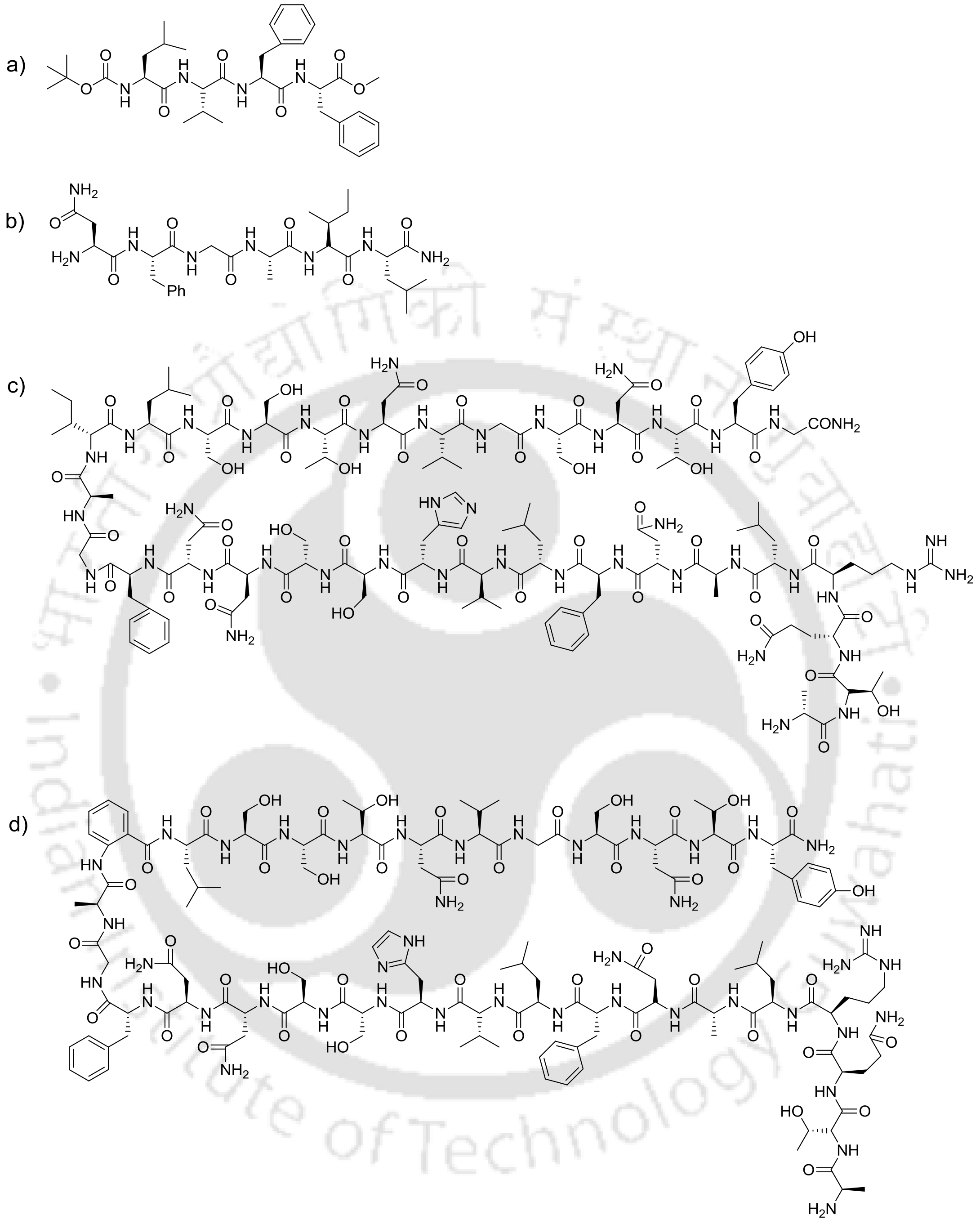


Figure 4.3.1. *Sequences of the peptide synthesized:* ***a****, Boc-LVFF-OMe in solution* ***b****, NFGAIL* ***c****, ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-*NH_2 ***d****, ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTY-*NH_2 *using SPPS strategy.*

We synthesized IAPP (8-37) peptide (IAPP, islet amyloid polypeptide: ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-NH_2 (Figure 4.3.3**c**) and IAPP

(8-37) peptide with one β-breaker element: ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTY-NH_2 (Figure 4.3.4**d**) by stepwise coupling of constituent amino acids on Rink amide resin following the Fmoc/tBu orthogonal protection strategy. 1.5 equiv of Fmoc amino acids, 0.8 equiv of **I** and 7 equiv of DIPEA were used for each coupling steps. For Fmoc-Gly-OH, 1 equiv of **I** was required for complete coupling. We achieved the synthesis of these two peptides in good yield (25%, 13 mg for IAPP (8-37) and 15%, 8 mg for IAPP (8-37) fragment with one β-breaker element) after chromatographic purification with respect to resin loading (Figure S24-S27).

4.4. Plausible mechanism

As described in chapter 3 section 3.4

4.5. Conclusion

Here, we have synthesized peptides and polypeptides using sub-stoichiometric amount of *o*-NosylOXY both in solution as well as on solid support. This method is compatible with common *N*-protecting groups, such as Fmoc, Cbz, and Bz in solution phase. Further, we demonstrated the utility of this protocol for synthesizing biologically important polypeptide i.e. fragment of Amylin peptide (known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes) and fragment of the amyloid *β* peptide (which is responsible for Alzheimer's disease). Thus, the use of the sub-stoichiometric amount of *o*-NosylOXY reduces the chemical waste generation that leads to environment-friendly process.

4.6. Experimental Section

4.6.1. Materials and methods

As described in chapter 2 section 2.4.1

4.6.2. General procedure for the synthesis of dipeptide:

o-NosylOXY (0.2 equiv) was added to a solution of *N*-protected amino acid (1 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 5 min for preactivation followed by the addition of methyl ester of amino acid (1.5 equiv) and DIPEA (2 equiv) in 1 ml of DCM. The reaction mixture was stirred at room temperature for more 30-120 min. After completion of the reaction, the reaction mixture was diluted with 50 ml of ethyl acetate, the organic phase was washed with 5% citric acid (3×20 ml), 5% $NaHCO_3$ (3×20 ml), brine and dried over anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered and the solvent was evaporated. The product was purified by silica gel column chromatography.

4.6.3. Solution Phase Synthesis of Boc-LVFF-OMe:

o-NosylOXY (0.2 equiv) was added to a solution of Boc-phenylalanine (1 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 5 min for preactivation. In another RB, methyl ester of phenylalanine (1.5 equiv) was taken in DCM and DIPEA was added to it till basic pH was reached. Finally, this solution was added to the above solution and stirring continued until completion of the reaction. Then, the reaction mixture was diluted by 50 ml of EtOAc, washed by 5% $NaHCO_3$ solution (2 × 5 ml) and washed by 5% citric acid solution (2 × 5 ml). Finally, the combined organic layer was dried using anhydrous Na_2SO_4. The solid product (Boc-FF-OMe) was obtained after evaporation of EtOAc by rotary vacuum evaporator.

In 50 ml RB, solid product (Boc-FF-OMe) was taken and TFA/DCM (1:1) mixture was added and stirring continued up to 2.5 h. After that, TFA was evaporated by rotary vacuum evaporator, the solution was washed 3-4 times with diethyl ether and finally a white solid (H_2N-FF-OMe) was obtained. After Boc deprotection, the resulting FF-OMe was coupled with Boc-V-OH following the above-mentioned procedure to obtain Boc-VFF-OMe. Another cycle of Boc-deprotection and coupling with Boc-L-OH, resulted in white solid Boc-LVFF-OMe, which was characterized by reversed phase HPLC; retention time 11 min on a linear gradient of 0 to 70%, 0-18 min then 70 to 90%, 18-20 min and then 90 to 100%, 20-30 min, CH_3CN in H_2O with 0.1% formic acid, symmetry C8 analytical column. HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{35}H_{51}N_4O_7$ 639.3758, found 639.3766. The yield was 70% with respect to starting material Boc-phenylalanine.

4.6.4. Solid Phase Synthesis of NFGAIL-NH_2:

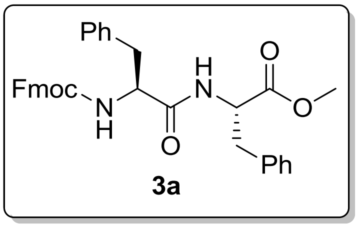
The hexapeptide was manually assembled stepwise on Fmoc Rink Amide MBHA resin using Fmoc/tBu orthogonal protection strategy. Fmoc amino acids (1.5 equiv), *o*-NosylOXY (0.2 equiv) and DIPEA (3 equiv) were kept for preactivation for 5 min. Then, amino acid coupling was performed for 2-4 h. Fmoc deprotection was carried out with the use of Piperidine/DMF (1:1) mixture for 2.5 h. The final peptide was cleaved from the resin by TFA/DCM mixture. Purification of the peptide was carried out by preparative HPLC and a linear gradient of 0 to 5%, 0-18 min then 5 to 100%, 18-20 min, CH_3CN in H_2O with 0.1% formic acid, symmetry C8 analytical column and afforded the final peptide.

4.6.5. Solid Phase Synthesis of IAPP (8-37) peptide and IAPP (8-37) peptide with one β-breaker element:

The two long Amylin peptides were synthesized. Amino acids were manually assembled stepwise on Fmoc Rink Amide MBHA resin using Fmoc/tBu protection strategy. Fmoc amino acids (1.5 equiv), *o*-NosylOXY (0.8 equiv) and DIPEA (7 equiv) were kept for preactivation for 5 min. Then, amino acid coupling was performed for 2-4 h. Fmoc deprotection was carried out with the use of Piperidine/DMF (1:1) mixture for 2.5 h. In each step, we used 10% anisole solution with DMF to reduce the hydrophobicity of peptide. The final peptide was cleaved from the resin by TFA/DCM mixture. Purification of the peptide was carried out by preparative HPLC. A linear gradient of 0 to 5%, 0-18 min then 5 to 100%, 18-20 min, CH_3CN in H_2O with 0.1% formic acid, and a symmetry C8 analytical column was used. After lyophilization the final peptide was obtained.

4.7. Characterization data

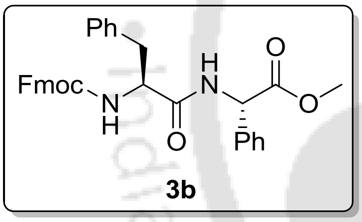
(*S*)-Methyl-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate, 3a



Solid; (488 mg, 89%); mp 171-173 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.54-7.51 (t, *J* = 7.8

Hz, 2H), 7.42-7.39 (t, *J* = 7.8 Hz, 2H), 7.32-7.24 (m, 6H), 7.19-7.18 (d, *J* = 7.2 Hz, 2H), 6.25 (br, 1H), 5.31 (br, 1H), 4.79-4.76 (m, 1H), 4.43-4.41 (m, 2H), 4.29 (br, 1H), 4.19-4.17 (t, *J* = 6.6 Hz, 1H), 3.67 (s, 3H), 3.09-3.00 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 170.5, 155.8, 143.9, 141.5, 135.7, 129.6, 129.4, 128.9, 128.7, 128.0, 127.3, 127.2, 125.2, 120.2, 67.3, 56.1, 53.5, 52.5, 47.3, 38.5, 38.1; FT-IR (KBr, cm^{-1}): 2924, 1738, 1697, 1644, 1535, 1444, 1257, 1033, 738, 698; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{34}H_{33}N_2O_5$ 549.2389, found 549.2287.

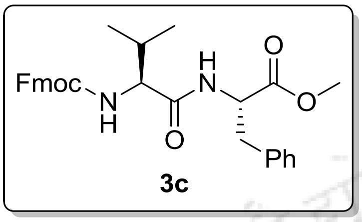
(*S*)-Methyl- 2-((S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-2-phenylacetate, 3b



3b

Solid; (491 mg, 92%); mp 176-178 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.54-7.51(t, *J* = 7.2 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.30-7.25 (m, 10H), 7.18-7.17 (m, 2H), 6.78 (br, 1H), 5.46-5.45 (d, *J* = 6.6 Hz, 1H), 5.37 (br, 1H), 4.52 (br, 1H), 4.42-4.37 (m, 1H), 4.32-4.31 (d, *J* = 6.6 Hz, 1H), 4.18-4.16 (t, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 3.18-3.01 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 170.4, 156.1, 144.0, 141.5, 136.1, 129.1, 128.9, 128.8, 127.9, 127.4, 127.3, 125.2, 120.2, 67.3, 56.8, 56.1, 53.0, 47.3, 38.8; FT-IR (KBr, cm^{-1}): 3313, 2948, 1739, 1690, 1655, 1534, 1451, 1283, 1253, 730; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{33}H_{31}N_2O_5$ 535.2233, found 535.2144.

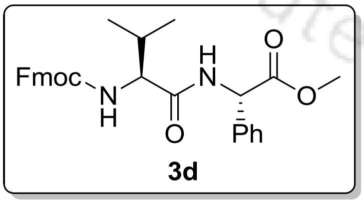
(*S*)-Methyl- 2-((S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-phenylpropanoate, 3c



3c

Solid; (410 mg, 82%); mp 169-172 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.78-7.76 (d, *J* = 7.8 Hz, 2H), 7.61-7.60 (d, *J* = 6.6 Hz, 2H), 7.41-7.39 (m, 2H), 7.33-7.30 (t, *J* = 7.2 Hz, 2H), 7.26-7.23 (m, 3H), 7.09-7.07 (d, *J* = 7.2 Hz, 2H), 6.34 (br, 1H), 5.43 (br, 1H), 4.90-4.86 (m, 1H), 4.45-4.42 (m, 1H), 4.35-4.32 (t, *J* = 7.2 Hz, 1H), 4.23-4.21 (t, *J* = 7.2 Hz, 1H), 3.99-3.97 (t, *J* = 6.6 Hz, 1H), 3.72 (s, 3H), 3.14-3.09 (m, 2H), 2.08-2.05 (m, 1H), 0.93-0.89 (dd, *J* = 7.2 Hz, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.8, 171.3, 144.0, 141.5, 135.7, 129.4, 128.8, 127.9, 127.3, 127.2, 125.3, 125.2, 120.2, 120.1, 67.3, 60.4, 53.3, 52.5, 47.3, 38.1, 31.4, 19.2; FT-IR (KBr, cm^{-1}): 3291, 2922, 1743, 1699, 1651, 1534, 1448, 1249, 1031, 740, 697; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{30}H_{33}N_2O_5$ 501.2389, found 501.2300.

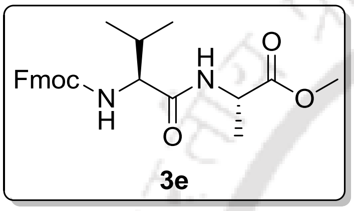
(*S*)-Methyl -2-((S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-2-phenylacetate, 3d



3d

Solid; (423 mg, 87%); mp 190-192 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.57-7.56 (t, *J* = 7.2 Hz, 2H), 7.41-7.38 (m, 2H), 7.33-7.29 (m, 7H), 6.79 (br, 1H), 5,54-5.52 (d, *J* = 7.2 Hz, 1H), 5.40 (br, 1H), 4.41-4.38 (t, *J* = 7.8 Hz, 1H), 4.35-4.32 (t, *J* = 6.6 Hz, 1H), 4.21-4.18 (t, *J* = 7.2 Hz, 1H), 4.07-4.06 (t, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 2.17-2.13 (m, 1H), 1.01-0.96 (dd, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.2, 170.9, 156.6, 144.1, 141.5, 136.0, 129.3, 128.9, 127.9, 127.5, 127.3, 125.3, 120.2, 67.3, 60.4, 56.8, 53.1, 47.3, 31.6, 18.1; FT-IR (KBr, cm^{-1}): 3064, 2957, 1738, 1691, 1653, 1536, 1450, 1389, 1289, 1174, 1034, 732, 647; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{29}H_{31}N_2O_5$ 487.2233, found 487.2135.

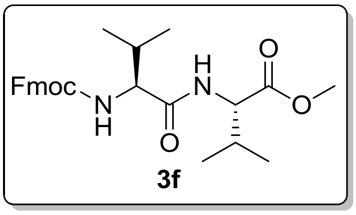
(*S*)-Methyl-2-((S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanoate, 3e



3e

Solid; (386 mg, 91%); mp 206-208 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.60-7.59 (t, *J* = 6.6 Hz, 2H), 7.41-7.38 (m, 2H), 7.33-7.30 (m, 2H), 6.39 (br, 1H), 5.45 (br, 1H), 4.61-4.56 (m, 1H), 4.44-4.41 (t, *J* = 7.2 Hz, 1H), 4.37-4.34 (t, *J* = 7.2 Hz, 1H), 4.23-4.21 (t, *J* = 7.2 Hz, 1H), 4.02-4.00 (t, *J* = 7.2 Hz, 1H), 3.75 (s, 3H), 2.13-2.10 (m, 1H), 1.42-1.41 (d, *J* = 7.2 Hz, 3H), 0.99-0.95 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.9, 171.1, 156.3, 143.6, 141.1, 127.6, 126.9, 125.0, 119.8, 116.6, 66.8, 59.8, 52.2, 48.0, 47.0, 31.3, 18.9, 17.6; FT-IR (KBr, cm^{-1}): 3065, 2954, 1745, 1693, 1650, 1536, 1451, 1295, 1116, 1029, 740; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{24}H_{29}N_2O_5$ 425.2076, found 425.2042.

(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-methylbutanoate, 3f

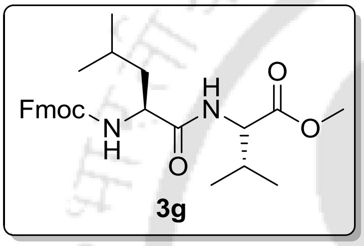


3f

Solid; (362 mg, 80%); mp 139-141 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.60-7.59 (d, *J* = 7.2 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.32-7.30 (t, *J* = 7.2 Hz, 2H), 6.34 (br, 1H), 5.47 (br, 1H), 4.55-4.53 (m, 1H), 4.44-4.38 (m, 2H), 4.24-4.21 (t, *J* = 7.2 Hz, 1H), 4.02-4.00 (t, *J* = 6.6 Hz, 1H), 3.74 (s, 3H), 2.17-2.09 (m, 2H), 0.98-

0.89 (m, 12H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.3, 171.4, 156.6, 144.0, 141.4, 127.8, 127.3, 125.3, 120.1, 67.3, 60.7, 57.4, 52.4, 47.3, 31.3, 29.9, 19.1, 18.0; FT-IR (KBr, cm^{-1}): 3294, 2961, 1740, 1693, 1652, 1541, 1450, 1388, 1250, 1140, 1030, 739; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{26}H_{33}N_2O_5$ 453.2389, found 453.2297.

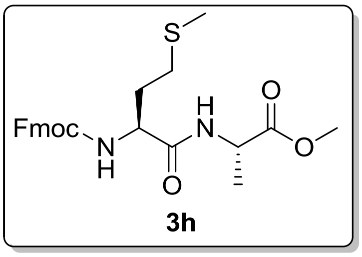
(*R*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanamido)-3-methylbutanoate, 3g



3g

Solid; (382 mg, 82%); mp 131-133 ºC; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.59-7.57 (d, *J* = 7.8 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.32-7.28 (t, *J* = 7.2 Hz, 2H), 6.48 (br, 1H), 5.25 (br, 1H), 4.55-4.51 (m, 1H), 4.42-4.40 (d, *J* = 7.2 Hz, 2H), 4.23-4.20 (t, *J* = 7.2 Hz, 2H), 3.73 (s, 3H), 2.18-2.14 (m, 1H), 1.69-1.65 (m, 2H), 1.59-1.52 (m, 1H), 0.95-0.88 (m, 12H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 172.2, 156.4, 143.9, 141.5, 127.9, 127.3, 125.2, 120.2, 67.3, 57.3, 53.7, 52.4, 47.3, 41.5, 31.4, 24.8, 23.1, 19.1; FT-IR (KBr, cm^{-1}): 2962, 1740, 1725, 1648, 1534, 1448, 1252, 743; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{27}H_{35}N_2O_5$ 467.2546, found 467.2535.

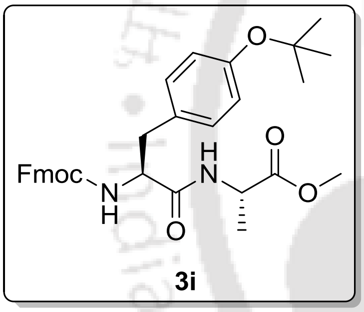
(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-(methylthio)butanamido)propanoate, 3h



3h

Solid; (369 mg, 81%); mp 148-150 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.76-7.74 (d, *J* = 7.8 Hz, 2H), 7.59-7.57 (m, 2H), 7.40-7.37 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (m, 2H), 6.87 (br, 1H), 5.76 (br, 1H), 4.58-4.54 (m, 1H), 4.44- 4.35 (m, 3H), 4.21-4.19 (t, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 2.61-2.58 (m, 2H), 2.11 (s, 3H), 2.07-1.97 (m, 2H), 1.41-1.40 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 171.0, 156.2, 144.0, 141.4, 127.9, 127.2, 125.2, 120.2, 67.3, 53.6, 52.6, 48.3, 47.3, 29.7, 18.2, 15.3; FT-IR (KBr, cm^{-1}): 2925, 1743, 1652, 1533, 1450, 1397, 1258, 1082, 739; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{24}H_{29}N_2O_5S$ 457.1797, found 457.1800.

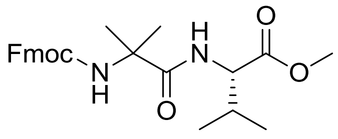
(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(tert-butoxy)phenyl)propanamido)propanoate, 3i



3i

Solid; (381 mg, 70%); mp 172-174 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.75 (d, *J* = 7.2 Hz, 2H), 7.57-7.55 (t, *J* = 6.6 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.32-7.30 (t, *J* = 7.2 Hz, 2H), 7.10-7.09 (d, *J* = 7.2 Hz, 2H), 6.91-6.89 (d, *J* = 8.4 Hz, 2H), 6.36 (br, 1H), 5.48 (br, 1H), 4.50-4.46 (m, 1H), 4.40-4.32 (m, 3H), 4.20-4.18 (t, *J* = 7.2 Hz, 1H), 3.71 (s, 3H), 3.09-2.96 (m, 2H), 1.34-1.33 (d, *J* = 7.2 Hz, 3H), 1.31 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 170.7, 156.1, 154.6, 143.9, 141.4, 130.0, 127.9, 127.2, 125.3, 125.2, 124.5, 120.1, 78.6, 67.3, 56.2, 52.6, 47.2, 38.2, 28.9, 18.4; FT-IR (KBr, cm^{-1}): 3064, 2921, 1740, 1689, 1653, 1532, 1450, 1365, 1257, 1163, 730; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{32}H_{37}N_2O_6$ 545.2652, found 545.2653.

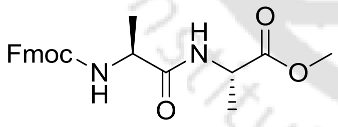
(*S*)-Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanamido)-3-methylbutanoate, 3j



3j

Solid; (350 mg, 80%); mp 109-111 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.60-7.58 (t, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 6.78 (br, 1H), 5.37 (br, 1H), 4.53-4.52 (t, *J* = 7.2 Hz, 1H), 4.42-4.41 (m, 2H), 4.22-4.20 (t, *J* = 7.2 Hz, 1H), 3.71 (s, 3H), 2.19-2.16 (m, 1H), 1.60 (s, 6H), 0.93-0.86 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 172.4, 155.0, 143.8, 141.3, 127.7, 127.0, 125.0, 124.9, 120.0, 66.7, 57.2, 57.0, 52.1, 47.1, 31.3, 25.8, 19.0, 17.6; FT-IR (KBr, cm^{-1}): 3281, 2963, 1730, 1658, 1536, 1446, 1387, 1255, 1084, 740; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{31}N_2O_5$ 439.2233, found 439.2234.

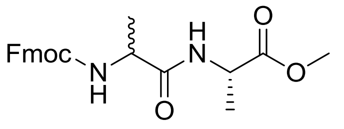
(*S*)-Methyl- 2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanoate, 3k



3k

Solid; (356 mg, 90%); mp 141-143 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.60-7.58 (d, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 6.43 (br, 1H), 5.35 (br, 1H), 4.59-4.56 (t, *J* = 7.2 Hz, 1H), 4.41 (br, 2H), 4.23-4.21 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 1.42-1.41 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 172.0, 156.1, 144.0, 141.5, 127.9, 127.3, 125.2, 120.2, 67.3, 52.7, 50.6, 48.3, 47.3, 19.0, 18.5; FT-IR (KBr, cm^{-1}): 2927, 1741, 1688, 1650, 1530, 1451, 1259, 1050, 758; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_5$ 397.1763, found 397.1754.

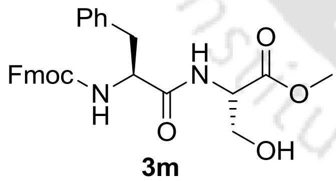
DL-(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanoate, 3l



3l

Solid; (348 mg, 88%); mp 141-143 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.60-7.59 (d, *J* = 6.6 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.30 (t, *J* = 7.2 Hz, 2H), 6.47 (br, 1H), 5.37 (br, 1H), 4.60-4.55 (m, 1H), 4.41 (br, 2H), 4.28-4.21 (m, 2H), 3.75, 3.73 (s, 3H), 1.42-1.41 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.5, 173.3, 173.0, 172.9, 156.4, 143.7, 143.6, 141.2, 127.6, 127.0, 124.9, 119.9, 66.9, 52.3, 52.2, 50.2, 50.0, 48.1, 48.0, 47.0, 18.2, 17.3, 17.2; FT-IR (KBr, cm^{-1}): 2927, 1741, 1688, 1650, 1530, 1451, 1259, 1050, 758; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_5$ 397.1763, found 397.1754.

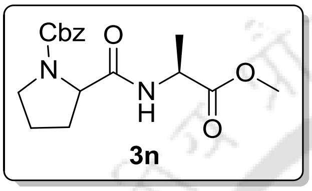
(*S*)-Methyl- 2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoate, 3m



3m

Solid; (439 mg, 90%); mp 191-193 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.53-7.51 (t, *J* = 7.2 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (t, *J* = 7.2 Hz, 5H), 7.19-7.18 (d, *J* = 6.6 Hz, 2H), 6.84 (br, 1H), 5.45 (br, 1H), 4.58 (s, 1H), 4.46-4.38 (m, 2H), 4.33-4.30 (t, *J* = 7.2 Hz, 1H), 4.18-4.15 (t, *J* = 7.2 Hz, 1H), 3.90 (s, 2H), 3.73 (s, 3H), 3.10-3.09 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 170.6, 156.4, 143.8, 141.5, 136.3, 129.5, 128.9, 128.0, 127.3, 127.2, 125.3, 125.2, 120.2, 67.5, 63.0, 56.4, 55.1, 53.0, 47.2, 38.6; FT-IR (KBr, cm^{-1}): 2962, 1734, 1698, 1658, 1544, 1450, 1260, 1028, 739, 698; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{29}N_2O_6$ 489.2026, found 489.2017.

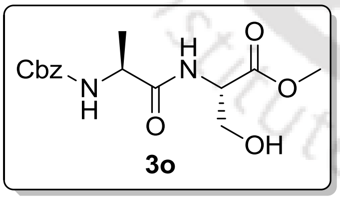
Benzyl 2-(((*S*)-1-methoxy-1-oxopropan-2-yl) carbamoyl) pyrrolidine-1-carboxylate, 3n



3n

Solid; (267 mg, 80%); mp 60-62 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.32 (m, 5H), 6.44 (br, 1H), 5.16 (s, 2H), 4.50 (s, 1H), 4.35-4.29 (m, 1H), 3.73 (s, 3H), 3.57-3.43 (m, 2H), 1.72-1.67 (m, 4H), 1.38-1.37 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 171.5, 155.9, 136.5, 128.5, 128.0, 127.9, 67.3, 60.3, 52.4, 48.2, 47.0, 28.6, 24.5, 18.0; FT-IR (KBr, cm^{-1}): 3322, 2961, 1760, 1702, 1657, 1541, 1423, 1359, 1161, 992, 768; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{23}N_2O_5$ 335.1607, found 335.1610.

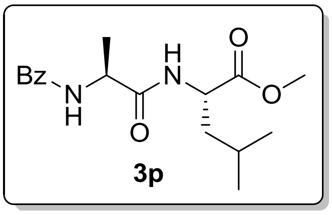
(*S*)-Methyl-2-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-3-hydroxypropanoate, 3o



3o

Solid; (279 mg, 86%); mp 132-134 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.30 (m, 5H), 7.01 (br, 1H), 5.47-5.46 (d, *J* = 7.2 Hz, 1H), 5.12-5.05 (m, 2H), 4.64-4.63 (m, 1H), 4.28-4.25 (t, *J* = 7.2 Hz, 1H), 3.96-3.94 (m, 2H), 3.78 (s, 3H), 1.41-1.40 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 171.0, 156.5, 136.2, 128.7, 128.3, 128.1, 67.2, 62.7, 54.9, 52.8, 50.7, 18.8; FT-IR (KBr, cm^{-1}): 2935, 1744, 1644, 1533, 1437, 1337, 1263, 1129, 1055, 746, 699; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{21}N_2O_6$ 325.1400, found 325.1420.

(*S*)-Methyl-2-((*S*)-2-benzamidopropanamido)-4-methylpentanoate, 3p



3p

Solid; (282 mg, 88%); mp 102-104 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.81-7.80 (d, *J* = 7.2 Hz, 2H), 7.51-7.49 (t, *J* = 7.2 Hz, 1H), 7.43-7.41 (t, *J* = 7.2 Hz, 2H), 7.09 (br, 1H), 4.87-4.83 (m, 1H), 4.62-4.56 (m, 1H), 3.66 (s, 3H), 1.71-1.57 (m, 4H), 1.51-1.50 (d, *J* = 7.2 Hz, 2H), 0.94-0.88 (m, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 173.2, 172.9, 167.2, 133.8, 131.6, 128.4, 127.2, 52.2, 50.9, 49.1, 40.8, 24.8, 22.8, 18.9; FT-IR (KBr, cm^{-1}): 2956, 1750, 1657, 1551, 1315, 1205, 1157, 729, 697; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{17}H_{25}N_2O_4$ 321.1814, found 321.1817.

4.8. Selected spectra and chromatograms

4.8.1. NMR (^{1}H and ^{13}C) and Mass spectra of peptides

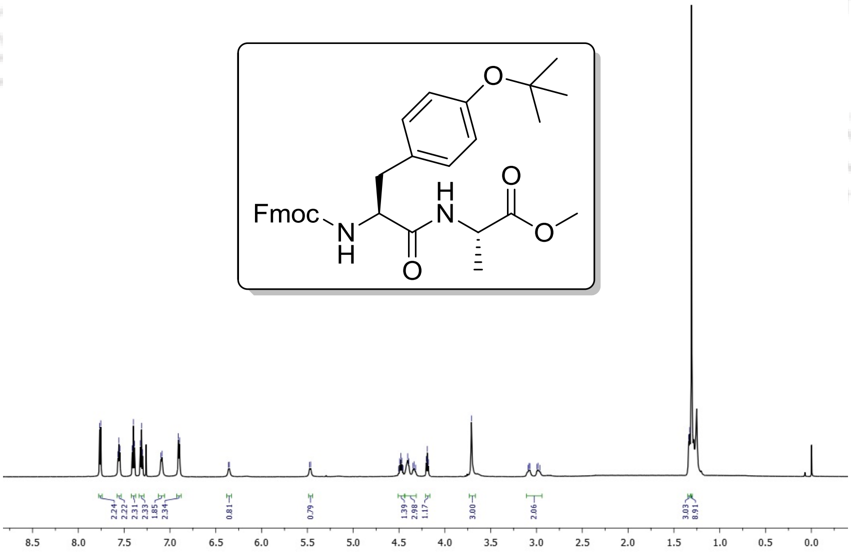


Figure S1. ^{1}H NMR spectra of compound **3i**

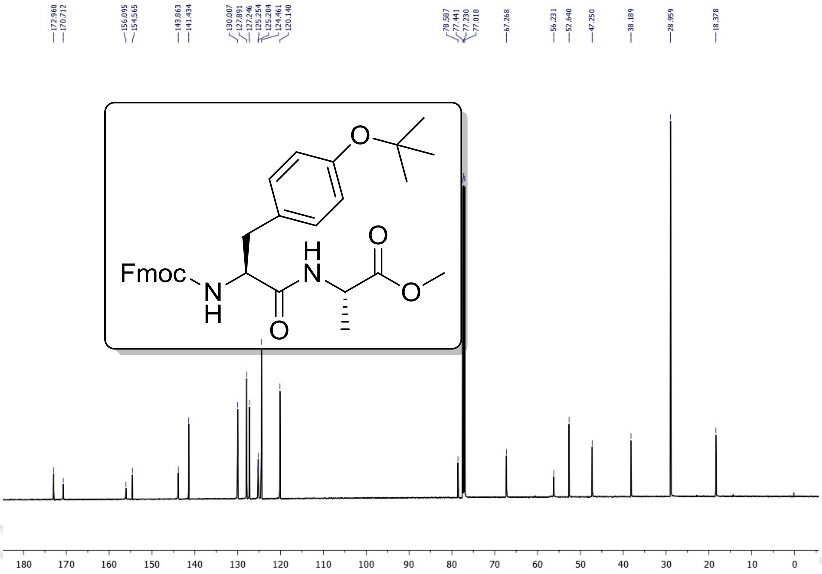


Figure S2. ^{13}C NMR spectra of compound **3i**

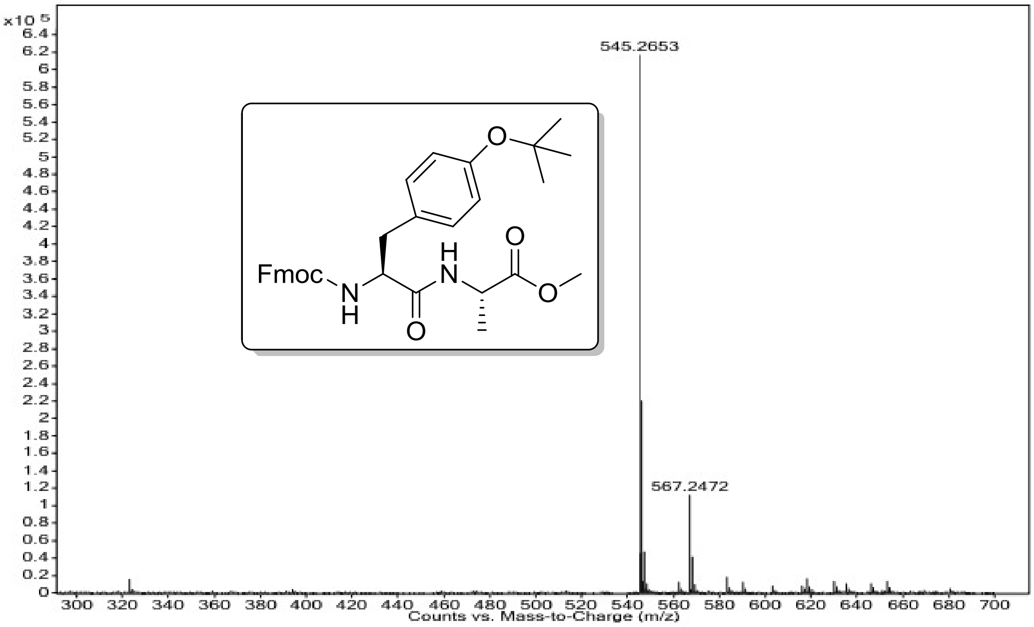


Figure S3. Mass spectra of compound **3i**

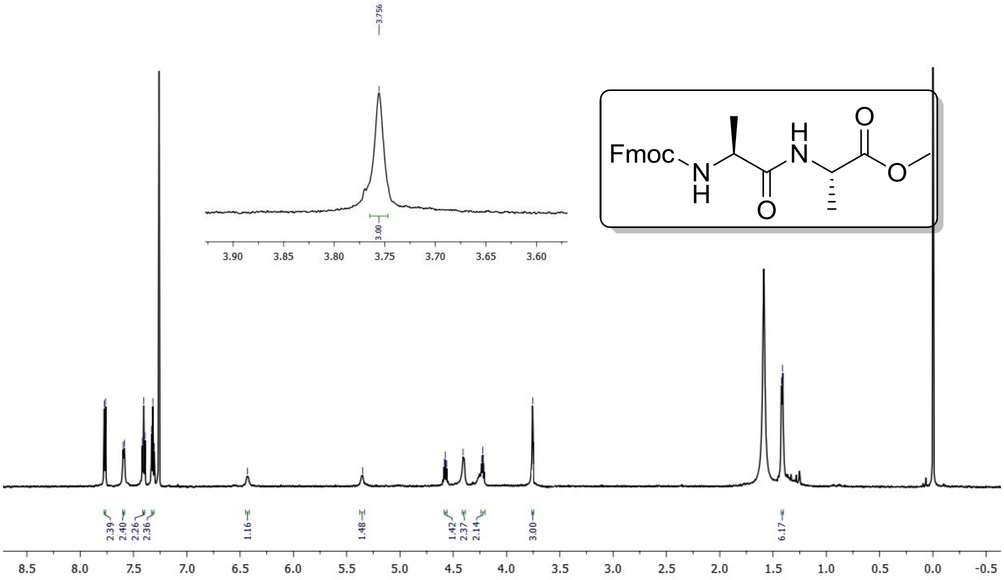


Figure S4. ^{1}H NMR spectra of compound **3k**

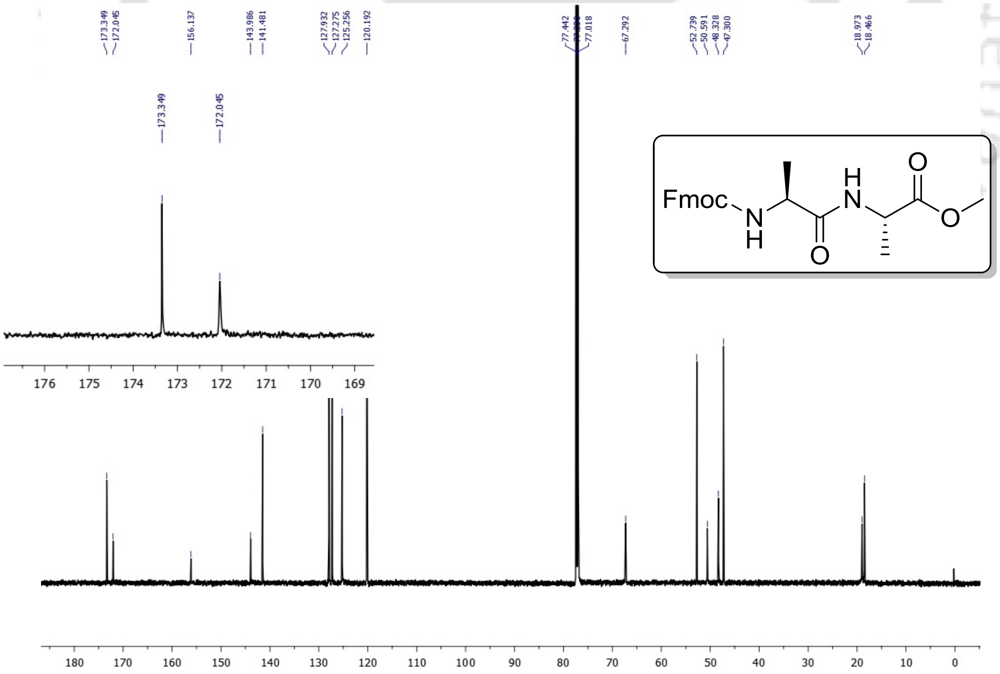


Figure S5. ^{13}C NMR spectra of compound **3k**

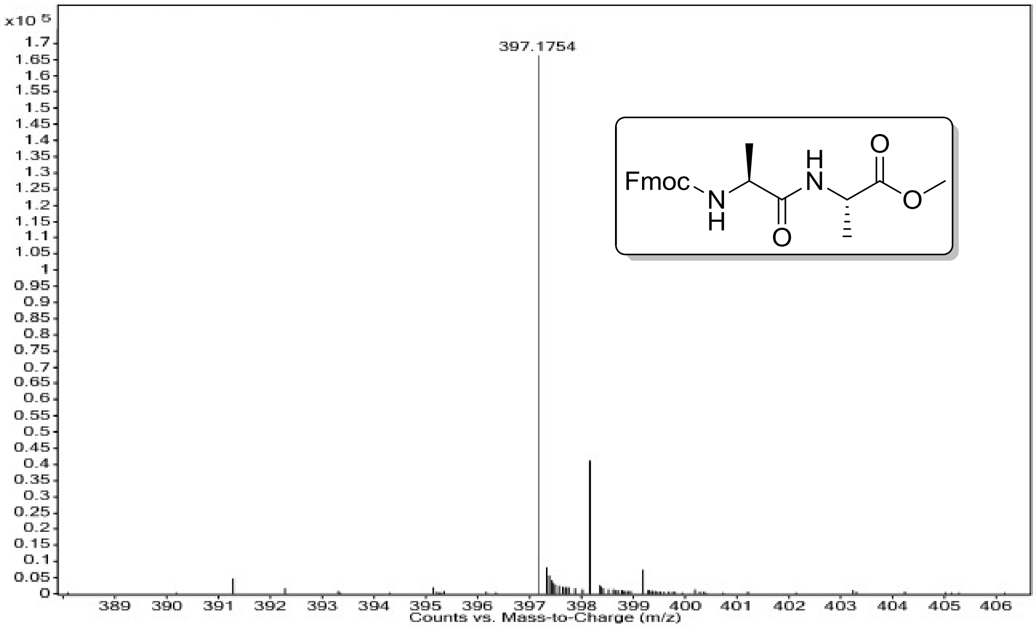


Figure S6. Mass spectra of compound **3k**

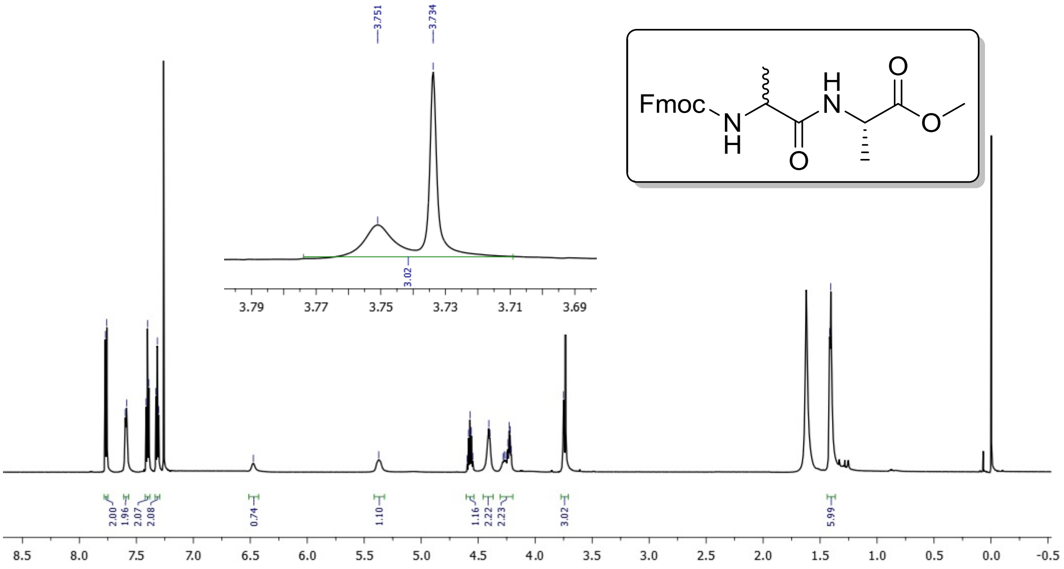


Figure S7. ^{1}H NMR spectra of compound **3l**

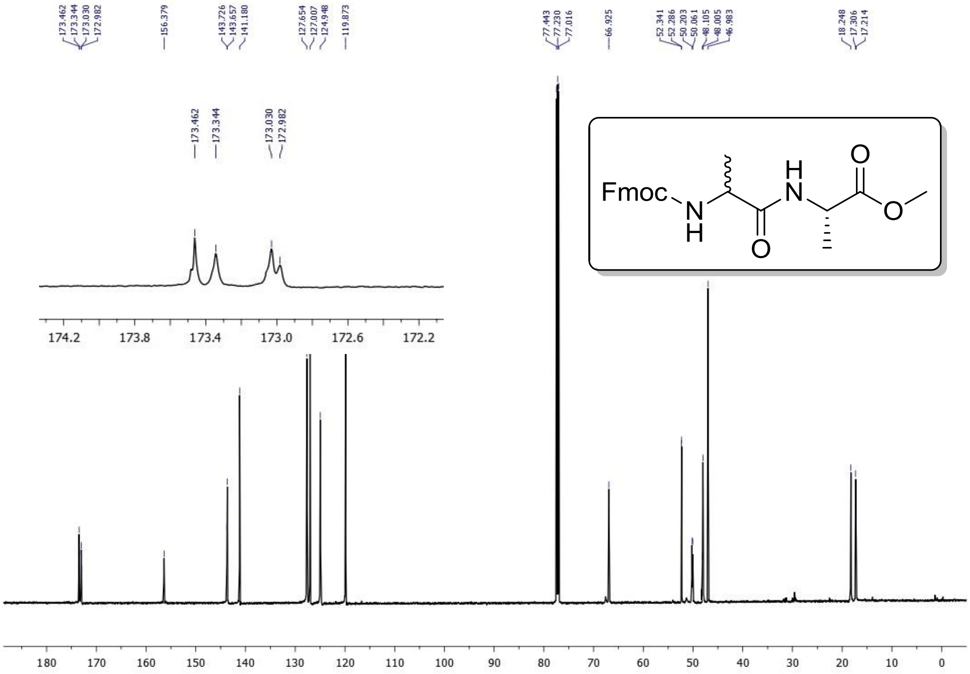


Figure S8. ^{13}C NMR spectra of compound **3l**

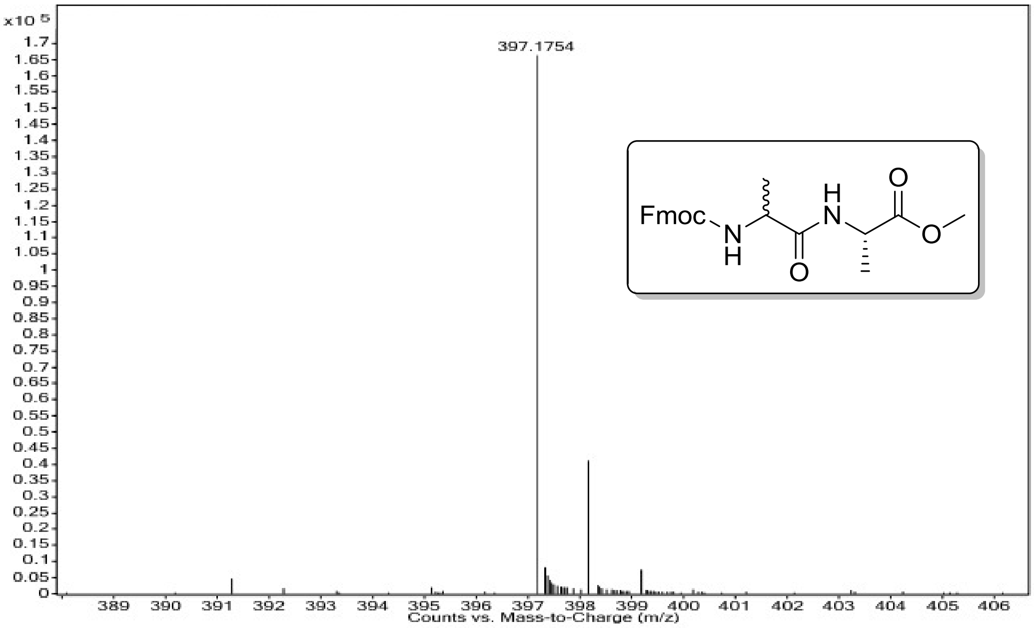


Figure S9. Mass spectra of compound **3l**

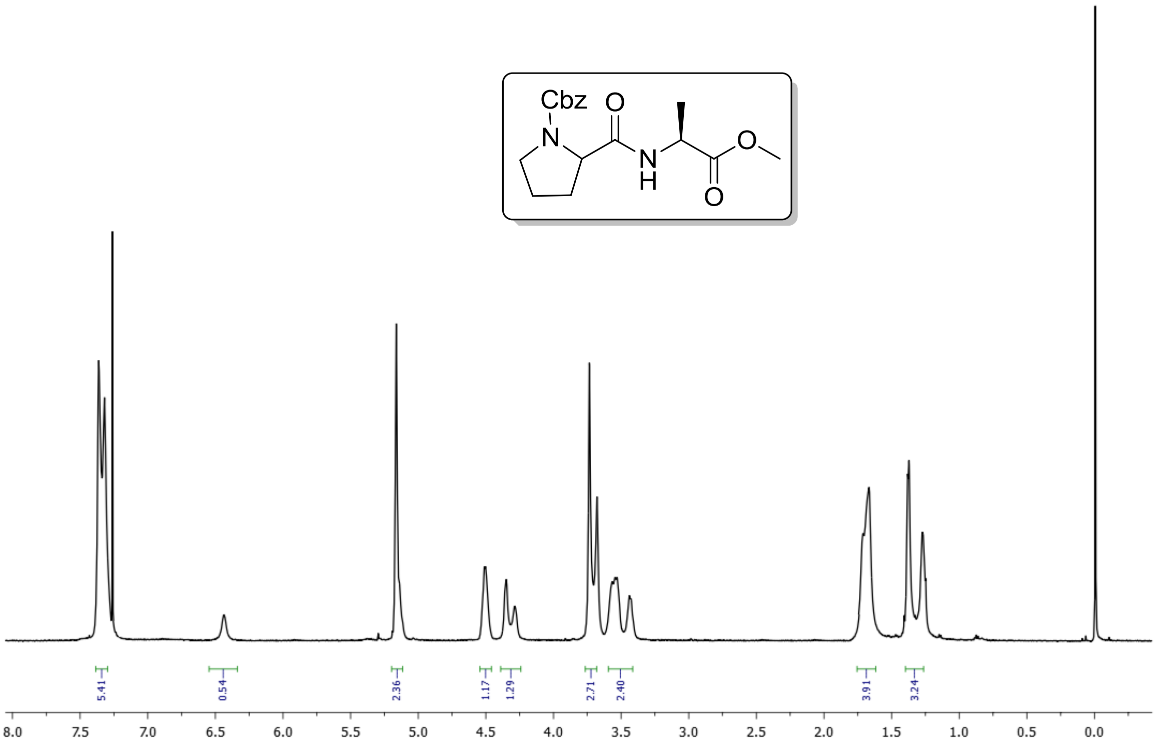


Figure S10. ^{1}H NMR spectra of compound **3n**

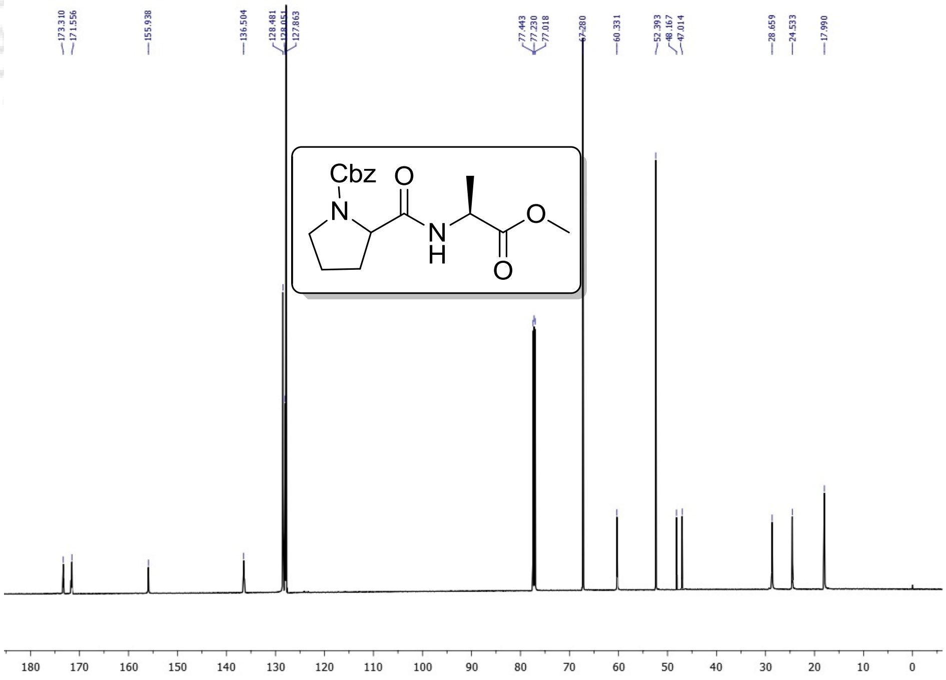


Figure S11. ^{13}C NMR spectra of compound **3n**

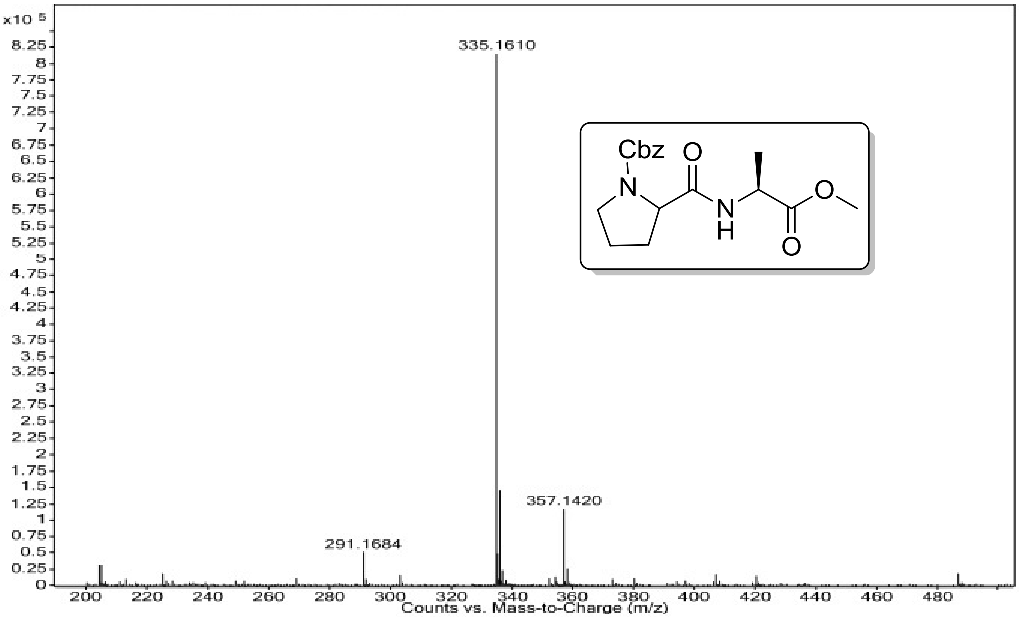


Figure S12. Mass spectra of compound **3n**

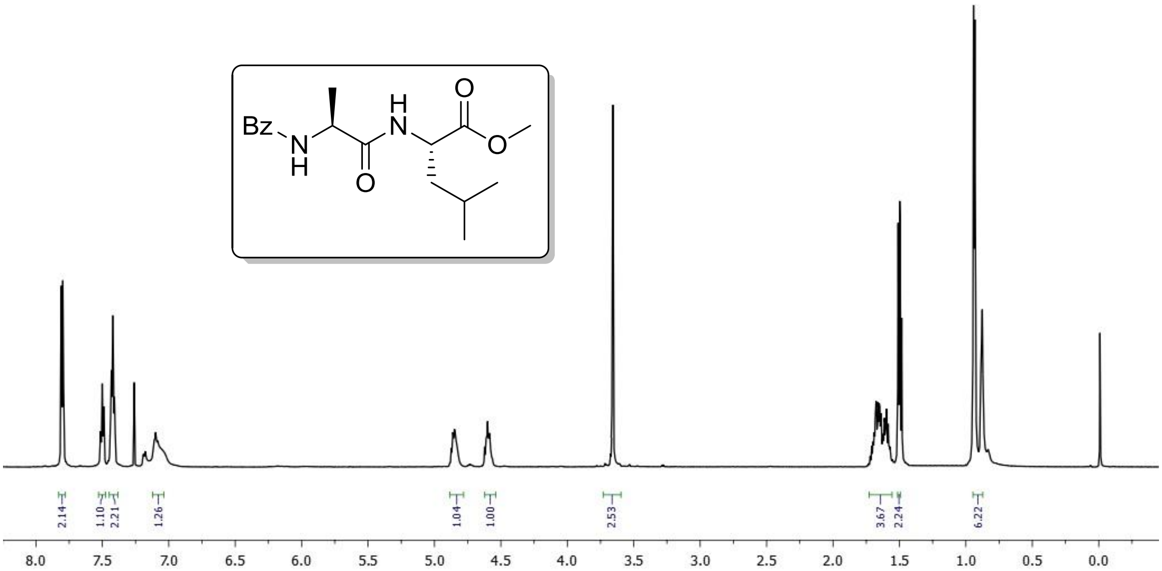


Figure S13. ^{1}H NMR spectra of compound **3p**

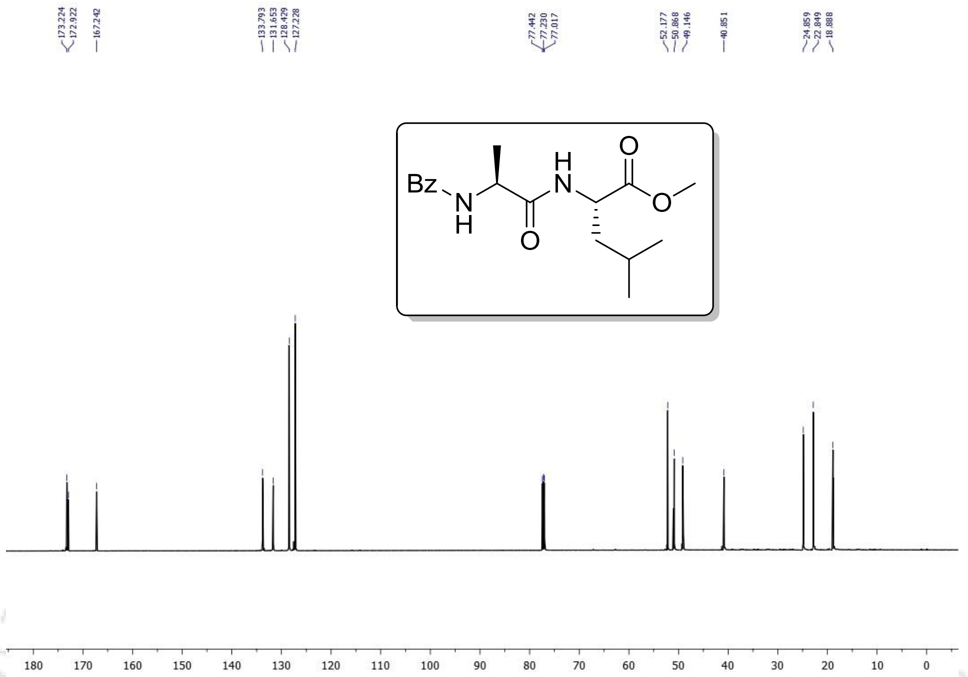


Figure S14. ^{13}C NMR spectra of compound **3p**

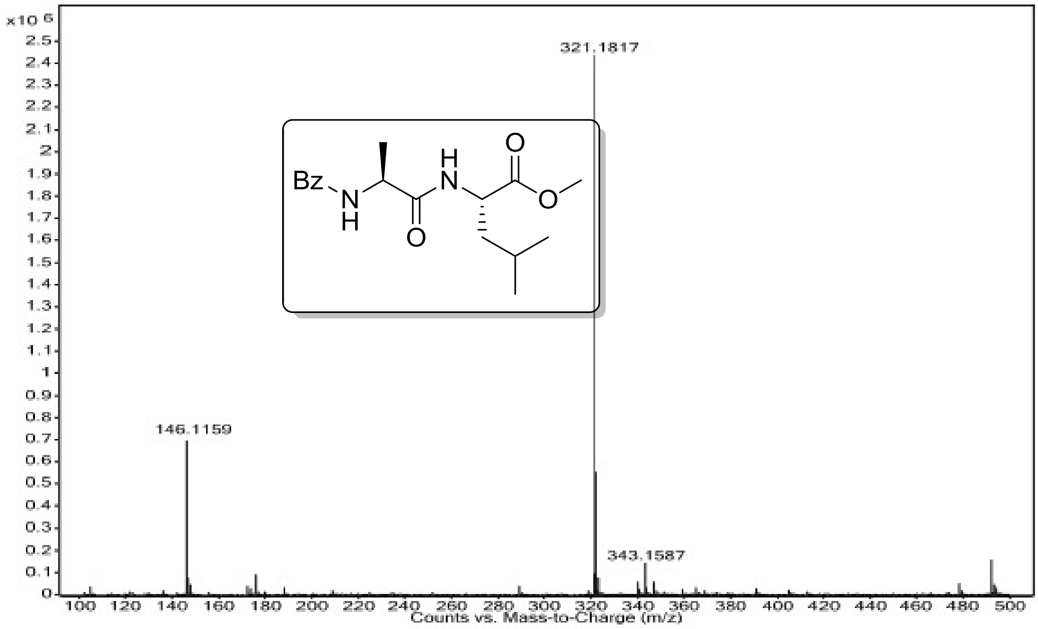


Figure S15. Mass spectra of compound **3p**

4.8.2. HPLC Data for racemization test

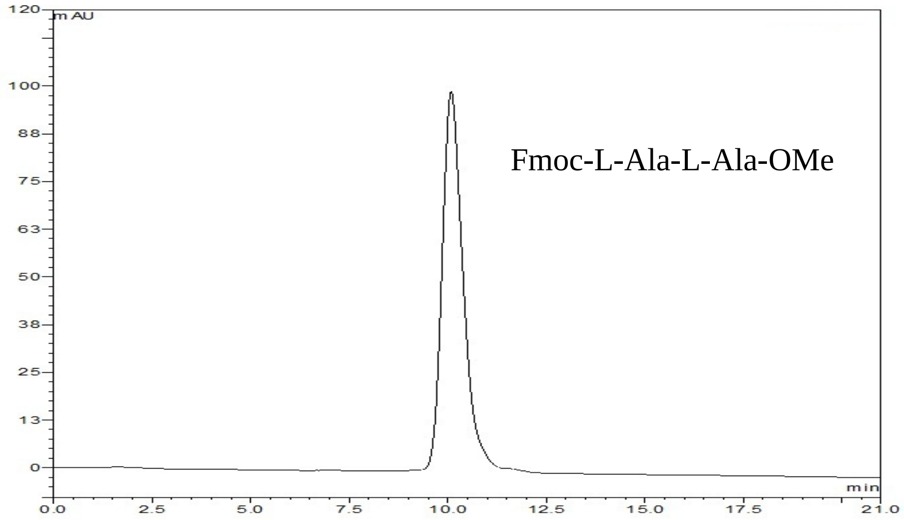


Figure S16. HPLC chromatogram of Fmoc-L-Ala-L-Ala-OMe dipeptide, in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min then 90 to 100%, up to 21 min, CH_3CN in H_2O with 0.1% formic acid (Scheme 4.1.1, entry 3k).

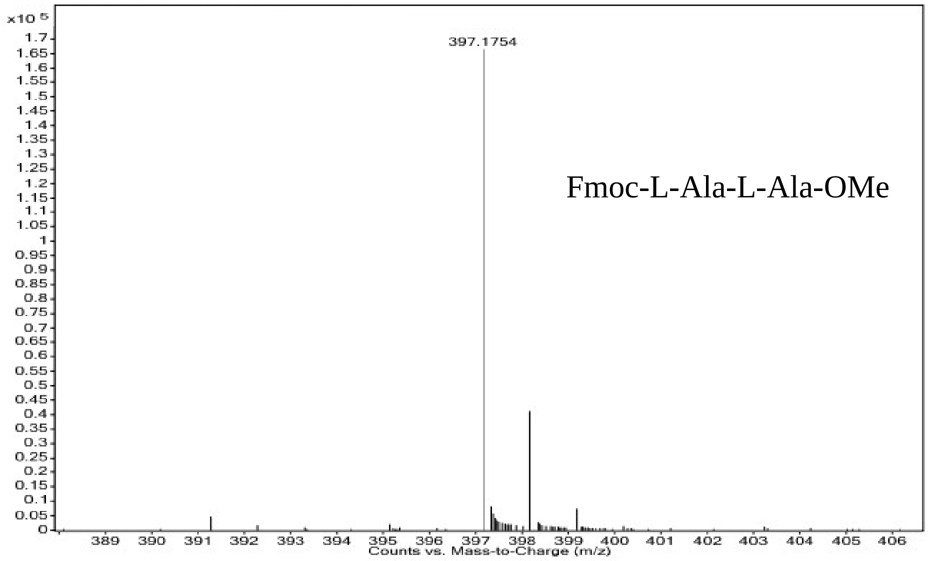


Figure S17. ESI-MS spectrum of Fmoc-L-Ala-L-Ala-OMe dipeptide (Scheme 4.1.1, entry 3k).

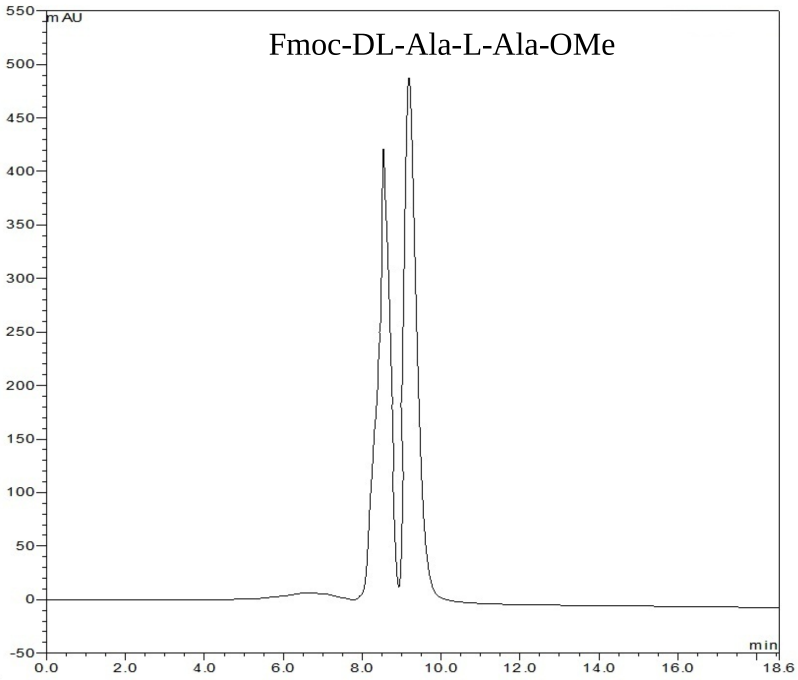


Figure S18. HPLC chromatogram of Fmoc-DL-Ala-L-Ala-OMe dipeptide, in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min then 90 to 100%, CH_3CN in H_2O with 0.1% formic acid (Scheme 4.1.1, entry 3l).

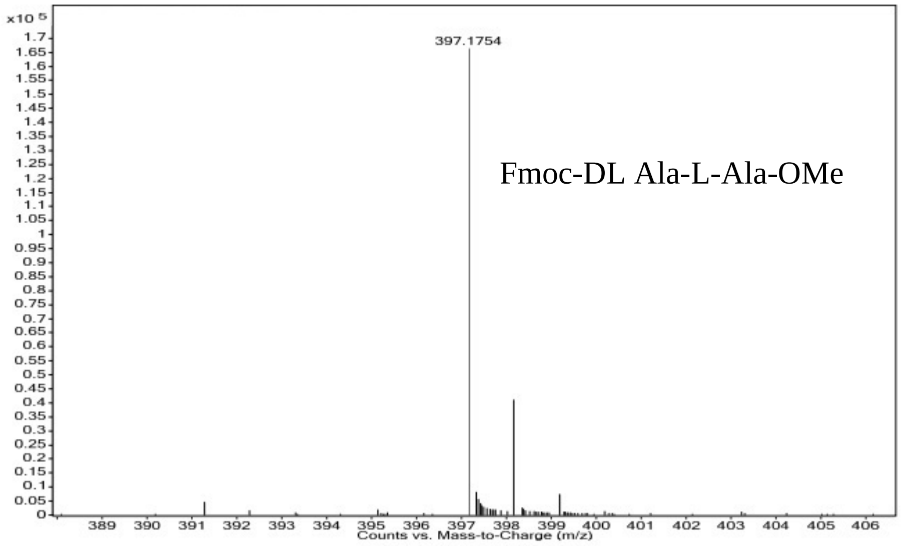


Figure S19. ESI-MS spectrum of Fmoc-DL-Ala-L-Ala-OMe dipeptide (Scheme 4.1.1, entry 3l)

4.8.3. HPLC chromatogram and mass spectra of Boc-LVFF-OMe via solution phase synthesis

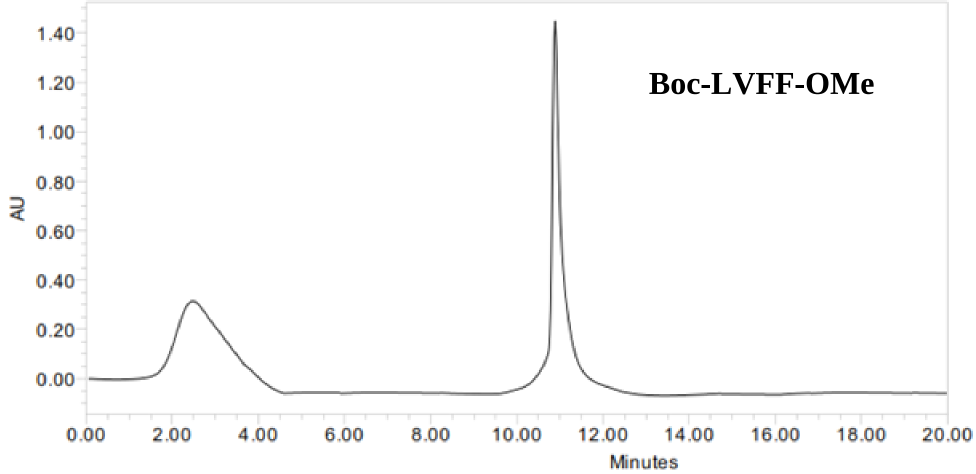


Figure S20. Crude HPLC profile of Tetrapeptide (Boc-LVFF-OMe).

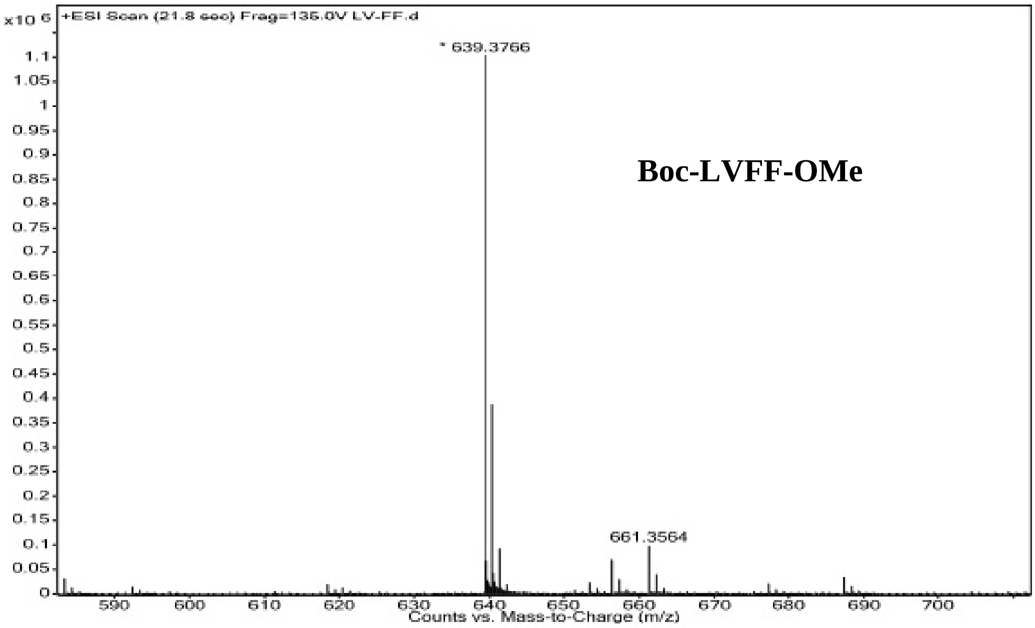


Figure S21. Mass spectra of Tetrapeptide (Boc-LVFF-OMe).

4.8.4. HPLC chromatogram and mass spectra of NFGAIL-NH_2 via solid phase synthesis

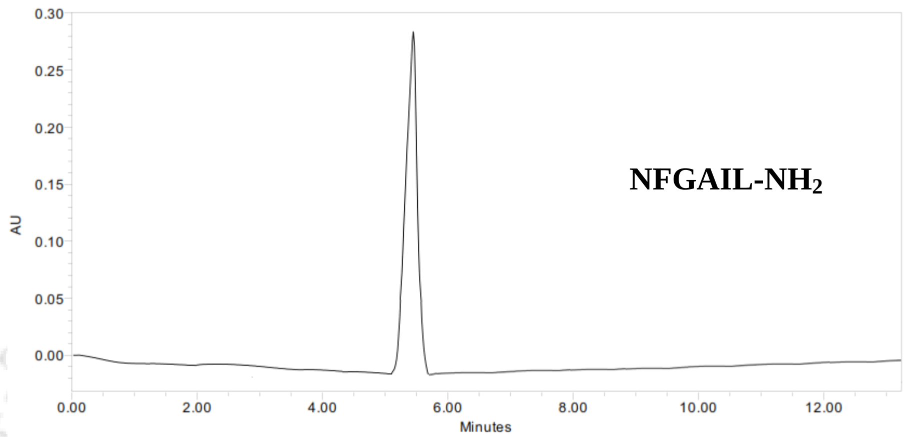


Figure S22. HPLC profile of NFGAIL (22-27) peptide

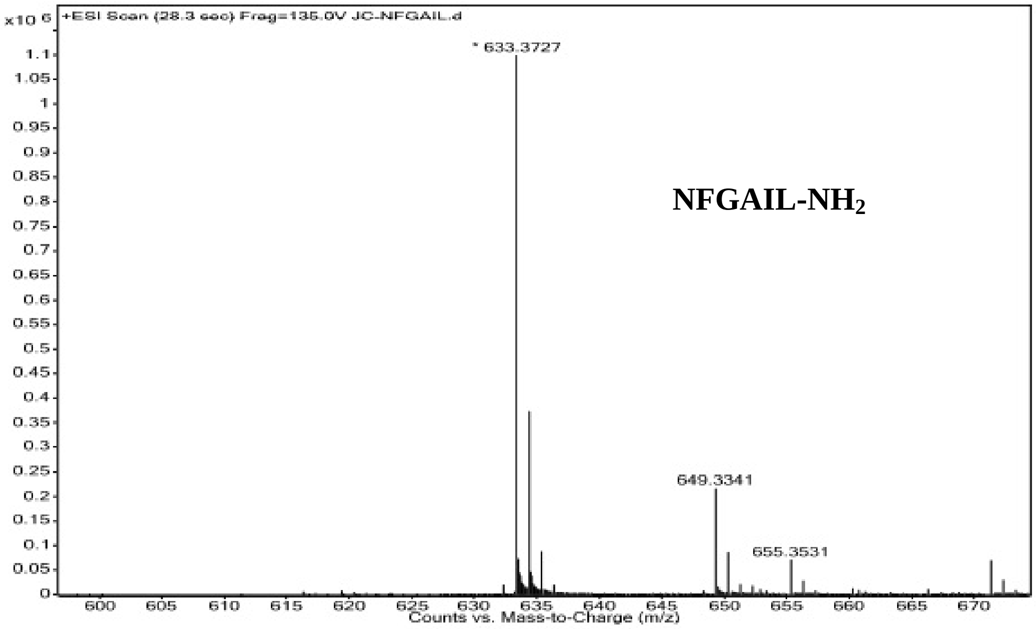


Figure S23. Mass spectra of NFGAIL (22-27) peptide

4.8.5. HPLC chromatogram and mass spectra of ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-NH_2 via solid phase synthesis

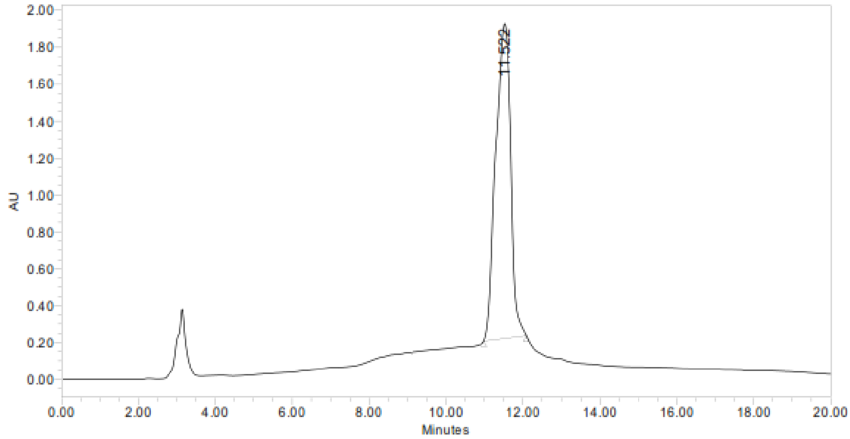


Figure S24. HPLC profile of ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-NH_2

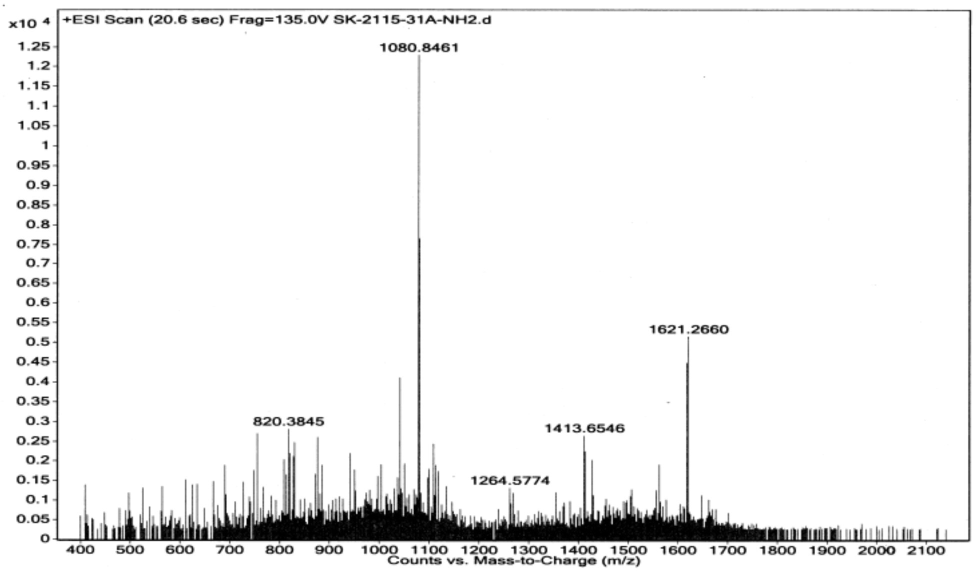


Figure S25. Mass spectra of ATQRLANFLVHSSNNFGAILSSTNVGSNTYG-NH_2 (IAPP, 8-37 peptide).

4.8.6. HPLC chromatogram and mass spectra of ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTYG-NH$_2$ via solid phase synthesis

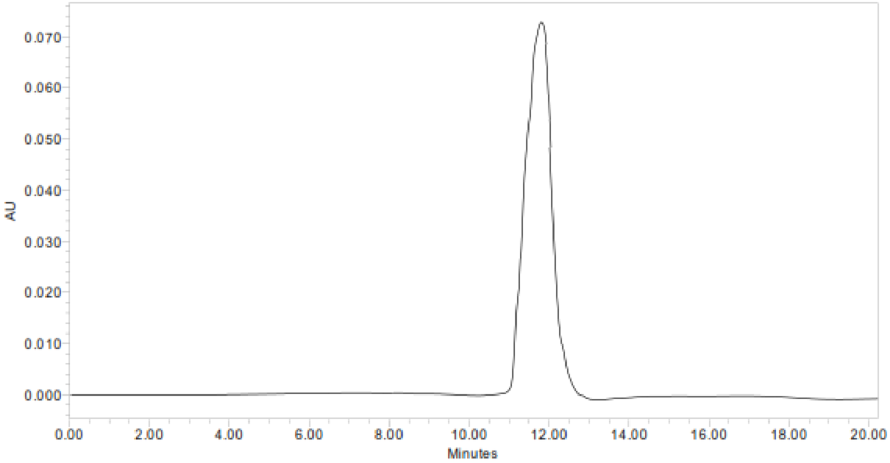


Figure S26. HPLC profile of ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTYG-NH$_2$ (IAPP, 8-37 peptide with β-breaker).

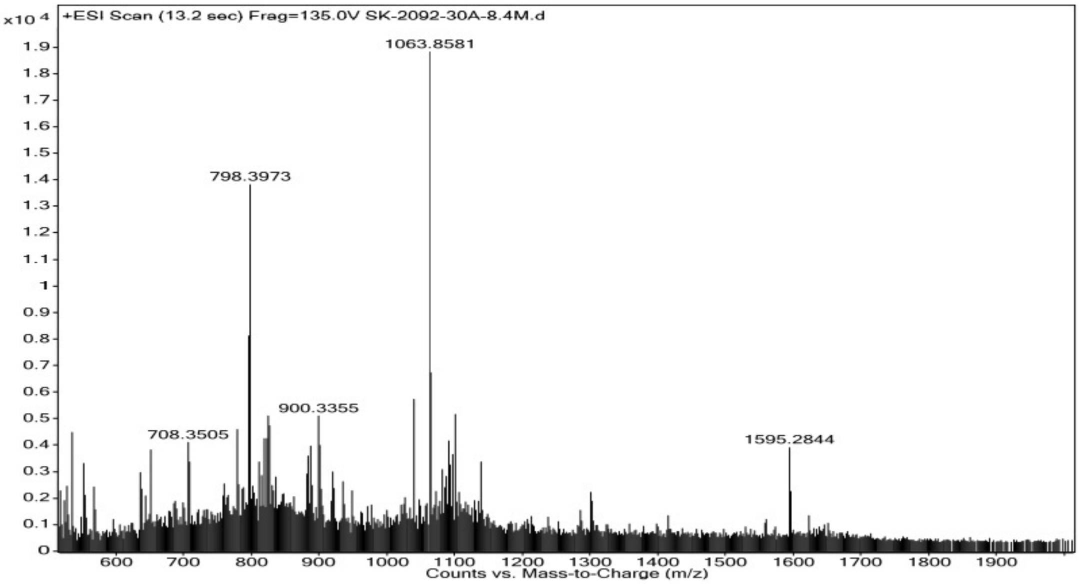


Figure S27. Mass spectra of ATQRLANFLVHSSNNFGA-Ant-LSSTNVGSNTYG-NH$_2$ (IAPP, 8-37 peptide with β-breaker).

Chapter 5: Synthesis of 1,2,4-Oxadiazoles by Using the Catalytic Amount of *o*-NosylOXY

In the previous chapter, we described the catalytic approach of *o*-NosylOXY for the synthesis of esters, amides, peptides and polypeptides. In continuation to these syntheses, we wanted to extend this catalytic approach for further organic transformations. In this chapter, we discussed the synthesis of 1,2,4-oxadiazole using sub-stoichiometric amount of *o*-NosylOXY.

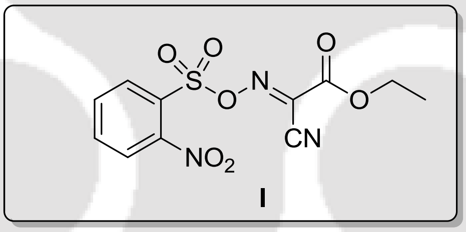
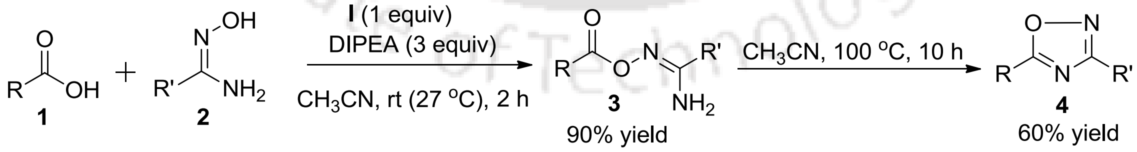


Figure 5.1. *Ethyl 2-cyano-2-(4-nitrophenylsulfonyloxyimino) acetate, (**I**, ortho-NosylOXY)*

1,2,4-oxadiazoles are a class of heterocyclic compounds containing two nitrogen, two carbon and one oxygen atoms have been found to possess a variety of biological activities and also CNS (central nervous system) related activities (Chapter 1, section 1.3.5).[1] Most of the nitrogen and oxygen-containing heterocyclic compounds are also used in medicines for the treatment of different types of fungal and bacterial infections[2] along with the treatment of gastric ulcer, cancer, *etc.*, which are mentioned in Chapter 1. These applications make this heterocycle an important structural motif in drug discovery. There are many synthetic methods reported for the synthesis of 1,2,4-oxadiazoles by using coupling reagents (Chapter 1, section 1.4.3). The major disadvantages of these coupling reagents (Chapter 1, section 1.5) were the usage of excess amount of coupling reagents, involves harsh reaction conditions, generation of undesired byproducts, chemical wastes as well as racemization. To address some of these drawbacks, we developed a catalytic synthesis of 1,2,4-oxadiazoles with wide substrate scope.

5.1. Reaction optimization and substrate scope for the synthesis of 1,2,4-oxadiazoles

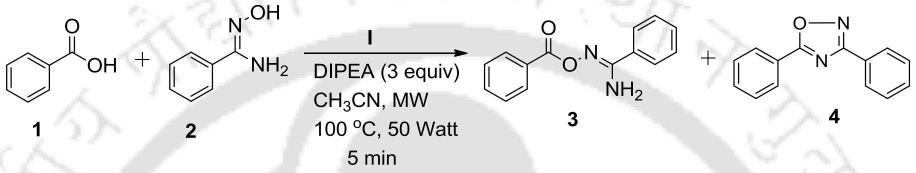
Initially, we optimized the reaction conditions with the synthesis of 3,5-diphenyl-1,2,4-oxadiazole as a model product. Benzoic acid (1 equiv) was dissolved in acetonitrile. To this, **I** (1 equiv), and Hunig's base (DIPEA, 3 equiv) were added and the reaction mixture was stirred for about 2-3 min. Then benzamidoxime (1 equiv) was added and the reaction mixture was allowed to stir at room temperature for 2 h. We did not observe the cyclized product (**4**), only intermediate **3** was formed exclusively with 90% yield. Further, this reaction mixture was refluxed for 10 h at 100 °C, afforded the cyclized 1,2,4-oxadiazole (**4**) with 60% yield (Scheme 5.1.1).



Scheme 5.1.1. Synthesis of 1, 2, 4-Oxadiazole under conventional heating condition.

When we performed the reaction under microwave irradiation, surprisingly, a higher conversion of **4** was obtained in a much shorter reaction time with 1 equiv of **I**. Next, we wanted to investigate whether the reaction works with catalytic amount of **I**. For that, we took the same model substrate with varied amount of **I** and treated under microwave irradiation. We observed that the reaction worked well with 0.1 equiv of **I**. However, in absence of **I**, no coupling product was noted (Table 5.1.1, entry **6**). We now wanted to understand the effect of **I** in the first and second step of the reaction, discretely.

Table 5.1.1. Optimization of the amount of reagent **I** under microwave irradiation.[a]

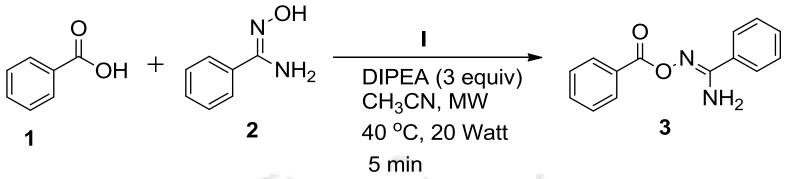


Entry	I	3 (Yield %)[b]	4 (Yield %)[b]
1	0.5 equiv	10	80
2	0.4 equiv	10	77
3	0.3 equiv	20	69
4	0.2 equiv	10	62
5	0.1 equiv	25	53
6	0.0 equiv	0	0

[a]*Reaction conditions: acid (1 mmol),* ***I*** *(varied amount), DIPEA (3 mmol), benzamidoxime (1 mmol) and* CH_3CN *(2 ml), microwave irradiation (temperature of reaction is 100 °C and upper limit of MW is 50 Watt), and the reaction time is 5 min.* [b]*Isolated yield.*

For the first step, we applied MW condition and observed only intermediate **3** with 1 equiv of **I** at 40 °C within 5 min with good yield.

Table 5.1.2. Optimization of the amount of reagent **I** under microwave irradiation for the synthesis of *O*-acylamidoxime intermediate.[a]



Entry	I	3 (Yield %)[b]
1	0.5 equiv	90
2	0.4 equiv	91
3	0.3 equiv	90
4	0.2 equiv	91
5	0.1 equiv	91
6	0.0 equiv	0

[a]*Reaction conditions: benzoic acid (1 mmol), o-NosylOXY (varied amount), DIPEA (3 mmol), benzamidoxime (1 mmol) and* CH_3CN *(2 ml), under microwave irradiation (upper limit of MW is 20 Watt, temperature of reaction is 40 °C) and the reaction time at 5 min.* [b]*Isolated yield.*

Then we wanted to confirm that the first step in the above-mentioned condition also works with a catalytic amount of **I**. Thus, we took a varied amount of **I** and performed the first step of the reaction in mild condition (40 °C and 20 Watt). We observed that 0.1 equiv of **I** is sufficient for producing the high yield of **3**. However, the absence of **I** does not produce **3** at similar conditions (Table 5.1.2, entry **6**). We also performed HPLC; in the absence of **I**, we did not observe the formation of **3**, only starting materials appeared

(Figure 5.1.1**a**). But, we observed only one peak corresponding to the intermediate **3** with 0.1 equiv of **I** (Figure 5.1.1**b**). Next, we wanted to investigate if there is any effect of **I** on the second step, i.e., dehydrative cyclization step, because, we observed clear and consistent alteration of the yield and amount of the existing intermediate in the results summarized in Table 5.1.2. For that, we have purified **3** and applied MW irradiation at 120 °C and 50 Watt for 5 min, and observed the yield of **4** was only 12% and 80% of **3** was unaltered (by HPLC) when **I** was absent (Figure 5.1.1**c**). But when we have used 0.1 equiv of **I**, the yield of **4** improved to be 85% (15% of **3** was left) (Figure 5.1.1**d**).

Later, we wanted to check the conversion of **3** to **4** under microwave irradiation with a varied amount of **I (**Figure 5.1.2**)**. For that we applied 100 °C under MW (upper limit of the MW energy was fixed at 50 Watt) on purified **3**; and observed no conversion to **4** in absence of **I**. However, when we have used 0.1 equiv of **I**, the yield of **4** was only 55% in the same conditions (blue curve).

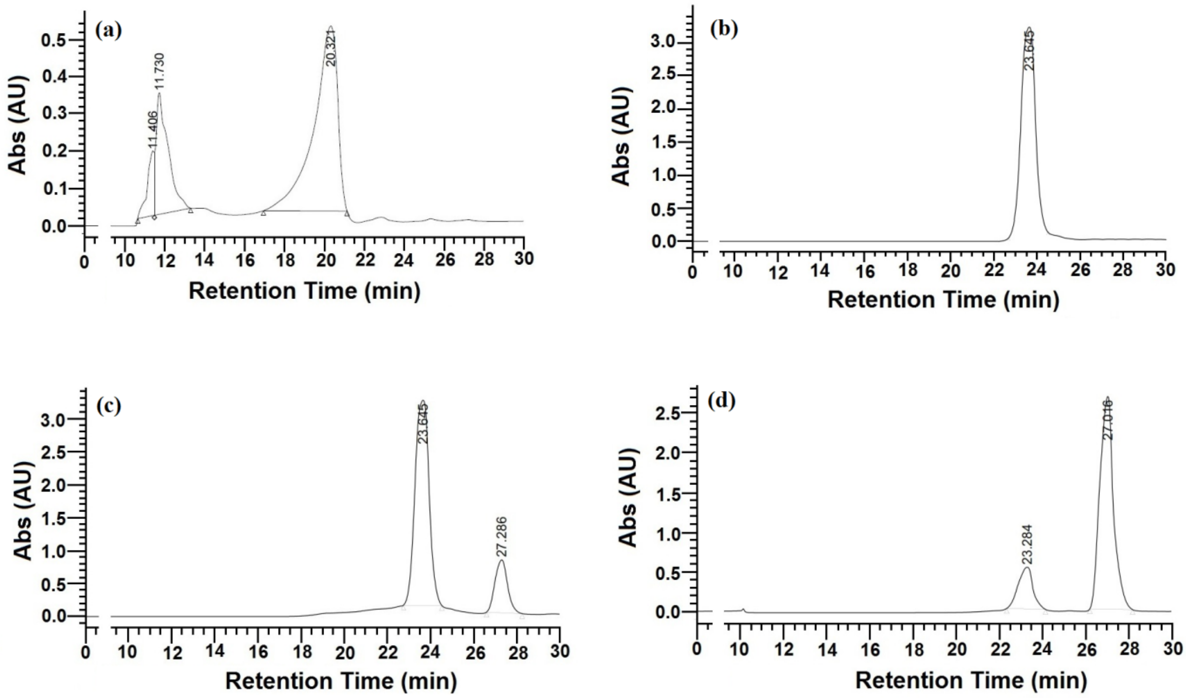


Figure 5.1.1. HPLC data: Benzoic acid (1 mmol), DIPEA (3 mmol), benzamidoxime (1 mmol) and CH_3CN (2 ml), in Microwave irradiation, time of the reaction was fixed to 5 min (a) Benzoic acid and benzamidoxime appeared (without reagent I, temp 40 °C, 20 Watt). (b) Formation of intermediate 3 (with 0.1 equiv of I, temp 40 °C, 20 Watt) (c) Conversion of 3 to 4 without reagent I (temp 120 °C, 50 watt). (d) Conversion of 3 to 4 with 0.1 equiv of I (temp 120 °C, 50 watt).

After that, we have increased the equivalence of **I** to improve the yield of **4**. For that we have used 0.5 equiv of reagent under same conditions, we observed the yield of the reaction was increased to 81% and starting material was 18% only. But when we have used 1 equiv of **I**, 100% conversion of **3** to **4** was observed. No evidence of the existence of the intermediate **3** was observed (Figure 5.1.2).

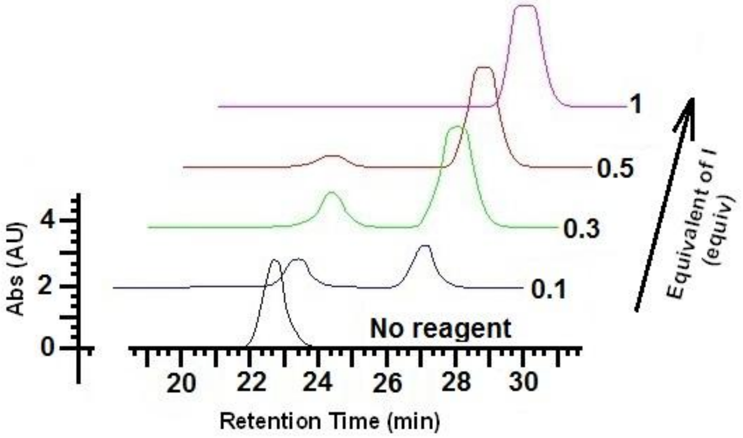


Figure 5.1.2. *HPLC data for conversion of **3** to **4** under microwave irradiation with a varied amount of **I**, time of reaction is 5 min (temp 100 °C, 50 Watt)*

Next question we wanted to answer is, how much time it takes for the conversion of **3** to **4** with one equivalent of **I** under MW (100 °C, 50 Watt). For that, we conducted a time-dependent HPLC analysis and the result is depicted in Figure 5.1.3 that confirmed that 5 min reaction time is necessary and sufficient for complete conversion in the said conditions.

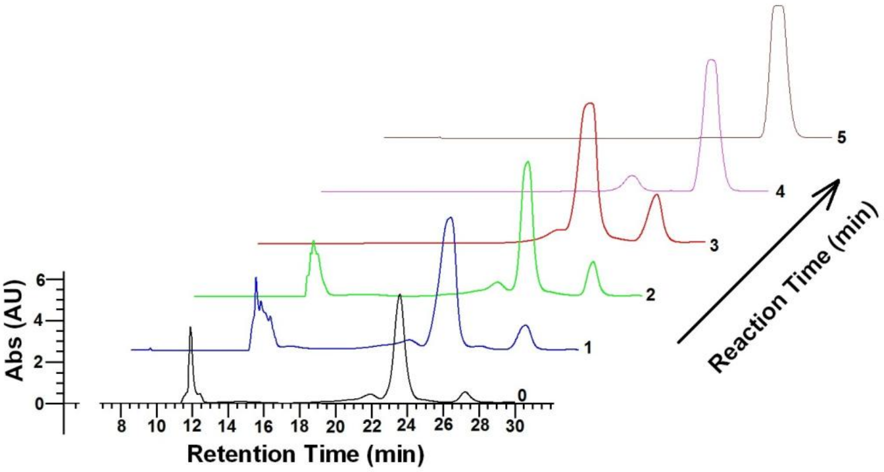
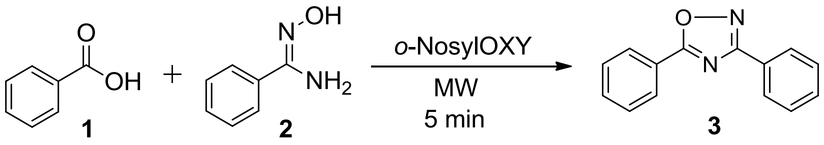


Figure 5.1.3. *Time-dependent HPLC data for conversion of **3** to **4** under microwave irradiation with 1 equiv of **I** (temp 100 °C, 50 Watt) and 3 equiv of DIPEA.*

As the same reagent is useful for both steps, we wanted to adopt a single pot protocol. We have used 1 equiv of **I** for final one-pot condensation/cyclization reaction for formation of 1,2,4-oxadiazole. The synthesis of 3,5-diphenyl-1,2,4-oxadiazole was used as a model reaction for optimization of the conditions. We mixed benzoic acid (1 equiv), **I** (1 equiv), and Hunig's base (iPr_2NEt, DIPEA, 3 equiv) in acetonitrile followed by the addition of benzamidoxime (1 equiv) and treated under microwave irradiation for 5 min. After completion of the reaction, the product was purified by column chromatography; an excellent yield (Table 5.1.3, entry **1**) was obtained.

Next, we screened the effect of solvent variation. When low boiling solvents, such as EtOAc, THF, and $CHCl_3$ were used, the yield of **4** was only 50%, 30%, and 10%, respectively (Table 5.1.3, entries **2**, **3**, and **4**) with 90% *O*-acylamidoxime intermediate **3**. Further, there was no reaction in MeOH and DCM (Table 5.1.3, entries **5** and **6**). But using high boiling solvents, e.g. Toluene, DMF, Dioxane, and DMSO (entries **7**, **8**, **9**, and **10**), the higher yield of **4** was obtained and 40% of *O*-acylamidoxime intermediate **3** was present along with that. However, in case of CH_3CN, **3** disappeared completely and **4** was obtained in better yield (Table 5.1.3, entry **1**). Thus, CH_3CN was found to be the best solvent.

Table 5.1.3. Optimization of the reaction conditions.[a]



Entry	Solvent	Temp (°C)	Base	Yield[b] (%)
1	**CH_3CN**	**100**	**DIPEA (3 equiv)**	**98**
2	EtOAc	100	DIPEA (3 equiv)	50
3	THF	100	DIPEA (3 equiv)	30
4	$CHCl_3$	100	DIPEA (3 equiv)	10
5	MeOH	100	DIPEA (3 equiv)	0
6	DCM	100	DIPEA (3 equiv)	0
7	Toluene	100	DIPEA (3 equiv)	80
8	DMF	100	DIPEA (3 equiv)	82
9	Dioxane	100	DIPEA (3 equiv)	76
10	DMSO	100	DIPEA (3 equiv)	75
11	CH_3CN	100	DBU (3 equiv)	85
12	CH_3CN	100	Et_3N (3 equiv)	82
13	CH_3CN	100	K_2CO_3 (3 equiv)	80

14	CH_3CN	100	DIPA (3 equiv)	50
15	CH_3CN	100	DABCO (3equiv)	30
16	CH_3CN	100	DMAP (3 equiv)	10
17	CH_3CN	100	DIPEA (1 equiv)	0
18	CH_3CN	100	DIPEA (2 equiv)	60
19	CH_3CN	90	DIPEA (3 equiv)	30
20	CH_3CN	110	DIPEA (3 equiv)	90
21	CH_3CN	120	DIPEA (3 equiv)	85
22	CH_3CN	130	DIPEA (3 equiv)	70
23	CH_3CN	140	DIPEA (3 equiv)	0

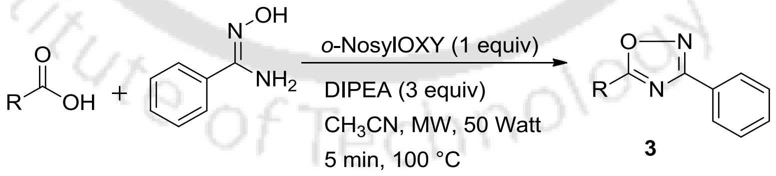
[a] *Reaction conditions: Acid (1 mmol), ortho-NosylOXY (1 mmol), Bases (varied amount), benzamidoxime (1 mmol) under microwave irradiation (upper limit of MW was fixed to 50 Watt), time of the reaction is 5 min.* [b] *Isolated yield.*

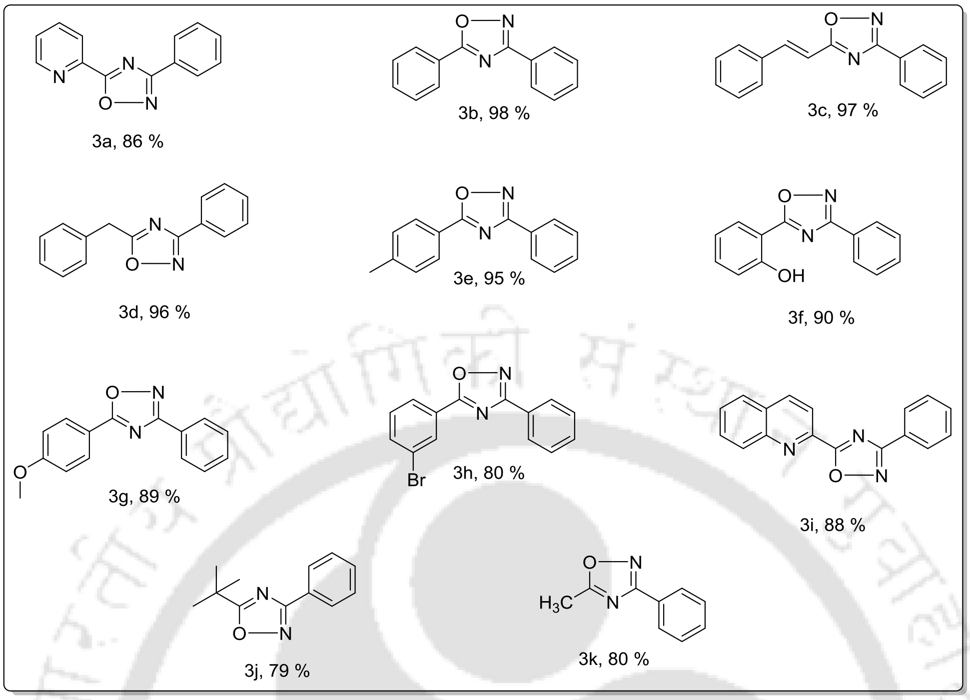
Next, the effect of variation of bases was investigated on oxadiazole formation. The product formation was good in presence of DBU, Et_3N, and K_2CO_3, (entries **11**, **12** and **13**), but bad in presence of DIPA, DABCO, and DMAP (entries **14**, **15**, and **16**) despite prolonged heating in the microwave. We found DIPEA afforded excellent yield and was accepted as a suitable base for the reaction. After that, the amount of DIPEA was optimized. The reaction did not proceed with 1 equiv of DIPEA (entry **17**) and product formation was moderate with 2 equiv (entry **18**) but worked much better with 3 equiv (entry **1**). Thus, 3 equiv of DIPEA was found to be necessary for the reaction.

After that effect of variation of temperature on the reaction was studied. We found the desired product with the better yield at 100 °C. The yield of the product was lower in both below 100 °C (entry **19**) and above 100 °C (entries **20**-**22**). At 140 °C, the product was not observed (entry **23**).

With optimized reaction conditions in hand, we proceeded to investigate the scope of reaction using a wide range of substituted carboxylic acid with benzamidoxime (Scheme 5.1.2). A broad range of carboxylic acid was tolerated under optimized conditions, including those bearing picolinic acid (**3a**), quinoline-2-carboxylic acid (**3i**) and halogen (**3h**) substituted at para position of the phenyl ring. Notably, pivalic acid reacted smoothly under optimized conditions to give the corresponding product (**3j**) in moderate yield.

Scheme 5.1.2. Scope of Carboxylic Acid.[a,b]

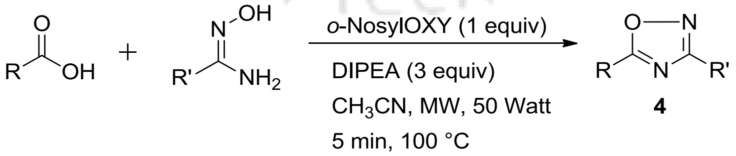


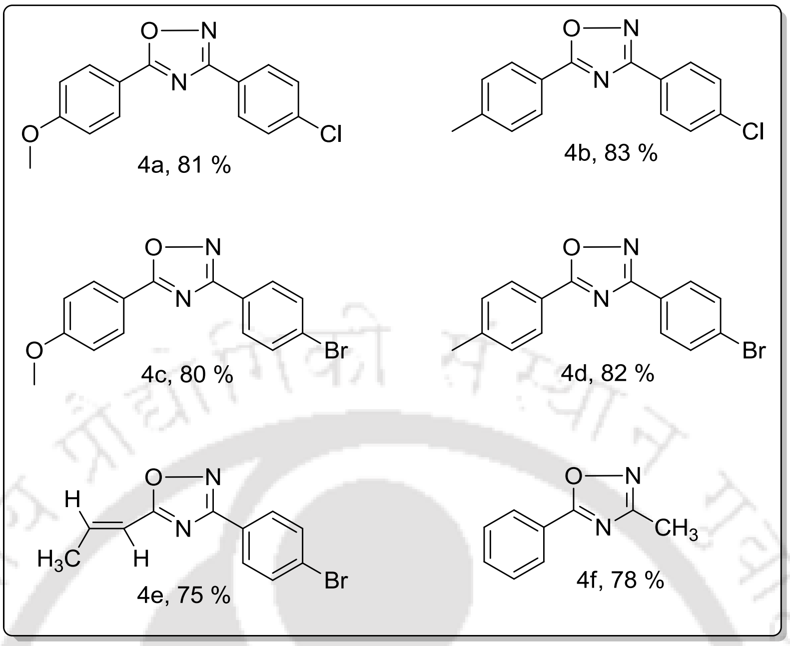


[a]*Reaction condition was similar to that mentioned in Table 5.1.3.* [b]*Isolated yield.*

The scope of the reaction was further expanded by reacting substituted carboxylic acid with different amidoximes. All of these substrates reacted smoothly under the optimized conditions to provide the desired products.

Scheme 5.1.3. Scope of amidoxime as substrates.[a,b]





[a]*Reaction condition was similar to that mentioned in Table 5.1.3.* [b]*Isolated yield.*

All the products have been characterized by NMR, IR, and ESI-MS. Among all substrates, the product of entry **4c** was confirmed with single crystal XRD (Figure 5.1.4).

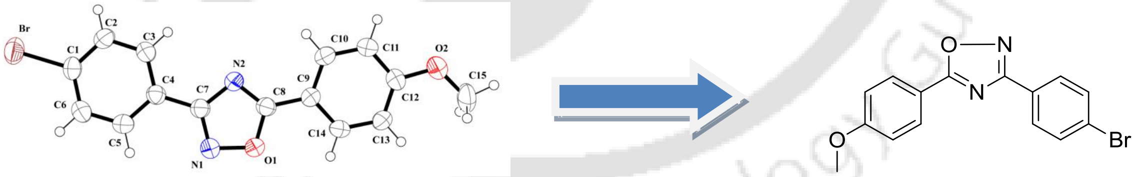
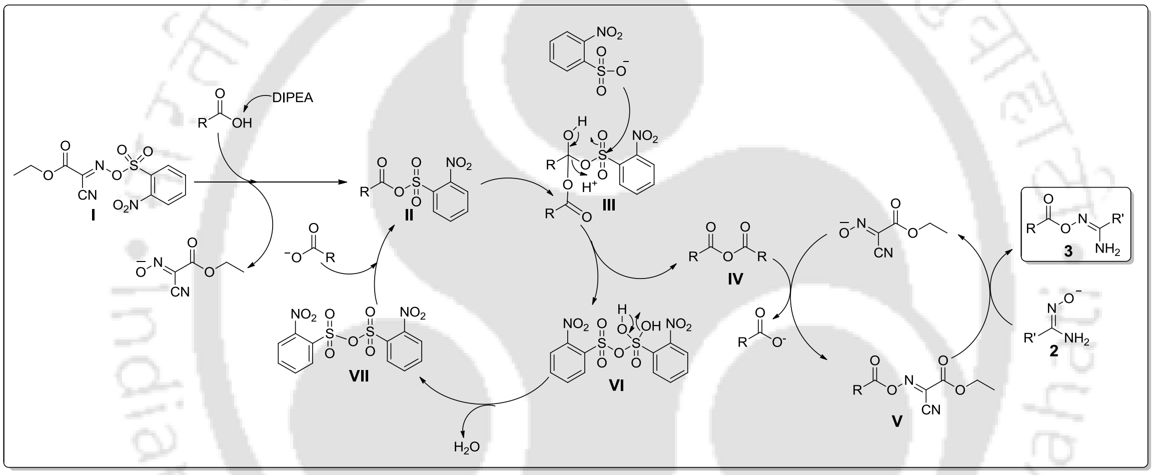


***Figure 5.1.4**. X-ray crystallographic structure of **4c** (ORTEP diagram with ellipsoid of 50% probability, CCDC No. 1043141).*

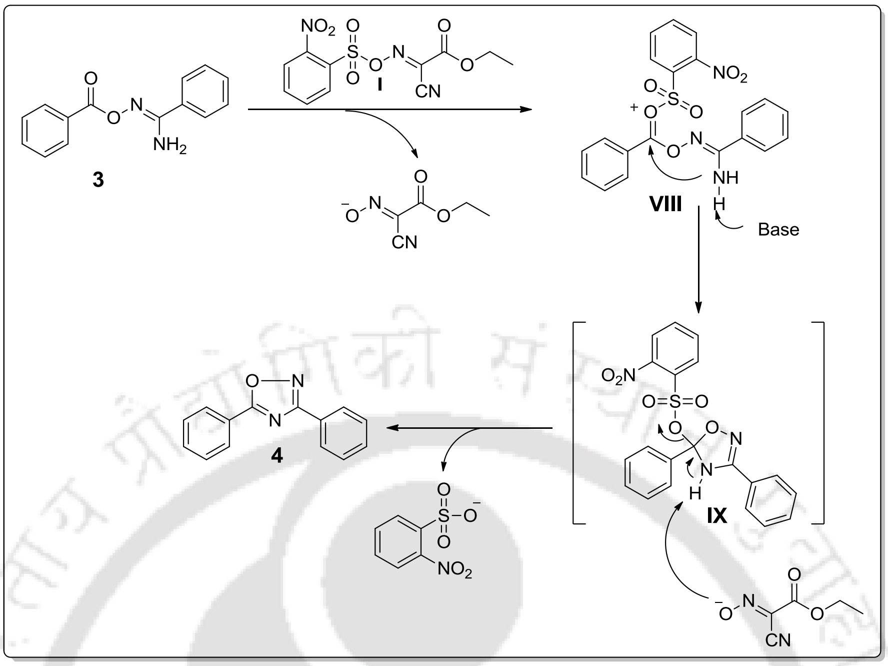
5.2. Plausible Mechanism

A plausible reaction mechanism for the formation of oxadiazole is explained through the formation of *O*-acylamidoxime intermediate (**3**) (Scheme 5.2.1). At first, activation of a carboxylic acid by **I** result in the formation of an acid anhydride of sulphonic acid (**II**) or formation of symmetric anhydride (**IV**). After that the intermediate **V** was generated by further attack of Oxyma anion to the intermediate **IV**. Then **V** reacts with amidoxime **2** and forms an *O*-acylamidoxime intermediate (**3**) which was isolated and characterized by ^{1}H, ^{13}C NMR, and HRMS (Figure S30-S32). According to the mechanistic analysis described in chapter 3, the role of **I** for the conversion of carboxylic acid to *O*-acylamidoxime intermediate (**3**) may be as an initiator. The plausible mechanism drawn in Scheme 5.2.1 is based on that.



Scheme 5.2.1. Catalytic formation of the O-acylamidoxime intermediate 3 using **I**

From the results demonstrated in Figure 5.1.2, we don't see any linear correlation between the amount of **I** and the yield of **4**. That indicates the role of **I** for the conversion of **3** to **4** may be dehydrative cyclization. A plausible mode of action of **I** for the conversion of **3** to **4** is depicted in Scheme 5.2.2. Initially, intermediate **3** reacts with reagent **I** and form intermediate **VIII** with release of Oxyma which was characterized by ESI-MS (Figure S33). After that Oxyma abstracts the hydrogen of intermediate **IX** and form the desired product **4** with release of 2-nitrobenzenesulphonic acid.



Scheme 5.2.2. *A plausible mechanism for the synthesis of 1, 2, 4- oxadiazole **4** from **3** using **I***

5.3. Conclusion

In summary, we have studied the effect of microwave irradiation on the reaction of carboxylic acids with different amidoximes for the synthesis of 1,2,4-oxadiazoles in the presence of **I**. All the substrates reacted smoothly and reaction completed within 5 min at 100 °C. In this method, we found that *ortho*-NosylOXY reagents act as a catalyst and that increases the yield of the reaction. Microwave technology not only increased the yield but also reduced the reaction times from hours to minutes.

5.4. Experimental Section

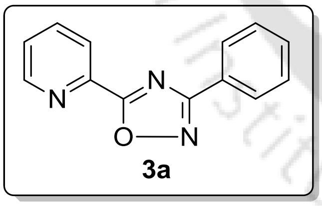
5.4.1. Materials and methods

As described in chapter 2 section 2.4.1

5.4.2. General procedure for the synthesis of 1, 2, 4-Oxadiazoles from carboxylic acids (one-pot protocol): *o*-NosylOXY (0.2 mmol) was added to a stirred solution of carboxylic acid (0.2 mmol), and DIPEA (0.6 mmol) in Acetonitrile (2 mL) at room temperature. To above solutions, Amidoximes (0.2 mmol) was added. The reaction vessel was sealed and heated in the microwave at 100 °C for 5 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary evaporator and then diluted with ethyl acetate, washed with 5% HCl (2×10 mL), 5% $NaHCO_3$ (2×10 mL), saturated NaCl solution (2×10 mL) and dried over anhydrous Na_2SO_4. The obtained residue was purified by silica gel column chromatography using ethyl acetate and hexane in the ratio of 2:8.

5.5. Characterization Data

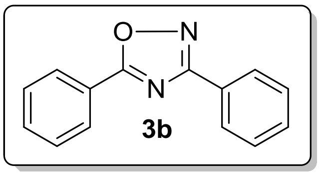
3-Phenyl-5-(pyridin-2-yl)-1,2,4-oxadiazole (3a)



3a

Solid; (192 mg, 86 %), mp 113 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86-8.85 (m, 1H), 8.30-8.20 (m, 3H), 7.94-7.90 (m,1H), 7.53-7.48 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 169.4, 150.8, 143.8, 137.5, 131.5, 129.0, 127.8, 126.8, 124.4; IR (KBr): 1647, 1569, 1446, 1364, 1175, 1072, 717, 693 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{10}N_3O$ 224.0824, found 224.0836.

3, 5-Diphenyl-1,2,4-oxadiazole (3b)

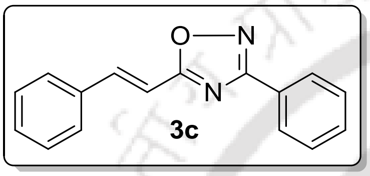


3b

Solid; (435 mg, 98 %), mp 108-109 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.08 (d, *J* = 7.6 Hz, 2H), 7.77-7.75 (d, *J* = 7.6

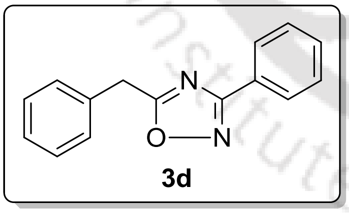
Hz, 2H), 7.61-7.58 (t, *J* = 7.2 Hz, 1H), 7.49-7.41 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 157.5, 133.2, 131.3, 129.7, 128.9, 128.7, 127.0; IR (KBr): 2985, 1636, 1542, 1353, 1243, 1130, 1032, 665 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{11}N_2O$ 223.0871, found 223.0880.

(*E*)-3-Phenyl-5-styryl-1,2,4-oxadiazole (3c)


3c

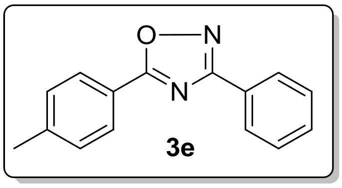
Solid; (313 mg, 97%); mp 95 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14-8.13 (m, 2H), 7.91- 7.88 (d, *J* = 16.2 Hz, 1H), 7.63-7.61 (m, 2H), 7.54-7.49 (m, 3H), 7.46-7.42 (m, 3H), 7.09-7.07 (d, *J* = 16.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.5, 169.0, 142.9, 134.7, 131.4, 130.7, 129.3, 129.1, 128.1, 127.7, 127.2, 110.5; IR (KBr): 2923, 1644, 1578, 1540, 1444, 1361, 1294, 1118, 963, 761, 695 cm^{-1}; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{16}H_{13}N_2O$ 249.1028, found 249.1080.

5-Benzyl-3-phenyl-1,2,4-oxadiazole (3d)


3d

Oil; (498 mg, 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-8.06 (d, *J* = 6.0 Hz, 2H), 7.47-7.25 (m, 8H), 4.29 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 163.5, 133.7, 131.3, 129.1, 129.0, 127.8, 127.6, 127.0, 33.2; IR (KBr): 2925, 1647, 1594, 1446, 1364, 1175, 1072, 717, 693 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{12}N_2O$ 237.1028, found 237.1029.

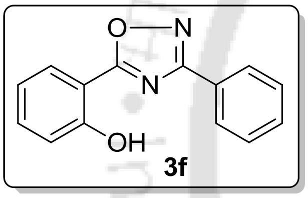
3-Phenyl-5-(p-tolyl)-1,2,4-oxadiazole(3e)



3e

Solid; (403 mg, 95%); mp 115-117 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 2H), 8.12-8.10 (d, *J* = 7.6 Hz, 2H), 7.51 (s, 3H), 7.36-7.34 (d, *J* = 7.6 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 169.1, 143.7, 131.3, 130.0, 129.0, 128.3, 127.7, 121.8, 22.0; IR (KBr): 1628, 1593, 1527, 1445, 1371, 1294, 1118, 968, 791, 698 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{13}N_2O$ 237.1028, found 237.1044.

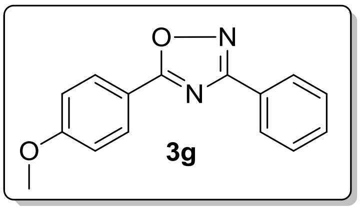
2-(3-Phenyl-1,2,4-oxadiazol-5-yl)phenol (3f)



3f

Solid; (385 mg, 90%); mp 155-156 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14-8.11 (m, 2H), 8.01-7.98 (m, 1H), 7.58-7.50 (m, 4H), 7.16- 7.14 (d, *J* = 8.0 Hz, 1H), 7.06-7.02 (t, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 167.2, 158.3, 135.4, 131.9, 129.2, 128.0, 127.7, 126.0, 120.3, 118.0, 108.3; IR (KBr): 1628, 1593, 1551, 1445, 1361, 1294, 1118, 963, 761, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{11}N_2O_2$ 239.0821, found 239.0830.

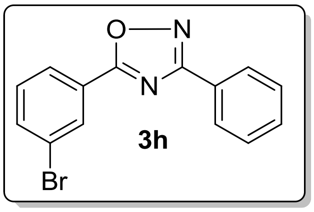
5-(4-Methoxyphenyl)-3-phenyl-1,2,4-oxadiazole (3g)



3g

Solid; (359 mg, 89%); mp 96-98 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17-8.15 (d, *J* = 6.4 Hz, 4H), 7.51 (s, 3H), 7.04-7.02 (d, *J* = 8.0 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 169.0, 163.4, 131.3, 130.3, 129.0, 127.7, 127.3, 117.1, 114.7, 55.7; IR (KBr): 2840, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 761, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found 253.0963.

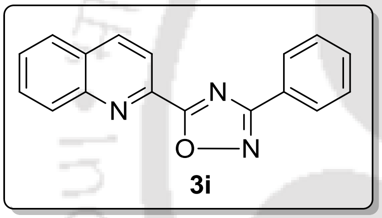
5-(3-Bromophenyl)-3-phenyl-1,2,4-oxadiazole (3h)



3h

Solid; (288 mg, 80%); mp 105-107 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39-8.38 (m, 1H), 8.18-8.15 (m, 3H), 7.75-7.73 (m, 1H), 7.54- 7.50 (m, 3H), 7.46- 7.43 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 169.3, 135.9, 131.6, 131.3, 130.9, 129.1, 127.8, 126.9, 126.3, 123.4; IR (KBr): 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 750, 695, 525 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{10}BrN_2O$ 300.9977, found 300.9982.

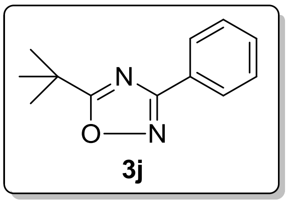
3-Phenyl-5-(quinolin-3-yl)-1,2,4-oxadiazole (3i)



3i

Oil; (408 mg, 88%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42-8.35 (m, 3H), 8.26-8.24 (m, 2H), 7.93-7.91 (d, *J* = 8.0 Hz, 1H), 7.86-7.82 (m, 1H), 7.70- 7.67 (t, *J* = 8.0 Hz, 1H), 7.54- 7.52 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.5, 148.3, 143.6, 137.9, 131.6, 131.0, 130.8, 129.3, 129.1, 129.0, 127.9, 126.8, 120.6; IR (KBr): 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 761, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{12}N_3O$ 274.0980, found 274.0980.

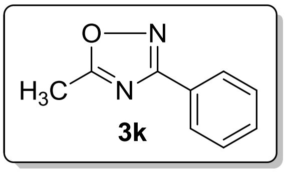
5-(tert-Butyl)-3-phenyl-1,2,4-oxadiazole (3j)



3j

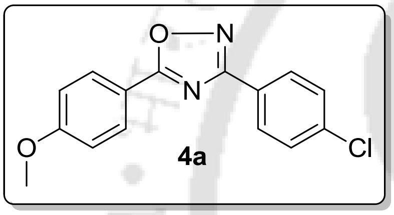
Oil; (239 mg, 79%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 2H), 7.38 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.4, 168.3, 131.1, 128.9, 127.6, 127.4, 33.8, 28.6; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 965, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{12}H_{15}N_2O$ 203.1184, found 203.1184.

5-Methyl-3-phenyl-1,2,4-oxadiazole (3k)



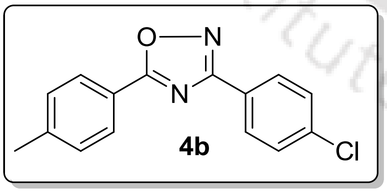
Solid; (384 mg, 80%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06-8.05 (d, *J* = 8.0Hz, 2H), 7.49-7.48 (m, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 168.7, 131.3, 129.1, 127.6, 127.0, 12.6; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1370, 1294, 1118, 1077, 965, 761, 690 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_9H_9N_2O$ 161.0715, found 161.0715.

3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-1,2,4-oxadiazole (4a)



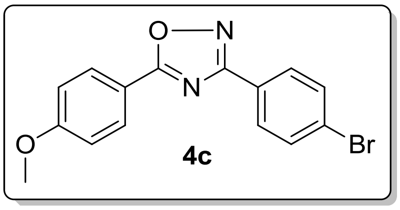
Solid; (370 mg, 81%); mp 142-150 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-8.08 (m, 4H), 7.48-7.46 (d, *J* = 8.4 Hz, 2H), 7.04-7.02 (d, *J* = 8.4 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 168.2, 163.5, 137.4, 130.3, 129.3, 129.0, 125.9, 116.9, 114.7, 55.7; IR (KBr): 2840, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 756, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{12}ClN_2O_2$ 287.0587, found 287.0560.

3-(4-Chlorophenyl)-5-(p-tolyl)-1,2,4-oxadiazole (4b)



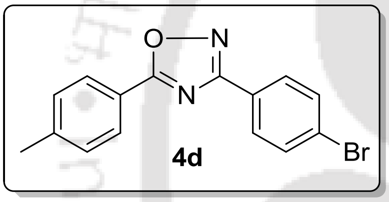
Solid; (157 mg, 83%); mp 126-131 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12-8.08 (t, *J* = 8.0 Hz, 4H), 7.49-7.47 (d, *J* = 8.4 Hz, 2H), 7.36- 7.34 (d, *J* = 8.0 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 168.3, 143.9, 137.5, 130.1, 129.4, 129.0, 128.4, 125.8, 121.6, 22.0; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 756, 695 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{12}ClN_2O$ 271.0638, found 271.0631.

3-(4-Bromophenyl)-5-(4-methoxyphenyl)-1,2,4-oxadiazole (4c)


4c

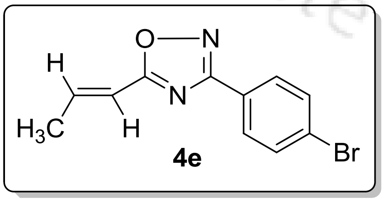
Oil; (264 mg, 80%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15-8.13 (d, *J* = 8.0 Hz, 2H), 8.04-8.02 (d, *J* = 7.6 Hz, 2H), 7.64-7.62 (d, *J* = 8.4 Hz, 2H), 7.04-7.02 (d, *J* = 8.0 Hz, 2H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 176.0, 168.3, 163.5, 132.3, 130.3, 129.2, 126.3, 125.8, 116.9, 114.8, 55.7; IR (KBr): 2840, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 761, 695, 525 cm^{-1}.

3-(4-Bromophenyl)-5-(p-tolyl)-1,2,4-oxadiazole (4d)


4d

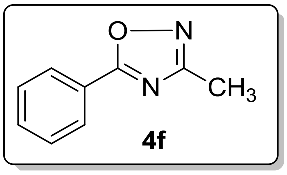
Solid; (180 mg, 82%); mp 146 ºC; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.02-7.96 (m, 4H), 7.58-7.56 (d, *J* = 8.4 Hz, 2H), 7.29-2.27 (d, *J* = 8.0 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 176.3, 168.4, 143.9, 132.3, 130.1, 129.2, 128.4, 126.3, 125.9, 121.6, 22.0; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 963, 761, 695, 525 cm^{-1}; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{15}H_{12}BrN_2O$ 315.0133, found 315.0124.

(*E*)-3-(4-Bromophenyl)-5-(prop-1-en-1-yl)-1,2,4-oxadiazole (4e)


4e

Solid; (198 mg, 75%); mp 143 ºC; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.96-7.94 (m, 2H), 7.63-7.60 (m, 2H), 7.19-7.13 (m, 1H), 6.48-6.45 (m, 1H), 2.05-2.03 (m, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 175.2, 168.0, 143.4, 132.3, 129.1, 126.2, 125.8, 115.1, 19.1; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1361, 1294, 1118, 965, 761, 695, 525 cm^{-1}; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{10}BrN_2O$ 264.9977, found 264.9971.

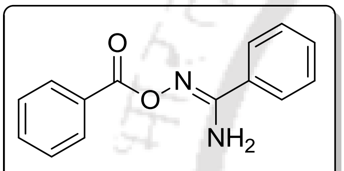
3-Methyl-5-phenyl-1,2,4-oxadiazole (4f)



4f

Oil; (199 mg, 78%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03-8.01 (d, *J* = 8.0 Hz, 2H), 7.51-7.41 (m, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 167.9, 132.8, 129.2, 128.1, 124.3, 11.8; IR (KBr): 2870, 1618, 1567, 1528, 1445, 1370, 1294, 1118, 965, 761, 695 cm^{-1}; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_9H_8N_2NaO$ 183.0534, found 183.1127.

(*Z*)-*N'*-(Benzoyloxy) benzimidamide, Intermediate 3



Intermediate **3**

Solid; (216 mg, 90%); mp = 95-97 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.08 (d, *J* = 7.6 Hz, 2H), 7.77-7.75 (d, *J* = 7.6 Hz, 2H), 7.61-7.58 (t, *J* = 7.2 Hz, 1H), 7.49-7.41 (m, 5H), 5.21 (br, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.2, 157.5, 133.2, 131.3, 131.2, 131.1, 129.8, 129.7, 129.0, 128.9, 128.7, 127.0, 126.9; IR (KBr): 3380, 1729, 1613, 1583, 1268, 1092, 702 cm^{-1}; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_2$ 241.0977, found 241.0975.

5.6. References

1. (a) Rebecca F Poulain, Andre L Tartar and Benoit P Deprez. Parallel synthesis of 1,2,4-oxadiazoles from carboxylic acids using an improved, uronium-based, activation. *Tetrahedron Lett*. **2001**, *42,* 1495-1498; (b) F. Eloy and R. Lenaers. The Chemistry of Amidoximes and Related Compounds. *Chem. Rev*. **1962**, *62*, 155-183.

2. J. T. Palmer, R. M. Rydzewski, R. V. Mendonca, D. Sperandio, J. R. Spencer, B. L. Hirschbein, J. Lohman, J. Beltman, M. Nguyen and L. Liu. Design. Synthesis of selective keto-1,2,4-oxadiazole-based tryptase inhibitors. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 3434-3439.

5.7. Selected spectra and chromatograms

5.7.1. ^{1}H NMR and ^{13}C NMR of selected compounds

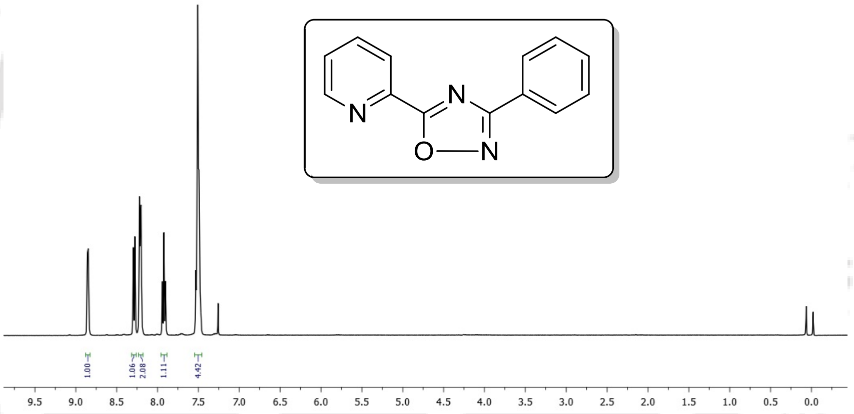


Figure S1. ^{1}H NMR spectra of compound 3a

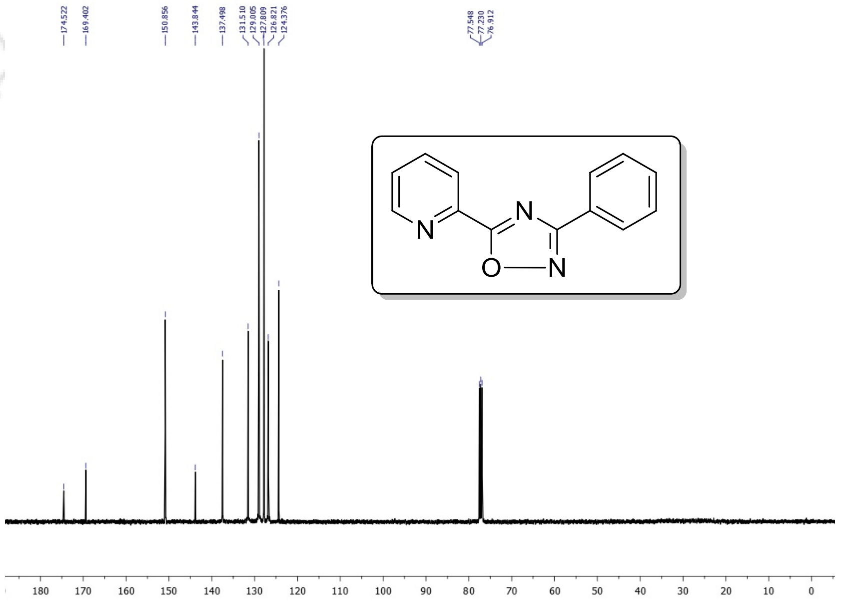


Figure S2. ^{13}C NMR spectra of compound 3a

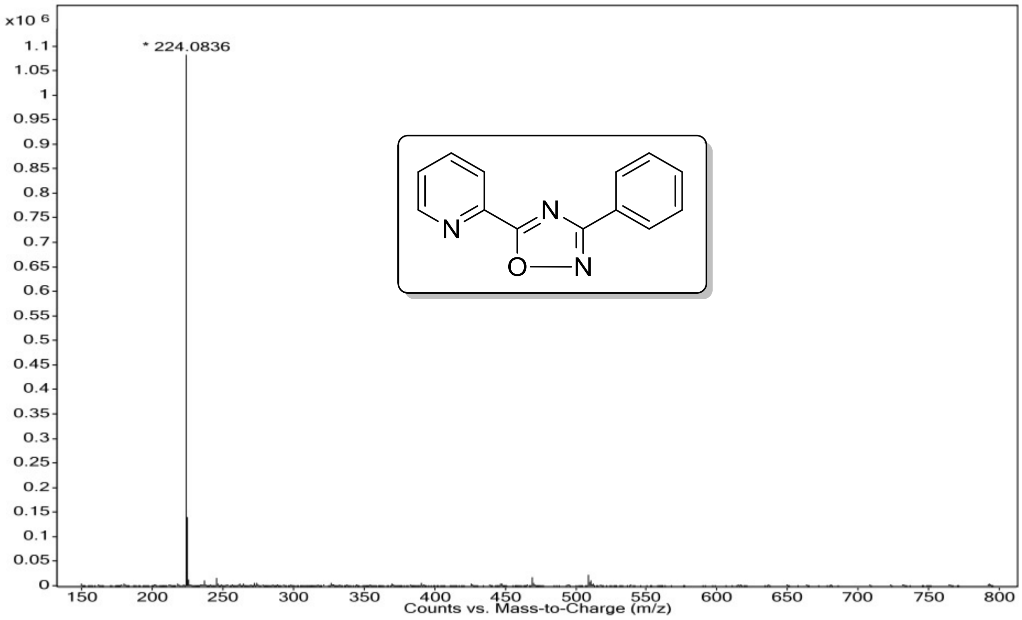


Figure S3. Mass spectra of compound 3a

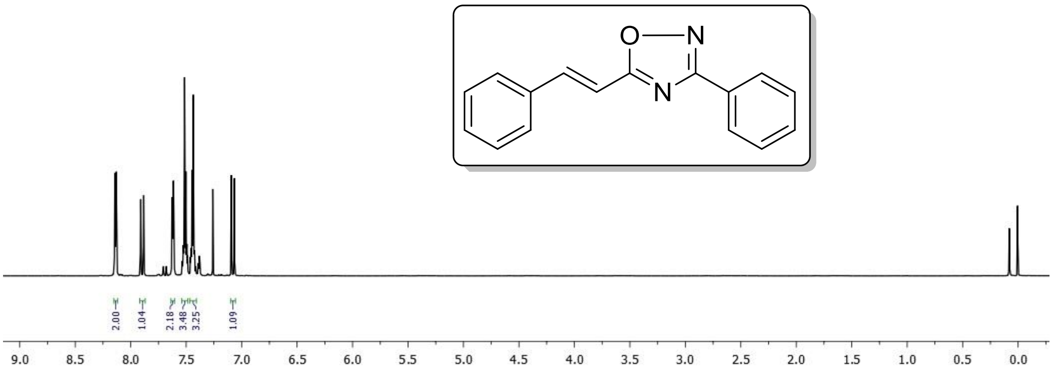


Figure S4. ^{1}H NMR spectra of compound 3c

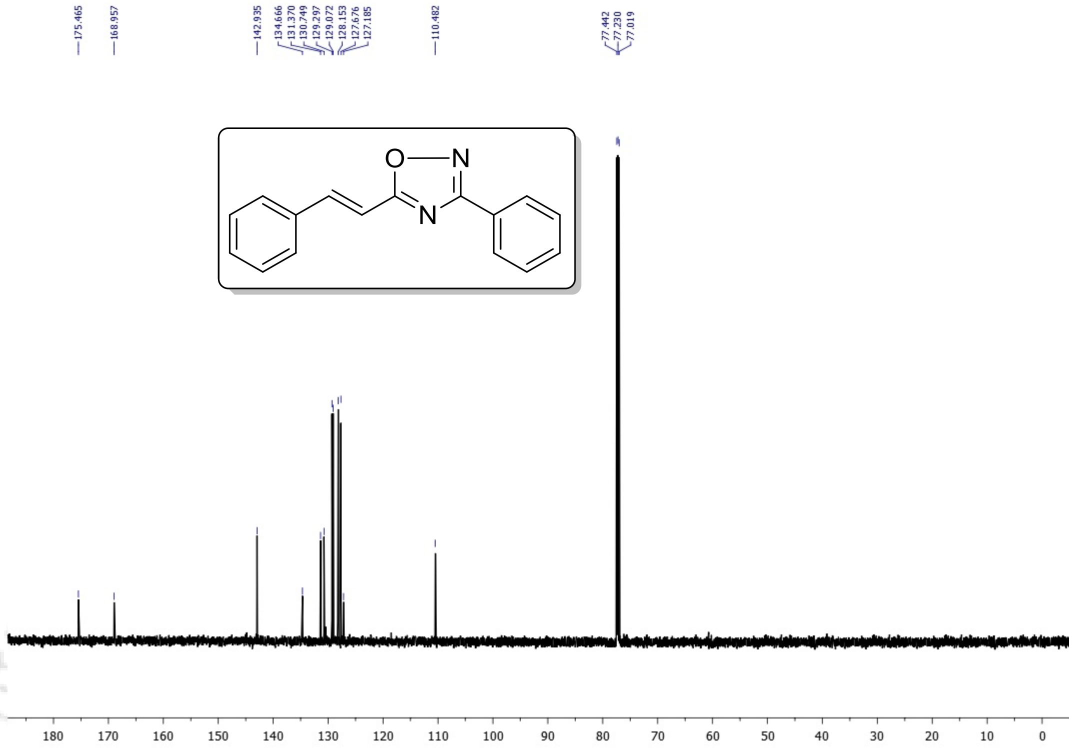


Figure S5. ^{13}C NMR spectra of compound 3c

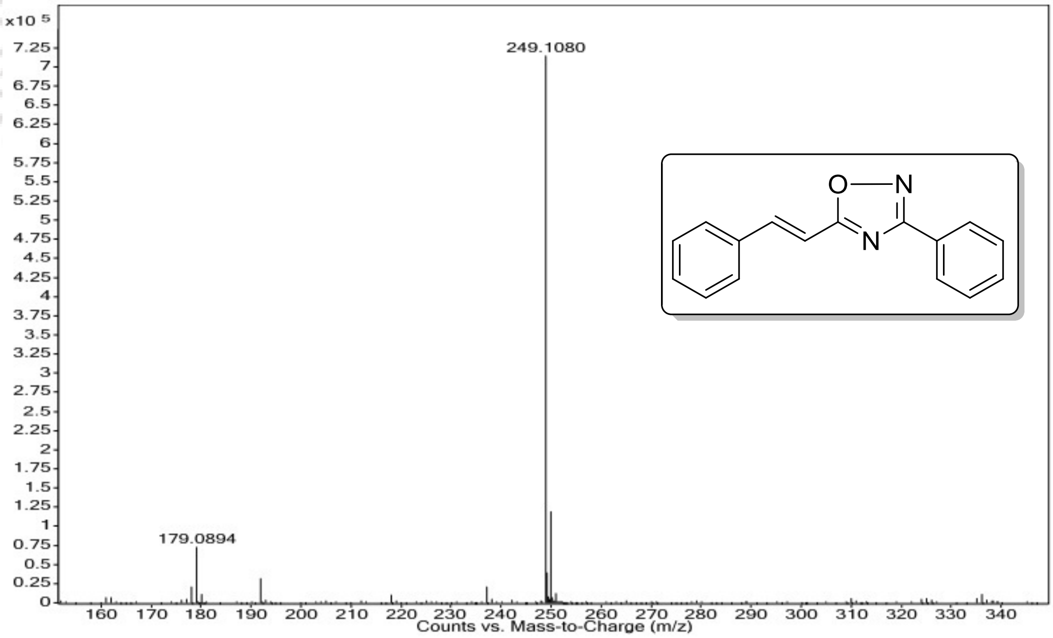


Figure S6. Mass spectra of compound 3c

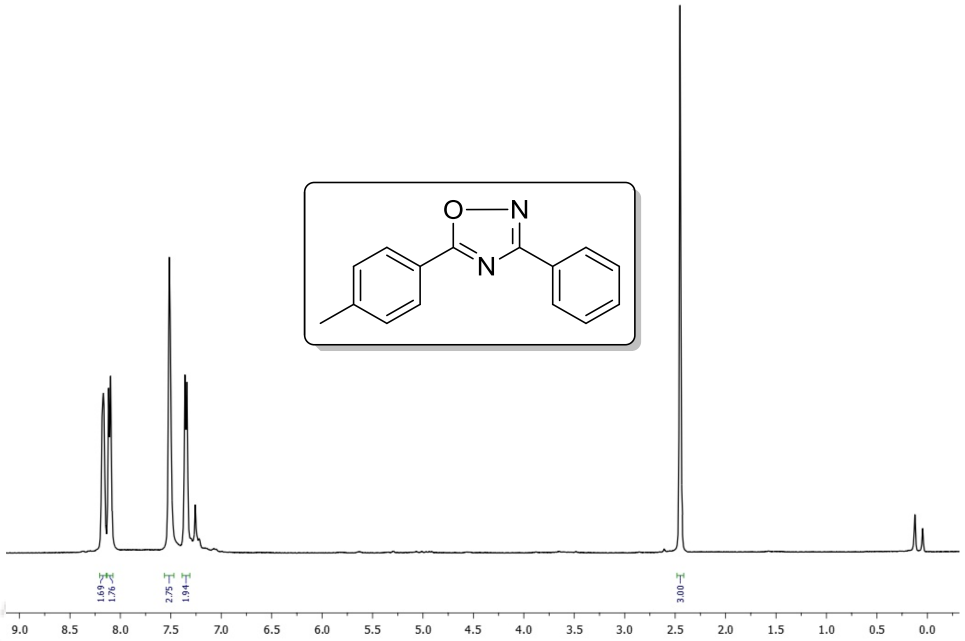


Figure S7. ^{1}H NMR spectra of compound 3e

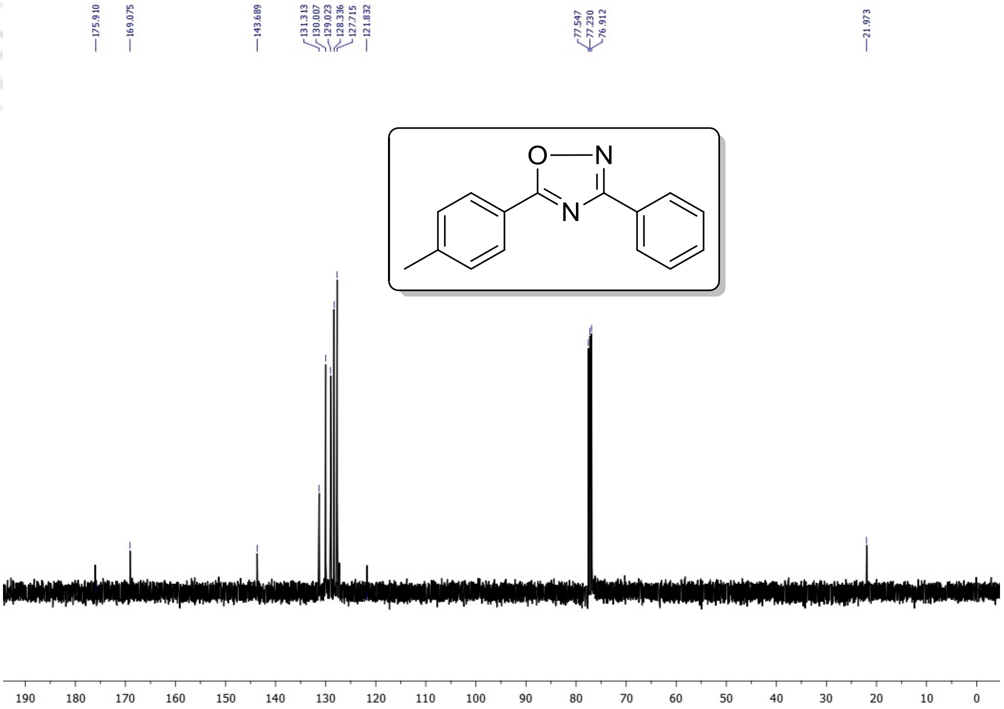


Figure S8. ^{13}C NMR spectra of compound 3e

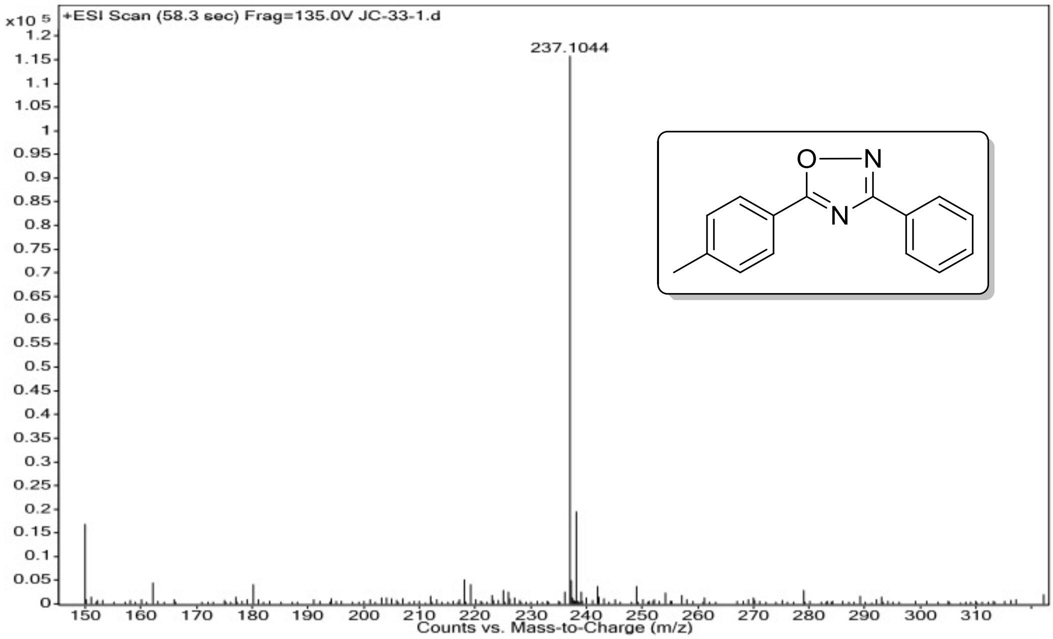


Figure S9. Mass spectra of compound 3e

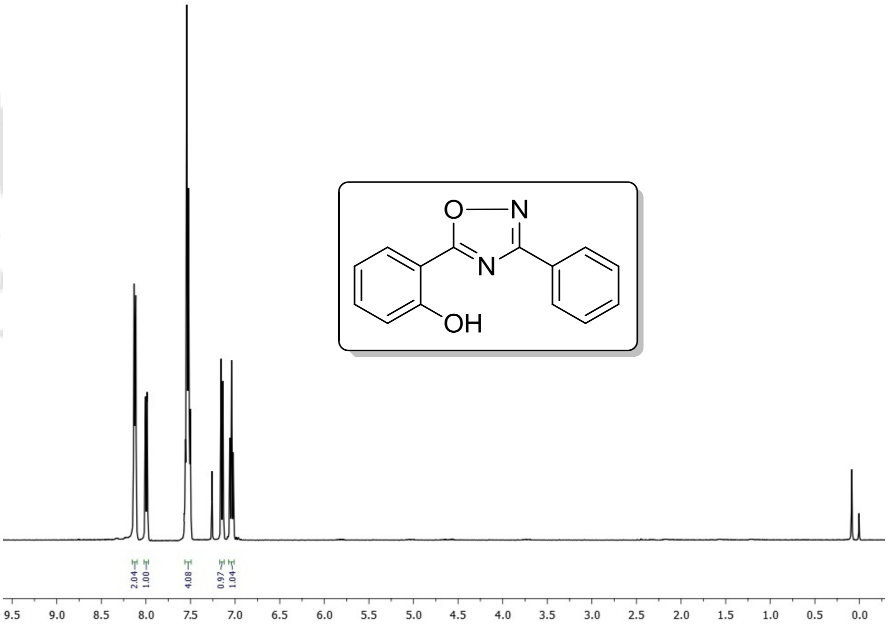


Figure S10. ^{1}H NMR spectra of compound 3f

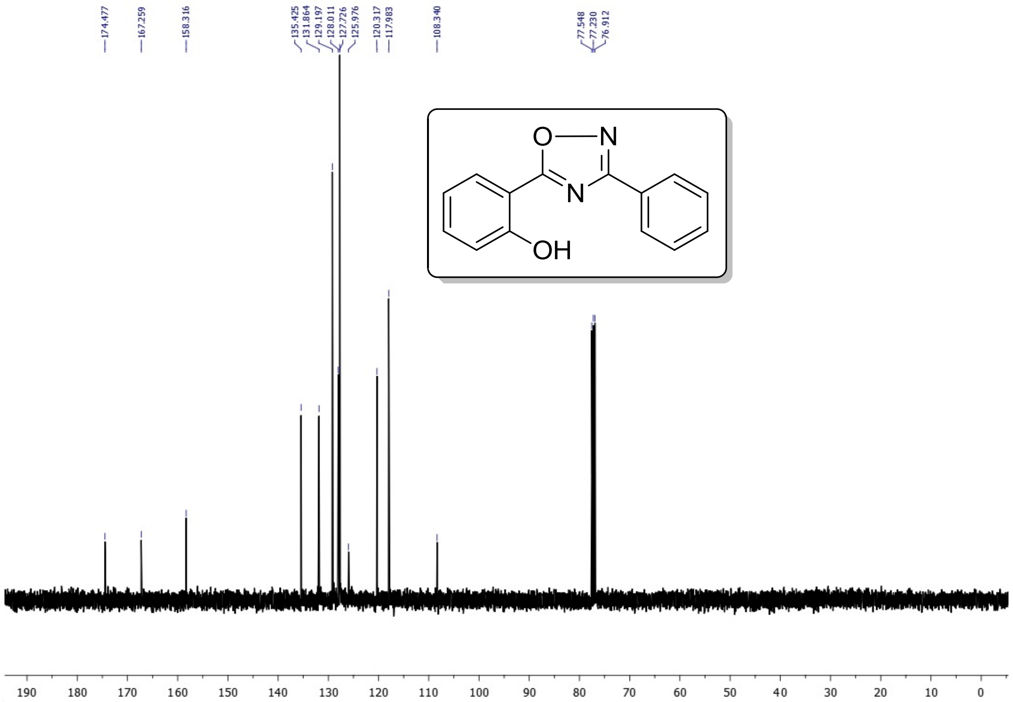


Figure S11. ^{13}C NMR spectra of compound 3f

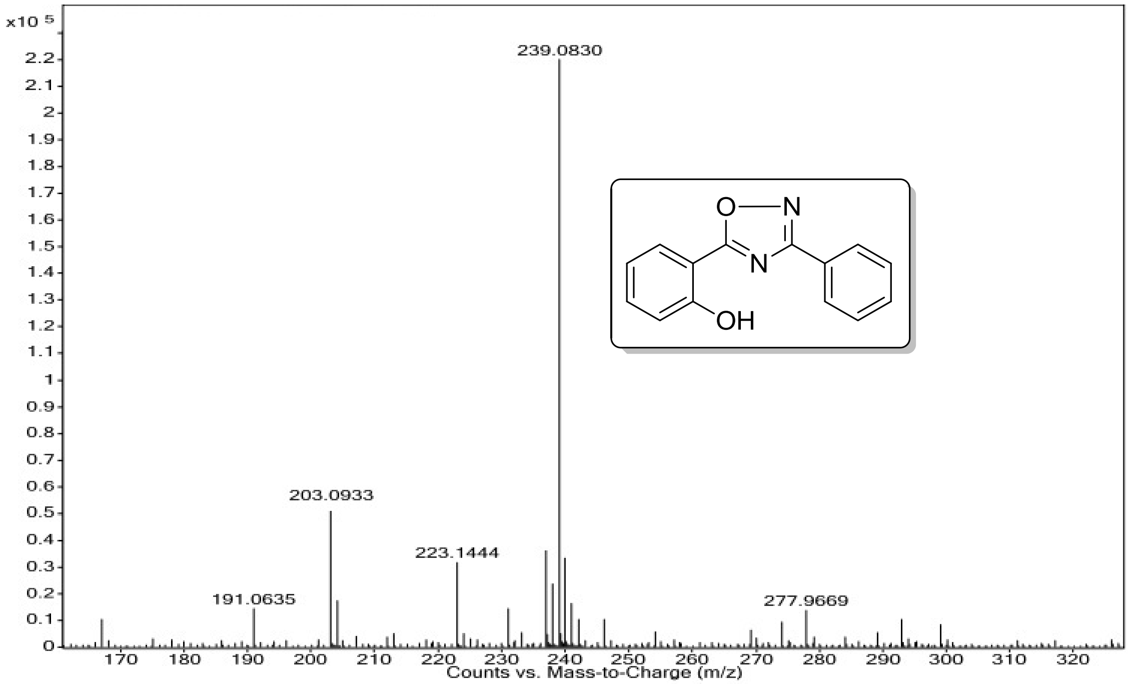


Figure S12. Mass spectra of compound 3f

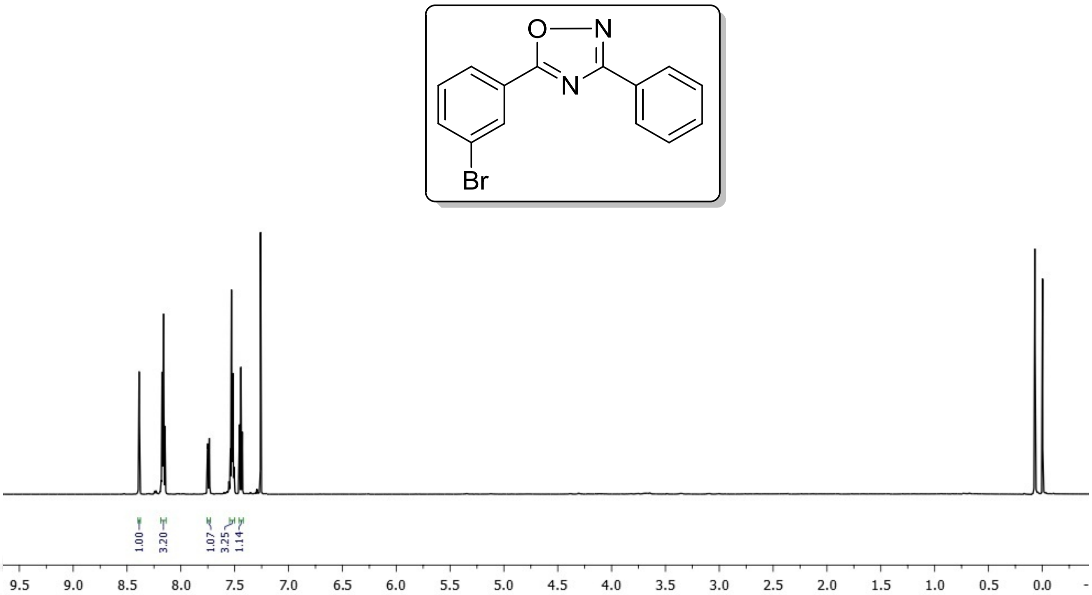


Figure S13. ^{1}HNMR spectra of compound 3h

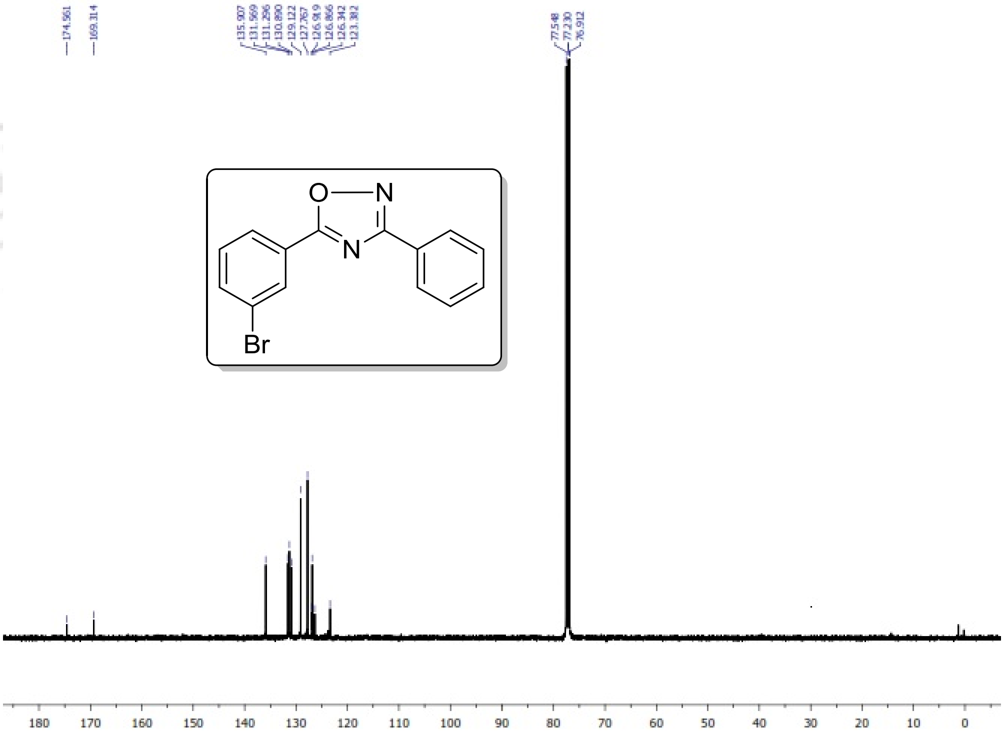


Figure S14. ^{13}C NMR spectra of compound 3h

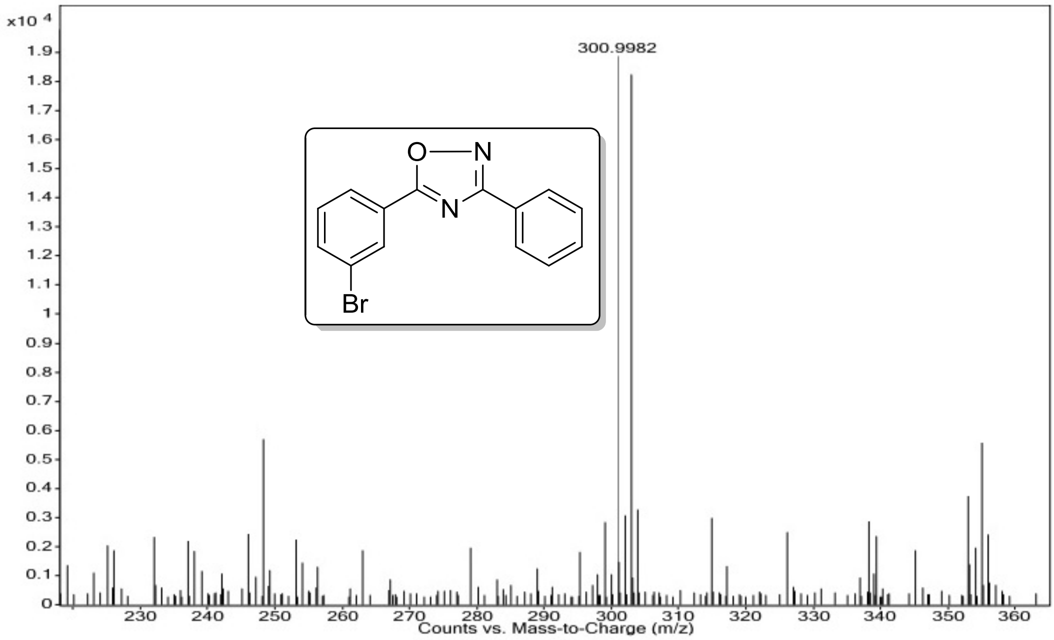


Figure S15. Mass spectra of compound 3h

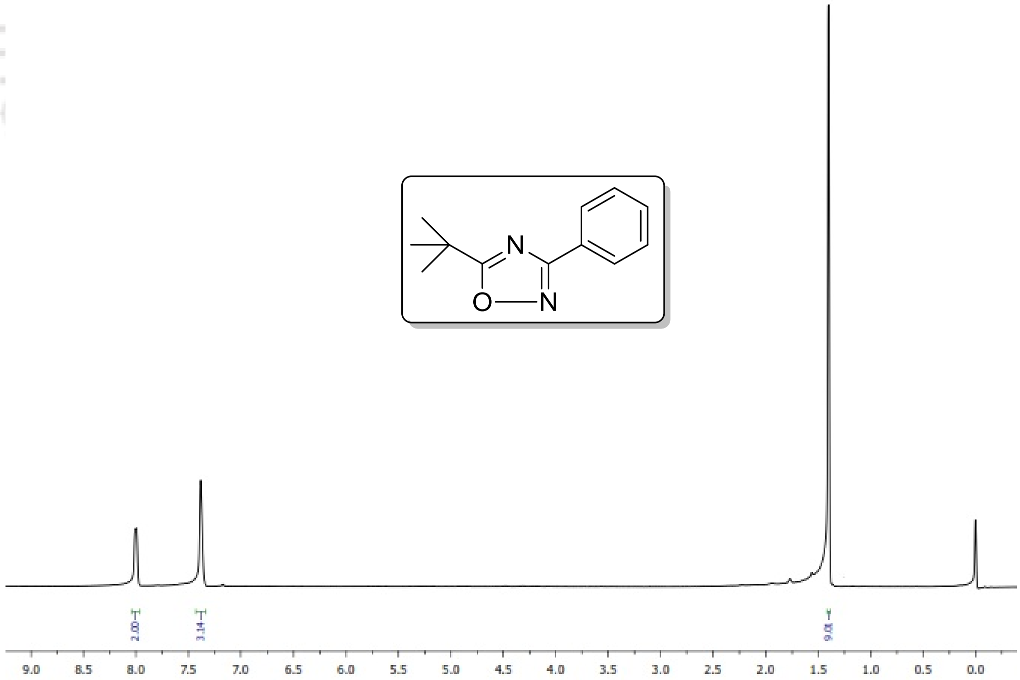


Figure S16. ^{1}H NMR spectra of compound 3j

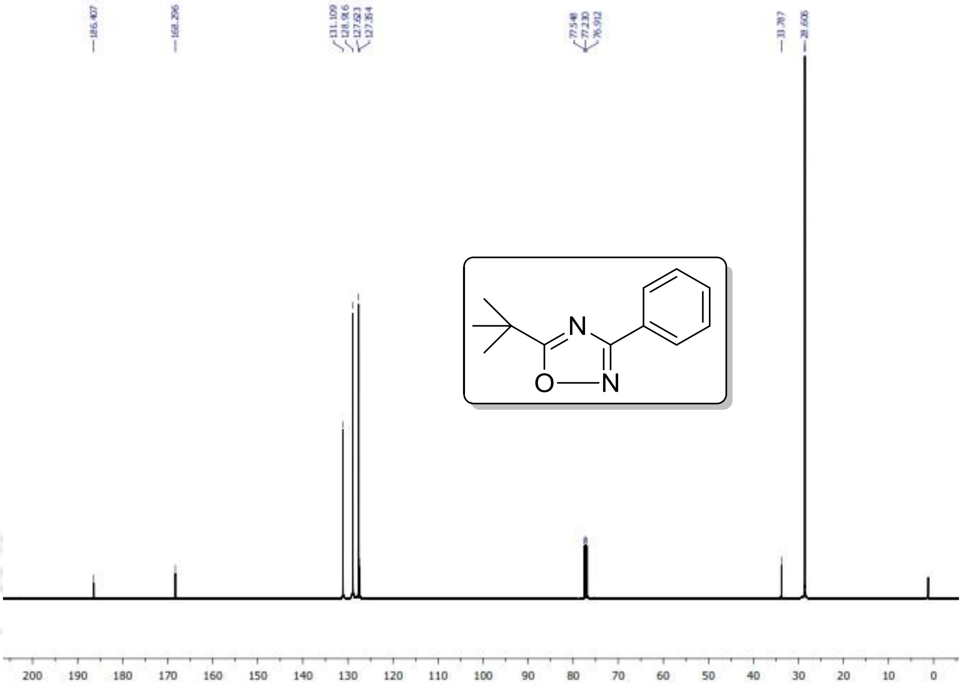


Figure S17. ^{13}C NMR spectra of compound 3j

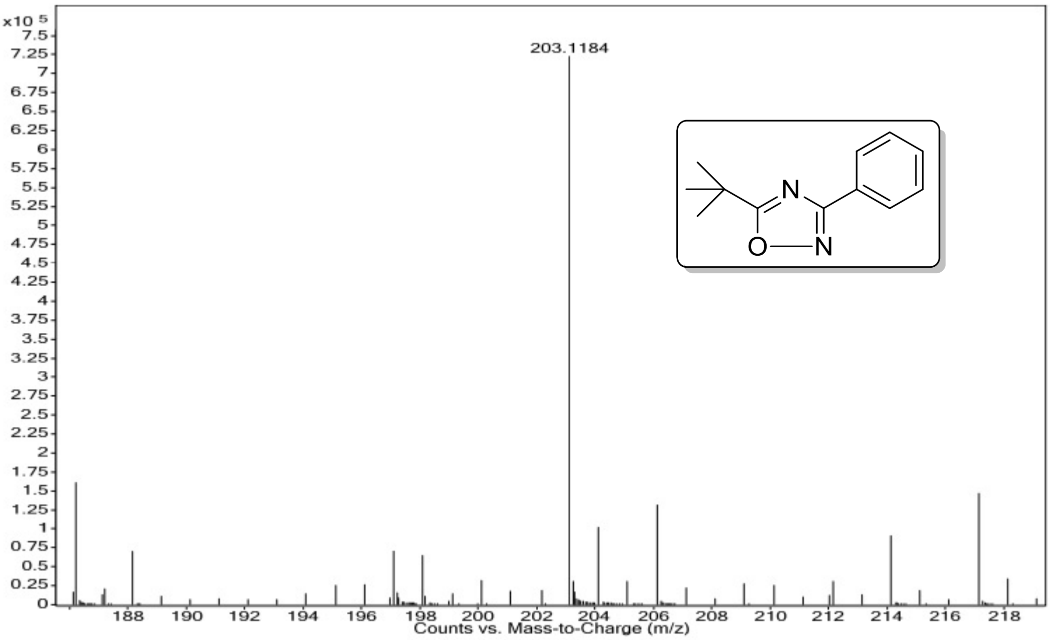


Figure S18. Mass spectra of compound 3j

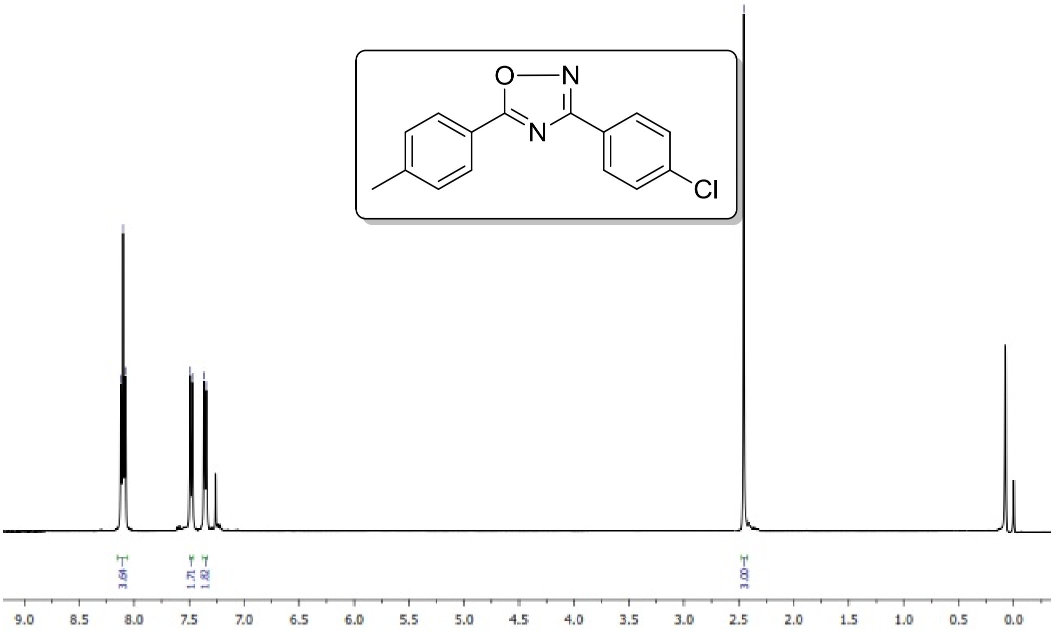


Figure S19. ^{1}H NMR spectra of compound 4b

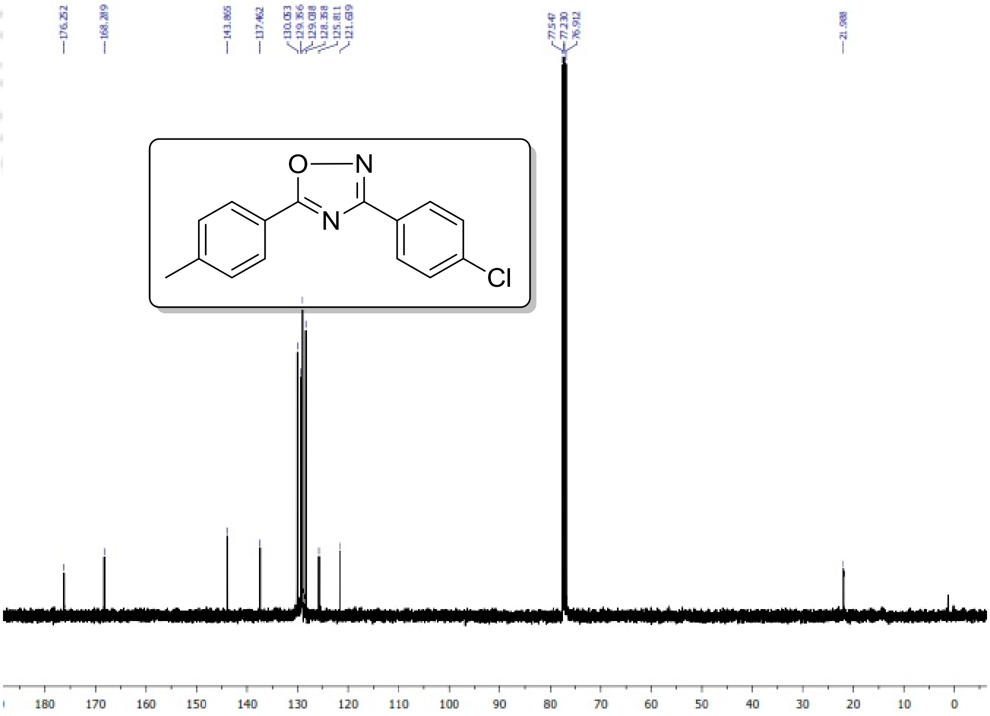


Figure S20. ^{13}C NMR spectra of compound 4b

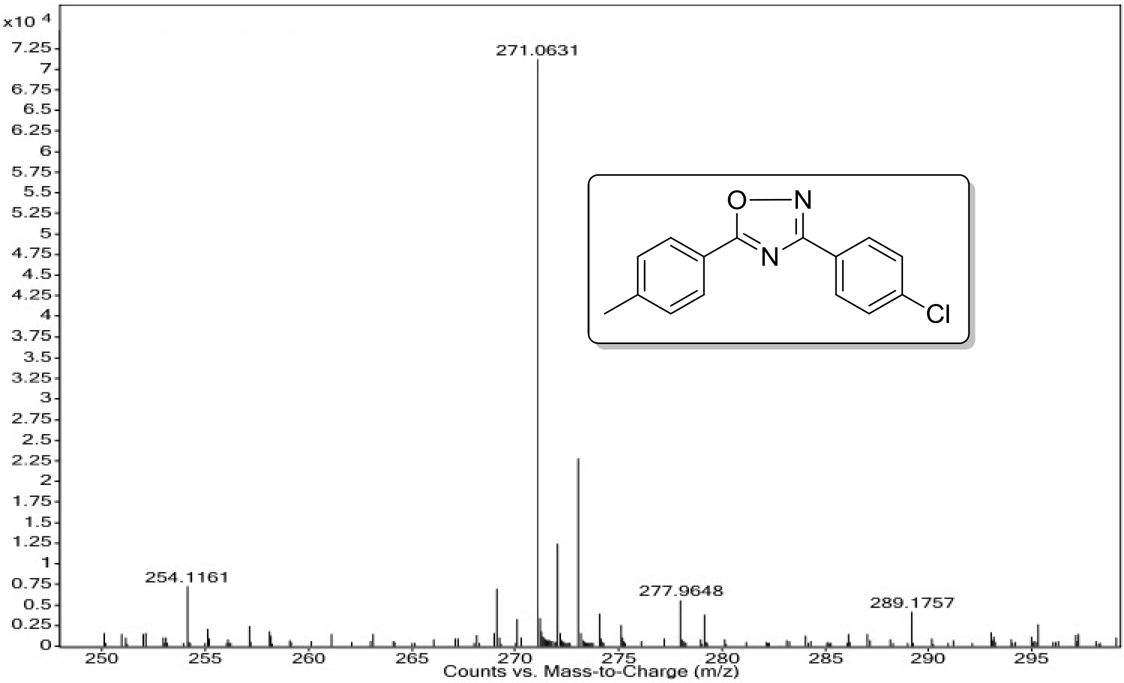


Figure S21. Mass spectra of compound 4b

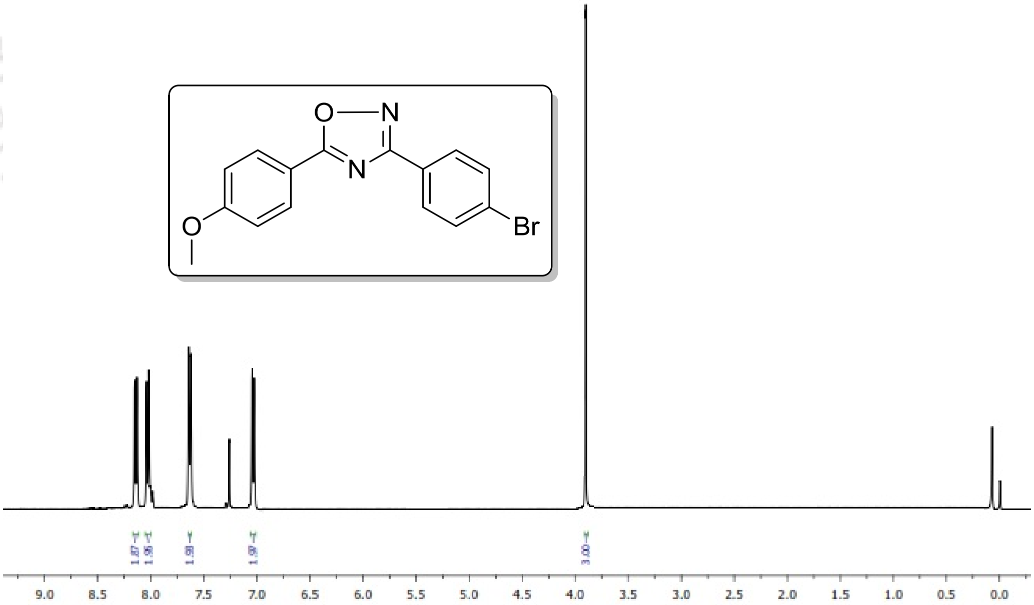


Figure S22. ^{1}H NMR spectra of compound 4c

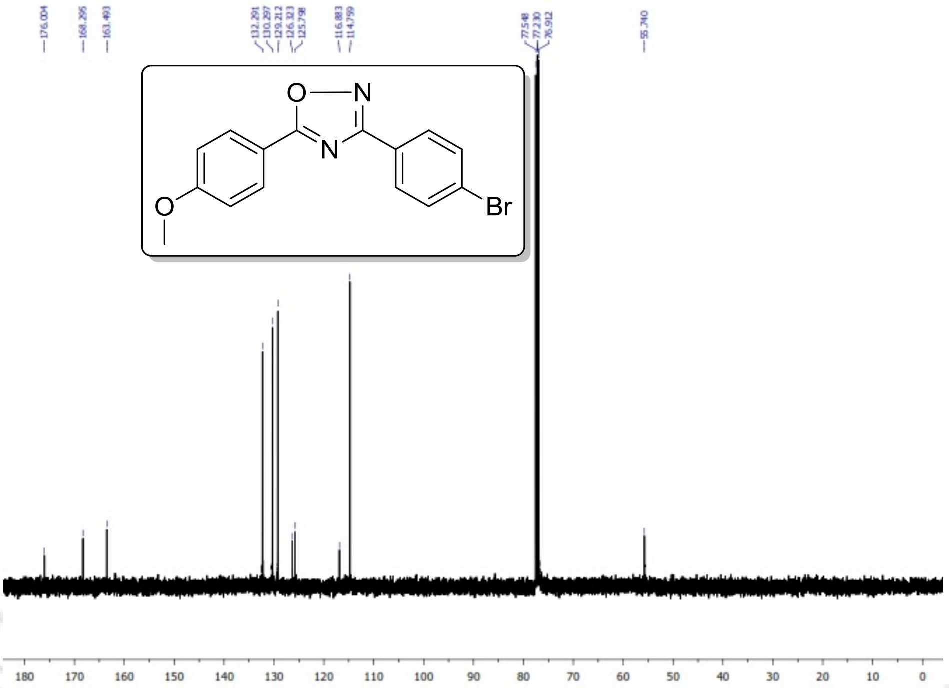


Figure S23. ^{13}C NMR spectra of compound 4c

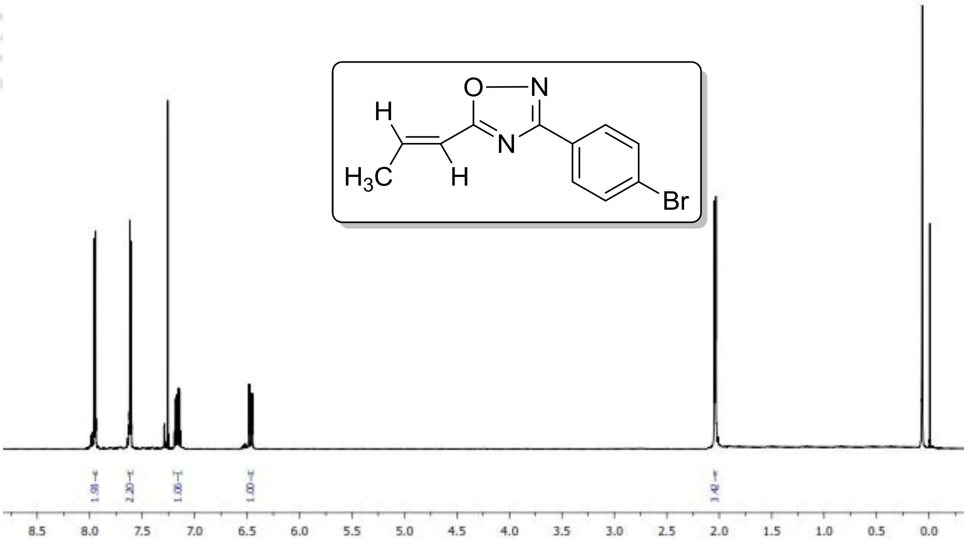


Figure S24. ^{1}H NMR spectra of compound 4e

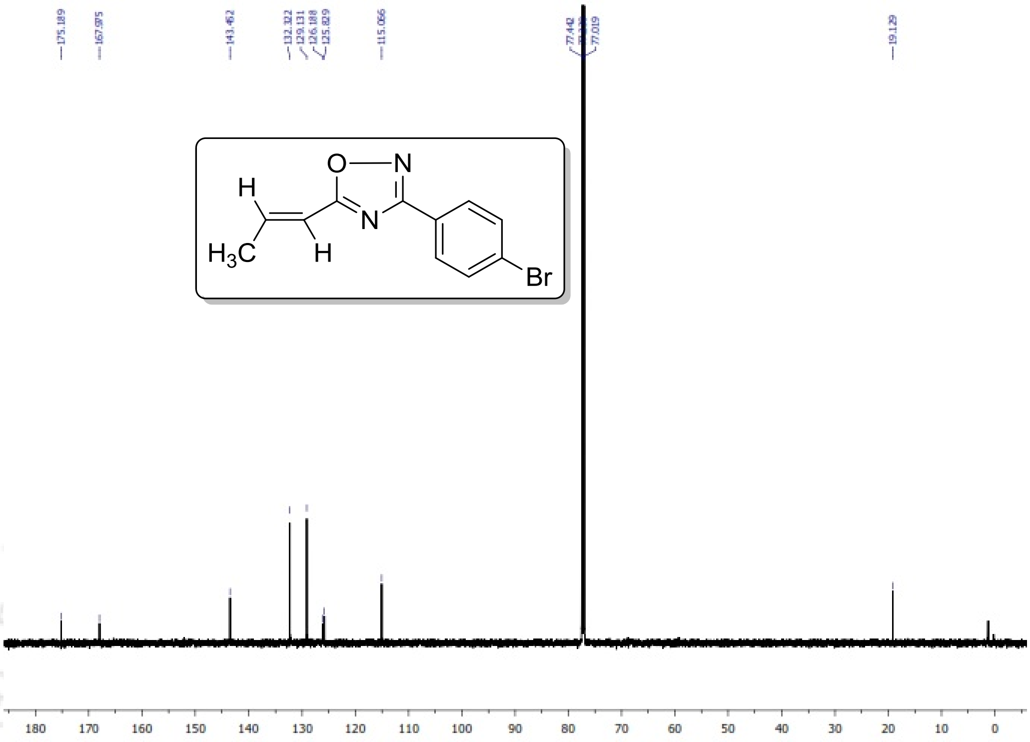


Figure S25. ^{13}C NMR spectra of compound 4e

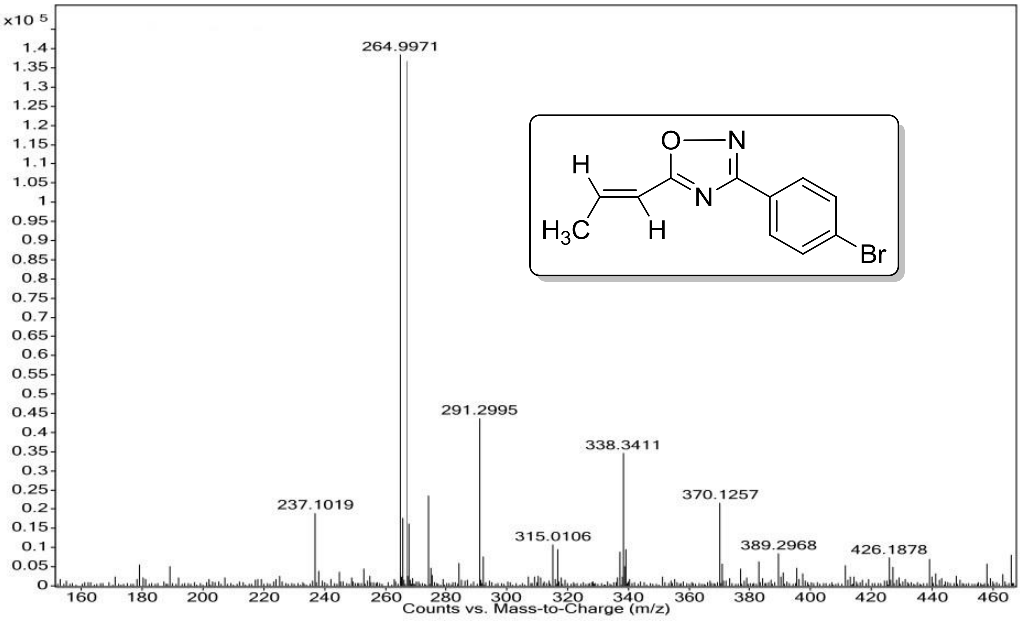


Figure S26. Mass spectra of compound 4e

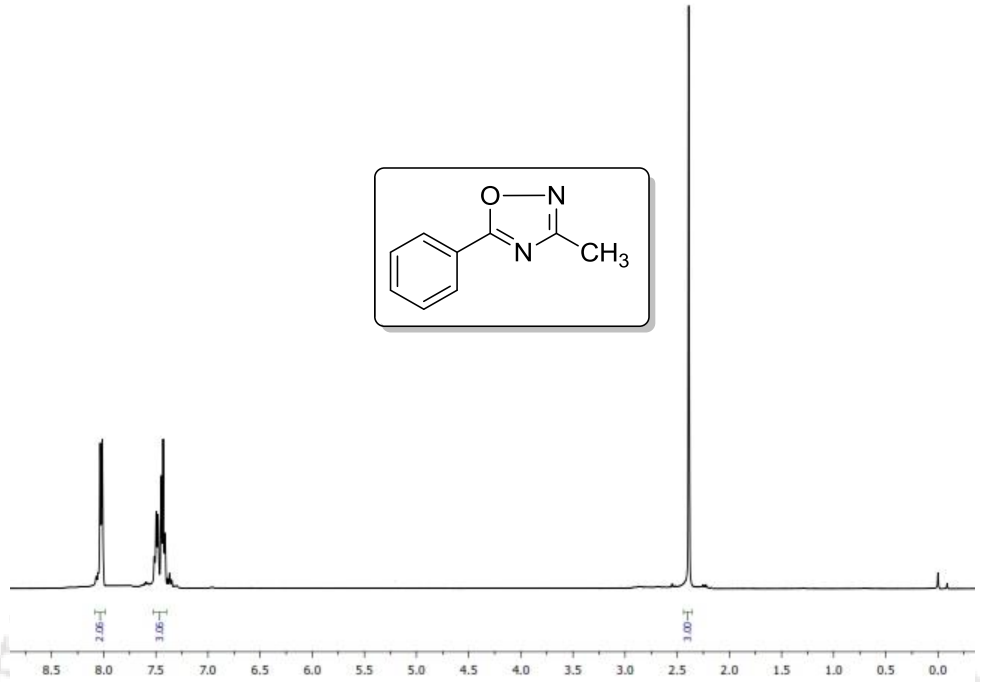


Figure S27. ^{1}H NMR spectra of compound 4f

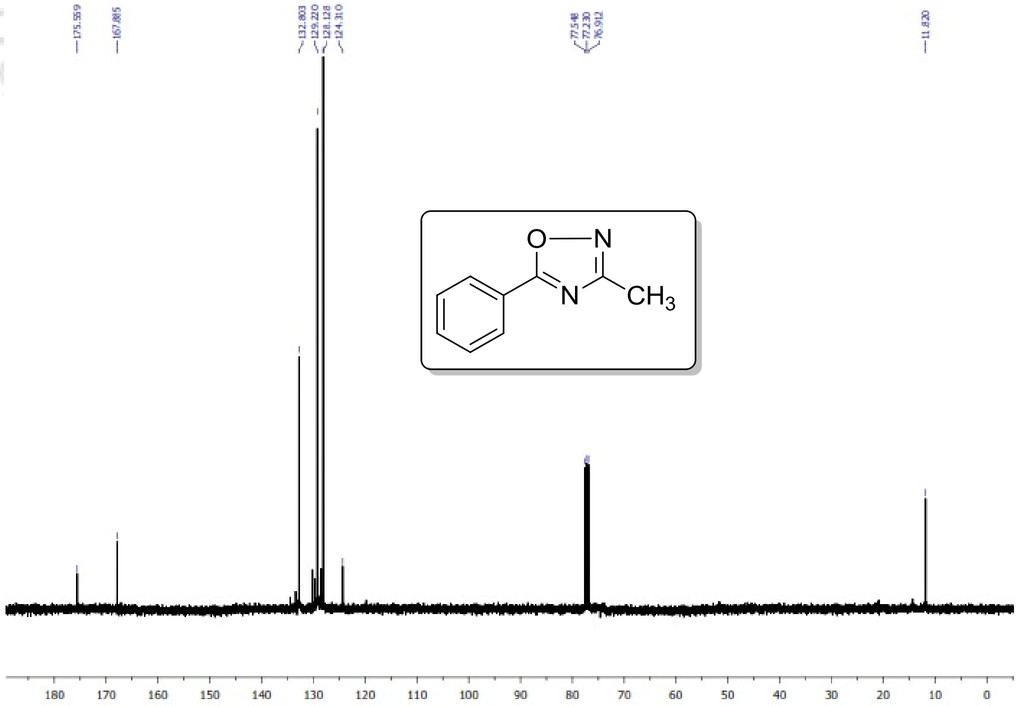


Figure S28. ^{13}C NMR spectra of compound 4f

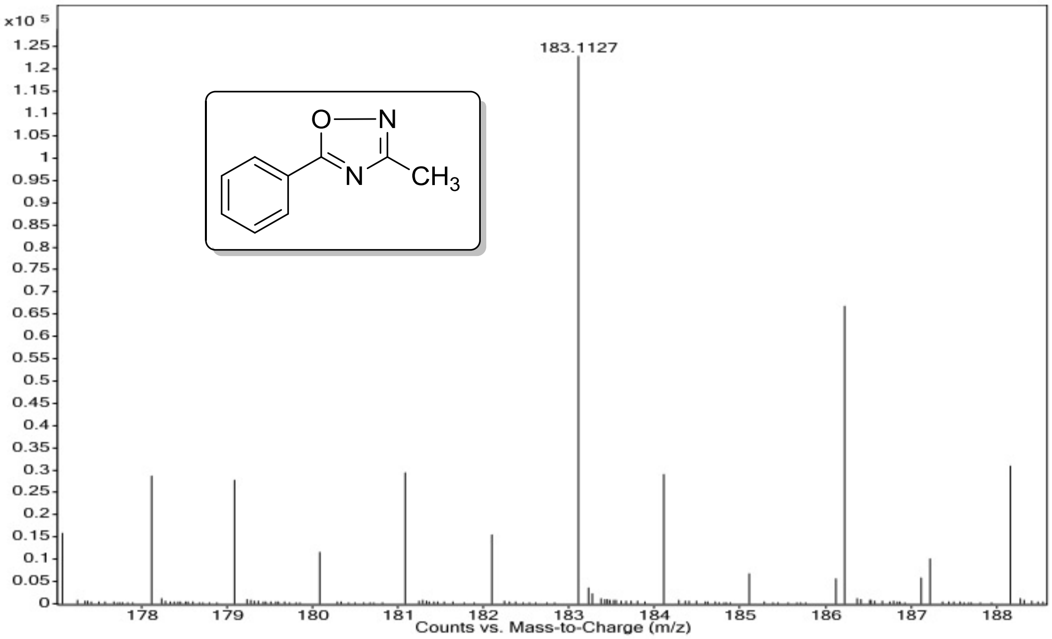


Figure S29. Mass spectra of compound 4f

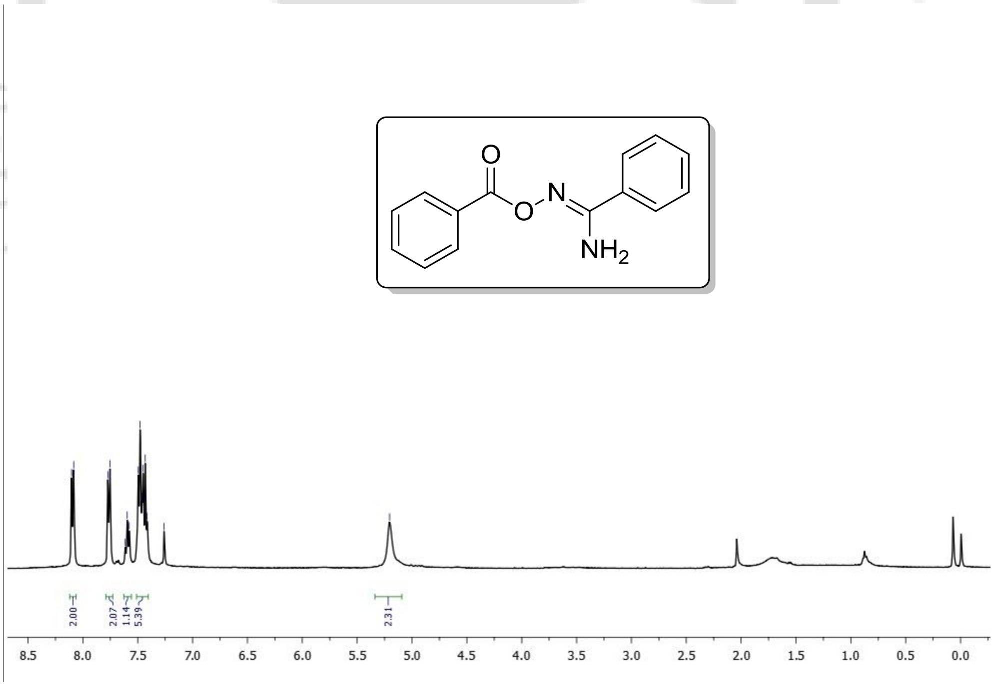


Figure S30. ^{1}H NMR spectra of Intermediate **3**

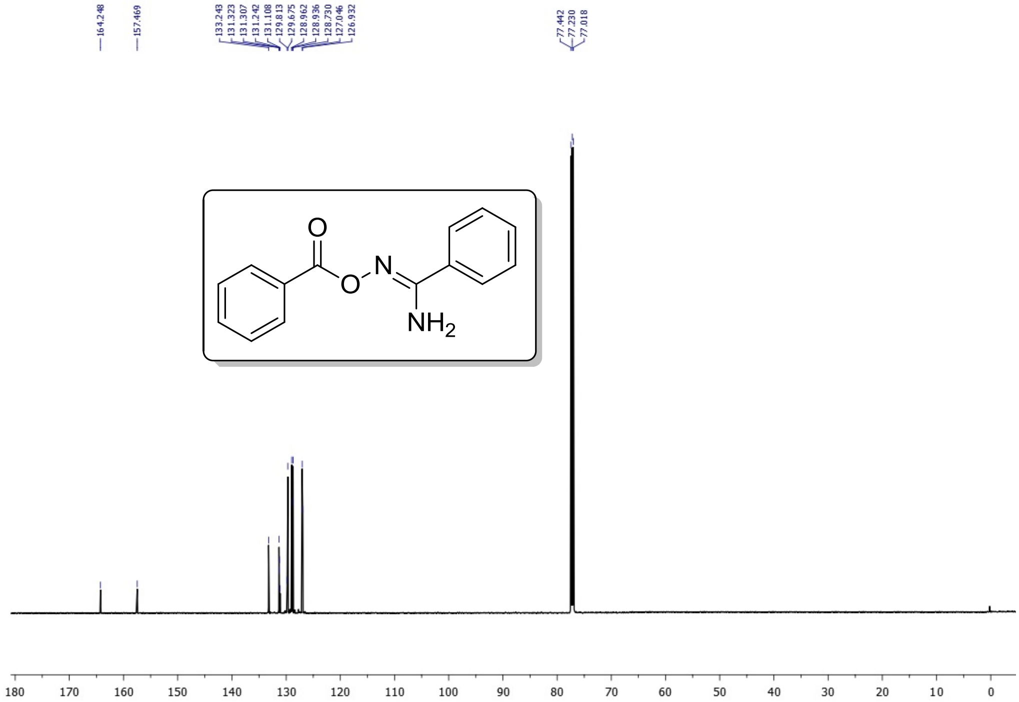


Figure S31. ^{13}C NMR spectra of Intermediate **3**

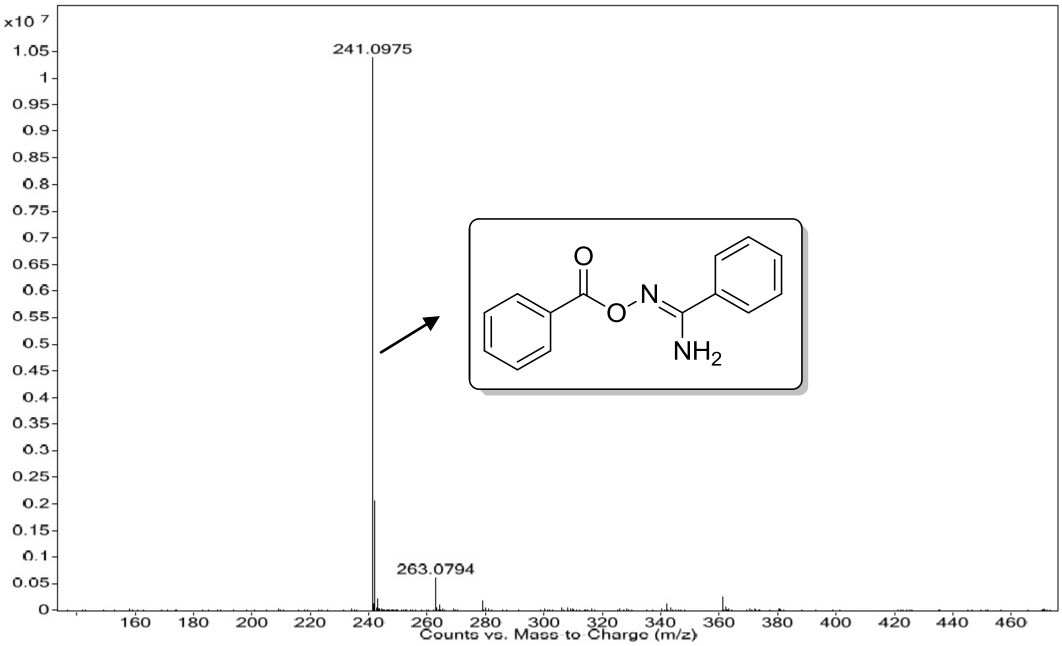


Figure S32. Mass spectra of Intermediate **3**.

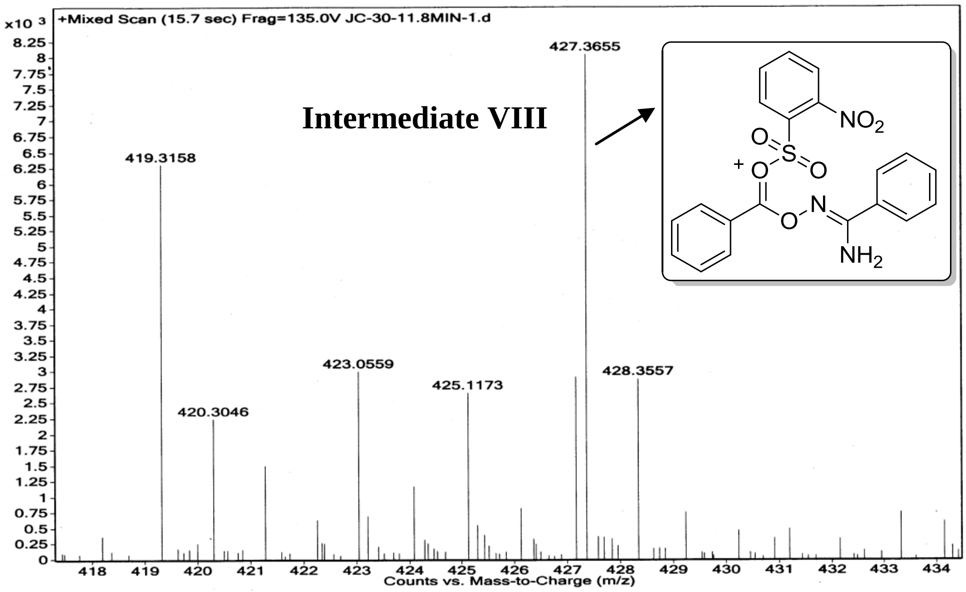


Figure S33. Mass spectra of Intermediate **VIII**.

Chapter 6: Development of a New Recyclable Coupling Reagent, (*E*)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY) for Synthesis of Esters, Thioesters, Amides and Peptides

In above mentioned chapters, we explored the organic transformation with *o*-NosylOXY. In this chapter, we developed another new recyclable Oxyma based coupling reagent, (*E*)-ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY, **II**), which was successfully applied for the synthesis of amides, peptides, esters, and thioesters in good yield with broad substrate scope.

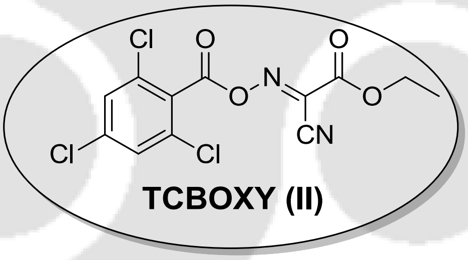


Figure 6.1. *(E)-Ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (TCBOXY,* ***II****)*

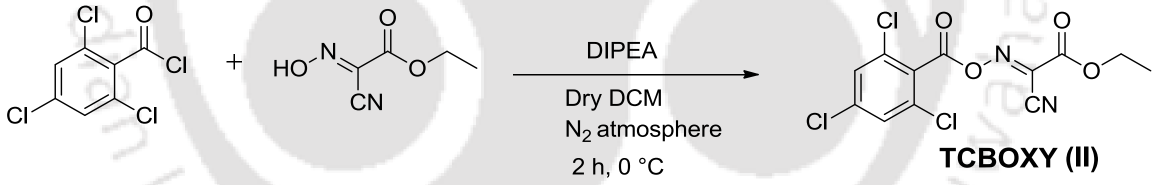
6.1. TCBOXY as a new coupling reagent for racemization free amidation and peptide synthesis

Amides and peptides are present in many compounds of biological and pharmaceutical importance.[1] In the survey, it has found that these functional groups present in 25% of all known pharmaceuticals.[2] Coupling reactions comprise of activation of carboxylic acids into an activated form that undergo acylation to produce esters, amides, and peptides. These are extremely important reactions for academia and industry. To date, numerous coupling reagents have been developed for activation of carboxylic acids including carbodiimides, phosphonium, and uronium/aminium salts[3] to achieve esters, amides, and peptides. On the other hand, Yamaguchi and coworkers have reported a coupling reagent i.e. 2,4,6-trichlorobenzoyl chloride[4] for the synthesis of carboxylic esters and lactones. Later, Shiina and coworkers developed a reagent similar to the Yamaguchi reagent i.e. 2-methyl-6-nitrobenzoic anhydride[5] for effective esterification process. A catalytic amount of DMAP and aromatic anhydrides or 2,4,6-trichlorobenzoyl chloride is used as coupling reagent to improve the regioselectivity.[6] In 2014, one more modified Yamaguchi reagent was reported by Yoshinori and coworkers. In this literature, they used 2,4,6-trichlorobenzoyl-4-dimethylamino pyridinium chloride (TCB-DMAP)[7] as a coupling reagent for esterification process. These mentioned coupling reagents are capable to afford the reactions, but not effective in the suppression of any undesired side product and racemization. Thus, the yield of the desired product decreases, chemical waste generation and complicacy of purification as well as racemization increases. Till date, HOBt (hydroxybenzotriazole) and HOAt (hydroxyazabenzotriazole) have been used frequently as an additive with peptide coupling reagents[8] to prevent the racemization in peptide synthesis. Recently, Oxyma (ethyl 2-hydroxyimino-2-cyanoacetate) has been used as a racemization suppressant in DIC (diisopropylcarbodiimide)[9] and TFFH (tetramethylfluoroformamidinium hexafluorophosphate)[10] mediated peptide synthesis and has gained popularity over HOBt and HOAt due to the explosive nature of HOBt and HOAt. The objective of the invention is to find a novel, greener and racemization free method for esterification, thioesterification, amidation and peptide synthesis.

To the best of our knowledge, there is no report discussing the racemization free amide and peptide synthesis using Yamaguchi and modified Yamaguchi reagent. We herein report a novel reagent, (*E*)-ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate (**TCBOXY**) and its application for racemization free synthesis of esters, thioesters, amides, and peptides.

6.1.1. Reaction optimization and substrate scope for ester, amide and peptide synthesis using TCBOXY

The reagent, **TCBOXY** (**II**) can easily be prepared by the reaction of Oxyma (ethyl 2-hydroxyimino 2-cyanoacetate) with 2, 4, 6-trichlorobenzoyl chloride in the presence of DIPEA under nitrogen atmosphere and dry DCM as solvent at 0 °C for 2 h (Scheme 6.1.1.1). The reagent **II** is stable at room temperature (25 °C) and can be stored for long period of time. Time-dependent HPLC studies indicated no change of **II** until 2 months (Figures S4-S5).



Scheme 6.1.1.1**. Preparation of reagent **II

To check the coupling efficiency of the newly developed coupling reagent **II**, we first optimized the reaction condition (Table 6.1.1.1). For optimization, we first investigated the reaction between benzoic acid, cyclohexylamine and **II** in the presence of DIPEA. We observed only 30% yield of the desired product and 60% yield of the side product, which is the corresponding amide of 2,4,6-trichlorobenzoic acid coming from **II**. To avoid the side product formation, we used a catalytic amount of DMAP that acted as an acyl transfer reagent. It is also known to be a stronger nucleophile and react regioselectively at the less hindered carbon center as known for Steglich esterification.[11]

We screened a varied amount of DMAP using the same model reaction in DCM (Table 6.1.1.1). We observed a maximum of 91% yield of the desired product with 0.3 equiv of DMAP (entry **3**). No change in the yield was observed by the enhancement of the amount of DMAP above 0.3 (entries **1** and **2**). But, as we decreased the equivalence of DMAP below 0.3, the yield of product was decreased (entries **4** and **5**). Further, the effect of variation of several solvents on the reaction was studied using reagent **II** (1 equiv) with the catalytic amount of DMAP (0.3 equiv). We found that dichloromethane, acetonitrile, ethyl acetate, chloroform and DMF afforded very good yield (entries **3, 7, 8, 9** and **10,** respectively). But there was no reaction in methanol and water (entries **13** and **14,** respectively). Therefore, 0.3 equiv of DMAP and DCM as a solvent were accepted as optimized conditions for the reaction (entry **3**).

Table 6.1.1.1. Optimization of reaction conditions.[a]

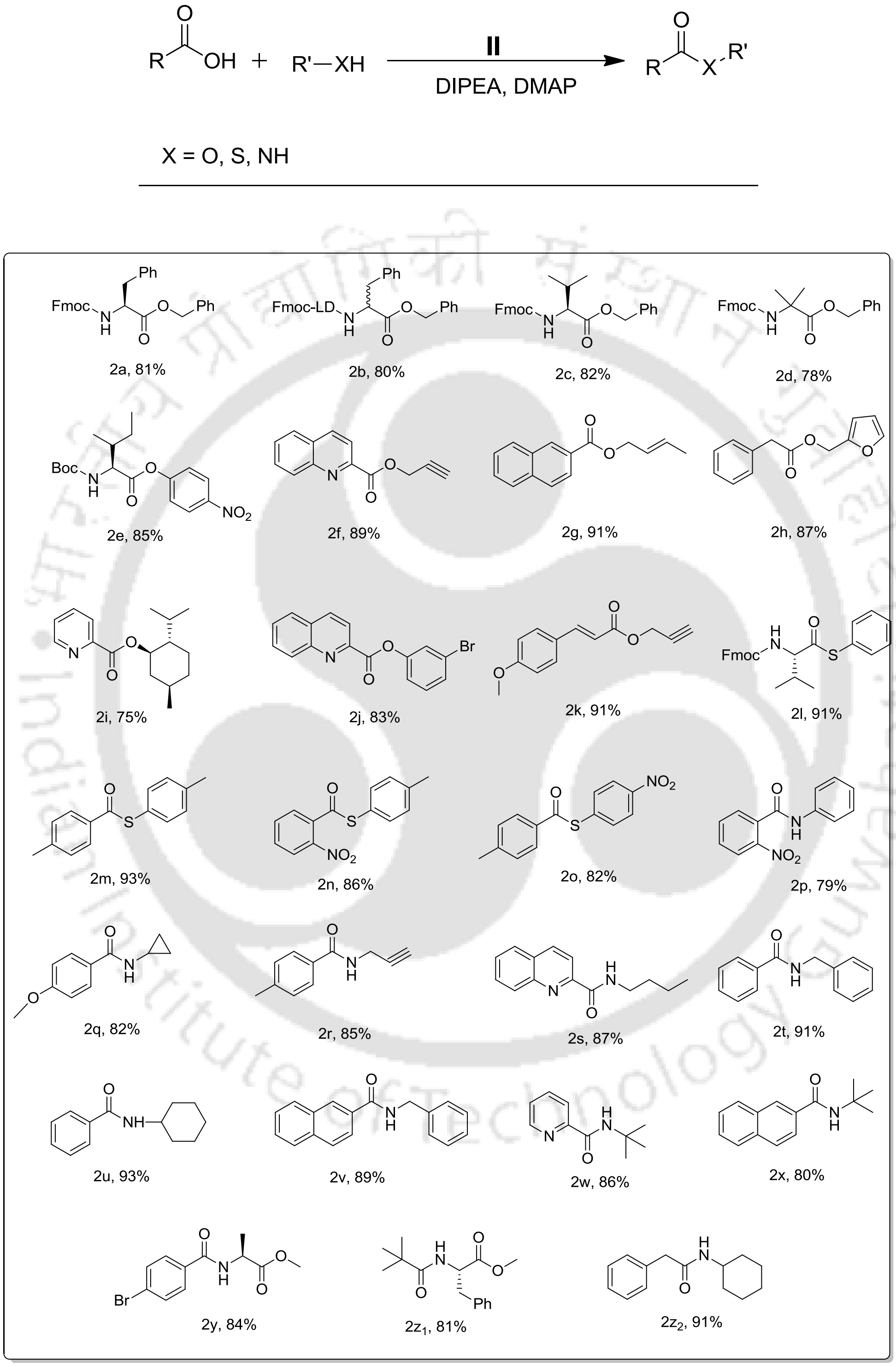
Entry	Solvents	DMAP (equiv)	Yield[b] (%)
1	DCM	0.8	91
2	DCM	0.4	91
3	**DCM**	**0.3**	**91**
4	DCM	0.2	80
5	DCM	0.1	65

6	DCM	0.0	30
7	CH_3CN	0.3	80
8	EtOAc	0.3	72
9	$CHCl_3$	0.3	75
10	DMF	0.3	85
11	THF	0.3	59
12	Toulene	0.3	40
13	MeOH	0.3	0
14	H_2O	0.3	0

[a]*Reaction conditions: Benzoic acid (1 mmol), TCBOXY (1 mmol), DIPEA (1.5 mmol), DMAP (varied amount), cyclohexylamine (1.2 mmol) at room temperature and the reaction time is 15 min.* [b]*Isolated yield*.

With the optimized conditions in hand, we proceeded to investigate the scope of esterification using various carboxylic acids and alcohols to explore the applicability of **II** as the coupling reagent. The reaction worked well with the sterically hindered amino acids (Scheme 6.1.1.2, entries **2a-2e**) as well as with heterocyclic, aromatic and aliphatic carboxylic acids (entries **2f-2k**) with good yields. On the other hand, **TCBOXY** also has efficiency for esterification with secondary alcohol (entry **2i**) in very good yield. We also extended this protocol for thioesterification (entries **2l-2o**). A broad range of carboxylic acids was tolerated, including those bearing electron donating group (EDG) (entries **2m** and **2o**) and electron withdrawing group (EWG) (entry **2n**) as well as Fmoc-protected amino acid (entry **2l**) with aromatic thiols bearing neutral, electron donating and electron withdrawing substituents. Further, we extended this protocol for investigation of the scope of **II** for amidation reactions under optimized conditions by reacting a variety of amines with aromatic acids and aliphatic acids.

Scheme 6.1.1.2. Esterification, thioesterification, and amidation by using **II**[a,b]

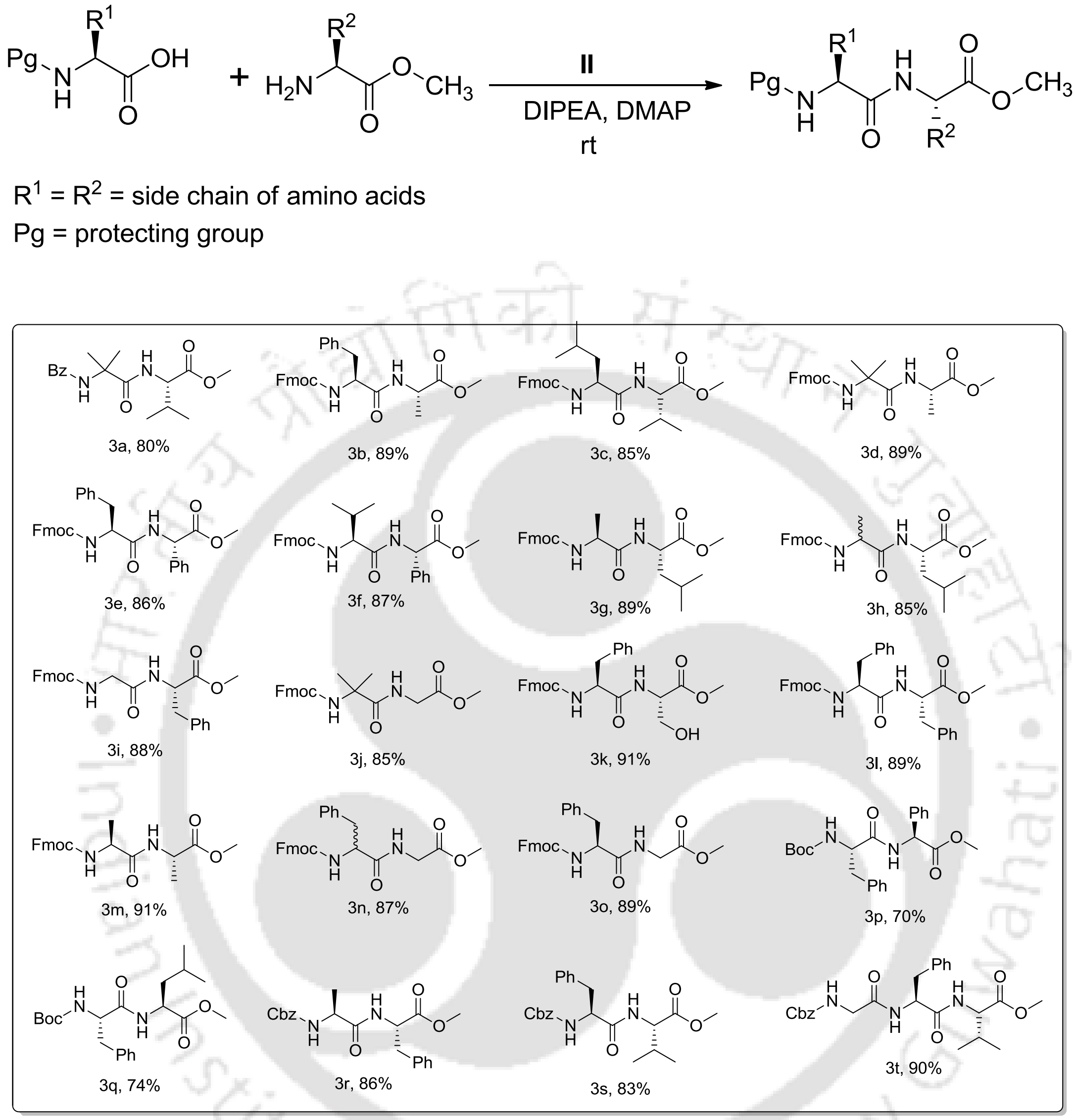


[a] *Reaction conditions: Acid (1 mmol), TCBOXY (1 mmol), DIPEA (1.5 mmol), DMAP (0.3 mmol) and alcohol or thiol or amine (1.2 mmol) at room temperature and the reaction time is 5-30 min.* [b] *Isolated yield.*

Interestingly, the reaction worked well with the aromatic carboxylic acids (Scheme 6.1.1.2, entries **2p-2y**), aliphatic carboxylic acids (entry **$2z_1$-$2z_2$**), aromatic amines (entry **2p**), aliphatic amines (entries **2q-2x**, **$2z_2$**), and *C*-protected amino acids (entries **2y** and **$2z_1$**).

We further explored the applicability of **II** for solution phase peptide synthesis with *N*-protected amino acid and *C*-protected amino acid (Scheme 6.1.1.3). The reaction worked well with common *N*-protections such as Bz (entry **3a**), Fmoc (entries **3b-3o**), Boc (entries **3p** and **3q**), Cbz (entries **3r-3t**), and sterically hindered amino acids with good to excellent yields.

Scheme 6.1.1.3. Wide scope of the synthesis of peptide in solution using **II**[a,b]



[a]Reaction conditions: N-protected amino acid (1 mmol), TCBOXY (1 mmol), DIPEA (1.5 mmol), DMAP (0.3 mmol) and C-protected amino acid(1.5 mmol) at room temperature and reaction time is 20-120 min. [b]Isolated yield.

We further synthesized long peptide in solution as well as in solid phase using reagent **II**. Firstly, we synthesized Boc-Val-Val-Ile-Ala-OMe, the *C*-terminal fragment of amyloid *β*-peptide.[12] This is a very difficult hydrophobic sequence with steric hindrance (Figure 6.1.1.1**a**). The solution phase methodology was performed in DCM in each step using

Boc-chemistry. A simple workup led to the isolation of 6.1.1.1**a** in 75% yield and purity of crude peptide was found to be 99% as determined by RP-HPLC analysis (Figures S67-S68). Later, we synthesized ACP (24-33) peptide fragment (ACP, acyl carrier protein: Asp-Asn-Ala-Ser-Phe-Val-Glu-Asp-Leu-Gly-OH, Figure 6.1.1.1**b**) and highly aggregating elastin peptide fragment: (Pro-Gly-Val-Gly-Val-$)_2$-NH_2 (Figure 6.1.1.1**c**) by stepwise coupling of amino acids on Rink amide MBHA resin following Fmoc protection strategy and final peptide was cleaved from the resin using TFA/DCM, precipitated using cold ether, to obtain a *C*-terminal amide.

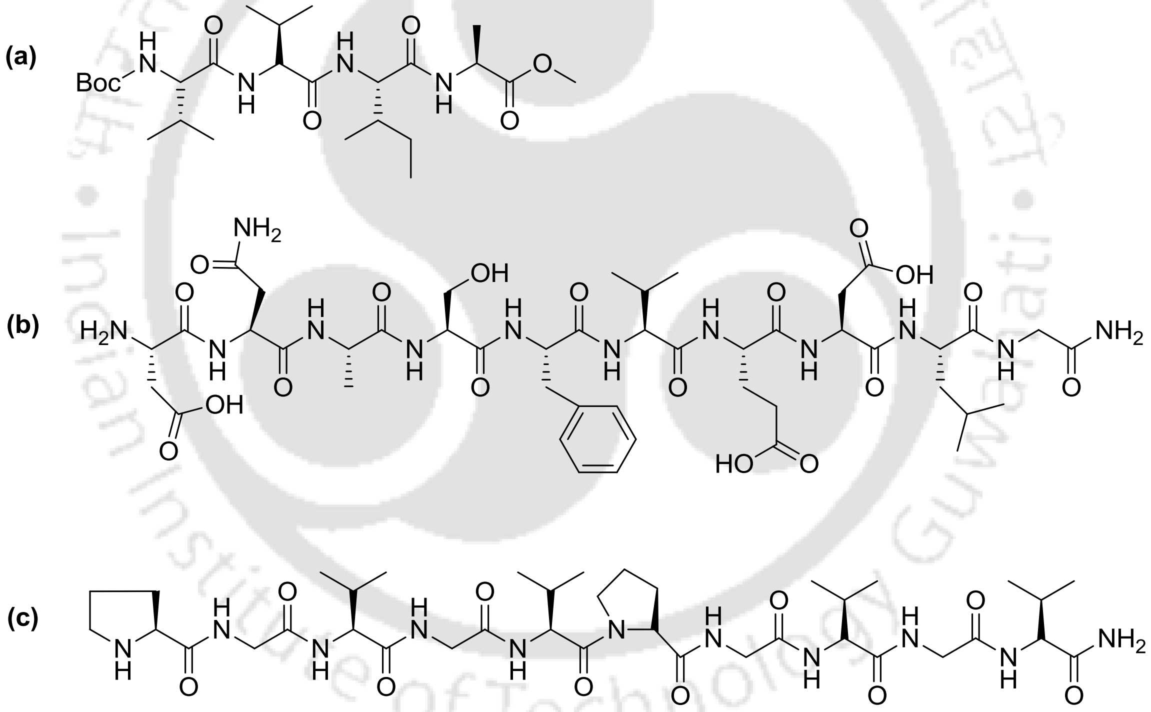


Figure 6.1.1.1. *Sequences of the synthesized peptides: (a) Boc-VVIA-OMe, in solution; (b) DNASFVEDLG-NH_2 and (c) PGVGVPGVGV-NH_2 using SPPS strategy.*

The ESI-MS data of each fragment during the synthesis of ACP (24-33) are mentioned in Table 6.1.1.2. The final peptides were then purified using preparative HPLC with good yield (21% for ACP (24-33) and 30% for elastin protein) with respect to resin loading.

The HPLC profile and ESI-MS spectrum of purified peptides are provided in the Figures S69-S70 and S71-S72, respectively.

Table 6.1.1.2. The ESI-MS data of each fragment during the synthesis of ACP (24-33)

Entry	Peptide	Expected mass (m/z)	Observed mass (m/z)
1	Fmoc-LG-NH_2	$[M+H]^+$ 410.2080	410.2124
2	Fmoc-DLG-NH_2	$[M+H]^+$ 525.2349	525.2368
3	Fmoc-EDLG-NH_2	$[M+H]^+$ 654.2775	654.2936
4	Fmoc-VEDLG-NH_2	$[M+H]^+$ 753.3459	753.3514
5	Fmoc-FVEDLG-NH_2	$[M+H]^+$ 900.4143	900.4296
6	Fmoc-SFVEDLG-NH_2	$[M+H]^+$ 987.4464	987.4435
7	Fmoc-ASFVEDLG-NH_2	$[M+H]^+$ 1058.4835	1058.4956
8	Fmoc-NASFVEDLG-NH_2	$[M+H]^+$ 1172.5264	1172.242
9	DNASFVEDLG-NH_2	$[M+H]^+$ 1065.4853	1065.5051

We further synthesized fragments of gramicidin **A**, **B**, and **C** on Wang resin with general structure Xaa–D-Leu-L-Trp-D-Leu-L-Trp-OH, where side chain of the Xaa varies (Figure 6.1.1.2). We achieved these peptides in good yield, i.e. 23%, 28%, 26% of gramicidin **A**, **B** and **C** fragments, respectively, with respect to the resin loading after purification using RP-HPLC (Figures S73-S78).

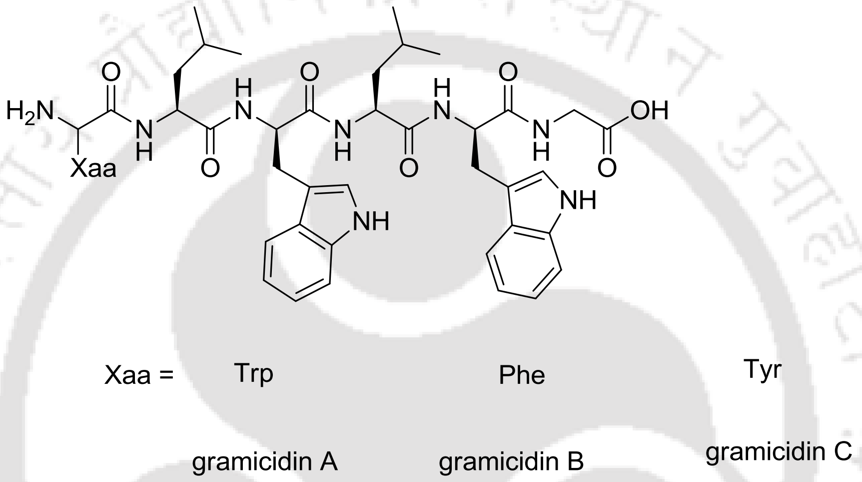


Figure 6.1.1.2. *Synthesized fragments of gramicidin **A, B**, and **C** using SPPS strategy.*

6.2. Racemization study

6.2.1. Racemization suppression efficiency of II for esters synthesis

To estimate the epimerization potential, we first synthesized Fmoc-DL-Phe-OBn using **II** and passed through a chiral column. Two distinct peaks appeared which correspond to the two enantiomers (Figure 6.2.1.1 and Figure S47). Next, we synthesized Fmoc-L-Phe-OBn using Yamaguchi reagent, its related reagents and **II**. All products were passed through a chiral column; we observed 12 to 24% epimerization with Yamaguchi and its related reagent (Figure 6.2.1.1 and Figure S50-S54). But with **II,** we observed only single enantiomeric product (Figure 6.2.1.1 and Figure S55). This indicates that **II** did not cause racemization during the coupling reaction.

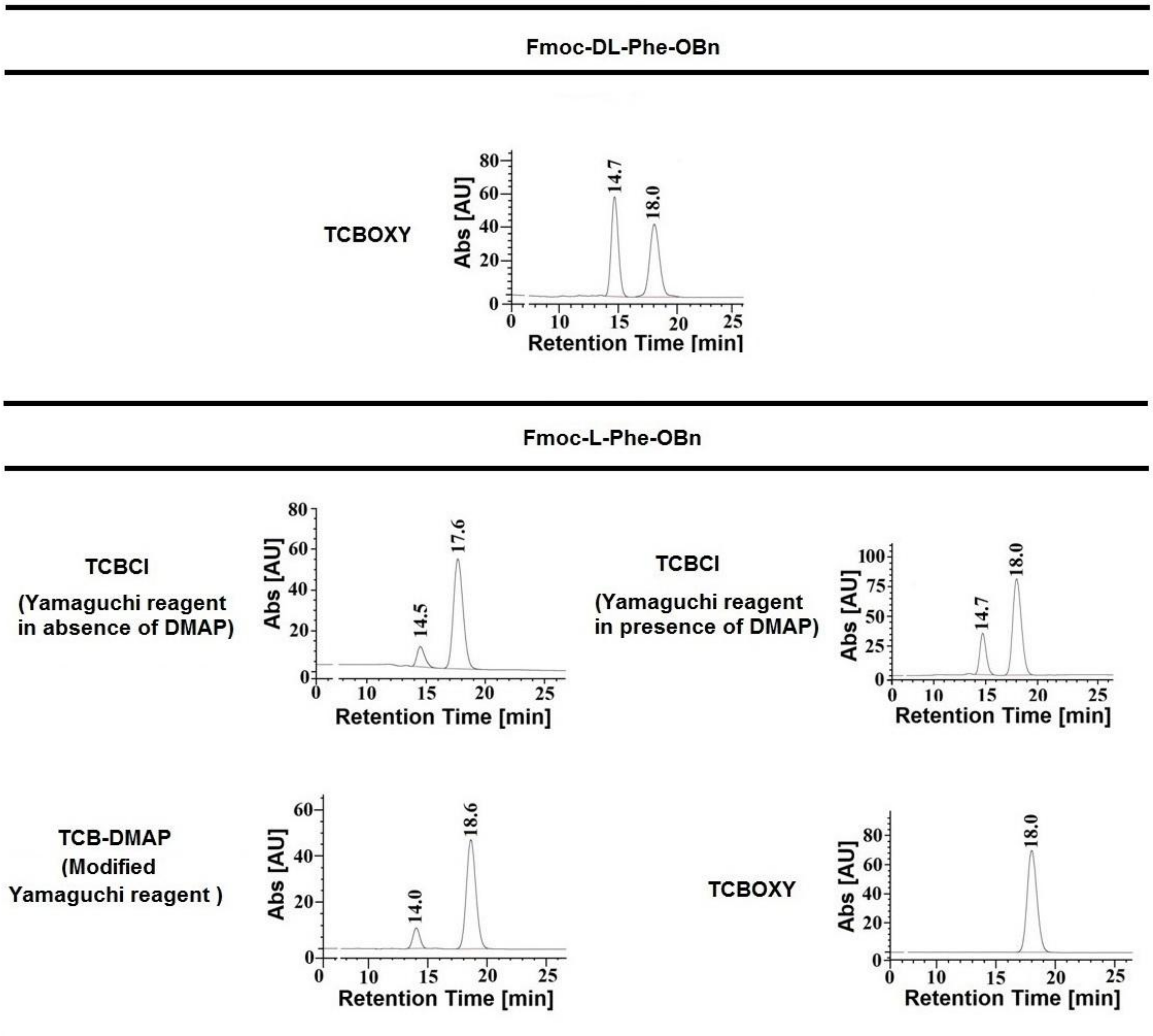


Figure 6.2.1.1. *HPLC chromatograms to compare the epimerization caused by relevant coupling reagents.*

6.2.2. Racemization suppression efficiency of II for peptide synthesis

For racemization study, we synthesized Fmoc-L-Ala-L-Leu-OMe (Scheme 6.1.3, entry **3g**) and Fmoc-DL-Ala-L-Leu-OMe dipeptides (entry **3h**) using **II** and compare their HPLC profiles. The appearance of a single peak in the HPLC profile of Fmoc-L-Ala-L-Leu-OMe corresponds to the single stereoisomeric product whereas the presence of the twin peak of

Fmoc-DL-Ala-L-Leu-OMe indicates two diastereomeric products (Figure 6.2.2.1 and Figures S57-S61). Then we compared the ^{1}H NMR and ^{13}C NMR spectra of **3g** & **3h** (Figure 6.2.2.1 and Figures S27-S32), we found one singlet at δ =3.72 ppm for the methoxy proton in the ^{1}H NMR and two peaks at δ =173.4, 172.3 ppm in ^{13}C NMR corresponding to the two carbonyls of the amide and the ester groups indicating the presence of the single diastereomer of **3g**. Whereas in case of **3h**, we found two singlets at δ = 3.70 and 3.68 ppm for the methoxy proton in ^{1}H NMR and four peaks at δ = 173.4, 173.3, 172.5, 172.4 ppm in ^{13}C NMR indicates the presence of two diastereomeric products. This indicates no racemization occurred during synthesis.

We also synthesized one more DL and L forms of Fmoc-Phe-Gly-OMe dipeptides (Scheme 6.1.3, **3n** & **3o**, respectively) and compared their HPLC profiles (Figure 6.2.2.2). We observed racemization free dipeptides. Also, for all the remaining L, L-dipeptides depicted in scheme 5.1.3, we observed single peak corresponding to the single stereoisomeric product which confirmed occurrence of no detectable racemization during peptide synthesis by **II**.

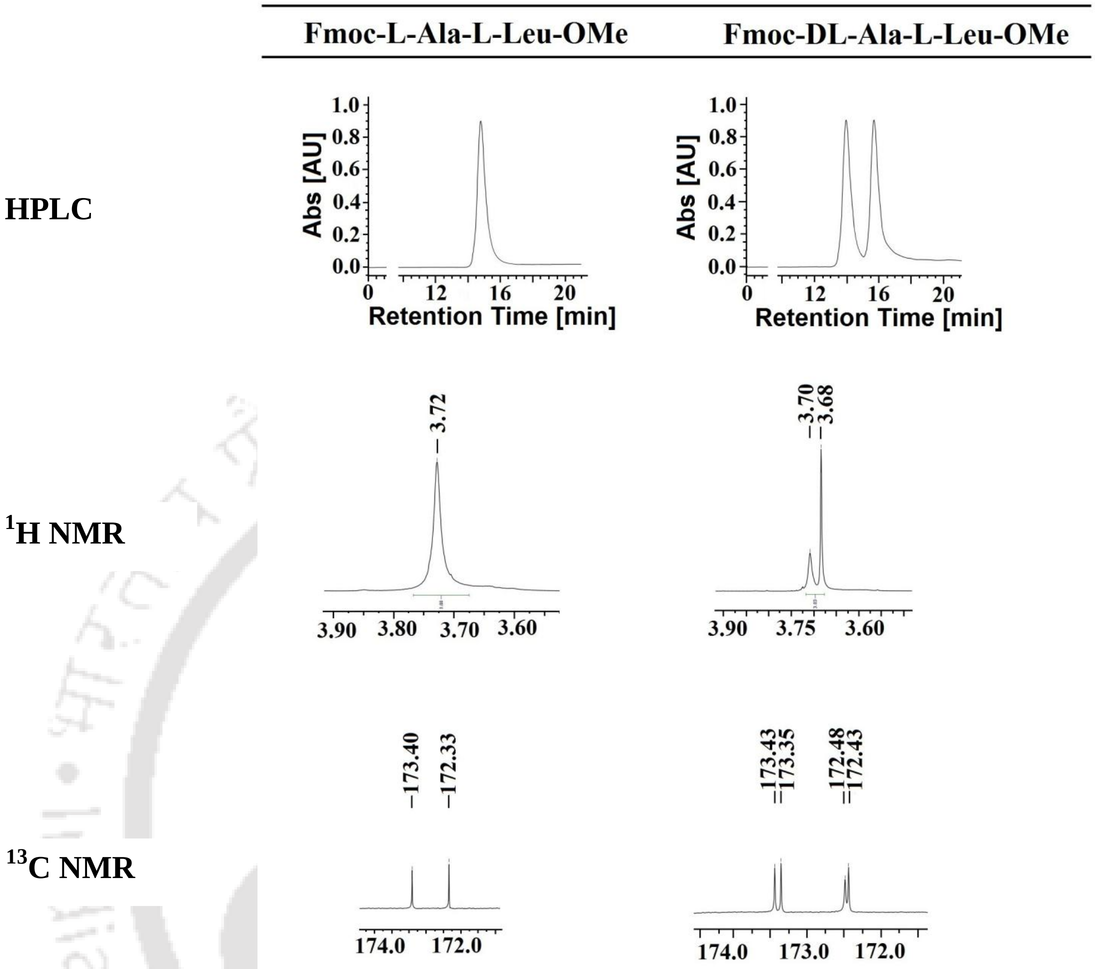


Figure 6.2.2.1. *Comparison study of racemization of 3g & 3h by HPLC,* ^{1}*H NMR and* ^{13}*C NMR*

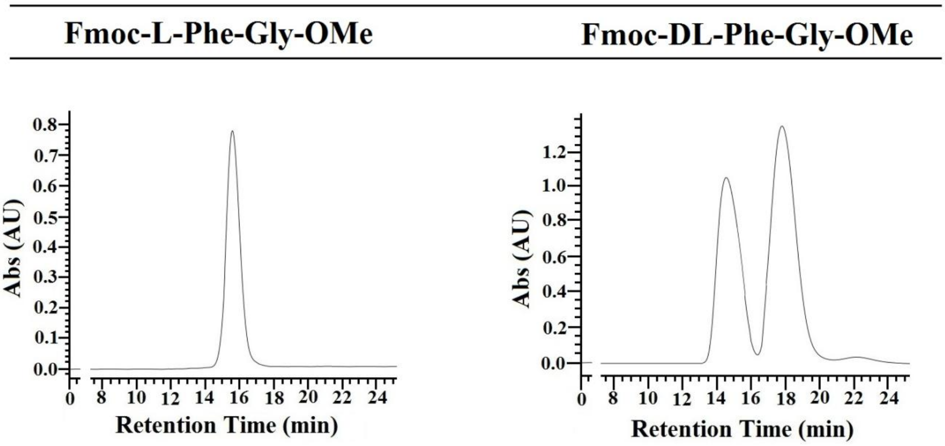


Figure 6.2.2.2. *Comparison study of racemization of* ***3o*** *(left panel) &* ***3n*** *(right panel) by HPLC*

Next, we went for tripeptide synthesis, Z-Gly-Phe-Val-OMe (Scheme 6.1.3, entry **3t**) in solution phase using **II** and compared the results to those reported well-known coupling reagents such as HATU, HDMA, HDMB, and HBTU.[13] We observed no racemization while using **II** (Table 6.2.2.1) by NMR and HPLC techniques unlike other coupling reagents (Figures S36-S38 and S62-S63).

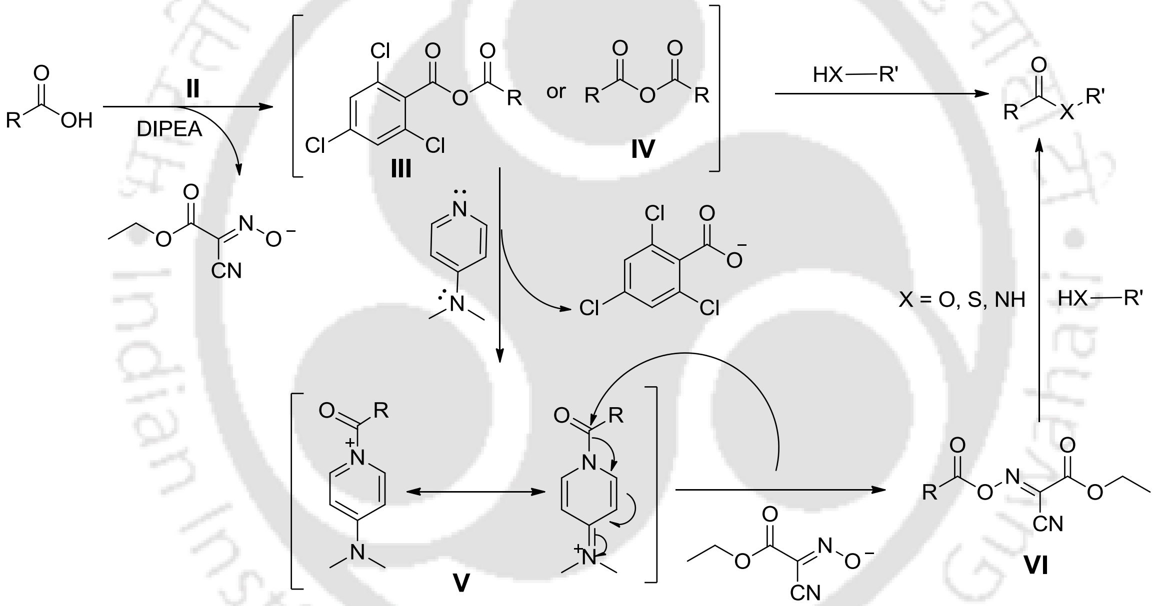
Table 6.2.2.1. Comparison of the yield and racemization of Z-Gly-Phe-Val-OMe, synthesized using various coupling reagents.

Entry	**Coupling reagent**	**Yield (%)**	**Racemization (%)**
1	HDMB	90	2.9
2	HDMA	90	0.7
3	HBTU	89	5.9
4	HATU	90	1.6
5	TCBOXY	90	n.d[*a*]

[a]No racemization could be detected by HPLC, ^{1}H and ^{13}C NMR.

6.3. Plausible mechanism

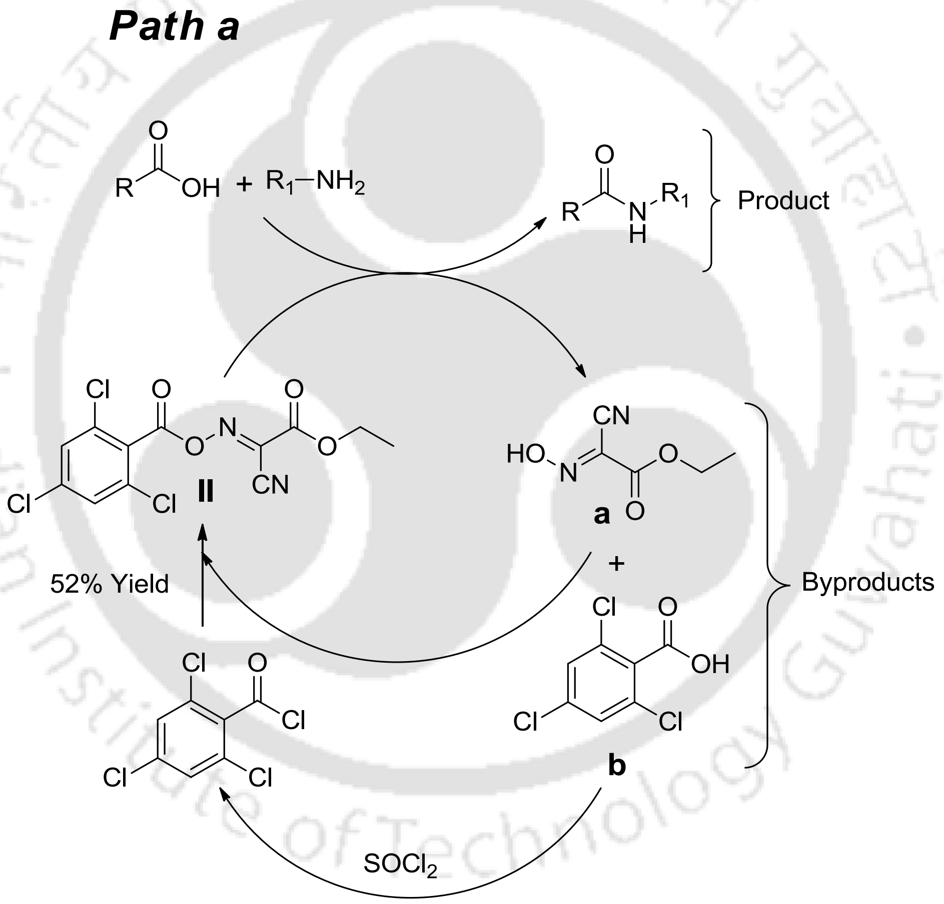
Having demonstrated the applicability of **II** as coupling reagent in esterification, thioesterification, amidation and peptide synthesis, we turned our attention to the elucidation of the mechanism of the reaction. We have drawn a plausible mechanistic pathway which is depicted in Scheme 6.3.1. Initially, the nucleophile generated by the deprotonation of the carboxylic acid in the presence of DIPEA attacks the carbonyl carbon of **II**, forming a mixed anhydride (**III**)[4] and releases the Oxyma. After that DMAP attacks regioselectively at less hindered carbon center of **III** or **IV**, forming *N*-acyl pyridinium salt (**V**).[7] The released Oxyma then attacks at carbonyl carbon of *N*-acyl pyridinium salt resulting in the formation of the intermediate **VI**, i.e. Oxyma ester of the carboxylic acid. Intermediate **VI** then undergoes nucleophilic substitution to produce the corresponding esters, thioesters, amides, and peptides. The intermediate **VI** was isolated and characterized by using ^{1}H NMR, ^{13}C NMR (Figures S45-S46).



Scheme 6.3.1. *A plausible mechanism of the coupling reaction mediated by* ***II****.*

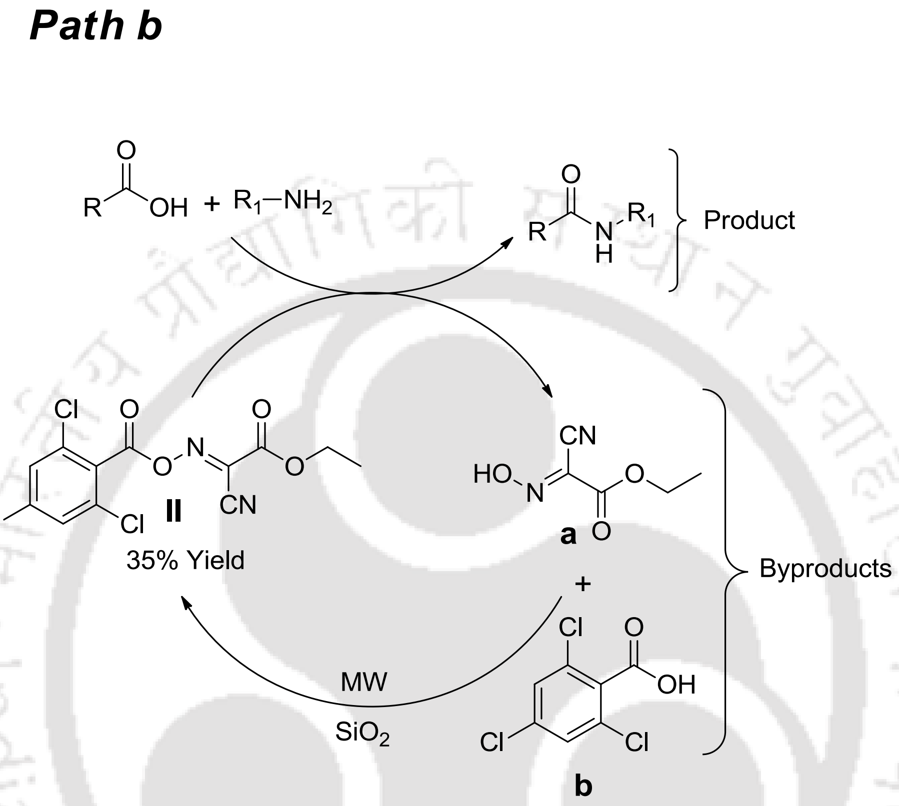
6.4. Recyclability of TCBOXY

Next, we wanted to recover the byproducts and recombine them to form the coupling reagent, **II**. For that, we took the substrate (Scheme 6.1.2, entry **2w**) and continued the reaction at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and washed with 5% HCl solution (3 × 5 mL). Then the whole reaction mixture was loaded over a silica gel column and eluted with a mixture of ethyl acetate and hexane. The recovered by-products **a** and **b** was as pure as commercial samples (Figures S53-S58). The recovered **b** was chlorinated with thionyl chloride by heating at 110-114 °C in toluene for 3 h and mixed with recovered Oxyma (**a**) in the presence of DIPEA to obtain **II** with 52% overall yield with respect to the initial **II** used (Scheme 6.4.1, ***path a***).



Scheme 6.4.1.*Recyclability of **II** through Path a.*

Alternatively, the recovered **a** and **b** were recombined by simply heating in presence of silica gel under microwave irradiation[14] (Scheme 6.4.2, ***path b***) based dehydration without using hazardous thionyl chloride. By this way, we were able to recover byproducts **a** and **b** and recombined to recycle the coupling reagent easily.



***Scheme 6.4.2.** Recyclability of **II** through Path b*

6.5. Conclusion

We have designed and synthesized a novel coupling reagent, TCBOXY for racemization free synthesis of esters, thioesters, amides, peptides in both solution phase as well as in solid phase with very good to excellent yield. The advantages of this coupling reagent are: (a) it can be easily prepared by a single step reaction and does not require multistep reaction pathways, (b) suppresses racemization during the coupling reaction, (c) does not generate any side products, (d) 2,4,6-trichlorobenzoic acid and Oxyma are generated as by-product which are nontoxic and easily recovered to generate the same coupling reagent, Thus, the described method for the synthesis of mentioned compounds using this new reagent is more eco-friendly and green process.

6.6. Experimental section

6.6.1. Materials and methods

As described in chapter 2 section 2.4.1

6.6.2. Procedure for synthesis of coupling reagent, (*E*)-ethyl 2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino) acetate (TCBOXY, II):

DIPEA (1 equiv) was added to a solution of Oxyma (1 equiv) in 2 ml of DCM under nitrogen. The reaction mixture was cooled up to 0 °C and followed by dropwise addition of 2,4,6-trichlorobenzoyl chloride. The reaction mixture was stirred for another 2 h at room temperature. After completion of the reaction, the reaction mixture was diluted with 10 ml of DCM and washed with 5% of HCl (3×5 ml). Finally organic layer dried by anhydrous $CaCl_2$ and recrystallized with hexane. R_f : 0.50 (EtOAc:Hexane, 1:9); Yield 326 mg, 94%; white crystalline solid, mp 83-85 °C; ^1H NMR (600 MHz, $CDCl_3$): δ 7.47 (s, 2H), 4.54-4.51 (q, 2H, CH_2), 1.47-1.44 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 156.6, 138.6, 134.0, 132.8, 128.7, 127.9, 106.6, 65.0, 14.1;IR (KBr) 3076, 1806, 1732, 1577, 1375, 1220, 1150, 1087, 990 cm^{-1}; HRMS (ESI) m/z: [M+Na]+Calcd for $C_{12}H_7C_{l3}N_2NaO_4$ 370.9369, found 370.9367.

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

TCBOXY (1 equiv) was added to a solution of carboxylic acid (1 equiv), DMAP (0.3 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 3-5 min for preactivation followed by the addition of alcohol, thiol or amine (1.2 equiv). The reaction mixture was stirred at room temperature for 5-30 min. After completion of the reaction, the reaction mixture was diluted with 20 ml of ethyl acetate; the organic phase was washed with 5% HCl (3×5 ml), 5% $NaHCO_3$ (3×5ml) and dried by anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the product which was purified by column chromatography.

6.6.4. General procedure for the synthesis of peptides:

TCBOXY (1 equiv) was added to a solution of *N*-protected amino acid (1 equiv), DMAP (0.3 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 5 min for preactivation followed by the addition of methyl ester of amino acid (1.5 equiv) and DIPEA (1.5 equiv) in 1 ml of DCM. The reaction mixture was stirred at room temperature for more 20-120 min. After completion of the reaction, the reaction mixture was diluted with 20 ml of ethyl acetate, the organic phase was washed with 5% HCl (3×5 ml), 5% $NaHCO_3$ (3×5 ml), brine and dried over anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered and the solvent was evaporated. The product was purified by silica gel column chromatography using ethyl acetate and hexane in the ratio of 4:6.

6.6.5. Solution Phase Synthesis of Boc-VVIA-OMe:

TCBOXY (1 equiv) was added to a solution of Boc-isoleucine (1 equiv), DMAP (0.3 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 5 min for preactivation. In another RB, methyl ester of alanine (1.2 equiv) was taken in DCM and DIPEA was added to it till basic pH was reached. Finally this solution was added to the above solution and stirring continued until completion of the reaction. Then, the reaction mixture was diluted by 20 ml of EtOAc, washed by 5% $NaHCO_3$ solution (2 × 5 ml) and washed by 5% citric acid solution (2 × 5 ml). Finally, combined organic layer was dried using anhydrous Na_2SO_4. Solid product (Boc-IA-OMe) was obtained after evaporation of EtOAc by rotary vacuum evaporator.

In 50 ml RB, solid product (Boc-IA-OMe) was taken and TFA/DCM (1:1) mixture was added and stirring continued at room temperature up to 2.5 h. After that, TFA was evaporated by rotary vacuum evaporator, the solution was washed 3-4 times with diethyl ether and finally a white solid (H_2N-IA-OMe) was obtained. After Boc deprotection, the resulting IA-OMe was coupled with Boc-V-OH following the above mentioned procedure to obtain Boc-VIA-OMe. Another cycle of Boc-deprotection and coupling with Boc-V-OH, resulted in white solid Boc-VVIA-OMe, which was characterized by reversed phase HPLC; retention time 4 min on a linear gradient of 0 to 70%, 0-10 min and then 70 to 100%, 10-25 min CH_3CN in H_2O with 0.1% formic acid, symmetry C8 analytical column. LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{47}N_4O_7$ 515.3445, found 515.3521. Yield was 75% with respect to starting material Boc-isoleucine.

6.6.6. Solid Phase Synthesis of H-PGVGVPGVGV-NH_2 and H-DNASFVEDLG-NH_2:

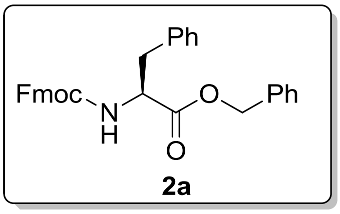
The decapeptide was manually assembled stepwise on Fmoc Rink Amide MBHA resin using Fmoc/tBu protection strategy. Fmoc amino acids (1.5 equiv), TCBOXY (1 equiv), DMAP (0.3 equiv) and DIPEA (3 equiv) were kept for preactivation for 5 min. Then, amino acid coupling was performed for 2-4 h. Fmoc deprotection was carried out with the use of piperidine/DMF (1:1) mixture for 2.5 h. Purification of the peptide was carried out by preparative HPLC and liophilization afforded the final peptide.

6.6.7. Procedure to identify the (*E*)-ethyl 9-cyano-1-(9*H*-fluoren-9-yl)-5-methyl-3,6-dioxo-2,7-dioxa-4,8-diazadec-8-en-10-oate (Intermediate VI, Scheme 6.3.1):

TCBOXY (1 equiv) was added to a solution of Fmoc-alanine (1 equiv), DMAP (0.3 equiv) and DIPEA (1.5 equiv) in 2 ml of DCM. The reaction mixture was stirred for 30 min at room temperature. After 30 min, a single spot was observed in TLC. The reaction mixture was diluted with 20 ml of ethyl acetate, the organic phase was washed with 5% HCl (3×5 ml), 5% $NaHCO_3$ (3×5 ml), brine and dried over anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the intermediate which were purified by column chromatography. R_f : 0.50 (EtOAc:Hexane, 2:8); Yield 357 mg, 82%; white solid, mp 95-97 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.61-7.59 (t, *J* = 6.6 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 5.38 (br s, 1H), 4.43-4.36 (m, 3H), 4.24-4.20 (m, 3H), 1.44-1.43 (d, *J* = 7.2 Hz, 3H), 1.29-1.28 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 155.8, 144.0, 141.5, 127.9, 127.3, 125.3, 120.2, 67.2, 61.8, 49.9, 47.4, 19.0, 14.3; IR (KBr) 2925, 1747, 1692, 1534, 1450, 1260, 1027, 738 cm^{-1}.

6.7. Characterization data

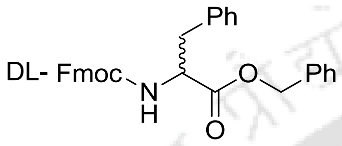
(*S*)-Benzyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanoate (2a)



2a

White solid; (386 mg, 81%); mp 107-109 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.76 (d, *J* = 7.8 Hz, 2H), 7.57-7.55 (t, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.8 Hz, 2H), 7.37-7.22 (m, 8H), 5.29 (br, 1H), 5.19-5.12 (m, 2H), 4.74-4.71 (m, 1H), 4.45-4.42 (m, 1H), 4.35-4.32 (m, 1H), 4.21-4.19 (t, *J* = 7.2 Hz, 1H), 3.15-3.08 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 155.7, 144.0, 143.9, 141.5, 135.7, 135.2, 129.5, 128.8, 128.7, 127.9, 127.3, 127.2, 125.3, 125.2, 120.1, 67.5, 67.1, 55.0, 47.3, 38.3; FT-IR (KBr, cm^{-1}): 2923, 1719, 1504, 1482, 1349, 1260, 1150, 1012, 740, 697; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{31}H_{28}NO_4$ 478.2018, found 478.2002.

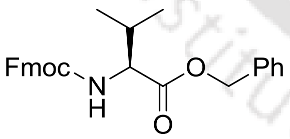
DL-(*S*)-Benzyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanoate (2b)



2b

White solid; (382 mg, 80%); mp 107-109 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.76 (d, *J* = 7.8 Hz, 2H), 7.57-7.55 (t, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.8 Hz, 2H), 7.37-7.22 (m, 8H), 5.29 (br, 1H), 5.20-5.13 (m, 2H), 4.74-4.71 (m, 1H), 4.45-4.42 (m, 1H), 4.35-4.32 (m, 1H), 4.21-4.19 (t, *J* = 7.2 Hz, 1H), 3.15-3.08 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 155.7, 143.9, 141.5, 135.7, 135.2, 129.6, 128.8, 127.9, 127.3, 127.2, 125.3, 125.2, 120.2, 67.5, 67.2, 55.0, 47.4, 38.4; FT-IR (KBr, cm^{-1}): 2923, 1719, 1504, 1482, 1349, 1260, 1150, 1012, 740, 697; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{31}H_{28}NO_4$ 478.2018, found 478.1996.

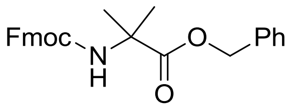
(*S*)-Benzyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoate (2c)



2c

White solid; (352 mg, 82%); mp 86-89 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.77 (d, *J* = 7.2 Hz, 2H), 7.61-7.60 (d, *J* = 7.2 Hz, 2H), 7.42-7.40 (t, *J* = 7.2 Hz, 2H), 7.36-7.31 (m, 3H), 5.35 (br, 1H), 5.23-5.15 (m, 2H), 4.41-4.36 (m, 3H), 4.25-4.22 (t, *J* = 7.2 Hz, 1H), 2.23-2.18 (m, 1H), 0.96-0.95 (d, *J* = 6.6 Hz, 3H), 0.88-0.87 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 156.4, 144.1, 143.9, 141.5, 135.5, 128.8, 128.7, 128.6, 127.9, 127.2, 125.3, 120.2, 67.3, 67.2, 59.2, 47.4, 31.5, 19.2, 17.7; FT-IR (KBr, cm^{-1}): 2925, 1744, 1689, 1530, 1449, 1270, 1156, 1030, 736, 696; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{27}H_{28}NO_4$ 430.2018, found 430.2018.

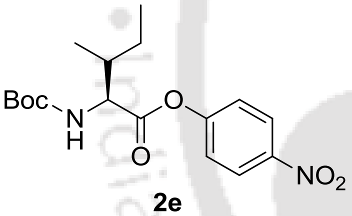
Benzyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanoate (2d)



2d

Yellow solid; (324 mg, 78%); mp 68-70 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.76 (d, *J* = 7.8 Hz, 2H), 7.59-7.58 (d, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.32-7.30 (t, *J* = 7.2 Hz, 3H), 5.45 (br, 1H), 5.18 (s, 2H), 4.35 (s, 2H), 4.19 (s, 1H), 1.59 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.6, 155.1, 144.1, 141.4, 135.7, 128.7, 128.4, 128.1, 127.8, 127.2, 125.2, 120.1, 67.4, 66.7, 56.7, 47.3, 25.2; FT-IR (KBr, cm^{-1}): 2987, 1736, 1693, 1537, 1450, 1386, 1277, 1143, 1107, 743, 696; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{26}H_{26}NO_4$ 416.1862, found 416.1864.

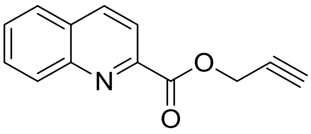
(2*S*, 3*R*)-4-Nitrophenyl 2-((tert-butoxycarbonyl)amino)-3-methylpentanoate (2e)



2e

Yellow Oil; (299 mg, 85%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29-8.28 (d, *J* = 9.0 Hz, 2H), 7.30-7.29 (d, *J* = 9.0 Hz, 2H), 5.09 (br, 1H), 4.51-4.48 (q, 1H), 1.58-1.54 (m, 1H), 1.47 (s, 9H), 1.33-1.29 (m, 2H), 1.08-1.07 (d, *J* = 7.2 Hz, 3H), 1.01-0.99 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6, 156.0, 155.3, 145.7, 126.4, 125.5, 122.6, 115.8, 80.8, 58.5, 38.0, 28.5, 25.4, 15.9, 11.8; FT-IR (KBr, cm^{-1}): 2973, 2879, 1765, 1691, 1583, 1367, 1162, 1045, 853, 629; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{25}N_2O_6$ 353.1713, found 353.1716.

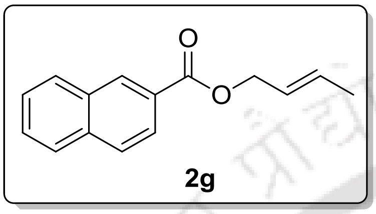
Prop-2-yn-1-yl quinoline-2-carboxylate (2f)



2f

Brown solid; (188 mg, 89%); mp 104-107 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34-8.31 (t, *J* = 7.8 Hz, 2H), 8.23-8.22 (d, *J* = 8.4 Hz, 1H), 7.90-7.89 (d, *J* = 7.8 Hz, 1H), 7.82-7.79 (t, *J* = 7.8 Hz, 1H), 7.68-7.66 (t, *J* = 7.2 Hz, 1H), 5.08 (s, 2H), 2.56-2.55 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.8, 147.8, 147.4, 137.6, 131.0, 130.6, 129.7, 129.1, 127.8, 121.3, 77.5, 75.7, 53.7; FT-IR (KBr, cm^{-1}): 2924, 2854, 2127, 1716, 1461, 1372, 1209, 1134, 781, 624; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{13}H_{10}NO_2$ 212.0712, found 212.1075.

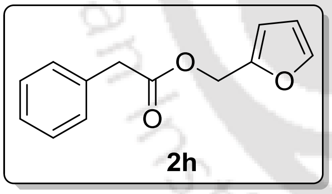
(*E*)-but-2-en-1-yl 2-naphthoate (2g)



2g

Yellow Oil; (206 mg, 91%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.08-8.07 (m, 1H), 7.96-7.95 (d, *J* = 7.8 Hz, 1H), 7.89-7.87 (d, *J* = 8.4 Hz, 2H), 7.60-7.53 (m, 2H), 5.95-5.90 (m, 1H), 5.79-5.75 (m, 1H), 4.83-4.82 (d, *J* = 6.6 Hz, 2H), 1.79-1.78 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 135.6, 132.6, 131.6, 131.2, 129.5, 128.3, 128.2, 127.9, 127.7, 126.7, 125.4, 125.3, 65.9, 18.0; FT-IR (KBr, cm^{-1}): 3024, 2939, 1716, 1631, 1510, 1446, 1281, 1227, 1195, 1094, 963, 779, 474; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{15}H_{15}O_2$ 227.1072, found 227.1075.

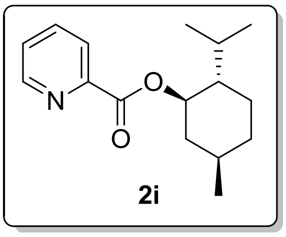
Furan-2-ylmethyl 2-phenylacetate (2h)



2h

Black Oil; (189 mg, 87%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40-7.39 (m, 1H), 7.32-7.24 (m, 5H), 6.37-6.32 (m, 2H), 5.06 (s, 2H), 3.62 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 149.4, 143.4, 133.8, 129.4, 128.7, 127.2, 110.8, 110.7, 58.5, 41.1; FT-IR (KBr, cm^{-1}): 3031, 2927, 1734, 1604, 1497, 1245, 1151, 700, 600; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{13}H_{13}O_3$ 217.0865, found 217.0863.

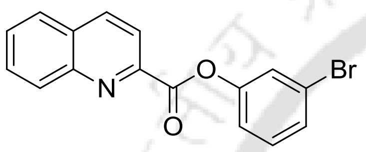
(1*R*, 2*S*, 5*R*)-2-Isopropyl-5-methylcyclohexyl picolinate (2i)



2i

Brown Oil; (196 mg, 75%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79-8.78 (d, *J* = 4.2 Hz, 1H), 8.11-8.09 (d, *J* = 7.8 Hz, 1H), 7.84-7.82 (t, *J* = 7.8 Hz, 1H), 7.47-7.46 (m, 1H), 5.03-4.99 (m, 1H), 1.96-1.91 (m, 1H), 1.72-1.52 (m, 4H), 1.26-1.07 (m, 4H), 0.90-0.87 (m, 6H), 0.77-0.75 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.8, 150.0, 148.6, 137.2, 126.9, 125.3, 76.1, 47.1, 40.9, 34.4, 31.7, 26.5, 23.6, 22.2, 20.9, 16.5; FT-IR (KBr, cm^{-1}): 2955, 2870, 1713, 1584, 1456, 1306, 1244, 1133, 1087, 748, 707, 619; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{16}H_{24}NO_2$ 262.1807, found 262.2001.

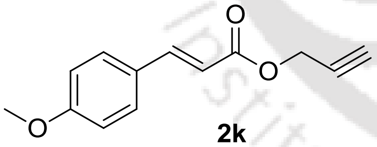
3-Bromophenyl quinoline-2-carboxylate (2j)



2j

Brown Oil; (271 mg, 83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43-8.41 (d, *J* = 8.4 Hz, 1H), 8.32-8.29 (m, 2H), 7.94-7.92 (d, *J* = 8.4 Hz, 1H), 7.81-7.77 (t, *J* = 7.2 Hz, 1H), 7.71-7.68 (t, *J* = 7.2 Hz, 1H), 7.38-7.36 (m, 2H), 7.25-7.21 (t, *J* = 7.6 Hz, 1H), 7.17-7.14 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 150.9, 147.1, 146.2, 138.9, 131.7, 130.9, 129.7, 128.0, 125.0, 123.6, 122.8, 121.5, 120.4, 119.0, 114.5; FT-IR (KBr, cm^{-1}): 2924, 1650, 1582, 1472, 1351, 1250, 1062, 994, 863, 770, 679.

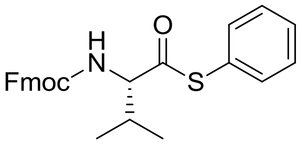
(*E*)-Prop-2-yn-1-yl 3-(4-methoxyphenyl)acrylate (2k)



2k

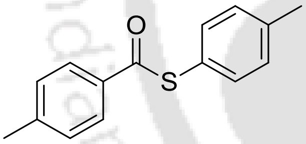
Yellowish Oil; (197 mg, 91%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64-7.60 (d, *J* = 16 Hz, 1H), 7.41-7.39 (d, *J* = 8.8 Hz, 2H), 6.84-6.82 (d, *J* = 8.8 Hz, 2H), 6.28-6.24 (d, *J* = 16 Hz, 1H), 4.75-4.74 (d, *J* = 2.4 Hz, 2H), 3.75 (s, 3H), 2.52-2.50 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 161.5, 145.5, 129.9, 126.8, 114.3, 78.0, 74.9, 55.3, 51.8; FT-IR (KBr, cm^{-1}): 3290, 2937, 2839, 2128, 1714, 1633, 1512, 1424, 1252, 1156, 1031, 828, 551; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{13}O_3$ 217.0865, found 217.0863.

(*S*)-*S*-Phenyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanethioate (2l)


2l

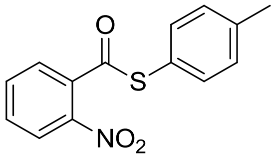
White solid; (392 mg, 91%); mp 127-129 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.75-7.74 (d, *J* = 7.2 Hz, 3H), 7.62-7.59 (t, *J* = 7.2 Hz, 2H), 7.40-7.36 (m, 5H), 7.31-7.28 (t, *J* = 7.2 Hz, 3H), 5.28 (br, 1H), 4.53-4.50 (m, 1H), 4.47-4.40 (m, 2H), 4.25-4.23 (t, *J* = 6.6 Hz, 1H), 2.36-2.30 (m, 1H), 1.01-1.00 (d, *J* = 6.6 Hz, 3H), 0.92-0.91 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 199.0, 156.4, 144.0, 143.9, 141.6, 134.8, 129.8, 129.5, 128.0, 127.3, 127.2, 125.3, 120.2, 67.4, 65.9, 47.5, 31.4, 19.7, 17.1; FT-IR (KBr, cm^{-1}): 2925, 1726, 1674, 1527, 1450, 1225, 1104, 1009, 741, 689; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{26}H_{26}NO_3S$ 432.1633, found 432.1620.

S-p-Tolyl 4-methylbenzothioate (2m)


2m

White solid; (225 mg, 93%); mp 121-123 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.93-7.91 (d, *J* = 7.8 Hz, 2H), 7.39-7.38 (d, *J* = 7.8 Hz, 2H), 7.28-7.25 (t, *J* = 7.2 Hz, 4H), 2.42 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 190.4, 144.7, 139.9, 135.3, 134.3, 130.3, 129.6, 127.7, 124.1, 21.9, 21.6; FT-IR (KBr, cm^{-1}): 2920, 1666, 1602, 1401, 1204, 1172, 904, 811, 717, 624; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{15}H_{15}OS$ 243.0844, found 243.0843.

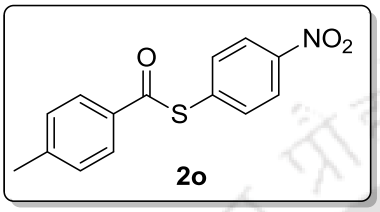
S-p-Tolyl 2-nitrobenzothioate (2n)


2n

Brown solid; (235 mg, 86%); mp 112-115 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.09-8.08 (d, *J* = 7.8 Hz, 1H), 7.74-7.72 (t, *J* = 7.8 Hz, 1H), 7.68-7.64 (m, 2H), 7.46-7.44 (d, *J* = 8.4 Hz, 2H), 7.28-7.27 (d, *J* = 7.8 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (150

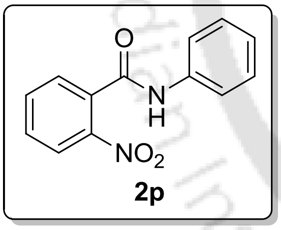
MHz, $CDCl_3$) δ 190.9, 146.3, 140.7, 135.0, 134.9, 133.8, 131.7, 130.5, 128.6, 124.9, 123.2, 21.6; FT-IR (KBr, cm^{-1}): 2879, 1694, 1532, 1350, 1204, 1104, 904, 618; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{12}NO_3S$ 274.0538, found 274.0534.

S-(4-Nitrophenyl) 4-methylbenzothioate (2o)



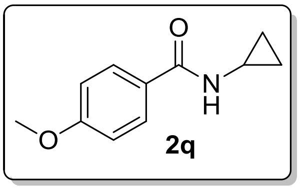
White solid; (224 mg, 82%); mp 118-120 ºC; ^1H NMR (600 MHz, $CDCl_3$) δ 8.29-8.28 (d, *J* = 8.4 Hz, 2H), 7.92-7.91 (d, *J* = 8.4 Hz, 2H), 7.71-7.70 (d, *J* = 9.0 Hz, 2H), 7.32-7.31 (d, *J* = 7.8 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.7, 148.4, 145.6, 136.6, 135.6, 133.6, 129.8, 127.9, 124.1, 22.0; FT-IR (KBr, cm^{-1}): 2923, 2852, 1673, 1598, 1518, 1345, 1261, 1025, 904, 811, 742, 636; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{12}NO_3S$ 274.0538, found 274.0533.

2-Nitro-N-phenylbenzamide (2p)



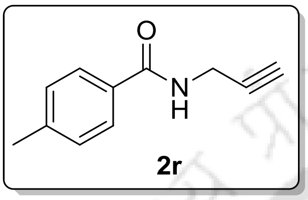
Solid; (191 mg, 79%); mp 140-143 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03-8.01 (d, *J* = 8.0 Hz, 1H), 7.67-7.63 (t, *J* = 7.2 Hz, 1H), 7.56-7.54 (d, *J* = 7.6 Hz, 4H), 7.33-7.29 (t, *J* = 7.6 Hz, 2H), 7.15-7.11 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 146.2, 137.8, 134.0, 133.0, 130.6, 129.1, 128.9, 125.0, 124.5, 120.6, 120.5; FT-IR (KBr, cm^{-1}): 2924, 2854, 1655, 1599, 1442, 1346, 1258, 856, 788, 588; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{11}N_2O_3$ 243.0770, found 243.0769.

N-Cyclopropyl-4-methoxybenzamide (2q)



White solid; (157 mg, 82%); mp 123-125 ºC; ^1H NMR (600 MHz, $CDCl_3$) δ 7.72-7.70 (d, *J* = 8.4 Hz, 2H), 6.86 (br s, 1H), 6.81-6.79 (d, *J* = 8.8 Hz, 2H), 3.76 (s, 3H), 2.83-2.78 (m, 1H), 0.76-0.71 (m, 2H), 0.59-0.55 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.7, 162.1, 128.9, 126.8, 113.6, 55.4, 23.2, 6.6; FT-IR (KBr, cm^{-1}): 3005, 2839, 2058, 1624, 1504, 1324, 1311, 1176, 958, 845, 770; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{14}NO_2$ 192.1025, found 192.1026.

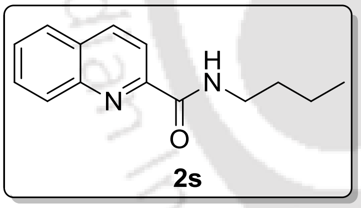
4-Methyl-*N*-(prop-2-yn-1-yl)benzamide (2r)



2r

White solid; (147 mg, 85%); mp 117-119 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70-7.68 (d, *J* = 8.4 Hz, 2H), 7.19-7.17 (d, *J* = 8.0 Hz, 2H), 6.82 (br s, 1H), 4.21-4.19 (q, 2H), 2.36 (s, 3H), 2.25-2.23 (t, *J* = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 142.3, 131.0, 129.3, 127.2, 79.9, 71.7, 29.8, 21.6; FT-IR (KBr, cm^{-1}): 2926, 1919, 1639, 1545, 1416, 1309, 1258, 1122, 840, 751, 654, 565; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{12}NO_2$ 174.0919, found 174.0914.

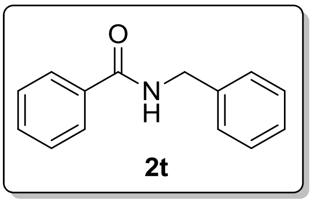
***N*-Butylquinoline-2-carboxamide (2s)**



2s

Brown Oil; (198 mg, 87%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31-8.28 (m, 2H), 8.10-8.09 (d, *J* = 8.4 Hz, 1H), 7.87-7.85 (d, *J* = 7.8 Hz, 1H), 7.76-7.74 (t, *J* = 7.2 Hz, 1H), 7.61-7.59 (t, *J* = 7.2 Hz, 1H), 3.55-3.51 (m, 2H), 1.70-1.65 (m, 2H), 1.49-1.42 (m, 2H), 0.98-0.96 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.5, 150.0, 146.5, 137.5, 130.1, 129.7, 129.3, 127.9, 127.8, 118.9, 39.4, 31.9, 20.3, 13.9; FT-IR (KBr, cm^{-1}): 2926, 2856, 1643, 1547, 1369, 1133, 856, 725, 587; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{14}H_{17}N_2O$ 229.1341, found 229.1340.

***N*-Benzylbenzamide (2t)**

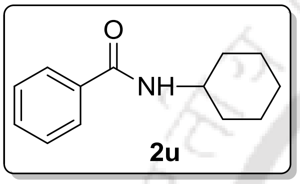


2t

White solid; (192 mg, 91%); mp 80-82 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79-7.78 (d, *J* = 7.2 Hz, 2H), 7.51-7.48 (t, *J* =

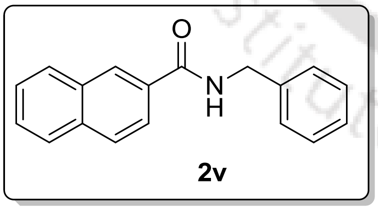
7.2 Hz, 1H), 7.43-7.41 (t, *J* = 7.2 Hz, 2H), 7.35-7.34 (d, *J* = 4.2 Hz, 4H), 7.32-7.28 (m, 1H), 6.59 (br, 1H), 4.64-4.63 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.9, 138.3, 134.3, 131.7, 128.8, 128.7, 128.0, 127.7, 127.2, 44.2; FT-IR (KBr, cm^{-1}): 2954, 1637, 1552, 1416, 1316, 1260, 985, 805, 725, 695, 461; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{14}H_{14}NO$ 212.1075, found 212.1075.

_N_-Cyclohexylbenzamide (2u)


2u

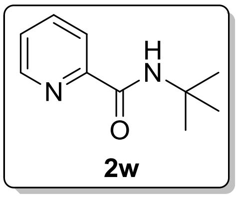
White solid; (189 mg, 93%); mp 152-154 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75-7.74 (d, *J* = 7.2 Hz, 2H), 7.49-7.46 (t, *J* = 7.2 Hz, 1H), 7.43-7.40 (t, *J* = 7.8 Hz, 2H), 6.02 (br s, 1H), 4.00-3.94 (m, 1H), 2.04-2.01 (m, 2H), 1.76-1.73 (m, 3H), 1.66-1.64 (m, 1H), 1.45-1.38 (m, 2H), 1.26-1.18 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 134.8, 131.4, 128.5, 126.9, 48.9, 33.0, 32.9, 25.5, 25.0; FT-IR (KBr, cm^{-1}): 2927, 2850, 1624, 1574, 1452, 1261, 1082, 700; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{18}NO$204.1388,, found 204.1395.

_N_-Benzyl-2-naphthamide (2v)


2v

White solid; (232 mg, 89%); mp 138-140 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.88-7.85 (m, 4H), 7.59-7.50 (m, 2H), 7.40-7.29 (m, 5H), 6.86 (br s, 1H), 4.70-4.68 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 138.4, 134.8, 132.7, 131.7, 129.1, 128.9, 128.5, 128.0, 127.8, 127.7, 127.6, 127.5, 126.8, 123.8, 44.3; FT-IR (KBr, cm^{-1}): 2923, 2853, 1637, 1547, 1414, 1320, 1264, 1146, 1048, 835, 780, 694, 478; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{18}H_{16}NO$262.1232, found 262.1235.

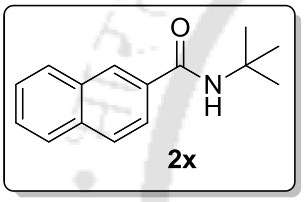
N-(tert-Butyl)picolinamide (2w)



2w

Brown Oil; (153 mg, 86%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.51-8.50 (d, *J* = 4.8 Hz, 1H), 8.18-8.16 (d, *J* = 11.0 Hz, 1H), 8.01 (br s, 1H), 7.84-7.80 (t, *J* = 7.6 Hz, 1H), 7.41-7.37 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.6, 151.0, 147.9, 137.9, 126.0, 121.9, 51.0, 28.9; FT-IR (KBr, cm^{-1}): 2966, 1679, 1522, 1461, 1365, 1231, 998, 751, 621; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{10}H_{15}N_2O$179.1184,, found 179.1188.

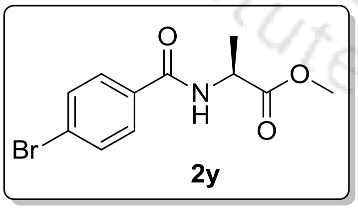
***N*-(tert-Butyl)-2-naphthamide (2x)**



2x

White solid; (182 mg, 80%); mp 162-164 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.21 (s, 1H), 7.90-7.89 (d, *J* = 7.8 Hz, 1H), 7.86-7.84 (t, *J* = 6.6 Hz, 2H), 7.79-7.77 (m, 1H), 7.55-7.51 (m, 2H), 6.23 (br s, 1H), 1.52 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 167.2, 134.7, 133.3, 132.8, 129.0, 128.5, 127.8, 127.6, 127.1, 126.8, 123.8, 51.9, 29.1; FT-IR (KBr, cm^{-1}): 2963, 1638, 1571, 1452, 1318, 834, 474; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{15}H_{18}NO$ 228.1388, found 228.1384.

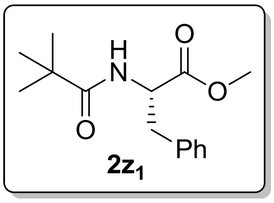
(*S*)-Methyl 2-(4-bromobenzamido) propanoate (2y)



2y

White solid; (239 mg, 84%); mp 104-106 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.64-7.62 (d, *J* = 8.4 Hz, 2H), 7.51-7.50 (d, *J* = 8.4 Hz, 2H), 6.99 (br s, 1H), 4.76-4.72 (m, 1H), 3.75 (s, 3H), 1.48-1.47 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 173.8, 166.1, 132.8, 131.9, 128.9, 126.6, 52.8, 48.7, 18.6; FT-IR (KBr, cm^{-1}): 274958, 2853, 1918, 1748, 1637, 1531, 1462, 1365, 1224, 1170, 845, 762, 620, 552; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{11}H_{13}BrNO_3$ 286.0079, found 286.9795.

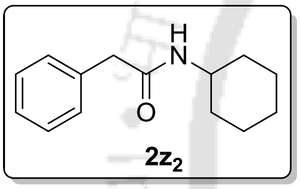
(*S*)-Methyl 3-phenyl-2-pivalamidopropanoate ($2z_1$)



$2z_1$

White solid; (213 mg, 81%); mp 88-90 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.25 (m, 3H), 7.09-7.07 (d, *J* = 7.2 Hz, 2H), 6.06 (br s, 1H), 4.88-4.85 (m, 1H), 3.74 (s, 3H), 3.19-3.08 (m, 2H), 1.15 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.0, 172.4, 136.0, 129.4, 128.6, 127.2, 53.0, 52.4, 38.7, 37.8, 27.4; FT-IR (KBr, cm^{-1}): 2973, 1754, 1731, 1634, 1531, 1202, 1038, 754, 702; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{15}H_{22}NO_3$ 264.1600, found 264.1608.

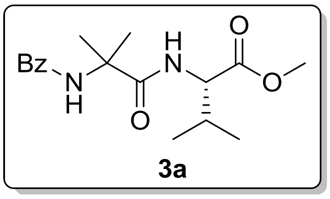
***N*-Cyclohexyl-2-phenylacetamide ($2z_2$)**



$2z_2$

White solid; (197 mg, 91%); mp 131 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.34 (t, *J* = 7.2 Hz, 2H), 7.31-7.24 (m, 3H), 5.24 (br s, 1H), 3.78-3.72 (m, 1H), 3.55 (s, 2H), 1.84-1.81 (m, 2H), 1.62-1.55 (m, 3H), 1.35-1.25 (m, 2H), 1.12-0.97 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 135.3, 129.4, 128.9, 127.2, 48.3, 43.9, 32.9, 25.5, 24.8; FT-IR (KBr, cm^{-1}): 2966, 1679, 1522, 1461, 1365, 1231, 998, 751, 621; LRMS (ESI) m/z: [M+H]+ calcd for $C_{14}H_{20}NO$ 218.1545, found 218.1625.

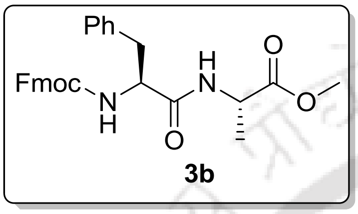
(*S*)-Methyl 2-(2-benzamido-2-methylpropanamido)-3-methylbutanoate (3a)



3a

White solid; (256 mg, 80%); mp 109-111 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78-7.77 (d, *J* = 7.8 Hz, 2H), 7.51-7.49 (t, *J* = 7.2 Hz, 1H), 7.44-7.42 (t, *J* = 7.2 Hz, 2H), 7.01 (br, 1H), 6.91 (br, 1H), 4.56-4.54 (m, 1H), 3.73 (s, 3H), 2.24-2.18 (m, 1H), 1.73-1.71 (d, *J* = 8.4 Hz, 6H), 0.97-0.90 (dd, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.7, 172.6, 167.4, 134.9, 131.9, 128.8, 127.1, 57.9, 57.6, 52.4, 31.5, 25.7, 19.2; FT-IR (KBr, cm^{-1}): 2960, 1745, 1655, 1532, 1314, 1194, 1021, 694; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{17}H_{25}N_2O_4$ 321.1814, found 321.1813.

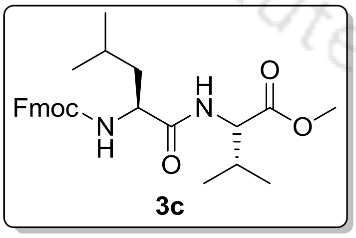
(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-3-phenylpropanoate (3b)



3b

White solid; (420 mg, 89%); mp 185-187 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.77-7.76 (d, *J* = 7.8 Hz, 2H), 7.55-7.52 (t, *J* = 7.8 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.31-7.19 (m, 8H), 6.38 (br, 1H), 5.42 (br, 1H), 4.51-4.49 (t, *J* = 7.2 Hz, 1H), 4.44-4.41 (m, 2H), 4.33-4.30 (t, *J* = 6.6 Hz, 1H), 4.19-4.17 (t, *J* = 7.2 Hz, 1H), 3.71 (s, 3H), 3.12-3.03 (m, 2H), 1.35-1.33 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 173.0, 170.5, 156.1, 143.8, 141.5, 136.4, 129.6, 128.9, 127.9, 127.3, 125.2, 120.2, 67.3, 56.2, 52.7, 48.4, 47.3, 38.8, 18.5; FT-IR (KBr, cm^{-1}): 2923, 2852, 1737, 1696, 1649, 1538, 1105, 619; HRMS (ESI) m/z: [M+H]+ calcd for $C_{28}H_{29}N_2O_5$ 473.2076, found 473.2076.

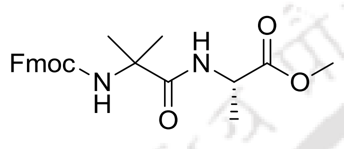
(*R*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanamido)-3-methylbutanoate (3c)



3c

White solid; (396 mg, 85%); mp 130-132 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.77-7.75 (d, *J* = 7.8 Hz, 2H), 7.58-7.57 (d, *J* = 6.6 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.32-7.29 (t, *J* = 6.6 Hz, 2H), 6.52 (br, 1H), 5.30 (br, 1H), 4.54-4.52 (m, 1H), 4.43-4.37 (m, 2H), 4.26-4.20 (m, 2H), 3.73 (s, 3H), 2.18-2.15 (m, 1H), 1.69-1.63 (m, 2H), 1.58-1.53 (m, 1H), 0.96-0.94 (m, 6H), 0.91-0.88 (m, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.4, 172.3, 156.4, 143.4, 141.4, 127.9, 127.2, 125.2, 120.1, 67.2, 57.3, 53.7, 52.3, 47.3, 41.5, 31.4, 24.8, 23.1, 22.2, 19.1,17.9; FT-IR (KBr, cm^{-1}): 3257, 2962, 1724, 1649, 1535, 1252, 743, 619; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{27}H_{35}N_2O_5$ 467.2546, found 467.2537.

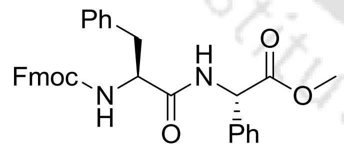
(*S*)-Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanamido)propanoate (3d)



3d

Yellow Oil; (365 mg, 89%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79-7.76 (d, *J* = 7.8 Hz, 2H), 7.62-7.61 (d, *J* = 6.6 Hz, 2H), 7.43-7.39 (m, 2H), 7.35-7.31 (m, 2H), 7.09 (br, 1H), 6.04 (br, 1H), 4.43 (s, 2H), 4.22-4.21 (m, 1H), 4.10-4.07 (m, 1H), 3.73 (s, 3H), 1.50-1.38 (m, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 173.4, 155.1, 143.8, 141.3, 127.7, 127.1, 125.0, 120.0, 66.6, 56.8, 52.4, 48.3, 47.2, 25.5, 18.1; FT-IR (KBr, cm^{-1}): 2984, 1732, 1652, 1255, 739, 620; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{23}H_{27}N_2O_5$ 411.1920, found 411.1929.

(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-2-phenylacetate (3e)

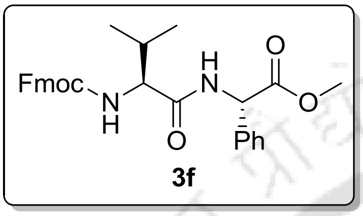


3e

White solid; (459 mg, 86%); mp 176-178 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.54-7.51 (t, *J* = 7.2 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.30-7.25 (m, 10H), 7.18-7.17 (m, 2H), 6.78 (br, 1H), 5.46-5.45 (d, *J* = 6.6 Hz, 1H), 5.37 (br, 1H), 4.52 (br, 1H), 4.42-4.37 (m, 1H), 4.32-4.31 (d, *J* = 6.6 Hz, 1H), 4.18-4.16 (t, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 3.18-3.01 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 170.4, 156.1, 144.0, 141.5, 136.1, 129.1, 128.9, 128.8, 127.9, 127.4, 127.3, 125.2, 120.2, 67.3, 56.8, 56.1, 53.0, 47.3, 38.8; FT-IR (KBr, cm^{-1}):

2921, 1739, 1690, 1654, 1535, 1283, 730; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{33}H_{31}N_2O_5$ 535.2233, found 535.2211.

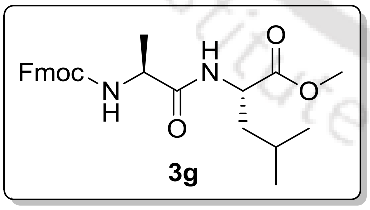
(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-2-phenylacetate (3f)



3f

White solid; (423 mg, 87%); mp 190-192 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.57-7.56 (t, *J* = 7.2 Hz, 2H), 7.41-7.38 (m, 2H), 7.33-7.29 (m, 7H), 6.79 (br, 1H), 5,54-5.52 (d, *J* = 7.2 Hz, 1H), 5.40 (br, 1H), 4.41-4.38 (t, *J* = 7.8 Hz, 1H), 4.35-4.32 (t, *J* = 6.6 Hz, 1H), 4.21-4.18 (t, *J* = 7.2 Hz, 1H), 4.07-4.06 (t, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 2.17-2.13 (m, 1H), 1.01-0.96 (dd, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 170.9, 156.6, 144.1, 141.5, 136.0, 129.3, 128.9, 127.9, 127.5, 127.3, 125.3, 120.2, 67.3, 60.4, 56.8, 53.1, 47.3, 31.6, 18.1; FT-IR (KBr, cm^{-1}): 3064, 2957, 1738, 1691, 1653, 1536, 1450, 1389, 1289, 1174, 1034, 732, 647; LRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{29}H_{31}N_2O_5$ 487.2233, found 487.2135.

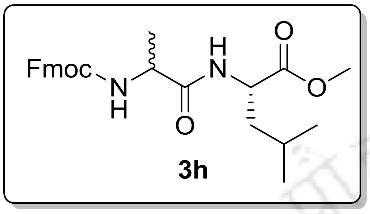
(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-methylpentanoate (3g)



3g

White solid; (390 mg, 89%); mp 126-128 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.75 (d, *J* = 7.8 Hz, 2H), 7.59-7.58 (t, *J* = 7.2 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.32-7.30 (t, *J* = 7.2 Hz, 2H), 6.41 (br, 1H), 5.45 (br, 1H), 4.64-4.59 (m, 1H), 4.39-4.38 (d, *J* = 6.6 Hz, 2H), 4.32-4.26 (m, 1H), 4.22-4.20 (t, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 1.67-1.61 (m, 2H), 1.56-1.53 (m, 1H), 1.41-1.40 (d, *J* = 7.2 Hz, 3H), 0.91-0.89 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.4, 172.3, 157.0, 144.0, 141.5, 127.9, 127.2, 125.2, 120.2, 67.1, 54.6, 52.2, 47.3, 41.4, 24.8, 23.0, 17.6; FT-IR (KBr, cm^{-1}): 2955, 1744, 1691, 1653, 1537, 1448, 1258, 738; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{31}N_2O_5$ 439.2233, found 439.2226.

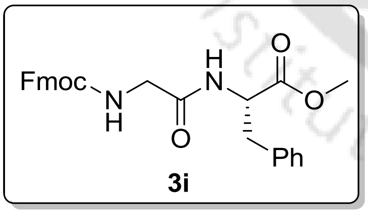
DL-(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-methylpentanoate (3h)



3h

White solid; (372 mg, 85%); mp 126-128 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.74 (d, *J* = 7.2 Hz, 2H), 7.59-7.58 (d, *J* = 7.8 Hz, 2H),7.40-7.37 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (t, *J* = 7.2 Hz, 2H), 6.91 (br, 1H), 6.77 (br, 1H), 5.75-5.72 (t, *J* = 7.8 Hz, 1H), 4.64-4.60 (m, 1H), 4.40-4.34 (m, 3H), 4.24-4.20 (m, 1H), 3.71, 3.68 (s, 3H), 1.67-1.62 (m, 2H), 1.58-1.53 (m, 1H), 1.42-1.40 (d, *J* = 7.2 Hz, 3H), 0.91-0.88 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.4, 173.3, 172.5, 172.4, 156.8, 156.2, 143.8, 141.4, 127.9, 127.2, 125.2, 120.1, 67.3, 52.5, 52.4, 50.9, 47.2, 41.4, 25.0, 22.9, 22.0, 21.9, 19.1; FT-IR (KBr, cm^{-1}): 2955, 1744, 1691, 1653, 1537, 1448, 1258, 738; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{31}N_2O_5$ 439.2233, found 439.2200.

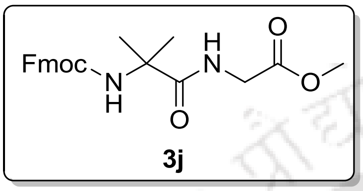
(*S*)-Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)acetamido)-3-phenylpropanoate (3i)



3i

White solid; (403 mg, 88%); mp 175-178 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77-7.75 (d, *J* = 7.6 Hz, 2H), 7.59-7.57 (d, *J* = 7.2 Hz, 2H), 7.42-7.38 (t, *J* = 7.2 Hz, 2H), 7.32-7.28 (t, *J* = 7.6 Hz, 2H), 7.23-7.19 (m, 3H), 7.08-7.06 (d, *J* = 7.2 Hz, 2H), 6.59 (br, 1H), 5.57 (br, 1H), 4.91-4.86 (m, 1H), 4.39-4.37 (d, *J* = 7.2 Hz, 2H), 4.22-4.19 (t, *J* = 7.2 Hz, 1H), 3.88-3.85 (t, *J* = 8.0 Hz, 1H), 3.71 (s, 3H), 3.16-3.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 168.7, 156.5, 143.9, 141.5, 135.7, 128.8, 127.9, 127.4, 127.3, 125.2, 120.2, 67.2, 60.2, 53.3, 47.1, 44.4, 37.8; FT-IR (KBr, cm^{-1}): 2951, 1734, 1668, 1525, 1449, 1216, 1047, 741; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{27}H_{27}N_2O_5$ 459.1920, found 459.1921.

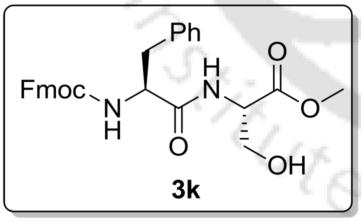
Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpropanamido)acetate (3j)



3j

Yellow solid; (337 mg, 85%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.59-7.58 (d, *J* = 7.2 Hz, 2H), 7.41-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.30 (t, *J* = 7.2 Hz, 2H), 6.73 (br, 1H), 5.30 (br, 1H), 4.45 (s, 2H), 4.21-4.19 (t, *J* = 6.6 Hz, 1H), 4.02 (s, 2H), 3.74 (s, 3H), 1.53 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.0, 170.4, 155.1, 143.8, 141.3, 127.7, 127.0, 125.0, 120.0, 66.5, 60.4, 52.2, 47.2, 41.4, 25.3; FT-IR (KBr, cm^{-1}): 2925, 2854, 1717, 1664, 1524, 1450, 1260, 1090, 739; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{22}H_{25}N_2O_5$ 397.1763, found 397.1765.

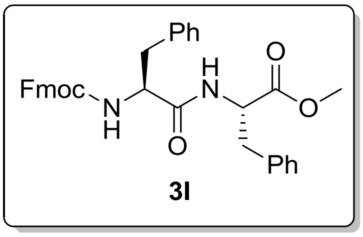
Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoate (3k)



3k

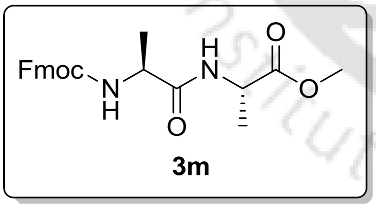
White solid; (444 mg, 91%); mp 191-193 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.53-7.51 (t, *J* = 7.2 Hz, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (t, *J* = 7.2 Hz, 5H), 7.19-7.18 (d, *J* = 6.6 Hz, 2H), 6.84 (br, 1H), 5.45 (br, 1H), 4.58 (s, 1H), 4.46-4.38 (m, 2H), 4.33-4.30 (t, *J* = 7.2 Hz, 1H), 4.18-4.15 (t, *J* = 7.2 Hz, 1H), 3.90 (s, 2H), 3.73 (s, 3H), 3.10-3.09 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 170.6, 156.4, 143.8, 141.5, 136.3, 129.5, 128.9, 128.0, 127.3, 127.2, 125.3, 125.2, 120.2, 67.5, 63.0, 56.4, 55.1, 53.0, 47.2, 38.6; FT-IR (KBr, cm^{-1}): 2925, 1733, 1662, 1542, 1450, 1292, 738, 699; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{28}H_{29}N_2O_6$ 489.2026, found 489.2002.

(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)-3-phenylpropanoate (3l)


3l

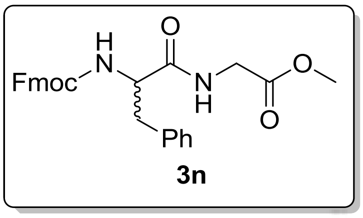
White solid; (488 mg, 89%); mp 171-173 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.54-7.51 (t, *J* = 7.8 Hz, 2H), 7.42-7.39 (t, *J* = 7.8 Hz, 2H), 7.32-7.24 (m, 6H), 7.19-7.18 (d, *J* = 7.2 Hz, 2H), 6.25 (br, 1H), 5.31 (br, 1H), 4.79-4.76 (m, 1H), 4.43-4.41 (m, 2H), 4.29 (br, 1H), 4.19-4.17 (t, *J* = 6.6 Hz, 1H), 3.67 (s, 3H), 3.09-3.00 (m, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.5, 170.5, 155.8, 143.9, 141.5, 135.7, 129.6, 129.4, 128.9, 128.7, 128.0, 127.3, 127.2, 125.2, 120.2, 67.3, 56.1, 53.5, 52.5, 47.3, 38.5, 38.1; FT-IR (KBr, cm^{-1}): 2924, 1738, 1697, 1644, 1535, 1444, 1257, 1033, 738, 698; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{34}H_{33}N_2O_5$ 549.2389, found 549.2388.

(*S*)-Methyl-2-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanoate (3m)


3m

White solid; (360 mg, 91%); mp 141-143 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.77-7.76 (d, *J* = 7.2 Hz, 2H), 7.60-7.58 (d, *J* = 7.2 Hz, 2H), 7.42-7.39 (t, *J* = 7.2 Hz, 2H), 7.33-7.31 (t, *J* = 7.2 Hz, 2H), 6.43 (br, 1H), 5.35 (br, 1H), 4.59-4.56 (t, *J* = 7.2 Hz, 1H), 4.41 (br, 2H), 4.23-4.21 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 1.42-1.41 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 173.3, 172.0, 156.1, 144.0, 141.5, 127.9, 127.3, 125.2, 120.2, 67.3, 52.7, 50.6, 48.3, 47.3, 19.0, 18.5; FT-IR (KBr, cm^{-1}): 2927, 1741, 1688, 1650, 1530, 1451, 1259, 1050, 758; HRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{22}H_{25}N_2O_5$ 397.1763, found 397.1754.

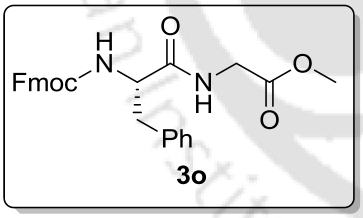
DL-Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)acetate (3n)



3n

White solid; (398 mg, 87%); mp 143-146 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.54-7.51 (m, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (m, 4H), 7.25-7.20 (m, 3H), 6.37 (br, 1H), 5.39 (br, 1H), 4.48-4.33 (m, 3H), 4.19-4.17 (t, *J* = 7.2 Hz, 1H), 4.04-3.91 (m, 2H), 3.72 (s, 3H), 3.10 (s, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.3, 170.0, 156.2, 143.9, 141.5, 136.5, 129.5, 128.9, 127.9, 127.3, 127.2, 125.2, 120.2, 67.3, 56.2, 52.6, 47.3, 41.4, 38.6; FT-IR (KBr, cm^{-1}): 3299, 2921, 1749, 1692, 1649, 1540, 1439, 1260, 1032, 739; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{27}H_{27}N_2O_5$ 459.1920, found 459.1874.

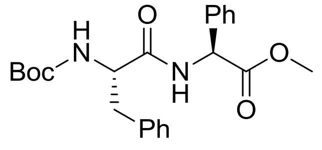
Methyl-2-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)acetate (3o)



3o

White solid; (408 mg, 89%); mp 143-146 ºC; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.76-7.75 (d, *J* = 7.2 Hz, 2H), 7.54-7.51 (m, 2H), 7.41-7.38 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (m, 4H), 7.25-7.20 (m, 3H), 6.41 (br, 1H), 5.41 (br, 1H), 4.48-4.33 (m, 3H), 4.19-4.17 (t, *J* = 7.2 Hz, 1H), 4.05-3.91 (m, 2H), 3.72 (s, 3H), 3.10 (s, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 171.3, 170.0, 156.2, 143.9, 141.5, 136.5, 129.5, 128.9, 127.9, 127.3, 127.2, 125.2, 120.2, 67.3, 56.2, 52.6, 47.3, 41.4, 38.6; FT-IR (KBr, cm^{-1}): 3299, 2921, 1749, 1692, 1649, 1540, 1439, 1260, 1032, 739; LRMS (ESI) m/z: [M+H]$^+$ calcd for $C_{27}H_{27}N_2O_5$ 459.1920, found 459.1876.

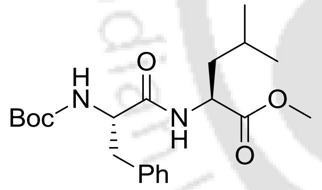
(*S*)-Methyl-2-((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-2-phenylacetate (3p)



3p

White solid; (288 mg, 70%); mp 126-128 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (s, 3H), 7.28-7.11 (m, 7H), 6.93 (br, 1H), 5.48 (br, 1H), 4.98 (br, 1H), 4.42 (br, 1H), 3.69 (s, 3H), 3.12-3.00 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 170.9, 155.6, 136.6, 136.4, 129.6, 129.5, 129.1, 129.0, 128.9, 128.7, 127.4, 127.1, 80.5, 56.7, 55.7, 53.0, 38.3, 28.4; FT-IR (KBr, cm^{-1}): 2979, 1750, 1648, 1535, 1269, 1168, 1017, 699; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_5$ 413.2076, found 413.2077.

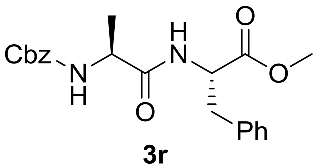
(*S*)-Methyl-2-((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-4-methylpentanoate (3q)



3q

White solid; (290 mg, 74%); mp 86-88 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30-7.28 (t, *J* = 7.2 Hz, 2H), 7.25-7.20 (m, 3H), 6.28 (br, 1H), 5.02 (br, 1H), 4.57-4.55 (t, *J* = 8.4 Hz, 1H), 4.35-4.34 (d, *J* = 6.0 Hz, 1H), 3.69 (s, 3H), 3.07-3.06 (m, 2H), 1.60-1.55 (m, 2H), 1.49-1.45 (m, 1H), 1.41 (s, 9H), 0.91-0.85 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 171.3, 155.6, 136.7, 129.5, 129.4, 128.7, 128.6, 127.0, 80.3, 55.7, 52.4, 50.9, 41.6, 38.2, 28.4, 24.8, 22.8; FT-IR (KBr, cm^{-1}): 2959, 2870, 1751, 1691, 1510, 1367, 1172, 702; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{21}H_{33}N_2O_5$ 393.2389, found 393.2389.

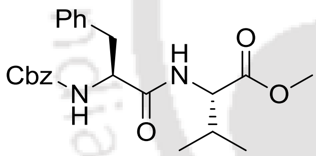
(*S*)-Methyl-2-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-3-phenylpropanoate (3r)



3r

White solid; (330 mg, 86%); mp 99-101 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.31 (m, 5H), 7.27-7.18 (m, 3H), 7.10-7.08 (d, *J* = 6.8 Hz, 2H), 6.80 (br, 1H), 5.56 (br, 1H), 5.12-5.03 (m, 2H), 4.87-4.82 (m, 1H), 4.28 (br, 1H), 3.69 (s, 3H), 3.15-3.02 (m, 2H), 1.32-1.31 (d, *J* = 10.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.9, 156.0, 136.3, 135.8, 129.4, 128.6, 128.3, 128.1, 127.2, 67.1, 53.4, 52.5, 50.5, 37.9, 18.6; FT-IR (KBr, cm^{-1}): 2925, 1757, 1691, 1537, 1451, 1265, 1072, 734, 695; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{21}H_{25}N_2O_5$ 385.1763, found 385.1758.

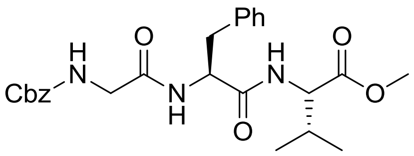
(*S*)-Methyl-2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-methylbutanoate (3s)



3s

White solid; (342 mg, 83%); mp 103-105 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.18 (m, 10H), 6.33 (br, 1H), 5.39 (br, 1H), 5.11-5.06 (m, 2H), 4.46-4.43 (m, 2H), 3.68 (s, 3H), 3.08-3.04 (m, 2H), 2.10-2.06 (m, 1H), 0.85-0.71 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.9, 170.9, 156.1, 136.5, 136.3, 129.5, 129.4, 129.0, 128.9, 128.7, 128.4, 128.3, 128.2, 127.3, 127.2, 67.3, 57.5, 56.4, 52.3, 38.5, 31.4, 19.0, 17.9; FT-IR (KBr, cm^{-1}): 2956, 1741, 1655, 1528, 1286, 750, 699; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_5$ 413.2076, found 413.2081.

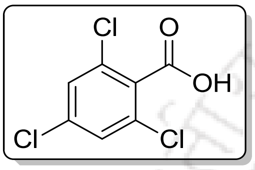
Methyl 8-benzyl-11-isopropyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (3t)



3t

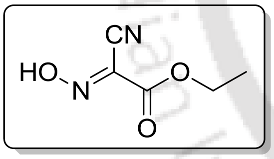
White solid; (422 mg, 90%); mp 101 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32-7.30 (m, 5H), 7.21-7.13 (m, 5H), 5.87 (br, 1H), 5.08 (s, 2H), 4.82 (br, 1H), 4.42-4.40 (m, 1H), 3.89-3.80 (m, 1H), 3.65 (s, 3H), 3.06-3.00 (m, 2H), 2.07-2.04 (m, 1H), 0.83-0.82 (d, *J* = 7.2 Hz, 3H), 0.80-0.79 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 171.2, 169.5, 156.8, 136.5, 136.4, 129.5, 128.7, 128.3, 128.2, 127.1, 67.3, 57.6, 54.6, 52.2, 44.5, 38.5, 31.2, 19.0, 18.0; FT-IR (KBr, cm^{-1}): 3406, 1728, 1677, 1651, 1646, 763, 543; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{25}H_{32}N_3O_6$ 470.2291, found 470.2295.

Recovered 2,4,6-Trichlorobenzoic acid (b)



White solid; mp 162-164 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.98 (br s, 1H), 7.39 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.5, 136.9, 132.8, 131.3, 128.4; FT-IR (KBr, cm^{-1}): 3449, 1717, 1579, 1390, 1281, 1126, 849; LRMS (ESI) m/z: $[M-H]^+$ calcd for $C_6H_3Cl_3O_2$ 222.9121, found 222.9214.

Recovered (*E*)-Ethyl 2-cyano-2-(hydroxyimino)acetate (a)



White solid; mp 130-134 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.41-4.37 (q, 2H), 1.37-1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.0, 126.1, 107.9, 63.8, 14.0; FT-IR (KBr, cm^{-1}): 1729, 1433, 1314, 1071, 849,767; LRMS (ESI) m/z: $[M-H]^+$ calcd for $C_4H_6N_2O_3$ 141.0300, found 141.0510.

6.8. References

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6.9. Selected spectra and chromatograms

6.9.1. NMR (^{1}H and ^{13}C), Mass spectra and HPLC chromatogram of TCBOXY:

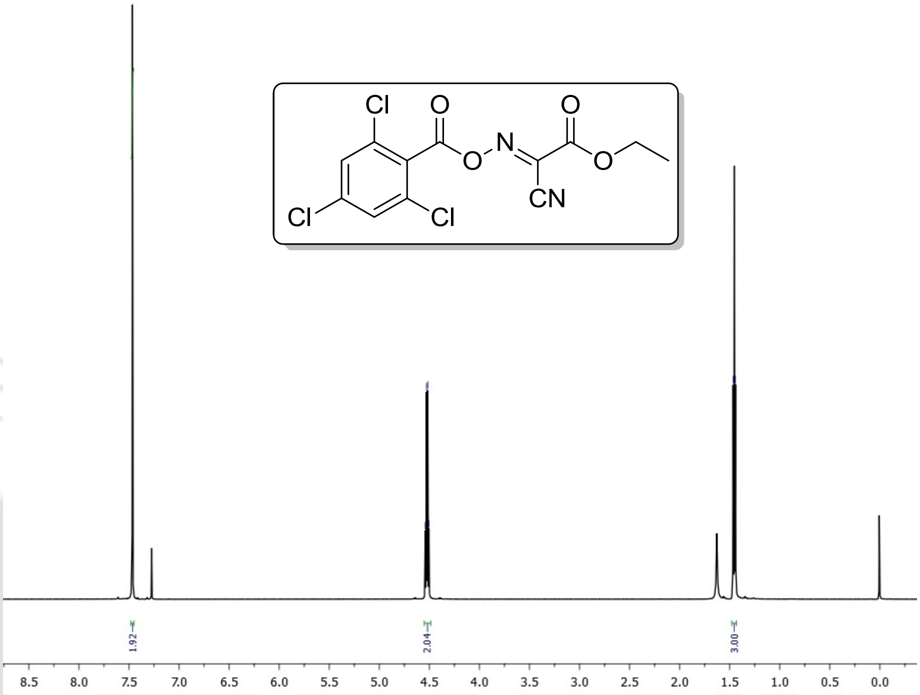


Figure S1. ^{1}H NMR spectra of TCBOXY

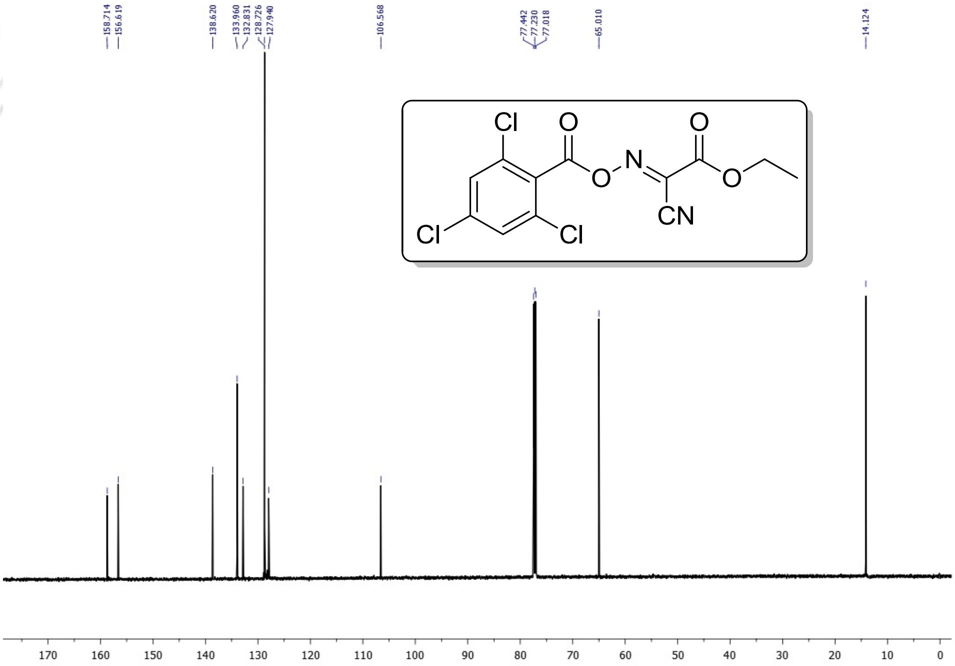


Figure S2. ^{13}C NMR spectra of TCBOXY

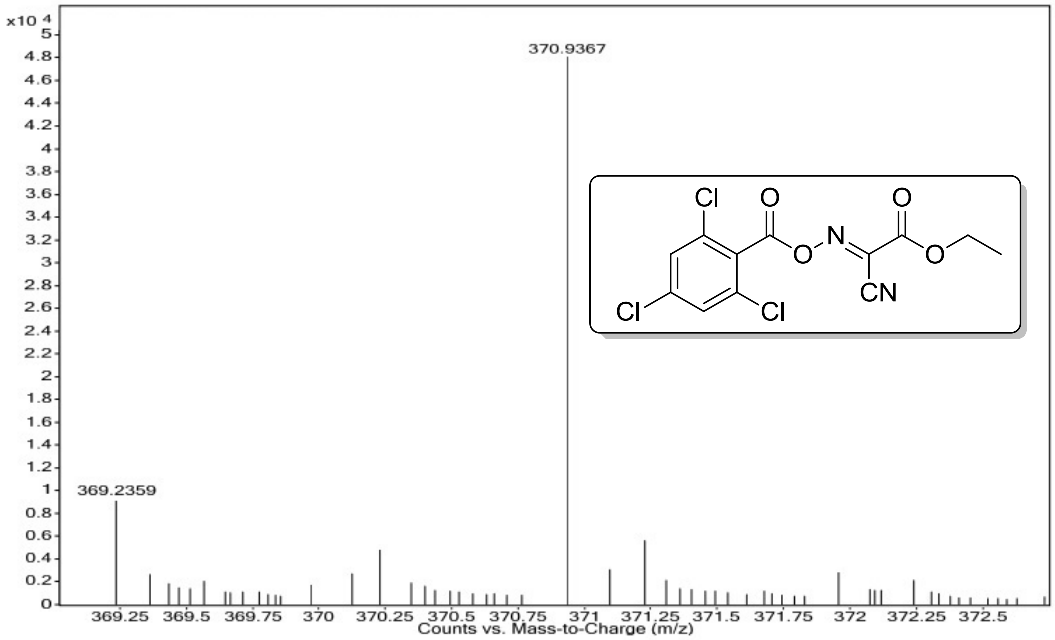


Figure S3. Mass spectra of TCBOXY

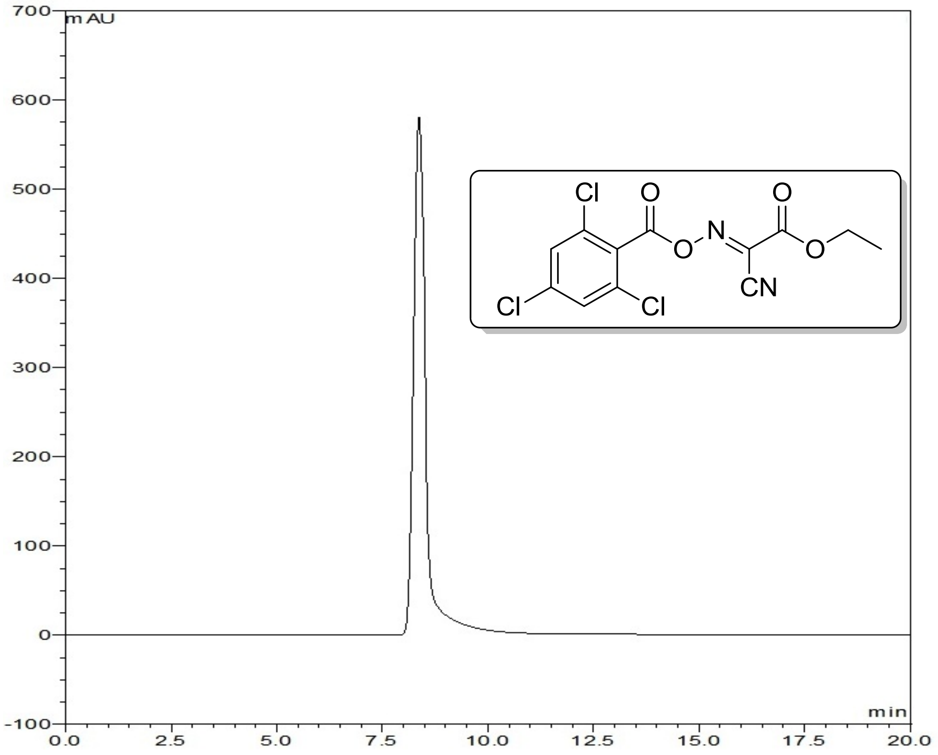


Figure S4. HPLC chromatogram of TCBOXY, first day of synthesis, run in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min, then 90 to 100% upto 20 min., CH_3CN in H_2O with 0.1% formic.

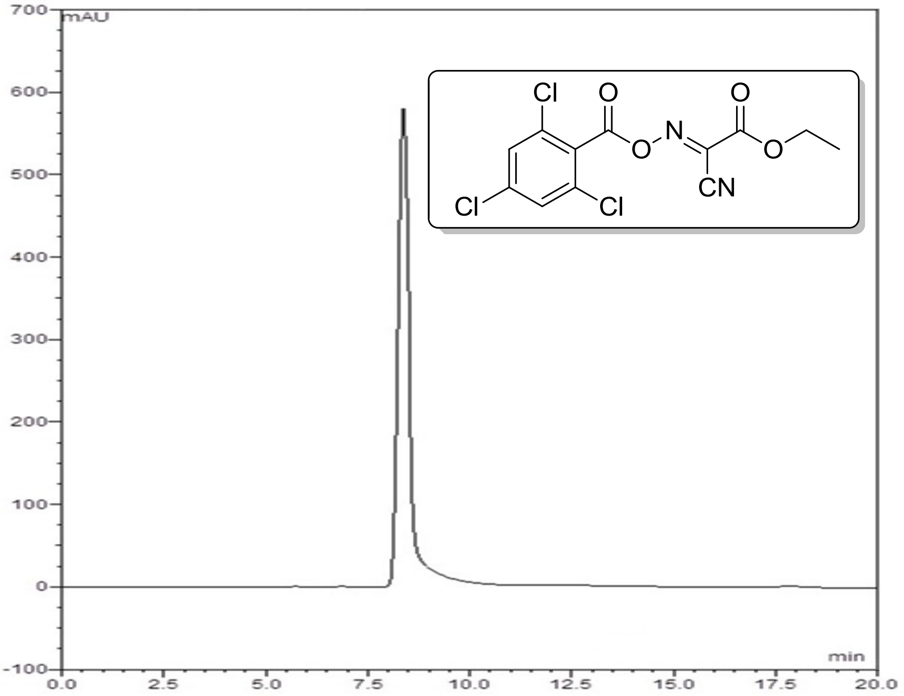


Figure S5. HPLC chromatogram of TCBOXY, after 2 months of synthesis, run in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min, then 90 to 100% upto 20 min., CH_3CN in H_2O with 0.1% formic.

6.9.2. NMR (^{1}H and ^{13}C) and Mass spectra of esters and thioesters

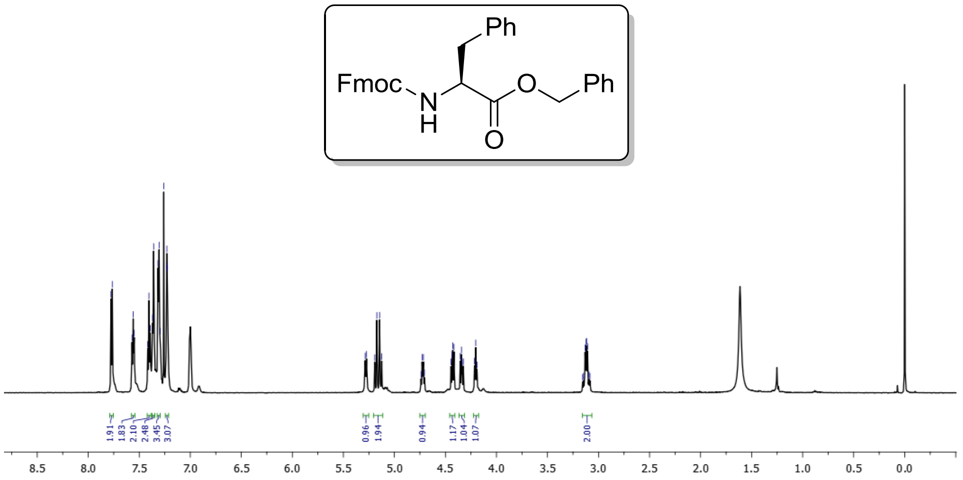


Figure S6. ^{1}H NMR spectra of compound 2a

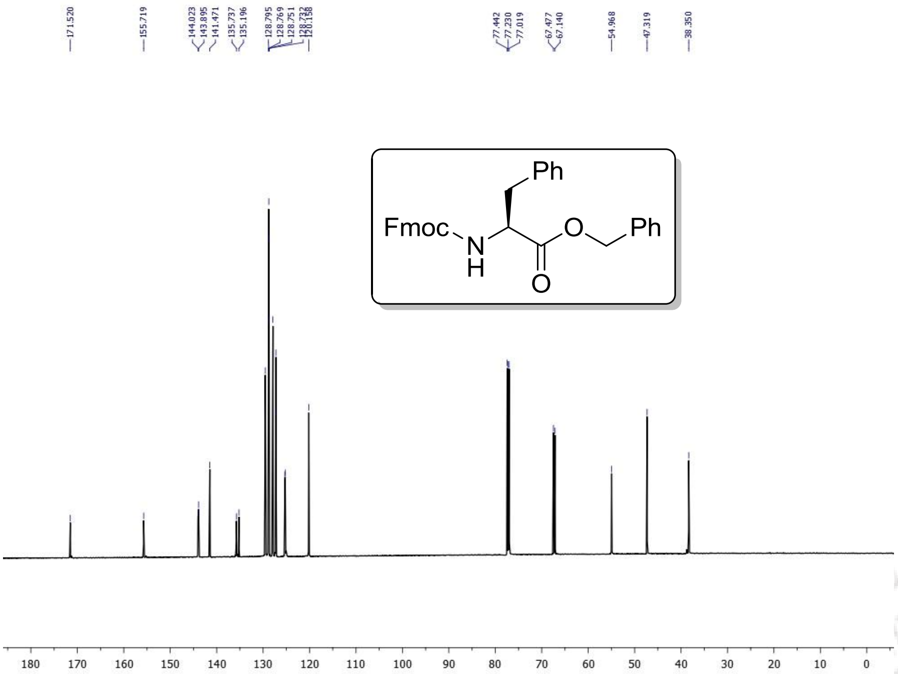


Figure S7. ^{13}C NMR spectra of compound 2a

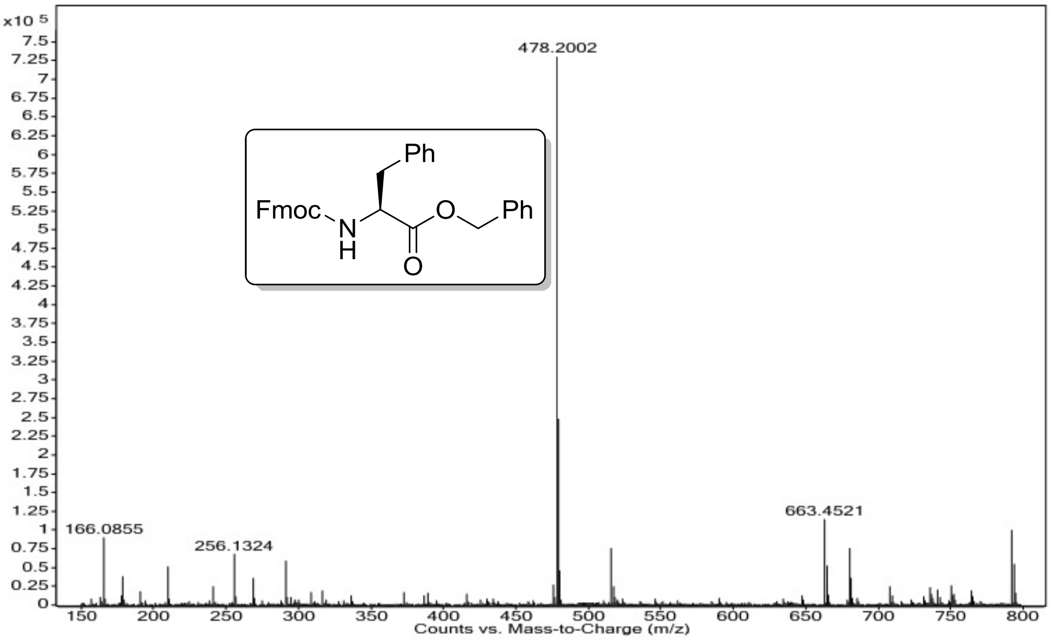


Figure S8. Mass spectra of compound 2a

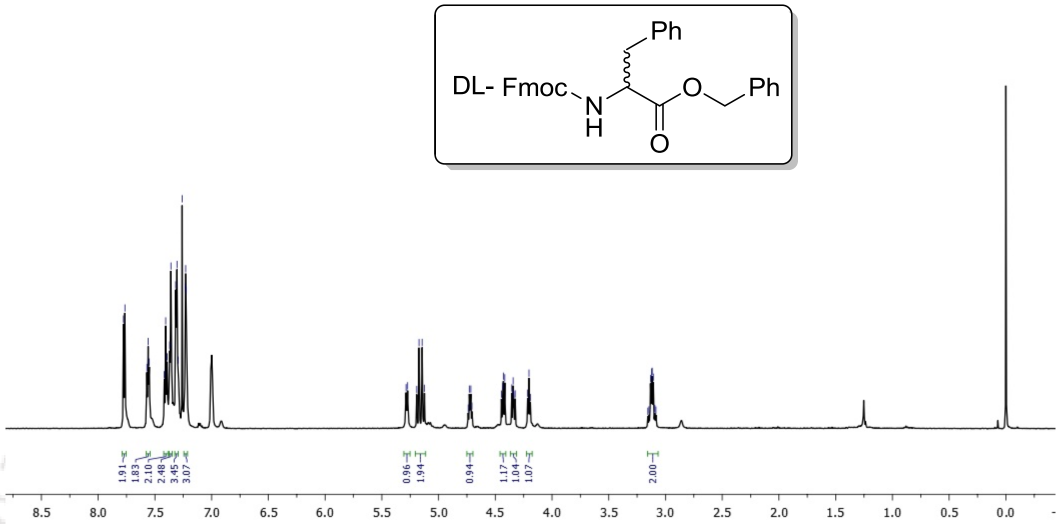


Figure S9. ^{1}H NMR spectra of compound 2b

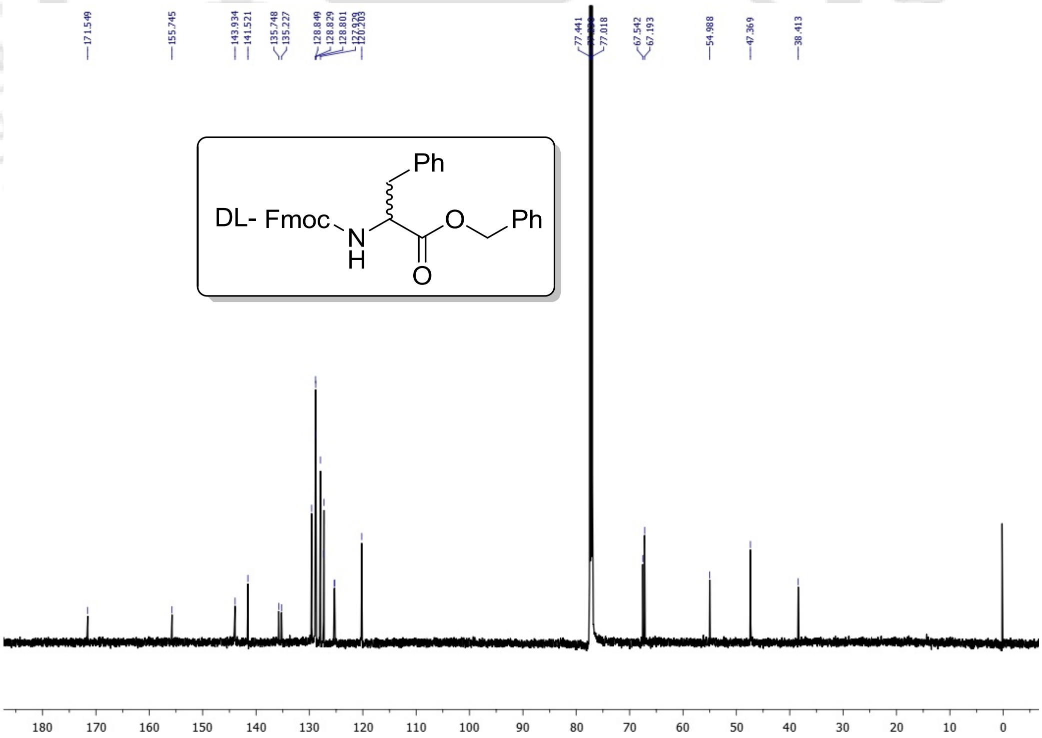


Figure S10. ^{13}C NMR spectra of compound 2b

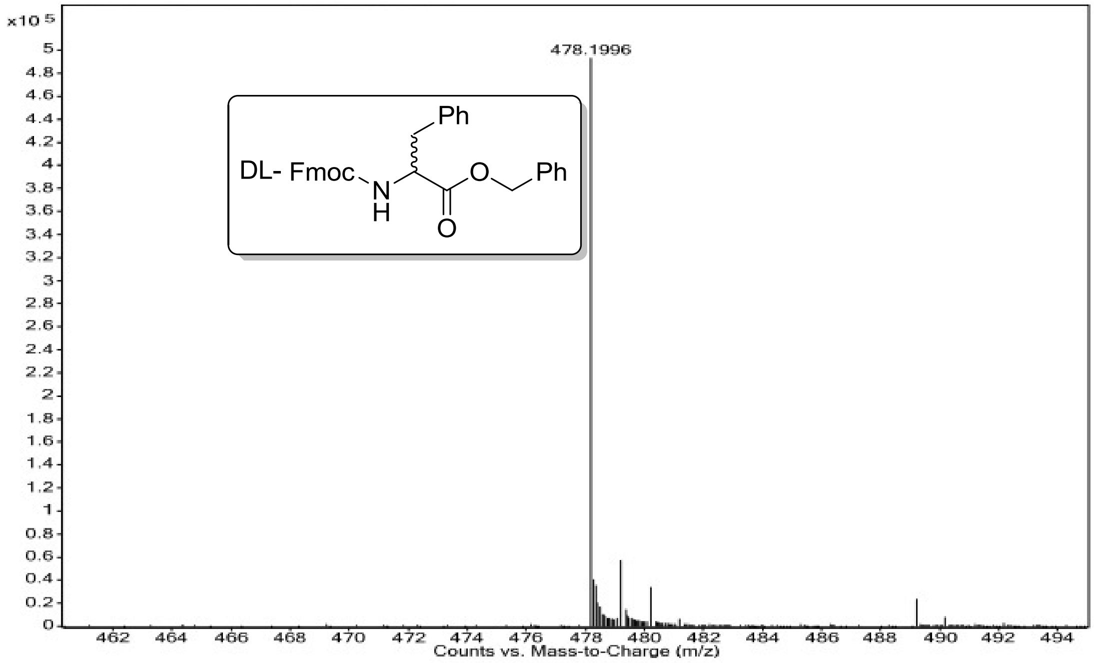


Figure S11. Mass spectra of compound 2b

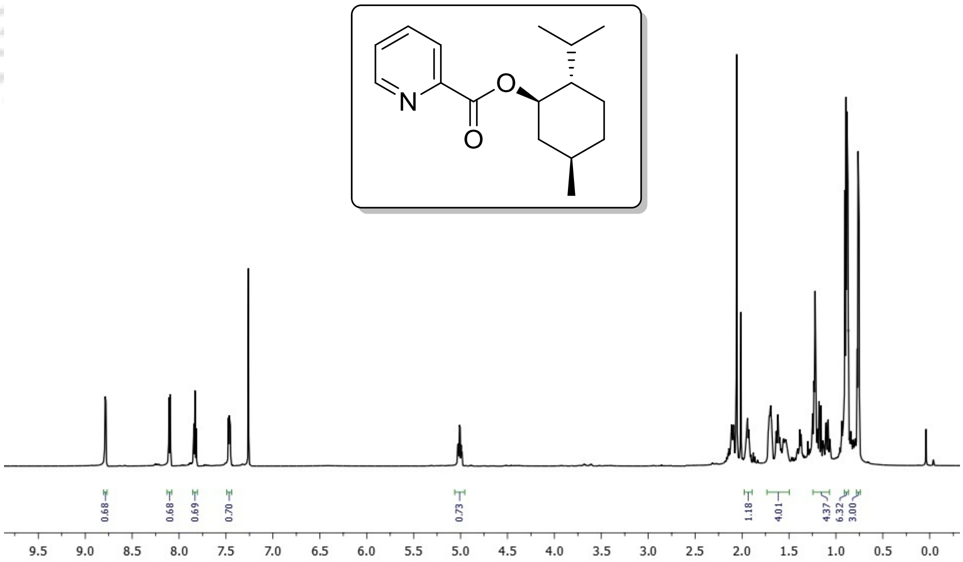


Figure S12. ^{1}H NMR spectra of compound 2i

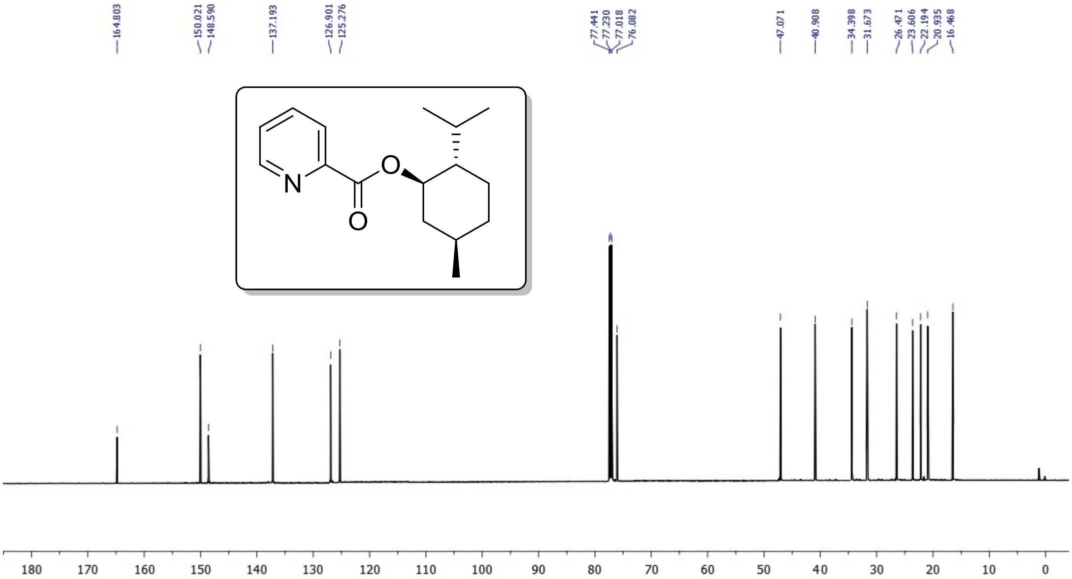


Figure S13. ^{13}C NMR spectra of compound 2i

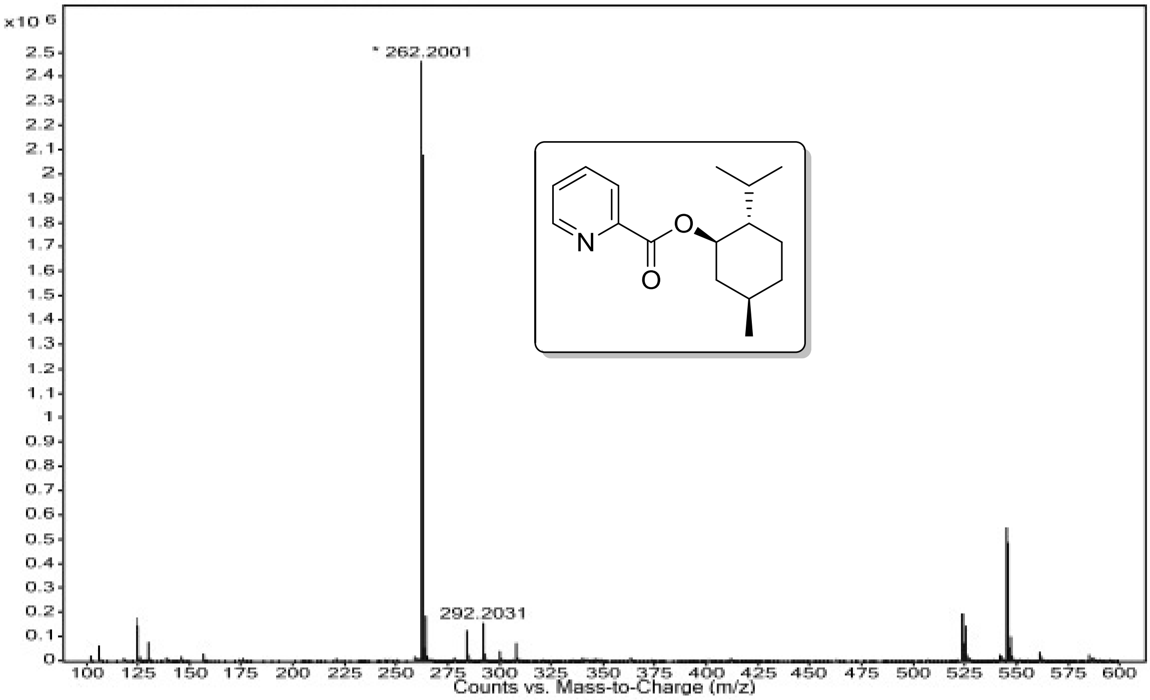


Figure S14. Mass spectra of compound 2i

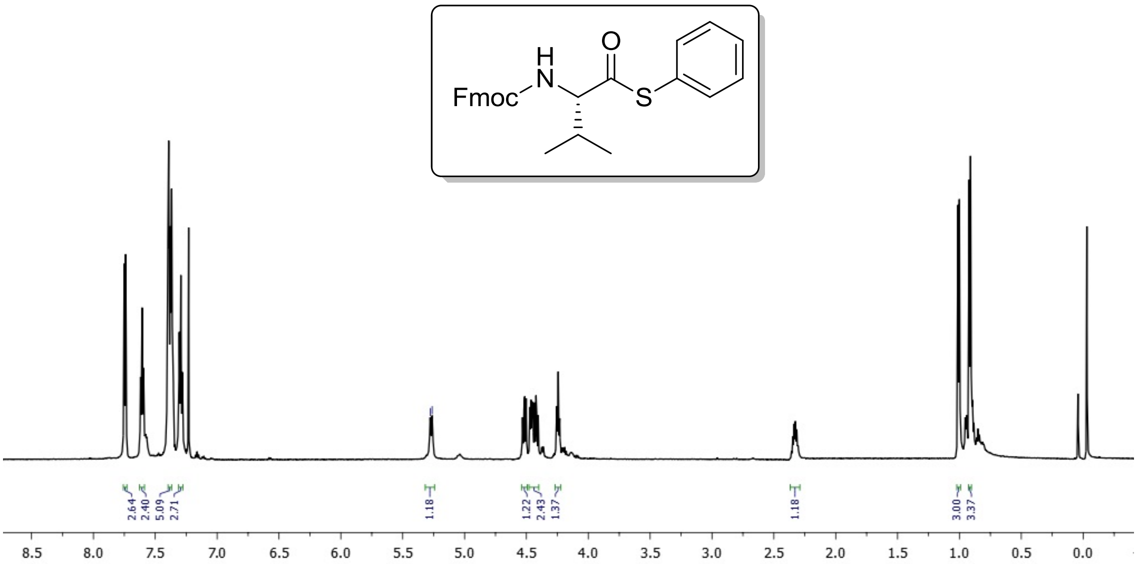


Figure S15. ^{1}H NMR spectra of compound 2l

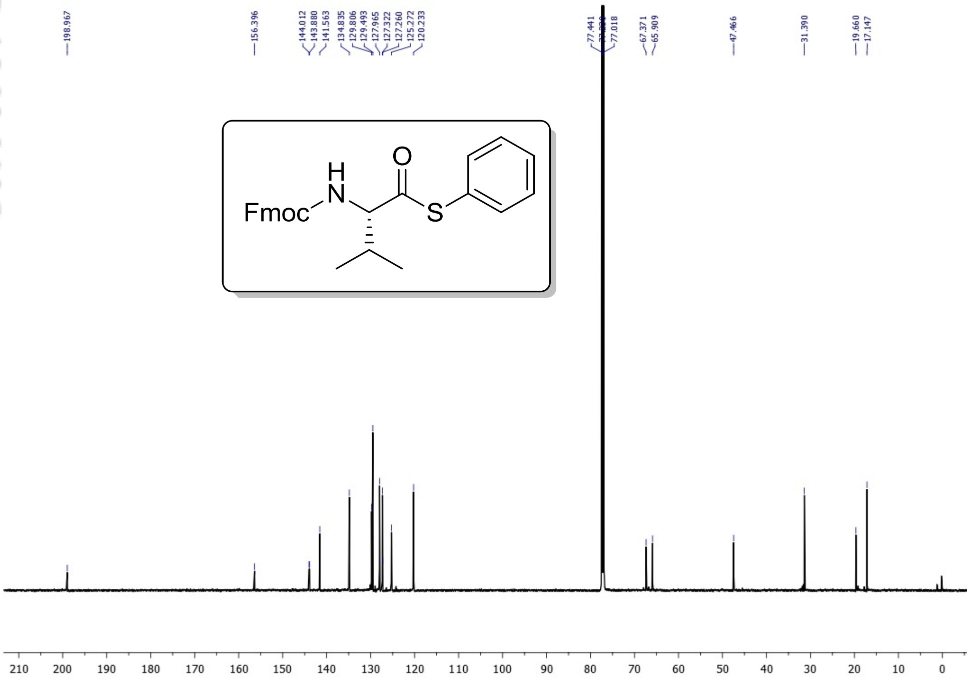


Figure S16. ^{13}C NMR spectra of compound 2l

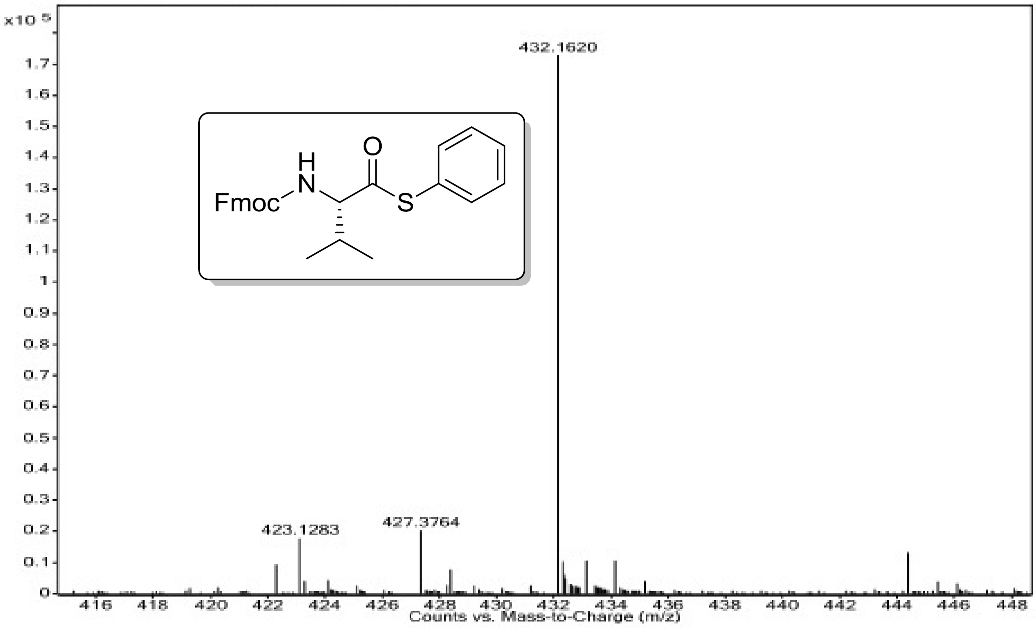


Figure S17. Mass spectra of compound 2l

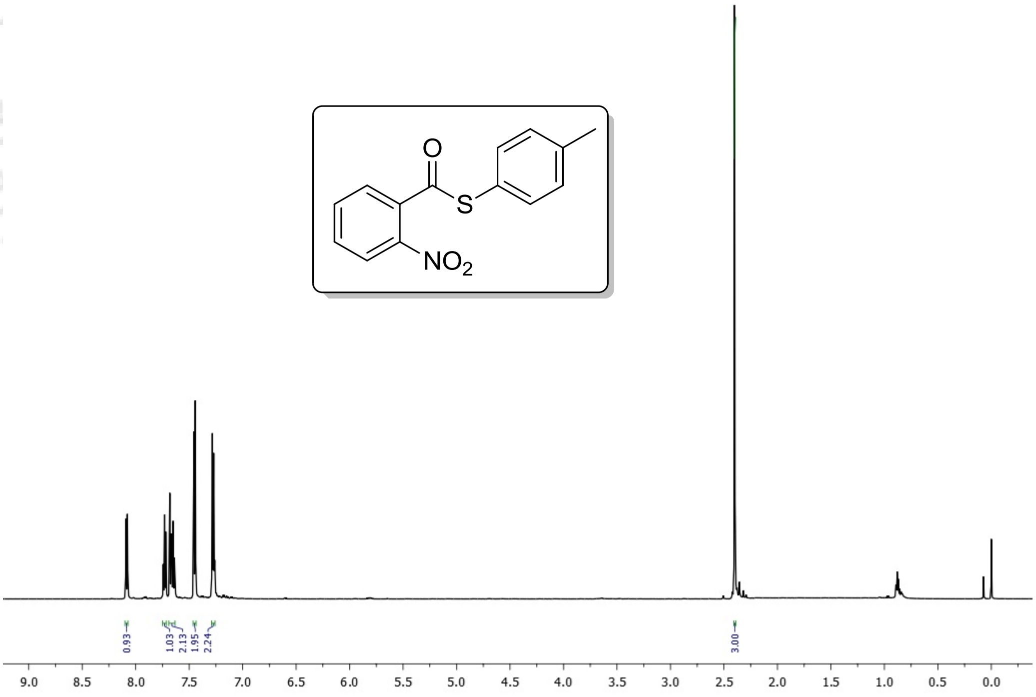


Figure S18. ^{1}H NMR spectra of compound 2n

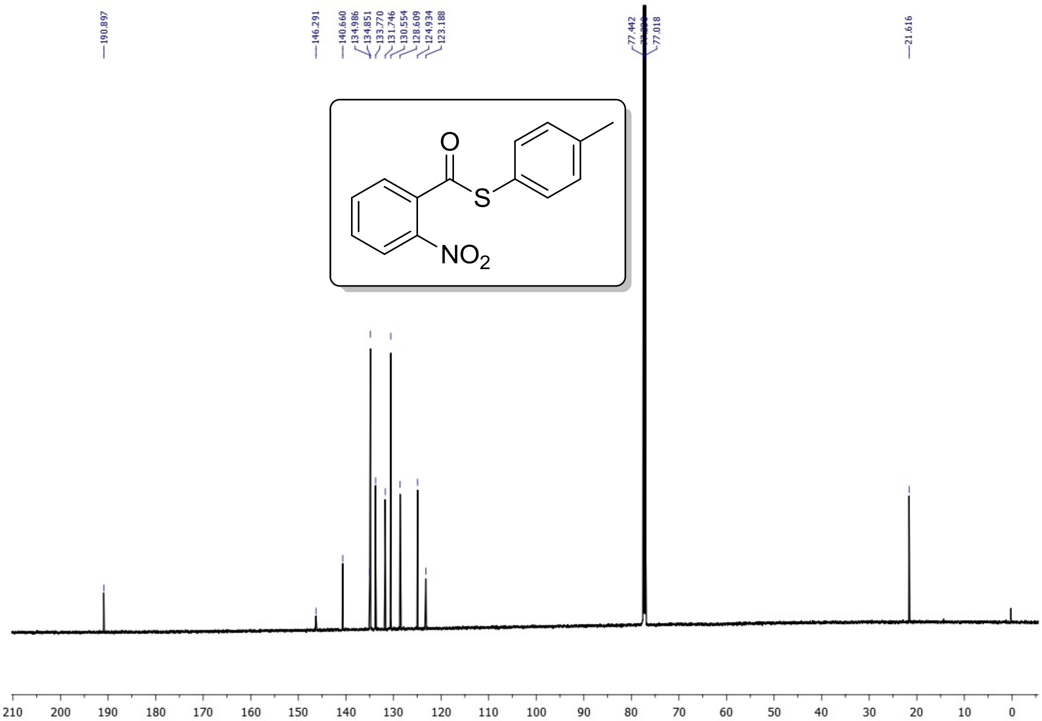


Figure S19. ^{13}C NMR spectra of compound 2n

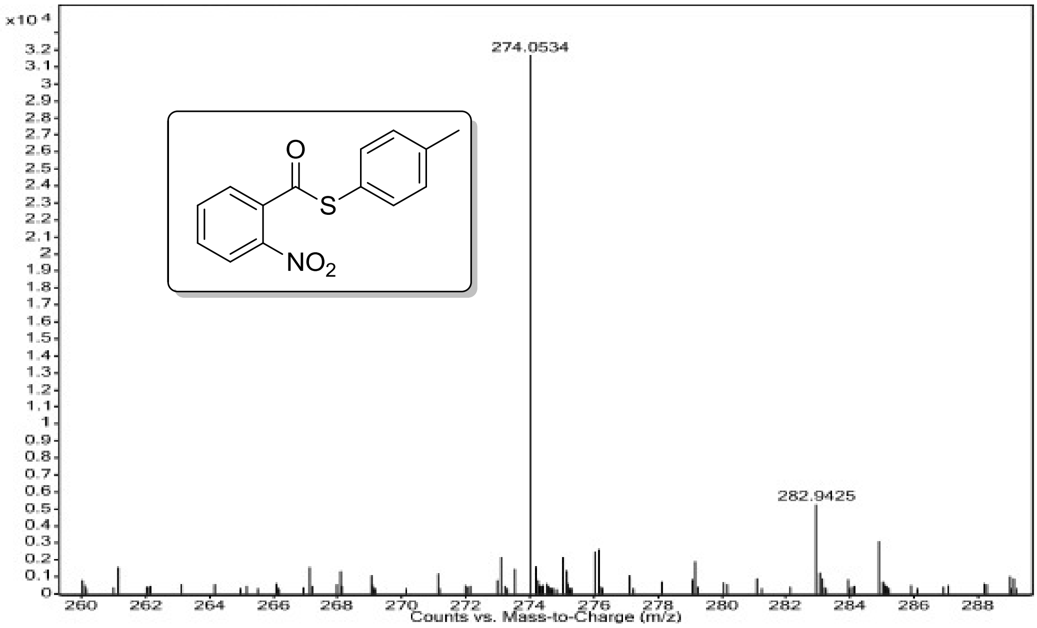


Figure S20. Mass spectra of compound 2n

6.9.3. NMR (^{1}H and ^{13}C) and Mass spectra of amides and peptides

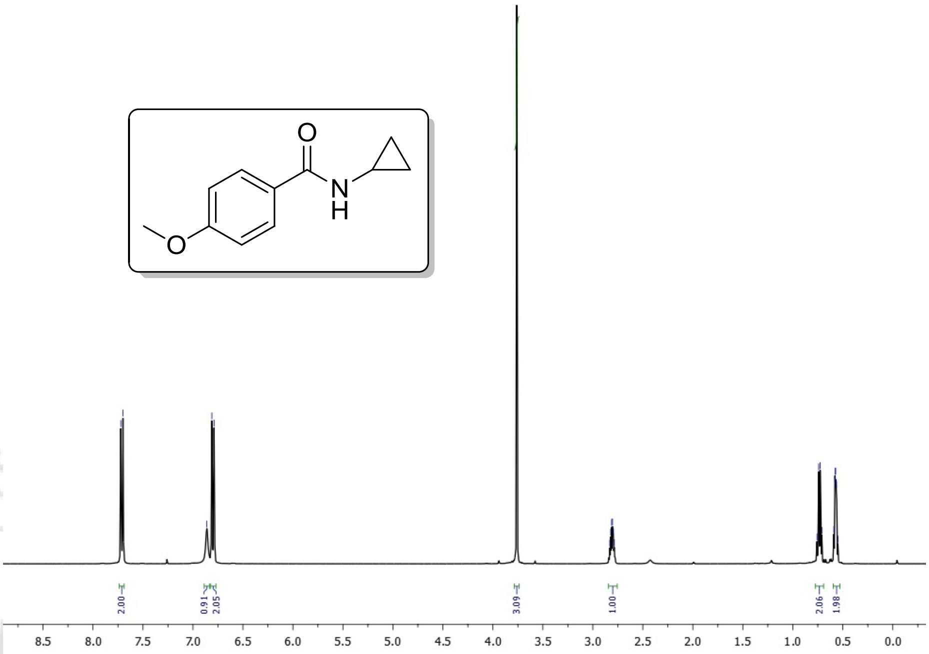


Figure S21. ^{1}H NMR spectra of compound 2q

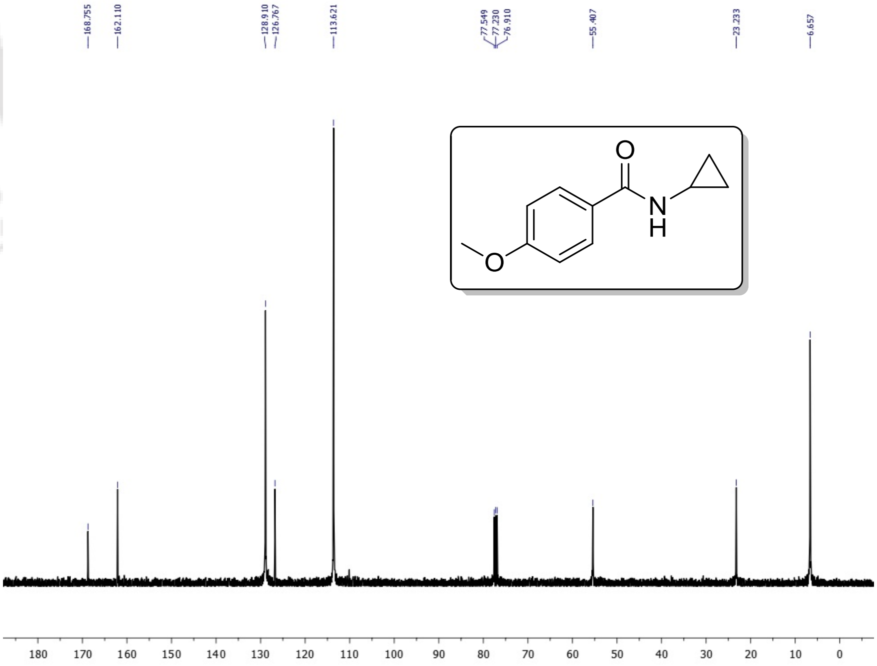


Figure S22. ^{13}C NMR spectra of compound 2q

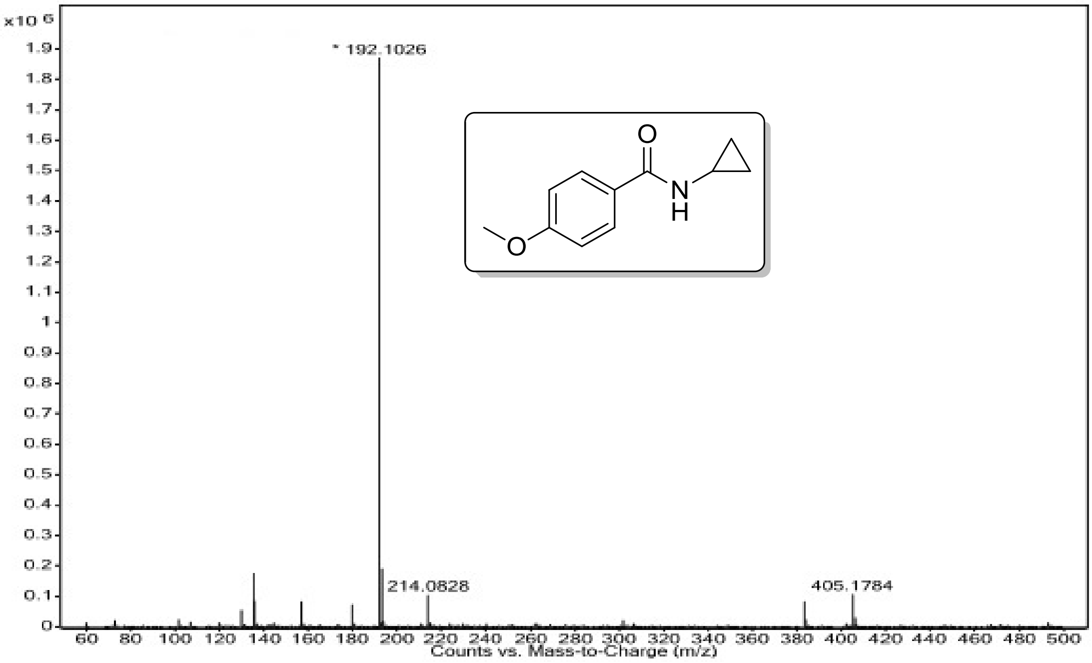


Figure S23. Mass spectra of compound 2q

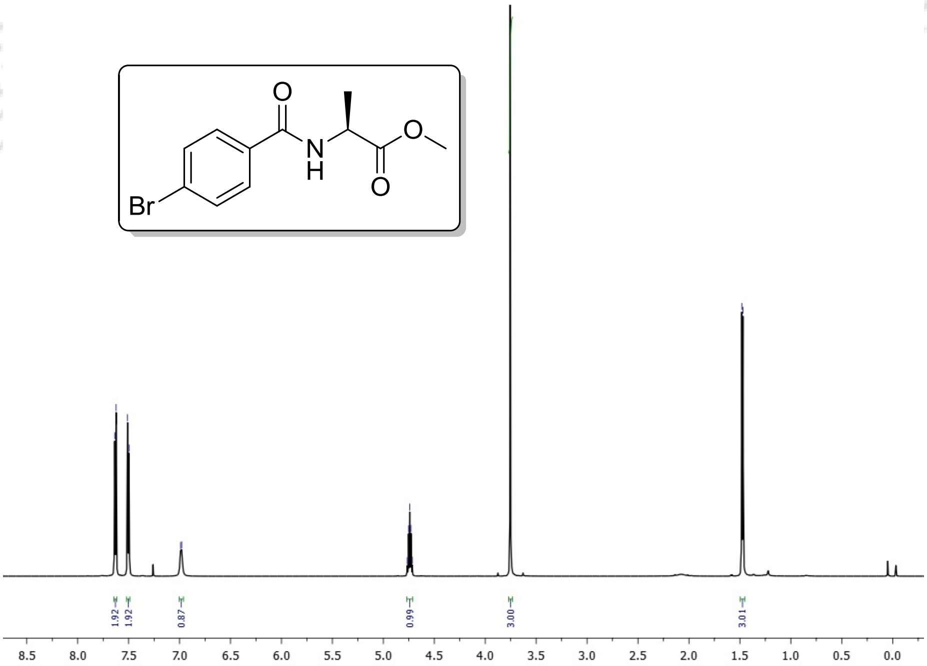


Figure S24. ^{1}H NMR spectra of compound 2y

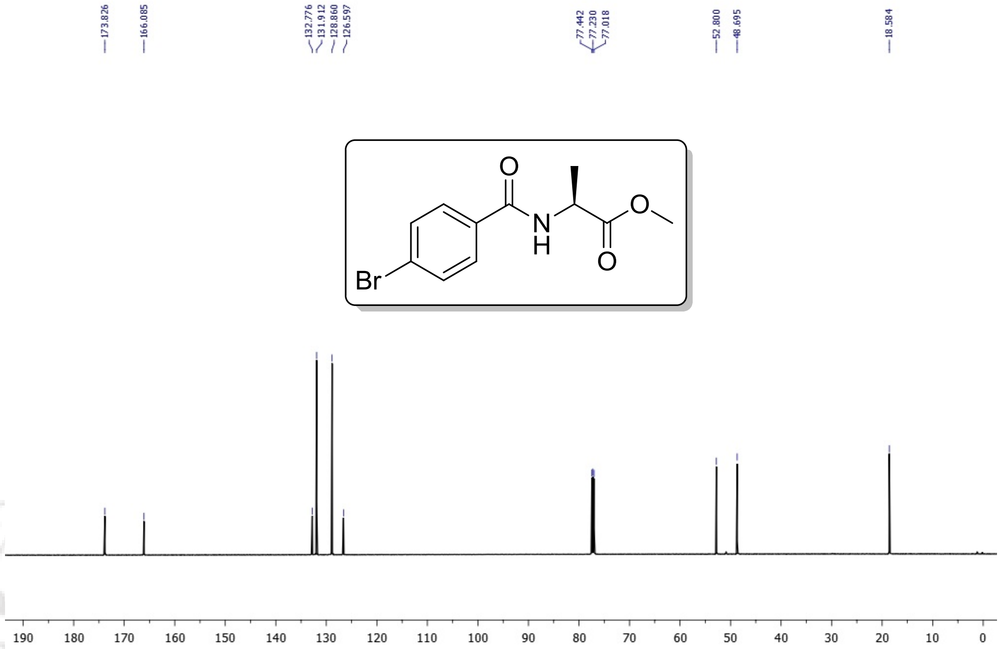


Figure S25. ^{13}C NMR spectra of compound 2y

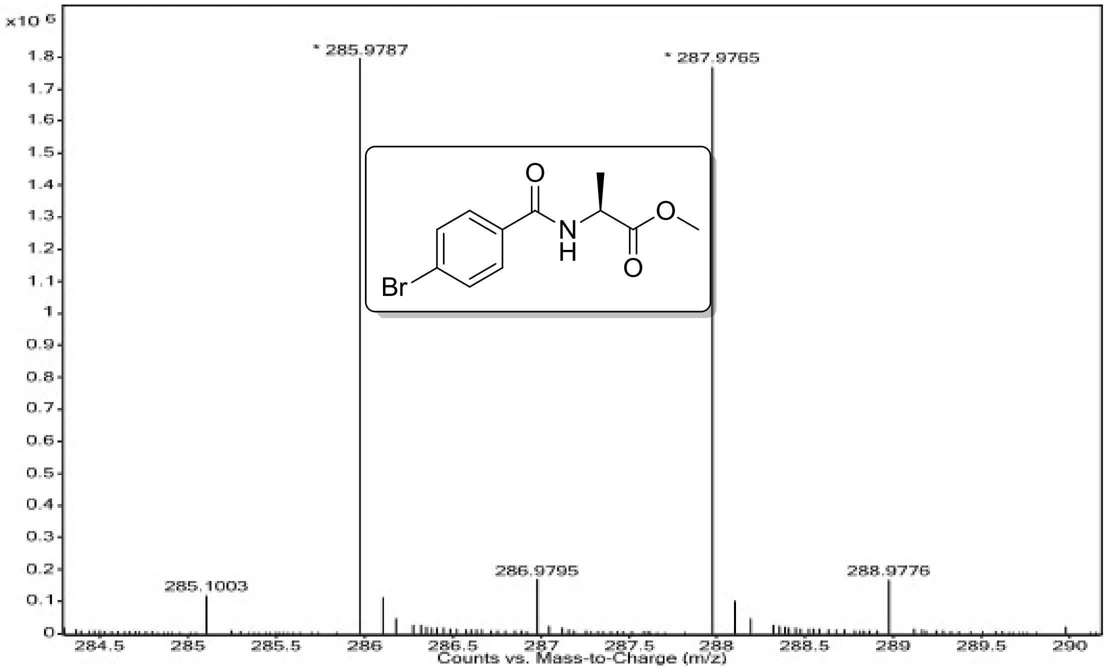


Figure S26. Mass spectra of compound 2y

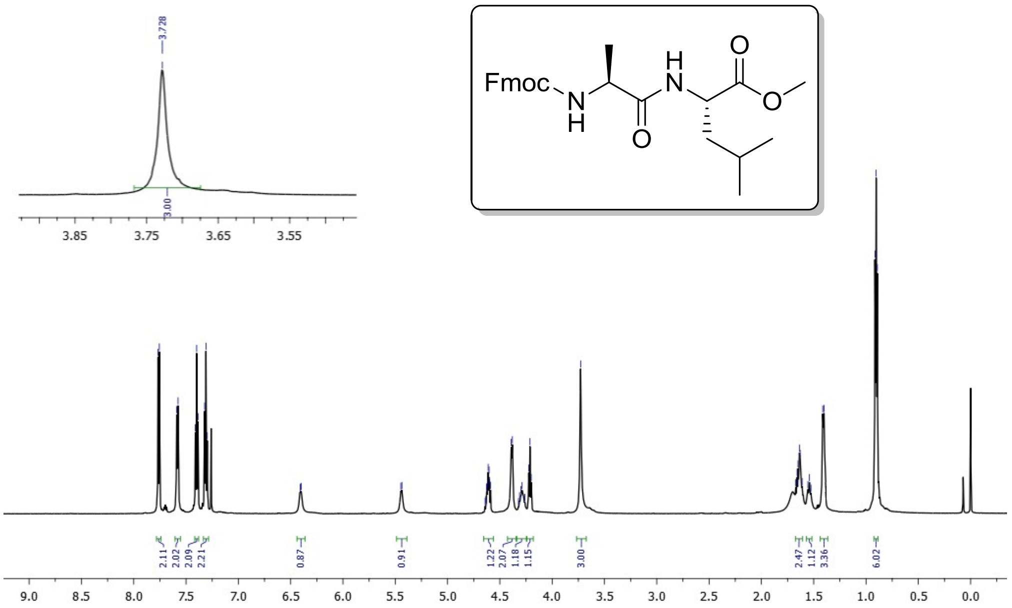


Figure S27. ^{1}H NMR spectra of compound 3g

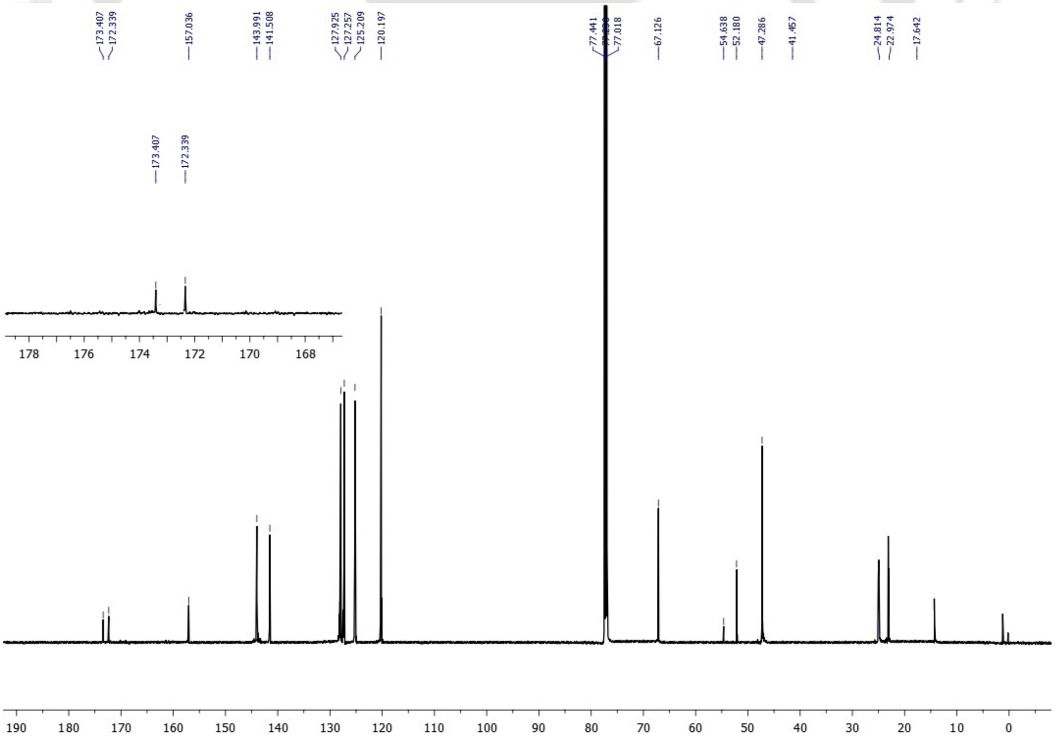


Figure S28. ^{13}C NMR spectra of compound 3g

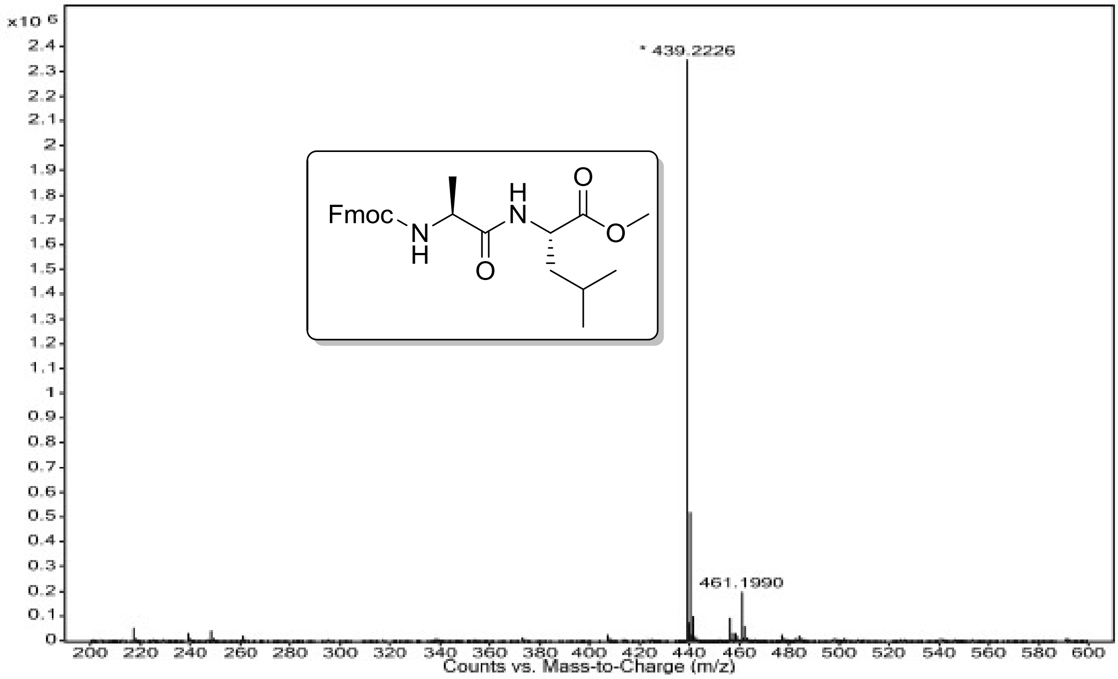


Figure S29. Mass spectra of compound 3g

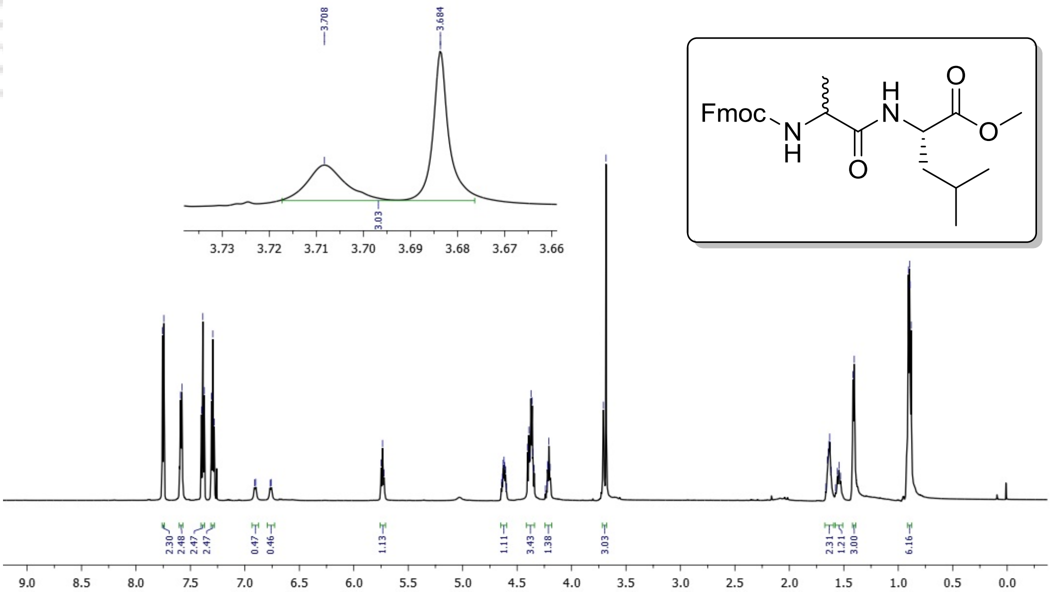


Figure S30. ^{1}H NMR spectra of compound 3h

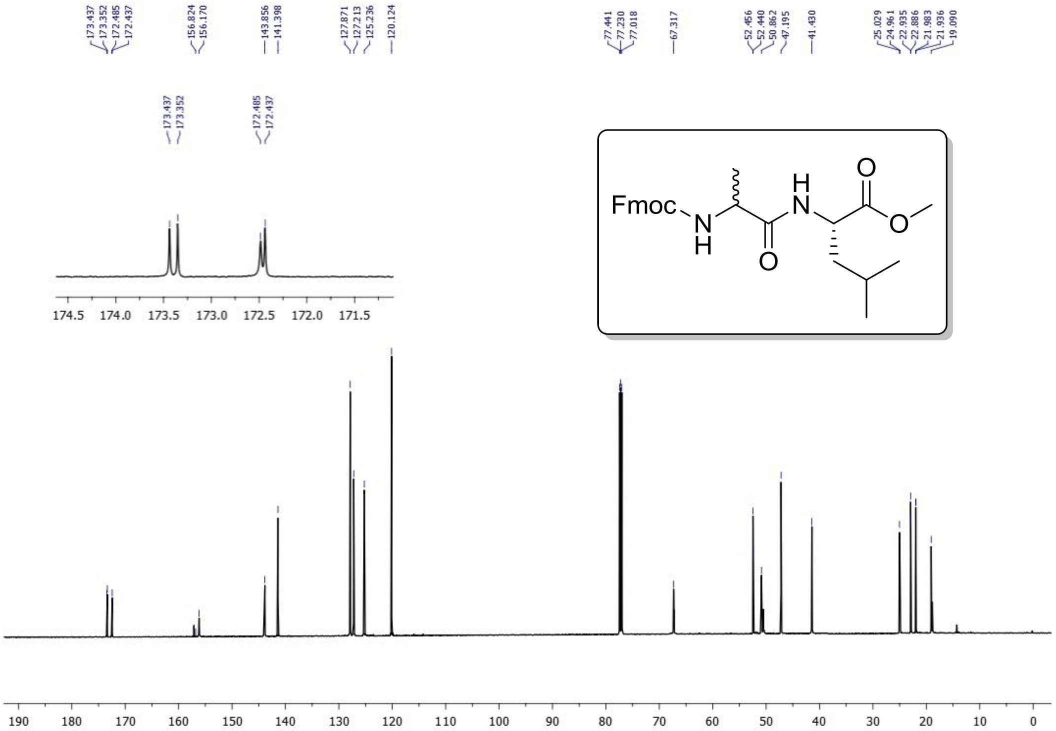


Figure S31. ^{13}C NMR spectra of compound 3h

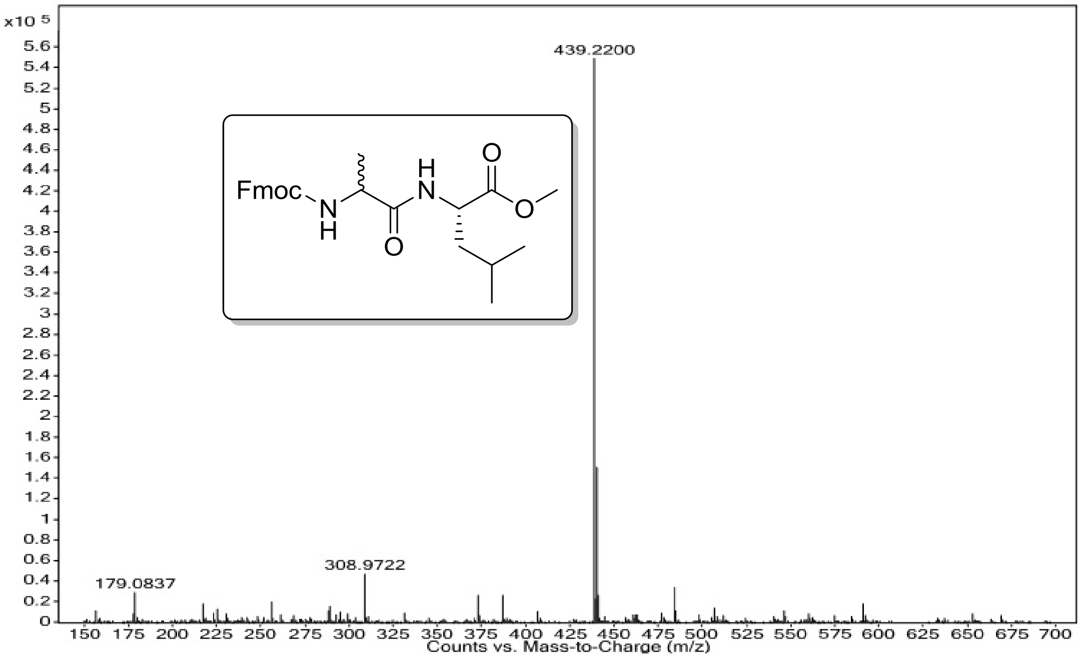


Figure S32. Mass spectra of compound 3h

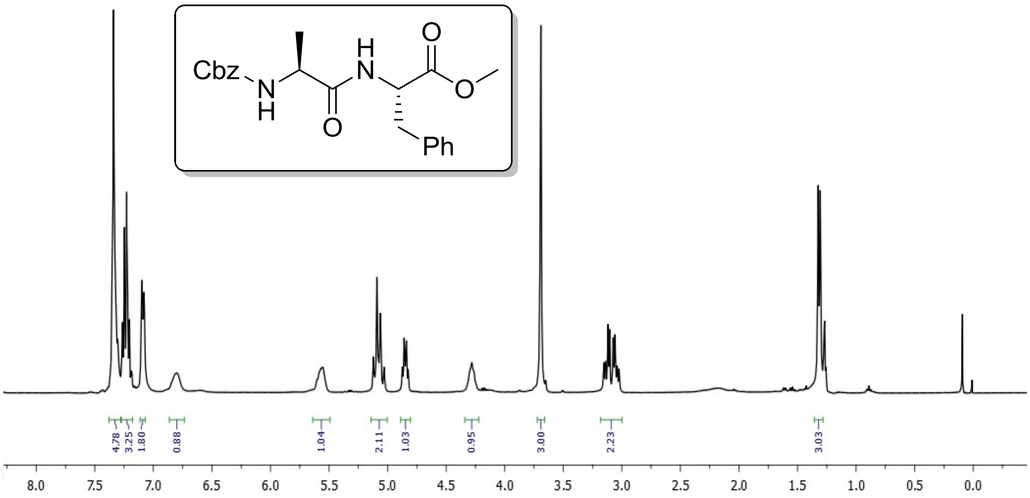


Figure S33. ^{1}H NMR spectra of compound 3r

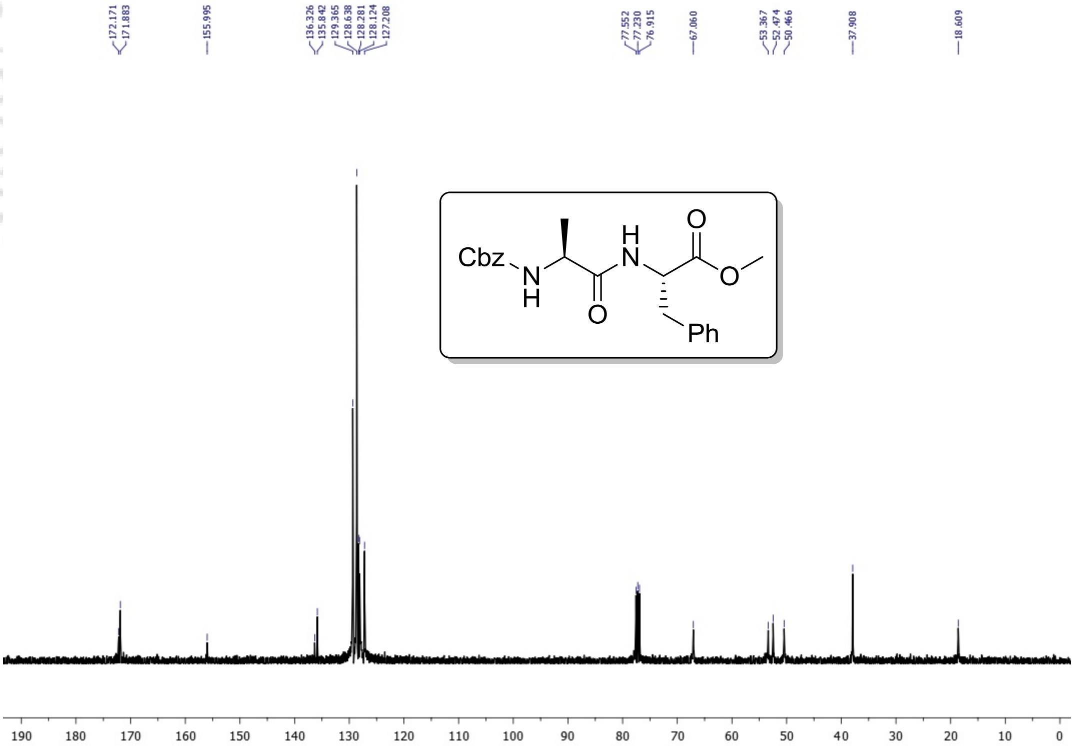


Figure S34. ^{13}C NMR spectra of compound 3r

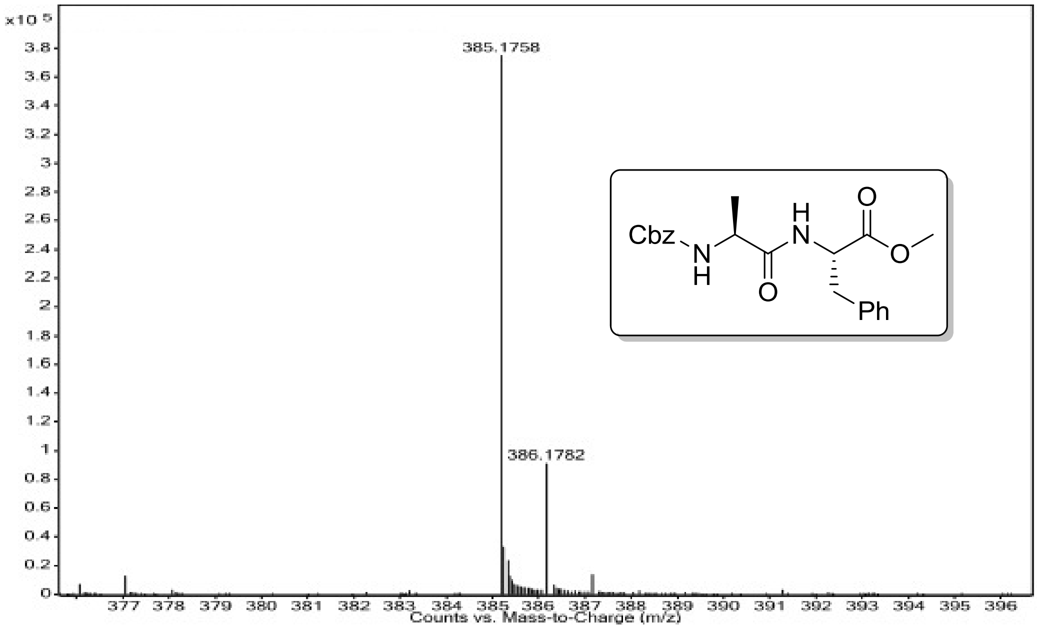


Figure S35. Mass spectra of compound 3r

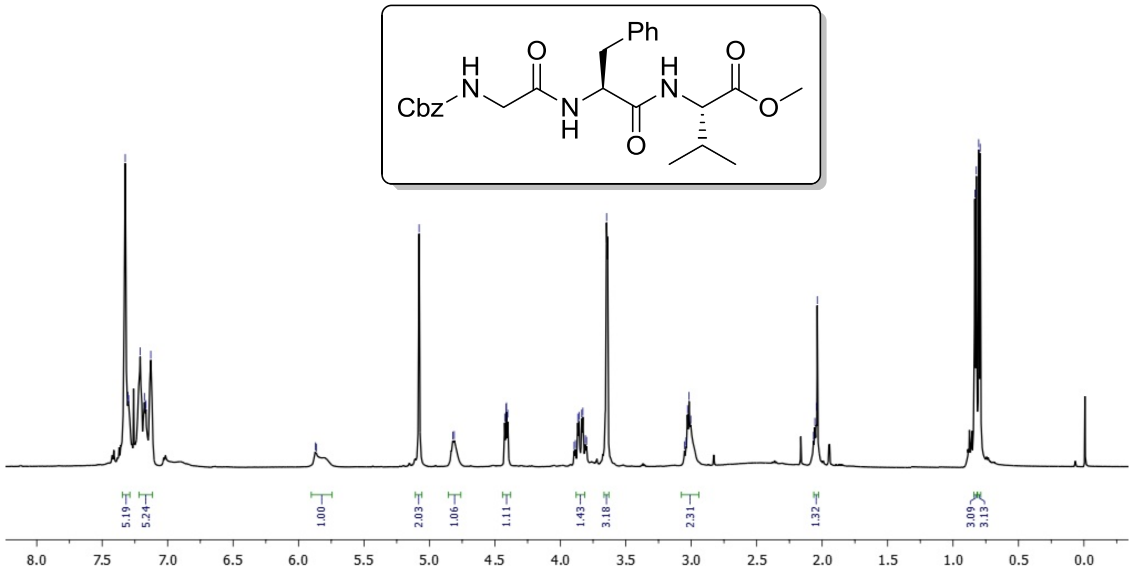


Figure S36. ^{1}H NMR spectra of compound 3t

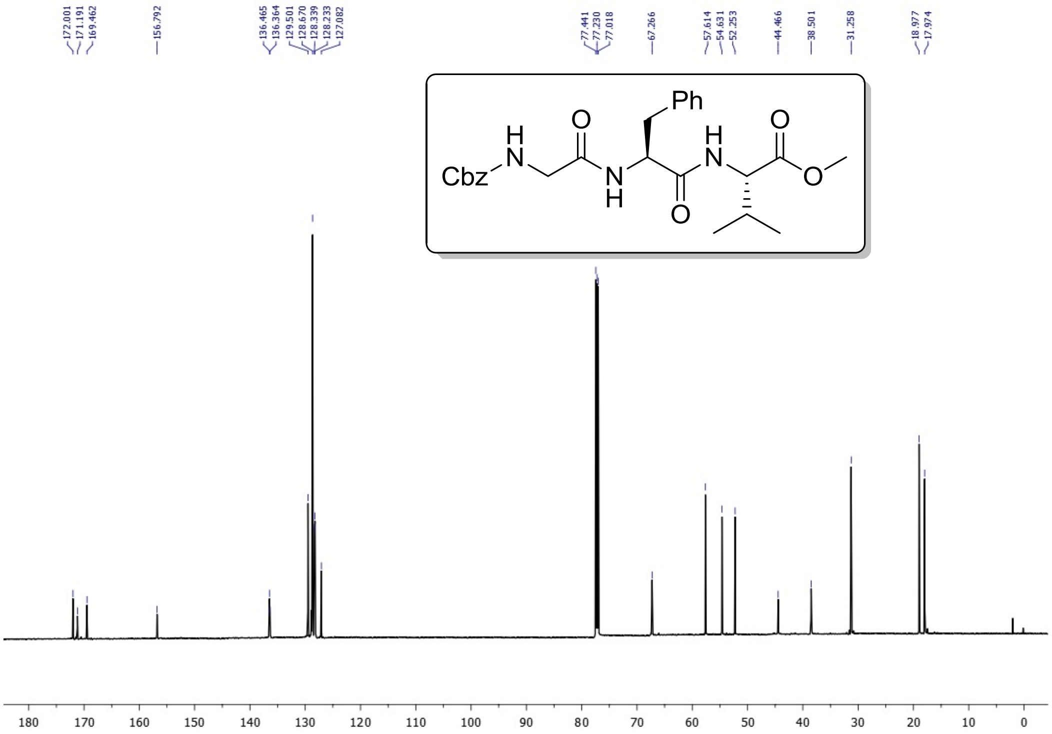


Figure S37. ^{13}C NMR spectra of compound 3t

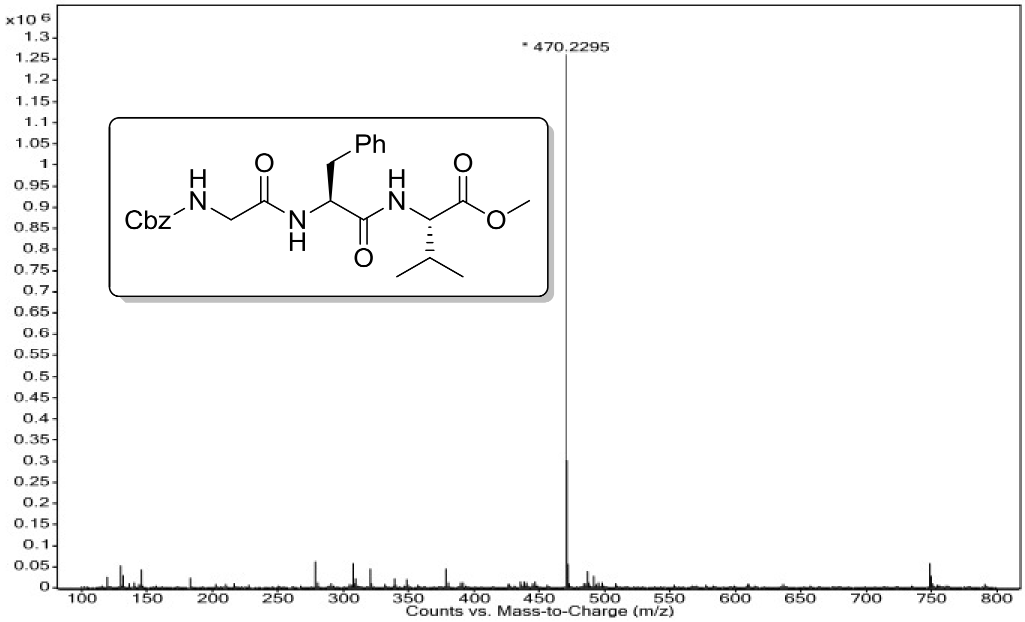


Figure S38. Mass spectra of compound 3t

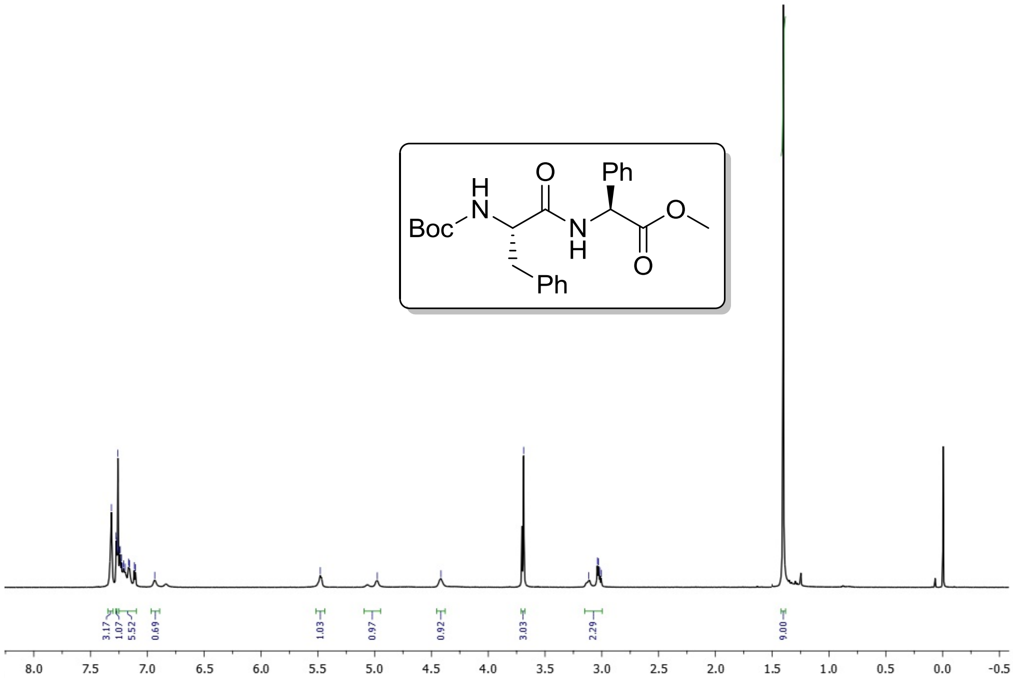


Figure S39. ^{1}H NMR spectra of compound 3p

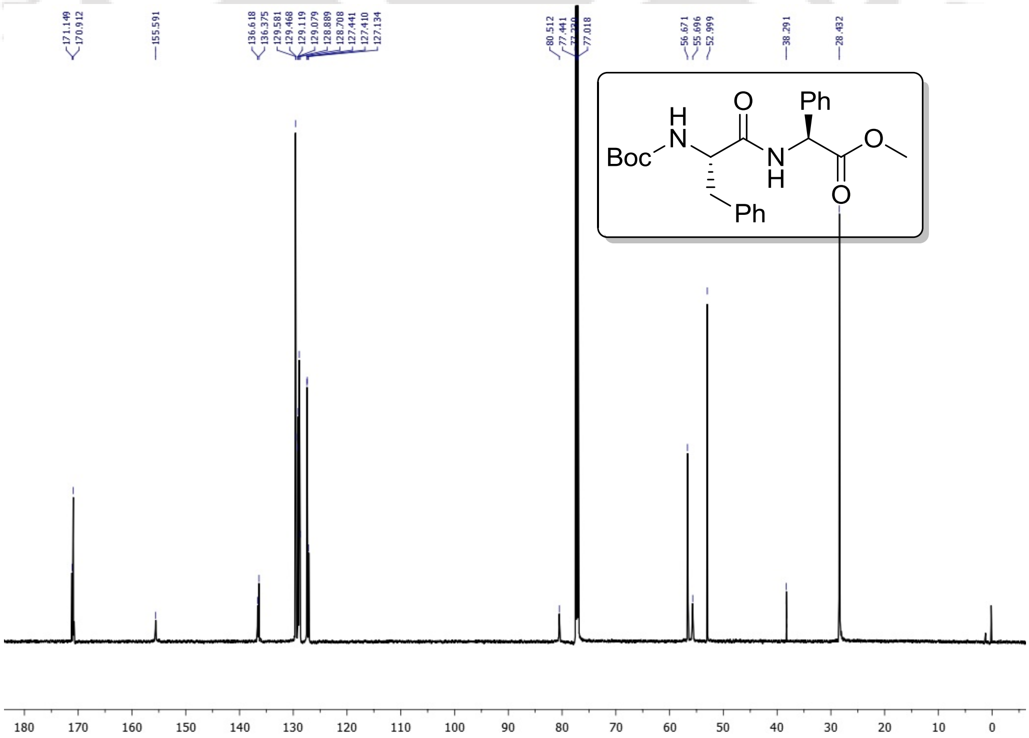


Figure S40. ^{13}C NMR spectra of compound 3p

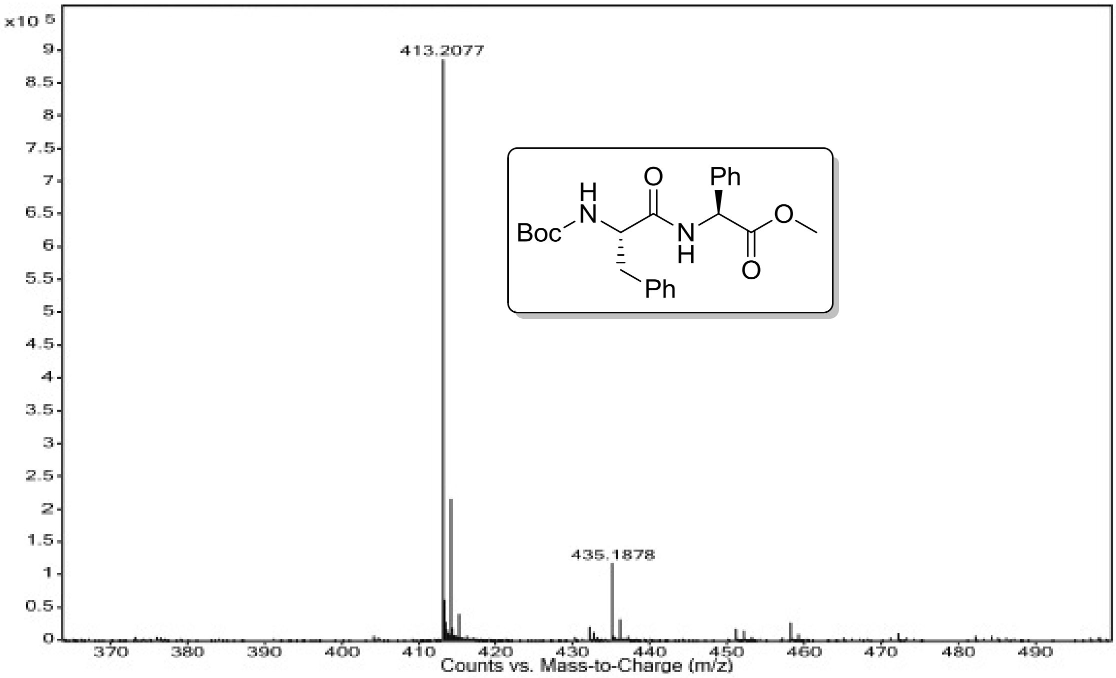


Figure S41. Mass spectra of compound 3p

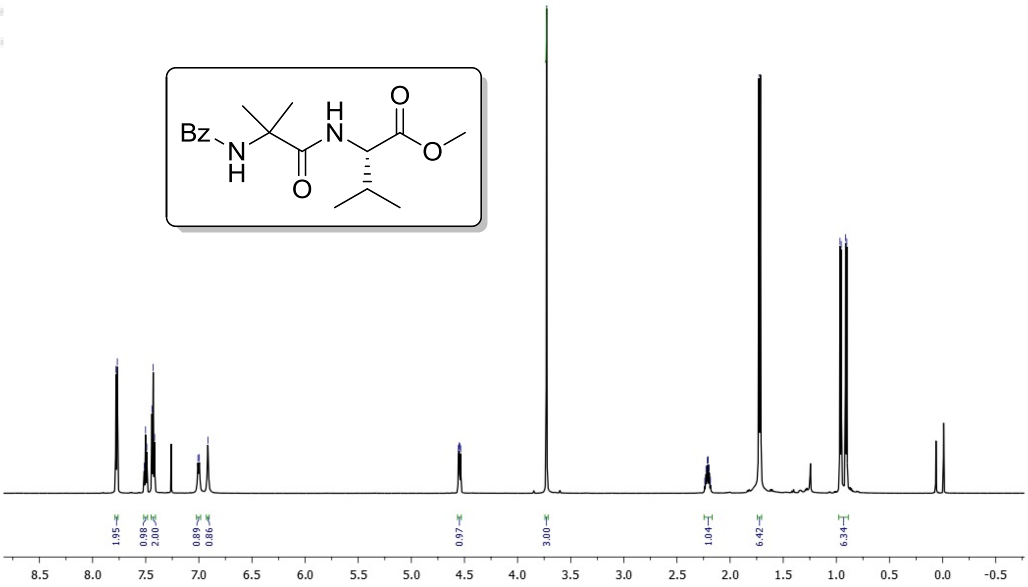


Figure S42. ^{1}H NMR spectra of compound 3a

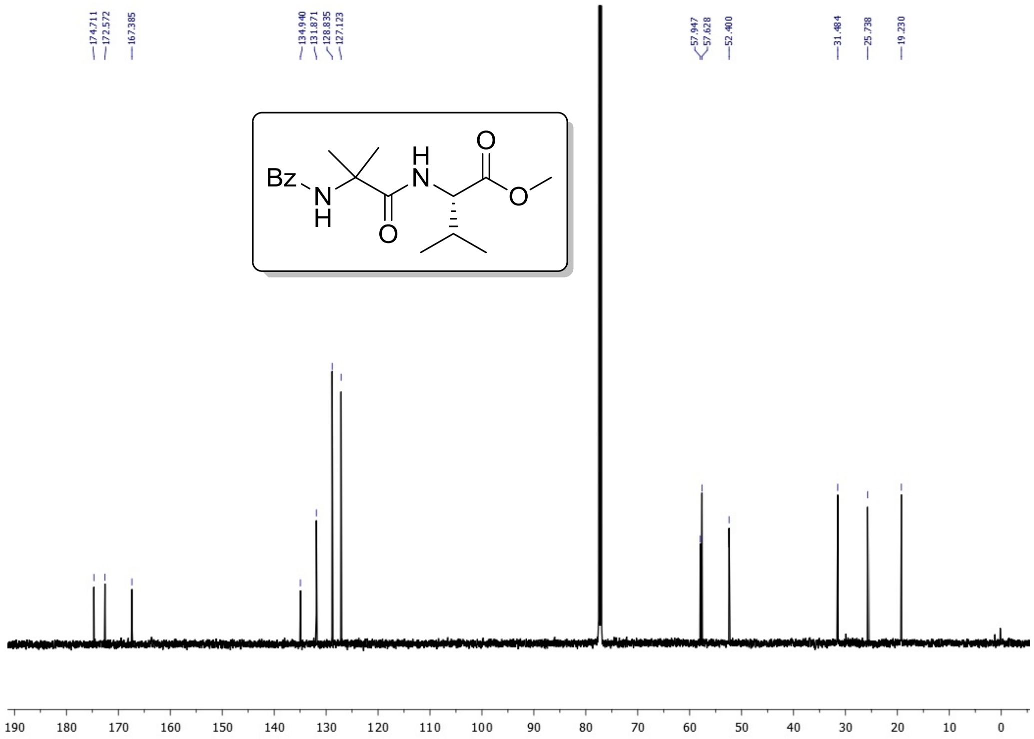


Figure S43. ^{13}C NMR spectra of compound 3a

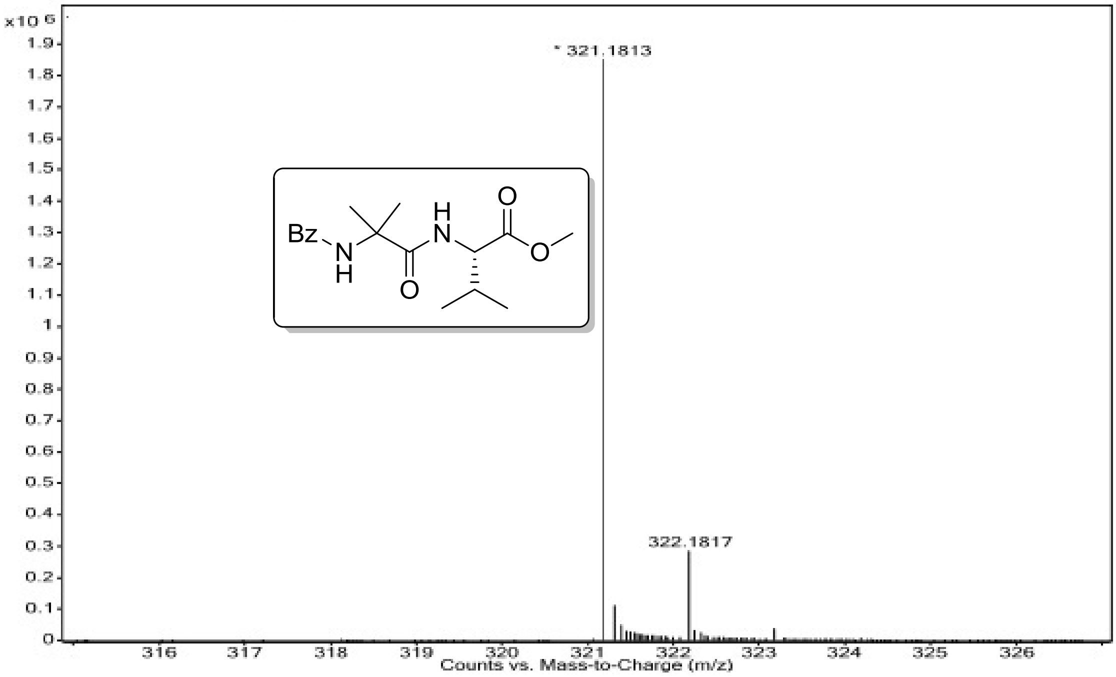


Figure S44. Mass spectra of compound 3a

6.9.4. NMR (^{1}H and ^{13}C) spectra of Intermediate, VI

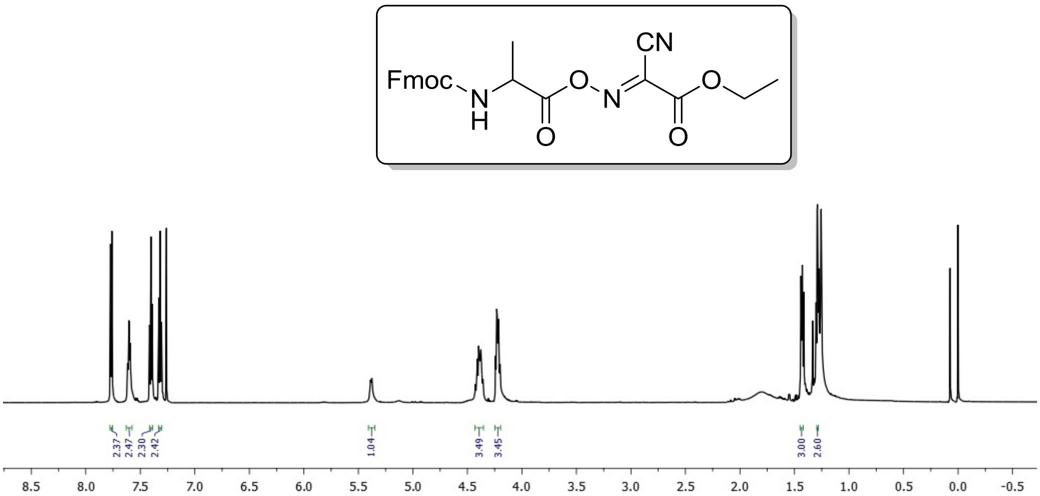


Figure S45. ^{1}H NMR spectra of Intermediate **VI** (Scheme 6.3.1)

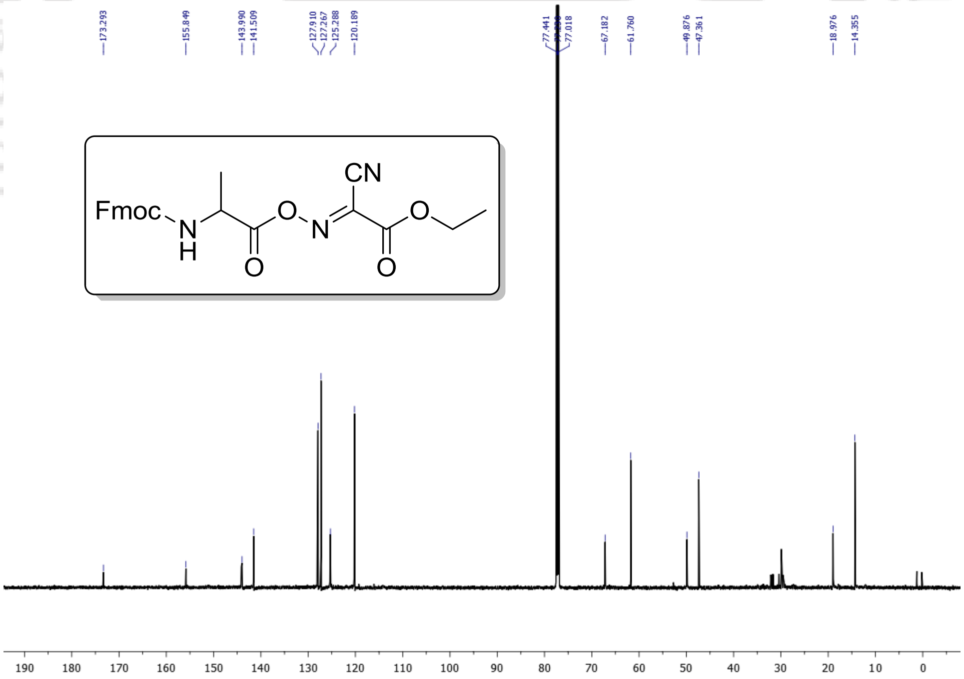
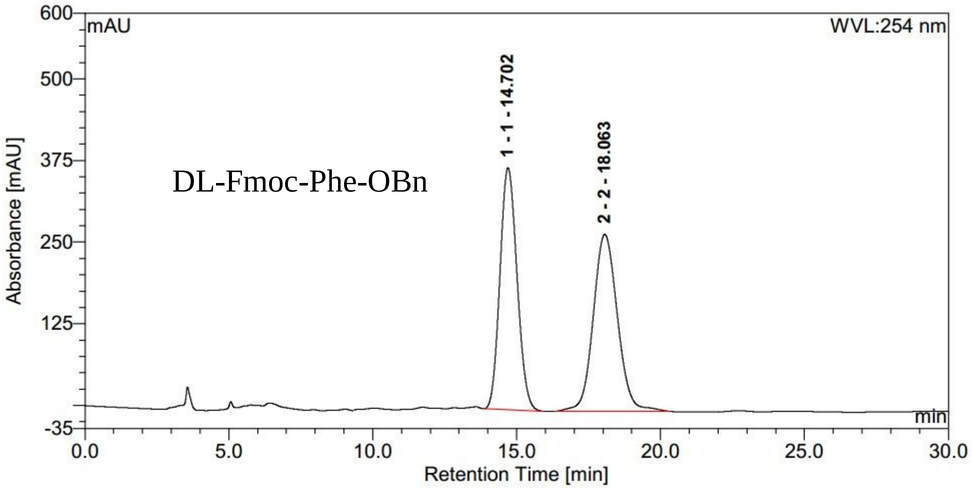


Figure S46. ^{13}C NMR spectra of Intermediate **VI** (Scheme 6.3.1)

6.9.5. Data for racemization test:



No.	Peak Name	Ret.Time (detected) min	Area mAU*min	Rel.Area(ident.) %	Height mAU	Amount
1	1	14.70	254.4198	49.03898656	370.2078	n.a.
2	2	18.06	264.391	50.96101344	270.928	n.a.

Figure S47. HPLC chromatogram of DL-Fmoc-Phe-OBn ester, CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Reaction was performed with TCBOXY reagent).

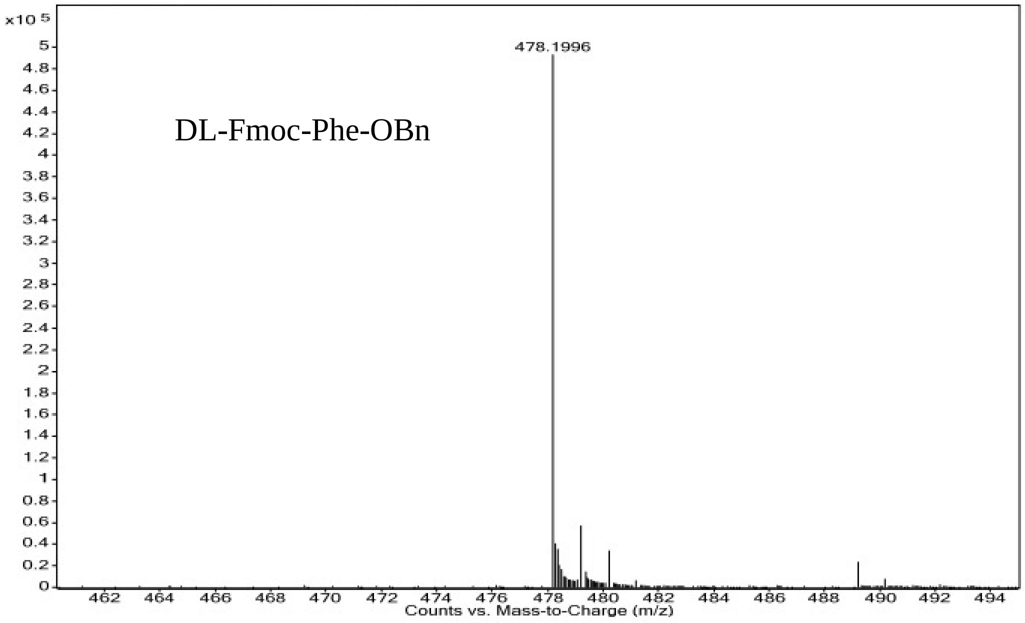


Figure S48. ESI-MS spectrum of DL-Fmoc-Phe-OBn ester eluted at 14.7 min (Reaction was performed with TCBOXY reagent).

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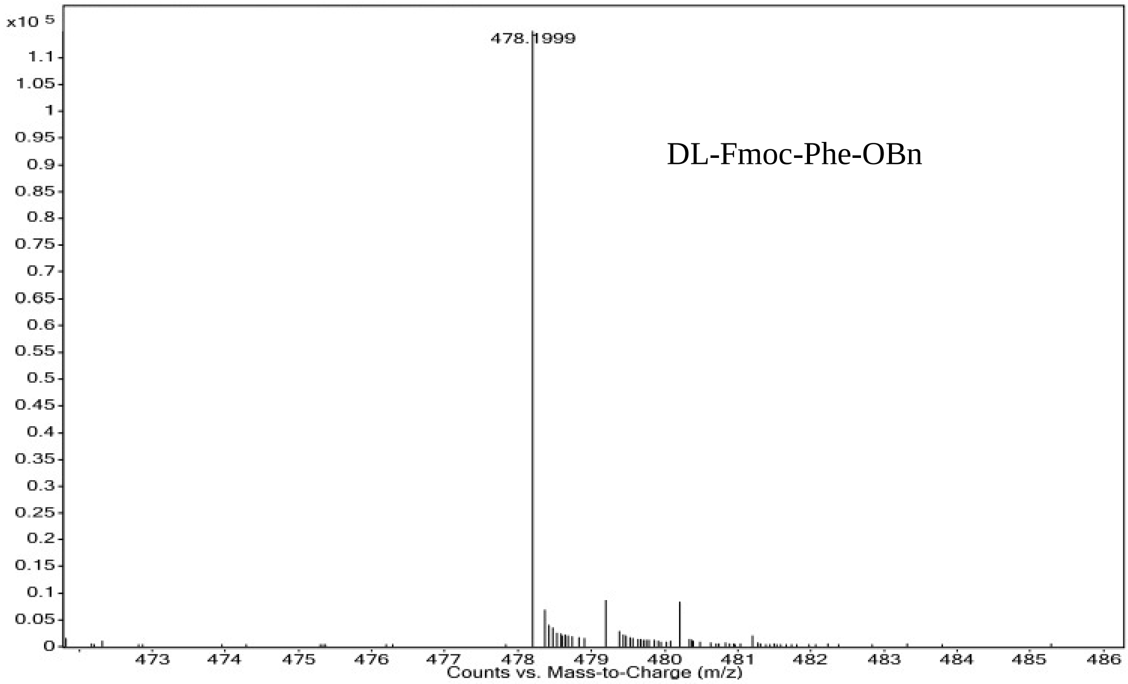
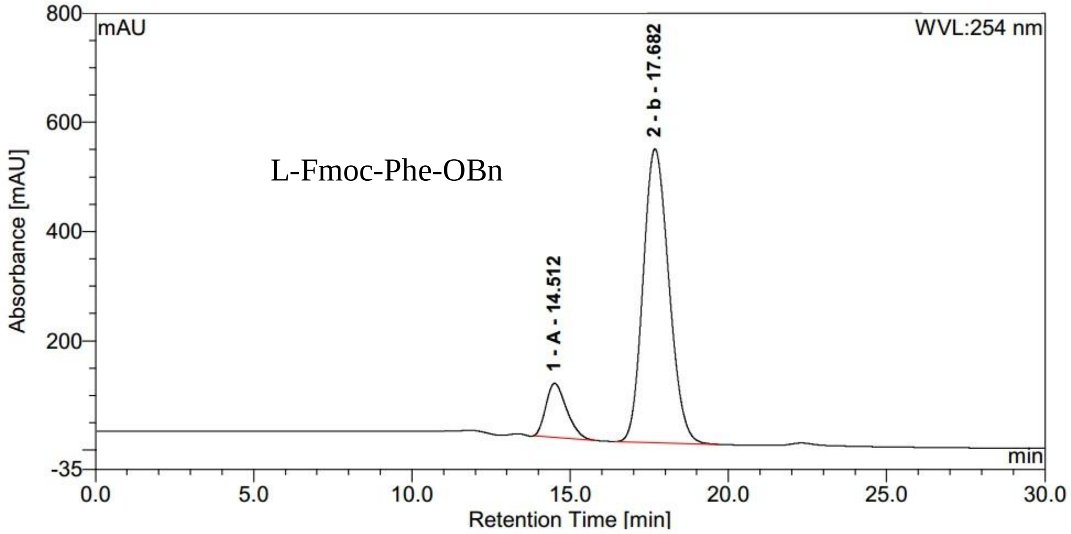
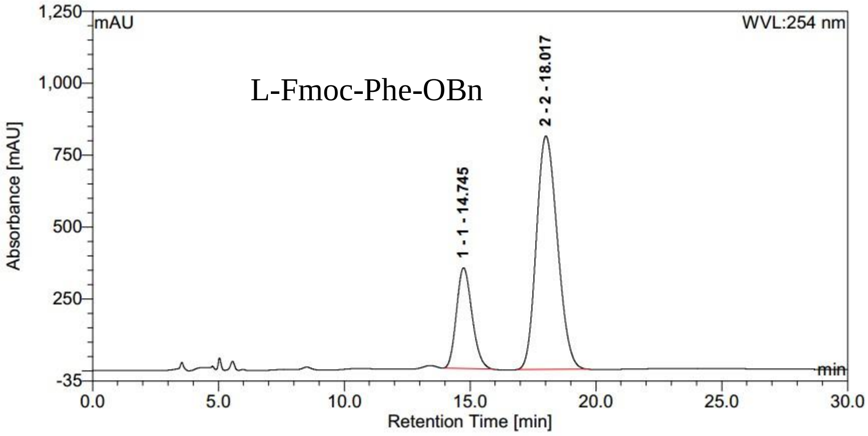


Figure S49. ESI-MS spectrum of DL-Fmoc-Phe-OBn eluted at 18 min (Reaction was performed with TCBOXY reagent).



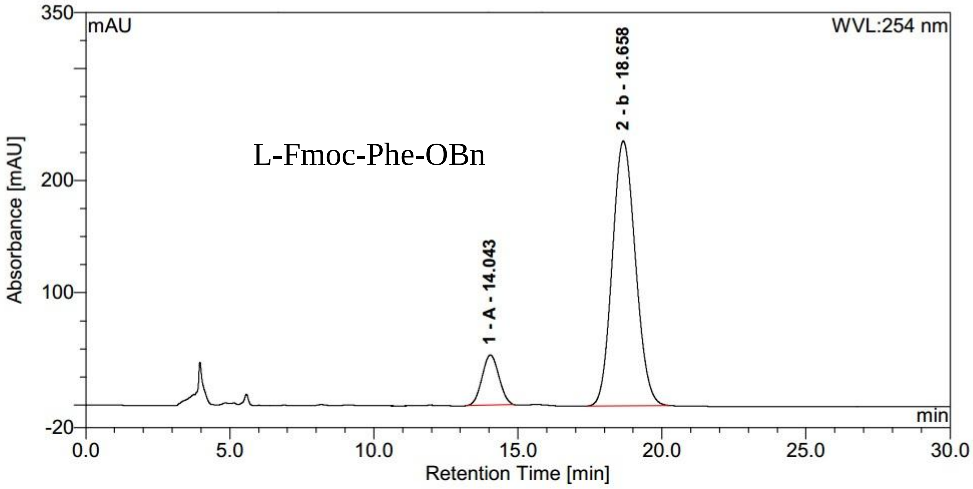
No.	Peak Name	Ret.Time (detected) min	Area mAU*min	Rel.Area(ident.) %	Height mAU	Amount
1	A	14.51	75.50556	12.93298899	99.2471	n.a.
2	b	17.68	508.316	87.06701101	538.030	n.a.

Figure S50. HPLC chromatogram of L-Fmoc-Phe-OBn, run time 30 min, in CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Reaction was performed with 2,4,6-trichlorobenzoyl chloride in the absence of DMAP).



No.	Peak Name	Ret.Time (detected) min	Area mAU*min	Rel.Area(ident.) %	Height mAU	Amount
1	1	14.75	250.2978	24.12568461	349.9076	n.a.
2	2	18.02	787.177	75.87431539	810.990	n.a.

Figure S51. HPLC chromatogram of L-Fmoc-Phe-OBn, run time 30 min, in CHIRAL PAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Reaction was performed with the 2,4,6-trichlorobenzoyl chloride in presence of DMAP).



No.	Peak Name	Ret.Time (detected) min	Area mAU*min	Rel.Area(ident.) %	Height mAU	Amount
1	A	14.04	30.33948	12.13473015	44.74646	n.a.
2	b	18.66	219.682	87.86526985	236.032	n.a.

Figure S52. HPLC chromatogram of L-Fmoc-Phe-OBn, run till 30 min, in CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Reaction was performed with modified Yamaguchi reagent, TCB-DMAP).

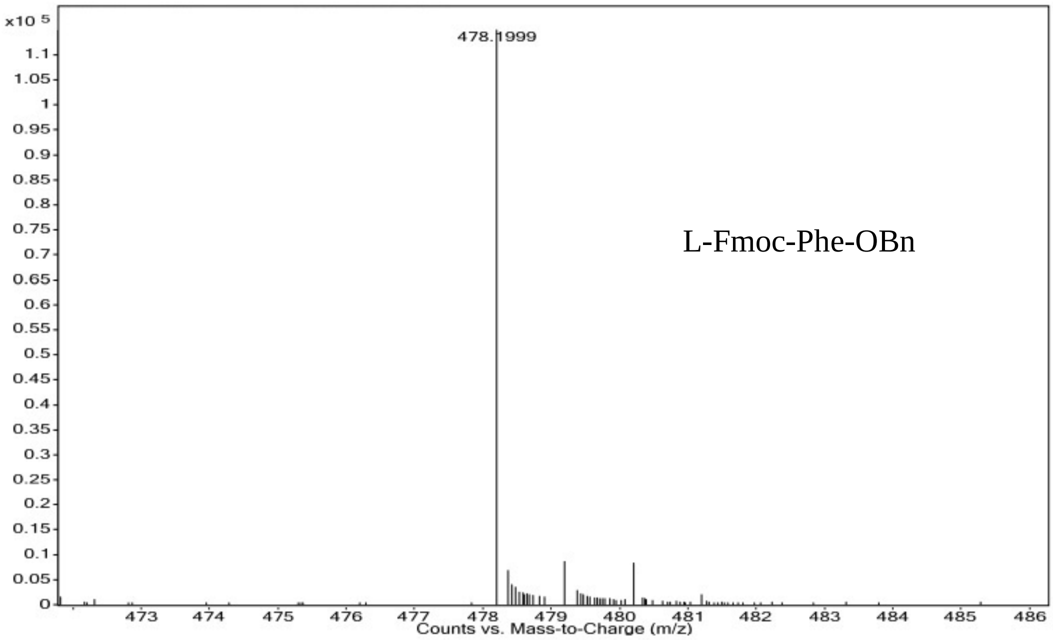


Figure S53. ESI-MS spectrum of L-Fmoc-Phe-OBn ester eluted at 14 min.

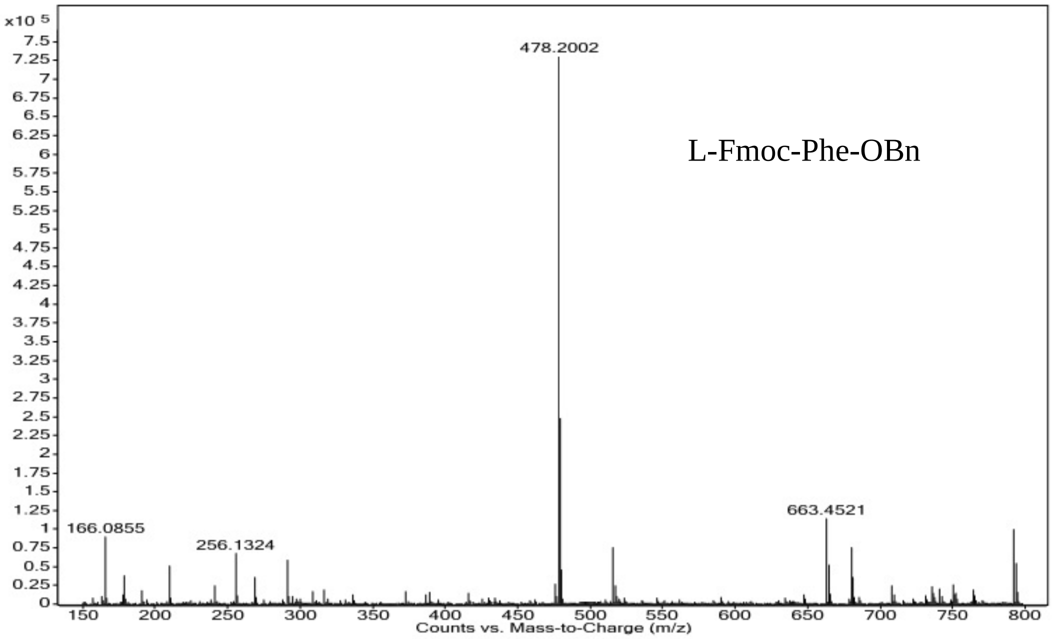
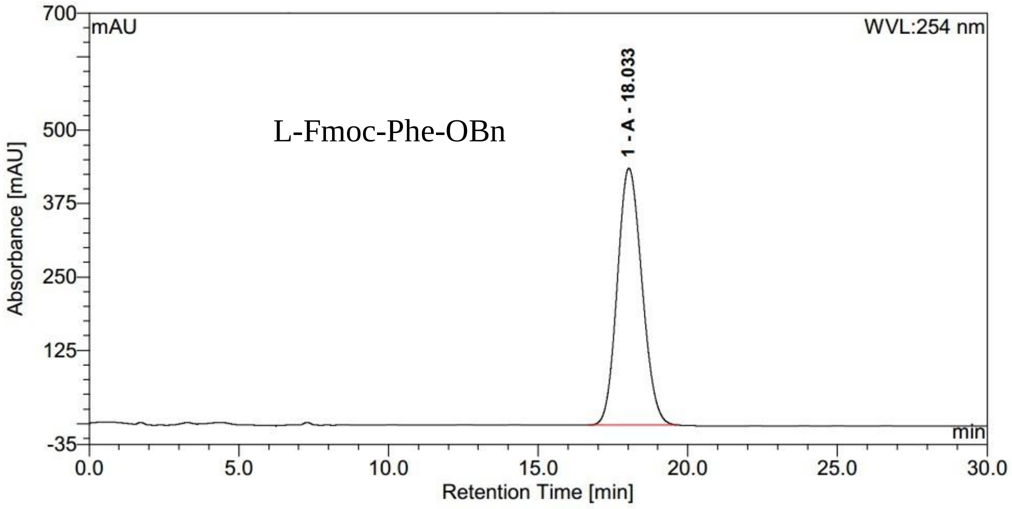


Figure S54. ESI-MS spectrum of L-Fmoc-Phe-OBn ester eluted at 18 min.



No.	Peak Name	Ret.Time (detected) min	Area mAU*min	Rel.Area(ident.) %	Height mAU	Amount
1	A	18.03	418.4887	100	438.1406	n.a.

Figure S55. HPLC chromatogram of L-Fmoc-Phe-OBn ester, CHIRALPAK® AS-H column, 5 µm, 4.6×250 mm, an isocratic gradient of 20% isopropanol in hexane was used (Reaction was performed with TCBOXY reagent).

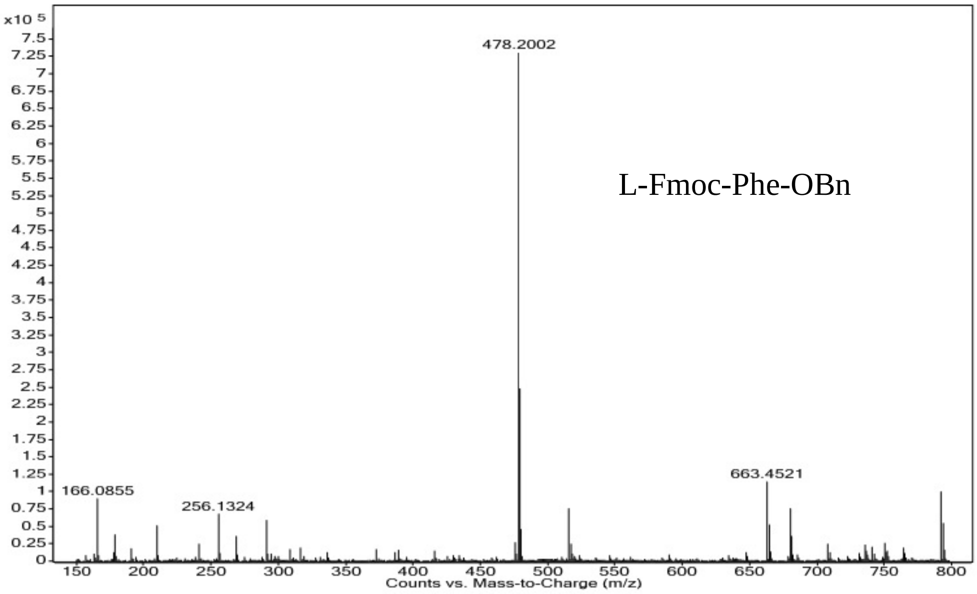


Figure S56. ESI-MS spectrum of L-Fmoc-Phe-OBn ester eluted at 18 min (Reaction was performed with TCBOXY reagent).

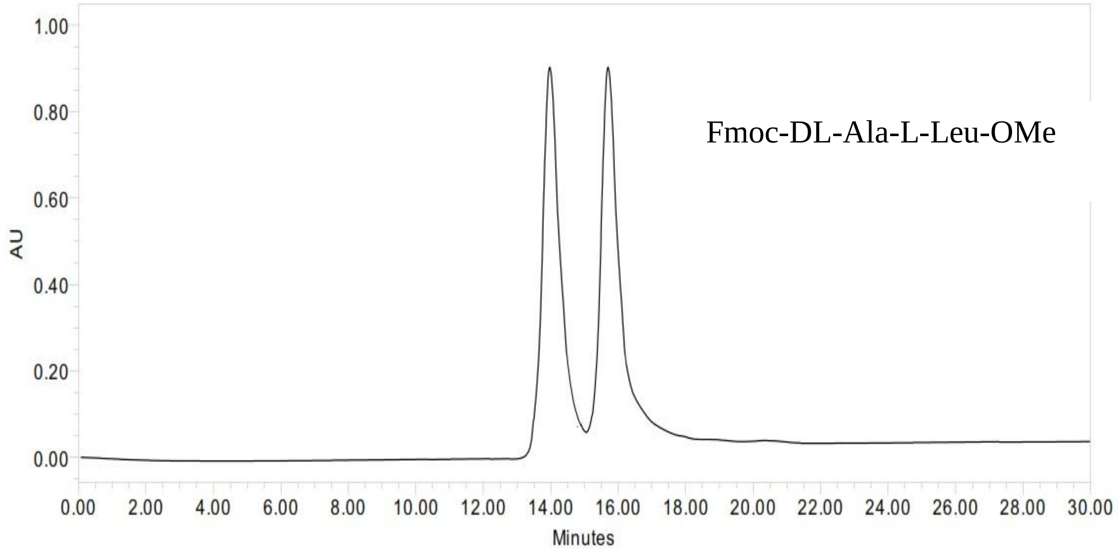


Figure S57. HPLC chromatogram of Fmoc-DL-Ala-L-Leu-OMe dipeptide run upto 30 min in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min then 90 to 100% up to 30 min, CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3h)..

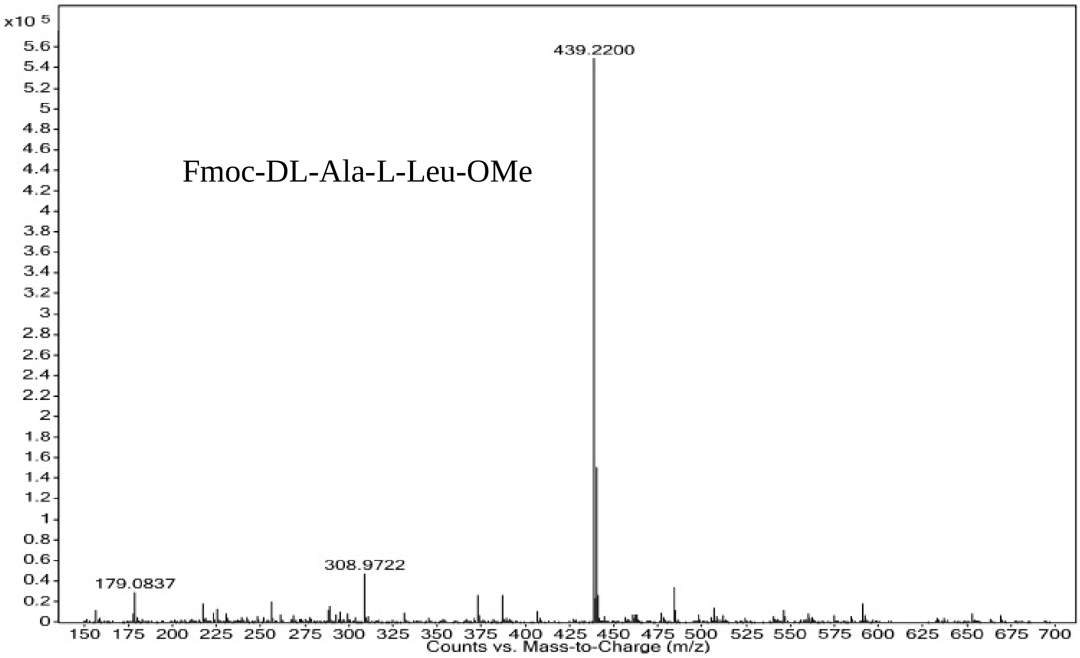


Figure S58. ESI-MS spectrum of Fmoc-DL-Ala-L-Leu-OMe dipeptide eluted at 14 min (Scheme 6.1.1.3, entry 3h).

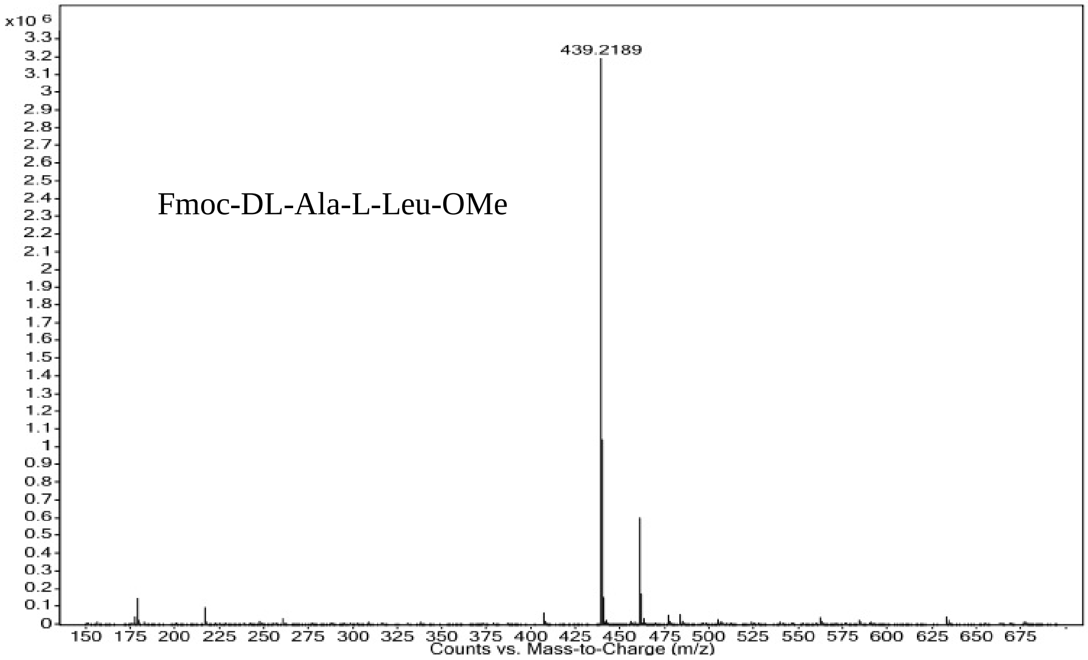


Figure S59. ESI-MS spectrum of Fmoc-DL-Ala-L-Leu-OMe dipeptide eluted at 16 min (Scheme 6.1.1.3, entry 3h).

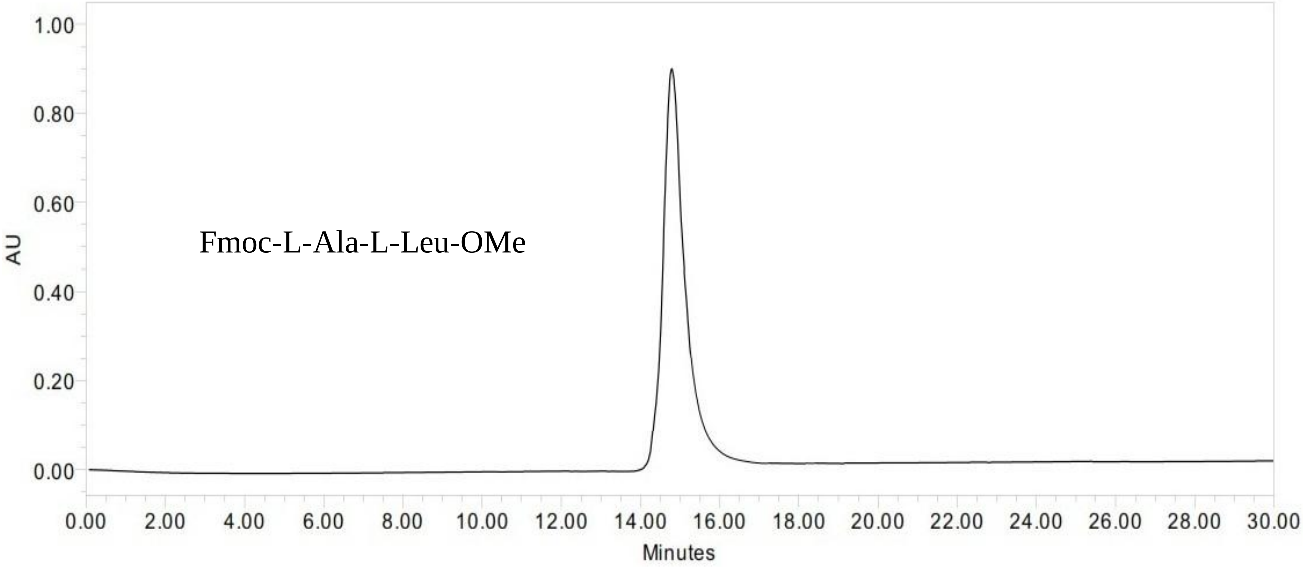


Figure S60. HPLC chromatogram of Fmoc-L-Ala-L-Leu-OMe dipeptide run upto 30 min in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min then 90 to 100%, up to 30 min, CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3g).

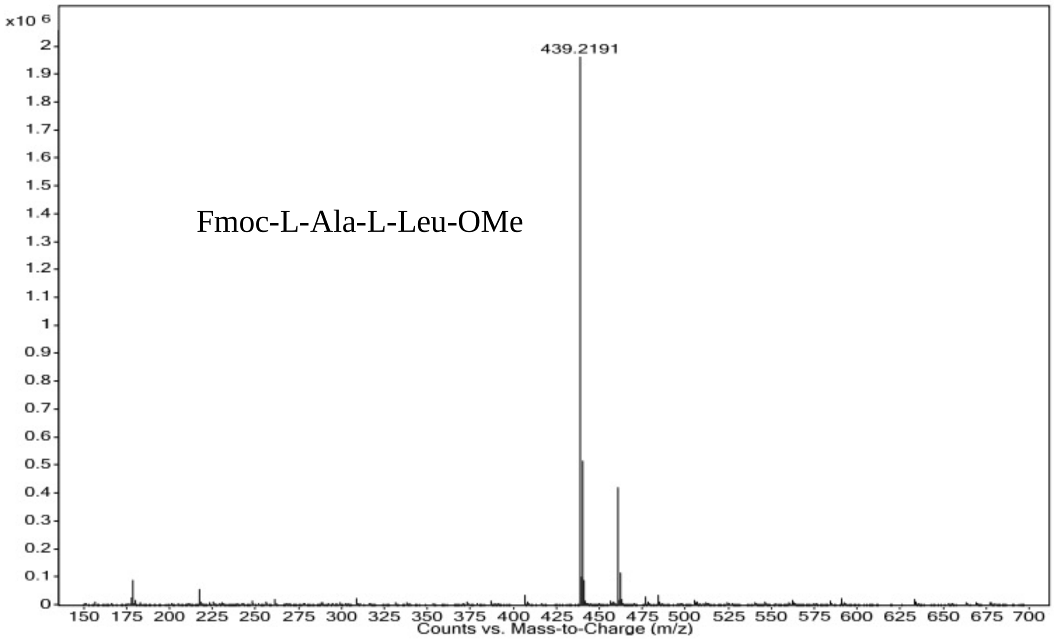


Figure S61. ESI-MS spectrum of Fmoc-L-Ala-L-Leu-OMe dipeptide eluted at 15 min (Scheme 6.1.1.3, entry 3g).

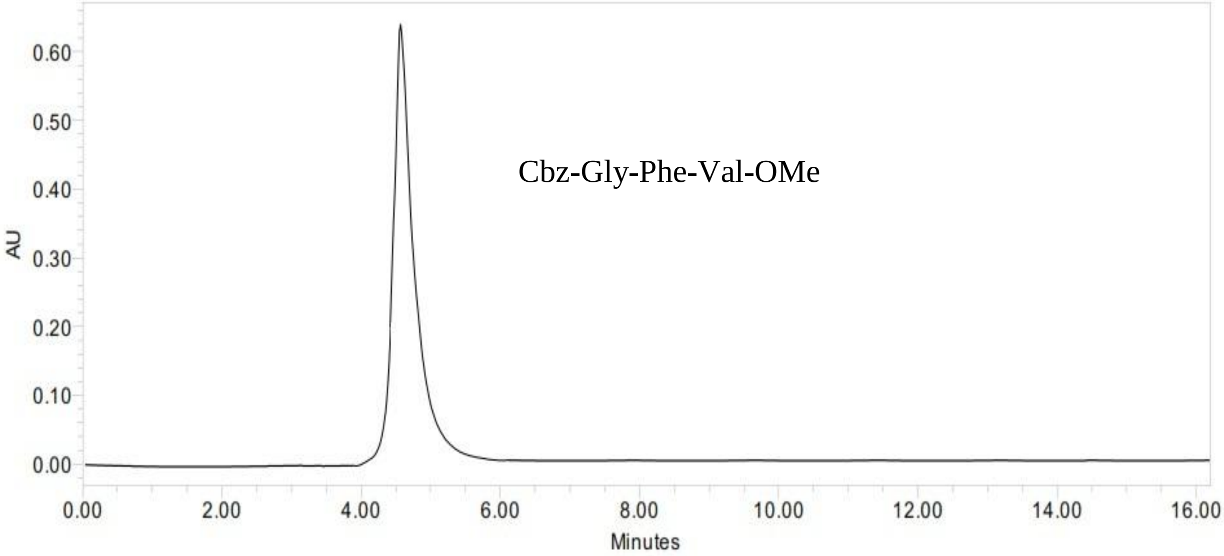


Figure S62. HPLC chromatogram of Cbz-Gly-Phe-Val-OMe tripeptide run upto 16 min in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 95%, upto 16 min, CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3t).

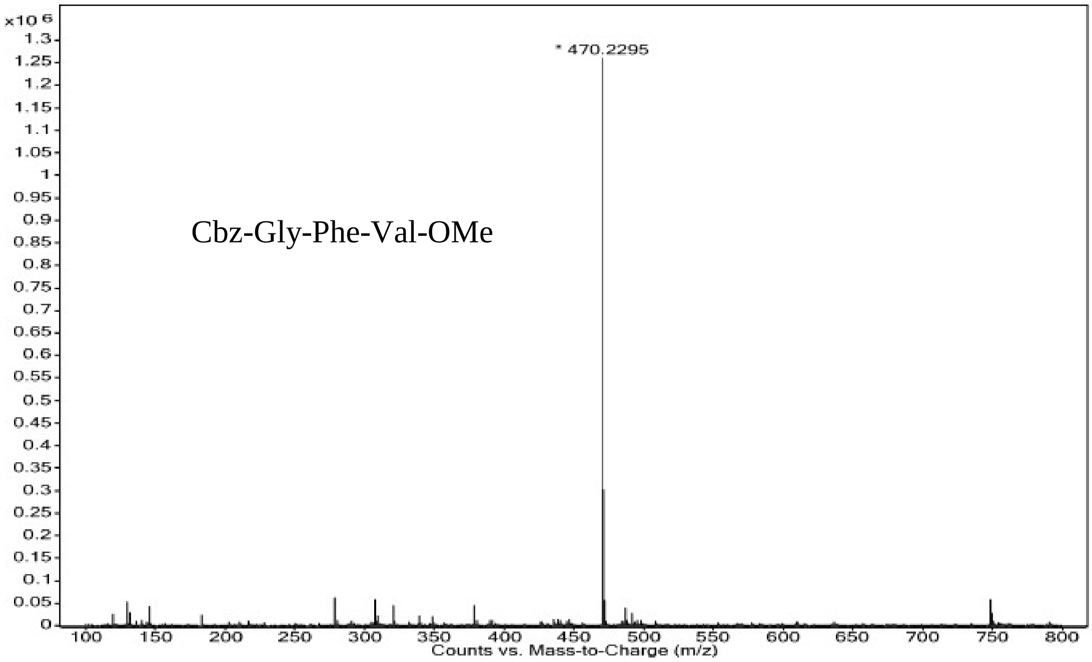


Figure S63. ESI-MS spectrum of Cbz-Gly-Phe-Val-OMe tripeptide (Scheme 6.1.1.3, entry 3t).

6.9.6. HPLC Chromatogram of dipeptides

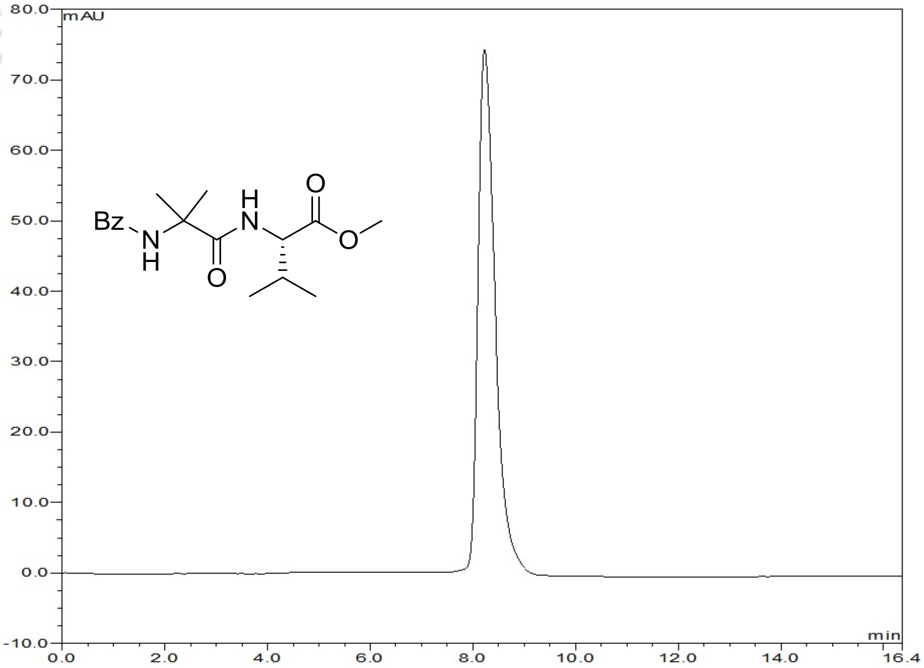


Figure S64. HPLC chromatogram of Bz-Aib-Val-OMe dipeptide run in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 95%, upto 17 min., CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3a).

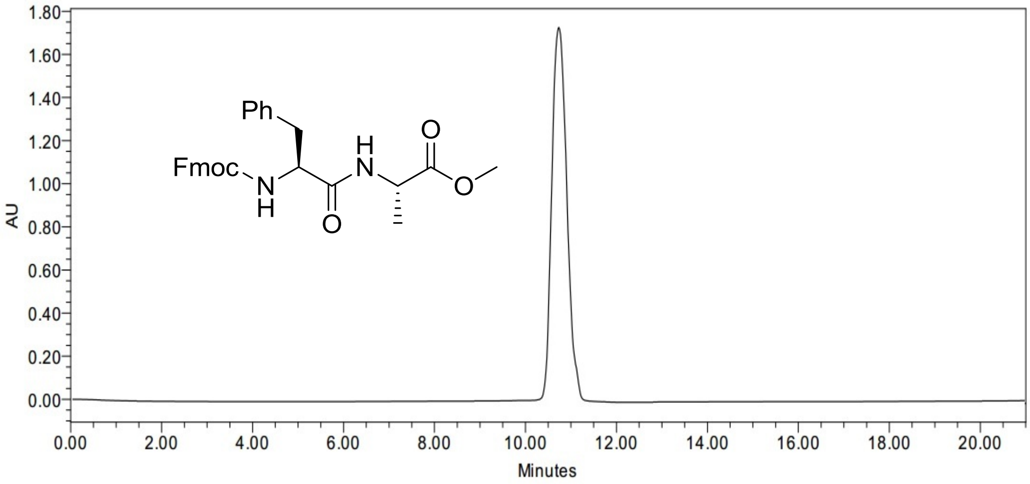


Figure S65. HPLC chromatogram of Fmoc-Phe-Ala-OMe dipeptide run in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 90%, 0-10 min, then 90 to 100% till 21 min., CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3b).

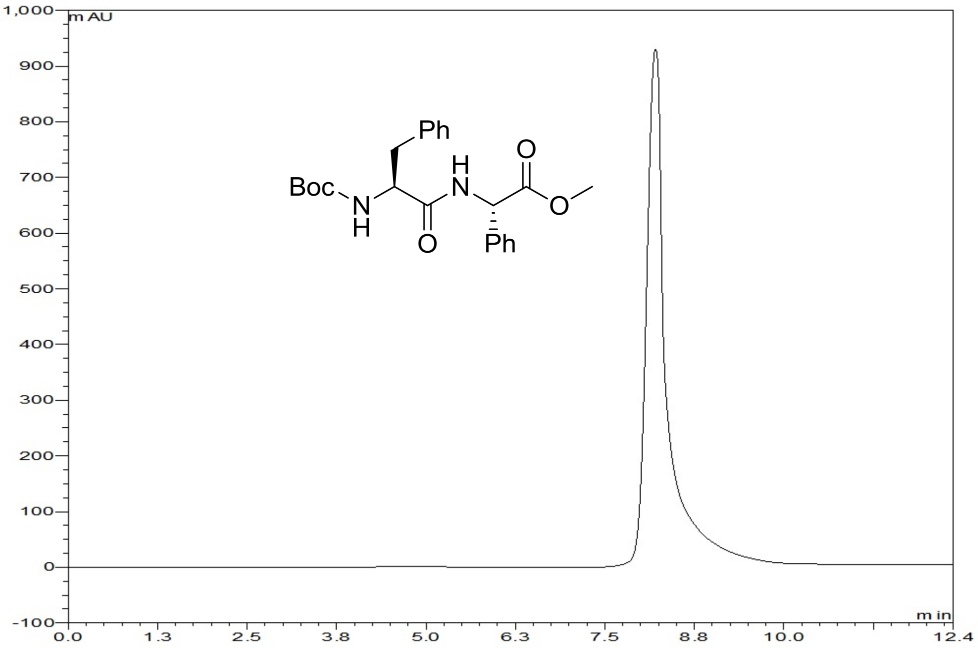


Figure S66. HPLC chromatogram of Boc-Phe-Phg-OMe dipeptide run in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 95% upto 13 min., CH_3CN in H_2O with 0.1% formic acid (Scheme 6.1.1.3, entry 3p).

6.9.7. HPLC Chromatogram and mass spectra of Boc-VVIA-OMe

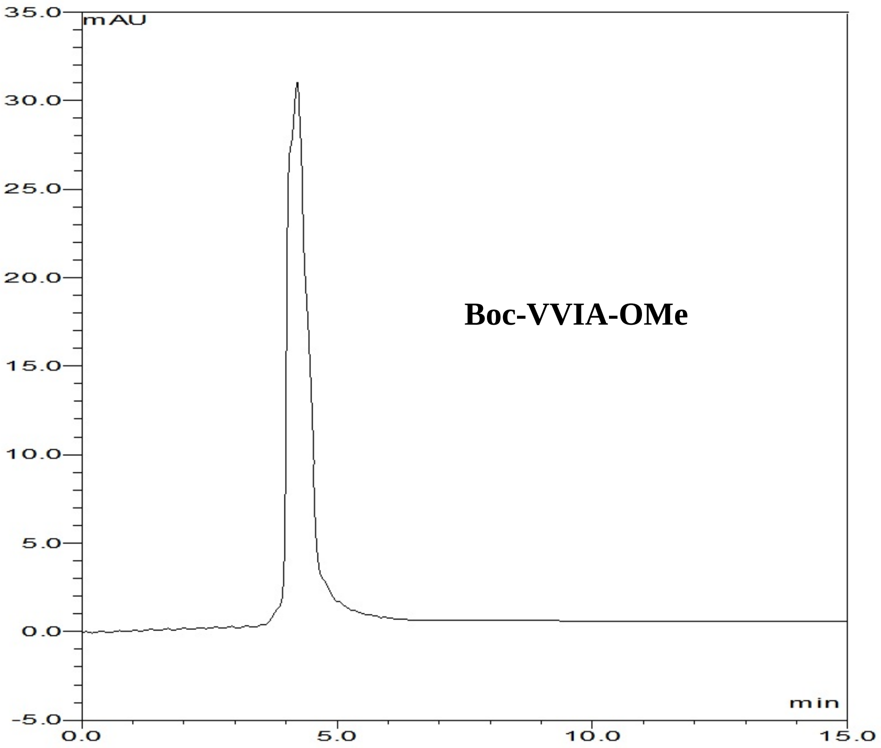


Figure S67. HPLC chromatogram of Boc-VVIA-OMe tetrapeptide run upto 15 min in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 95%, upto 15 min, CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.1a).

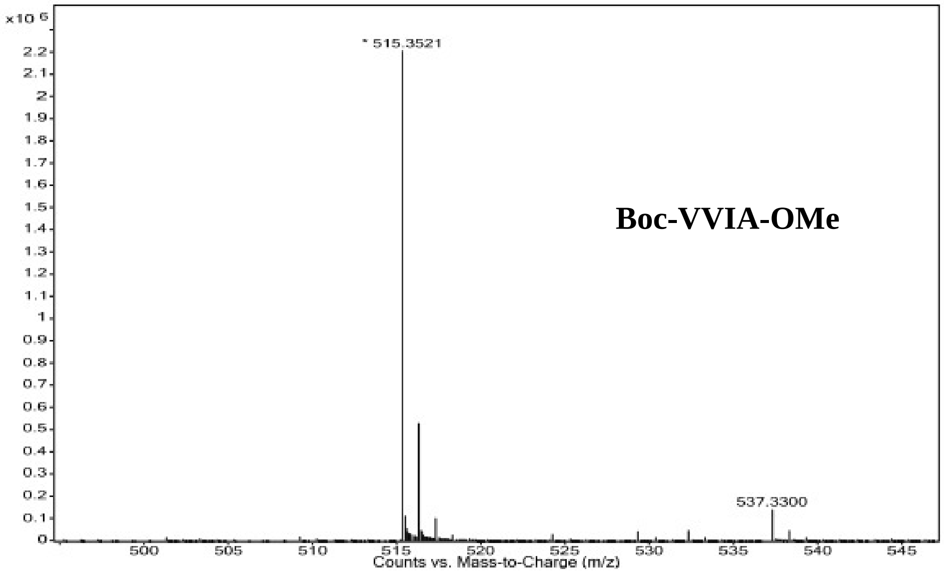


Figure S68. ESI-MS spectrum of Boc-VVIA-OMe (Figure 6.1.1.1a)

6.9.8. HPLC chromatogram and mass spectra of DNASFVEDLG-NH$_2$ peptide

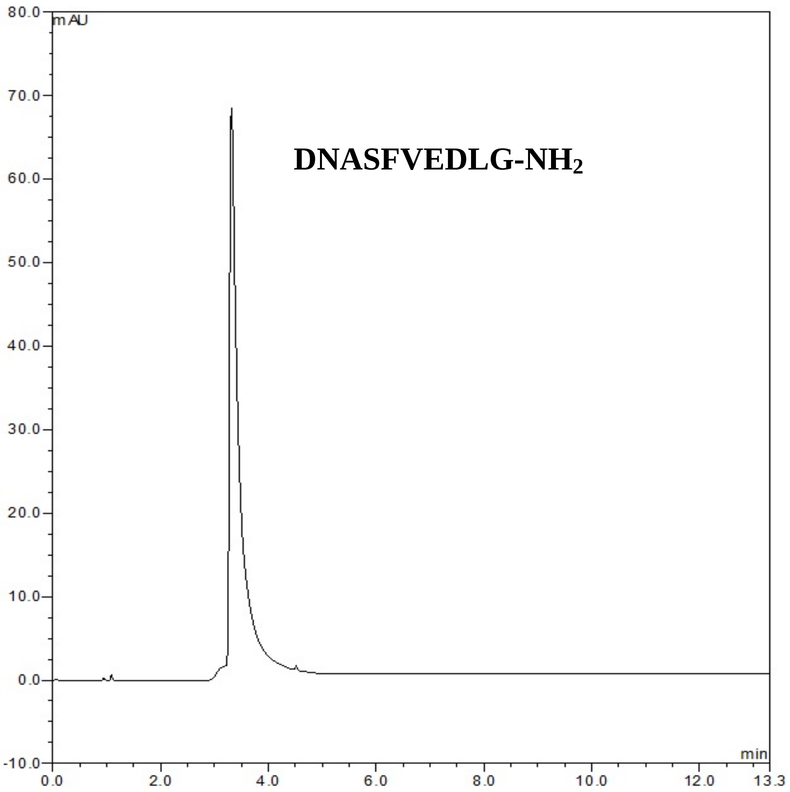


Figure S69. HPLC chromatogram of DNASFVEDLG-NH$_2$ run in Ascentis C18 reverse phase analytical column and a linear gradient of 70 to 100%, CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.1b)

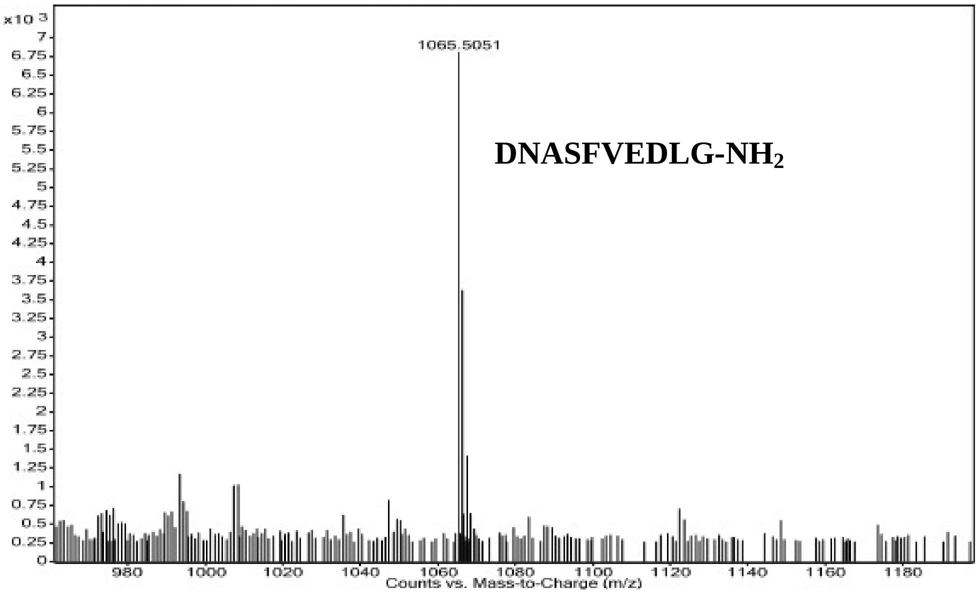


Figure S70. ESI-MS of DNASFVEDLG-NH$_2$ (Figure 6.1.1.1b)

6.9.9. HPLC chromatogram and mass spectra of PGVGVPGVGV-NH$_2$ peptide:

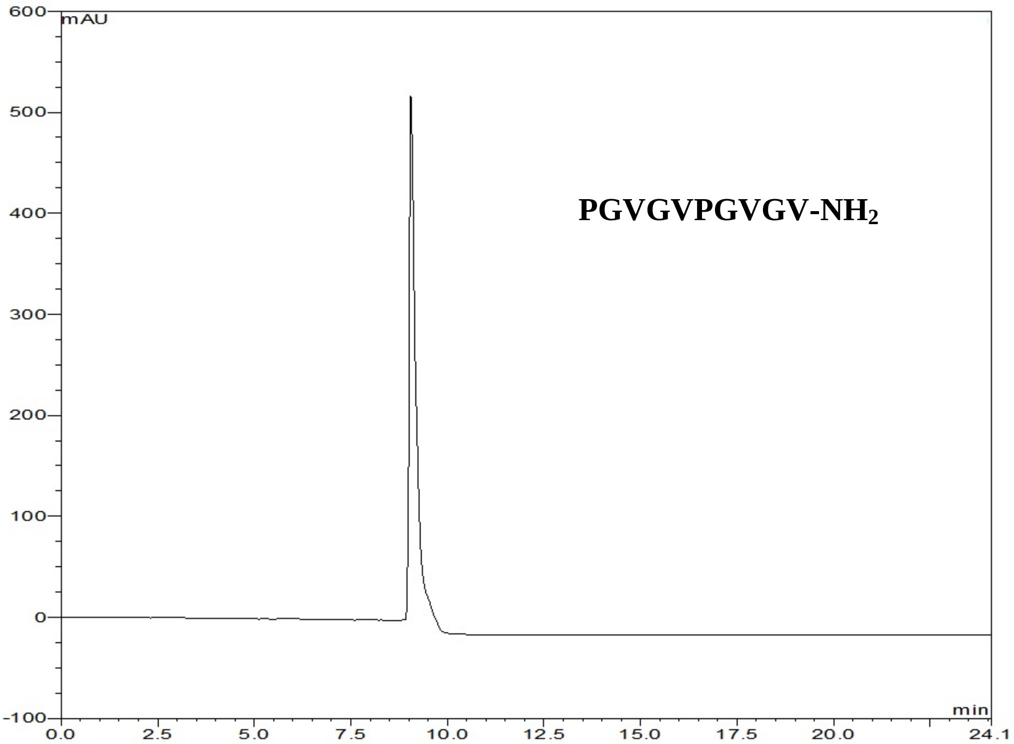


Figure S71. HPLC chromatogram of PGVGVPGVGV-NH$_2$ decapeptide run upto 24 min in Ascentis C18 reverse phase analytical column and a linear gradient of 0 to 70%, 0-15 min, then 70 to 100% upto 24 min., CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.1c)

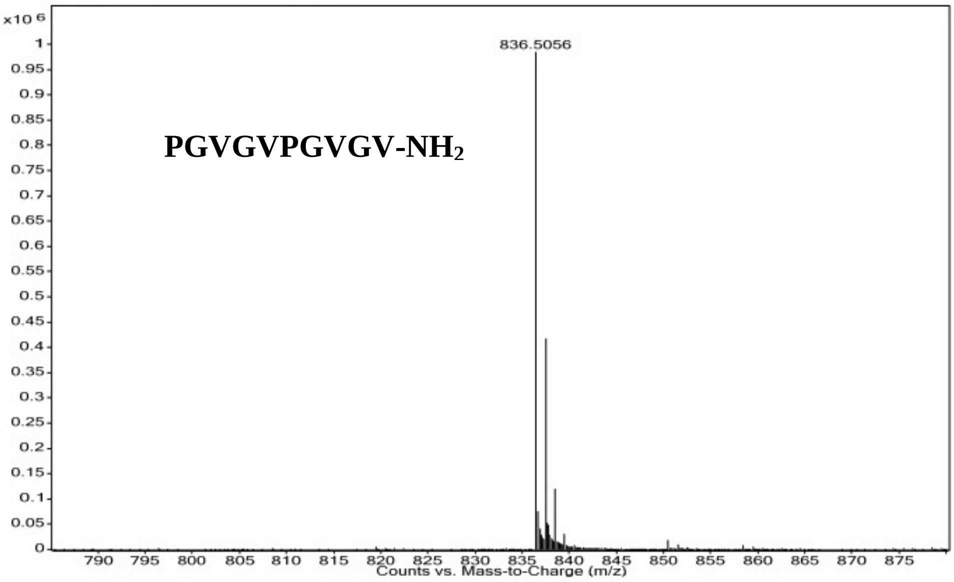


Figure S72. ESI-MS of PGVGVPGVGV-NH$_2$ (Figure 6.1.1.1c).

6.9.10. HPLC chromatogram and mass spectra of fragments of gramicidin via solid phase synthesis

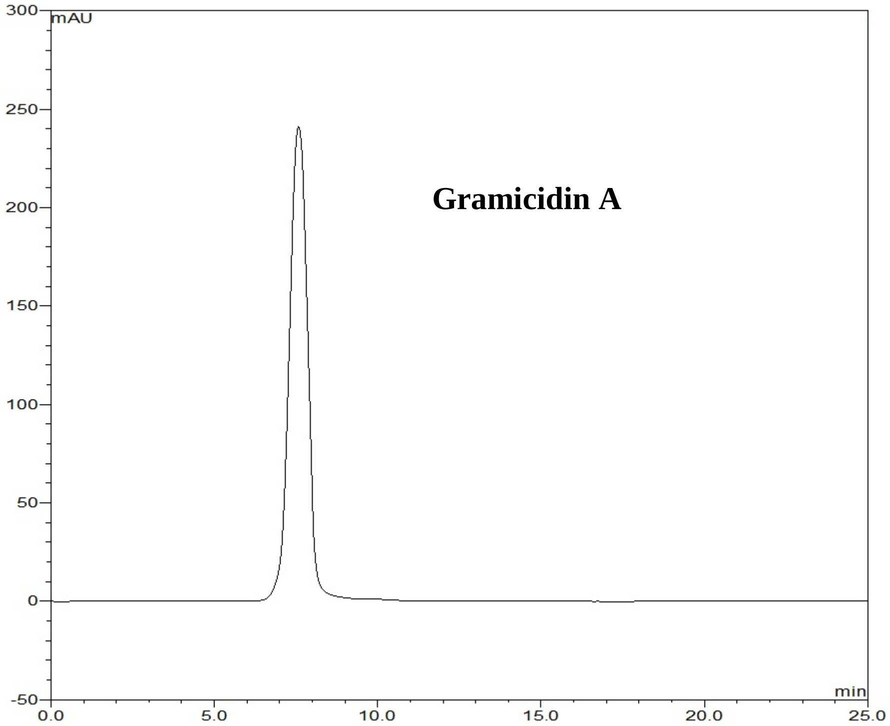


Figure S73. HPLC chromatogram of Gramicidin **A** fragment run in Ascentis C18 reverse phase analytical column and a linear gradient of 70 to 100%, CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.2)

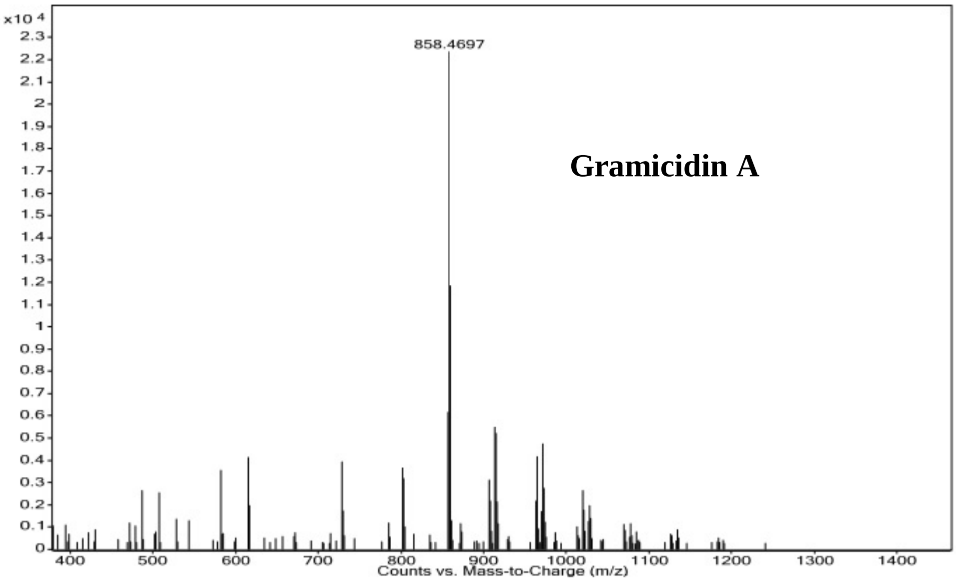


Figure S74. ESI-MS of Gramicidin **A** fragment (Figure 6.1.1.2)

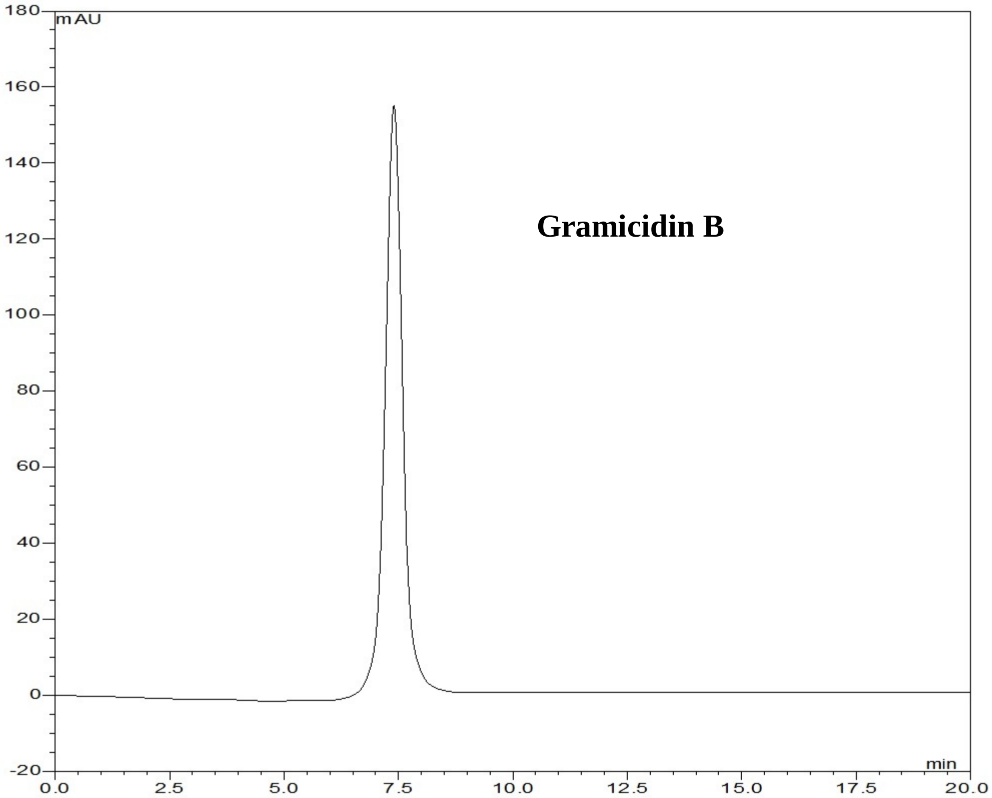


Figure S75. HPLC chromatogram of Gramicidin **B** fragment run in Ascentis C18 reverse phase analytical column and a linear gradient of 70 to 100%, CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.2)

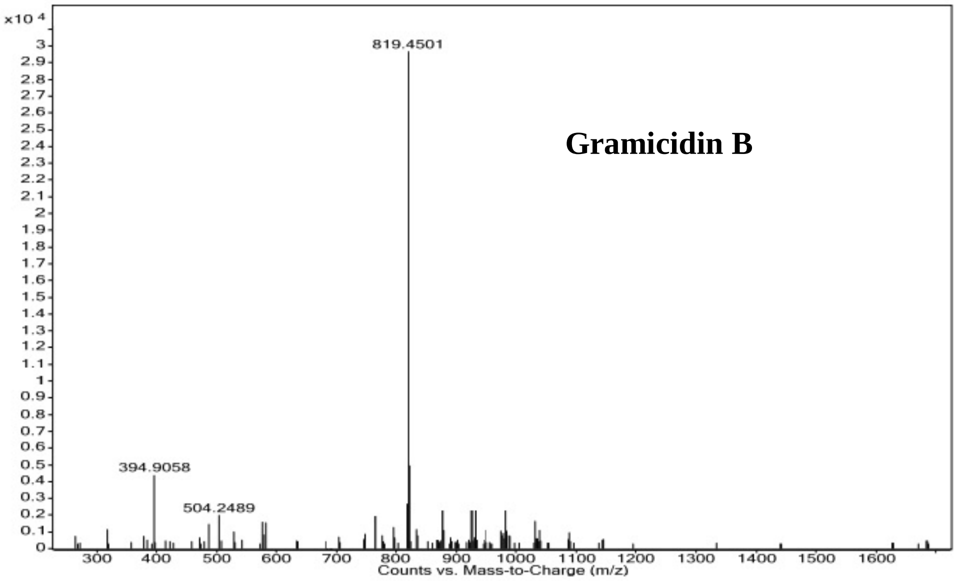


Figure S76. HPLC chromatogram of Gramicidin **B** fragment (Figure 6.1.1.2)

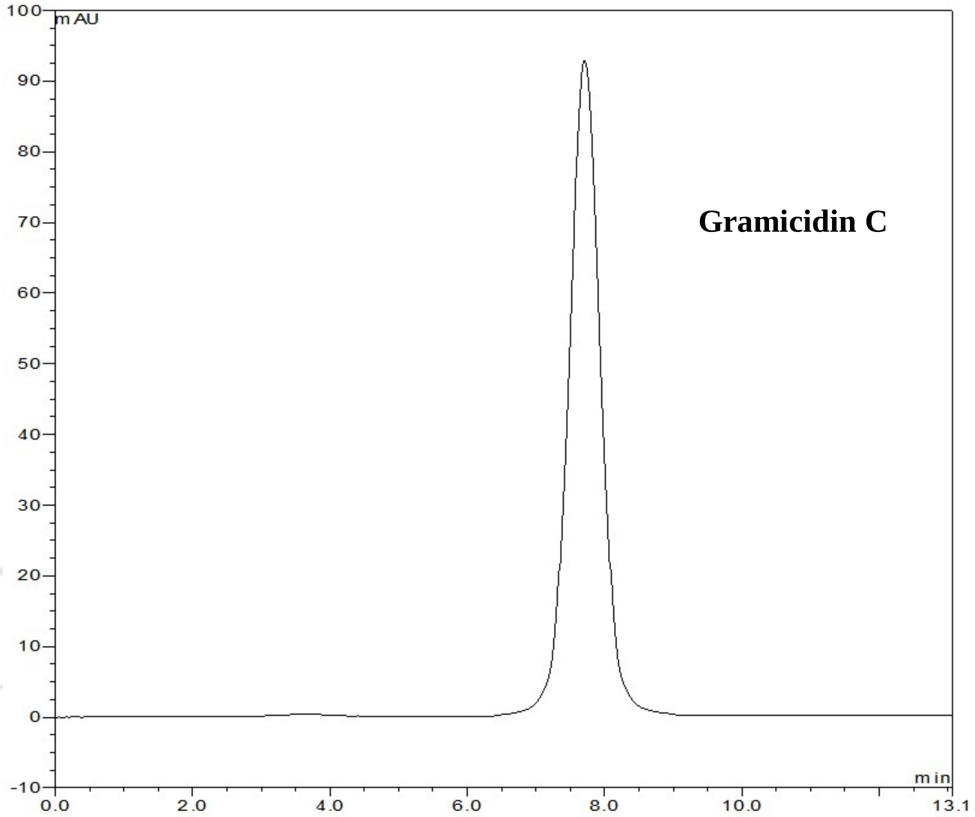


Figure S77. HPLC chromatogram of Gramicidin **C** fragment run in Ascentis C18 reverse phase analytical column and a linear gradient of 70 to 100%, CH_3CN in H_2O with 0.1% formic acid (Figure 6.1.1.2)

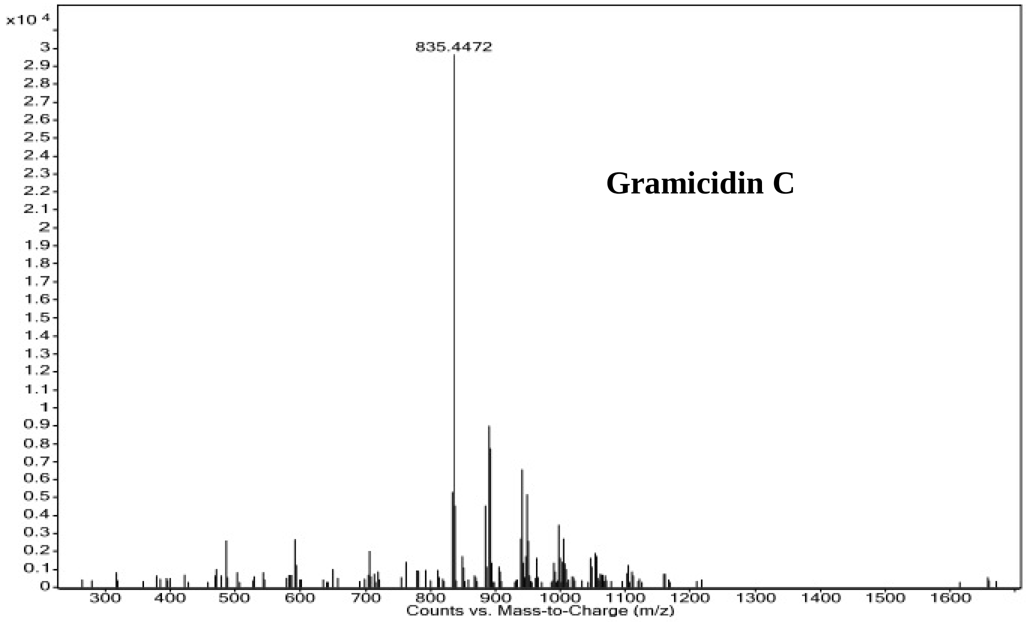
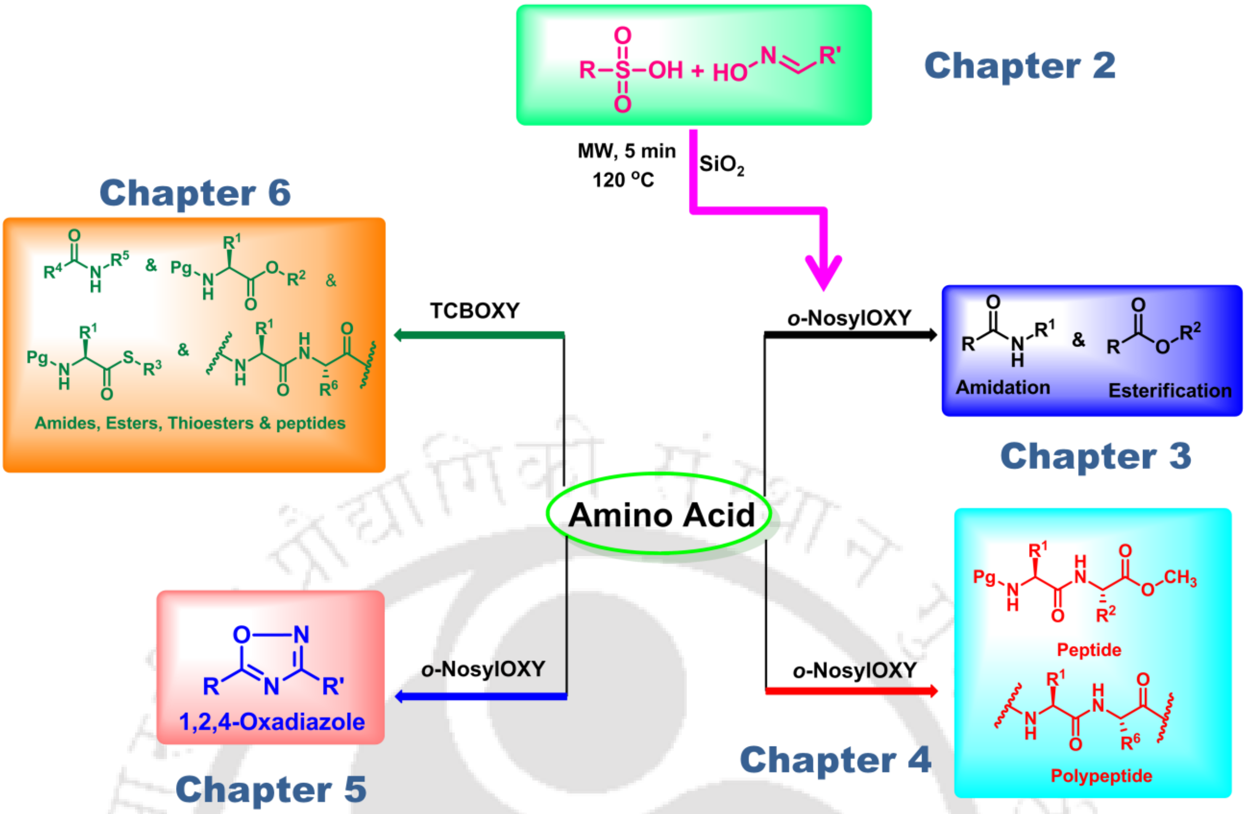


Figure S78. HPLC chromatogram of Gramicidin **C** fragment (Figure 6.1.1.2)

Conclusions and Future Directions

Conclusions

The work described in this thesis is mainly focused on the development of green methodologies for the synthesis of sulphonate esters, amides, esters, thioesters, peptides, polypeptides as well as 1,2,4-oxadiazole. Chapter 1 contain introduction. This introductory chapter briefly focuses on the importance of above-mentioned compounds, their existing synthetic methods and drawbacks associated with the existing methods. In chapter 2, we demonstrated the halogen free synthesis of sulphonate esters of alcohol, Oxyma-*O*-sulphonates, and oxime-*O*-sulphonates under microwave irradiation. In chapter 3, we discussed the simple and green approach towards synthesis of amides and esters using catalytic amount of *o*-NosylOXY. In chapter 4, we described the racemization free synthesis of peptides and polypeptides using sub-stoichiometric amount of *o*-NosylOXY. In chapter 5, we illustrated the synthesis of 1,2,4-oxadiazoles by using the catalytic amount of *o*-NosylOXY. In last chapter, i.e. chapter 6, we described the development of a new generation of efficient coupling reagent, TCBOXY, for the synthesis of esters, thioesters, amides, and peptides. The total content of the thesis is depicted in scheme 1.



Scheme 1. Thesis overview

Future directions

During the PhD tenure, we developed new coupling reagent, TCBOXY, which can further be used to explore for the synthesis of many other organic transformations from amino acids under milder conditions.

Research Outcome

Publications:

1. Manne, S. R.; **Chandra, J**.; Mandal, B. Synthesis of *o*-Nitroarylamines via Ipso Nucleophilic Substitution of Sulphonic Acids, *Org. Lett*. **2019**, 21, 636-639.
2. **Chandra, J**.; Manne, S. R.; Mondal, S.; Mandal, B. (*E*)-Ethyl-2-cyano-2-(((2,4,6-trichlorobenzoyl)oxy)imino)acetate: A Modified Yamaguchi Reagent for Enantioselective Esterification, Thioesterification, Amidation and Peptide Synthesis, *ACS Omega*. **2018**, *3*, 6120–6133.
3. Manne, S. R.; **Chandra, J**.; Giri, R. S.; Kalita, T.; Mandal, B. Synthesis of β-Amino Alcohols Using Ethyl 2-Cyano-2-(2-nitrobenzenesulfonyloxyimino)acetate (*o*-NosylOXY), *ChemistrySelect*. **2018**, *1*, 1-6.
4. **Chandra, J**.; Chaudhuri, R.; Manne, S. R.; Mondal, S.; Mandal, B. Direct Synthesis of Sulphonates of Alcohol, Oxyma-O-sulphonates and Oxime-O-sulphonates under Microwave Irradiation, *ChemistrySelect*. **2017**, *2*, 8471–8477.
5. Manne, S. R.; Thalluri, K.; Giri, R. S.; **Chandra, J.;** Mandal, B. Ethyl 2-(tert-Butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma): An Efficient Reagent for the Racemization Free Synthesis of Ureas, Carbamates and Thiocarbamates via Lossen Rearrangement, *Adv. Synth. Catal*. **2017**, *359*, 168–176.

6. Dev, D.; **Chandra, J**.; Palakurthy, N. B.; Kishore, T.; Kalita, T.; Mandal, B. Benzoxazole and Benzothiazole Synthesis from Carboxylic Acid in Solution and on Resin by Ethyl 2-cyano-2-(2-nitro-benzenesulfonyloxyimino) acetate and para-Toluenesulfonic Acid, *Asian J. Org. Chem.* **2016**, *5*, 663–675.
7. Dev, D.; Palakurthy, N. B.; Kishore, T.; **Chandra, J**.; Mandal, B. Ethyl 2-cyano-2-(2-nitrobenzenesulfonyloxyimino)acetate (*o*-NosylOXY): A Recyclable Coupling Reagent for Racemization free Synthesis of Peptide, Amide, Hydroxamate and Ester, *J. Org. Chem*, **2014**, *45*, 5420-5431.
8. **Chandra, J**.; Dev. D.; Manne, S. R.; Kalita, S.; Mondal, S.; Mandal, B. "Catalytic approach of *o*-NosylOXY for esterification, amidation, and peptide synthesis" (Manuscript to be submitted).
9. **Chandra, J**.; Manne, S. R.; Dev. D.; Mondal, S.; Mandal, B. "Synthesis of 1,2,4-oxadiazoles by using the catalytic amount of *o*-NosylOXY" (Manuscript to be submitted).

Patents

1. **Chandra, J.** and Mandal, B. "A METHOD FOR PREPARATION OF SULPHONATES OF ALCOHOL, OXIME-O-SULPHONATES AND THEIR DERIVATIVES" an Indian patent application No. 201731016281 (complete application filed on the 9th May **2017**).
2. **Chandra, J.** and Mandal, B. "A NOVEL COUPLING REAGENT FOR ESTERIFICATION, THIOESTERIFICATION, AMIDATION AND PEPTIDE SYNTHESIS" New Indian Patent Application No. 201731045011 (complete application filed on the 14th Dec **2017**).

Book Chapter

Thalluri, K.; Dev, D.; Palakurthy, N. B.; Manne, S. R.; **Chandra**, **J**.; Mandal, B.* "The Coupling Reagents the World in Need Indeed", in *Proceedings of International Conference on New Dimensions in Chemistry & Chemical Technologies – Applications in Pharma Industry, NDCT-2014*, 23th-25th June, 2014, JNTU Hyderabad. Prof. K. Mukkanti (Editor-in-Chief), Spectrum Publications, Hyderabad-500038, A. P. India. (ISBN 978-93-82829-90-4) **2014**, p 348-351**.**

Conference

1. **Chandra, J**.; Mandal, B.* *Direct Synthesis of Oxyma-O-sulphonates, Sulphonates of Alcohols and Oxime-O-sulphonates under Microwave Irradiation and its Application in Relevant Organic Transformation*, XIII J-NOST Conference for Research Scholars (J-NOST-2017), 9th November **2017**, Department of Chemistry, Banaras Hindu University, Varanasi, PP-14 (**Poster**).
2. **Chandra, J**.; Dev, D.; Mandal, B.* *o-NosylOXY: The Best Coupling Reagent for Peptide Synthesis*, Chemconvene, 25th July **2017**, Department of Chemistry, Indian Institute of Technology Guwahati, OP-04 (**oral**).
3. **Chandra, J**.; Dev, D.; Mandal, B.* *Synthesis of Benzoxazole and Benzothiazole using Ethyl 2-Cyano-2-(2-nitrobenzenesulfonyloxyimino) acetate (o-NosylOXY) both Solution and Solid Phase*, 20th CRSI National Symposium in Chemistry, Department of Chemistry, 3rd Feb **2017**, Gauhati University, Page 143, PP-141 (**Poster**).

4. **Chandra, J**.; Dev, D.; Mandal, B.* *Ethyl-2-cyano-2-(2-nitrobenzenesulfonyloxyimino) acetate (2-NBsOXY): a recyclable coupling reagent for synthesis of hydroxamate under mild condition*, Abstract book, Frontier in Chemical Sciences (FICS-2014), 4th-6th December, **2014**, IIT Guwahati, India, P-39, Page 86. (**Poster**).

Curriculum Vitae

JYOTI CHANDRA

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QUALIFICATION

07/2013–Present	**Ph.D. in Chemistry (CPI 8.02 out of 10)** Indian Institute of Technology Guwahati (IITG), Assam, India Thesis: Development of new strategies for peptide synthesis. Thesis advisor: Dr. Bhubaneswar Mandal
07/2010–05/2012	**M.Sc. in Chemistry (First Class)** Indian Institute of Technology Kharagpur, West Bengal, India Thesis: Influence of Chirality on Physical Gelation of Organic Liquids by Amino Acid Derived Amphiphiles. Thesis advisor: Prof. Joykrishna Dey
07/2007–05/2010	**B.Sc. in Chemistry Honours (First Class)** Patna University, Bihar, India

HONOURS/AWARDS

- Ranked in IIT JAM 2010 (AIR) 14.
- MHRD scholarship for pursuing PhD in IIT Guwahati.
- Received National Eligibility Test (NET-JRF) Dec-2012 (UGC-rank 107) in Chemical Science.
- Received Graduate Aptitude Test in Engineering (GATE) 2013 in Chemistry (score 311), (GATE) 2012 in Chemistry (score 197).

PATENTS

- **Chandra, J.** and Mandal, B. "A NOVEL COUPLING REAGENT FOR ESTERIFICATION, THIOESTERIFICATION, AMIDATION AND PEPTIDE SYNTHESIS" New Indian Patent Application No. 201731045011 (complete specification filed on the 14th Dec, 2017).

- **Chandra, J.** and Mandal, B. "A METHOD FOR PREPARTION OF SULPHONATES OF ALCOHOL, OXIME-O-SULPHONATES AND THEIR DERIVATIVES" an Indian patent application No. 201731016281 (complete specification filed on the 9th May, 2017).

PUBLICATIONS

1. Manne, S. R.; **Chandra, J**.; Mandal, B. ***Org. Lett***. 2019, 21, 636-639.
2. **Chandra, J**.; Manne, S. R.; Mondal, S.; Mandal, B. ***ACS Omega***. 2018, *3*, 6120–6133.
3. Manne, S. R.; **Chandra, J**.; Giri, R. S.; T.; Kalita.; Mandal, B. ***ChemistrySelect.*** 2018, *3*, 992–996.
4. **Chandra, J**.; Chaudhuri, R.; Manne, S. R.; Mondal, S.; Mandal, B. ***ChemistrySelect.*** 2017, *2*, 8471 – 8477
5. Manne, S. R.; Thalluri, K.; Giri, R. S.; **Chandra, J**.; Mandal, B. ***Adv. Synth. Catal.*** 2017, *359*, 168–176.
6. Dev, D.; **Chandra, J**.; Palakurthy, N. B.; Kishore, T.; Kalita, T.; Mandal, B. ***Asian J. Org. Chem.*** 2016, *5*, 663 – 675.
7. Dev, D.; Palakurthy, N. B.; Kishore, T.; **Chandra, J**.; Mandal, B. ***J. Org. Chem.*** 2014, *45*, 5420-5431.
8. **Chandra, J**.; Dev. D.; Manne, S. R.; Kalita, S.; Mondal, S.; Mandal, B. "Catalytic approach of *o*-NosylOXY for esterification, amidation, and peptide synthesis" (Manuscript to be submitted).
9. **Chandra, J**.; Manne, S. R.; Dev. D.; Mondal, S.; Mandal, B. "Synthesis of 1,2,4-oxadiazoles by using the catalytic amount of *o*-NosylOXY" (Manuscript to be submitted).

BOOK CHAPTER

Kishore Thalluri, Dharm Dev, Nani Babu Palakurthy, Srinivasa Rao Manne, **Jyoti Chandra** and Bhubaneswar Mandal*, "The Coupling Reagents the World in Need Indeed", in *Proceedings of International Conference on New Dimensions in Chemistry & Chemical Technologies – Applications in Pharma Industry, NDCT-2014*, 23th-25th June, 2014, JNTU Hyderabad. Prof. K. Mukkanti (Editor-in-Chief), Spectrum Publications, Hyderabad-500038, A. P. India. (ISBN 978-93-82829-90-4) 2014, p 348-351.

POSTER & ORAL PRESENTATION IN CONFERENCES

1. **Jyoti Chandra** and Bhubaneswar Mandal*, XIII J-NOST Conference for Research Scholars (J-NOST-2017), 9th November, **2017**, Department of Chemistry, Banaras Hindu University, Varanasi, PP-14 (**poster**).
2. **Jyoti Chandra**, Dharm Dev and Bhubaneswar Mandal* Chemconvene, 25th July **2017**, Department of Chemistry, Indian Institute of Technology Guwahati, OP-04 (**oral**).
3. **Jyoti Chandra**, Dharm Dev and Bhubaneswar Mandal*, 20th CRSI National Symposium in Chemistry, Department of Chemistry, 3rd Feb **2017**, Gauhati University, PP-141 (**poster**).
4. **Jyoti Chandra**, Dharm Dev and Bhubaneswar Mandal*, Abstract book, Frontier in Chemical Sciences (FICS-2014), 4th-6th December, **2014**, IIT Guwahati, India, PP-39 (**poster**).

RESEARCH & INSTRUMENTAL SKILLS

- I have expertise in conformational analysis of chiral compounds by various instrumental techniques including, NMR, HPLC, ESI-MS, FTIR, GCMS, *etc*.
- Expertise in design and execution of multistep organic reactions and peptide synthesis in both solution and solid phase

ACADEMIC & PROFESSIONAL SKILLS

- **Laboratory and Instrumentation:** Familiar with Synthetic Organic Chemistry, Peptide Synthesis (Solution Phase and Solid Phase), NMR Spectroscopy, Mass Spectrometry (ESI), GCMS, HPLC and FTIR.
- **Computer Knowledge:** Diploma in Computer Appication.
- **Software:** Origin, MS-Office and Chem Draw.
- **Teaching Experience:** Worked as Teaching Assistant (TA) in B.Tech and M.Sc Laboratory at IIT Guwahati, India.
- **Language:** English, Hindi and Bengali.