Site Selective Modification of Peptides and Proteins

A Thesis

submitted in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

Manish Kumar

(Reg. No. 20161012)

Indian Institute of Science Education and Research Pune

Dr. Homi Bhabha Road,

Pashan, Pune 411008, INDIA.

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Supervised by Prof. Hosahudya N. Gopi

Certificate

This is to certify that this dissertation entitled "**Site Selective Modification of Peptides and Proteins**" goes towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Manish Kumar** at Indian Institute of Science Education and Research (IISER), Pune under the supervision of Prof. Hosahudya N. Gopi, Professor, Department of Chemistry, during the academic year of 2020-2021.

31/07/2021

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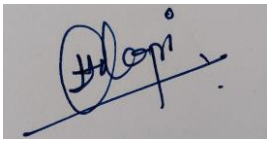


Prof. Hosahudya N. Gopi

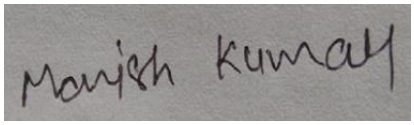
Professor,

Department of Chemistry

IISER Pune

Declaration

I hereby declare that the matter embodied in the report entitled "Site Selective Modification of Peptides and Proteins" are the results of the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research (IISER), Pune, under the supervision of Prof. Hosahudya N. Gopi and the same has not been submitted elsewhere for any other degree.



Manish Kumar

Date: 14/07/2021

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CONTENTS

Abbreviations:

PTM = Post translational modification

Boc = *tert*-Butoxycarbonyl

$(Boc)_2O$ = Di-*tert*-butyl-dicarbonate (Boc anhydride)

THF= Tetrahydrofuran

Leu = Leucine

DCM = Dichloromethane

DIPEA = Diisoproylethyl amine

DMF = N,N'-Dimethylformamide

Tyr = Tyrosine

EtOAc = Ethyl Acetate

EtOH = Ethanol

HOBt = 1-Hydroxybenzotriazole

HBTU=(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium

HPF_6 = Hexafluorophosphoric acid

Phe = Phenylalanine

Cys = Cysteine

TFA = Trifluoroacetic acid

TLC = Thin layer chromatography

HRMS = High resolution mass spectrometry

NMR = Nuclear magnetic resonance

MALDI = Matrix assisted laser desorption ionisation

ACN= Acetonitrile

Abstract

The diazo-coupling reactions of arenediazonium salts with Tyrosine containing peptides have shown a good selectivity to the Tyrosine residues over other competitive amino acids. In addition to that arenediazonium salts are highly reactive, which allows diazo-coupling reaction to happen even at very mild conditions. So, to develop a spectrophotometric detection of the Tyrosine containing peptides, we have synthesized a series of azo-dyes with Tyrosine and calculated their molar absorptivity coefficients. We observed Tyrosine and other aromatic amino acids containing peptides shows higher selectivity of diazo-coupling reaction on Tyrosine residue giving yield of 70-80%. We also studied the relative reactivity of different arenediazonium salts with Tyrosine by monitoring the reaction kinetics by using a *UV-Vis* spectrophotometer.

Introduction

Enzymatic modifications of protein happen after its biosynthesis, which is also called post-translational modifications (PTMs). These PTMs helps to make synthesized peptides and proteins into an active or mature form from their inactive form. There are several types of PTMs such as phosphorylations, glycosylations, lipidation, ubiquitination, formation of disulfide bridges from two cysteine groups etc[1]. Amino acid side chains are one of the most abundant targets for Post-translational modifications such as phosphorylation in serine, threonine, and tyrosine; phosphoester bond formation in histidine, lysine and arginine; phosphoramidate bond formation in aspartic acid and glutamic acid through an anhydride linkage. Systematic side chain modification of natural peptides and proteins provides valuable information on the structure and function of these important biologically active molecules. Selective modification and conjugation of individual amino acid without affecting any other amino acids in the

polypeptide sequence is an excellent way to design diagnostic tools and chemical probes. Diazonium salts of aromatic amines have emerged as a good class of arylating agents for side-chain modifications of especially Cysteine and Tyrosine. In 2016, Shi and co-workers have reported an attractive *S*-arylation of cysteine side chain using Au(I) complex catalysed reaction producing high yields (70-80%) in mild conditions.[2] Further in 2017 for *S*-arylation of cysteine side chain Noel and co-workers have used Ru complex as a photoredox catalyst.[3] They also have shown selective arylation by performing an arylating reaction on two dipeptides Boc-Leu-Cys-OMe and Boc-Phe-Cys-OMe and a pentapeptide containing polar residues.

Tyrosine is one of the non-essential aromatic amino acid and frequently found in proteins. Being exposed to the surface of proteins, tyrosines are an attractive target for conjugation and modification through its side chain.[4] Several methods for selective modification and conjugation of tyrosine have been developed which includes O-allylation catalysed by palladium, ene type reaction, Mannich reaction and diazo-coupling reactions using aryldiazonium salts. Since tyrosine has an electron-rich aromatic ring hence diazo-coupling reactions happen very fast and give coloured compounds which also extends its applications to selective labelling of peptides and proteins. Diazo-coupling reactions of tyrosine have been used for the study of the synthesis, structure elucidation and chemoselectivity of this bio-conjugation reaction. It was first reported by Pauly in 1915, but chemoselectivity of the reaction was first demonstrated by Francis and co-workers in 2004.[5] They have selectively modified the MS-2 interior capsid of bacteriophage with excellent yields of 95% in just 15 minutes. However, the reaction was only fast when they used the para nitro group in the benzene ring of diazonium salts compared to other diazonium salts. Further, they also have reported the bioconjugation of tobacco mosaic virus capsid with 90% yields where all of the surface exposed tyrosine was conjugated.[6] Despite, quick reactivity of aryl diazonium salts with tyrosine under basic conditions it was not

sufficient for pH sensitive peptides. In 2012, Barbas and co-workers have developed a hexafluorophosphate reagent for the isolation of diazonium salts which can be used any time as compared to the traditional way of immediately using diazonium salts after preparation.[7] They have used these diazonium salts for antibody labelling, cell surface labelling of tyrosine containing peptides. They also have introduced para formyl group on the benzene ring of diazonium salt as shown in **Fig. 1** for future functionalisation.

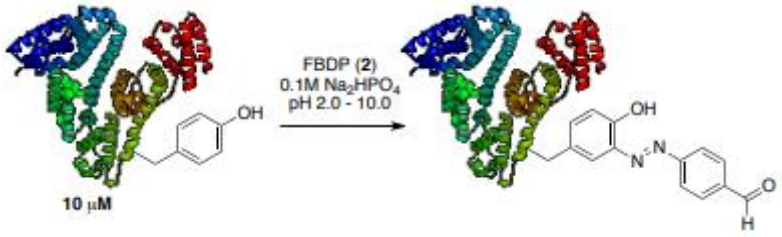
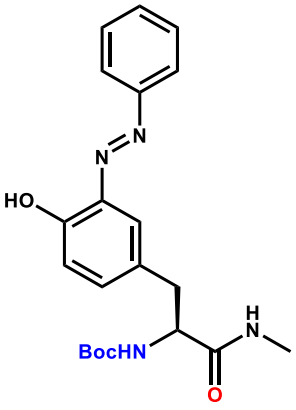


Fig. 1 Conjugation and functionalisation of tyrosine

Literature has shown that arenediazonium salts are very selective and reacts quickly to the Tyrosine residues of the peptide. So, to develop a spectrophotometric detection technique of the Tyrosine containing peptides, we have synthesized a series of azo-dyes **P1** to **P11** with different diazonium salts and calculated their molar absorptivity coefficients. To check the selectivity of the reaction toward tyrosine residue we synthesized a tripeptide containing aromatic amino acids phenylalanine, tyrosine and tryptophan and treated it with 1 equivalent of the diazonium salt. To check whether the reaction causing some racemization of the tyrosine residue we synthesized a dipeptide Boc-Tyr-Leu-OMe coupled with diazonium salt of toluidine and analysed by ^{1}H NMR and HPLC trace. Azo coupling reactions with tyrosine can also be done at physiological pH so, we have checked the relative reactivity of the different diazonium salts at pH 7.4.



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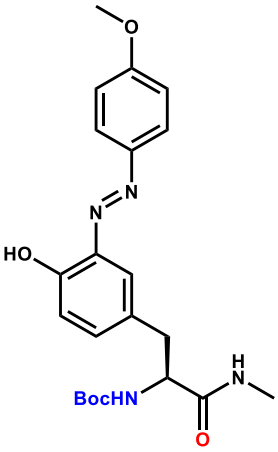

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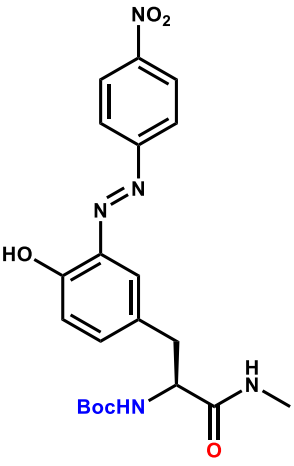

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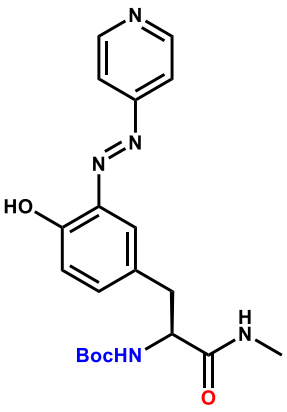

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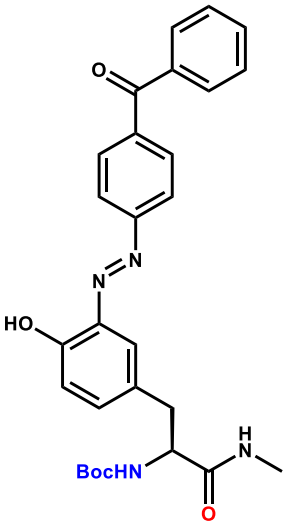

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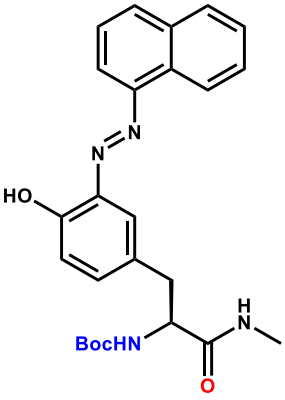

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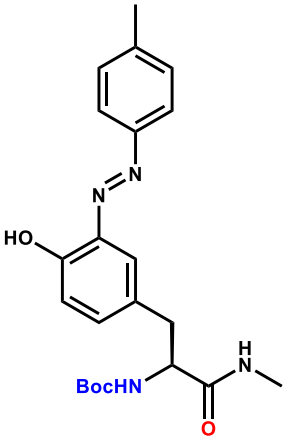

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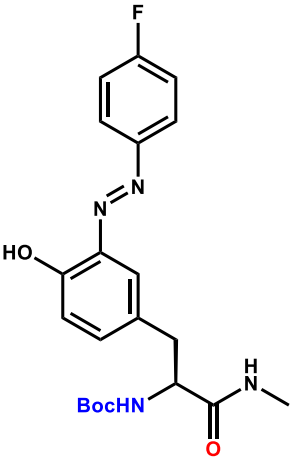

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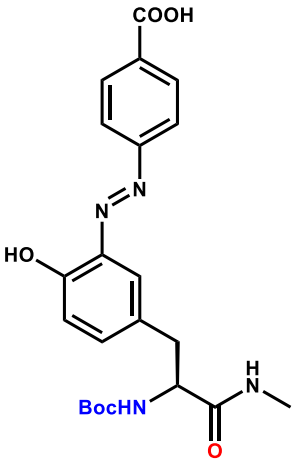

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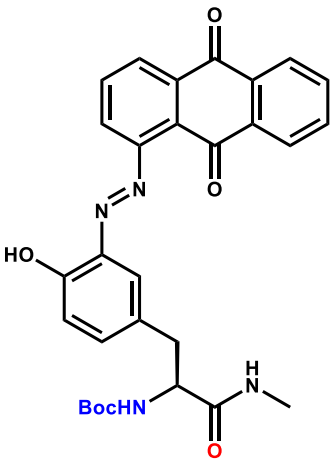

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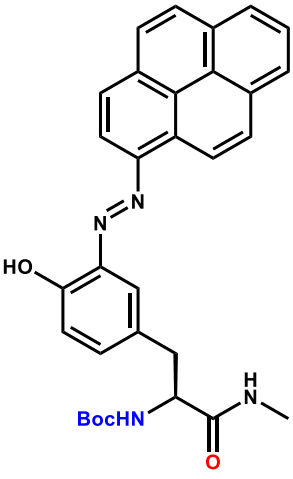

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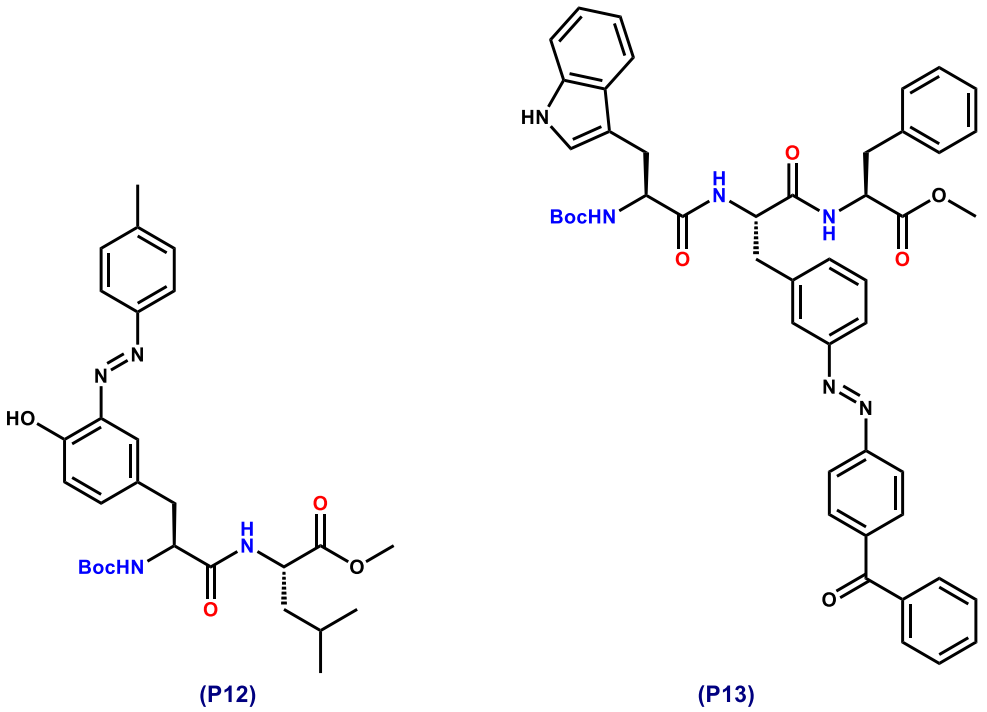


Fig. 2 Molecules/Peptide synthesized for study.

Experimental Section

Synthesis of Boc-Tyr-OH

Briefly, Tyrosine (9.05g, 50 mmol) was converted to Boc-Tyr-OH by treating it with 1 equiv. of di-tert butyl pyrocarbonate in THF and 10% Na_2CO_3 for 12 hr at RT. TLC method was used to monitor the progress of the reaction. After reaction completion, THF was evaporated in vacuum. The reaction mixture was acidified using 10% HCl. Using EtOAc (3×60 mL), the product was extracted. It was finally washed with brine solution (60 mL) and anhydrous Na_2SO_4 was used to dry the remaining water. The white colour compound was collected after concentrating the solution in a vacuum. Reaction yield was 95%

Synthesis of Boc-Tyr-NH-Me

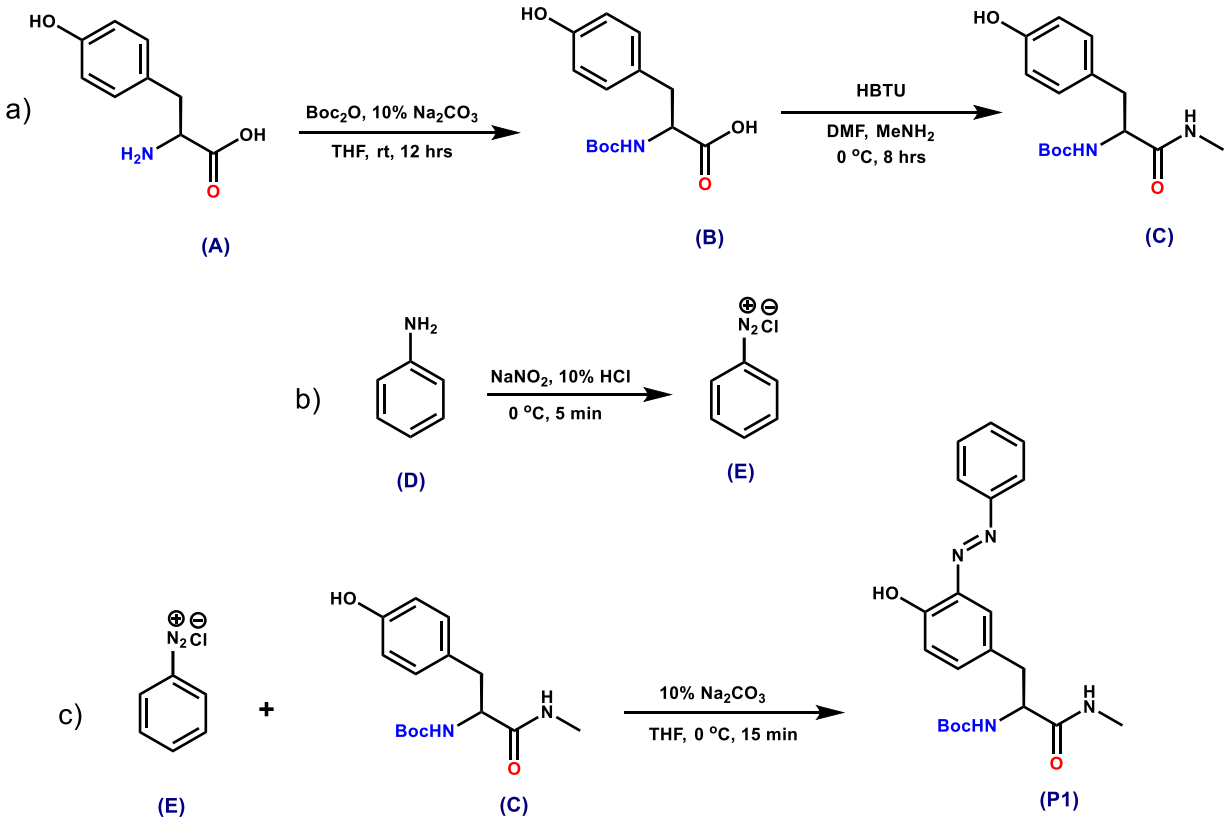
Boc-Tyr-OH (1.40g, 5 mmol) was converted to Boc-Tyr-NH-Me by treating it with 1 equiv. of HBTU and 1.2 equiv. of methylamine in DMF at 0 °C for 8hrs. TLC method was used to monitor the progress of the reaction. On reaction completion, the reaction mixture was acidified using 10% HCl. Using EtOAc (3×20 mL), the product was extracted. To remove the remaining acid 10% Na_2CO_3 (2×20 mL) was used to wash the organic layer. It was finally washed with brine solution (20 mL) and anhydrous Na_2SO_4 was used to dry the remaining water. A white colour compound was collected after concentrating the solution in a vacuum. The reaction yield was 93%.

A general method for Preparation of Diazonium salts

Aniline or aromatic amines (0.5 mmol) was converted to diazonium salt by treating with 2 equiv. of sodium nitrite ($NaNO_3$) in 10% HCl solution in ice cold conditions for 5 minutes. The obtained product was liquid and it was immediately used for the next reaction.

General Procedure for Synthesis of Azo dye with Boc-Tyr-NH-Me

Boc-Tyr-NH-Me (0.147g, 0.5 mmol) was converted to compound **P1** to **P11** by treating it with 1 equiv. of diazonium salts of aniline or aromatic amines in 10% Na_2CO_3 and THF for 15 min at ice cold conditions. TLC method was used to monitor the progress of the reaction. On reaction completion, THF was evaporated in a vacuum. The precipitated compound was filtered using the Büchner funnel. Further, it was purified using column chromatography by using EtOAc and hexanes as mobile phase. **Table1** shows the reaction yields.



Scheme 1 a) Synthesis of Boc-tyrosine-methylamine. b) Synthesis of the diazonium salt. c) Synthesis of azo dye with L-tyrosine.

Characterization of P1: Yellow orange solid; Yield 83%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.78 (s, 1H), 7.89 – 7.76 (m, 3H), 7.55 – 7.45 (m, 3H), 7.19 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 5.95 (d, *J* = 5.2 Hz, 1H), 5.12 (s, 1H), 4.33 (q, *J* = 7.5 Hz, 1H), 3.17 – 3.02 (m, 2H), 2.76 (d, *J* = 4.8 Hz, 3H), 1.40 (s, 9H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.86, 151.77, 150.61, 137.18, 134.36, 133.72, 131.44, 129.53, 128.47, 122.40, 118.53, 77.48, 76.84, 37.89, 28.42, 26.35.; HRMS (ESI-TOF) *m/z*: $[M+H]^{+}$ calculated for $C_{21}H_{26}N_4O_4H$ is 399.2032 found 399.2032.

Characterization of P2: Yellow orange solid; Yield 81%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.79 (s, 1H), 7.86 – 7.81 (m, 2H), 7.73 (d, *J* = 2.3 Hz, 1H), 7.14 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.05 – 6.99 (m, 2H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.87 (d, *J* = 5.5 Hz, 1H), 5.10 (s, 1H), 4.31 (q, *J* = 7.3 Hz, 1H), 3.90 (s, 3H), 3.08 (d, *J* =

7.0 Hz, 2H), 2.75 (d, *J* = 4.9 Hz, 3H), 1.41 (s, 9H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.86, 162.47, 151.67, 144.81, 137.13, 133.46, 133.12, 128.29, 124.21, 118.39, 114.75, 77.48, 76.84, 55.81, 37.89, 28.44, 26.36.; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{22}H_{28}N_4O_5Na$ is 451.1952 found 451.4128.

Characterization of P3: Yellow orange solid; Yield 95%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.48 (s, 1H), 8.44 – 8.37 (m, 2H), 8.04 – 7.97 (m, 2H), 7.85 (d, *J* = 2.3 Hz, 1H), 7.29 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.90 (s, 1H), 5.08 (s, 1H), 4.33 (q, *J* = 7.4 Hz, 1H), 3.19 – 3.05 (m, 2H), 2.78 (d, *J* = 4.9 Hz, 3H), 1.41 (s, 9H).; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{21}H_{25}N_5O_6Na$ is 444.1883 found 444.1886.

Characterization of P4: Yellow orange solid; Yield 69%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.48 (d, *J* = 2.0 Hz, 1H), 8.83 – 8.75 (m, 2H), 7.81 (d, *J* = 2.2 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.28 (d, *J* = 2.3 Hz, 1H), 6.98 (dd, *J* = 8.4, 1.4 Hz, 1H), 6.11 (s, 1H), 5.17 (s, 1H), 4.34 (q, *J* = 7.4 Hz, 1H), 3.48 (s, 1H), 3.10 (qd, *J* = 13.9, 6.9 Hz, 2H), 2.77 (d, *J* = 4.8 Hz, 3H), 1.40 (s, 9H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.73, 152.15, 151.63, 136.38, 134.48, 129.12, 118.84, 115.79, 77.48, 76.84, 37.78, 28.43, 26.38.; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{20}H_{25}N_5O_4Na$ is 400.1985 found 400.1982.

Characterization of P5: Yellow orange solid; Yield 92%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.69 (s, 1H), 8.02 – 7.91 (m, 4H), 7.87 – 7.80 (m, 3H), 7.63 (td, *J* = 7.3, 1.5 Hz, 1H), 7.52 (dd, *J* = 8.4, 7.0 Hz, 2H), 7.26 – 7.21 (m, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 5.94 (d, *J* = 5.3 Hz, 1H), 5.11 (s, 1H), 4.34 (q, *J* = 7.4 Hz, 1H), 3.11 (qd, *J* = 13.9, 6.9 Hz, 2H), 2.77 (d, *J* = 4.8 Hz, 3H), 1.41 (s, 9H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 134.15, 132.95, 131.44, 130.19, 128.61, 122.20, 118.73, 77.48, 76.84, 37.77, 28.43, 26.40.; HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ calculated for $C_{28}H_{30}N_4O_5H$ is 503.2294 found 503.2299.

Characterization of P6: Yellow orange solid; Yield 81%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 13.08 (s, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H),

7.98 – 7.91 (m, 2H), 7.89 (d, *J* = 2.2 Hz, 1H), 7.67 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 2H), 7.26 – 7.21 (m, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 5.91 (d, *J* = 5.1 Hz, 1H), 5.11 (s, 1H), 4.35 (d, *J* = 7.6 Hz, 1H), 3.13 (d, *J* = 7.0 Hz, 2H), 2.78 (d, *J* = 4.8 Hz, 3H), 1.42 (d, *J* = 1.4 Hz, 9H).; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{25}H_{28}N_4O_4Na$ is 471.2008 found 471.2011.

Characterization of P7: Yellow orange solid; Yield 85%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.66 (s, 1H), 8.25 – 8.15 (m, 2H), 7.95 – 7.87 (m, 2H), 7.82 (d, *J* = 2.2 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 5.87 (s, 1H), 5.07 (s, 1H), 4.32 (q, *J* = 7.5 Hz, 1H), 3.97 (s, 3H), 3.11 (h, *J* = 7.1 Hz, 2H), 2.77 (d, *J* = 4.8 Hz, 3H), 1.41 (s, 9H).; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{23}H_{28}N_4O_6Na$ is 479.1906 found 479.1910.

Characterization of P8: Yellow orange solid; Yield 87%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.54 (s, 1H), 7.93 – 7.81 (m, 2H), 7.76 (d, *J* = 2.3 Hz, 1H), 7.24 – 7.14 (m, 3H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.02 (d, *J* = 5.2 Hz, 1H), 5.15 (s, 1H), 4.34 (q, *J* = 7.4 Hz, 1H), 3.15 – 3.01 (m, 2H), 2.75 (d, *J* = 4.8 Hz, 3H), 1.40 (s, 9H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.86, 155.62, 151.63, 137.06, 134.39, 133.61, 128.57, 124.39, 124.30, 118.50, 116.72, 116.49, 77.48, 76.84, 37.86, 28.42, 26.36.; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{21}H_{25}FN_4O_4Na$ is 439.1757 found 439.1755.

Characterization of P9: Brown solid; Yield 81%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 13.64 (s, 1H), 8.47 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.39 – 8.33 (m, 1H), 8.31 – 8.25 (m, 1H), 8.22 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.88 (t, *J* = 7.9 Hz, 1H), 7.84 – 7.77 (m, 2H), 7.74 (d, *J* = 2.3 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 7.03 (d, *J* = 8.6 Hz, 1H), 6.03 (d, *J* = 4.9 Hz, 1H), 5.17 (s, 1H), 4.36 (q, *J* = 7.4 Hz, 1H), 3.16 – 3.03 (m, 2H), 2.78 (d, *J* = 4.8 Hz, 3H), 1.41 (s, 9H).; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{29}H_{28}N_4O_6Na$ is 551.1906 found 551.1912.

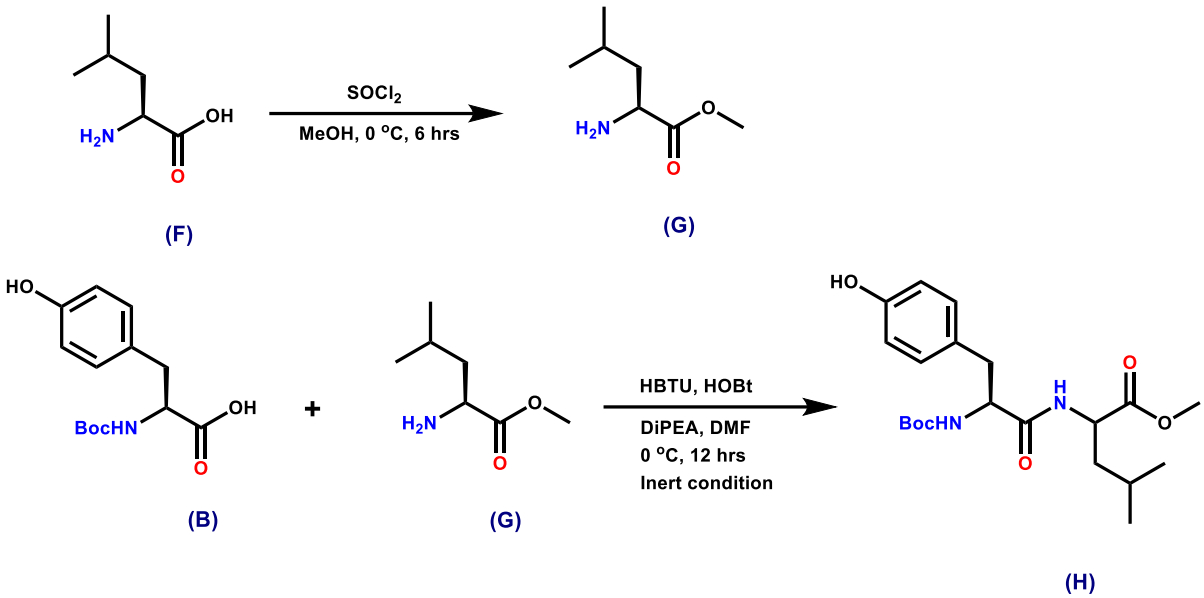
Characterization of P10: Yellow orange solid; Yield 89%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.83 (s, 1H), 7.81 – 7.71 (m, 3H), 7.35 – 7.29 (m, 2H), 7.22 – 7.11 (m, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 5.89 (s, 1H), 5.10 (s, 1H), 4.32 (q, *J* = 7.4 Hz, 1H), 3.09 (d, *J* = 6.9 Hz, 2H), 2.75 (d, *J* = 4.9 Hz, 3H), 2.44 (s, 3H), 1.40 (s,

9H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.91, 151.75, 148.66, 142.18, 137.13, 133.94, 133.47, 130.19, 128.35, 122.35, 118.44, 77.48, 76.84, 37.91, 28.42, 26.34, 21.67.; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{22}H_{28}N_4O_4Na$ is 435.2008 found 435.2007.

Characterization of P11: Brown solid; Yield 85%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 13.33 (s, 1H), 8.65 (d, *J* = 9.2 Hz, 1H), 8.50 (d, *J* = 8.5 Hz, 1H), 8.24 – 8.01 (m, 7H), 7.91 (d, *J* = 2.2 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.02 (s, 1H), 5.18 (s, 1H), 4.39 (d, *J* = 6.9 Hz, 1H), 3.14 (d, *J* = 7.1 Hz, 2H), 2.79 (d, *J* = 4.8 Hz, 3H), 1.43 (s, 9H).; ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.41, 130.46, 129.75, 129.06, 128.90, 127.40, 126.49, 126.41, 125.78, 122.18, 120.01, 113.80, 77.90, 55.81, 39.99, 39.31, 39.10, 38.89, 28.10, 25.59.; HRMS (ESI-TOF) *m*/*z*: $[M+Na]^+$ calculated for $C_{31}H_{30}N_4O_4Na$ is 545.2164 found 545.2166.

Synthesis of dipeptide Boc-Tyr-Leu-OMe

Leucine (0.65g, 5 mmol) was converted to Leu-OMe by treating it with $SOCl_2$ in methanol at 0 °C for 4 hrs. Methanol was evaporated in a vacuum to get the product. Boc-Tyr-OH (0.70g, 2.5 mmol) was converted to Boc-Tyr-Leu-OMe by treating it with 1 equiv. of HBTU, 1 equiv. of HOBt, 1 equiv of Leu-OMe and 5 equiv. of DIPEA in DMF at 0 °C for 10 hrs. TLC method was used to monitor the progress of the reaction. On reaction completion, the reaction mixture was acidified using 10% HCl. Using EtOAc (3×20 mL), the product was extracted. To remove the remaining acid 10% Na_2CO_3 (2×20 mL) was used to wash the organic layer. It was finally washed with brine solution (20 mL) and anhydrous Na_2SO_4 was used to dry the remaining water. The white colour compound was collected after concentrating the solution in a vacuum. The reaction yield was 91%. Further, it was purified using column chromatography by EtOAc and hexanes as mobile phase.

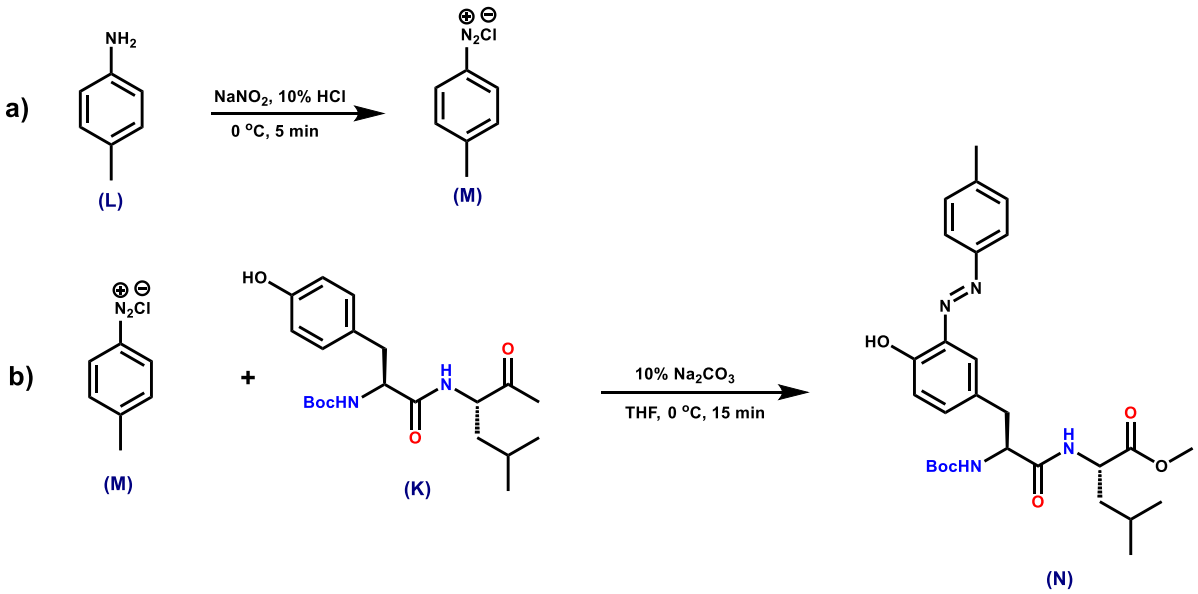


Scheme 2 Synthesis of dipeptide Boc-Tyr-Leu-OMe.

Characterization of dipeptide Boc-Tyr-Leu-OMe: White solid; Yield 91%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.01 (d, *J* = 8.3 Hz, 2H), 6.96 – 6.86 (m, 1H), 6.72 (d, *J* = 8.5 Hz, 2H), 6.45 (d, *J* = 8.2 Hz, 1H), 5.22 – 5.10 (m, 1H), 4.55 (q, *J* = 7.2 Hz, 1H), 4.34 – 4.21 (m, 1H), 3.68 (s, 3H), 2.95 (d, *J* = 6.9 Hz, 2H), 1.56 (qt, *J* = 11.0, 6.1 Hz, 2H), 1.47 (dd, *J* = 9.1, 7.5 Hz, 1H), 1.41 (s, 9H), 0.88 (t, *J* = 5.6 Hz, 6H).

Diazo-coupling reaction on dipeptide Boc-Tyr-Leu-OMe

Boc-Tyr-Leu-OMe (0.92 g, 2.27 mmol) was converted to compound **P1** by treating it with 1 equiv. of diazonium salts of toluidine in 10% Na_2CO_3 and THF for 30 min at 0 °C. TLC method was used to monitor the progress of the reaction. On reaction completion, THF was evaporated in a vacuum. The precipitated compound was filtered using the Büchner funnel. Further, it was purified using column chromatography by EtOAc and hexanes as mobile phase. The reaction yield was 78%.



Scheme 3 a) Synthesis of the diazonium salt. b) Diazo-coupling reaction on dipeptide Boc-Tyr-Leu-OMe.

Characterization of P12: Yellow orange solid; Yield 78%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.82 (s, 1H), 7.76 (d, *J* = 8.5 Hz, 3H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.18 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.36 (d, *J* = 8.2 Hz, 1H), 5.08 (s, 1H), 4.59 (td, *J* = 8.5, 4.9 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 1H), 3.66 (s, 3H), 3.10 (d, *J* = 6.7 Hz, 2H), 2.43 (s, 3H), 1.63 – 1.54 (m, 2H), 1.52 – 1.45 (m, 1H), 1.42 (s, 9H), 0.88 (dd, *J* = 10.0, 6.2 Hz, 6H).; ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.84, 170.88, 151.68, 148.55, 142.03, 137.03, 133.93, 133.51, 130.06, 122.23, 118.35, 52.30, 50.76, 41.66, 28.27, 24.69, 22.76, 21.88, 21.54.; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calculated for $C_{28}H_{38}N_4O_6Na$ is 549.2688 found 549.2692.

Synthesis of tripeptide Boc-Trp-Tyr-Phe-OMe

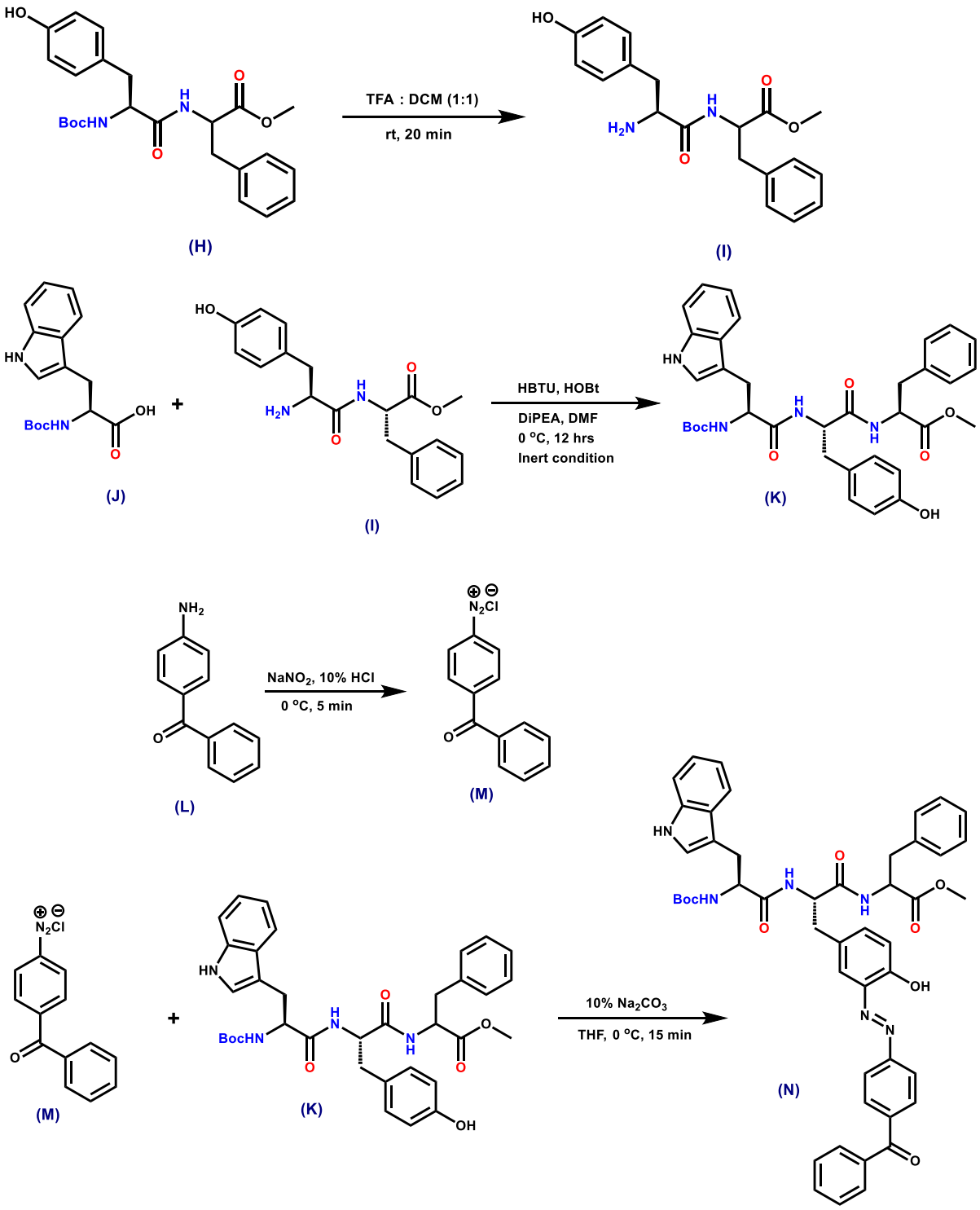
Boc-Tyr-Phe-OMe dipeptide was prepared by the method described in the scheme 2. Boc-Tyr-Phe-OMe (2.21g, 5 mmol) was converted to Tyr-Phe-OMe by treating it with a 1:1 (v/v) solution of TFA-DCM (11 ml) for 20 minutes at room temperature. TLC method was used to monitor the progress of the reaction. On reaction completion, DCM and the remaining TFA was evaporated in a vacuum to get the product. The reaction yield was 98%. Tyr-Phe-OMe (1.10g,

4.9 mmol) was converted to Boc-Trp-Tyr-Phe-OMe by treating it with 1 equiv. of HBTU, 1 equiv. of HOBt, 1 equiv. of Boc-Trp-OH and 5 equiv. of DIPEA in DMF at 0 °C for 10 hrs. TLC method was used to monitor the progress of the reaction. On reaction completion, the reaction mixture was acidified using 10% HCl. Using EtOAc (3×20 mL), the product was extracted. To remove the remaining acid 10% Na_2CO_3 (2×20 mL) was used to wash the organic layer. It was finally washed with brine solution (20 mL) and anhydrous Na_2SO_4 was used to dry the remaining water. The white colour compound was collected after concentrating the solution in a vacuum. The reaction yield was 88%. Further, it was purified using column chromatography by EtOAc and hexanes as mobile phase.

Diazo-coupling reaction on tripeptide Boc-Trp-Tyr-Phe-OMe

Boc-Trp-Tyr-Phe-OMe (0.92g, 2.27 mmol) was converted to compound **P1** by treating it with 1 equiv. of diazonium salts of 1-Aminobenzophenonene in 10% Na_2CO_3 and THF for 20 min at 0 °C. TLC method was used to monitor the progress of the reaction. On reaction completion, THF was evaporated in a vacuum. The reaction was quenched by using 10% HCL solution. The precipitate of compound P13 after reaction was filtered using the Büchner funnel. Further, it was purified using column chromatography by EtOAc and hexanes as mobile phase. The reaction yield was 72%.

Characterization of P13: Yellow orange solid; Yield 78%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.64 (s, 1H), 8.08 (s, 1H), 7.99 – 7.88 (m, 4H), 7.87 – 7.81 (m, 2H), 7.68 – 7.58 (m, 3H), 7.53 (t, *J* = 7.5 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 4.9 Hz, 3H), 7.16 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 7.09 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 6.99 (dd, *J* = 7.7, 1.8 Hz, 2H), 6.95 – 6.87 (m, 2H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.30 (d, *J* = 7.7 Hz, 2H), 5.07 (s, 1H), 4.71 (dt, *J* = 7.7, 6.3 Hz, 1H), 4.57 (q, *J* = 6.8 Hz, 1H), 4.43 (d, *J* = 6.5 Hz, 1H), 3.66 (s, 3H), 3.31 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.18 – 2.79 (m, 6H), 1.38 (s, 9H).; MALDI/TOF-TOF *m*/*z*: $[M+Na]^+$ calculated for $C_{48}H_{48}N_6O_8Na$ is 875.3165 found 875.3848.



Scheme 4 a) Synthesis of tripeptide Boc-Trp-Tyr-Phe-OMe. b) Synthesis of the diazonium salt. c) diazo-coupling reaction on tripeptide Boc-Trp-Tyr-Phe-OMe.

Calculation of Molar absorptivity coefficients (ε)

Wavelength (λ_{max}) was obtained using *UV-Vis* spectrophotometer as shown in the *UV* absorption profiles section from **Fig. 6** to **Fig. 16** and then at that λ_{max} absorbance was taken at different concentrations ranging 10-100 μM and then using Beer-lamberts law, graphs of absorbance *vs* Concentration were plotted as shown in graphs section fig12 to fig22. The slope of the linear fitted graphs yielded molar absorptivity coefficients (ε) of **P1** to **P11**.

Beer-lambert's law is given by,

$$\boldsymbol{A = \varepsilon c l}$$

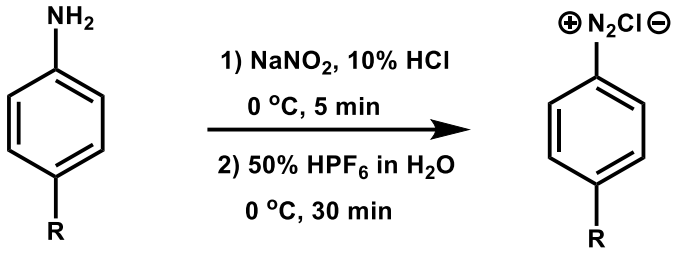
Where,

$\boldsymbol{A}$ = absorbance, $\boldsymbol{\varepsilon}$ = molar absorptivity coefficient, $\boldsymbol{c}$ = concentration (moles/L)

$\boldsymbol{l}$ = path length

General Procedure for isolation of diazonium salts

Aromatic amines (0.5 mmol) were converted to diazonium salt by treating with 2 equiv. of sodium nitrite ($NaNO_3$) in 10% HCl solution at 0 °C for 10-15 minutes. 1.5 equiv. of 50% HPF_6 in water was added to the reaction mixture and stirred for 30 minutes. White to brown colour precipitate was formed. The precipitated compound was filtered using a Büchner funnel by washing it with water followed by ethanol and diethyl ether. Reaction yield was 50-60%. Further, it was stored at 0 °C and used for reaction kinetics experiment.



Scheme 5 Isolation of diazonium salts.

Preparation of 100 µM phosphate buffer of pH 7.4

In 200 mL of distilled water, 5.054 g of Na_2HPO_4 and 0.848 g of NaH_2PO_4 was added. the pH of the final solution was adjusted to 7.4 using 1N HCl and 1N NaOH. Finally, add distilled water until volume is 0.25 L.

Evaluating second-order rate constants

Azo conjugates uniquely absorb at their characteristic λ_{max}, which was used to spectrophotometrically monitor their formation. The rate for all of the reactions were measured under pseudo-first-order reaction conditions and ACN as solvent, where the aryl-diazonium salt was used as the limiting reagent (10 µM) and concentrations of the amino acid (Boc-Tyr-OH) was used in significant excess (at least 10-fold). Reactions were performed in 100 mM phosphate buffer (pH 7.4) at room temperature. Pseudo-first order rate (k_B) constant was calculated by Beer-lamberts law and according to the following equation.

$$\ln[A] = \ln[A_o] - k_B t$$

The second-order rate constant (k_2) was then calculated using the following equation:

$$k_B = k_2 * [B]$$

Results and Discussion

In accordance with the proposed **Scheme 1**, we have successfully synthesized the compound **P1** which was yellow-orange in colour. The formation of compound **P1** was characterized using ^{1}H NMR. In order to compare molar absorptivity coefficients. A series of different compounds were synthesized changing the diazonium salts. **Table1** describes the synthesized compounds and their % yields. In the case of **P3,** we got maximum yield (95%), which may be due to the nitro group which is reducing the electron density in the ring and making diazonium salt more electrophilic. All of the compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. All of the compounds were almost yellow-orange in colour. To check the purity of the synthesized compounds HPLC traces were taken.

Entry	R	% yield
1	Phenyl (**P1**)	83
2	4-methoxy phenyl (**P2**)	81
3	4-nitro phenyl (**P3**)	95
4	4-pyridyl (**P4**)	69
5	Benzophenone (**P5**)	92
6	1-naphthyl (**P6**)	81
7	4-benzoate (**P7**)	85
8	4-fluoro phenyl (**P8**)	87
9	Anthraquinone (**P9**)	81
10	4-methyl phenyl (P**10**)	89
11	1-pyrenyl (**P11**)	85

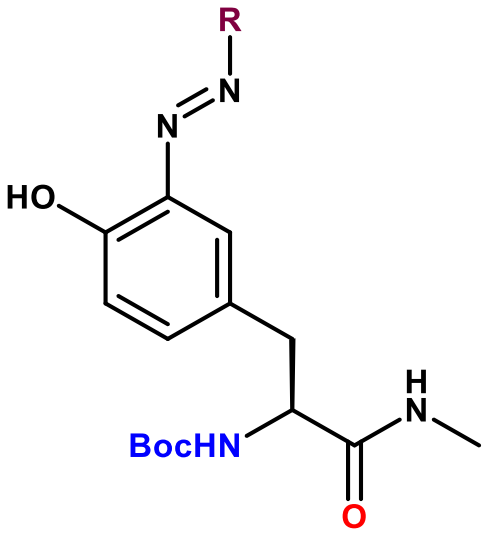


Table 1 Synthesized compounds and their yields.

λ_{max} was obtained using *UV-Vis* spectrophotometer and then at that λ_{max} absorbance was taken at different concentrations and then using linear fit method molar absorptivity coefficients (ε) of P1 to P6 were calculated as shown in

Table2. Out of P1 to P11, **P1** had a maximum molar absorptivity coefficient (25800 M^{-1} cm^{-1}).

Entry	R	Molar Absorptivity coefficient ($M^{-1}cm^{-1}$)
1	Phenyl (P1)	25880
2	4-methoxy phenyl (P2)	23910
3	4-nitro phenyl (P3)	23190
4	4-pyridyl (P4)	19390
5	Benzophenone (P5)	21790
6	1-naphthyl (P6)	15350
7	4-benzoate (P7)	10960
8	4-fluoro phenyl (P8)	19520
9	Anthraquinone (P9)	18950
10	4-methyl phenyl (P10)	19720
11	1-pyrenyl	23980

Table 2 Calculated Molar absorptivity Coefficients

To check whether the diazo-coupling reaction on peptide causing racemization, we have performed the reaction on a dipeptide Boc-Tyr-Leu-OMe as shown in **Scheme 3**. After purification through the column chromatography, we got the yield of 88% and purity was checked by HPLC. After analysing ^{1}H NMR we confirmed that there were no diastereomers present in the compound hereby, no racemization has happened during the course of reaction.

To study the selectivity of the diazo-coupling reaction over various aromatic amino acids, a tripeptide Boc-Trp-Tyr-Phe-OMe (**K**) was synthesized as shown in **scheme 4**. After performing a coupling reaction with 1 equiv. of diazonium salt of 1-aminobenzophenone, a yellow-orange compound with 77% yield was obtained. The purity of the compound was checked using HPLC. After analysing its MALDI spectra we confirmed that the coupling happened at only one site. By using UV absorption profile as shown in **Fig. 3**, we got to know that it happened

only on Tyrosine residue of the peptide, as it matches with the UV absorption profile of the chromophore synthesized by reaction of Boc-Tyr-NH-Me with diazonium salt of benzophenone (**P5**), which confirms its high selectivity towards tyrosine over other aromatic amino acids.

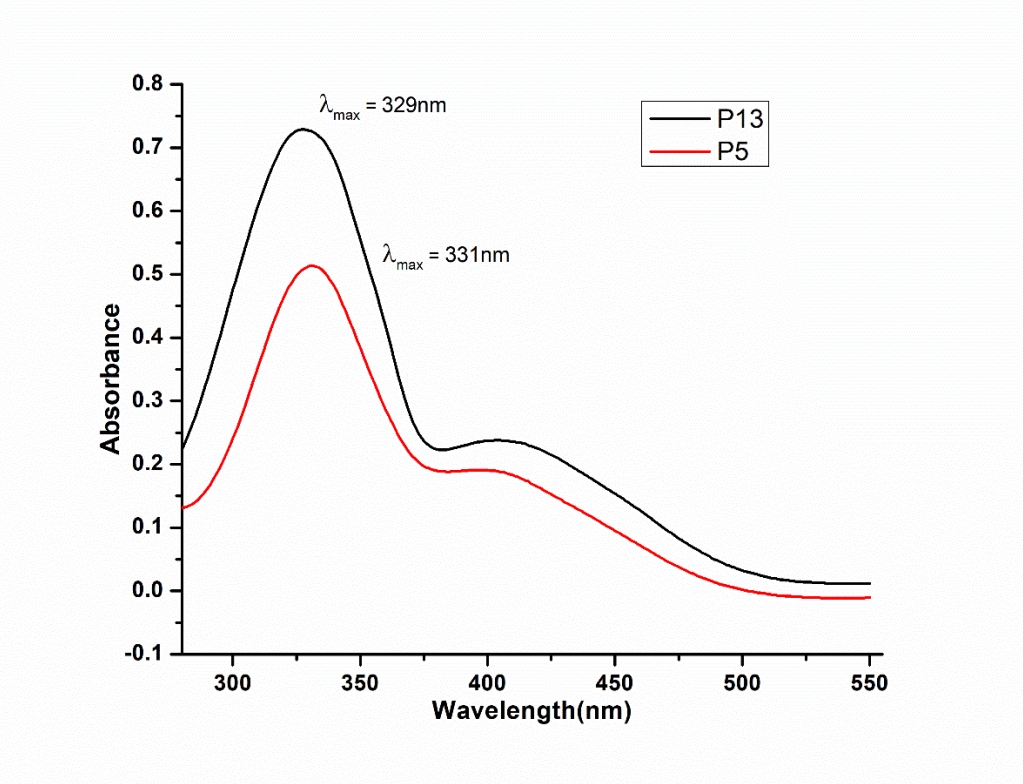


Fig. 3 UV absorption profile of P5 and P13

To study the reactivity of different diazonium salts at physiological pH 7.4. Phosphate buffer of pH 7.4 was prepared and diazonium salts were isolated using HPF_6 as shown in **Scheme 5** and characterised using ^1H NMR. **Table 3** shows their respective yields. Reaction kinetics of each diazonium salt with Boc-Tyr-OH were monitored by using *UV-Vis* spectrophotometer at their respective $\lambda_{max.}$ We observed that reaction was feasible only in the case of Para-nitro substituted diazonium salt at pH 7.4 and it was completed within 3 minutes. Reaction progress is shown in **Fig. 4**. The second order rate constant of the reaction was calculated using pseudo first order rate equation and it came out to 18 $M^{-1}S^{-1}$ as shown in **Fig. 5**. Others showed no change in absorbance at their respective λ_{max} even in 10 hrs of monitoring. However, at pH 9.0 reaction was possible with all

of the diazonium salts within 10-30 minutes. This shows the reaction at controlled pH increases the selectivity and specificity. Feasibility of the reaction at mild pH makes the reaction compatible for its application to protein modification by selective labelling.

Entry	R	% yield	Colour
1	Phenyl (M1)	63	White
2	4-methoxy phenyl (M2)	71	Light Brown
3	4-nitro phenyl (M3)	55	Brown
4	4-methyl phenyl (M4)	69	White
5	4-benzoate (M5)	62	Light Brown

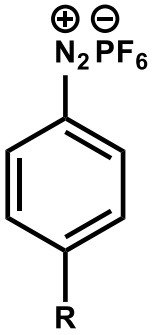


Table 3 Yields of Isolated diazonium salts.

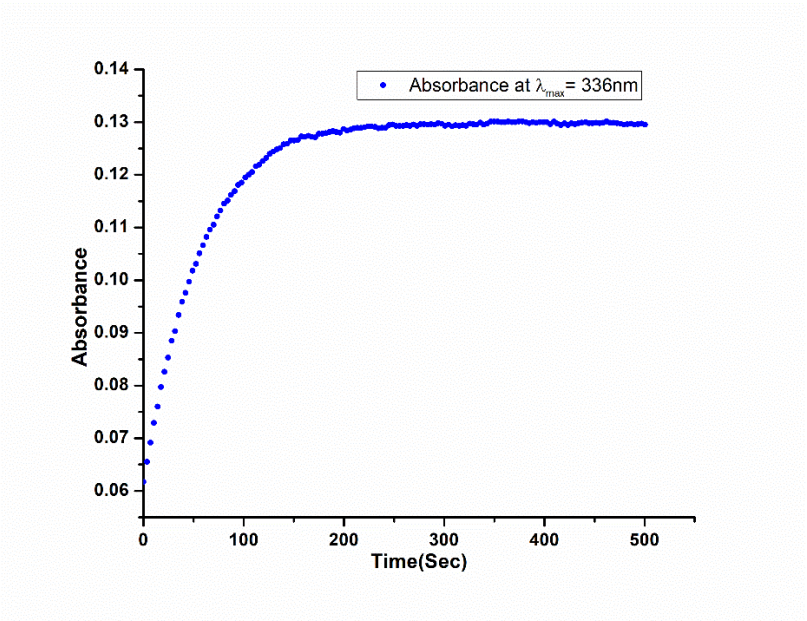


Fig. 4 Monitoring reaction progress.

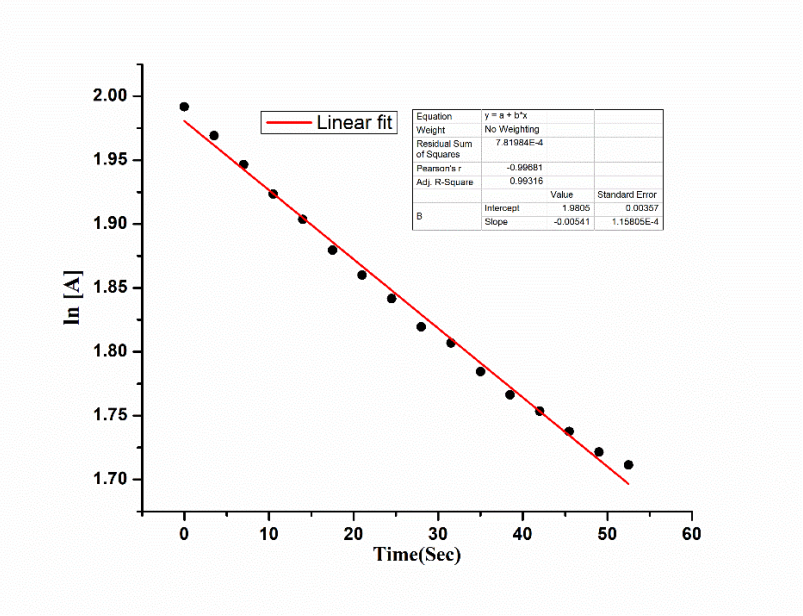


Fig. 5 Linear fit for calculating second-order rate constant.

Conclusion

We successfully synthesized and calculated molar absorptivity coefficients of the differently substituted diazonium salts coupled with Boc-Tyr-NH-Me with high yields of 70-95%. Using ^{1}H NMR and HPLC we proved that the reaction is not causing any racemization at the chiral centres present in the molecule. We also proved its selectivity on the tyrosine residue over the other aromatic amino acids. We have observed that in the case of para nitro substituted the reaction goes within 5 minutes even at very mild pH 7.4. However other diazonium salts did not show any reaction at pH 7.4.

Based on the observed high molar absorptivity coefficients, a number of applications can be possible for the reaction such as site selective modification and labelling of natural proteins. One can also identify the number of Tyrosine residues in the given polypeptide sequence. Since tyrosine is present in most of the cancer urinary metabolites so, a spectrophotometric detection technique can also be developed using highly reactive diazonium salts. By using the selectivity of the diazo-coupling reaction, we are developing a side chain directed cyclization of a beta hairpin peptide containing two Tyrosine at the both terminals of the peptide named as azo bridged peptide.

UV absorption profiles

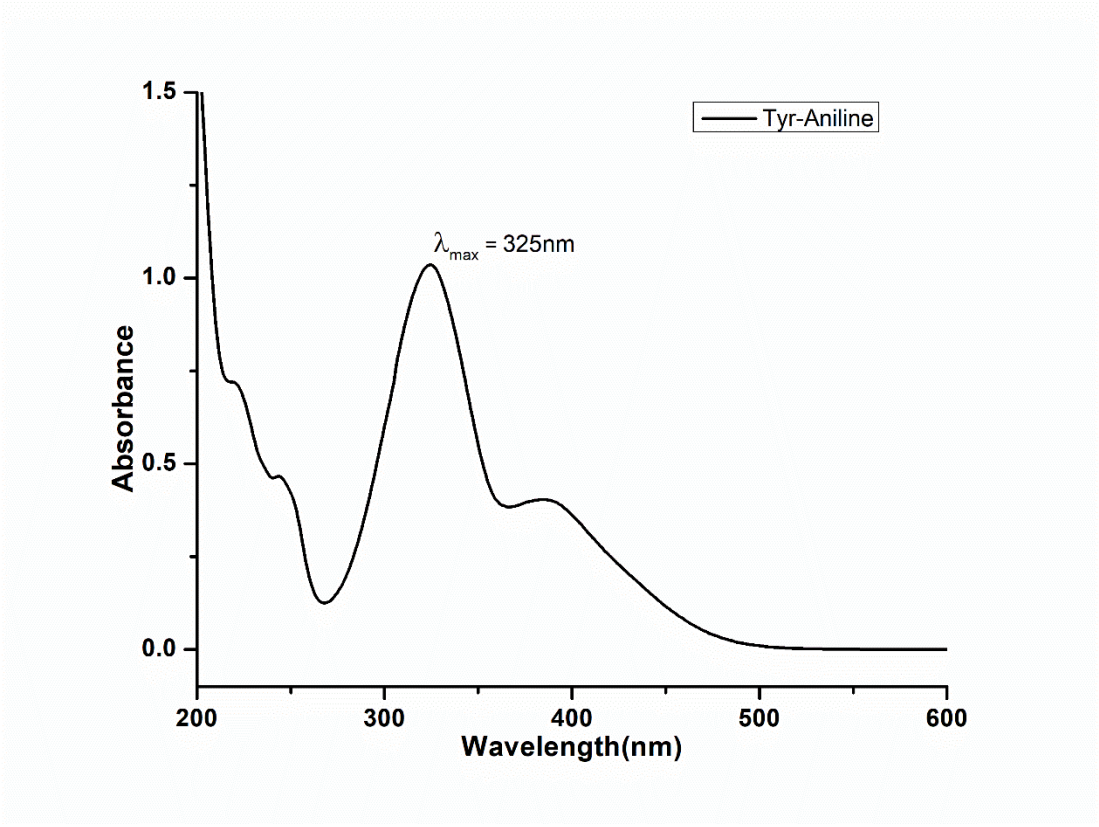


Fig. 6 UV absorption profile of P1

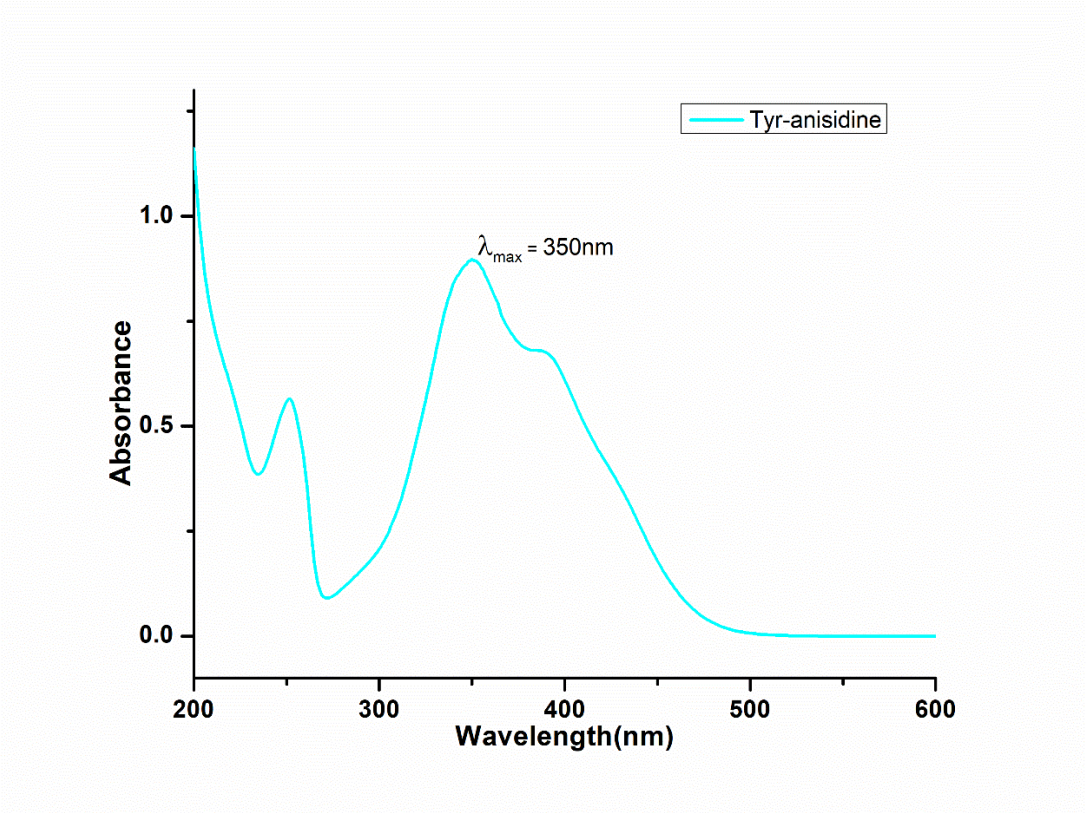


Fig. 7 UV absorption profile of P2

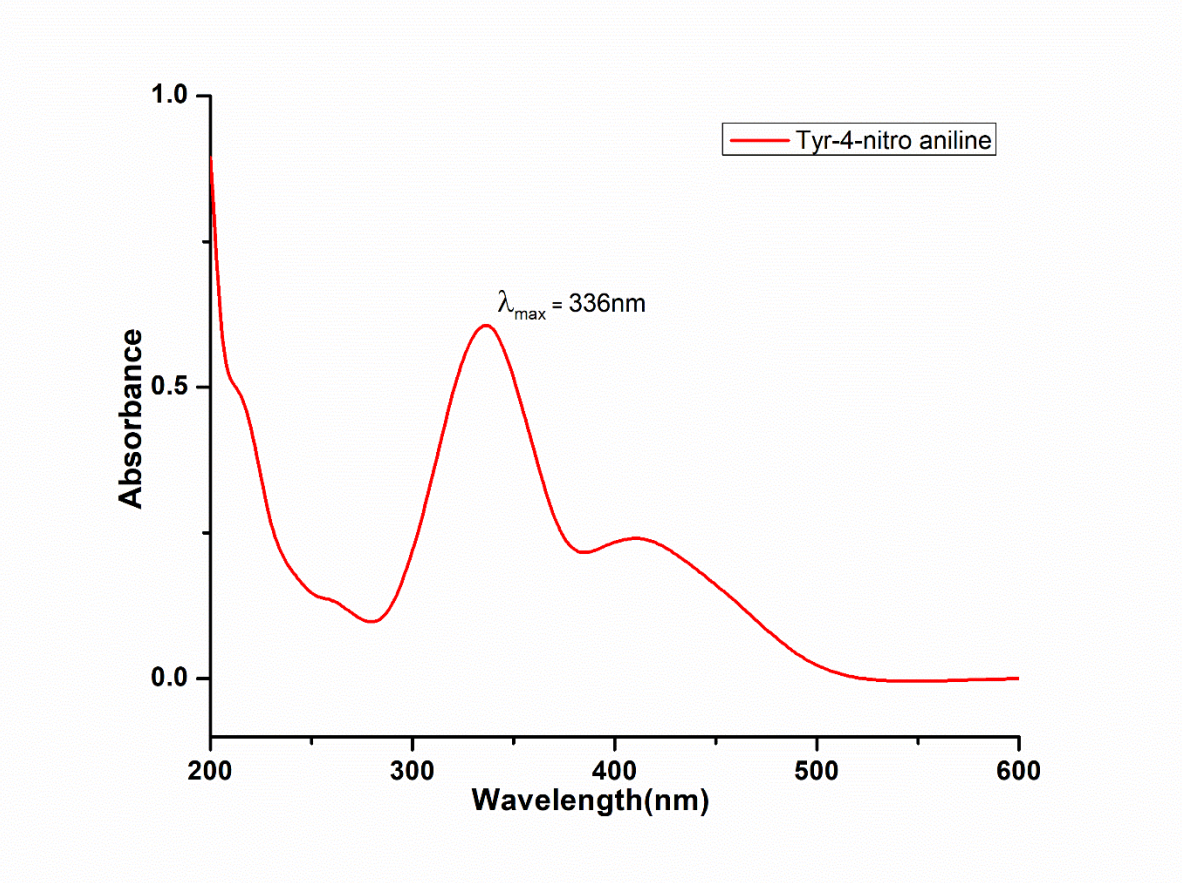


Fig. 8 UV absorption profile of P3

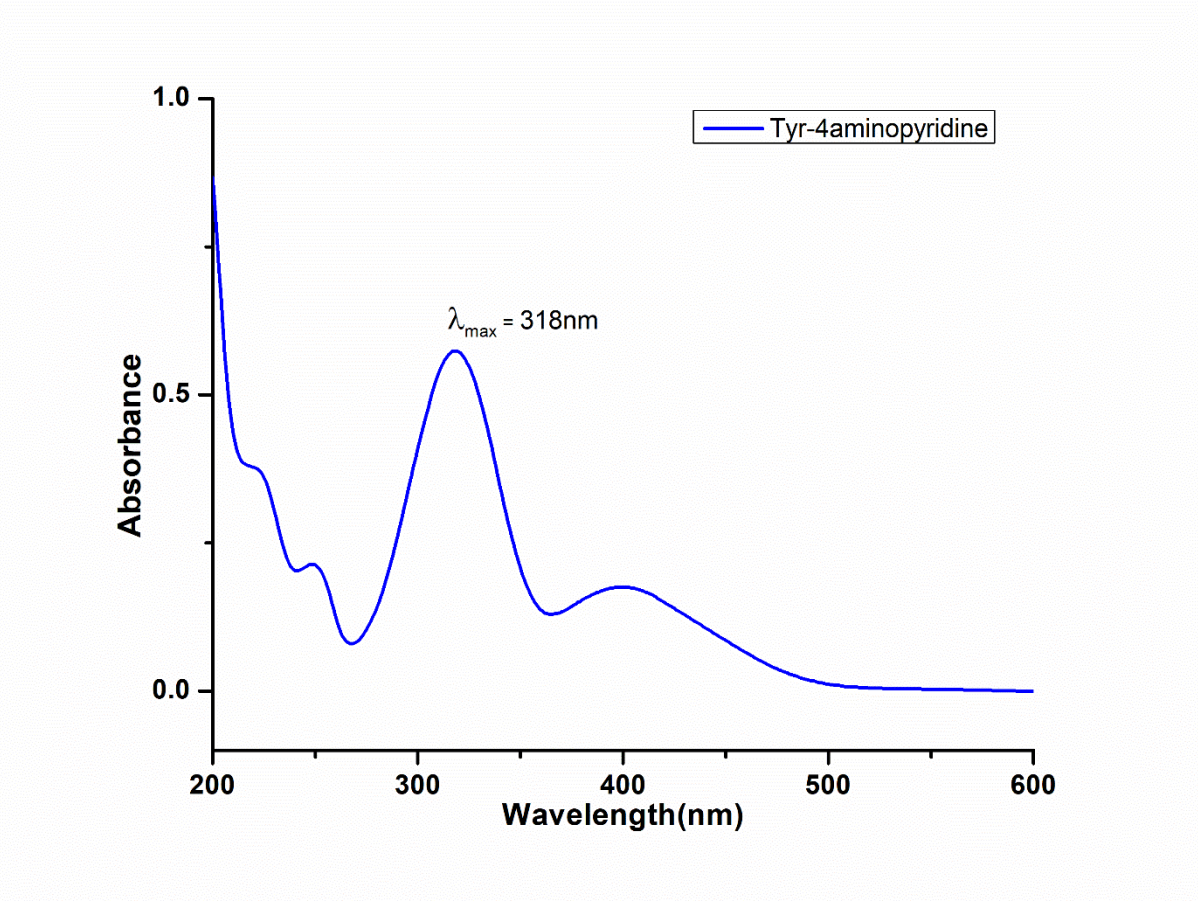


Fig. 9 UV absorption profile of P4

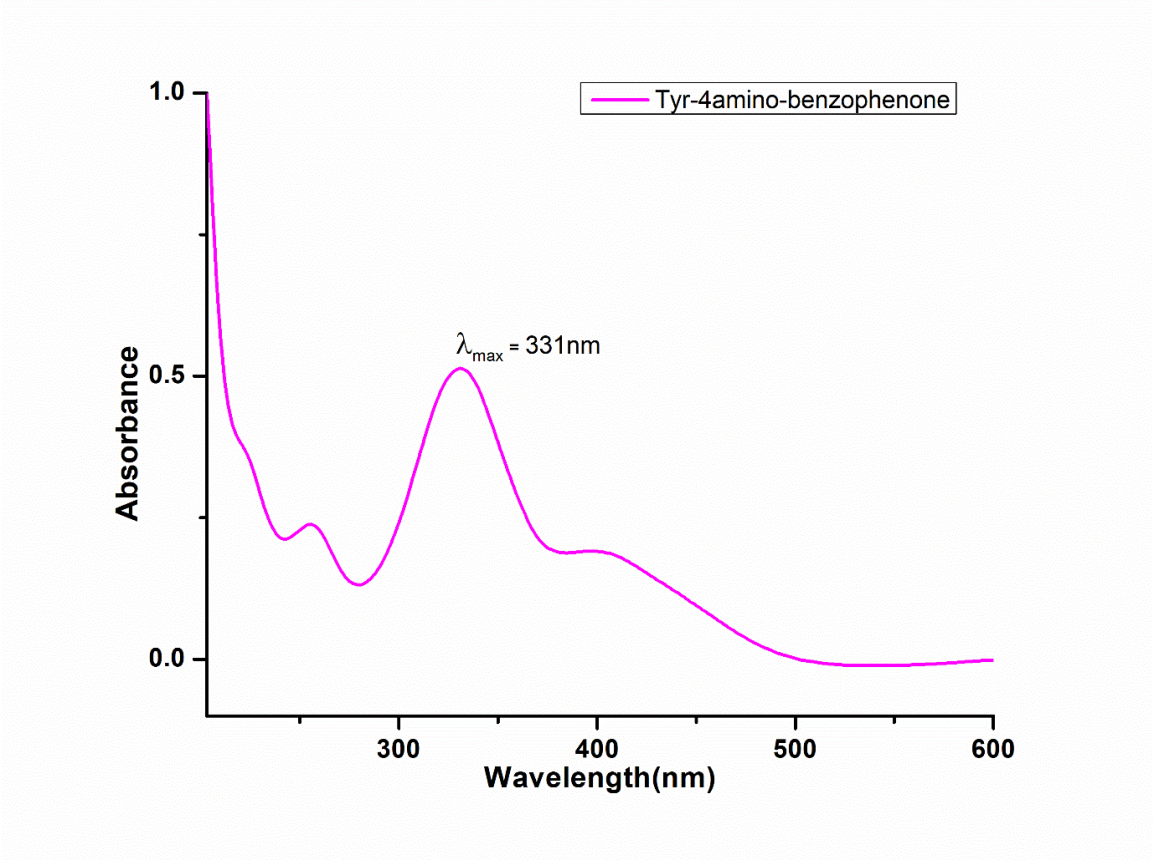


Fig. 10 UV absorption profile of P5

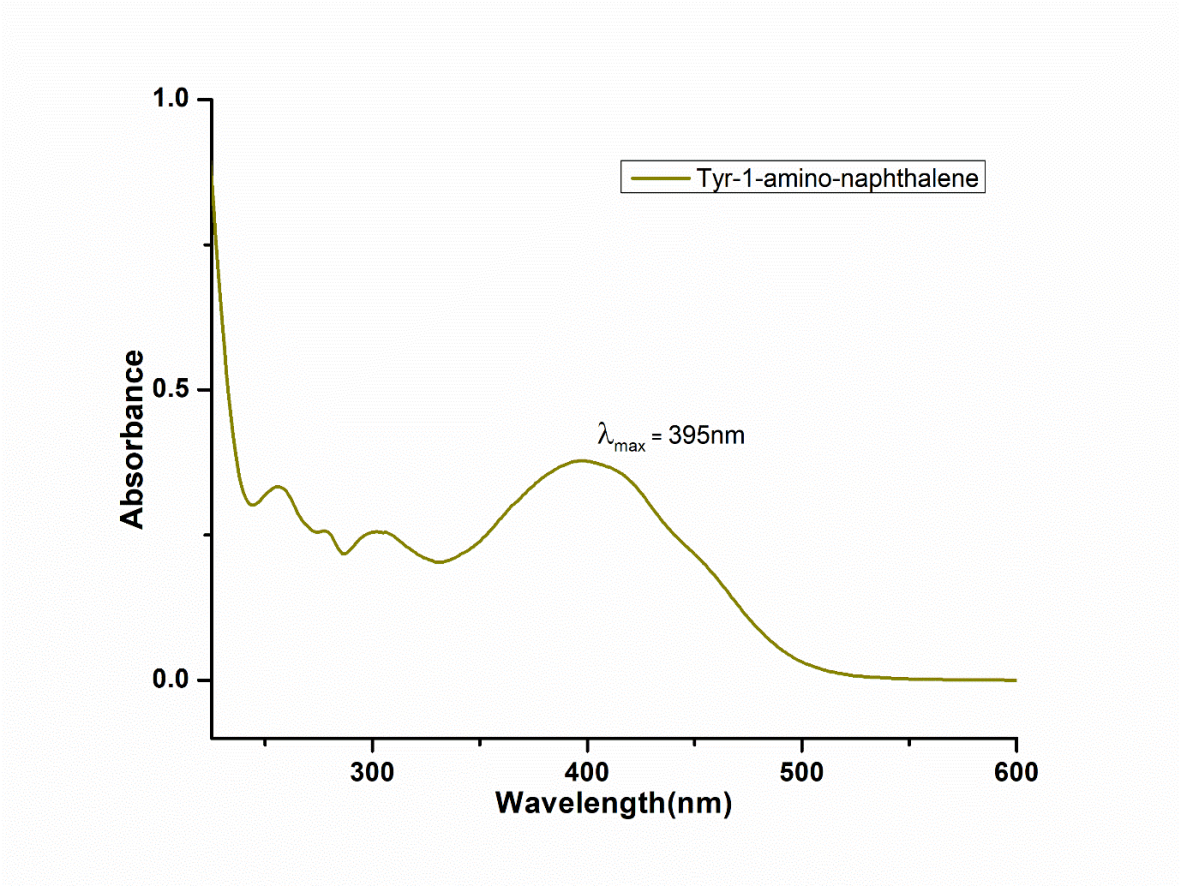


Fig. 11 UV absorption profile of P6

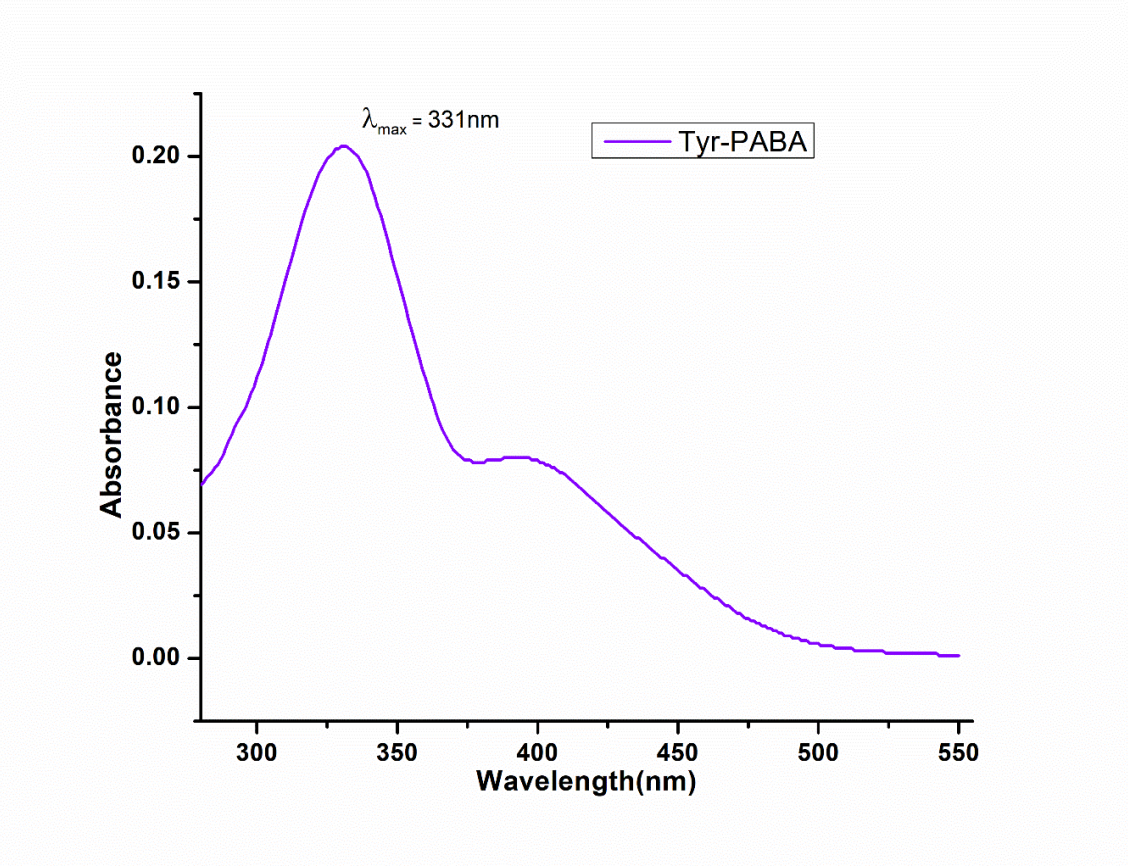


Fig. 12 UV absorption profile of P7

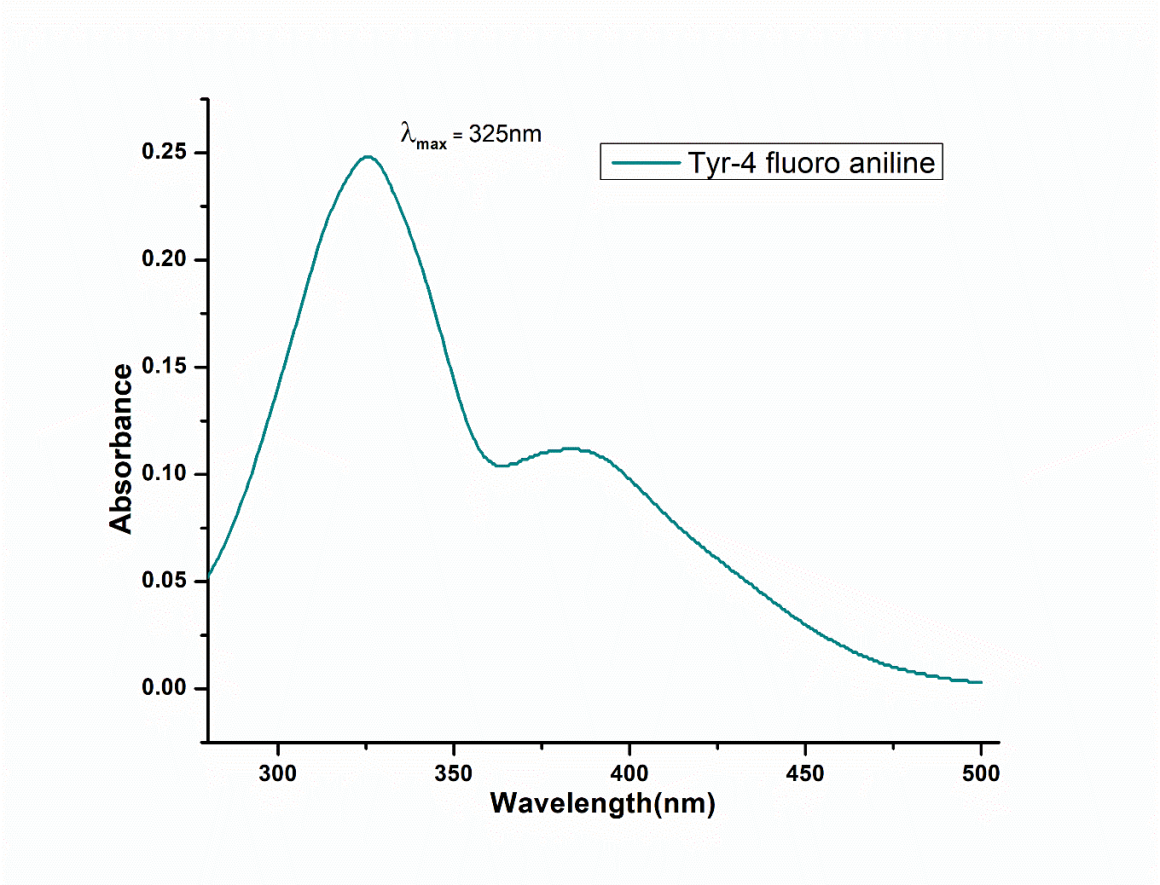


Fig. 13 UV absorption profile of P8

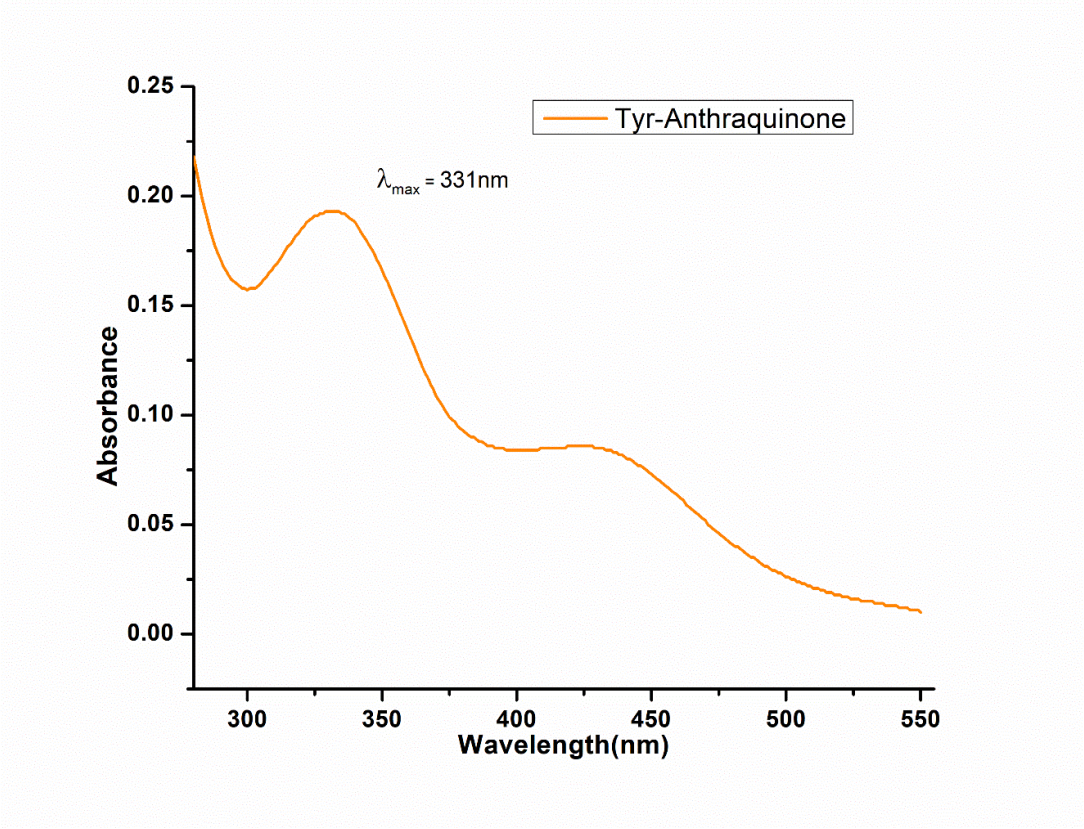


Fig. 14 UV absorption profile of P9

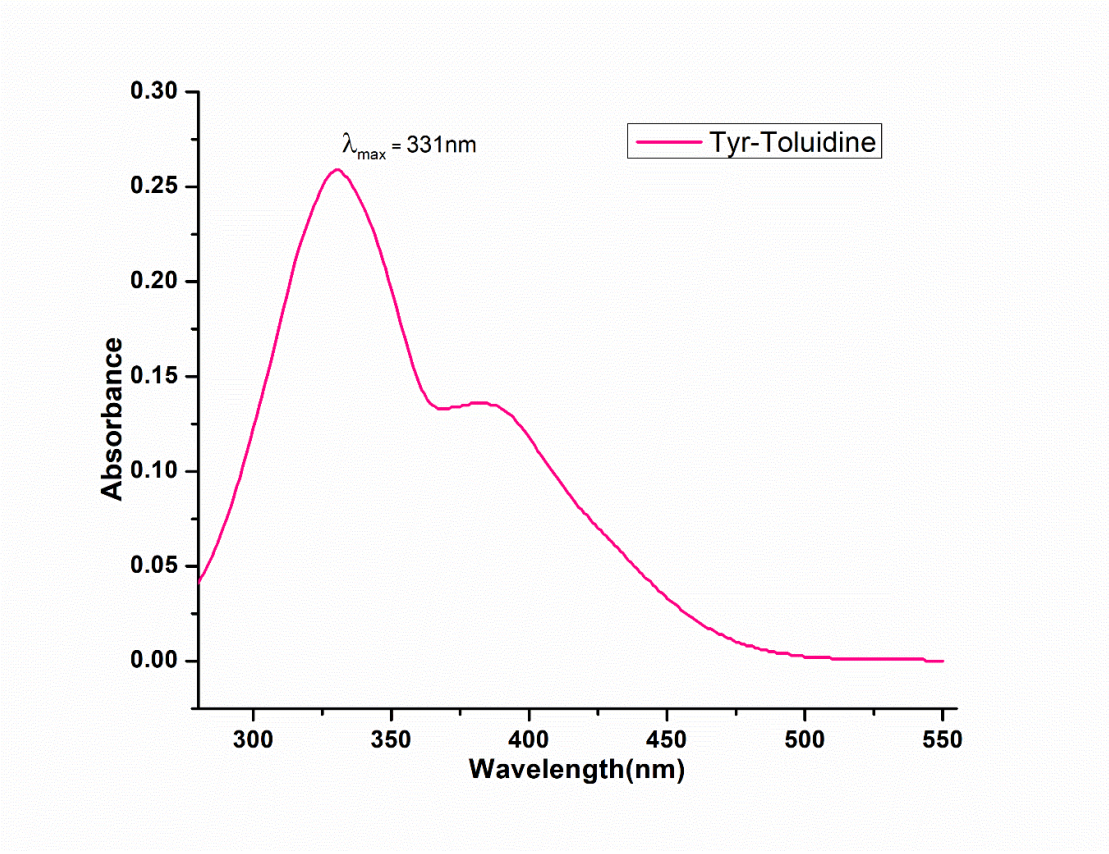


Fig. 15 UV absorption profile of P10

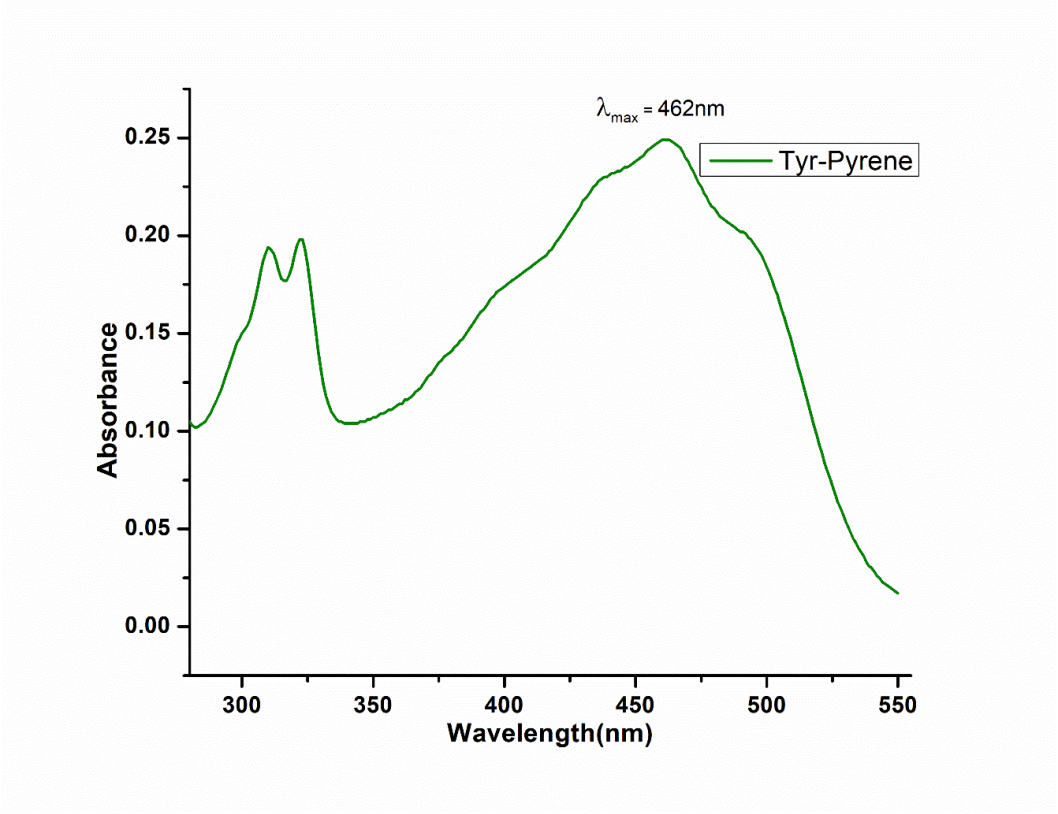


Fig. 16 UV absorption profile of P11

Graphs

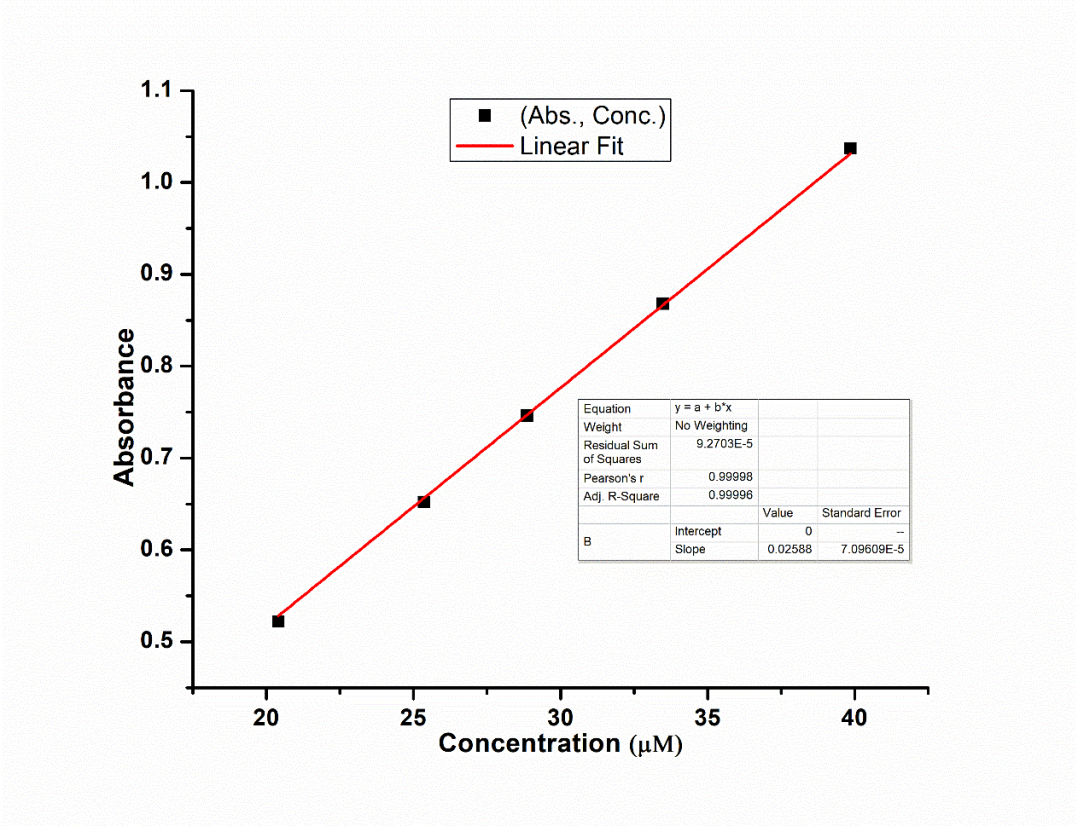


Fig. 17 Linear fit graph for P1

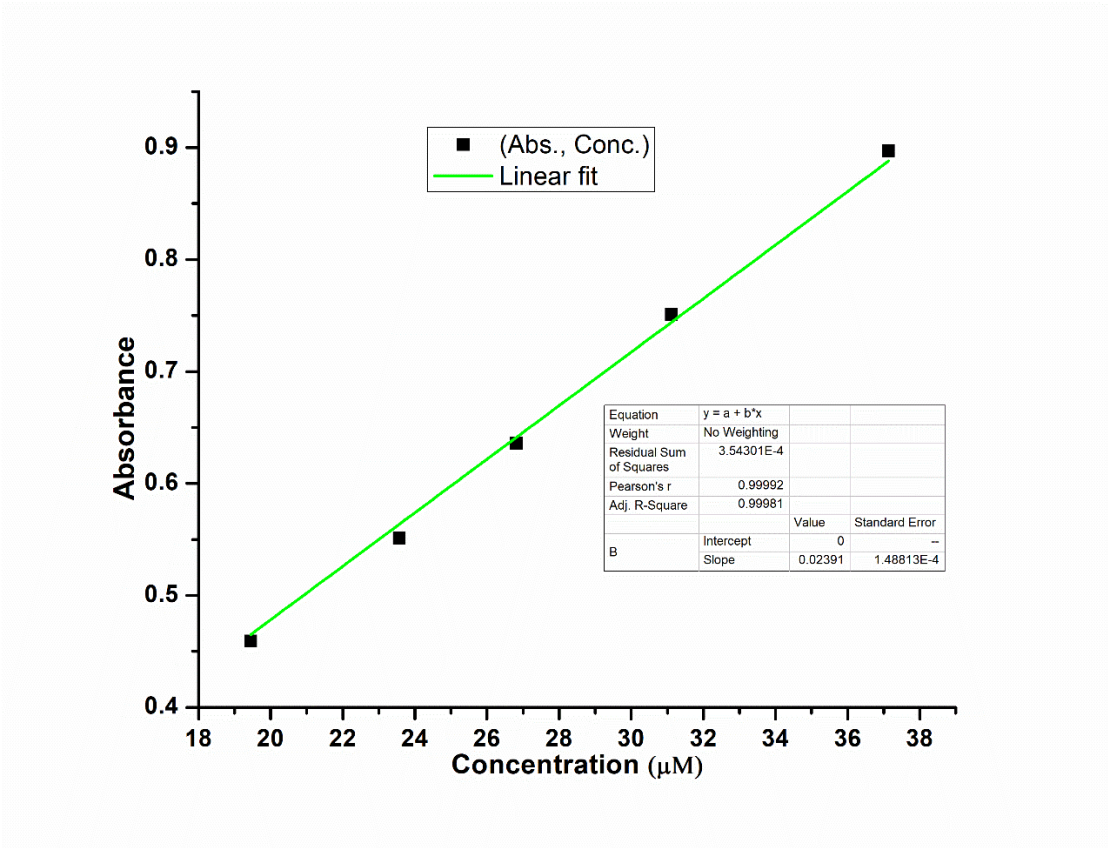


Fig. 18 UV absorption profile of P2

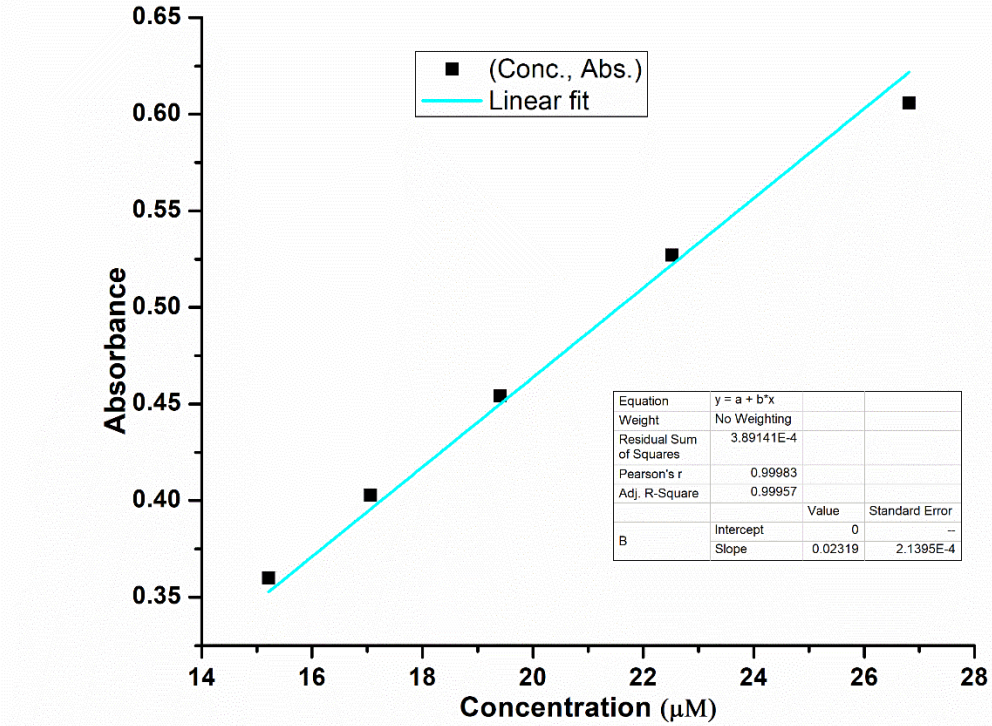


Fig. 19 UV absorption profile of P3

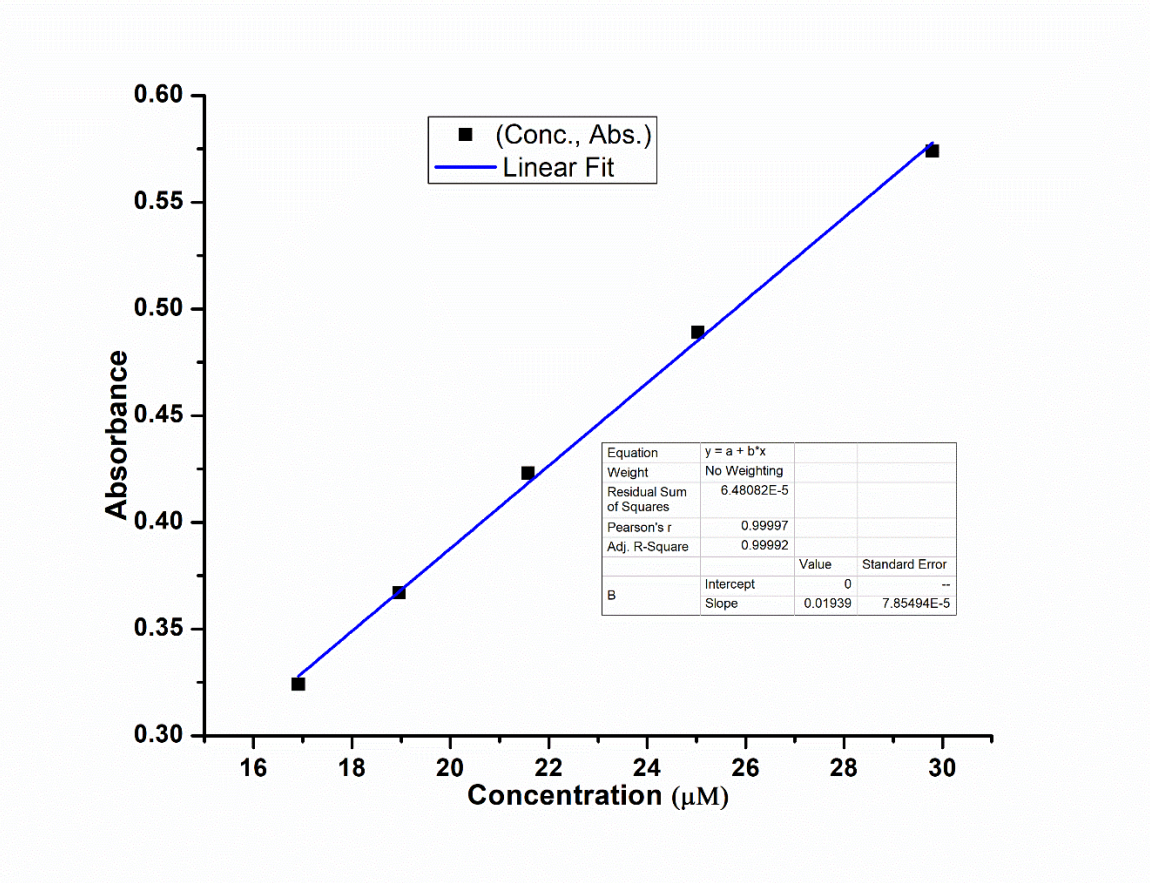


Fig. 20 UV absorption profile of P4

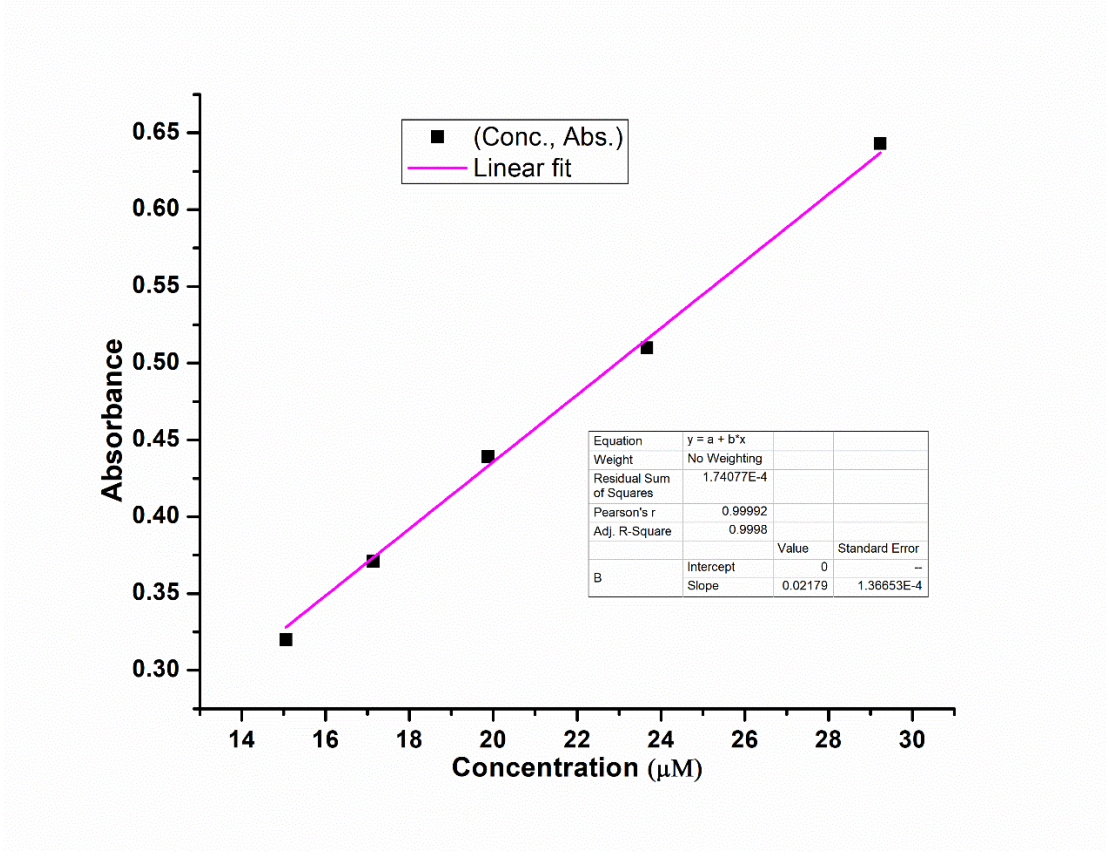


Fig. 21 UV absorption profile of P5

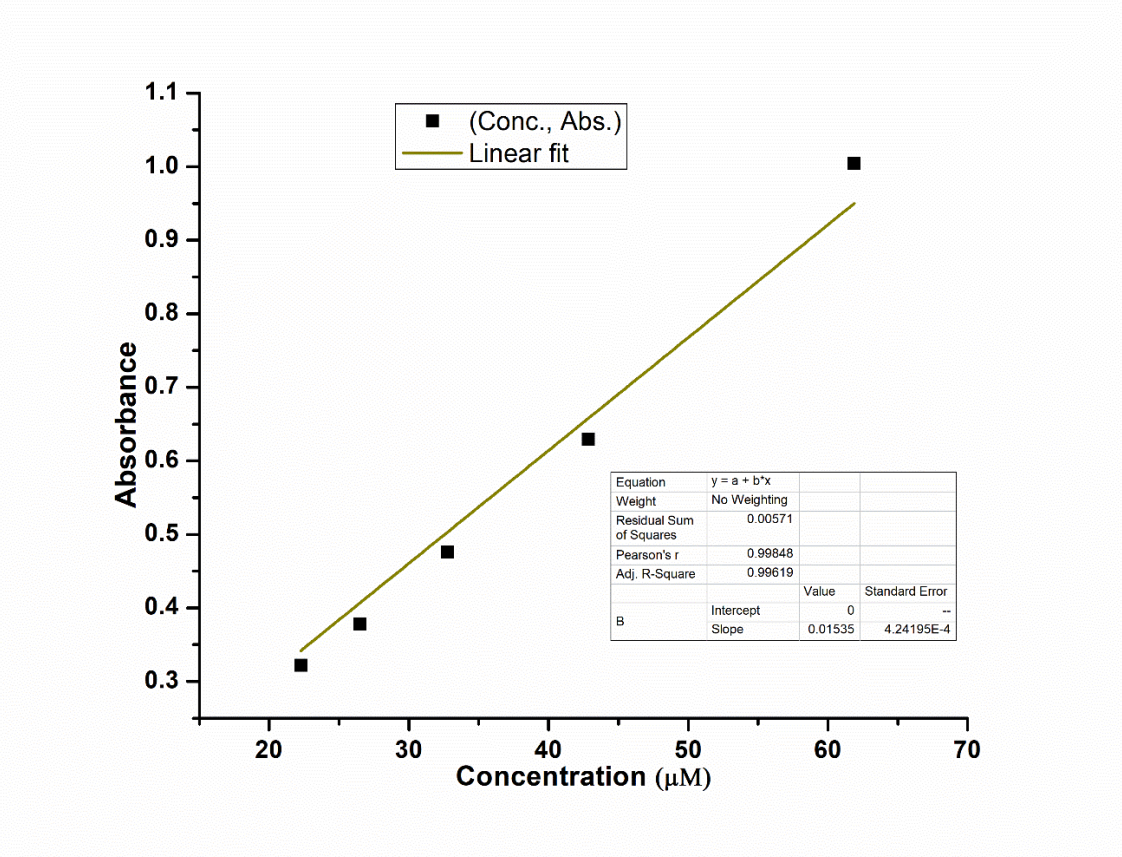


Fig. 22 UV absorption profile of P6

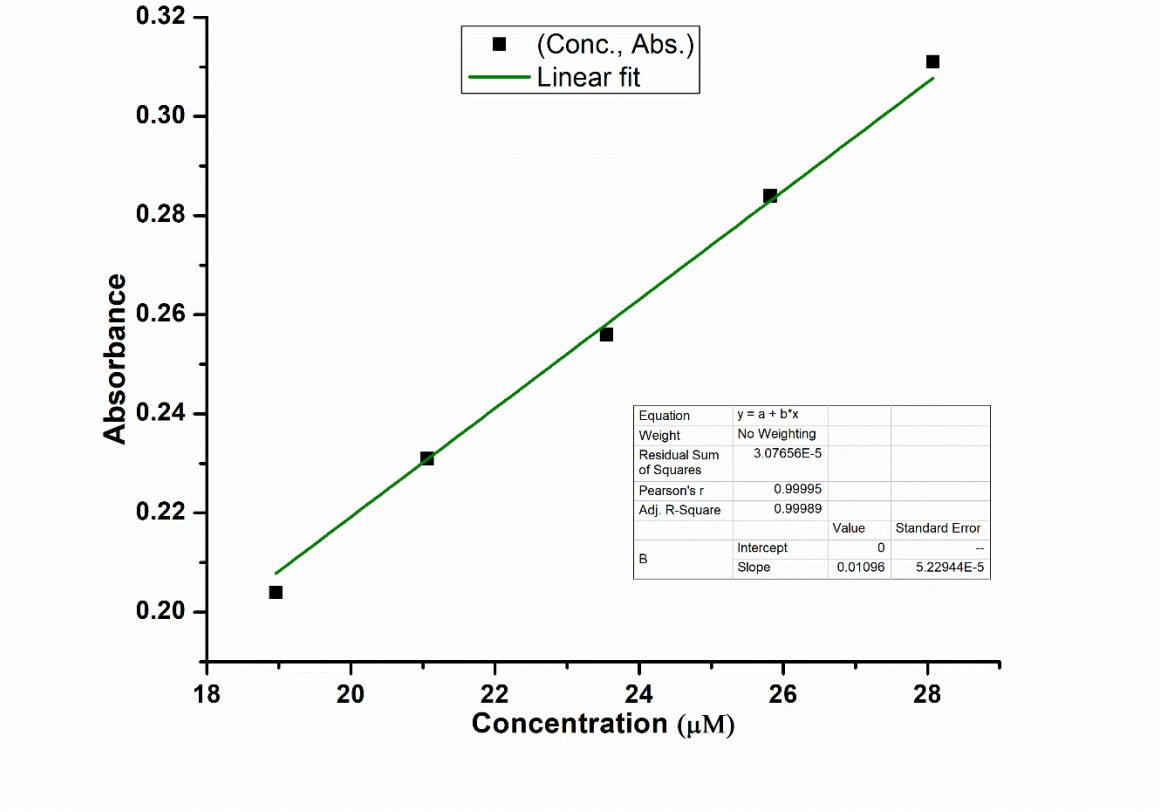


Fig. 23 UV absorption profile of P7

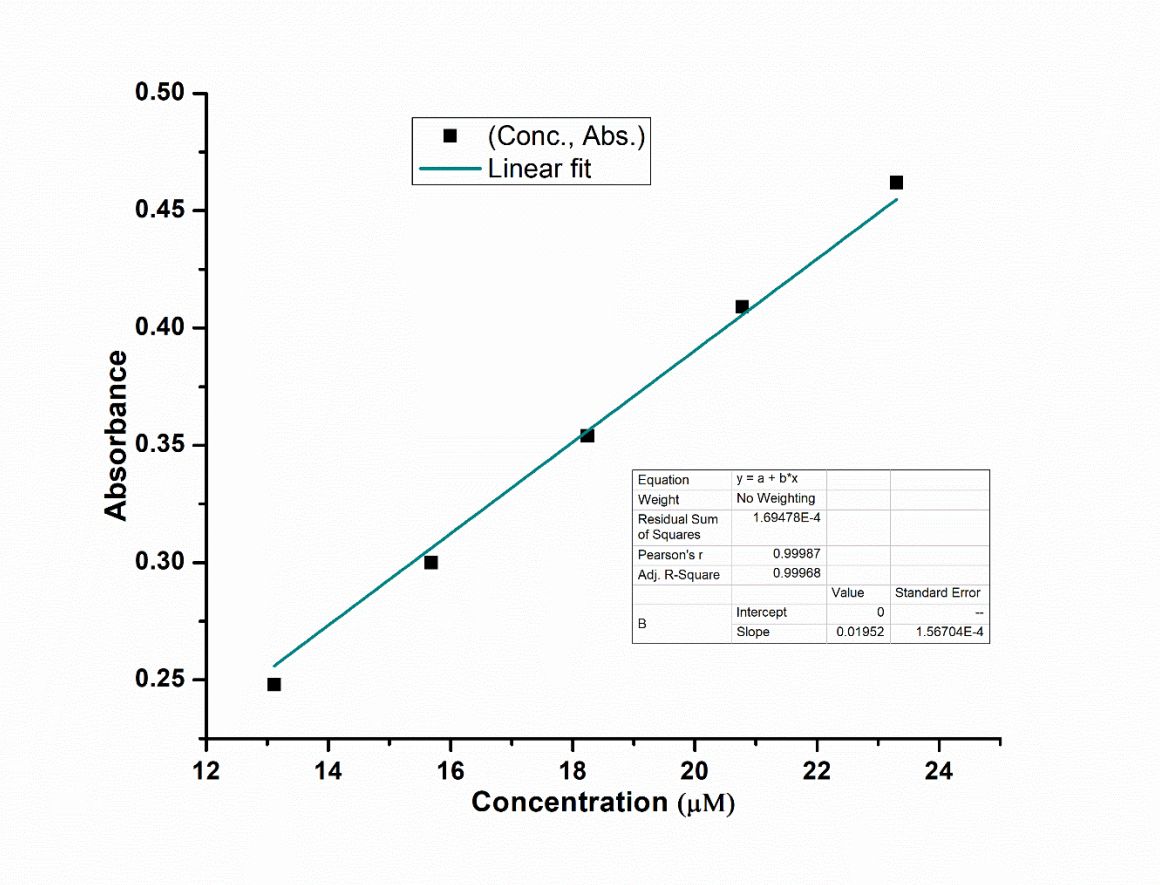


Fig. 24 UV absorption profile of P8

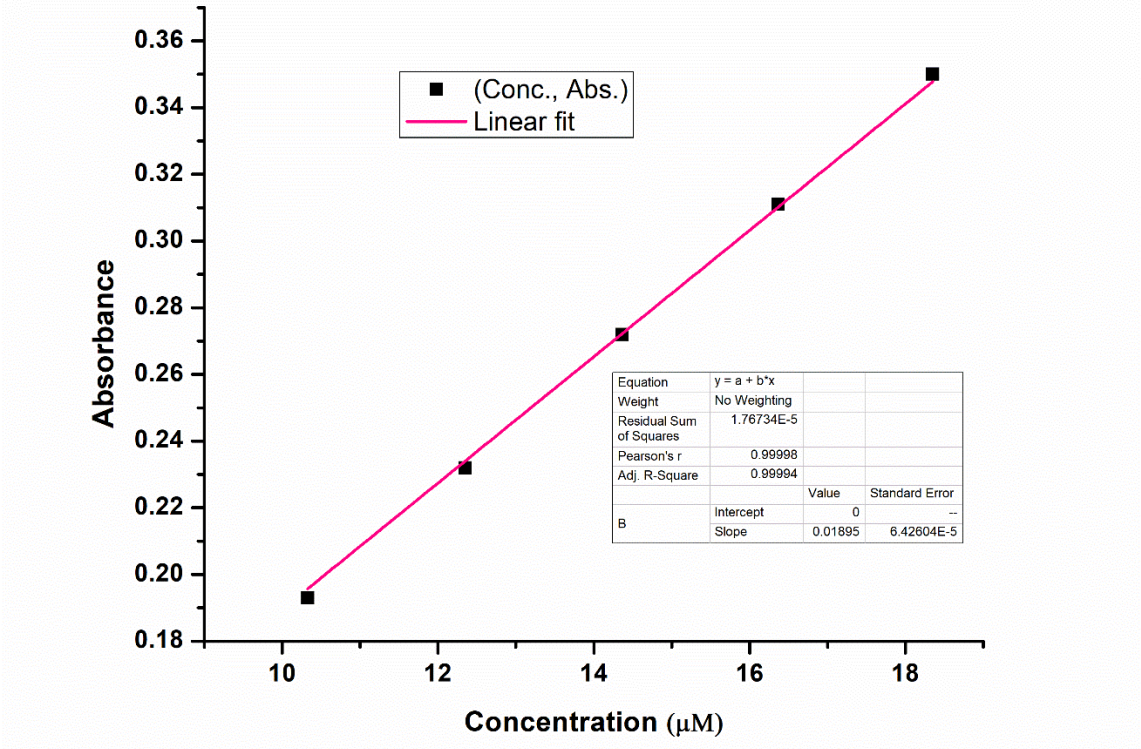


Fig. 25 UV absorption profile of P9

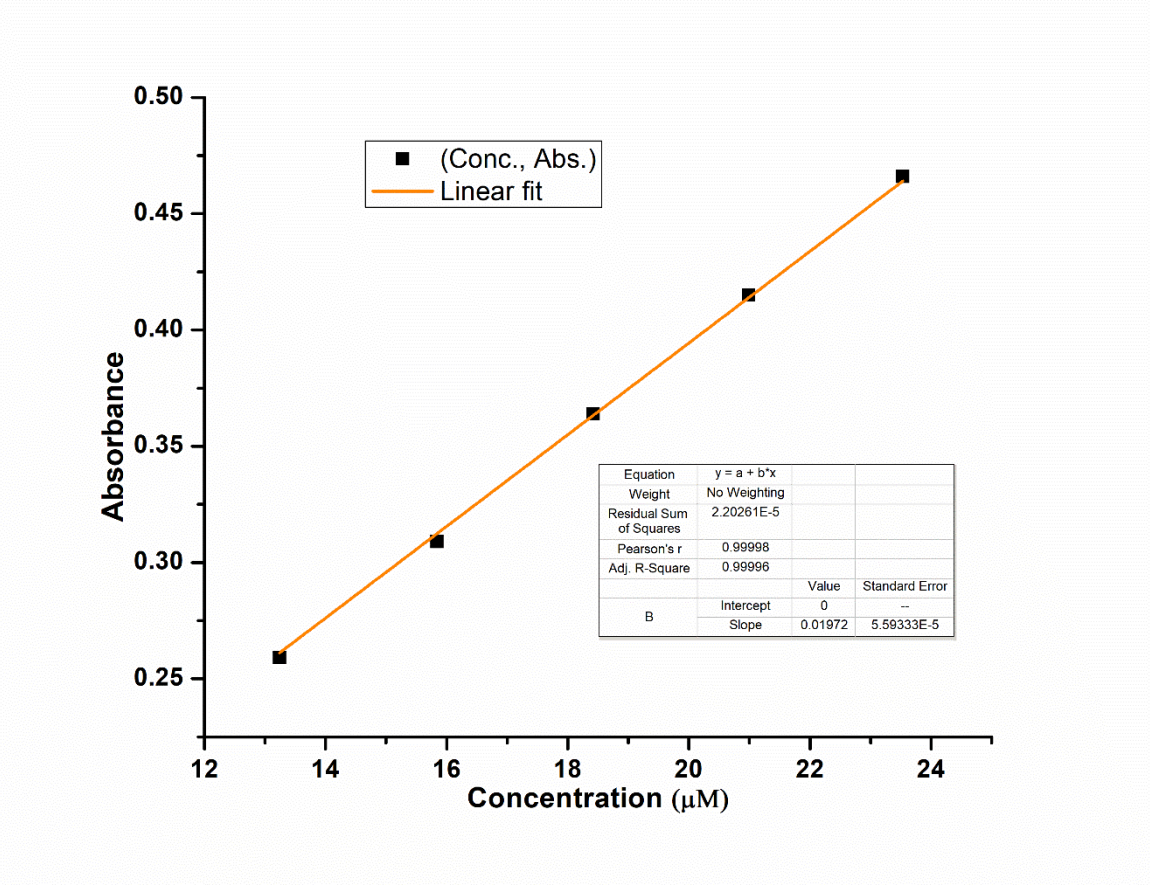


Fig. 26 UV absorption profile of P10

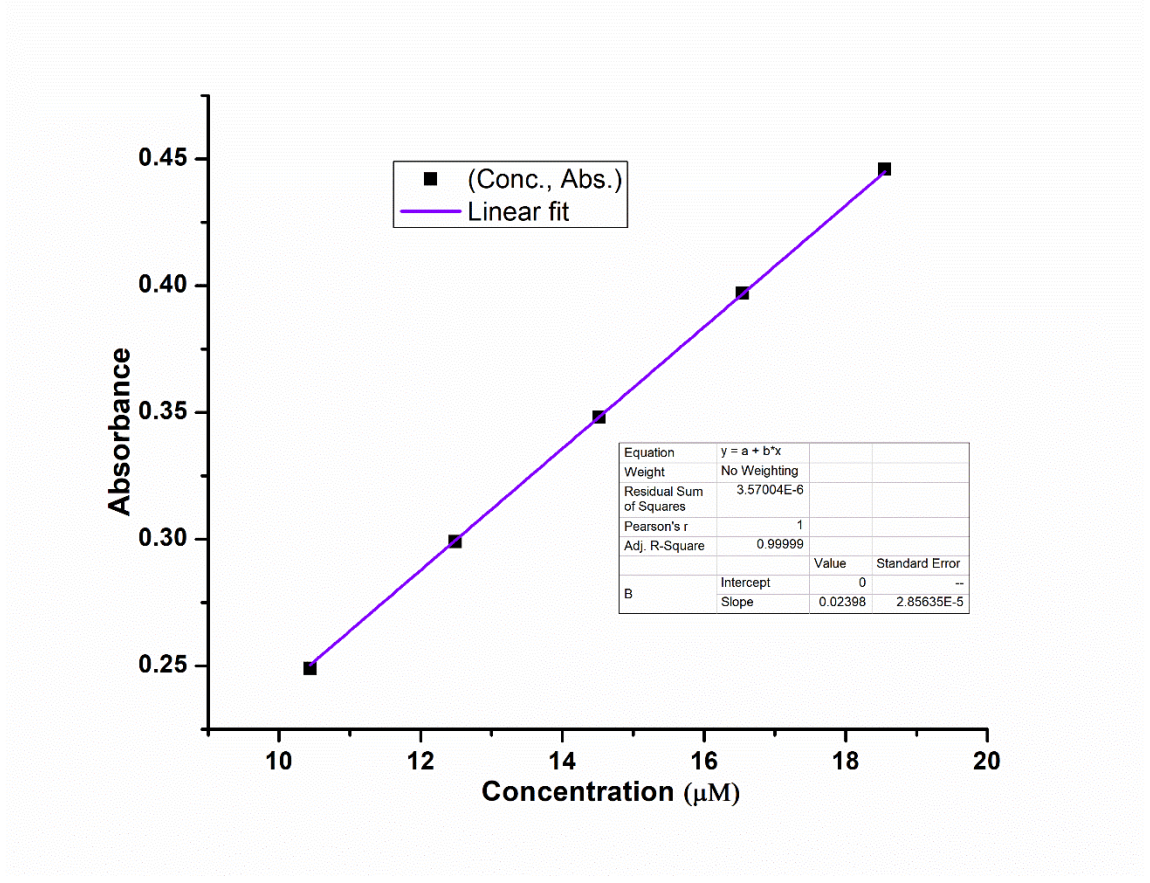
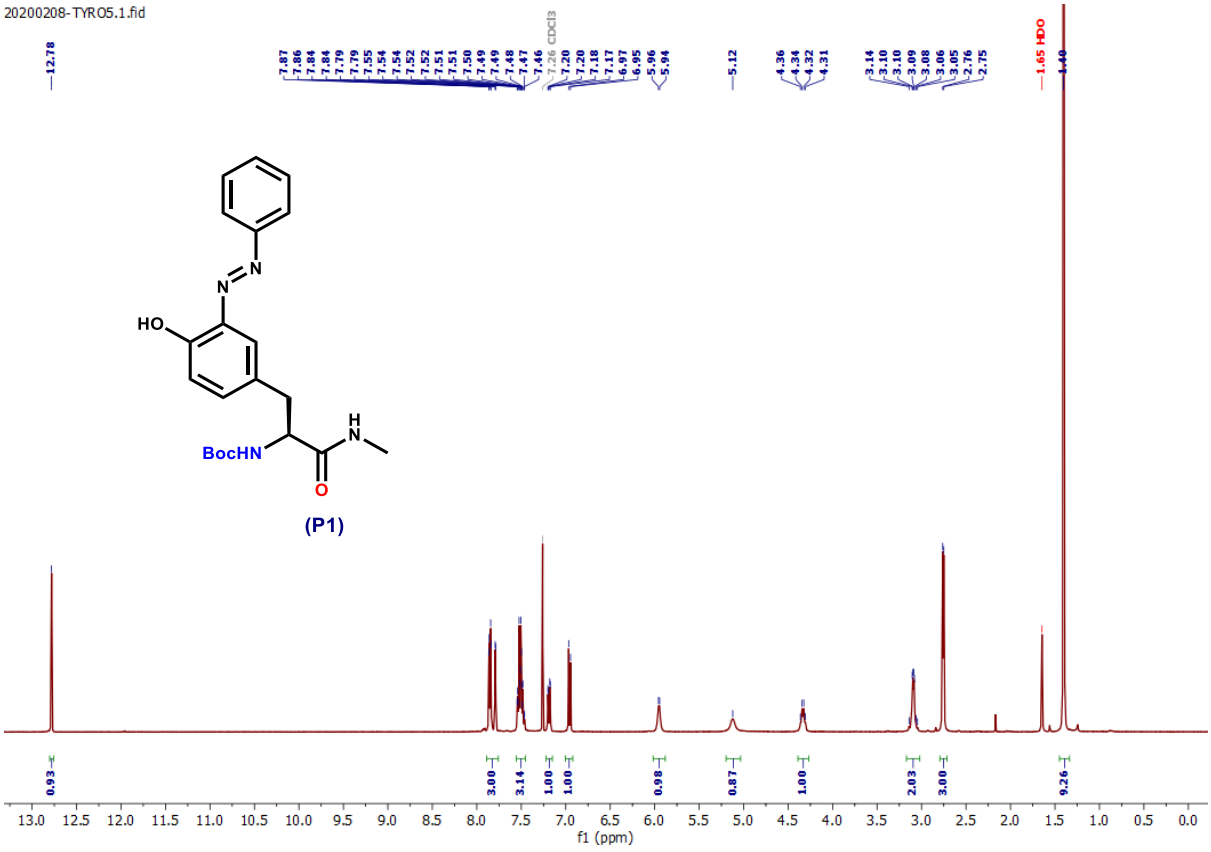
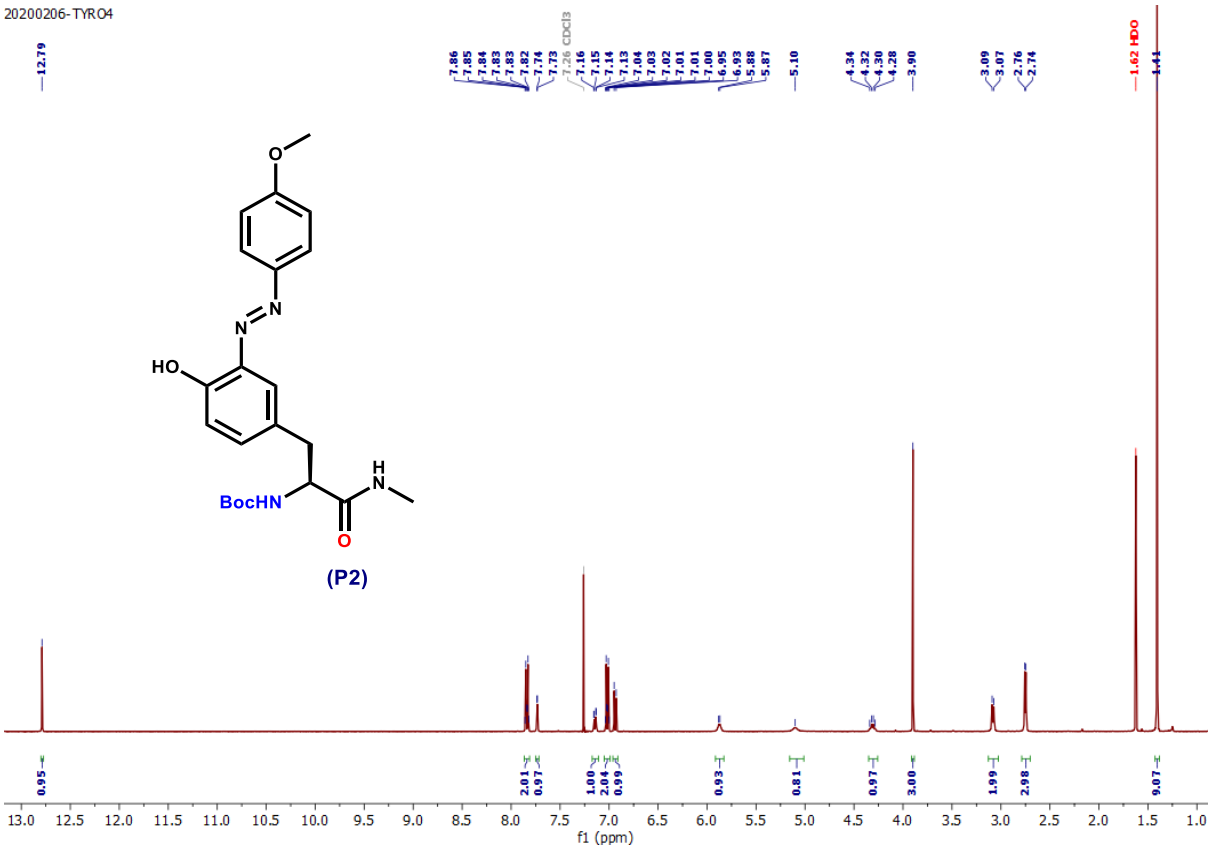
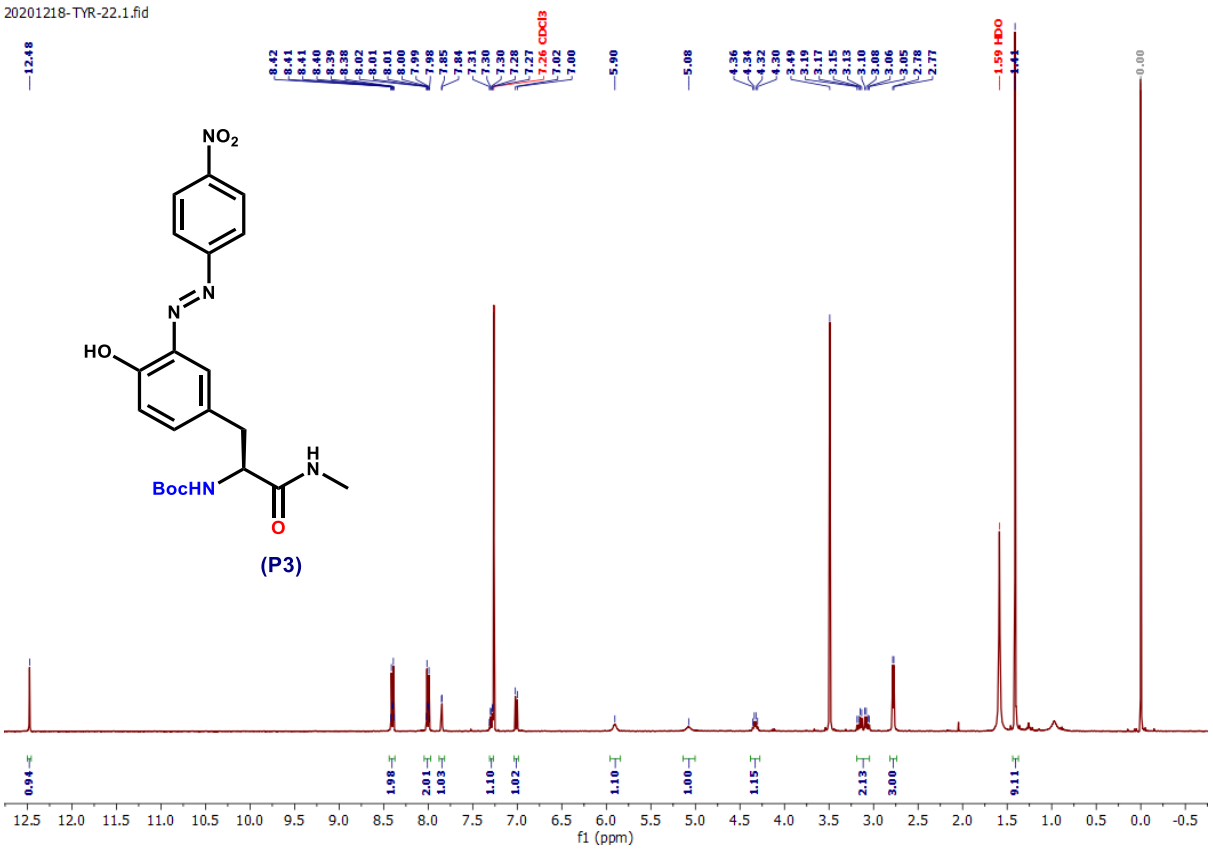


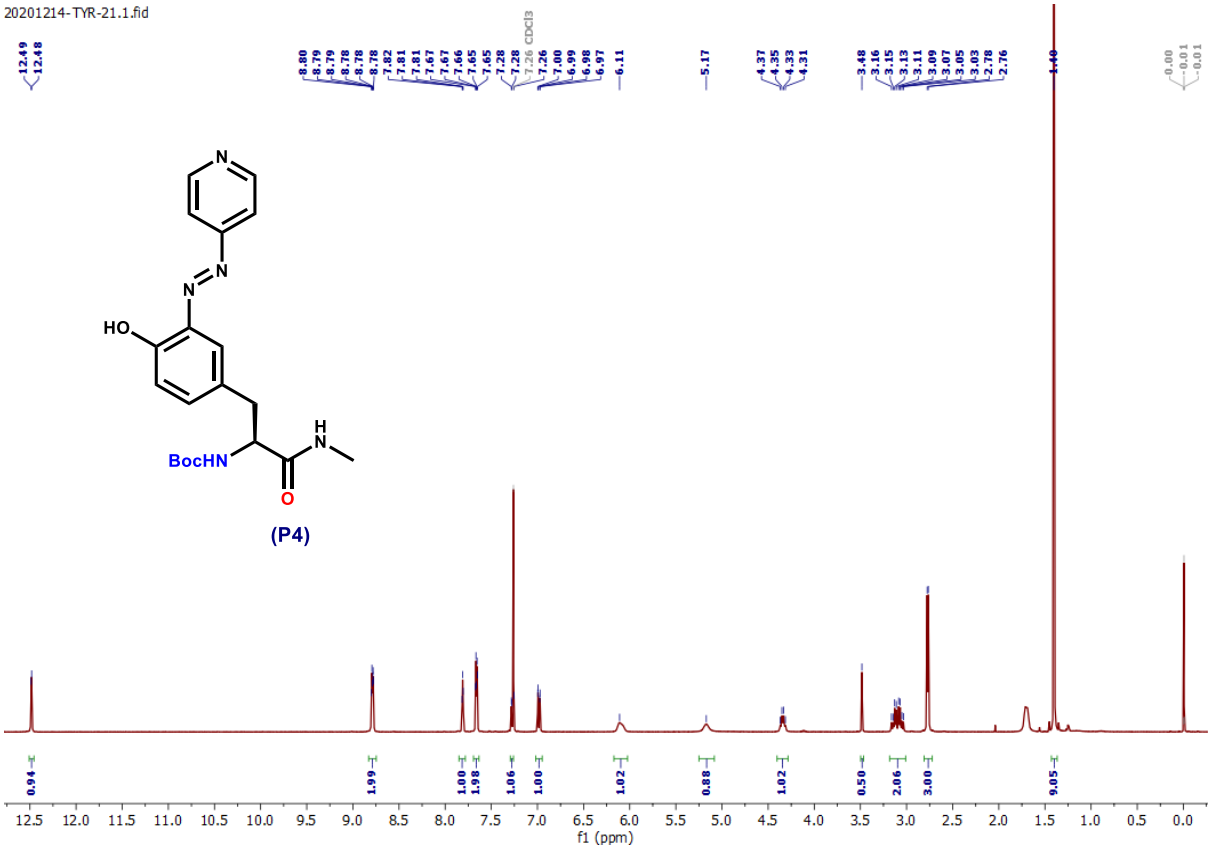
Fig. 27 UV absorption profile of P11

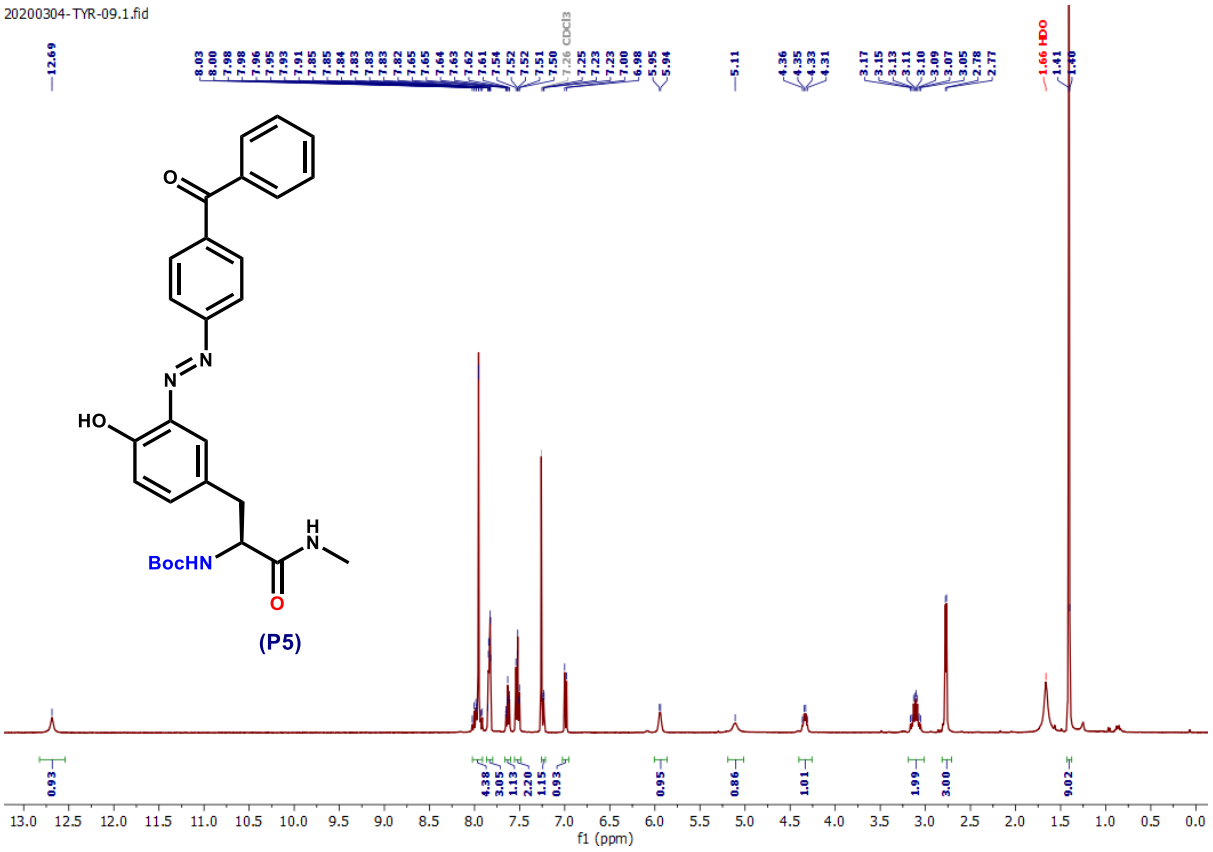
^{1}H NMR ^{13}C NMR and Mass Spectra of Compounds

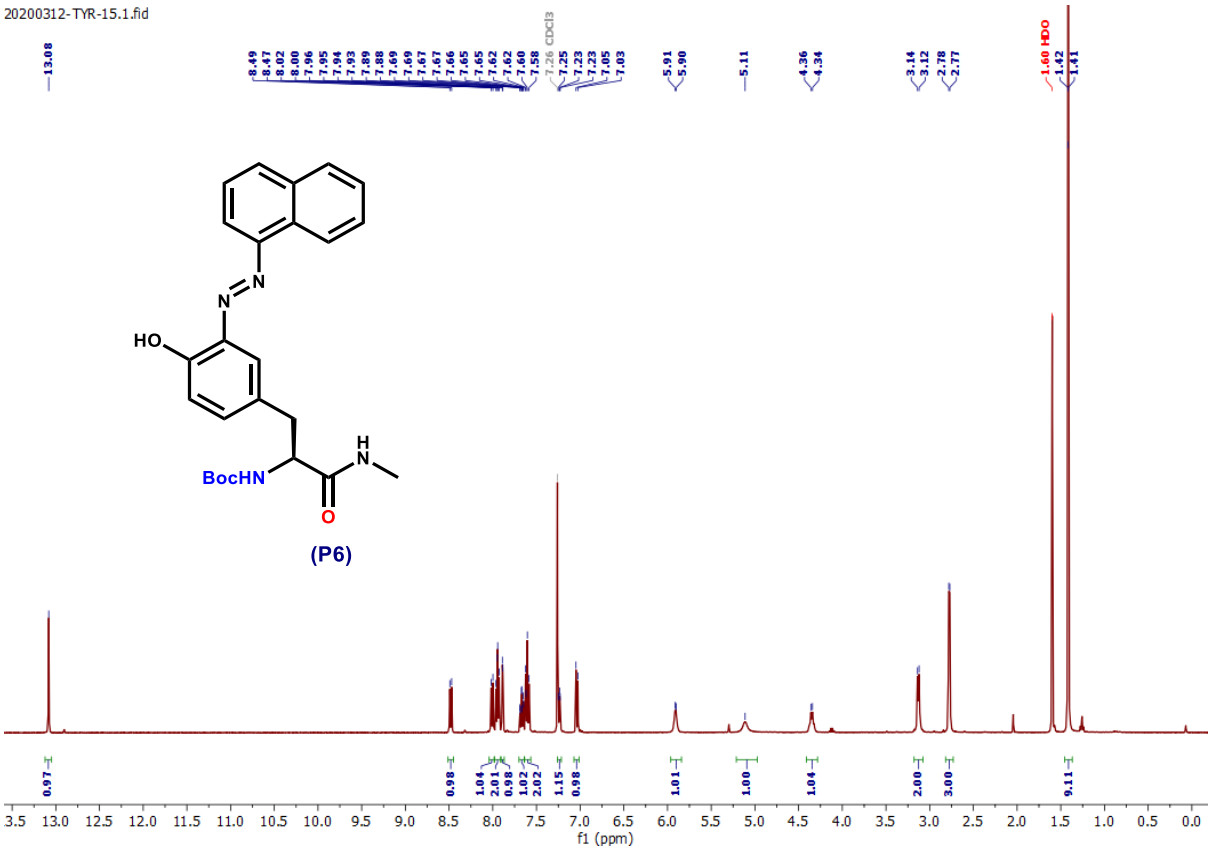


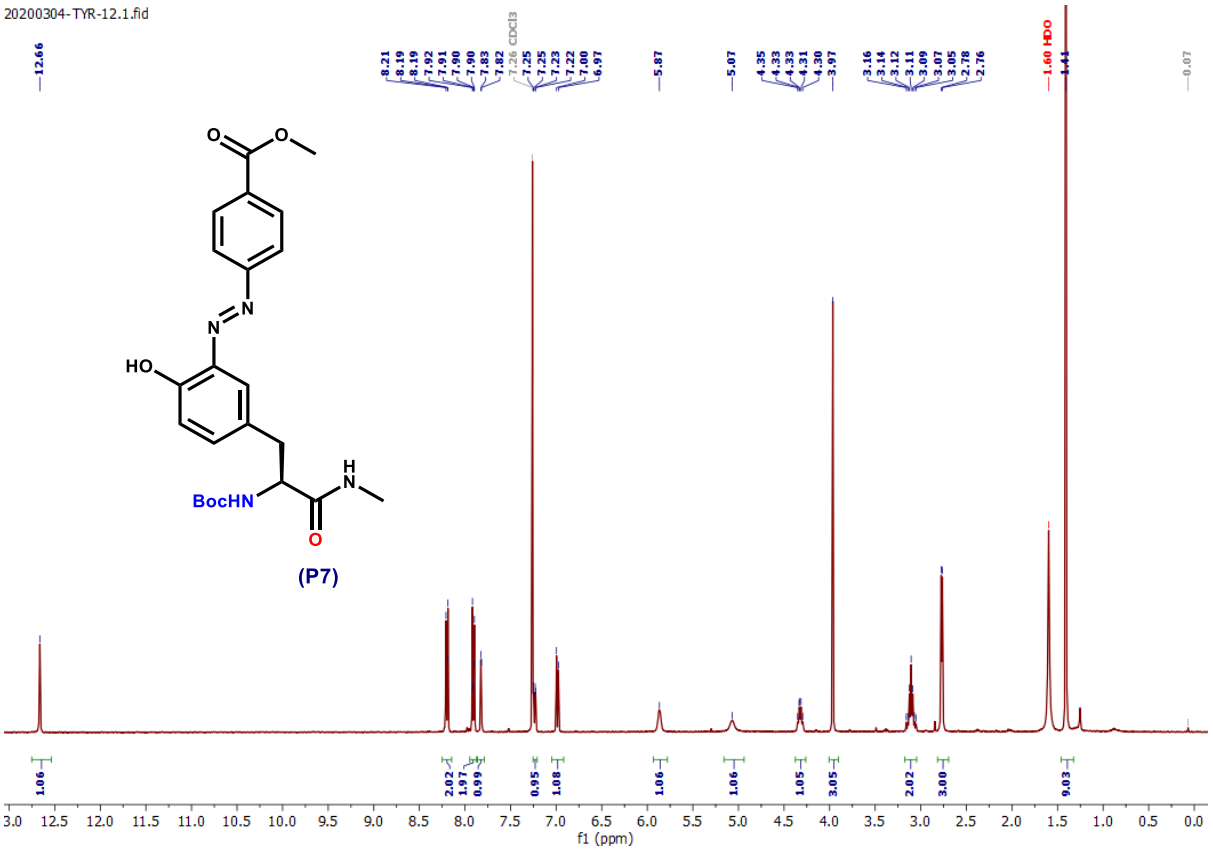


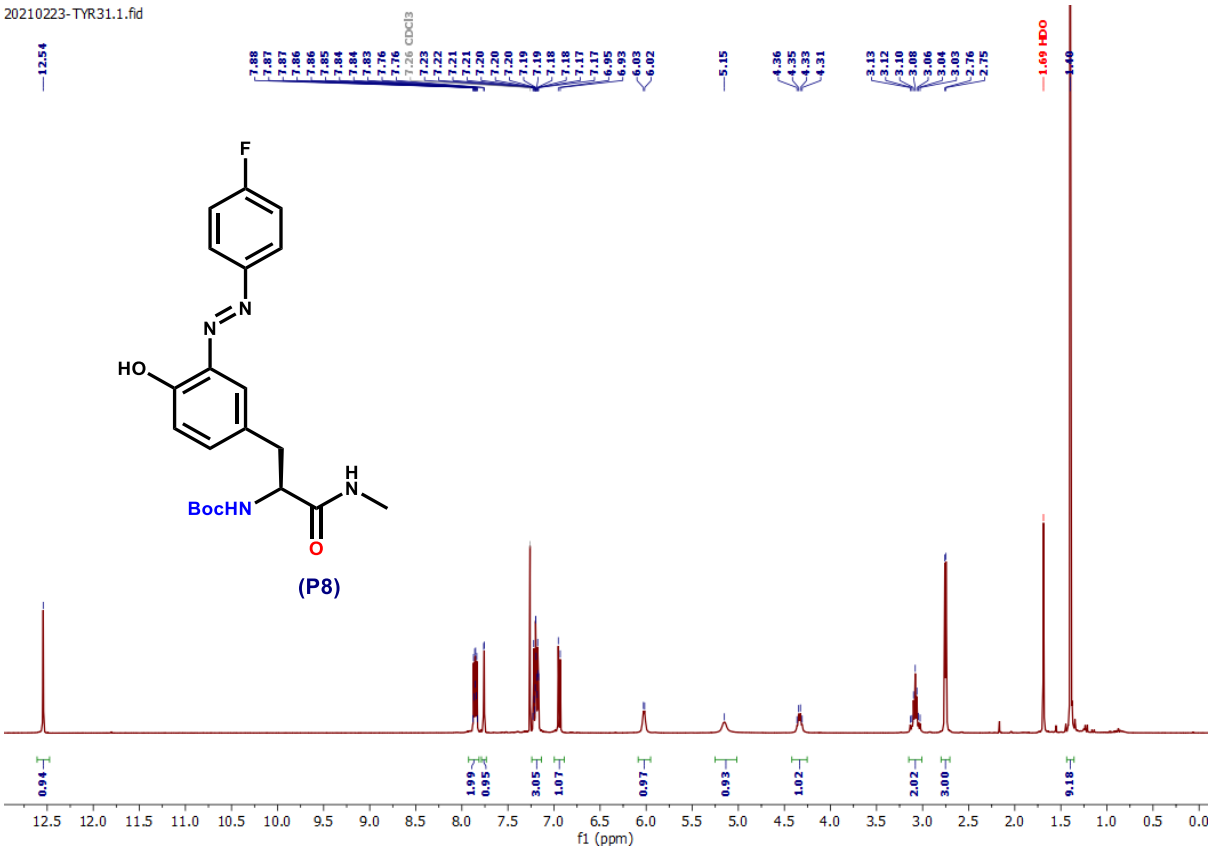

20201218-TYR-22.1.fid
NO2
N
N
HO
BocHN
H
N
O
(P3)
f1 (ppm)

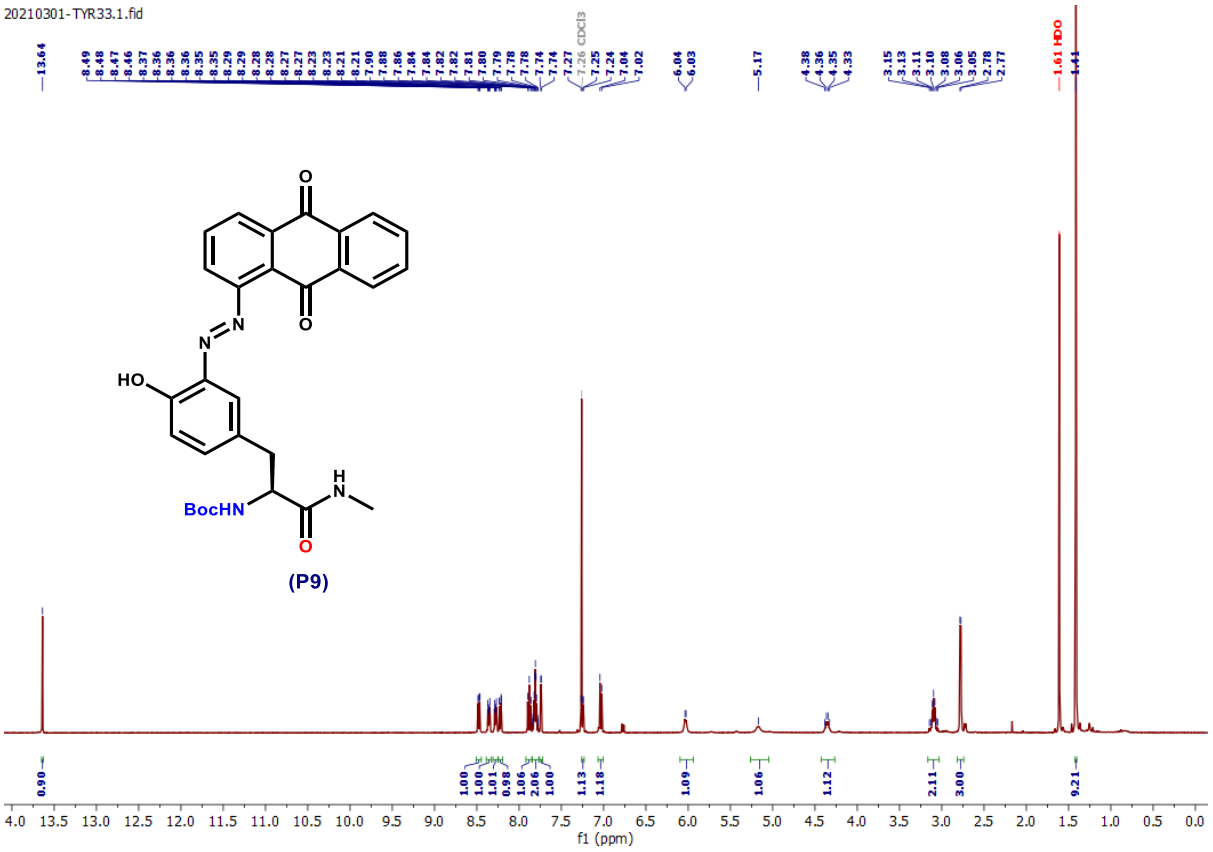

20201214-TYR-21.1.fid
N
N
HO
BocHN
H
N
O
(P4)
f1 (ppm)

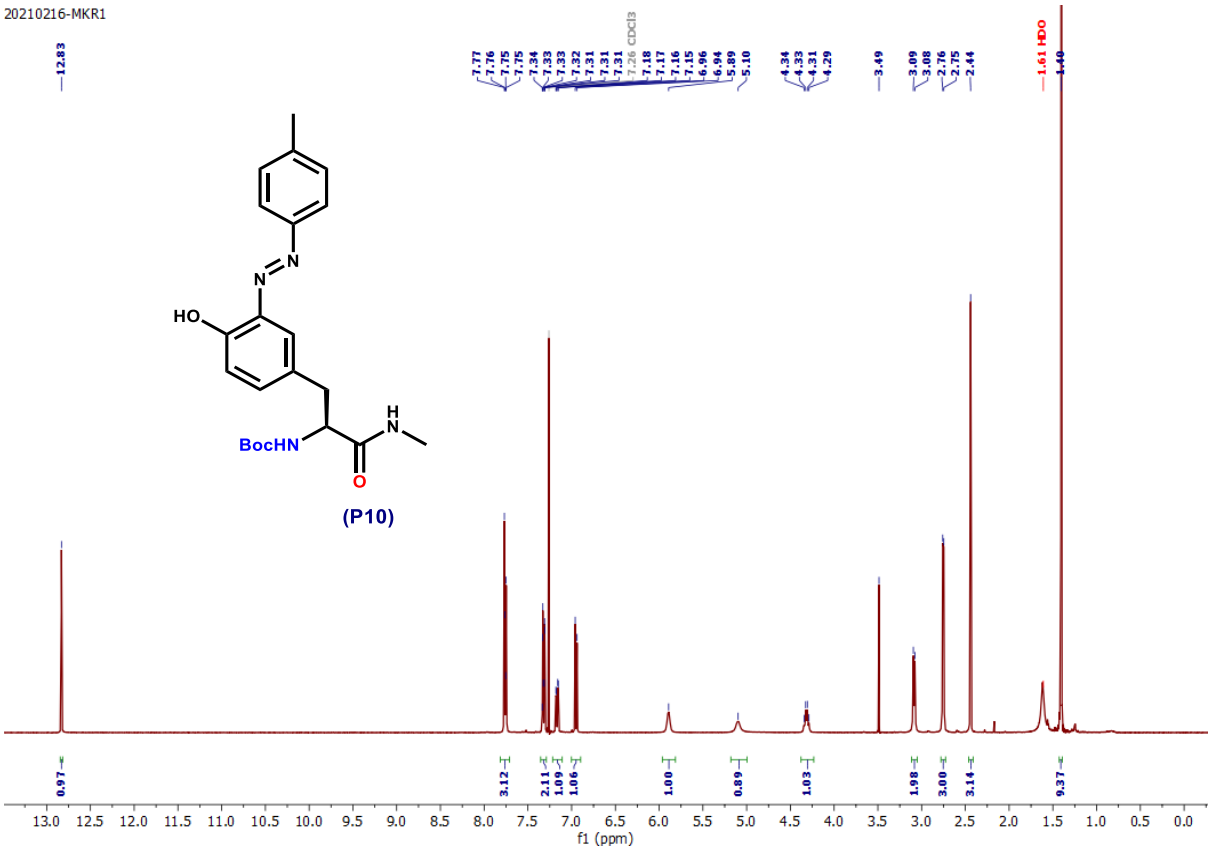

20200304-TYR-09.1.fid
O
N
N
HO
BocHN
H
N
O
(P5)
f1 (ppm)

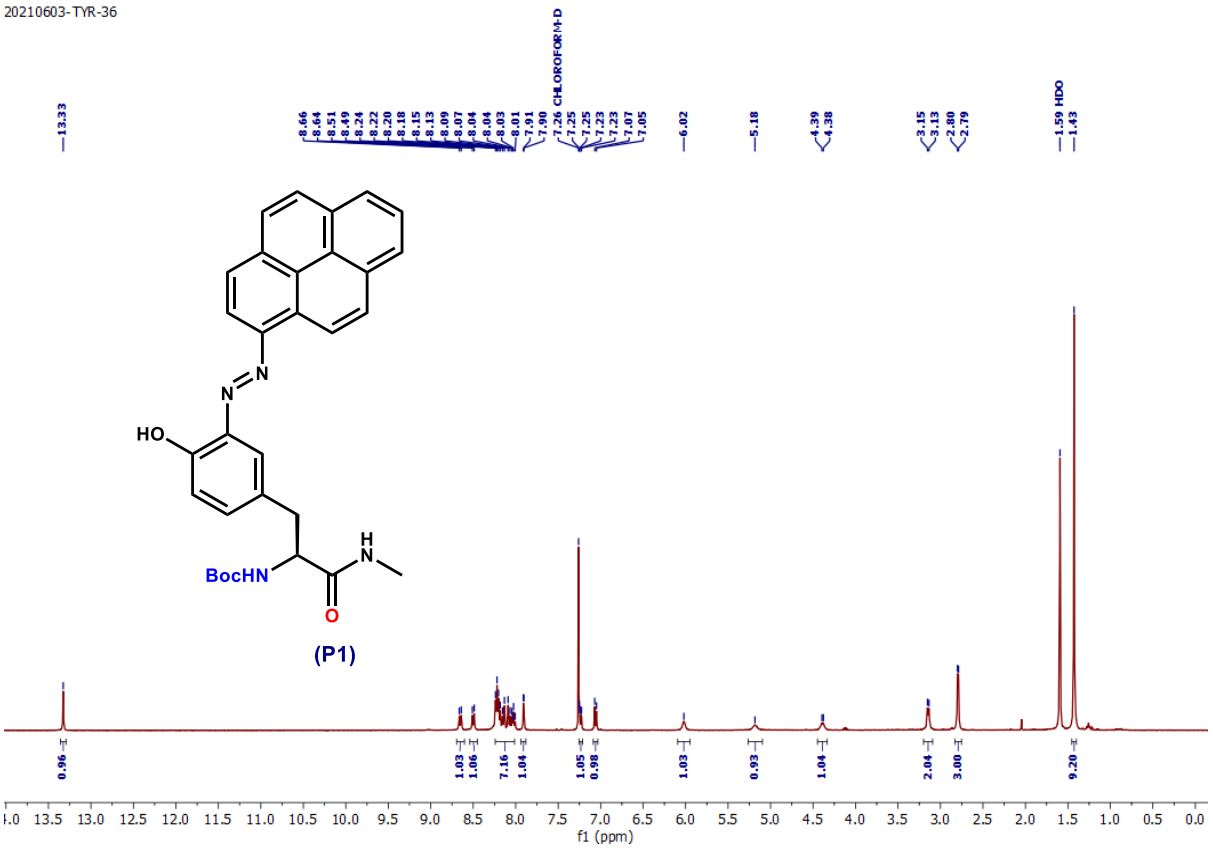

20200312-TYR-15.1.fid
N
N
HO
BocHN
H
N
O
(P6)
f1 (ppm)

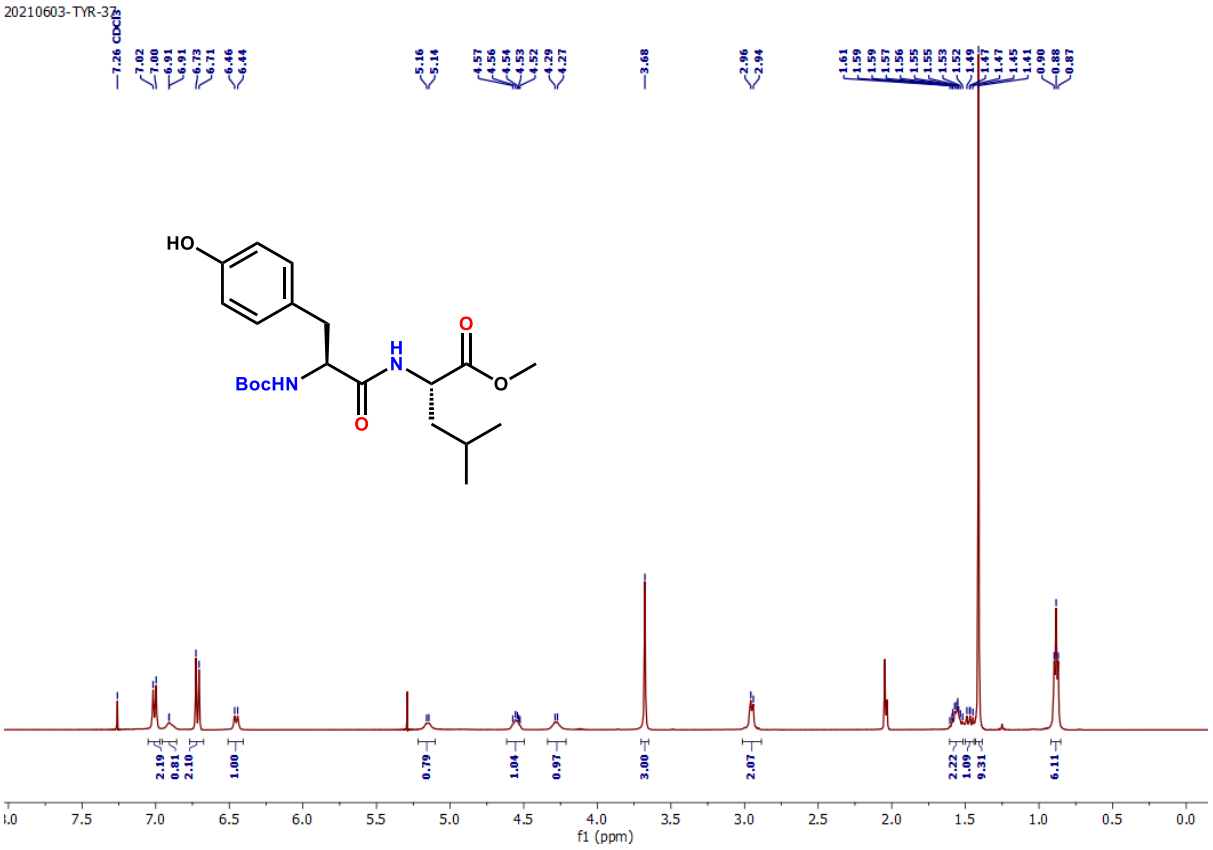

20200304-TYR-12.1.fid
HO
BocHN
(P7)
f1 (ppm)

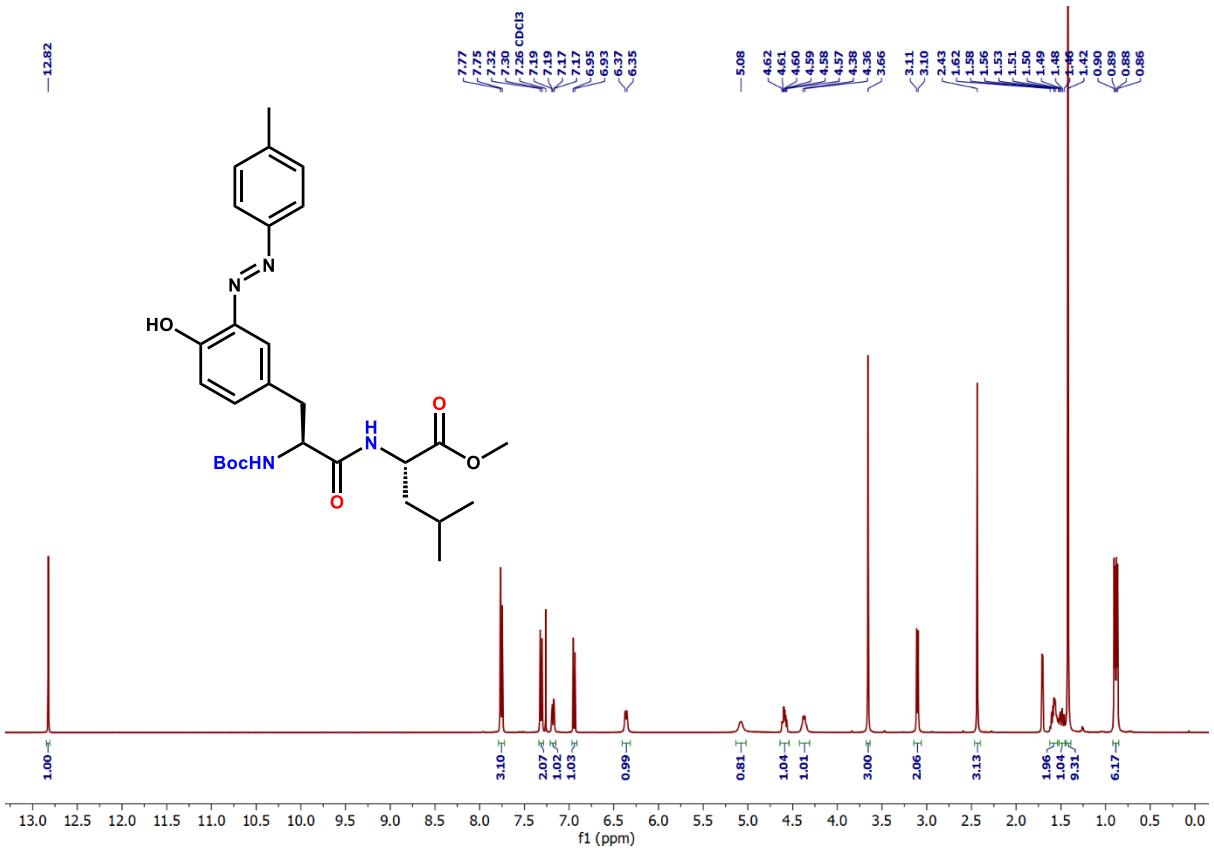

20210223-TYR31.1.fid
F
HO
BocHN
(P8)
f1 (ppm)

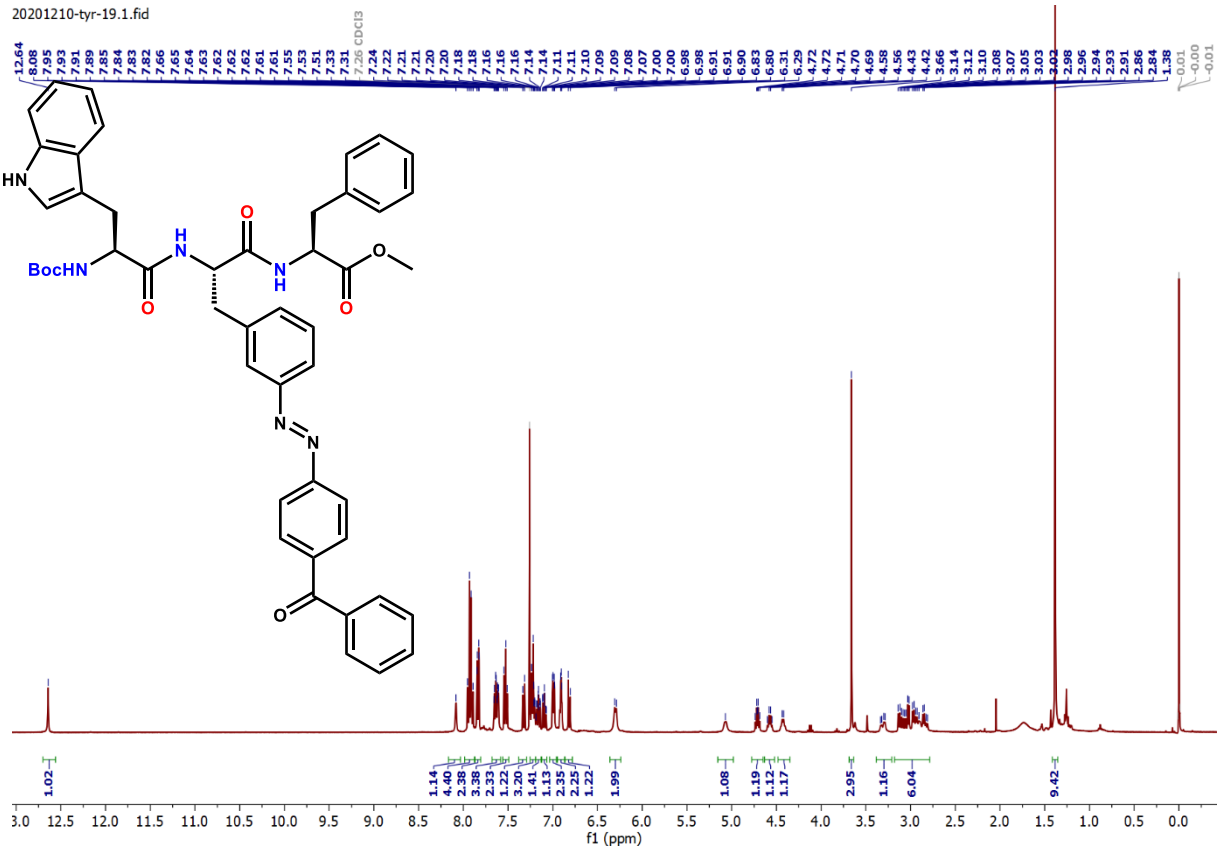

20210301-TYR33.1.fid
HO
BocHN
O
(P9)
f1 (ppm)


20210216-MKR1
HO
BocHN
O
(P10)
f1 (ppm)

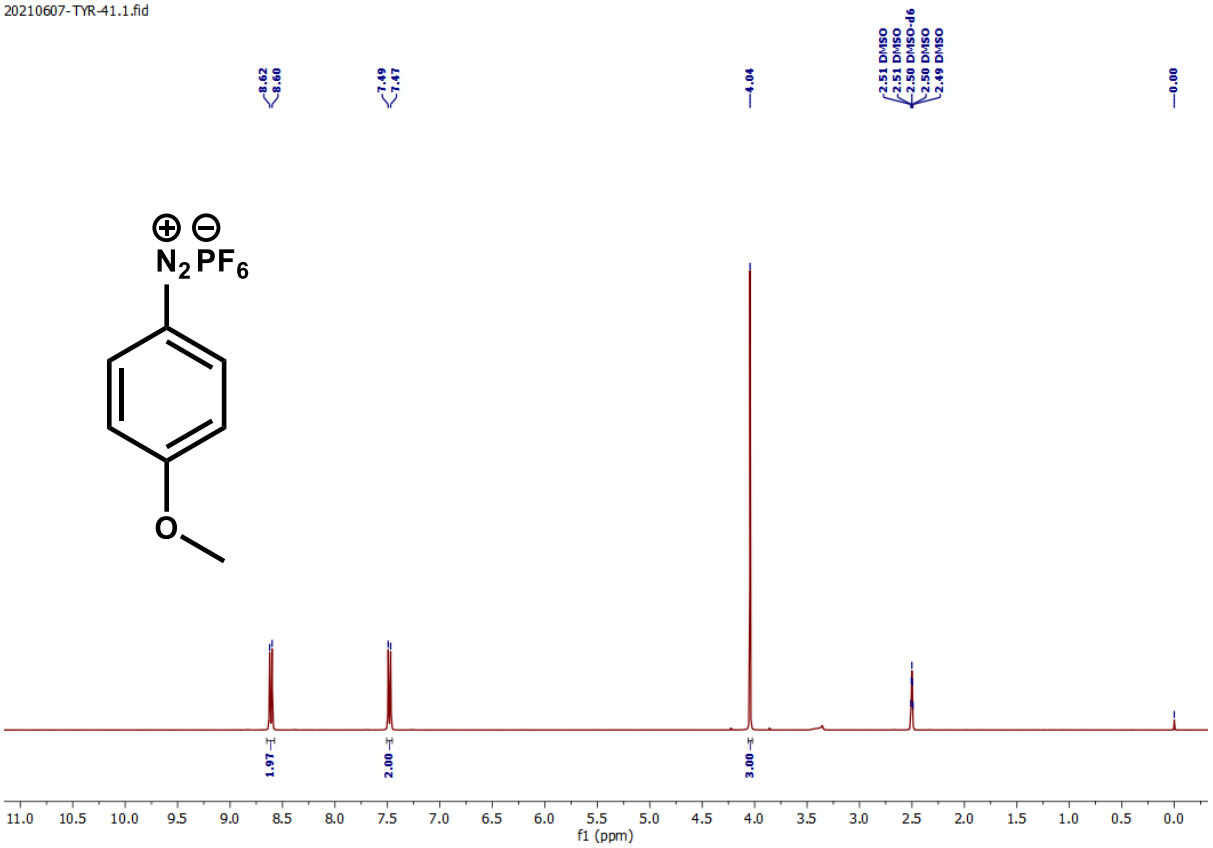

20210603-TYR-36
HO
N
N
BocHN
H
N
O
(P1)
f1 (ppm)


20210603-TYR-37
HO
BocHN
H
N
O
O
O
f1 (ppm)

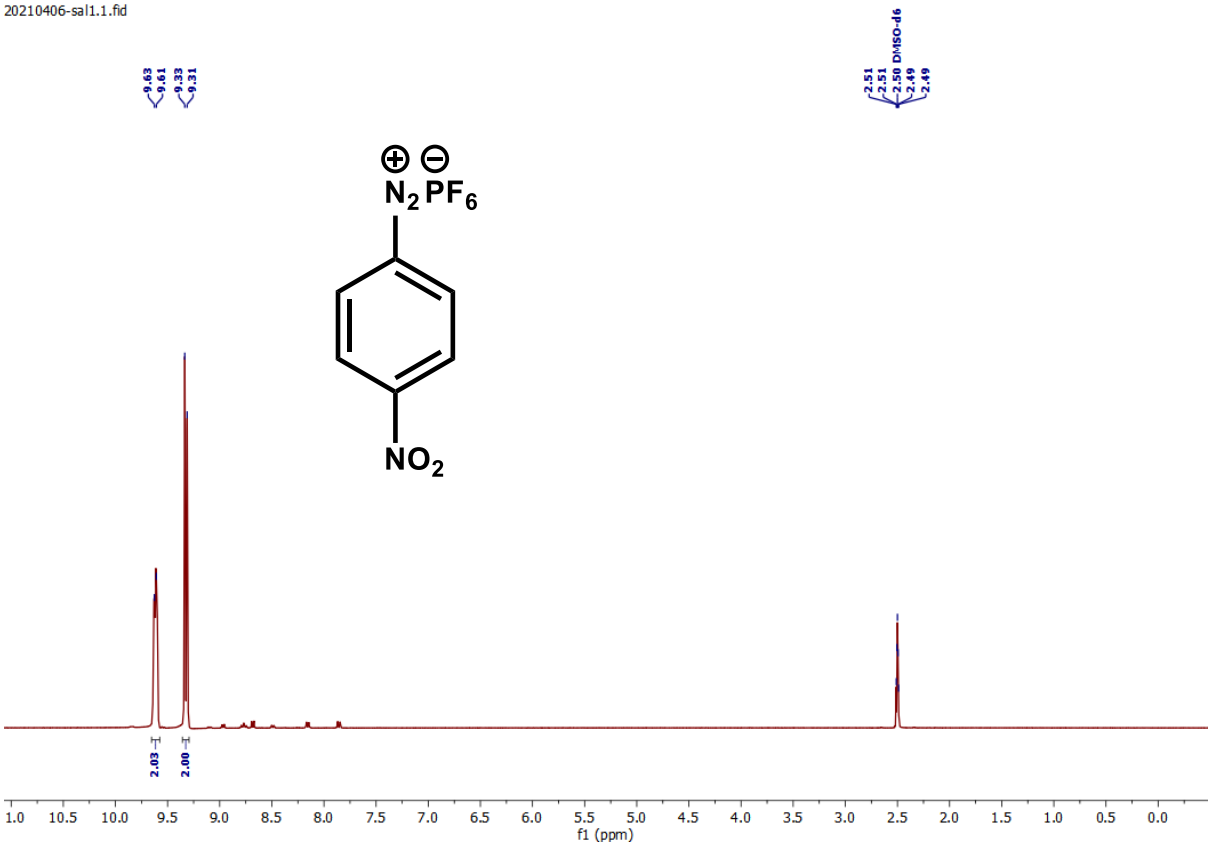

HO
BocHN
f1 (ppm)


20201210-tyr-19.1.fid
HN
BocHN
f1 (ppm)

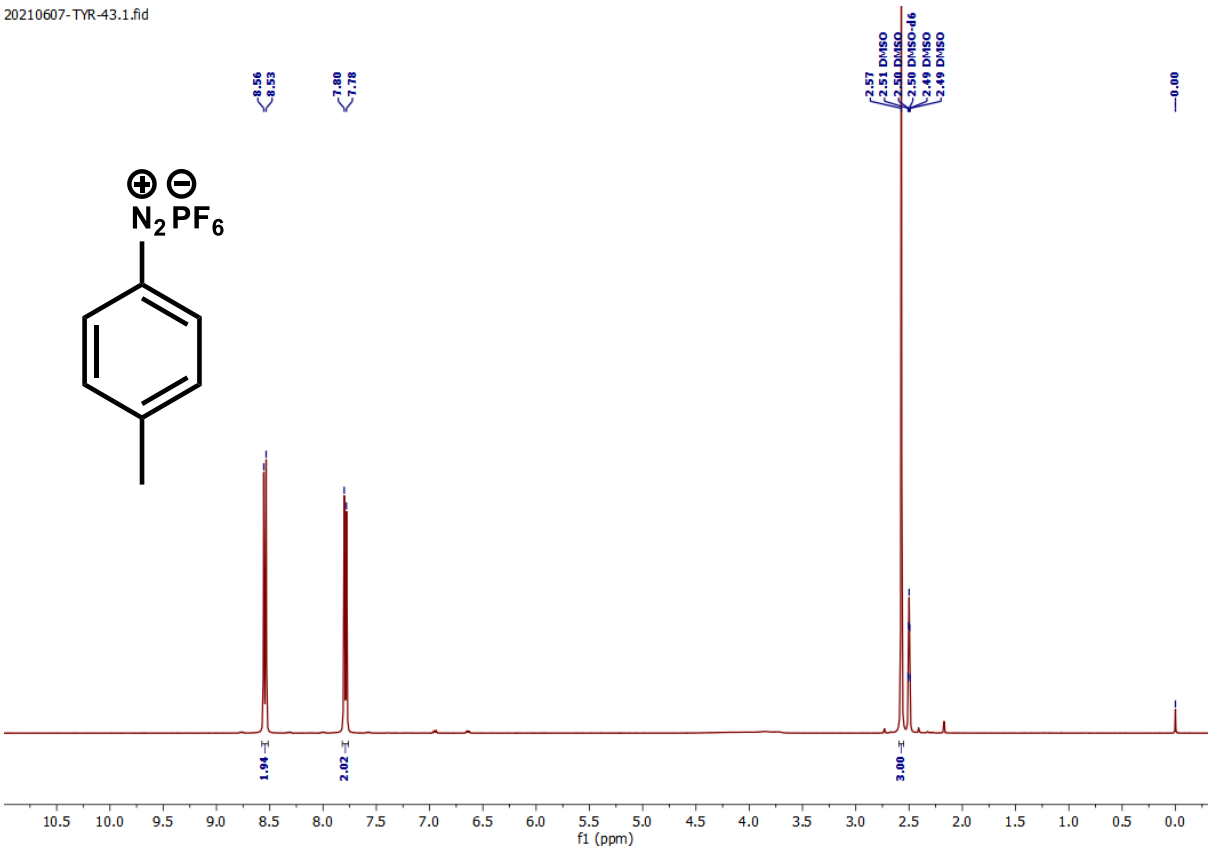
20210607-TYR-41.1.fid



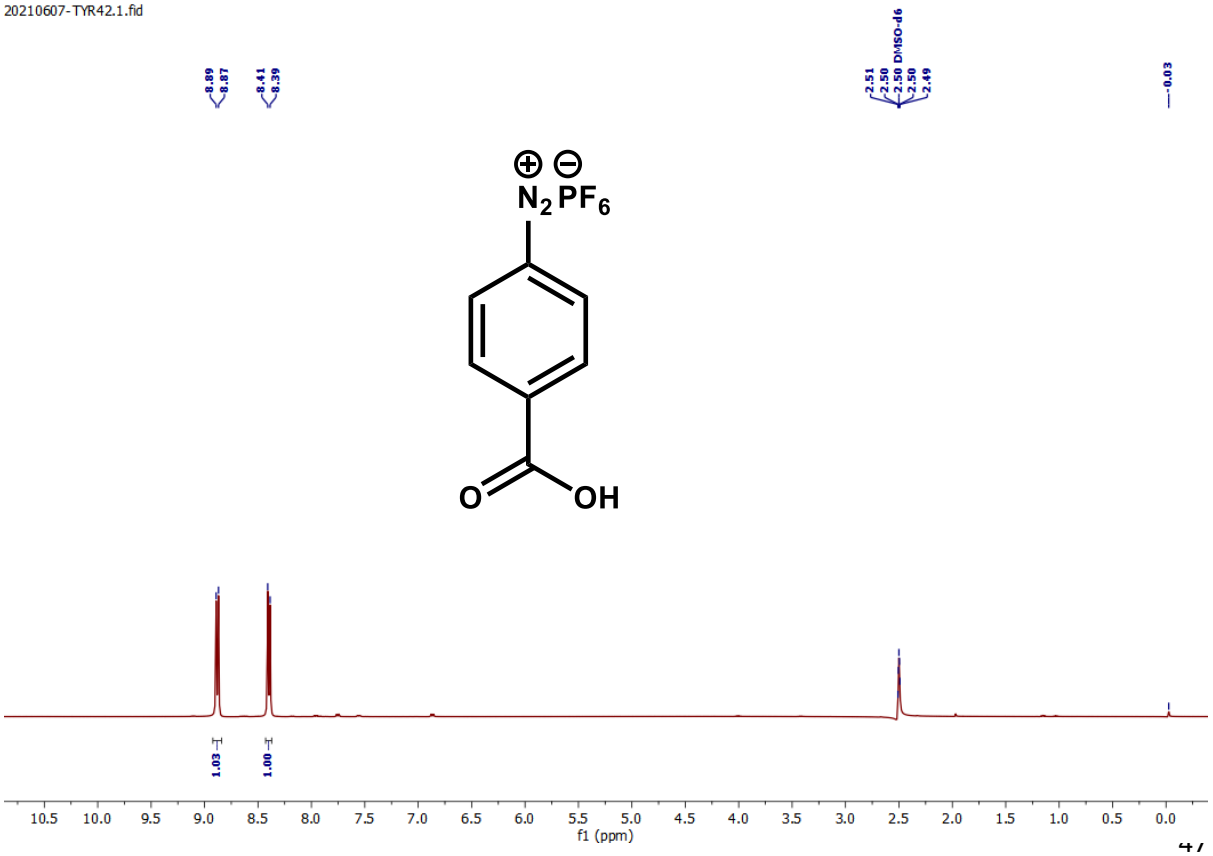
20210406-sal1.1.fid

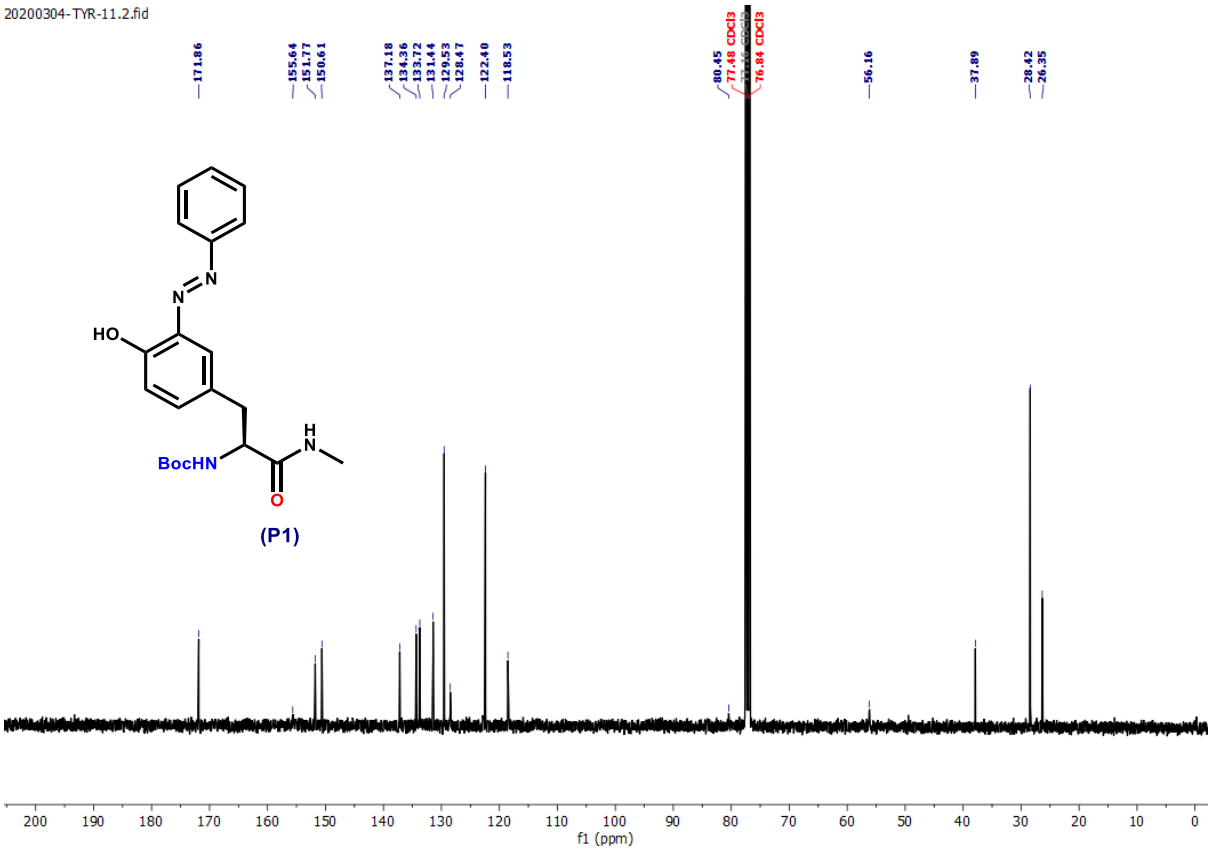


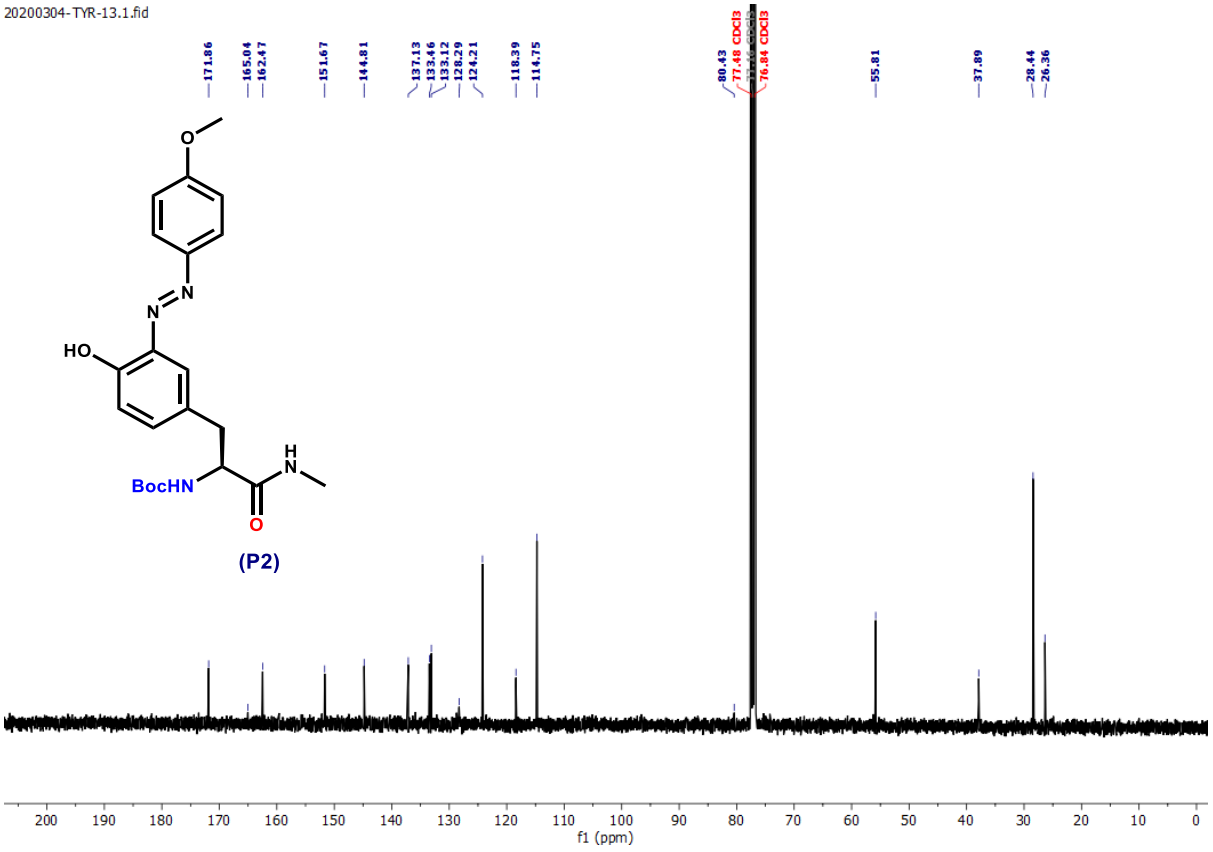
20210607-TYR-43.1.fid



20210607-TYR42.1.fid




20200304-TYR-11.2.fid
171.86
155.64
151.77
150.61
137.18
134.36
133.72
131.44
129.53
128.47
122.40
118.53
80.45
77.48 CDCl3
76.84 CDCl3
56.16
37.89
28.42
26.35
HO
N
N
BocHN
H
N
O
(P1)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


20200304-TYR-13.1.fid
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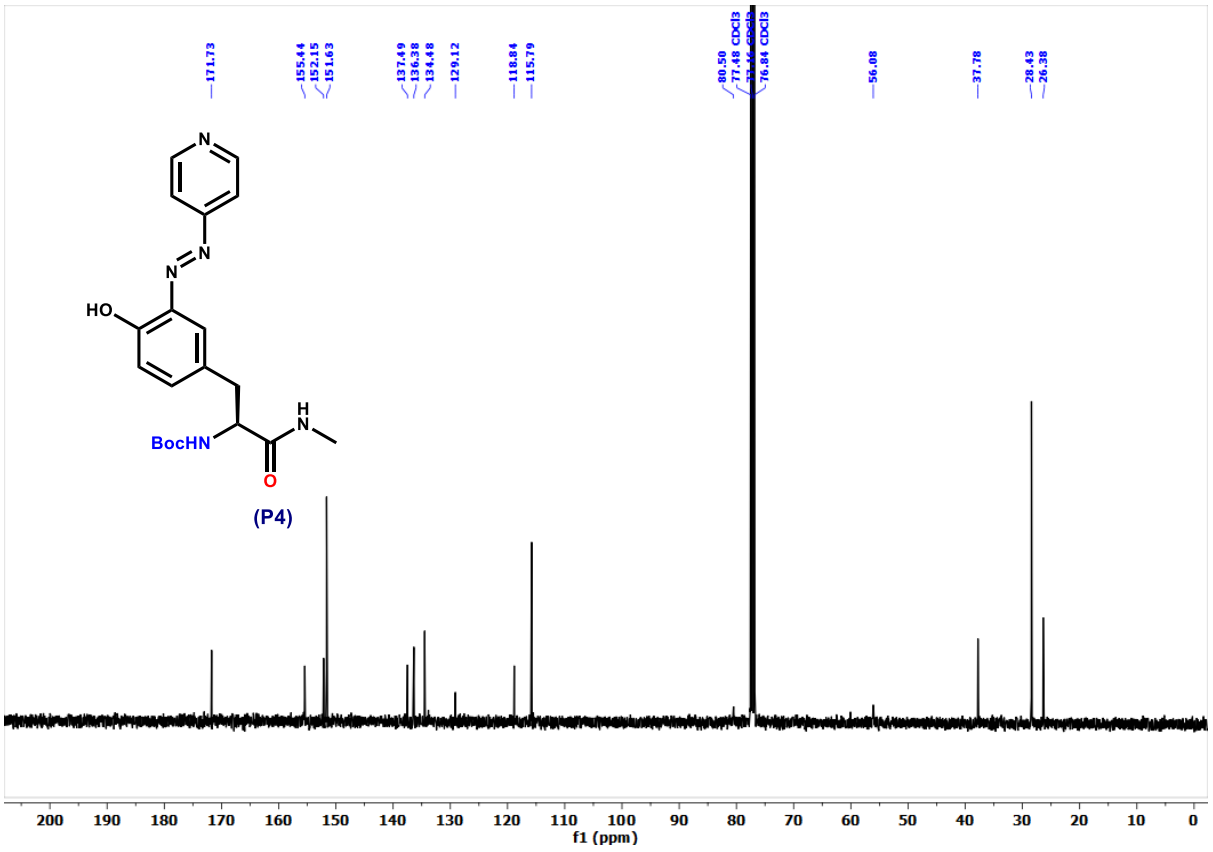
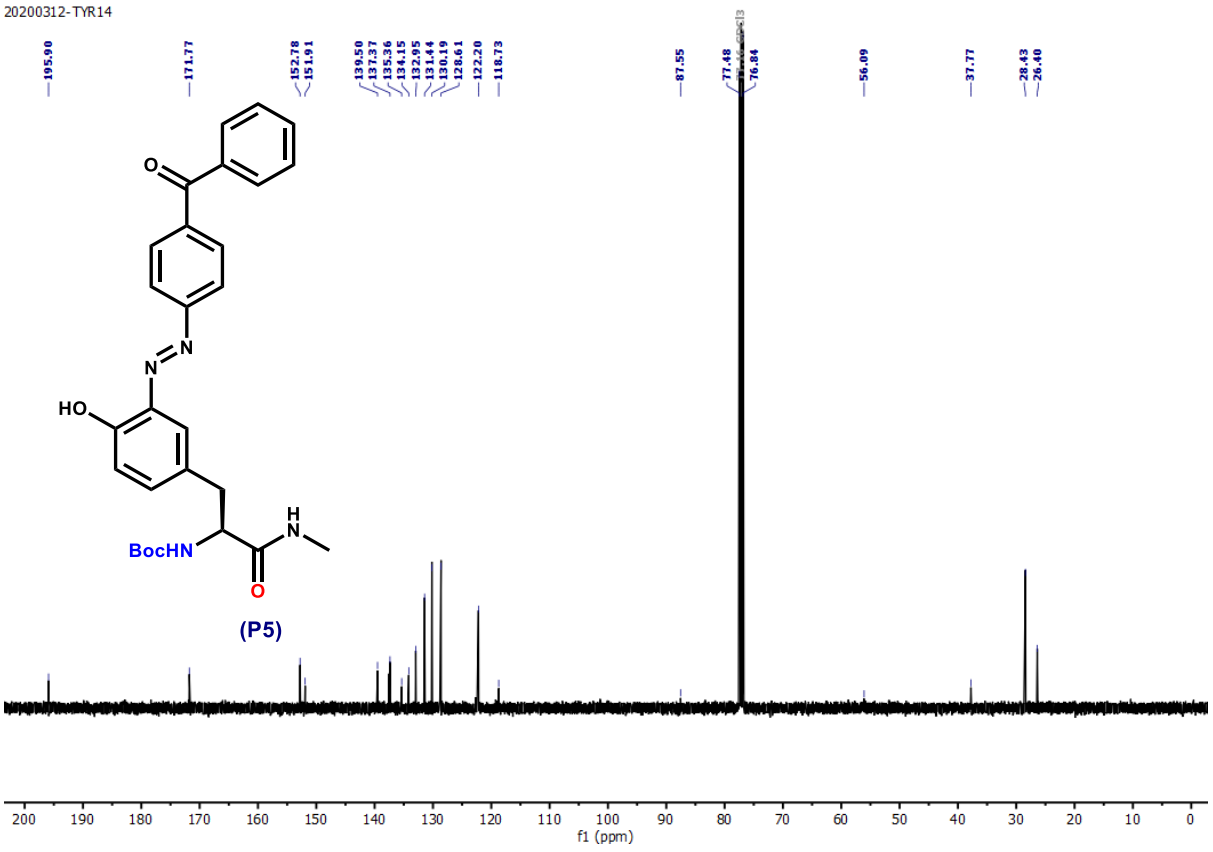
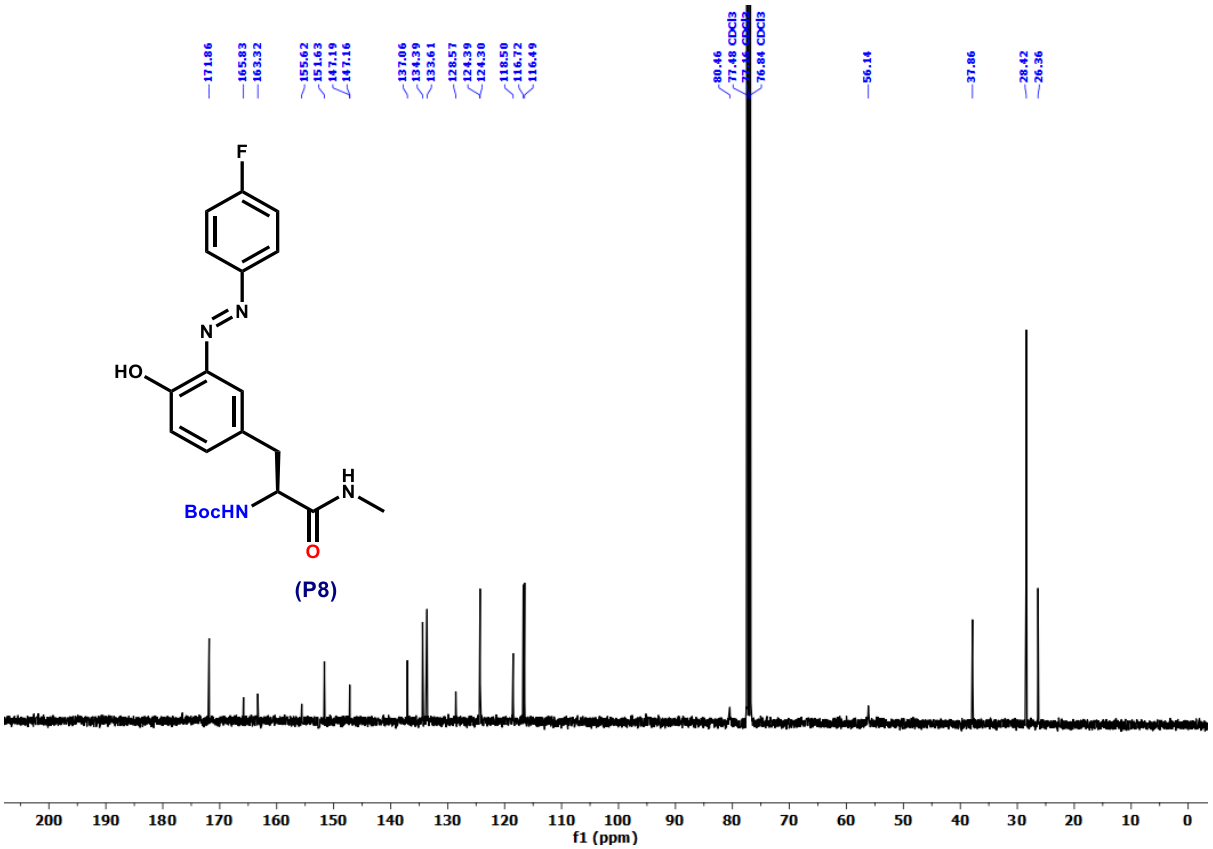
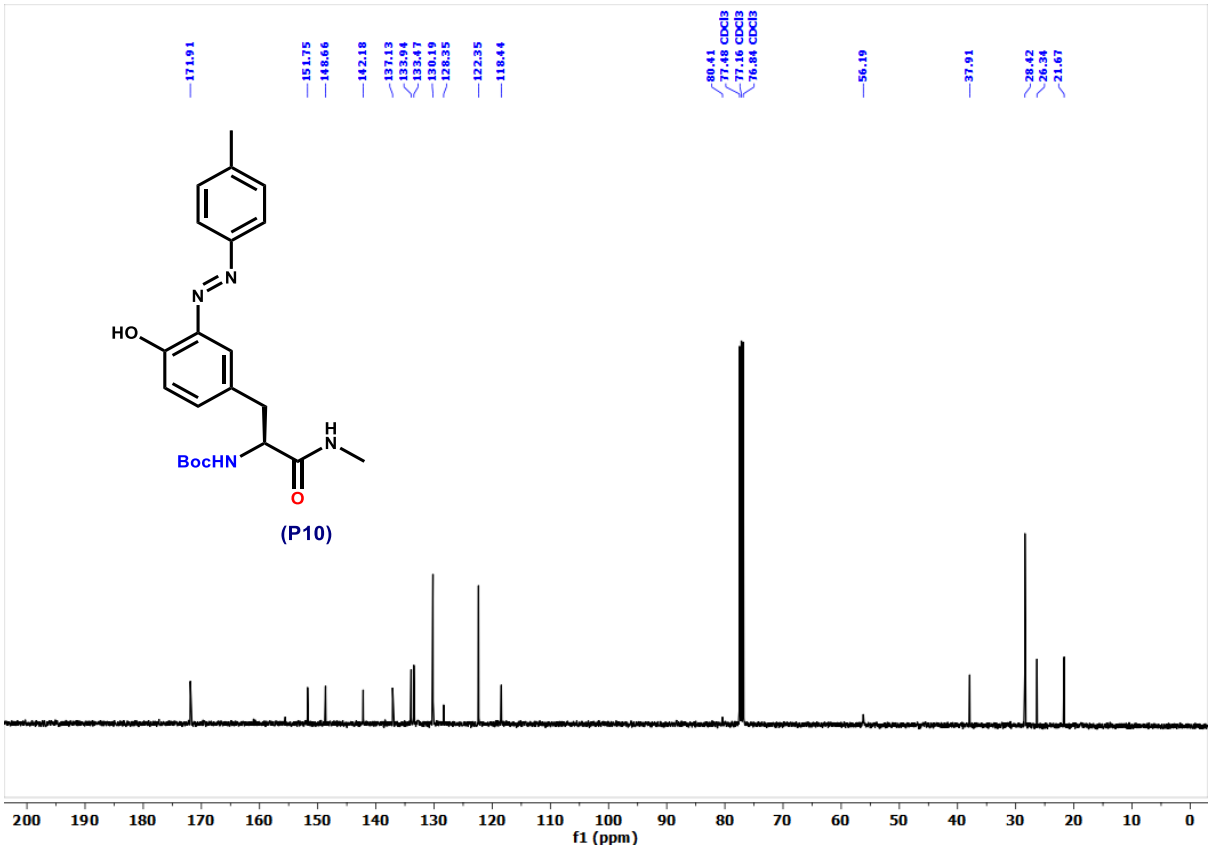
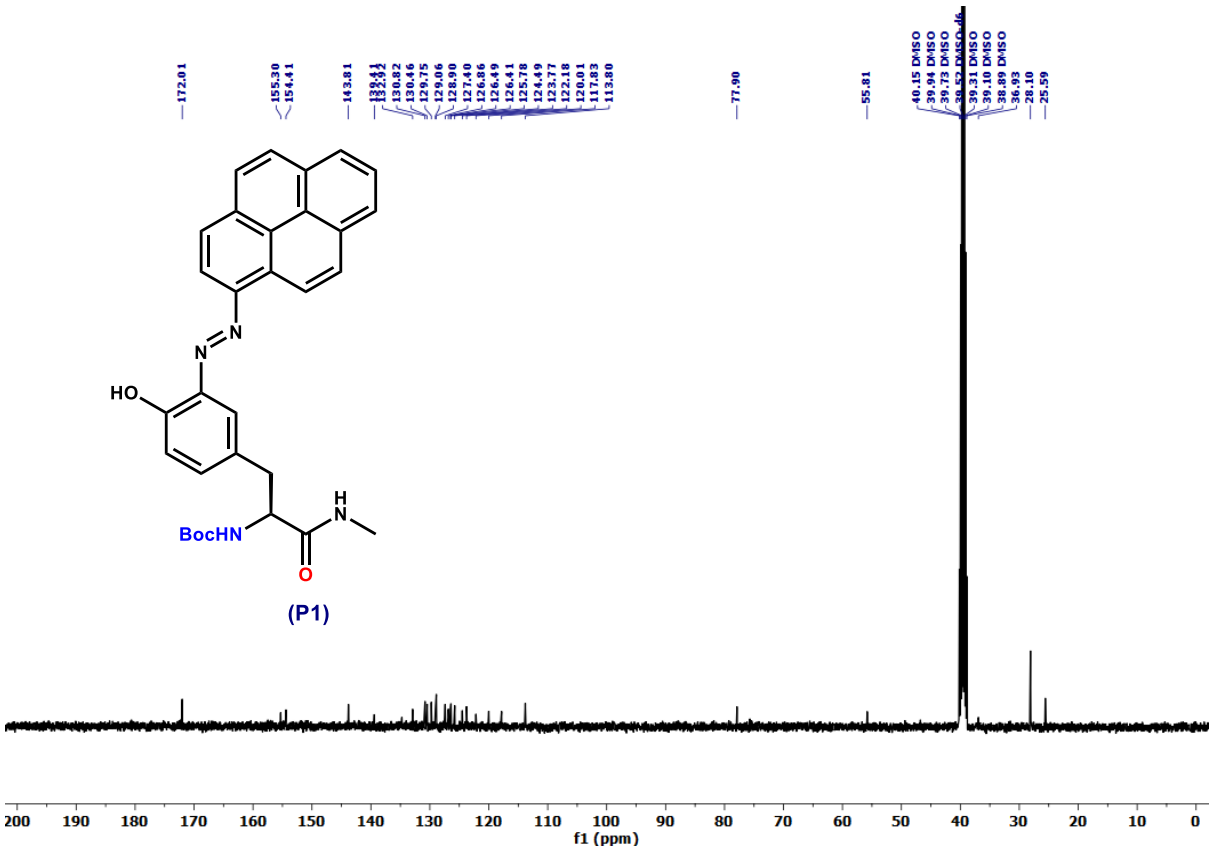
165.04
162.47
151.67
144.81
137.13
133.46
133.12
128.29
124.21
118.39
114.75
80.43
77.48 CDCl3
76.84 CDCl3
55.81
37.89
28.44
26.36
O
HO
N
N
BocHN
H
N
O
(P2)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

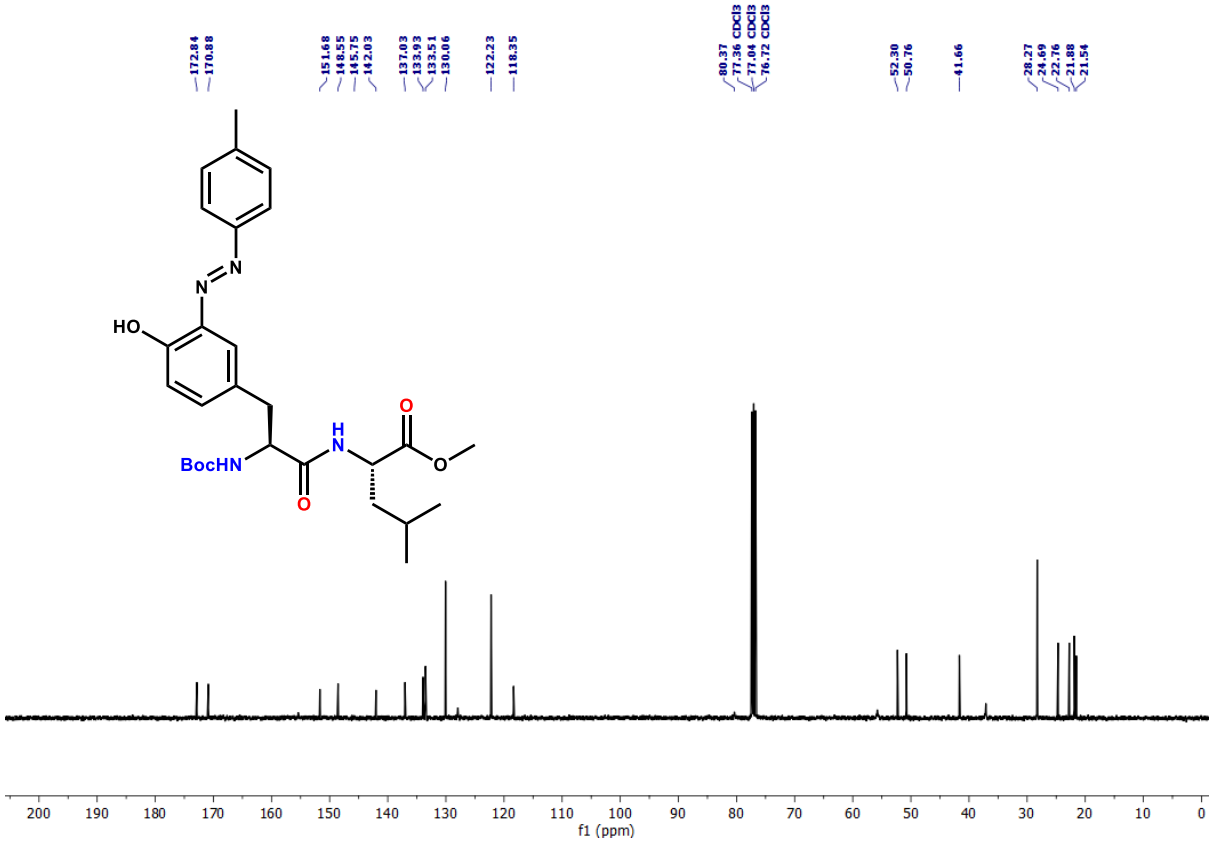

171.73
155.44
152.15
151.63
137.49
136.36
134.48
129.12
118.84
115.79
80.50 CDCl3
77.48 CDCl3
76.84 CDCl3
56.08
37.78
28.43
26.38
HO
BocHN
(P4)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

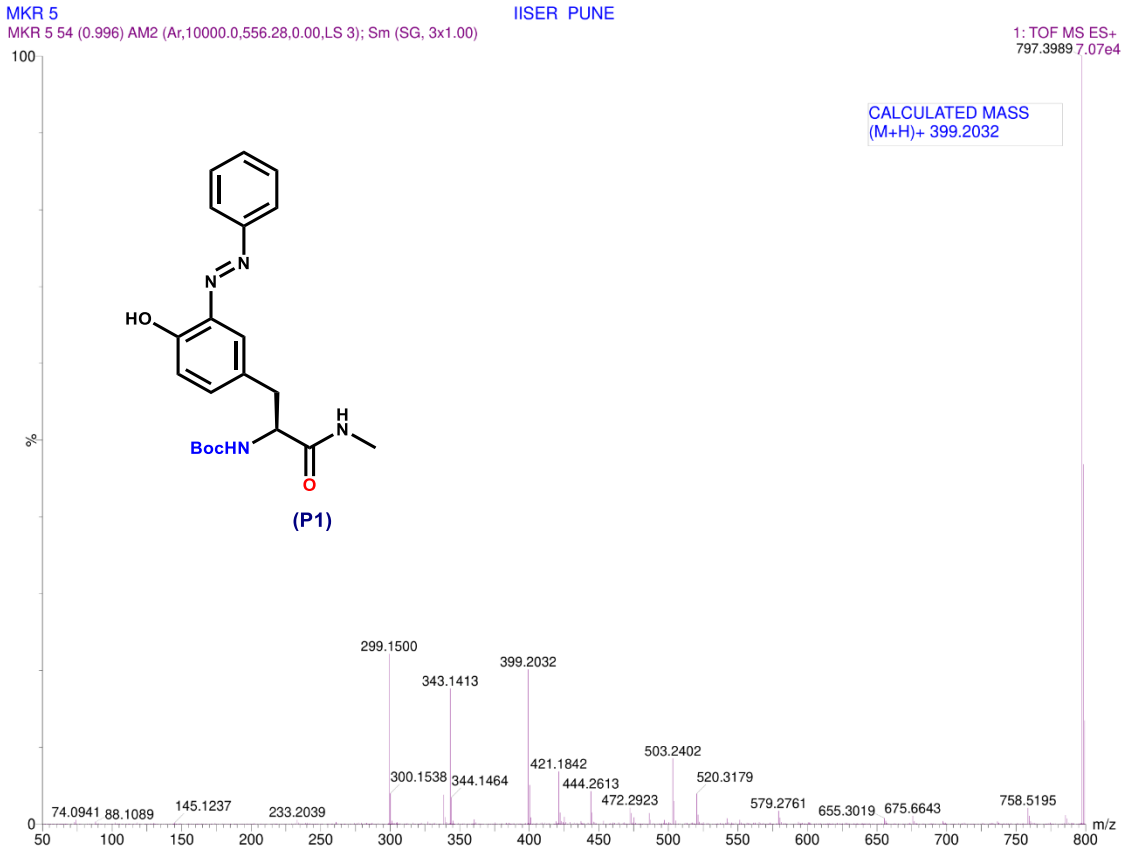

20200312-TYR14
195.90
171.77
152.78
151.91
139.50
137.37
135.36
134.15
132.95
131.44
130.19
128.61
122.20
118.73
87.55
77.48
76.84
56.09
37.77
28.43
26.40
HO
BocHN
(P5)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

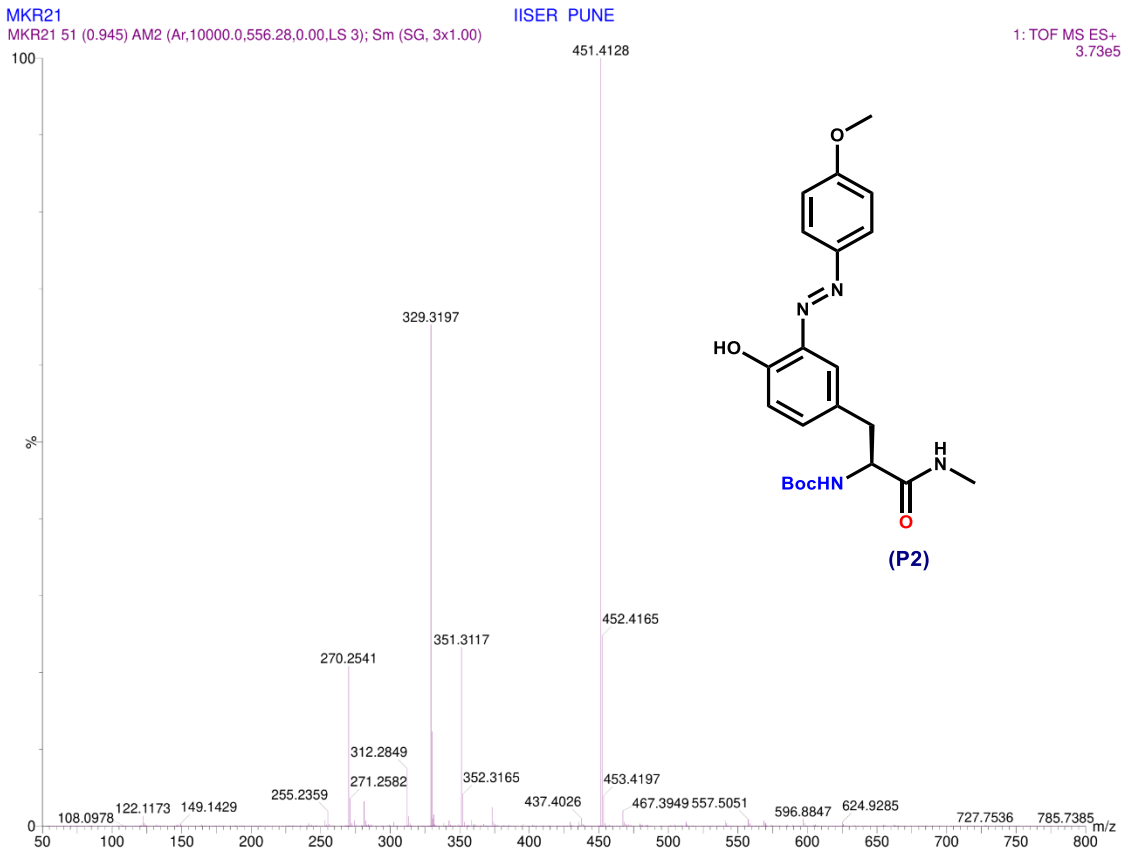

171.86
165.83
163.32
155.62
151.63
147.19
147.16
137.06
134.39
133.61
128.57
124.39
124.30
118.50
116.72
116.49
80.46
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
56.14
37.86
28.42
26.36
F
N
N
HO
H
N
BocHN
O
(P8)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

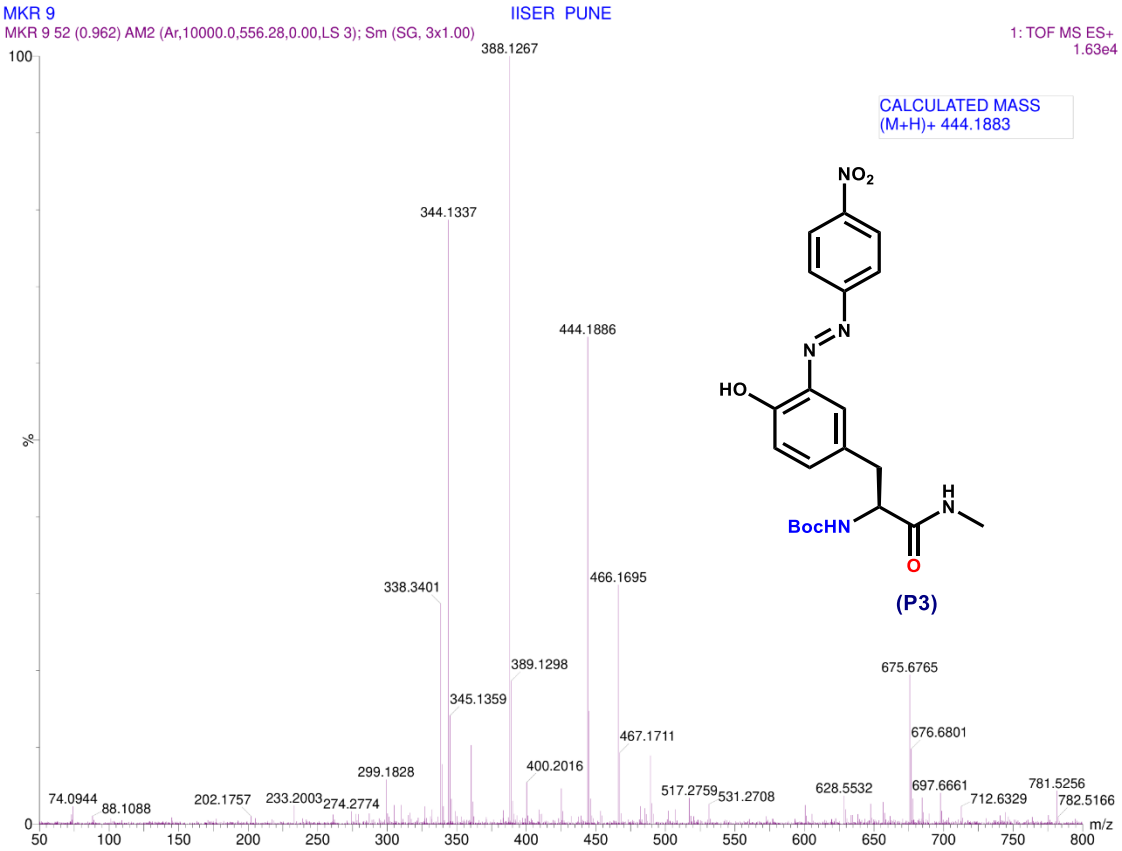

171.91
151.75
148.66
142.18
137.13
133.94
133.47
130.19
128.35
122.35
118.44
80.41
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
56.19
37.91
28.42
26.34
21.67
N
N
HO
H
N
BocHN
O
(P10)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

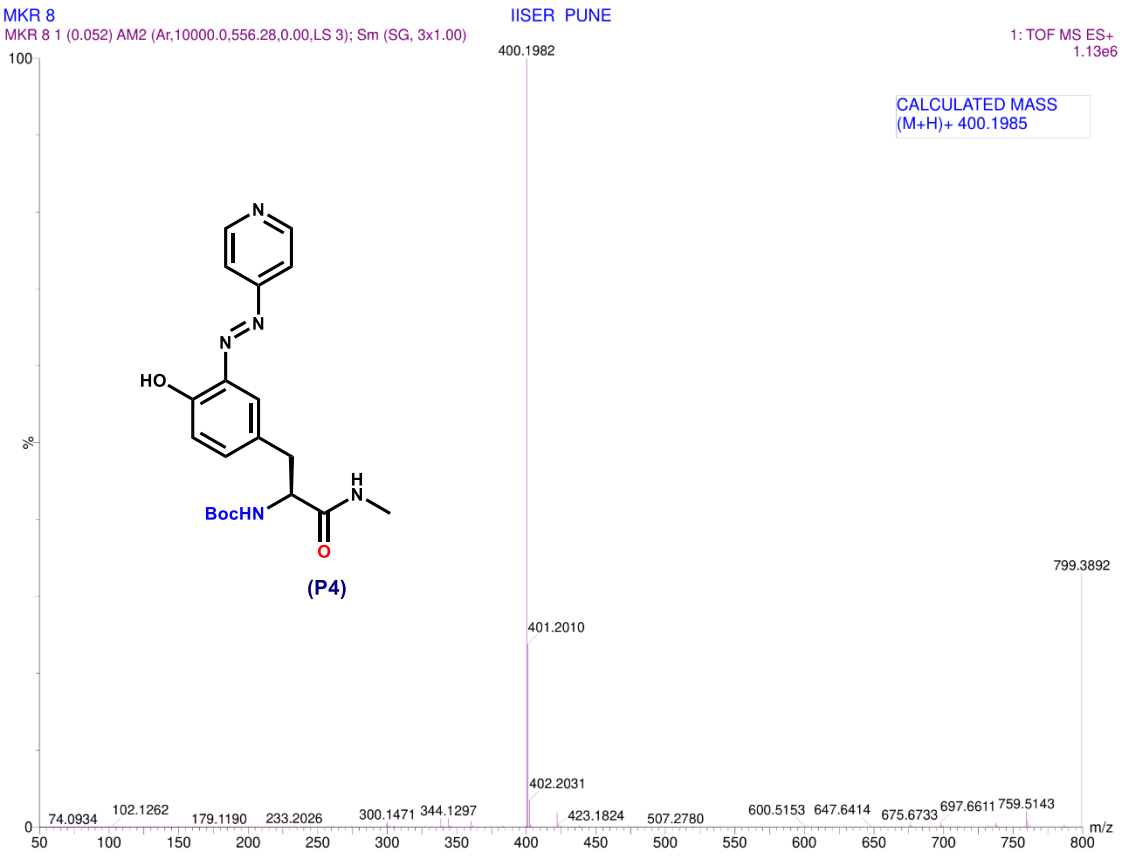

172.01
155.30
154.41
143.81
130.82
130.46
129.75
129.06
128.90
127.40
126.86
126.49
126.41
125.78
124.49
123.77
122.18
120.01
117.83
113.80
77.90
55.81
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
36.93
28.10
25.59
HO
N
N
H
N
BocHN
O
(P1)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

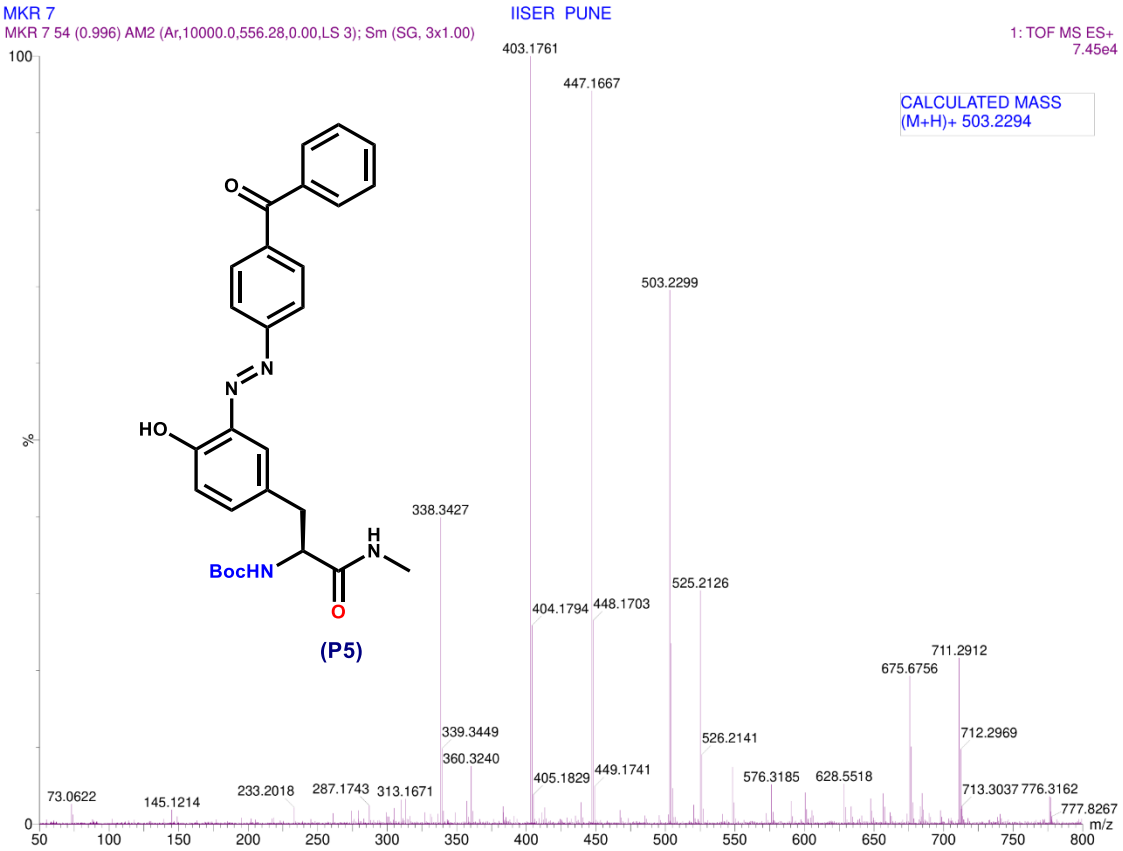

172.84
170.88
151.68
148.55
145.75
142.03
137.03
133.93
133.51
130.06
122.23
118.35
80.37
77.36 CDCl3
77.04 CDCl3
76.72 CDCl3
52.30
50.76
41.66
28.27
24.69
22.76
21.88
21.54
HO
N
N
H
N
BocHN
O
O
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

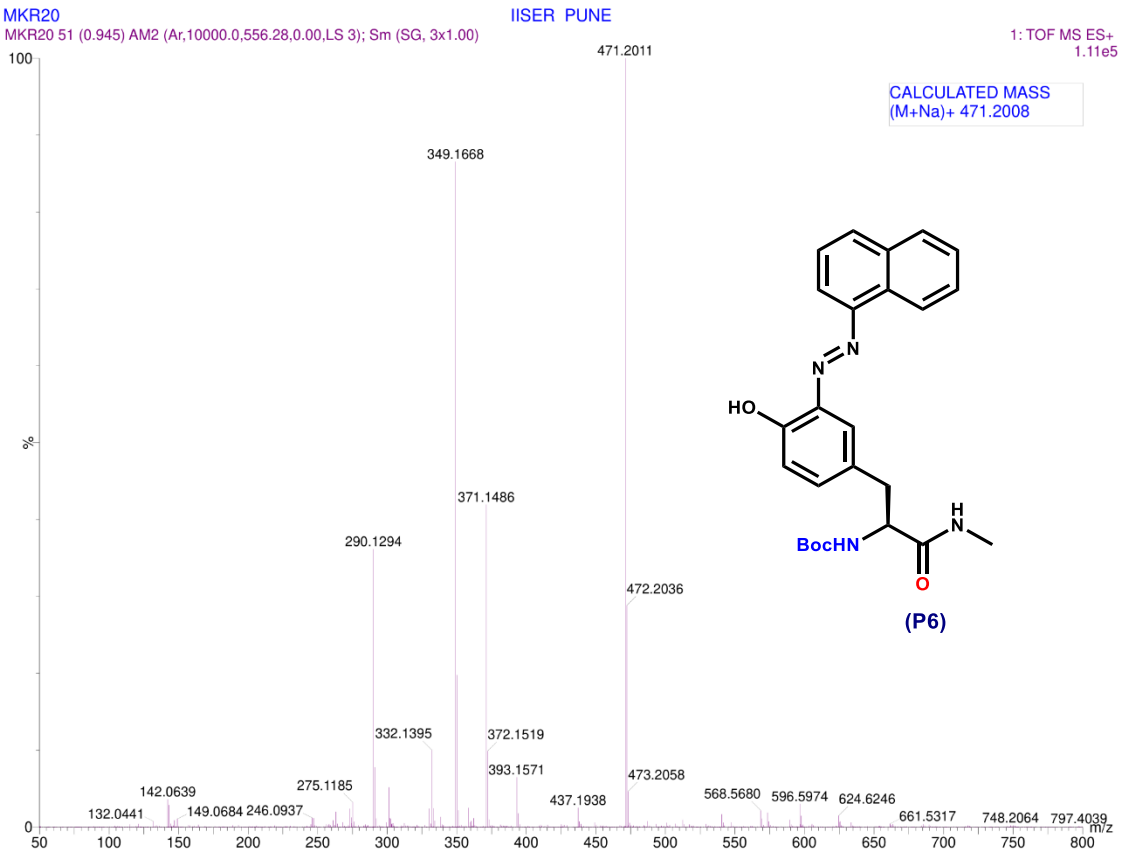

MKR 5
IISER PUNE
MKR 5 54 (0.996) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
797.3989 7.07e4
CALCULATED MASS
(M+H)+ 399.2032
HO
N
N
BocHN
H
N
O
(P1)
299.1500
399.2032
343.1413
503.2402
421.1842
300.1538
344.1464
444.2613
520.3179
472.2923
74.0941
88.1089
145.1237
233.2039
579.2761
655.3019
675.6643
758.5195
%
m/z

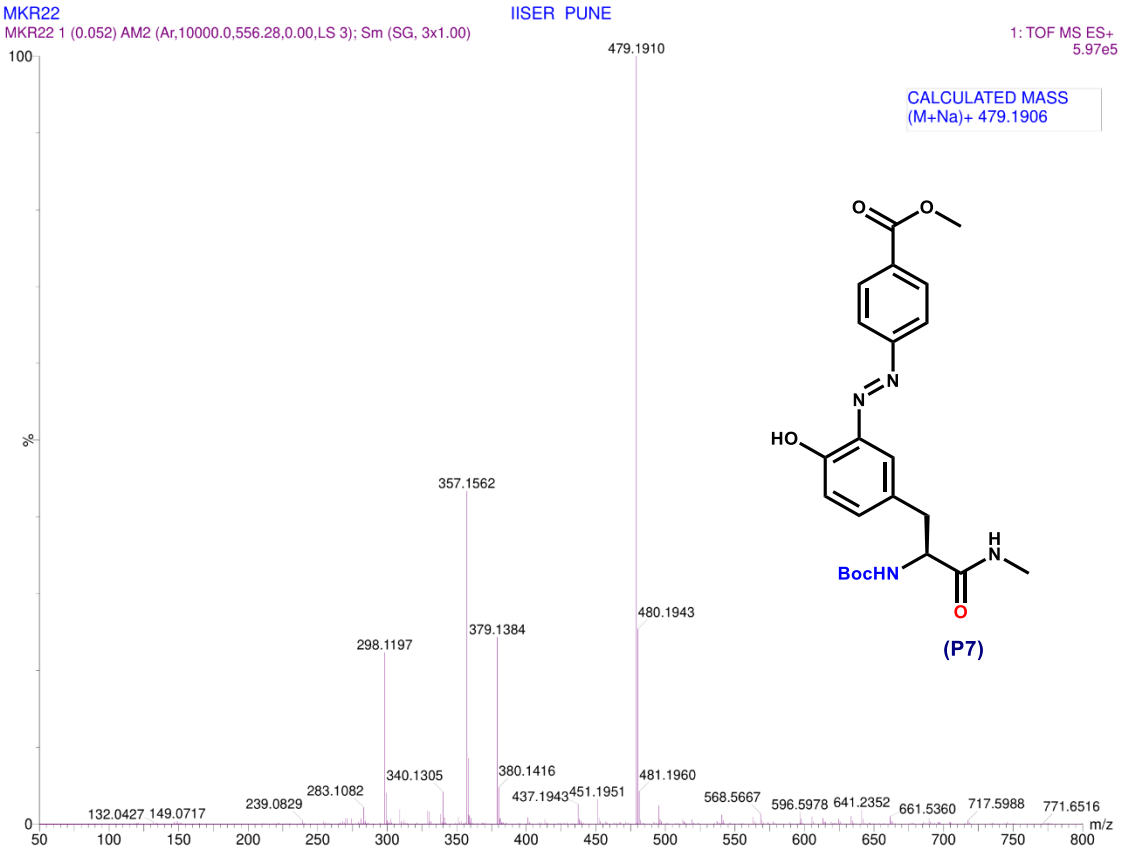

MKR21
IISER PUNE
MKR21 51 (0.945) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
3.73e5
451.4128
O
N
N
HO
BocHN
H
N
O
(P2)
329.3197
452.4165
351.3117
270.2541
312.2849
271.2582
352.3165
453.4197
255.2359
437.4026
467.3949
557.5051
596.8847
624.9285
108.0978
122.1173
149.1429
727.7536
785.7385
%
m/z

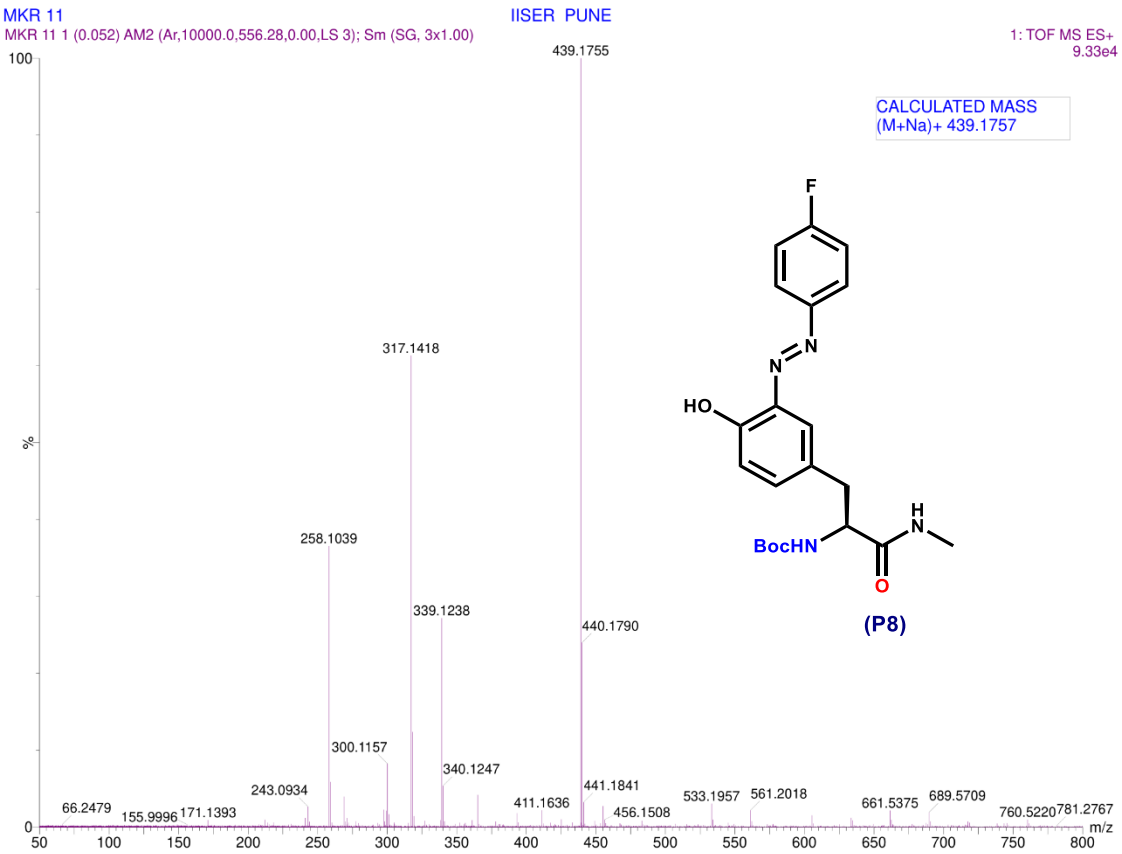

MKR 9
IISER PUNE
MKR 9 52 (0.962) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
1.63e4
CALCULATED MASS
(M+H)+ 444.1883
(P3)
m/z

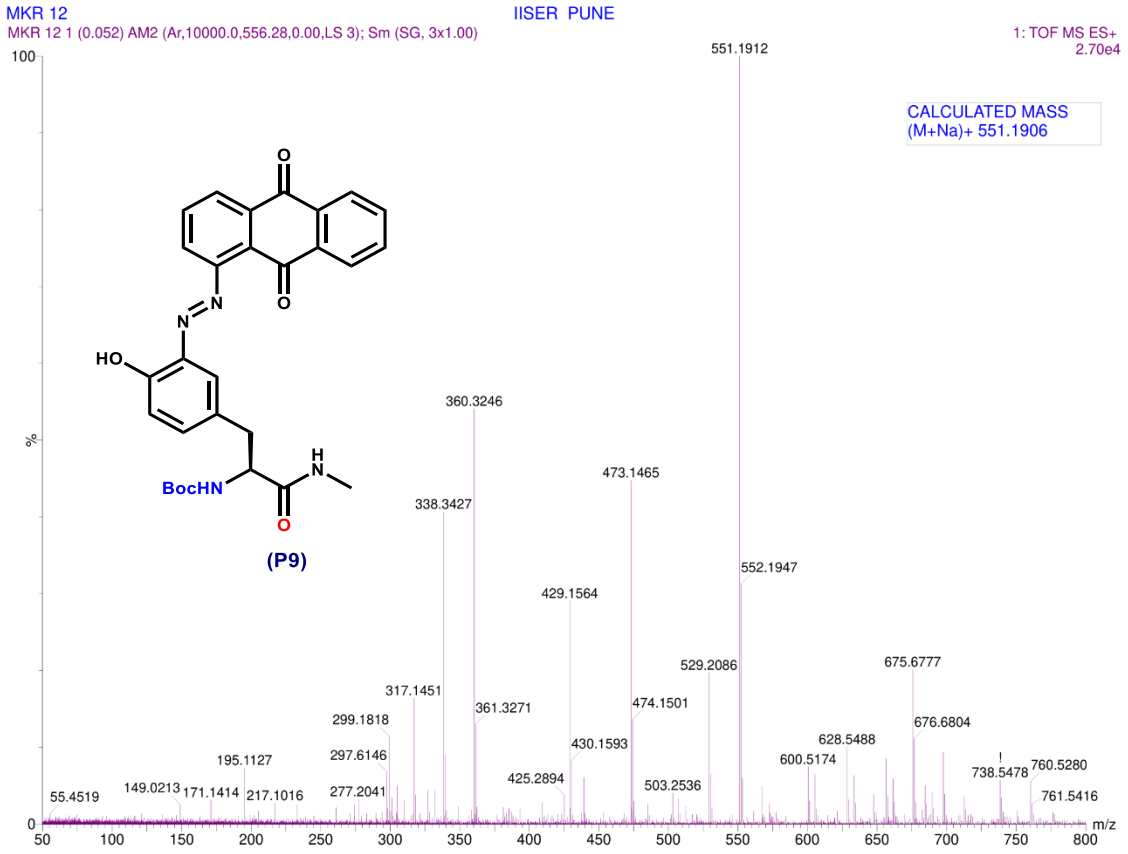

MKR 8
IISER PUNE
MKR 8 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
1.13e6
CALCULATED MASS
(M+H)+ 400.1985
(P4)
m/z

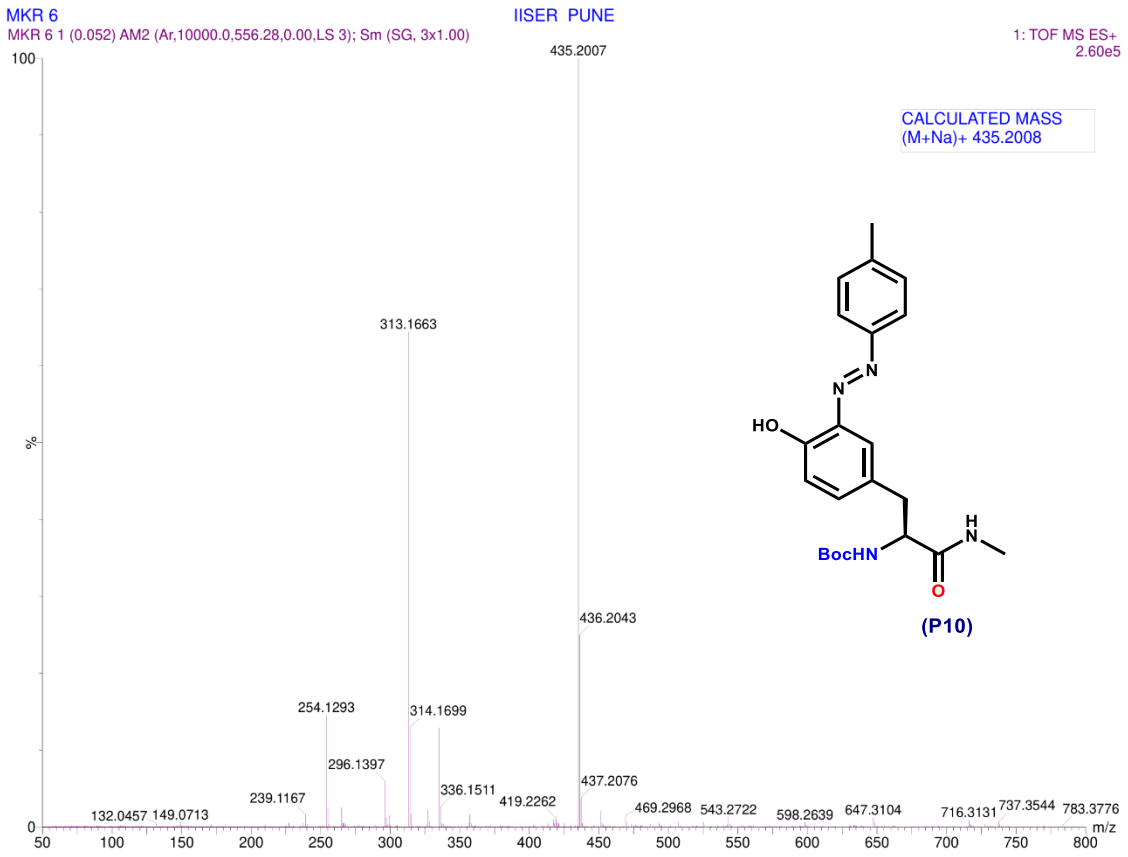

MKR 7
IISER PUNE
MKR 7 54 (0.996) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
7.45e4
CALCULATED MASS
(M+H)+ 503.2294
403.1761
447.1667
503.2299
338.3427
525.2126
404.1794
448.1703
711.2912
675.6756
339.3449
360.3240
526.2141
712.2969
405.1829
449.1741
576.3185
628.5518
713.3037
776.3162
73.0622
145.1214
233.2018
287.1743
313.1671
777.8267
(P5)
m/z

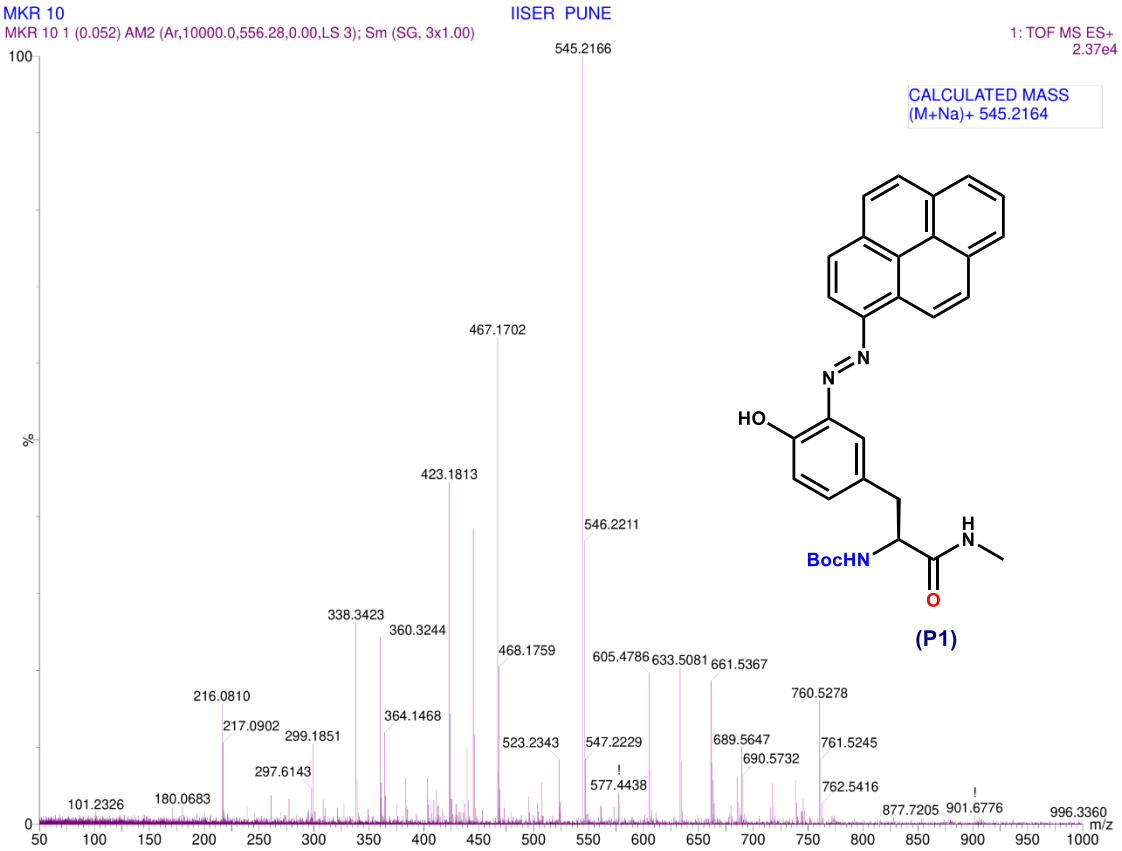

MKR20
IISER PUNE
MKR20 51 (0.945) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
1.11e5
CALCULATED MASS
(M+Na)+ 471.2008
471.2011
349.1668
371.1486
290.1294
472.2036
332.1395
372.1519
393.1571
473.2058
142.0639
275.1185
437.1938
568.5680
596.5974
624.6246
132.0441
149.0684
246.0937
661.5317
748.2064
797.4039
(P6)
m/z

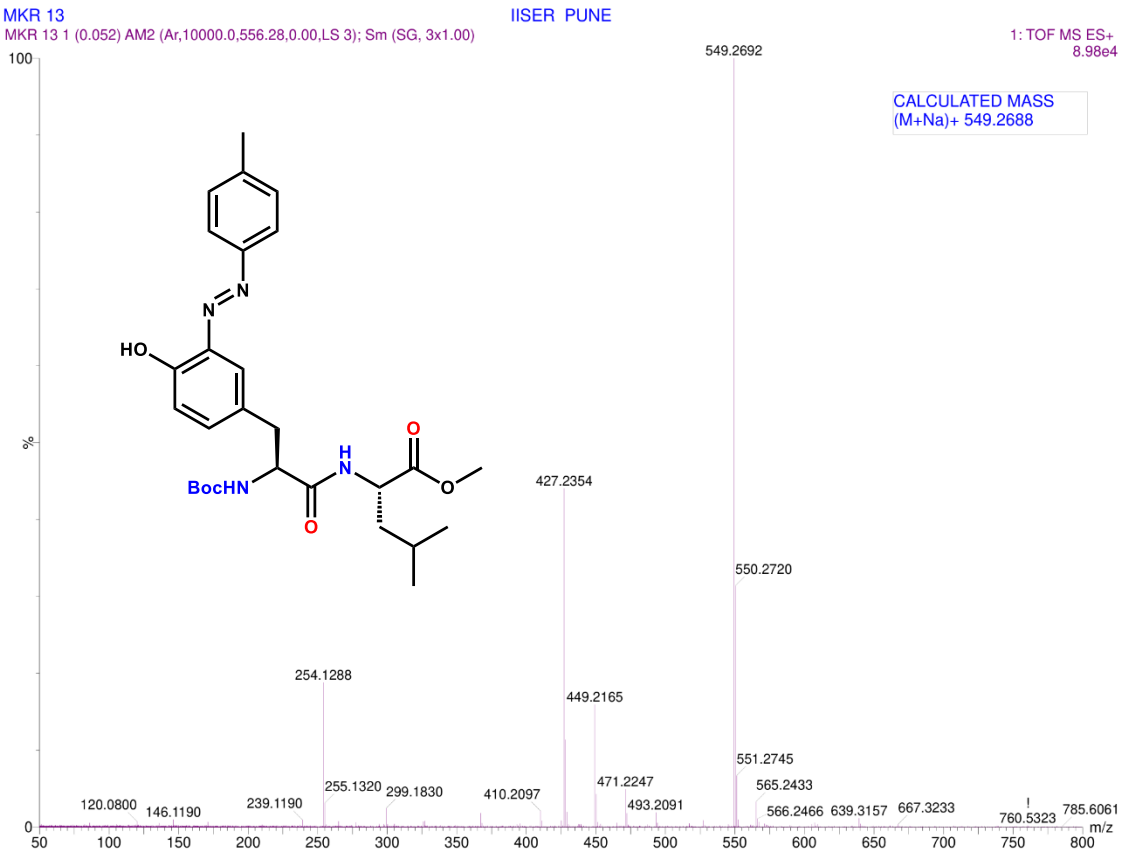

MKR22
IISER PUNE
MKR22 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
5.97e5
CALCULATED MASS
(M+Na)+ 479.1906
479.1910
357.1562
480.1943
379.1384
298.1197
340.1305
380.1416
481.1960
283.1082
239.0829
437.1943
451.1951
568.5667
596.5978
641.2352
661.5360
717.5988
771.6516
132.0427
149.0717
HO
BocHN
(P7)
%
m/z


MKR 11
IISER PUNE
MKR 11 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
9.33e4
CALCULATED MASS
(M+Na)+ 439.1757
439.1755
317.1418
258.1039
339.1238
440.1790
300.1157
340.1247
243.0934
441.1841
411.1636
456.1508
533.1957
561.2018
661.5375
689.5709
760.5220
781.2767
66.2479
155.9996
171.1393
F
HO
BocHN
(P8)
%
m/z


MKR 12
IISER PUNE
MKR 12 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.70e4
CALCULATED MASS
(M+Na)+ 551.1906
551.1912
360.3246
473.1465
338.3427
552.1947
429.1564
529.2086
675.6777
317.1451
361.3271
474.1501
299.1818
676.6804
628.5488
430.1593
297.6146
600.5174
195.1127
738.5478
760.5280
425.2894
503.2536
149.0213
171.1414
217.1016
277.2041
55.4519
761.5416
HO
BocHN
(P9)
%
m/z

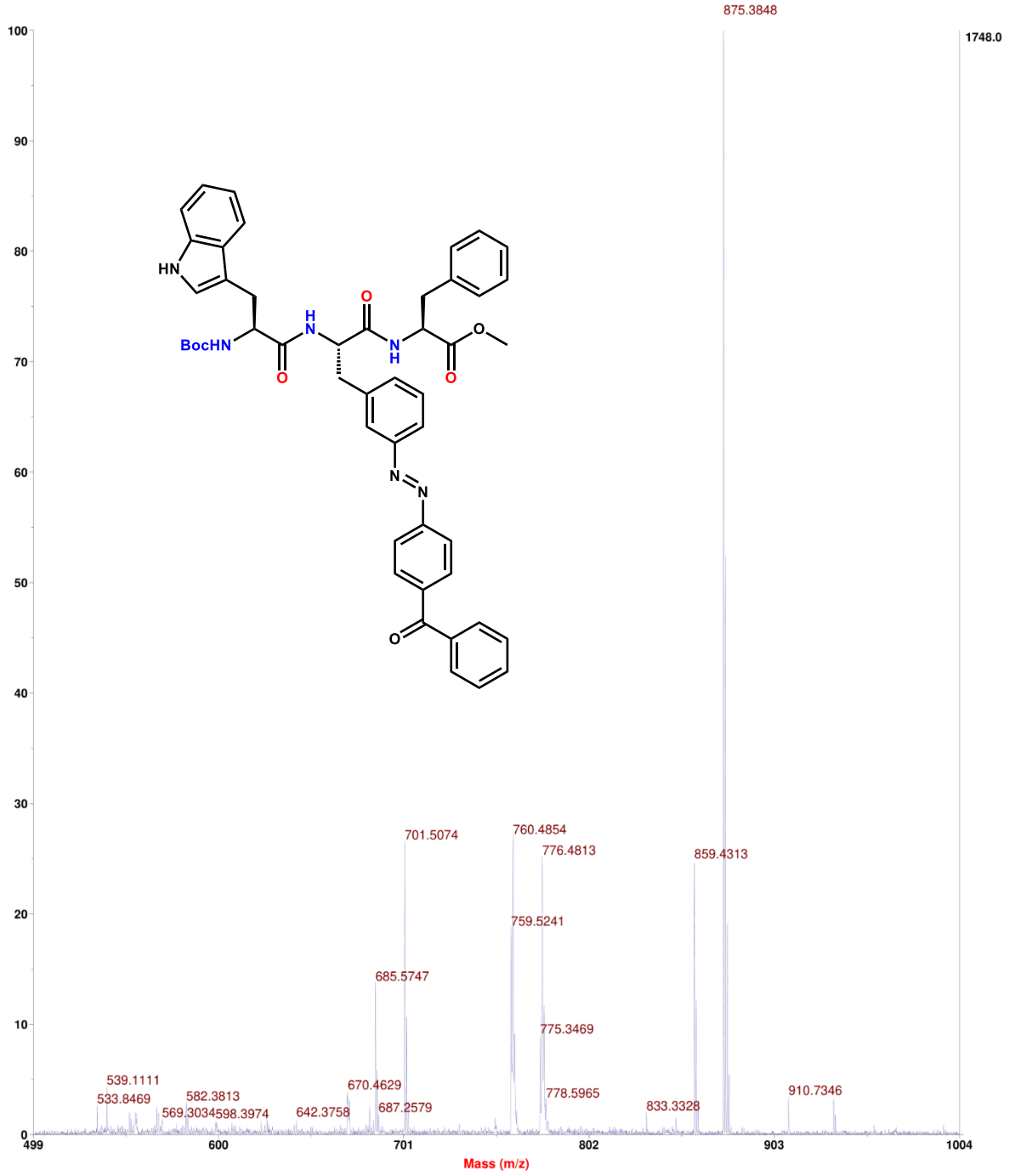

MKR 6
IISER PUNE
MKR 6 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.60e5
CALCULATED MASS
(M+Na)+ 435.2008
435.2007
313.1663
436.2043
254.1293
314.1699
296.1397
336.1511
437.2076
239.1167
419.2262
132.0457
149.0713
469.2968
543.2722
598.2639
647.3104
716.3131
737.3544
783.3776
HO
BocHN
(P10)
%
m/z


MKR 10
IISER PUNE
MKR 10 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.37e4
CALCULATED MASS
(M+Na)+ 545.2164
545.2166
467.1702
423.1813
546.2211
338.3423
360.3244
468.1759
605.4786
633.5081
661.5367
216.0810
760.5278
217.0902
364.1468
299.1851
523.2343
547.2229
689.5647
761.5245
690.5732
297.6143
577.4438
762.5416
101.2326
180.0683
877.7205
901.6776
996.3360
HO
N
BocHN
H
O
(P1)
%
m/z


MKR 13
IISER PUNE
MKR 13 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
8.98e4
549.2692
CALCULATED MASS
(M+Na)+ 549.2688
427.2354
550.2720
254.1288
449.2165
551.2745
565.2433
255.1320
299.1830
471.2247
410.2097
493.2091
120.0800
146.1190
239.1190
566.2466
639.3157
667.3233
760.5323
785.6061
HO
BocHN
H
O
%
m/z

Spectrum Report

Final - Shots 400 - IISER-96-1-2020; Label E3



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