Mild and Biocompatible Synthesis of Highly Symmetrical Tetra-Substituted Pyrazines from Amino acids and Peptides: A Novel Strategy for the Self-Stapling of Peptides

Thesis submitted towards the partial fulfillment of the BS-MS dual degree programme

By

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Under the guidance of

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CERTIFICATE

This is to certify that this dissertation entitled "***Mild and Biocompatible Synthesis of Highly Symmetrical Tetrasubstituted Pyrazines from Amino acids and Peptides: A Novel Strategy for the Self-Stapling of Peptides***" towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by "**Rupal Dinesh Bhaisare** at IISER Pune " under the supervision of "**Dr. Hosahudya N. Gopi**, Associate professor, Department of Chemistry, IISER Pune" during the academic year 2013-2014.

2nd April 2014

Dr. Hosahudya N. Gopi
Associate Professor
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DECLARATION

I hereby declare that the matter embodied in the report entitled "***Mild and Biocompatible Synthesis of Highly Symmetrical Tetrasubstituted Pyrazines from Amino acids and Peptides: A Novel Strategy for the Self-Stapling of Peptides***" are the results of the investigations carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune, under the supervision of **Dr. Hosahudya N. Gopi** and the same has not been submitted elsewhere for any other degree.

2nd April 2014

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Rupal

CONTENTS

ABBREVATION

Boc = tert-Butoxycarbonyl

$(Boc)_2O$ = Boc anhydride

DIPEA = Diisopropylethyl amine

DMF = Dimethyl formamide

EtOAc = Ethyl Acetate

Fmoc = 9-Fluorenylmethoxycarbonyl

Fmoc-OSu = *N*-(9-Fluorenylmethoxycarbonyloxy) succinimide

DCC = Dicyclohexyl carbodiimide

DCM = Dichloromethane

$LiAlH_4$ = Lithium Aluminum Hydride

HBTU = O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate

HCl = Hydrochloric acid

HOBt = Hydroxybenzotriazol

HPLC = High Performance Liquid Chromatography

$SnCl_2$ = Tin chloride

TFA = Trifluoroacetic acid

THF = Tetrahydrofuran

HRMS = High Resolution Mass Spectroscopy

Cbz = Carboxybenzyl

Val = Valine

Ala = Alanine

Na_2CO_3 = Sodium Carbonate

ABSTRACT

The utility of unnatural amino acids has been extensively explored in the construction of new building blocks, molecular scaffolds, and therapeutic leads. For instance, highly versatile γ-amino *β*-keto acids present in several biologically active peptides, has been used as precursor for the synthesis of many biologically relevant molecules such as statines, ketomethylene dipeptide isosteres, *β*-lactams, tricarbonyl compounds, rhodopeptins, substituted pyridines, fluorescent amino acid tags, cholecystokinin receptor antagonists, γ-amino α*,* *β*-unsaturated esters, and α-azo *β*-carbonyl compounds. Here we are demonstrating the utility of β-keto-γ-amino ester in the synthesis of highly symmetrical tetrasubstituted pyrazines through simple aerial oxidation. In addition we also exploited this mild and biocompatible protocol for the self-stapling of peptides. The easy synthesis, mild and biocompatible aromatization of the β-keto-γ-amino esters and peptide β-keto-esters may find application in biological and medicinal chemistry as well as in material science.

INTRODUCTION :

Pyrazines are a class of heteroaromatic compounds, which occurs almost ubiquitously in nature. Pyrazine are common structural units in a wide variety of natural products[1-6]. Many of the pyrazine containing natural products have showed excellent biological activities, including cytostatic[6], antitumor[3], antituberculosis[7], sedative hypnotics[8], antiviral[9], etc. Besides their biological activities, pyrazines have also been extensively used in the food and perfume industry because of their taste and aroma[10-11]. Pyrazine derivatives also served as female insect sex pheromones and many sexually deceptive orchid species produces pyrazines to attract male wasps as pollinators[12-13]. Further, pyrazine derivatives have been found to be function as releasers of alarm behavior in ponerine ants[14]. Many bacterial strains use various substituted pyrazines as a sole source of carbon and energy[15]. Some representative examples of naturally occurring pyrazine natural products are shown in Figure 1. Recently, Kutonovas *et al*. reported the catabolic pathway for the degradation of pyrazines[16].

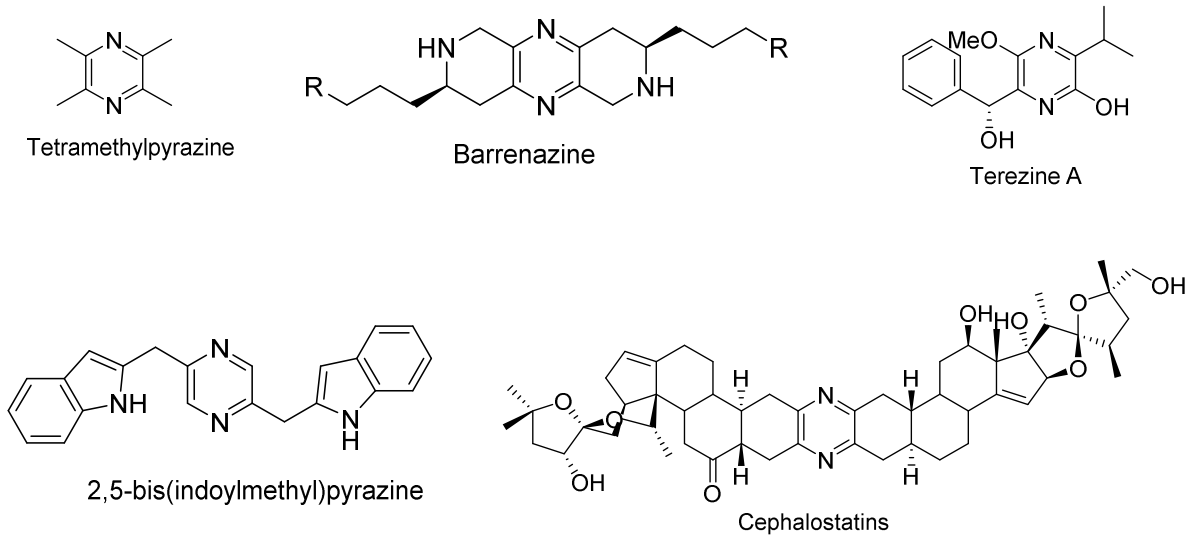
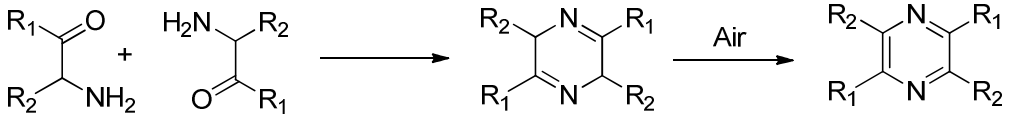


Figure 1: Representative examples of pyrazine containing natural products

As a consequence of ever increasing demand for the pyrazine derivatives in pharma, food and perfume industries various synthetic protocols have been developed. Generally, pyrazines are synthesized by the condensation of 1, 2-diamines and 1, 2–dicarbonyl compounds as well as self-condensation of α-amino ketones. Synthesis of pyrazines through the reaction between α-haloketones and ammonia is also well established reaction. The self condensation of α-amino ketones and subsequent aerial oxidation to pyrazines was discovered by German scientist Gutknecht[17]. The schematic representation of the transformation of α-amino ketones to pyrazines is shown in Scheme 1. Besides this popular method, various protocols including α-haloenol Acetates[18],



Scheme 1: Synthesis of pyrazines through self-condensation of α-amino ketones.

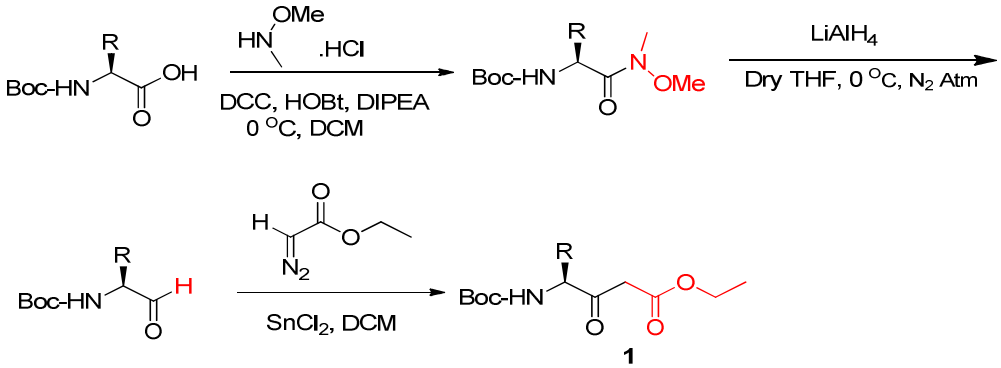
nitroepoxides[19], ruthenium catalyzed dehydrogenative coupling of β-amino alcohols[20], thermal treatment of azirine phosphonates and tosylketoximes[21] etc. is also known. Nature selectively utilizes α-amino acids to synthesize pyrazine derivatives. Recently, Sperry and colleagues have examined the self-condensation of various amino aldehydes and revealed the importance of Cbz-protection over the Boc- and Fmoc-protection of amino aldehydes[22].

We have been interested in the synthesis and conformational analysis of peptide foldamers containing naturally occurring nonribosomal γ-amino acids such as β-hydroxy γ-amino acids, α, β-unsaturated γ-amino acids and γ-amino acids. Recently, we reported the facile synthesis of β-keto-γ-amino esters and their utility in the synthesis of β-hydroxy-γ-amino acids and peptides as well as fluorescent coumarin amino acids[23-24].

The wide-spread applications of pyrazine motivated us to investigate whether β-keto-γ-amino esters can be exploited for the synthesis of highly symmetric tetrasubstituted pyrazines. In addition, we anticipate that by utilizing either amine or acid side-chain functionalized amino acids as starting materials it may be possible to connect peptides together through the formation of pyrazines. Herein, we are reporting the mild and biocompatible synthesis of the highly symmetric tetrasubstituted pyrazines and novel strategy for head-to-head stapling of peptides.

Results and Discussion:

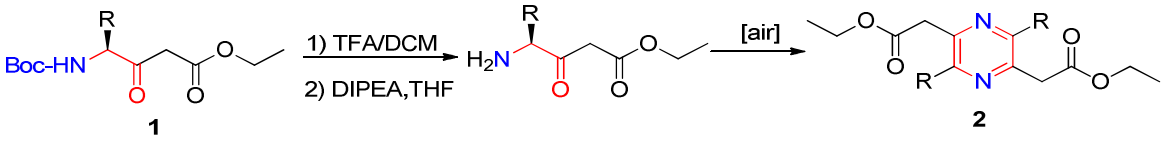
The ethyl esters of β-keto-γ-amino esters were synthesized starting from *N*-Boc amino aldehydes and ethyl diazoacetate in the presence of anhydrous tin chloride as reported earlier[23]. The schematic representation of the synthesis of β-keto-γ-esters starting from *N*-protected amino acid is shown in Scheme 2. All ethyl esters of β-keto-γ-amino esters were isolated in good yields after the column purification. In order to understand whether the β-keto-γ-amino esters can



Where R= (a) -CH_2-Ph; b) -Ph; c) -CH_2-$CH(CH_3)_2$; d) -CH_2-CH_2-S-CH_3, and e) -CH_2-NH-Cbz

Scheme 2: Synthesis of β-keto-γ-amino esters starting from amino acids

undergo self-condensation followed by aerial oxidation to give pyrazine derivatives, we initially subjected ethyl ester of β-keto-γ-phenylalanine (**1a**) to the self-condensation reaction in the open air at little above the neutral pH after the deprotection of *N*-Boc group. The schematic representation of the reaction is shown in Scheme 3. The complete transformation of γ-amino β-keto-ester into highly symmetrical tetrasubstituted pyrazine was achieved after stirring the reaction mixture in THF for 24h.



Where R= (a) -CH_2-Ph; b) -Ph; c) -CH_2-$CH(CH_3)_2$; d) -CH_2-CH_2-S-CH_3, and e) -CH_2-NH-Cbz

Scheme 3: Schematic representation of the synthesis of tetrasubstituted pyrazines starting from *N*-Boc-β-keto-γ-amino esters

The pure tetrasubstituted pyrazine **2a** was isolated after column chromatography. Further upon standing pure **2a** gave X-ray quality single crystals and its X-ray structure is shown in Figure 2.

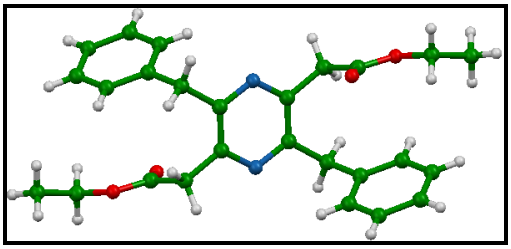
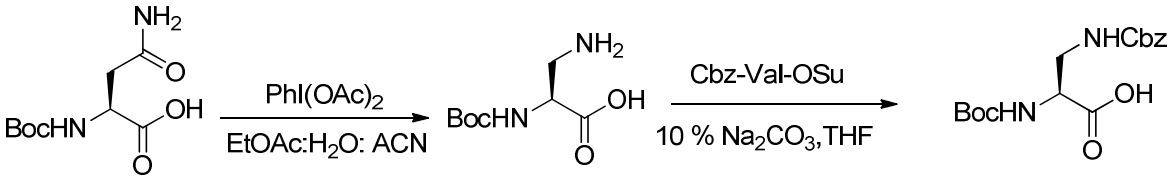


Figure 2: Single crystal structures of **2a**.

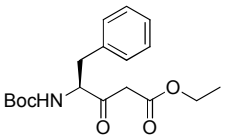
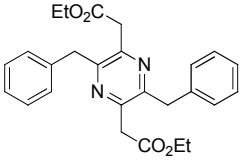
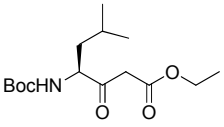
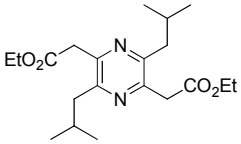
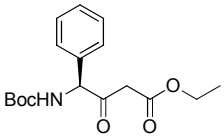
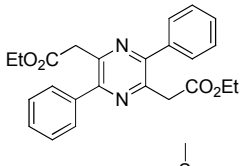
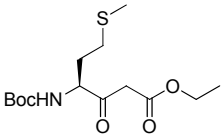
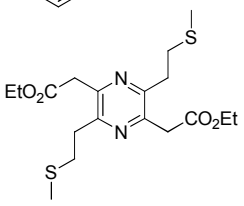
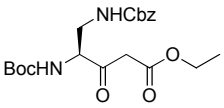
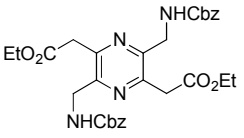
Inspired by the highly symmetric structure of **2a**, we subjected all other β-keto-γ-amino esters given in Scheme 2 for the pyrazine synthesis. All pyrazine products **2a** to **2e** were isolated in moderate to good yields (data given in the Table 1).

The β-keto-γ-amino esters with functional side chains **1d** and **1e** also undergoes cyclization similar to the other amino acids. Boc-Dap(Cbz)-COOH was synthesized starting from Boc-Asn through Hoffmann rearrangement mediated by the $PhI(OAc)_2$[25]. The free amino group obtained after the rearrangement was protected with Cbz-group the Schematic representation of the reaction is shown in the Scheme 4.

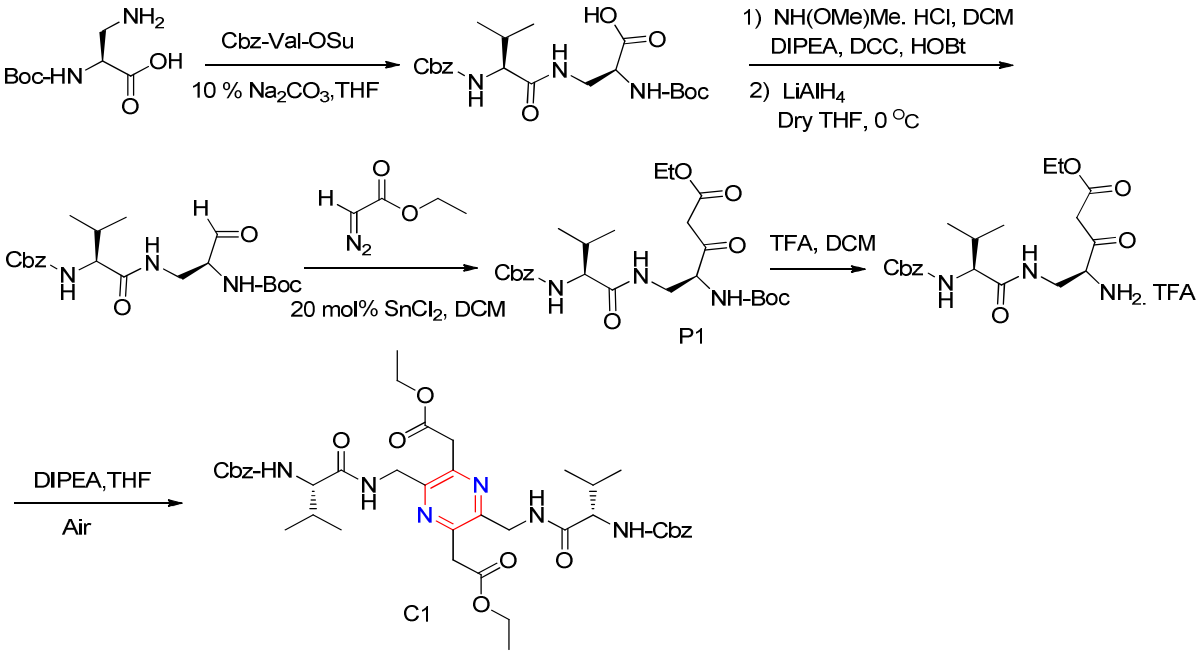


Scheme 4: Synthesis of Boc-Dap(Cbz)COOH starting from the Boc-Asn

Table 1: List of pyrazines synthesized starting from β-keto-γ-amino ester

Entry	Beta-keto amino ester (1)	Pyrazines (2)	Yield (%)
a			65
b			56
c			58
d			62
e			59

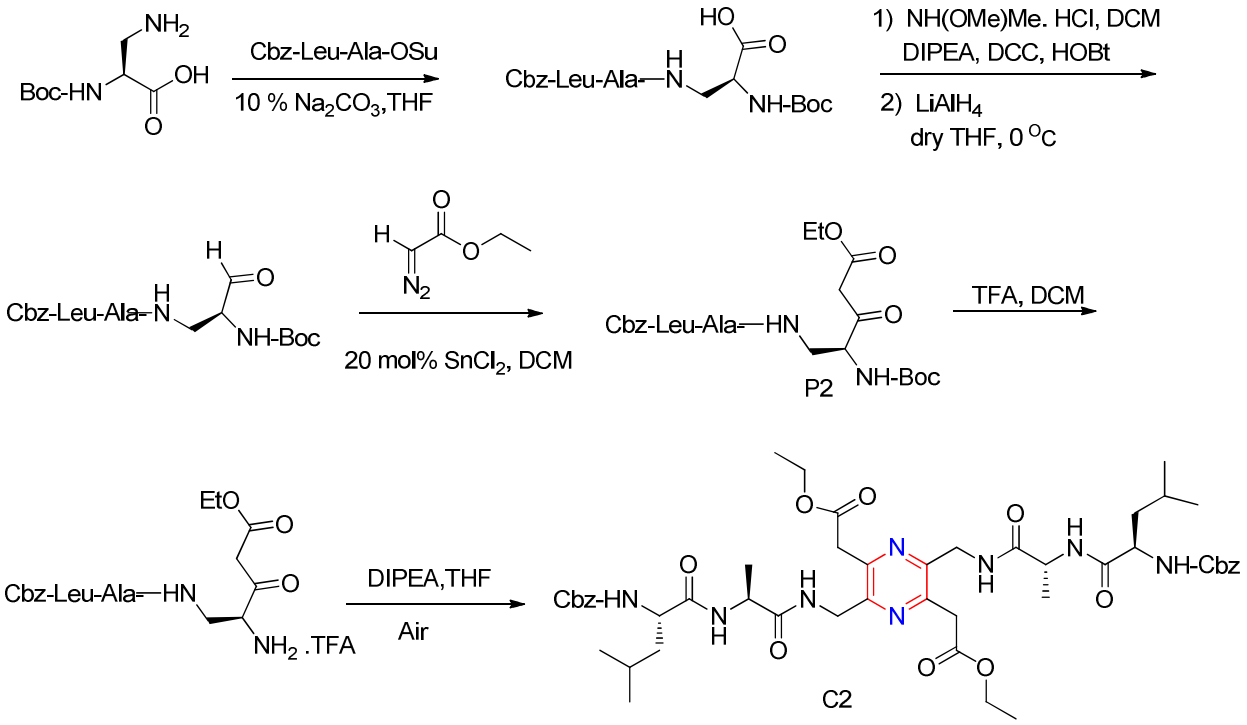
The cyclization of 1e to 2e motivated us to extend this cyclization strategy to peptides. Based on the cyclization of the 1e, we anticipate that if the Cbz-group is replaced with peptide it might be possible to cyclize the peptides at mild basic conditions. To understand whether the peptide β-keto esters can undergo cyclization similar to the β-keto-γ-amino esters, we designed new strategy for the synthesis of peptide β-keto-esters. The schematic representation of the synthesis of the dipeptide P1 is shown in the Scheme 5.



Scheme 5: Synthesis of the dipeptide β-keto-ester and subsequent pyrazine synthesis

The free amine obtained after the Hoffmann rearrangement of Boc-Asn was directly coupled to the *N*-hydroxysuccinimide ester of *N*-Cbz-Val-OH. The dipeptide carboxylic acid was isolated in good quantitative yield. The free carboxylic acid of the dipeptide was further coupled with the *N*,*O*-dimethylhydroxyamine hydrochloride using DCC/HOBt coupling conditions. The pure dipeptide Weinreb amide was isolated in good

yield and subjected to LAH reduction to give corresponding dipeptide aldehyde. The dipeptide aldehyde was converted to the β-keto-ester by the treatment of ethyl diazoacetate in the presence of tin (II) chloride. The pure dipeptide β-keto-ester was isolated in good yield after the column purification. The Boc-group of the dipeptide was selectively deprotected using 2:1 mixture of TFA and DCM. The TFA salt of the dipeptide β-keto-ester was precipitated using diethyl ether. Finally, the TFA salt was neutralized using DIPEA in THF solution. Later, the free amine was stirred for 24 hours in open air. The cyclization was monitored using TLC. The pure dipeptide pyrazine was isolated with moderate yield after the column chromatography. The formation of pyrazine product was confirmed by using ^{1}H, ^{13}C NMR and HRMS. Overall this strategy proved that dipeptides can be converted to the pyrazines under mild conditions.



Scheme 6: Transformation of tripeptide β-keto-ester into pyrazine

Inspired by this mild protocol, we extended the same strategy for tripeptides to understand whether this protocol is compatible for the high order peptides. The schematic representation of the synthesis of tripeptide β-keto-ester and subsequent transformation of β-keto-ester into pyrazines is shown in Scheme 6. The target

tripeptide was synthesized by direct coupling of *N*-hydroxysuccinimide ester of *N*-Cbz-protected dipeptide to the free amine of *N*-α-Boc-Dap. The C-terminal free carboxylic acid was converted to Weinreb amide as described earlier. The tripeptide was further treated with LAH to give corresponding tripeptide aldehyde. Further, the tripeptide aldehyde was converted to corresponding β-keto-ester after the treatment with ethyl diazoacetate in the presence of anhydrous tin (II) chloride in DCM. The pure tripeptide β-keto-ester obtained after the column purification, was subjected to aerial oxidation after the deprotection of the Boc-group as described earlier. The cyclization was monitored using TLC and complete conversion of β-keto-ester to corresponding pyrazine was observed after 24 hrs. The pyrazine coupled peptide was purified by reverse phase HPLC on C_{18} column using methanol/H_2O gradient system. The mass of the peptide was confirmed using MALDI-TOF/TOF. The HPLC profile of peptide P2 is shown in Figure 3.

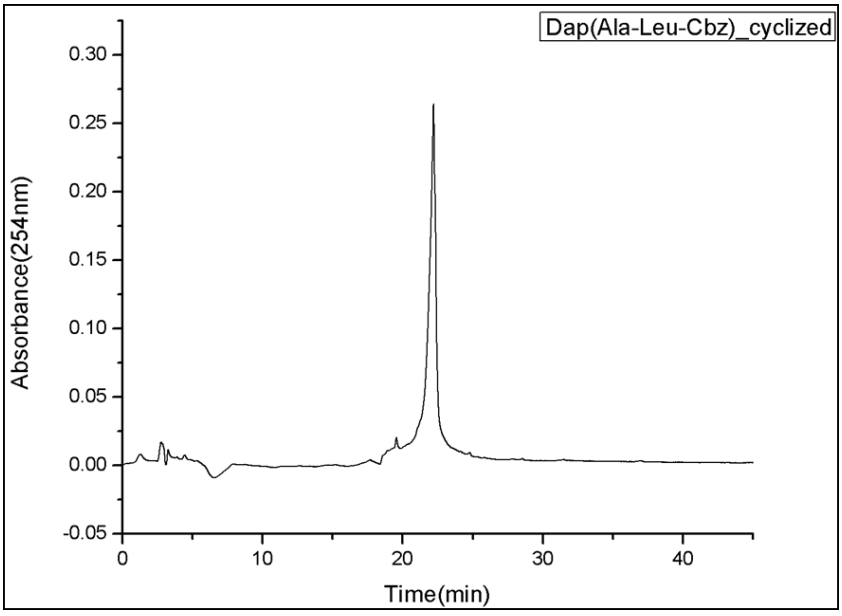


Figure 3: Reverse phase HPLC profile of peptide **P2**

Experimental Section

All amino acids, ethyl diazoacetate, LAH, DIPEA, tin(II) chloride and Cbz–Cl were purchased from Aldrich. THF, DCM and DMF were purchased from Merck. HBTU, HOBt, di-tert-butyl dicarbonate were obtained from spectrochem and used without further purification.THF and DiPEA were dried over sodium and distilled immediately prior to use. Column chromatography was performed on Merck silica gel (120–200 mesh). The ^{1}H spectra were recorded on *Jeol* 400 MHz (or 100 MHz for ^{13}C) spectrometers using residual solvent signals as an internal reference ($CDCl_3$ δ_H, 7.24 ppm, δ_C 77.0 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. High-resolution mass spectra were obtained from HRMS-ESI (Waters), MALDI TOF/TOF (Applied Biosciences).

General procedure for the synthesis of *N*-protected γ-amino β-keto-γ-amino-ester

N-Boc protected amino acid (10 mmol) was dissolved in 10 mL DCM. To this solution, *N*,*O*-dimethylhydroxyamine hydrochloride (13 mmol) was added. The reaction mixture was cooled to 0 ℃ and treated with DIPEA (20 mmol), HOBt (10 mmol) and DCC (10 mmol). The reaction mixture was stirred for another 10 h and progress of reaction was monitored by TLC. After the completion of reaction (indicated by TLC) the reaction mixture was diluted with ethyl acetate (60 mL) and DCU was filtered. The filtrate was washed with 10% HCl, 10% Na_2CO_3 and brine solution. The organic layer was dried over Na_2SO_4 and solvent was evaporated under reduced pressure. The crude *N*-Boc protected Weinreb amide obtained after evaporation was directly used for the next step without further purification.

The *N*-Boc protected Weinreb amide obtained from the above step was dissolved in dry THF (10 mL) under the nitrogen atmosphere. The solution was cooled to 0 ℃ and treated with $LiAlH_4$ (15 mmol). The reaction mixture was stirred for about 1 h. After the completion of reaction (monitored by TLC), excess $LiAlH_4$ was quenched by

adding 5% HCl (30 mL). The reaction mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give crude oily product which was used immediately for β-keto synthesis reaction.

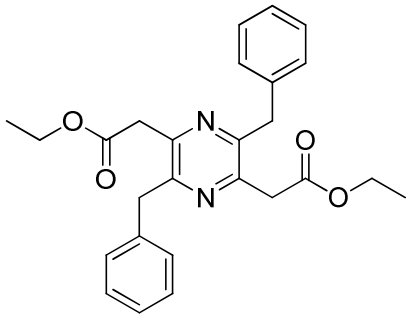
The *N*-protected amino aldehyde (2.0 mmol) obtained from the above step was dissolved in 10 mL of DCM at room temperature. Further, the amino aldehyde solution was treated with 0.07(20 mol%) of tin(II) chloride, followed by (2.1 mmol) of ethyl diazoacetate in toluene solution. Immediate gas evolution was observed. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by TLC. After completion of the reaction, it was quenched with 10 mL of 5% HCl and the reaction mixture was extracted with DCM (30 mL × 3). The combined organic layer was washed with 20 mL of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to get a greenish oily crude product which was further purified using column chromatography.

General procedure for synthesis of pyrazine from β-keto-γ-amino ester:

The *N*-Boc-protected β-keto-γ-amino ester (1 mmol) was dissolved in 2:1 mixture TFA and DCM at 0 ℃. The reaction mixture was stirred for about 1 hour and volatile organic solvents were evaporated under reduced pressure. The crude product was dissolved in THF and neutralized using DIPEA (2 mmol). The free amine solution was stirred for about 24 h. Completion of the reaction was monitored by TLC. After completion of the reaction, the crude product was dissolved in ethyl acetate and washed with 5% HCl and brine solution, dried over Na_2SO_4. The pure pyrazine was isolated in moderate to good yields after the column chromatography using ethyl acetate and petroleum ether solvent system.

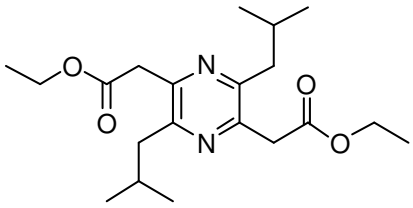
Spectroscopic data

Diethyl 2, 2'-(3,6-dibenzylpyrazine-2,5-diyl)-diacetate (2a)



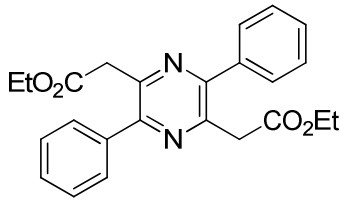
Yield = 65 % **UV** (λ_{max}) = 204 nm, 281 nm; **Fluoroscence** (λ_{ex}) = 281 nm, (λ_{em}) = 443 nm; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.27–7.14 (m, 10H), 4.18 (s, 4H), 4.07 (q, J = 7.1 Hz, 4H), 3.81 (s, 4H), 1.19 (t, J = 7.1 Hz, 6H).

Diethyl 2, 2'-(3, 6-diisobutylpyrazine-2, 5-diyl)-diacetate (2b)



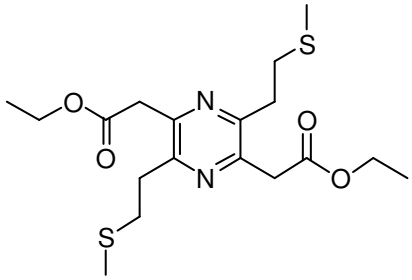
Yield = 56 % **UV** (λ_{max}) = 215 nm, 267 nm; **Fluoroscence (**λ_{ex}) = 267 nm, **(**λ_{em}) = 429nm; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.16 (*q*, J = 7.1 Hz, 4H), 3.85 (*s*, 4H), 2.61 (*d*, J = 7.3 Hz, 4H), 2.17 – 2.10 (*m*, 2H), 1.23 (*t*, J = 7.2 Hz, 6H), 0.92 (*d*, J = 6.7 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.2, 151.6,146.2, 61.0, 42.6, 40.8, 28.2, 22.4, 14.1; **HRMS** (ESI) m/z calculated for $C_{20}H_{32}N_2O_4$ $(M + H)^+$ = 365.2240 observed $(M + H)^+$ = 365.2240.

Diethyl 2, 2'-((3, 6-diphenylpyrazine) -2,5-diyl)-diacetate (2C)



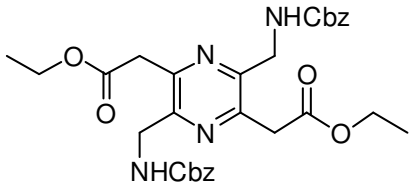
Yield = 58%; **UV** (λ_{max}) = 250 nm, 292 nm ; **Fluoroscence (**λ_{ex}) = 292 nm, **(**λ_{em}) = 371 nm ;**^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (*dd*, *J* = 7.8, 1.7 Hz, 4H), 7.52 – 7.42 (*m*, 6H), 4.13 (*q*, *J* = 7.1 Hz, 4H), 3.99 (*s*, 4H), 1.21 (*t*, *J* = 7.1 Hz, 6H) ; **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3, 151.0, 146.3, 61.2, 40.7, 33.7, 32.5, 15.8, 14.2 ; **HRMS (**ESI) m/z calculated for $C_{18}H_{28}N_2O_4S_2$ $(M + H)^+$ = 405.1814 observed $(M + H)^+$ = 405.1815.

Diethyl 2, 2'-(3,6-Bis(2-(methylthio)ethyl)pyrazine -2,5-diyl)-diacetate (2d)



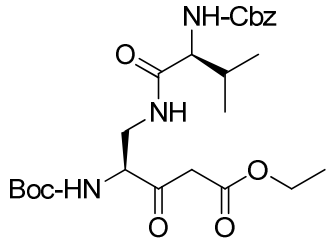
Yield = 62%; **UV** (λ_{max}) = 210 nm, 280 nm; **Fluoroscence (**λ_{ex}) = 280 nm, **(**λ_{em}) = 332 nm ;**^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.18 (*q*, *J* = 7.1 Hz, 1H), 3.89 (*s*, 1H), 3.07 – 3.00 (*m*, 1H), 2.93 – 2.86 (*m*, 1H), 2.12 (*s*, 2H), 1.27 (*t*, *J* = 7.2 Hz, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3, 151.0, 146.3, 61.2, 40.7, 33.7, 32.5, 15.8, 14.1; **HRMS** (ESI) m/z calculated for $C_{18}H_{28}N_2O_4S_2$ $(M + H)^+$ = 401.15689 observed $(M + H)^+$ = 401.1564.

Diethyl-2, 2'-(3, 6-bis ((((benzyloxy) carbonyl) amino) methyl) pyrazine-2, 5-diyl)-diacetate (2e)



Yield = 59%; **UV** (λ_{max}) = 216 nm, 276 nm ; **Fluoroscence** (λ_{ex}) = 267 nm, (λ_{em}) = 441 nm ; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 (*dd*, *J* = 12.8, 4.3 Hz, 10H), 6.03 (*s*, 4H), 5.13 (*s*, 4H), 4.52 (*s*, 4H), 4.17 (*q*, *J* = 7.0 Hz, 4H), 3.92 (*s*, 4H), 1.26 (*t*, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3, 156.2, 148.3, 145.6, 136.2,128.4, 128.1, 66.9, 61.5, 42.4, 39.8, 14.0; **HRMS (**ESI) m/z calculated for $C_{30}H_{34}N_4O_8$ $(M + H)^+$ = 579.2455 observed $(M + H)^+$ = 579.2455, $(M + Na)^+$=601.2274 observed $(M+ Na)^+$ = 601.2287.

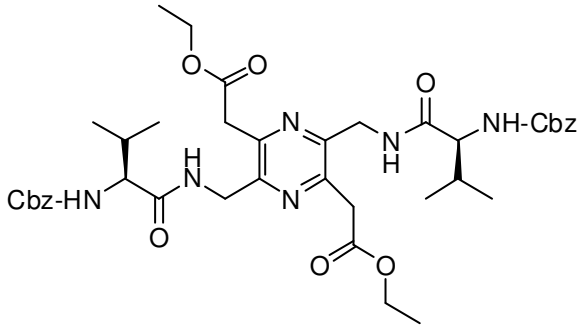
(*S*)-Ethyl 5-((*S*)-2-(((benzyloxy) carbonyl) amino)-3-methylbutanamido)-4-((tert-butoxycarbonyl) amino)-3-oxopentanoate (P1)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30(*s*, 5H), 7.00 (*s*, 1H), 5.95 (*s*, 1H), 5.68(*s*, 1H), 5.09 (*s*, 2H), 4.41 (*s*,1H), 4.16 (*q*, *J* = 8.0 Hz, 2H), 3.98(*s*, 1H), 3.67 (*m*, 2H), 3.18 (*s*, 1 H), 2.07 (*s*, 1H) ,1.41 (*s*, 9 H),1.24 (t, *J* = 8.0, 3H) , 0.88 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ

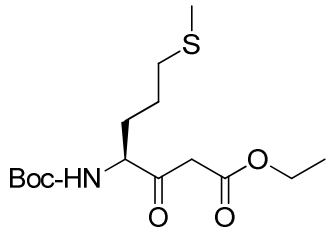
201.2,167.0, 156.9 ,155.5, 136.0 , 127.9, 127.8, 80.2, 66.8, 61.3, 60.1, 45.9, 41.3, 29.9, 28.0,13.8.

Diethyl-2, 2'-(3,6-Bis((((benzyloxy)amino)-3-methylbutaamido)methyl)pyrazine-2,5-diyl)diacetate (C1)



Yield = 37%; **UV** (λ_{max}) = 208 nm, 278 nm; **Fluoroscence** (λ_{ex}) = 276 nm, (λ_{em}) = 314 nm ;**^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 (*s*, 10H), 7.18 (*s*, 2H), 5.44 (*s*, 2H), 5.10 (*d*, *J* = 3.6 Hz, 4H), 4.57 (*s*, 4H), 4.24 – 4.15 (*q*, *J* = 7.1 Hz, 4H), 4.10 (*d*, *J* = 8.9 Hz, 2H), 3.91 (*s*, 4H), 2.14 (*d*, *J* = 19.4 Hz, 2H), 1.27 (*t*, *J* = 7.1 Hz, 6H), 0.93 (*dd*, *J* = 16.1, 6.8 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.2, 169.3, 156.3, 148.1, 145.1, 136.1, 128.5, 128.1, 128.0, 67.0, 61.6, 60.3, 40.9, 39.8, 31.2, 19.1, 17.72, 14.4; **HRMS (**ESI) m/z calculated for $C_{14}H_{52}N_6O_{10}$ $(M + H)^+$ = 777.3823 observed $(M + H)^+$ = 777.3875, $(M + Na)^+$ = 799.3643 observed $(M + Na)^+$ = 799.3701.

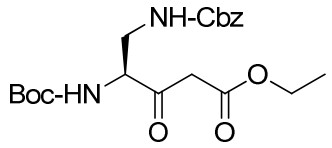
(*S*)-Ethyl 4-((tert-butoxycarbonyl) amino)-7-(methylthio)-3-oxoheptanoate (1d)



^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (*s*, 1H), 4.42 (*s*, 1H), 4.14 (*q*, *J* = 7.1 Hz, 3H), 3.54 (*d*, *J* = 4.6 Hz, 2H), 2.49 (*t*, *J* = 7.3 Hz, 3H), 2.15 (*s*, 1H), 2.04 (*s*, 3H), 1.80 (*dd*, *J* = 14.8,

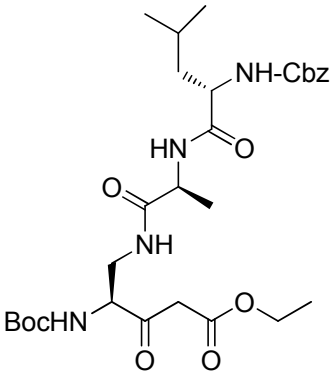
7.6 Hz, 1H), 1.39 (*s*, 9H), 1.23 (*t*, *J* = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* 201.8,166.8, 155.3, 80.1, 61.4 , 58.6, 46.1, 30.0, 29.9, 28.1, 15.3, 13.9.

(*S*)-Ethyl 5-(((benzyloxy) carbonyl)amino)-4-((tert-butoxycarbonyl)amino)-3-oxopentanoate (1e)



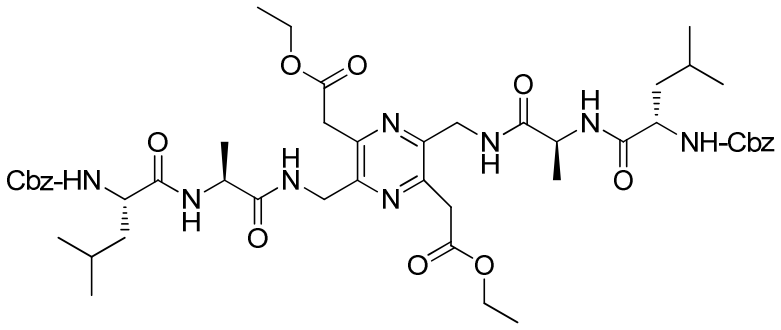
^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.30 (*s*, 5H), 5.54 (*s*, 1H), 5.05 (*s*, 2H), 4.41 (*s*,1H), 4.14 (*q*, *J* = 8.0 Hz, 2H), 3.68 (*d*, *J* = 8 Hz, 2H), 3.58 (*s*, 2H), 3.53 (*d*, *J* = 8 Hz, 2H),1.40 (*s*, 9H),1.23 (*t*, *J* = 4, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* 201.4,172.6, 167.2 , 156.4,155.7, 136.0, 128.4, 128.0, 127.8, 80.2 , 66.9, 62.0, 61.0, 60.4, 45.9, 39.7, 29.5, 28.1,19.1, 13.9**.**

(5S, 8S, 12S)-Ethyl-12-((tert-butoxycarbonyl) amino)-5-isobutyl-8-methyl-3, 6,9,13-tetraoxo-1-phenyl-2-oxa-4,7,10-triazapentadecan-15-oate (P2)



1H NMR (400 MHz, $CDCl_3$) δ 7.29 (*s*, 5H), 6.05(s, 2H), 5.09 (*s*, 2H), 4.43 (*s*,2H), 4.22-4.12 (*m*, 3H),3.65-3.43(*m*, 2H), 1.64-1.20 (*m*,16 H),0.88 (*s*, 6 H); **13C NMR** (100 MHz, CDCl3) δ 201.8, 173.4, 172.9, 167.3, 156.2, 136.4, 128.5, 128.1, 127.9, 80.4, 67.1, 61.4, 60.3, 53.8, 49.0, 46.1, 41.4, 39.9, 29.6, 28.1, 24.6, 23.0, 21.7, 14.0.

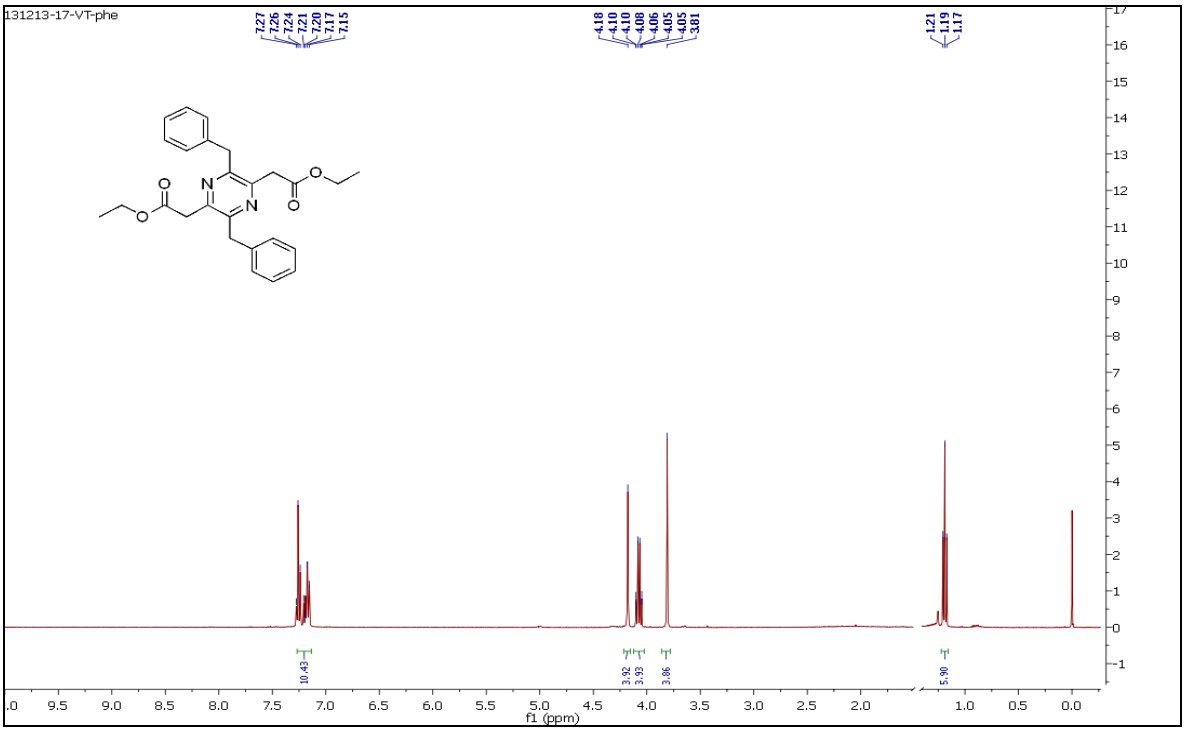
Diethyl 2, 2'-(3,6-bis((5S,8S)-5-isobutyl-8-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazaundecan-11-yl)pyrazine-2,5-diyl)diacetate (C2)



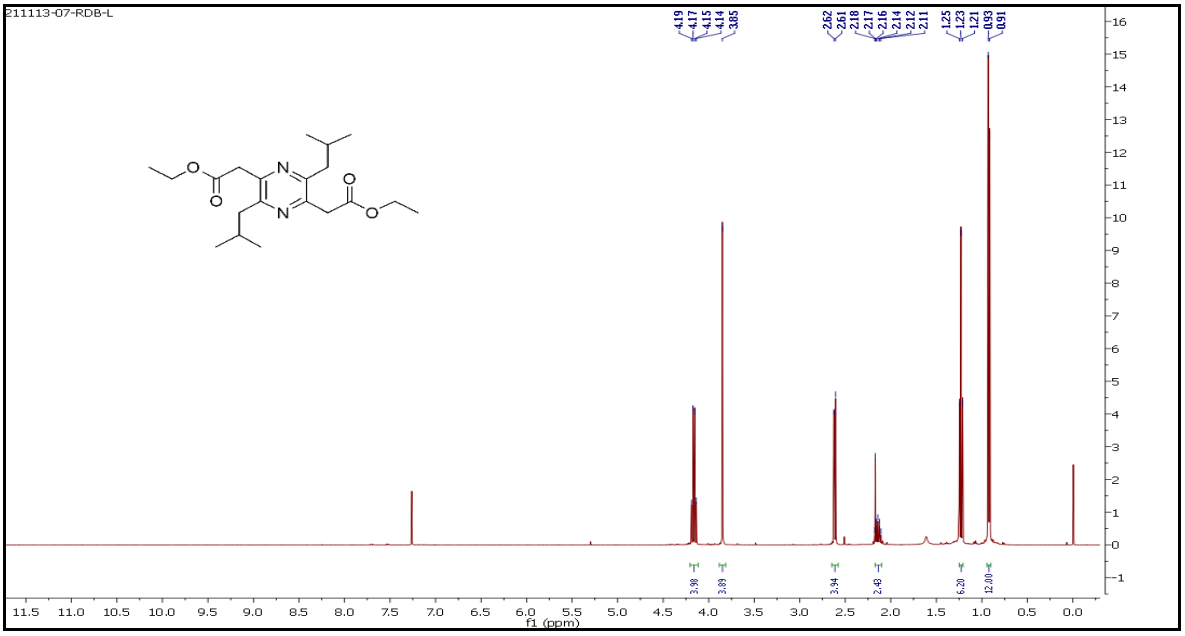
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.22(*m*, 14H), 6.06(*s*, 2H), 5.25-5.01 (*m*, 6H), 4.55 (*s*, 4H), 4.21-4.16 (*m*, 3H),3.93 (s, 4H), 1.64-1.20 (*m*,16H),1.67 (*s*, 8H), 1.39 (*s*, 4H), 1.39 (*s*, 4H), 1.28(*t*, 6H), 0.93 (*dd*, 12H); **MALDI-TOF/TOF** m/z calculated for $C_{48}H_{66}N_8O_{12}$ (M + Na)$^+$ = 969.4689 observed (M + Na)$^+$ = 969.6588.

Copies of ^{1}H and ^{13}C spectra

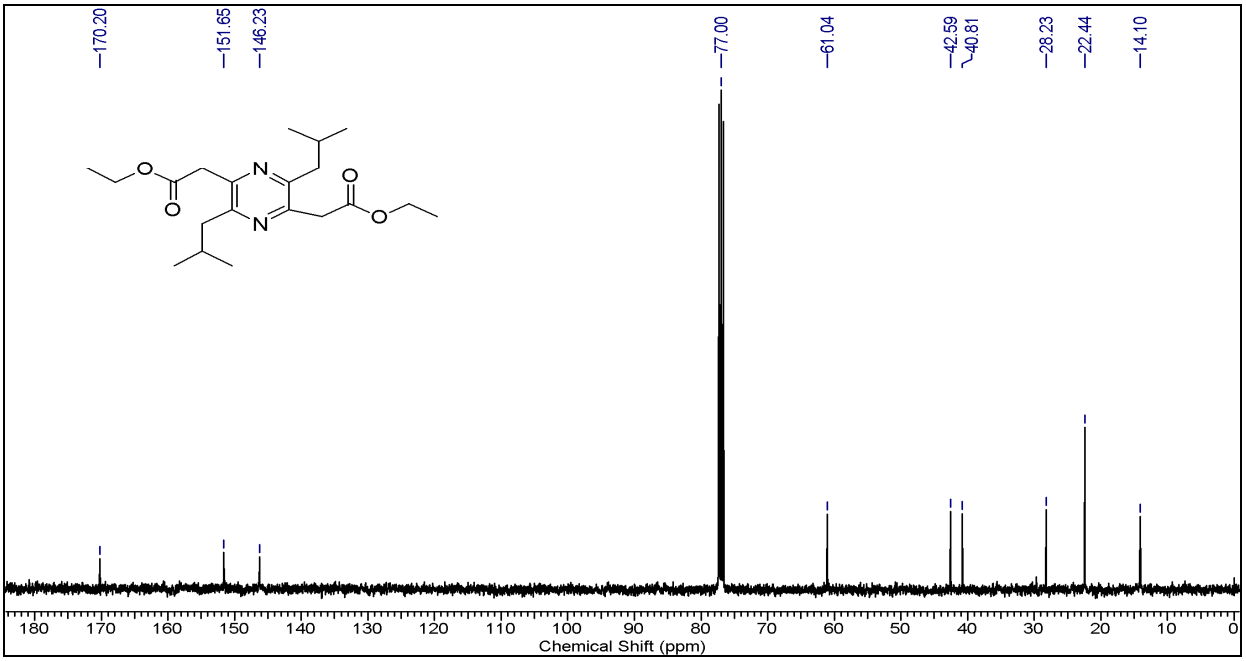
^{1}H NMR for compound 2a



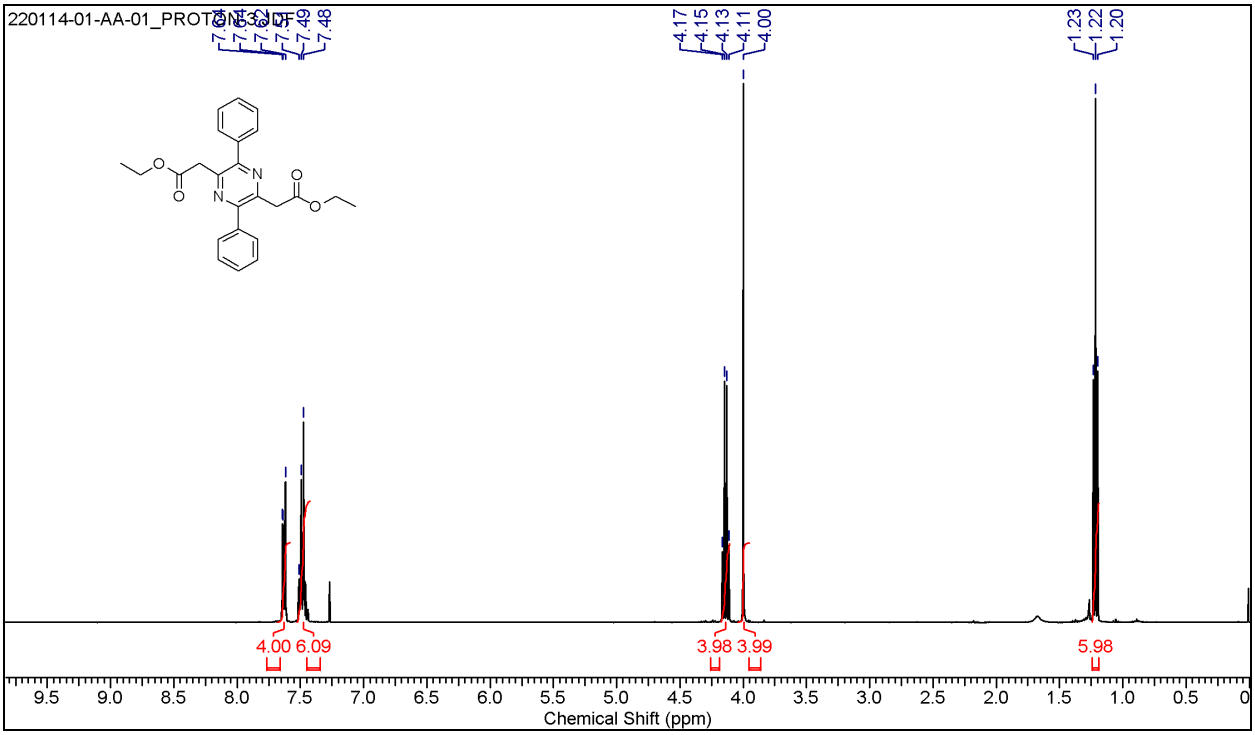
^{1}H NMR for compound 2b



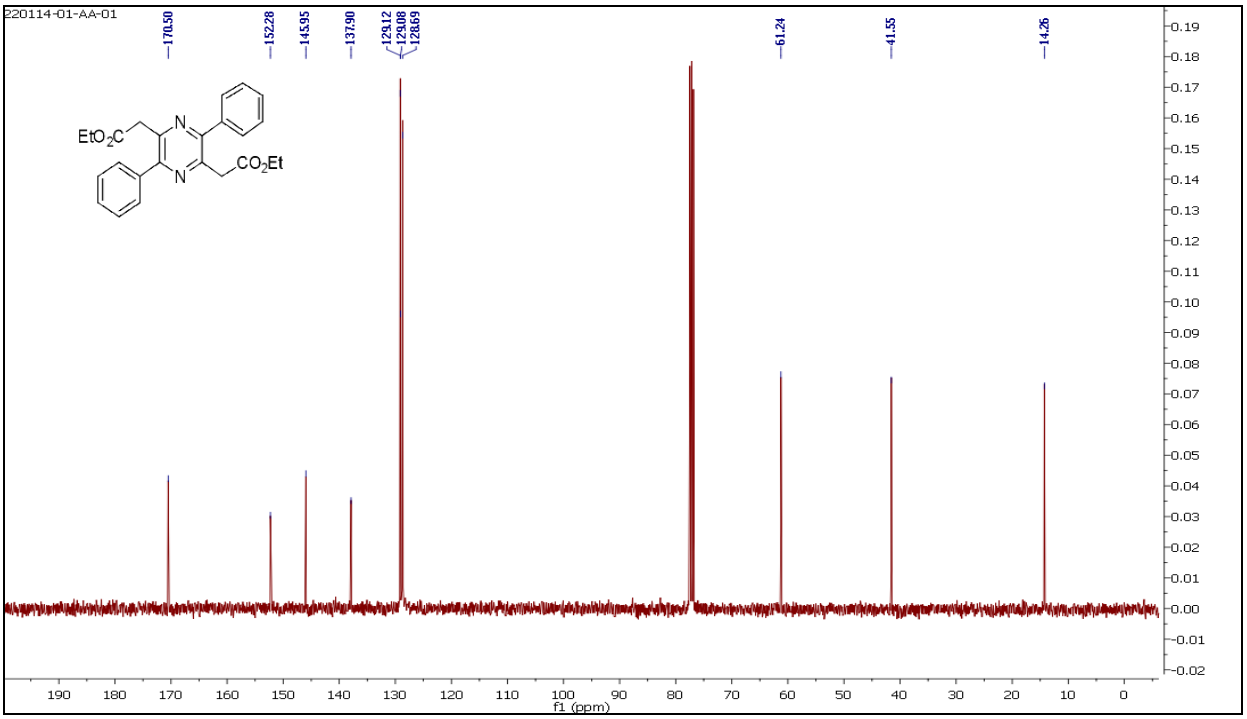
^{13}C NMR for compound 2b



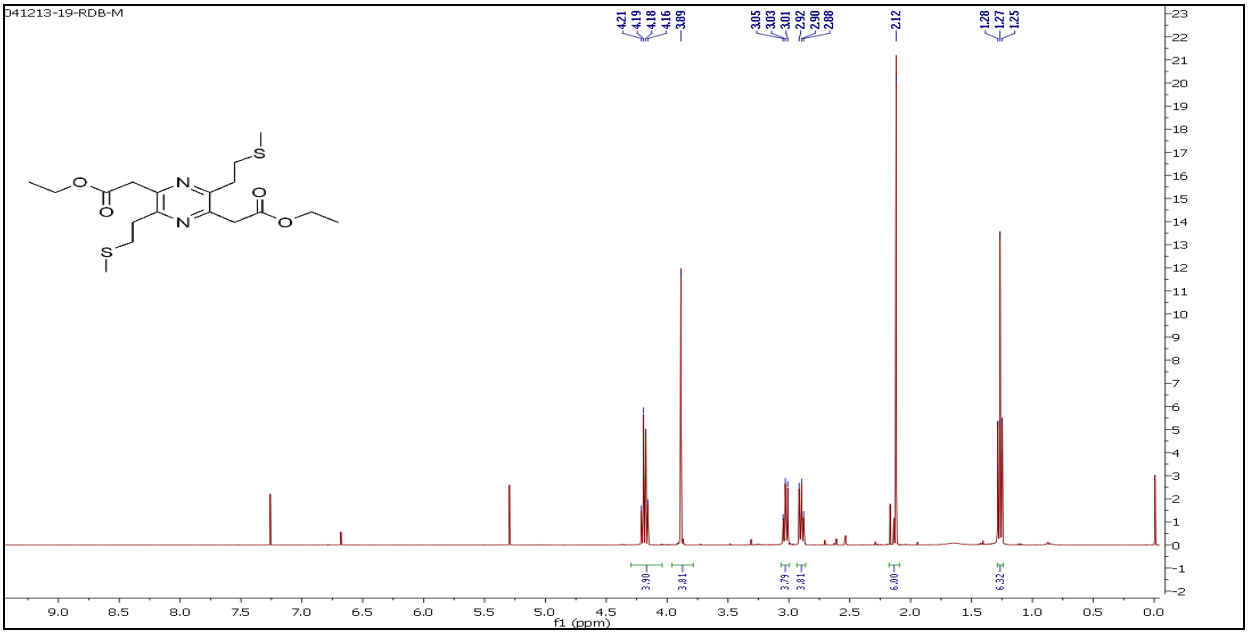
^{1}H NMR for compound 2c



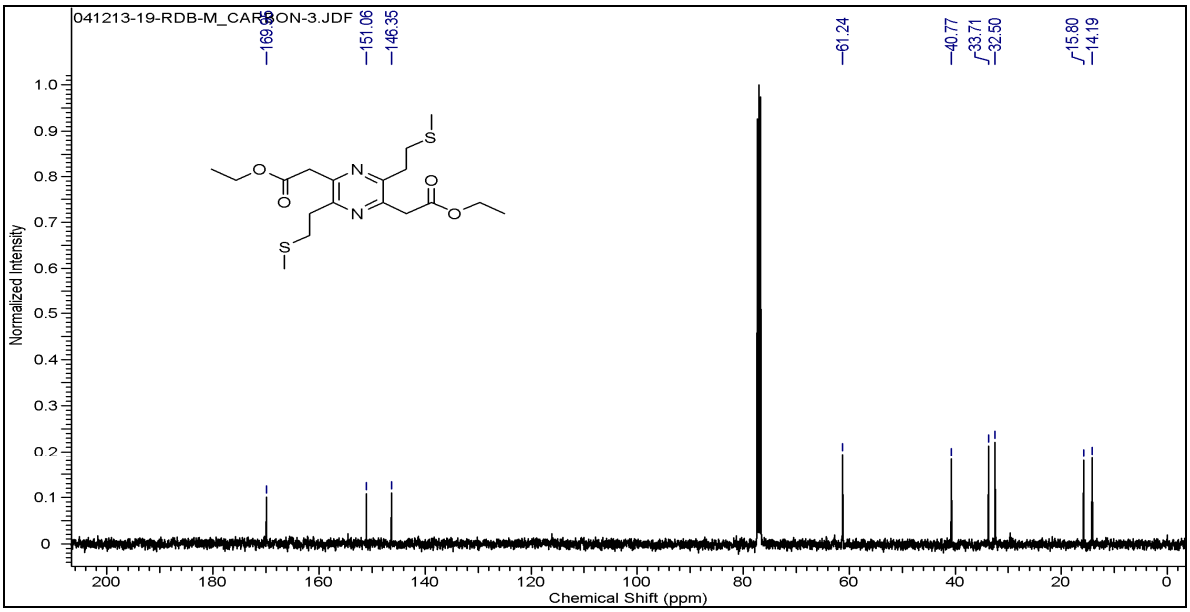
^{13}C NMR for compound 2c



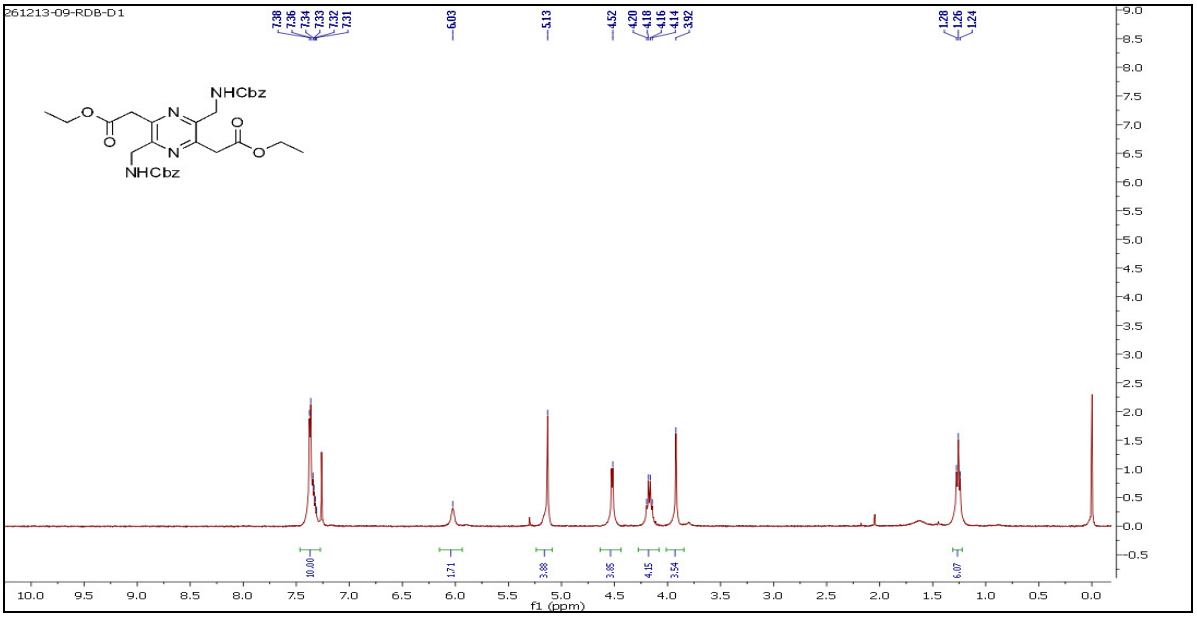
^{1}H NMR for compound 2d



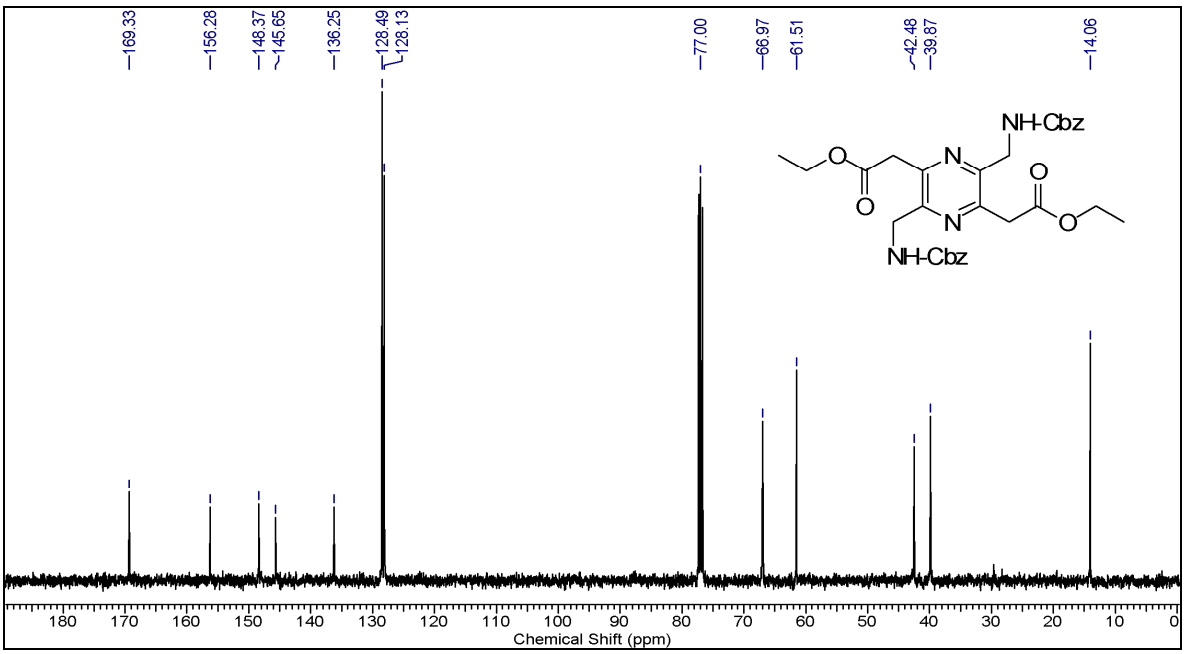
^{13}C NMR for compound 2d



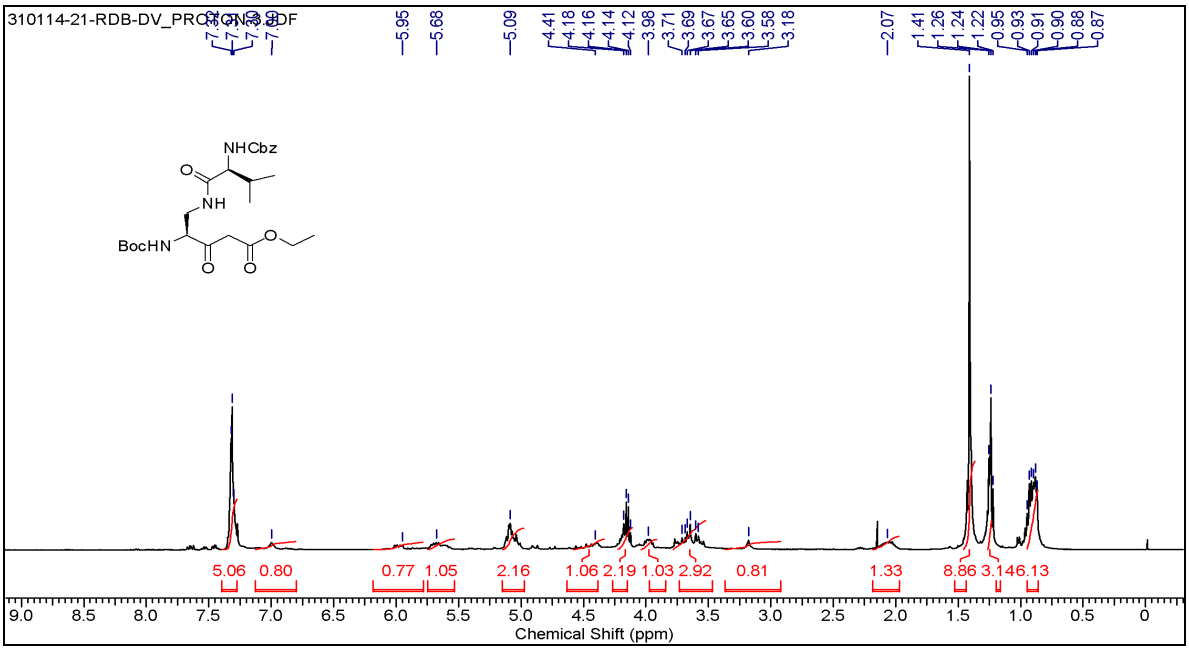
^{1}H NMR for compound 2e



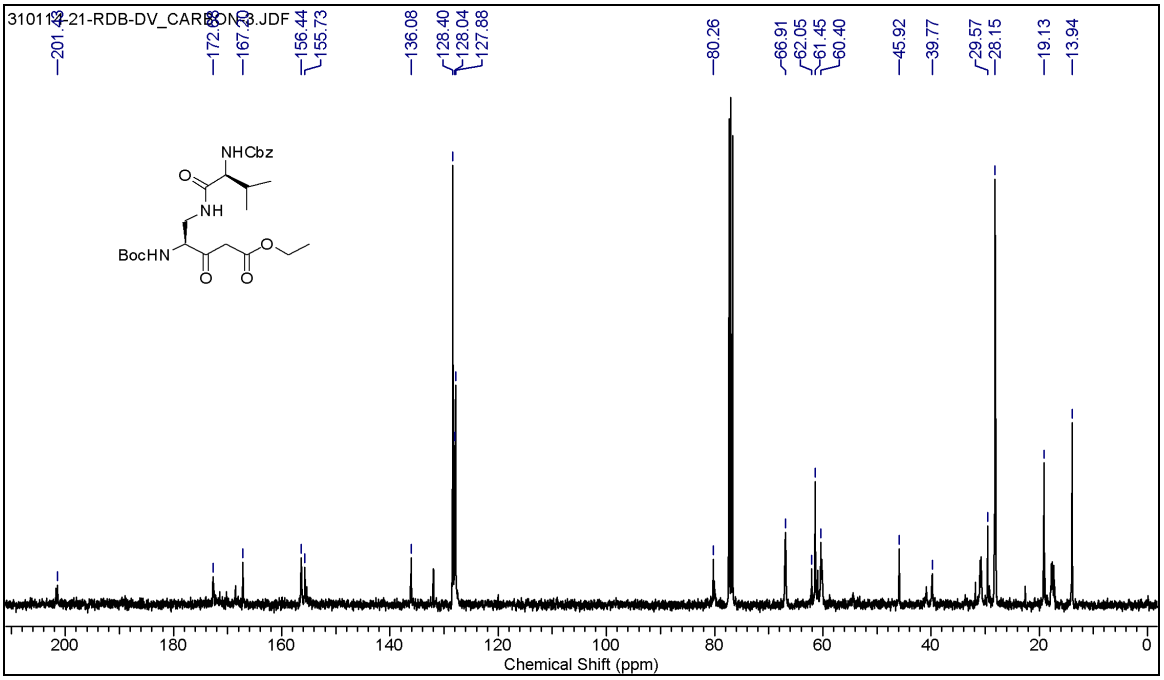
^{13}C NMR for compound 2e



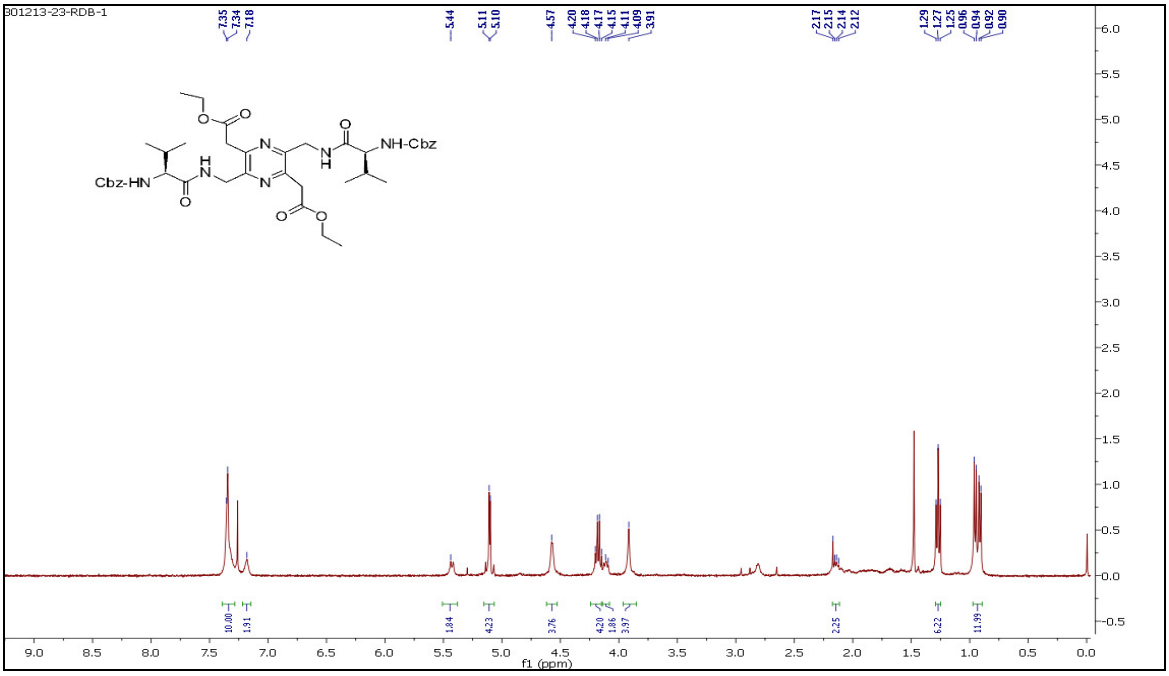
^{1}H NMR for compound P1



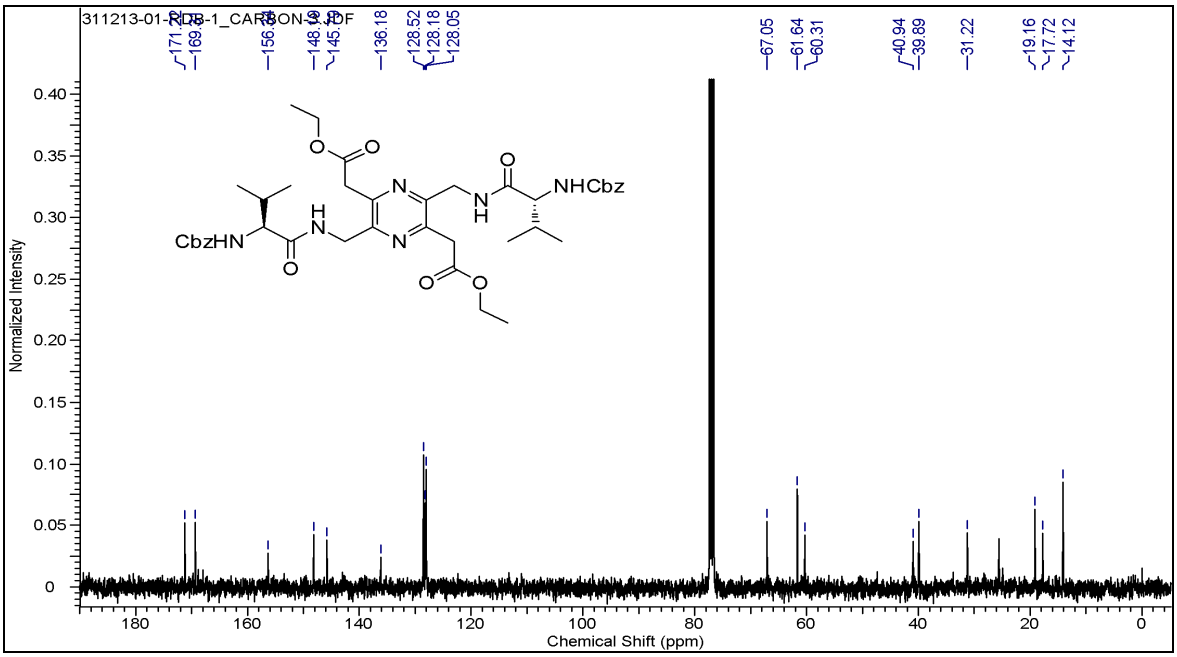
^{13}C NMR for compound P1



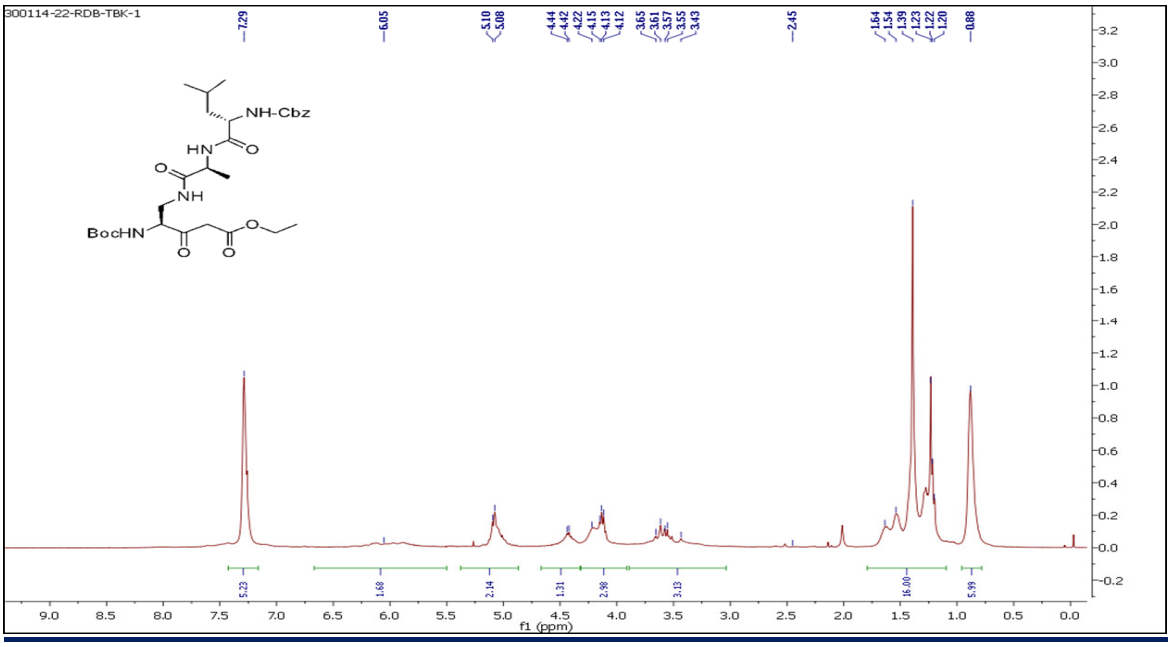
^{1}H NMR for compound C1



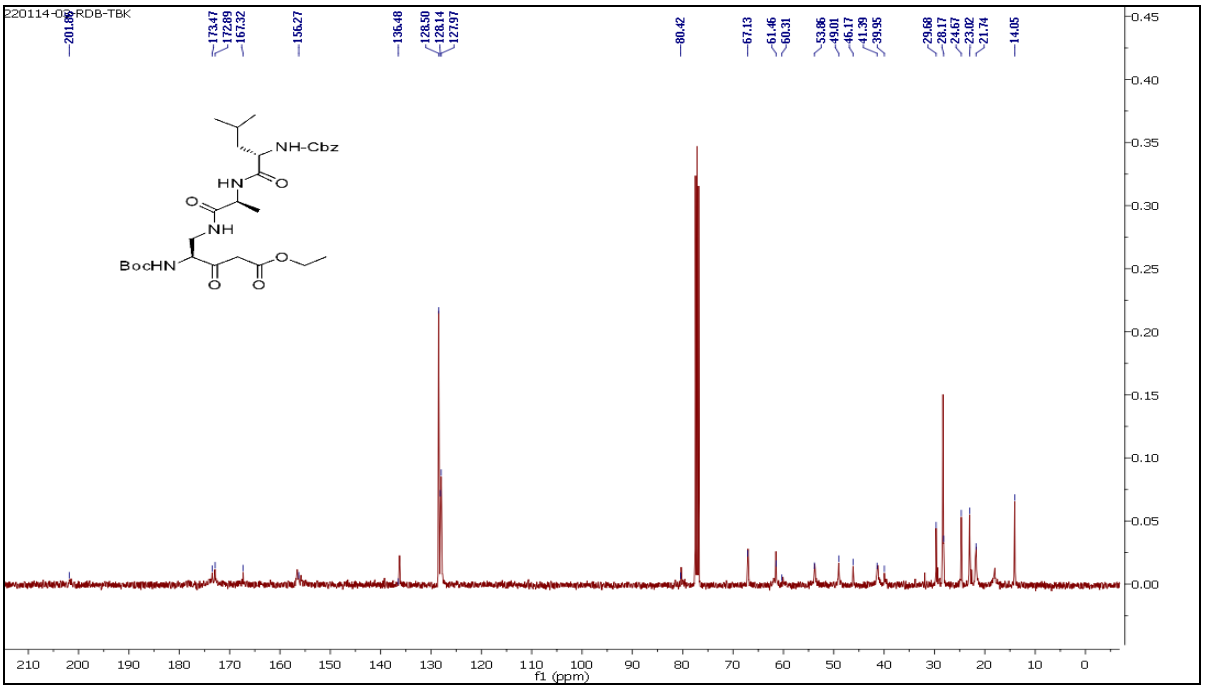
^{13}C NMR for compound C1



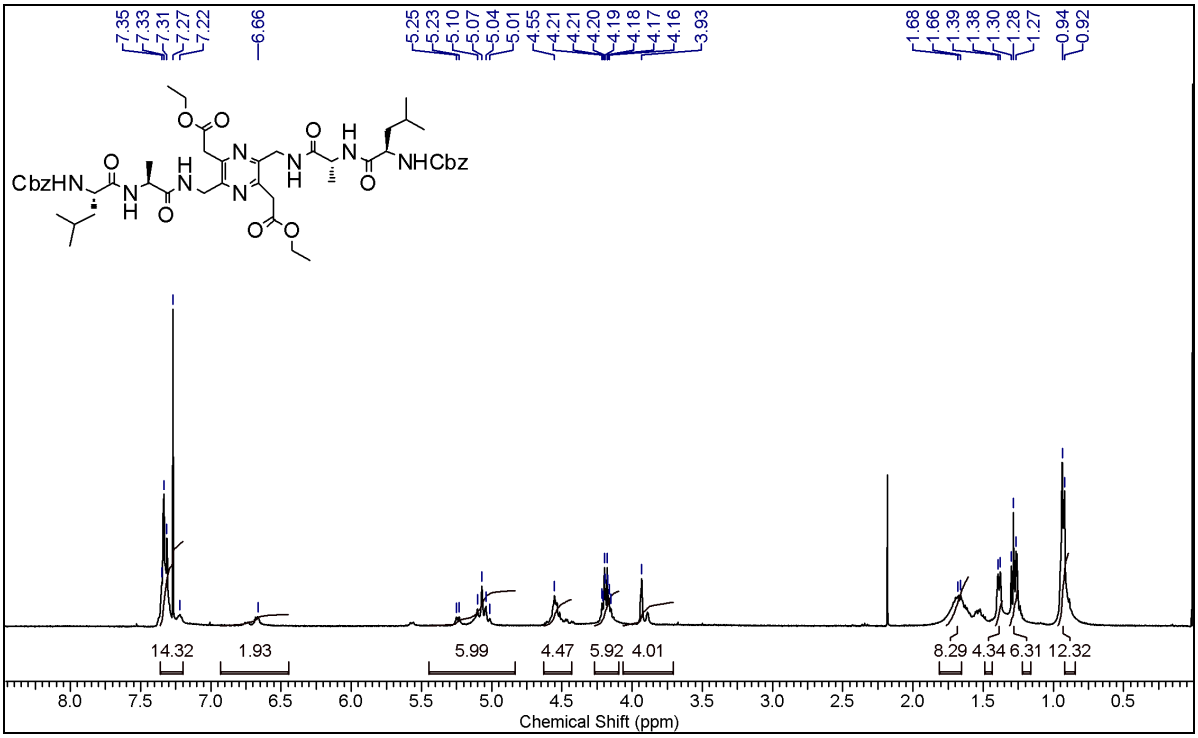
^{1}H NMR for compound P2



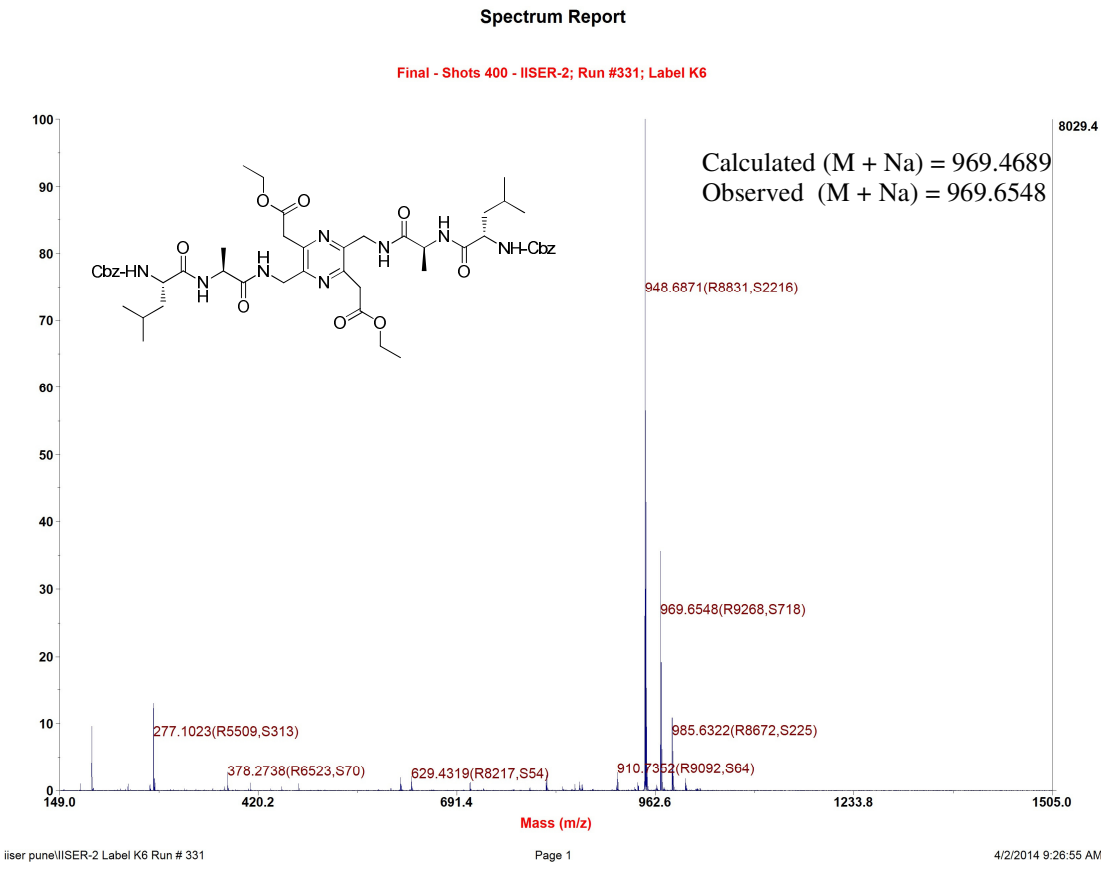
^{13}C NMR for compound P2



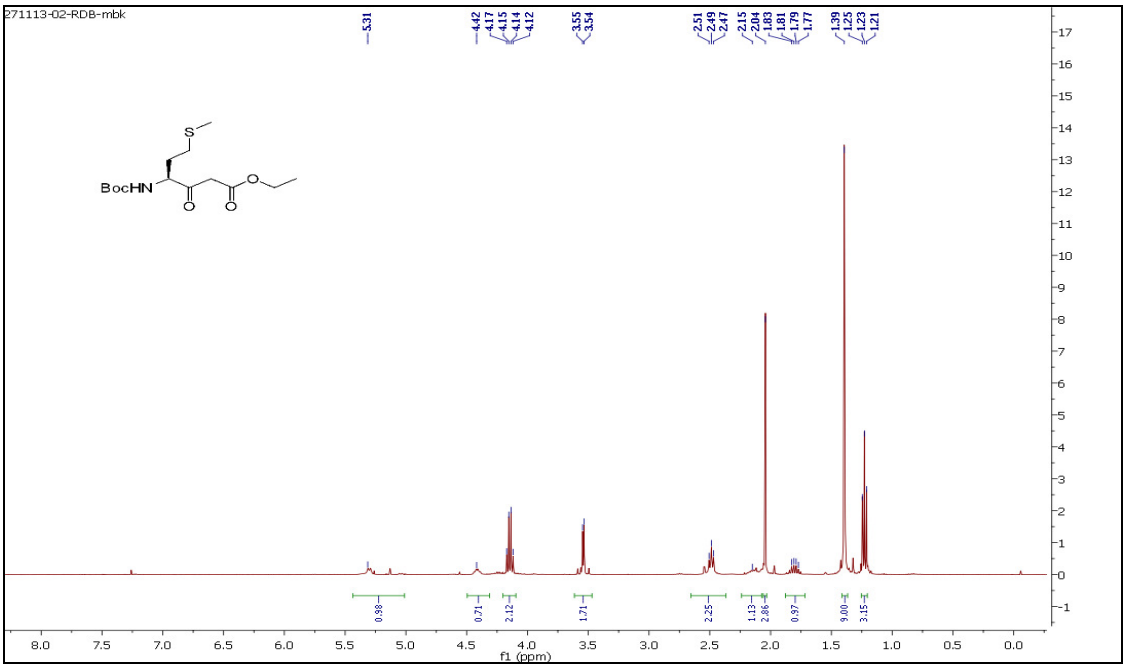
^{1}H NMR for compound C2



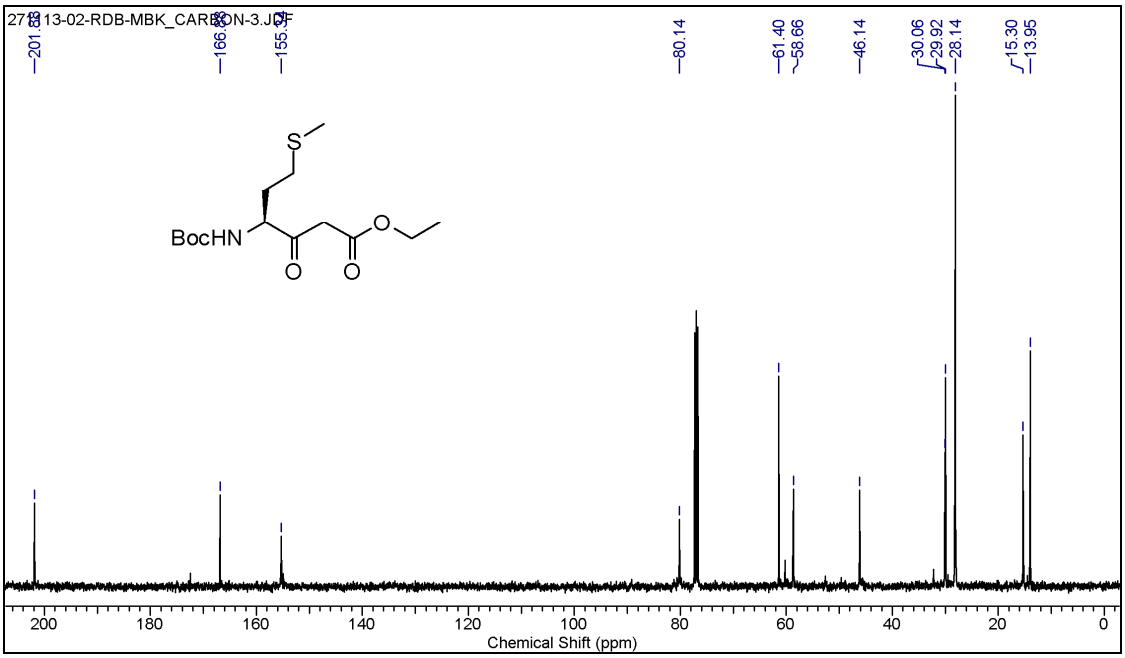
MALDI TOF/TOF mass spectra for compound C2



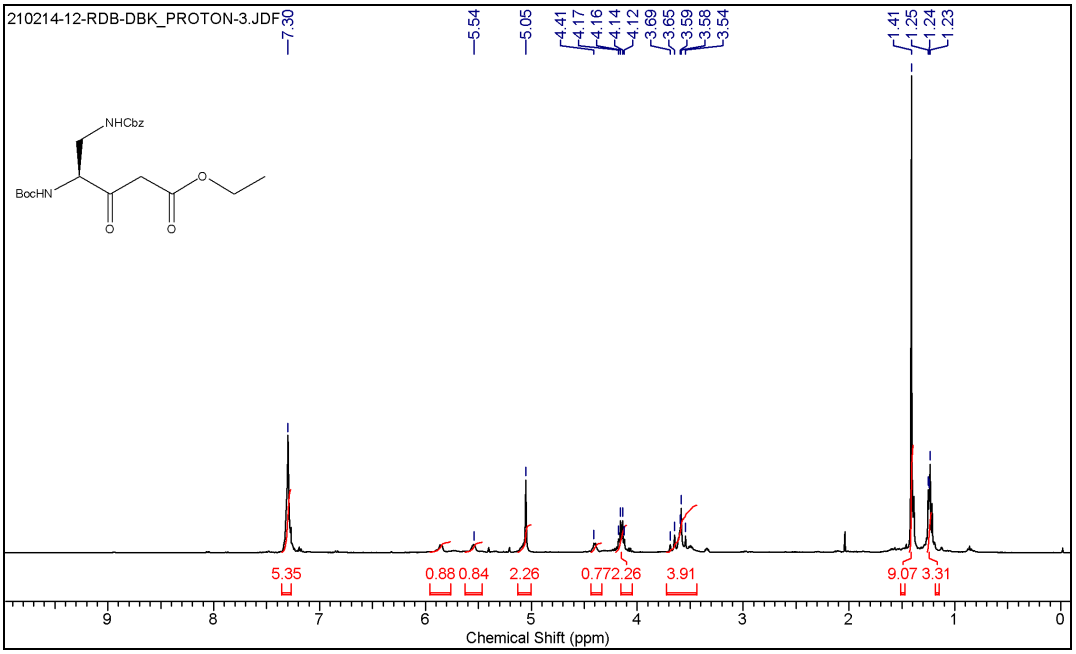
^{1}H NMR for compound 1d



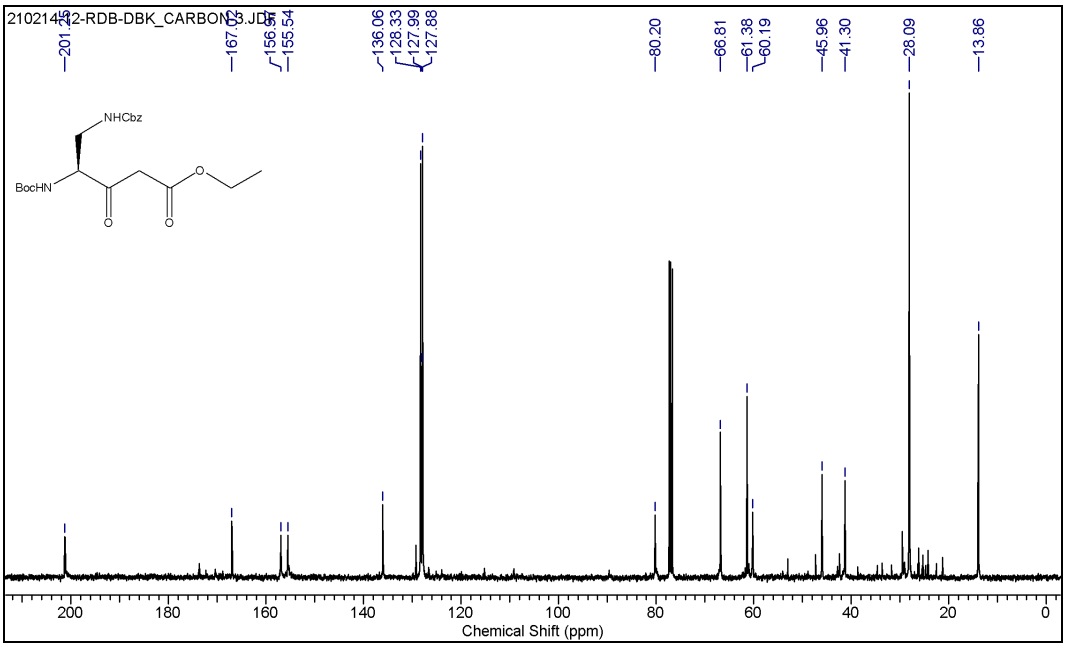
^{13}C NMR for compound 1d



^{1}H NMR for compound 1e



^{13}C NMR for compound 1e



Conclusion:

Overall, we have demonstrated the mild and biocompatible conversion of the ethyl ester of β-keto-γ-amino acids into highly symmetrical tetrasubstituted pyrazines. All these tetrasubstituted pyrazines were isolated in moderate to good yields. In addition to the β-keto-amino acids, we also demonstrated the synthesis of β-keto-esters of peptides and subsequent transformation of peptide β-keto-esters into highly symmetrical tetrasubtituted pyrazines. We are currently extending this novel head-to-head stapling strategy to larger peptides. The mild and biocompatible aromatization of β-keto-esters of amino acids and peptide may find applications in the design of inhibitors for protein-protein interactions as well as in design of biomaterials.

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