

Supplementary Material (ESI) for Chemical Communications

Acyl Hydrazides as Peptoid Sub-monomers

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Materials and Equipment

All of the chemical reagents and solvents from commercial sources were used without further purification. TentaGel S-NH₂ (160 μm, 0.51 mmol/g) resin was purchased from Rapp Polymere. Knorr Amide MBHA (0.69 mmol/g) resin was purchased from NovaBiochem. 5 mL and 10 mL Disposable Reaction Columns (Intavis AG) were used as reaction vessels for solid phase synthesis. Syntheses of peptoids under microwave conditions were performed in a 1550 W microwave oven (GE model JE 1860BH04) with 10% power. HPLC was carried out on Waters systems equipped with Waters 1525 binary HPLC pumps and a 2487 dual λ absorbance detector, or a 2998 photodiode array detector. The mobile phase comprised of buffer A (H₂O containing 5% CH₃CN and 0.1% trifluoroacetic acid (TFA)) and buffer B (CH₃CN containing 0.1% TFA). Analytical HPLC was conducted using a Vydac C-18 column (5 μm, 250 x 4.6 mm, Alltech, Deerfield, IL) at a flow rate of 1.0 mL/min with UV detection at 220 nm. HRMS was recorded on a commercial LTQ-Orbitrap FT/MS instrument (Thermo Electron Finnigan LTQ ion trap mass spectrometer). ¹H and ¹³C NMR spectra were recorded at 25 °C on Bruker 400 instrument operating at 400 MHz for ¹H and 100 MHz for ¹³C nuclei. ¹H and ¹³C NMR chemical shifts were referenced to residual solvent signals: δ_H 7.26 and δ_C 77.00 for CDCl₃. MS and MS/MS (MALDI-TOF) were performed on a 4800 Proteomics Analyzer (Applied Biosystems) with α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix.

General procedure for synthesis of **1a-1d**

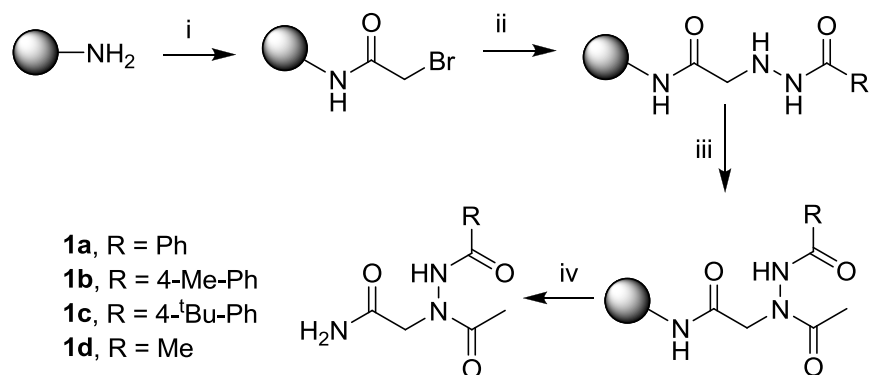


Fig. S1. Proposed sub-monomer synthesis of N-aza peptoids **1a-1d**. For 100 mg resin (i) Bromoacetic acid (2M, 1mL) and DIC (3.4 M, 1mL), 37 °C, 10 mins; (ii) Acyl hydrazide (2M, 2mL), 37 °C, 1h (iii) acetic acid (2M, 1mL), DIC (3.4M, 1mL), 30 mins (iv) TFA:TIS:water (96:2:2) (2mL).

To synthesize compounds **1a-1d**, Knorr Amide RAM resin (100 mg) in DMF (2 mL) was swollen at room temperature (23 °C) for 1 hour in the 5 mL disposable reaction column. Fmoc protecting group was then removed by incubation of the resin with 20 % piperidine in DMF (2 mL) for 30 mins. The resin was thoroughly washed with DMF. After Fmoc group was removed, 2-bromoacetic acid (1 mL, 2 M in DMF) and diisopropylcarbodiimide (DIC) (1 mL, 3.4 M in DMF) were added. The reaction vessel containing the resin was shaken for about 10 mins at 37 °C. The resin was then washed again with DMF. Then a solution of acyl hydrazide (2 mL, 2 M in N-methylpyrrolidinone (NMP)) was added and shaken at 37 °C for 1h. The resin was then washed with DMF and reacted with acetic acid (2M, 1mL) and (DIC) (3.4 M, 1 mL) for 30 mins at room temperature. The resin was then thoroughly washed with DMF and finally with DCM. The resin was then treated with the cleavage cocktail of 96% TFA, 2% TIS (triisopropylsilane) and 2% water for 1h at room temperature. The cleavage cocktail solution was then dried by flushing argon and cold ether was added to precipitate out the compounds **1a-1b**, which were then purified by reverse phase HPLC.

General procedure for synthesis of **2a-2b** and **3**.

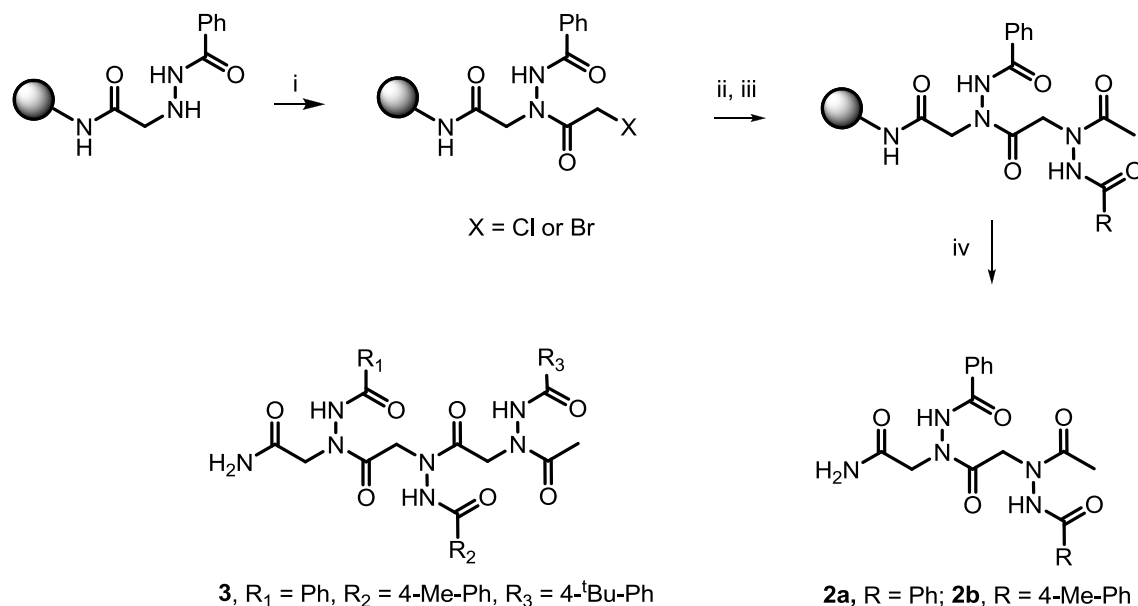


Fig. S2. Optimization of acylation and displacement reactions of acyl hydrazide. For 100 mg resin (i) Cl/Br-acetic acid (2M, 1mL) and DIC (3.4 M, 1mL), 37 °C, 10 mins; (ii) Benzoic hydrazide (2M, 2mL), 37 °C, 1h (iii) Acetic acid (2M, 1mL), DIC (3.4M, 1mL), 25 °C, 30 mins (iv) TFA:TIS:water (96:2:2) (2mL), 1h, 25 °C.

To synthesize compounds **2a-2b**, Knorr Amide RAM resin (100 mg) in DMF (2 mL) was swollen at room temperature (23 °C) for 1 hour in the 5 mL disposable reaction column. Fmoc protecting group was then removed by incubation of the resin with 20 % piperidine in DMF (2 mL) for 30 mins. The resin was thoroughly washed with DMF. After Fmoc group was removed, 2-bromoacetic acid (1 mL, 2 M in DMF) and DIC (1 mL, 3.4 M in DMF) were added. The reaction vessel containing the resin was shaken for about 10 mins at 37 °C. The resin was then washed again with DMF. Then a solution of acyl hydrazide (2 mL, 2 M in NMP) was added and shaken at 37 °C for 1h. Then the above acylation reaction with 2-bromoacetic acid and displacement reaction with a second acyl hydrazide were repeated for the synthesis of **2a** and **2b**, whereas the acylation and displacement steps were repeated twice for the synthesis of compound **3**. The resin was then washed with DMF and reacted with acetic acid (2M, 1mL) and (DIC) (3.4

M, 1 mL) for 30 mins at room temperature. The resin was then thoroughly washed with DMF and finally with DCM and was treated with the cleavage cocktail of 96% TFA, 2% TIS and 2% water for 1h at room temperature. The cleavage cocktail solution was then dried by flushing argon and cold ether was added to precipitate out the compounds, which were then purified by reverse phase HPLC.

General procedure for synthesis of **4a-4c**.

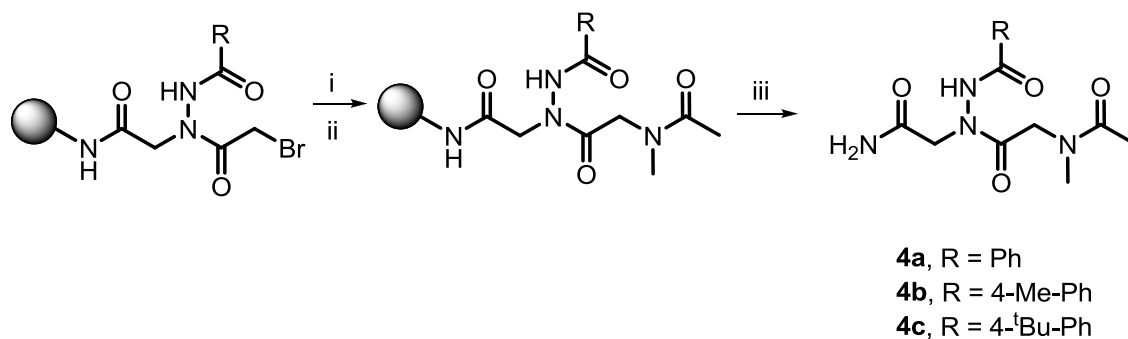
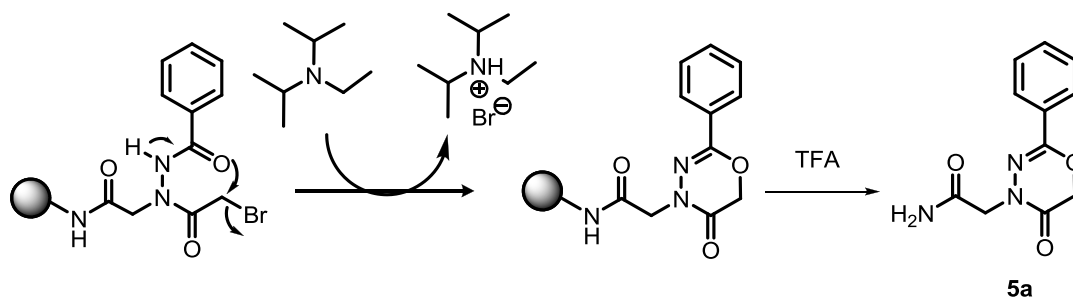


Fig. S3. Synthesis of compounds **4a-4c**. For 100 mg resin (i) CH_3NH_2 (40% v/v in water) (1 mL in 1 mL DMF), 37 °C, 1h (ii) acetic acid (2M, 1mL), DIC (3.4M, 1 mL), 25 °C, 30 mins (iii) TFA:TIS:water (96:2:2) (2mL), 25 °C, 1h.

To synthesize compounds **4a-4c**, Knorr Amide RAM resin (100 mg) in DMF (2 mL) was swollen at room temperature (23 °C) for 1 hour in the 5 mL disposable reaction column. Fmoc protecting group was then removed by incubation of the resin with 20 % piperidine in DMF (2 mL) for 30 mins. The resin was thoroughly washed with DMF. After Fmoc group was removed, 2-bromoacetic acid (1 mL, 2 M in DMF) and DIC (1 mL, 3.4 M in DMF) were added. The reaction vessel containing the resin was shaken for about 10 mins at 37 °C. The resin was then washed again with DMF. Then a solution of methylamine (1mL, 40% v/v in water) and 1mL DMF was added and shaken at 37 °C for 1h. The resin was then washed with DMF and reacted with acetic acid (2M, 1mL) and DIC (3.4 M, 1 mL) for 30 mins at room temperature. The resin was then thoroughly washed with DMF and finally with DCM and was treated with the cleavage cocktail of 96% TFA, 2% TIS and 2% water for 1h at room temperature. The cleavage cocktail solution was then dried by flushing argon and cold ether was added to precipitate out the compounds, which were then purified by reverse phase HPLC. From these reactions, compounds

4a-4c were isolated in ~75% along with ~25% of **5a-5c**. The compound **6** was synthesized similarly to **4** by using 2 mL of 2M solution of isopropylamine instead of methylamine.

Synthesis of **5a**.



To synthesize compounds **5a**, Knorr Amide RAM resin (100 mg) in DMF (2 mL) was swollen at room temperature (23 °C) for 1 hour in the 5 mL disposable reaction column. Fmoc protecting group was then removed by incubation of the resin with 20 % piperidine in DMF (2 mL) for 30 mins. The resin was thoroughly washed with DMF. After Fmoc group was removed, 2-bromoacetic acid (1 mL, 2 M in DMF) and DIC (1 mL, 3.4 M in DMF) were added. The reaction vessel containing the resin was shaken for about 10 mins at 37 °C. The resin was then washed again with DMF. Then a solution of diisopropylethylamine (DIEA) (2mL, 2M) was added and shaken at room temperature for 2h. The resin was then thoroughly washed with DMF and finally with DCM and was treated with the cleavage cocktail of 96% TFA, 2% TIS and 2% water for 1h at room temperature. The cleavage cocktail solution was then dried by flushing argon and cold ether was added to precipitate out the compounds, which were then purified by reverse phase HPLC.

Synthesis of the tetramer and pentamer acyl hydrazide libraries by split and pool strategy:

The synthesis of the acyl hydrazide libraries were carried out on TentaGel Macrobeads NH₂ (1 g, 160 μm, 0.48 mmol/g) resin. The beads were incubated with anhydrous DMF for 1h and Fmoc-L- methionine (10 equiv.) was coupled using O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (10 equiv.), hydroxybenzotriazole (HOBt) monohydrate (10 equiv.) and N-methylmorpholine (NMM) (20 equiv.) for 2h. The beads were then washed

thoroughly with DMF and the Fmoc group was deprotected by using 20 mL of 20% piperidine. After Fmoc deprotection, α -bromoacetic acid (10 mL, 2M) and DIC (10 mL, 3.4 M) was added to the resin, microwave-assisted acylation reaction was carried out (15 seconds, 10% power, 2 \times). The resin was then washed thoroughly by DMF and reacted with 2M solution of N-Boc-diamminobutane to displace the resin-bound bromide. The acylation and the displacement steps were further repeated two times. After three N-Boc-diamminobutane have been introduced, the beads were reacted with α -bromoacetic acid (10 mL, 2 M) and DIC (10 mL, 3.4 M) and microwave-assisted acylation reaction was carried out (15 seconds, 10% power, 2 \times). After the acylation reaction was over, the beads were split to ten reaction vessels in roughly equal amount, and each vessel was treated with an acyl hydrazide (2 mL, 2 M in NMP) and the bromide displacement reactions were carried out for 1h at 37 °C. The beads were then washed extensively with DMF and pooled in a 25 mL reaction vessel and treated with α -bromoacetic acid (10 mL, 2 M) and DIC (10 mL, 3.4 M) and the acylation reaction was carried out for 10 mins at 37 °C. The bromide displacement and acylation reactions were repeated until the tetramer aza-peptoid was obtained. Once the tetramer library was obtained, half the beads (~500 mg) were separated and one more acylation step was carried out by using α -bromoacetic acid (5 mL, 2 M) and DIC (5 mL, 3.4 M) at 37 C for 10 mins. The beads were then washed with DMF and then split in ten different vessels and reacted with ten acyl hydrazides and a pentamer library of acyl hydrazide was obtained. The beads were then washed with DMF and treated with α -bromoacetic acid (5 mL, 2 M) and DIC (5 mL, 3.4 M) at 37 C for 10 mins. Once the acylation reaction was over, the beads were washed with DMF and incubated with diisopropylethyl amine (DIEA) (2M, 10 mL) overnight (~12h) to obtain the pentamer library with ten different 4-substituted 2-aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-ones in the 5th position.

50 mg of each of the resin-bound peptoid library was treated with 1mL TFA cleavage cocktail (96% TFA: 2% TIS:2% Water) and few beads were picked up for CNBr cleavage. Each bead was isolated into a single well of a 96-well plate and treated with 20 μ L of a CNBr solution (30 mg/mL in 5:4:1 CH₃CN/acetic acid/H₂O). The cleavage mixture was removed by evaporation and the resulting residue was dissolved in CH₃CN/H₂O (50:50) and submitted to tandem MALDI mass spectrometry for sequencing analysis

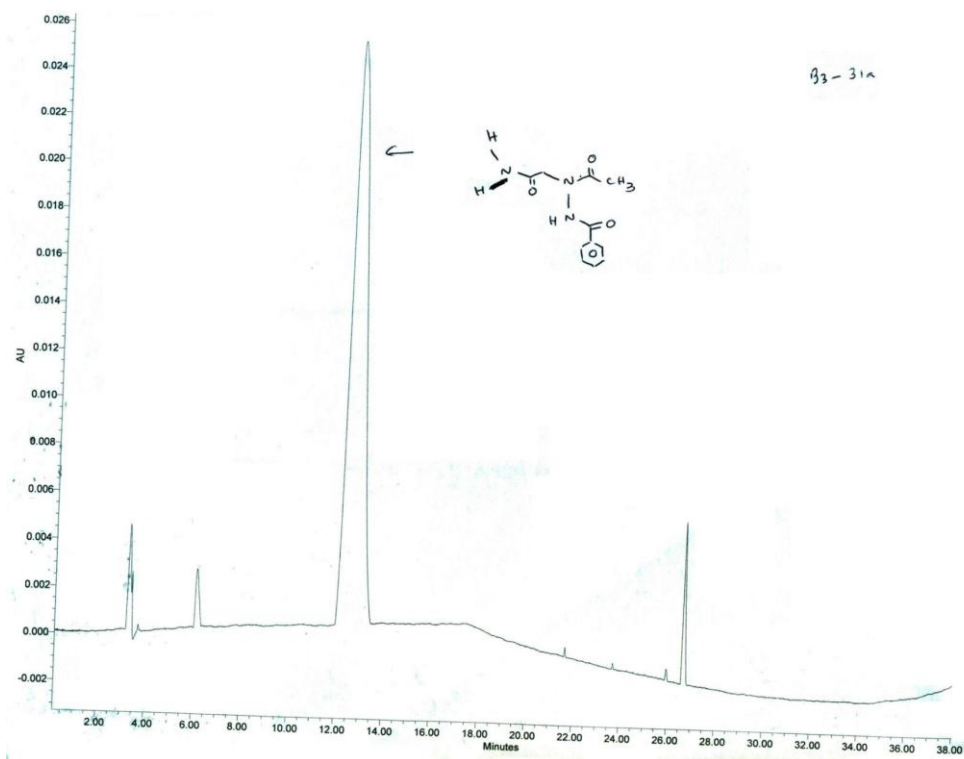


Fig. S4. HPLC chromatogram of crude product **1a**. Gradient of water and acetonitrile was used as eluent.

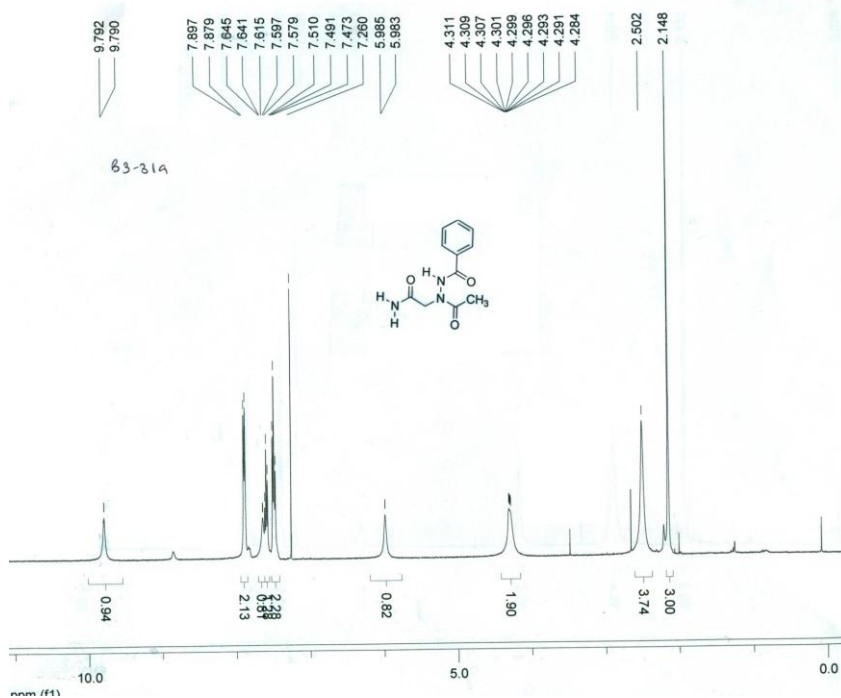


Fig. S5. ^1H NMR spectra of **1a** in CDCl_3 .

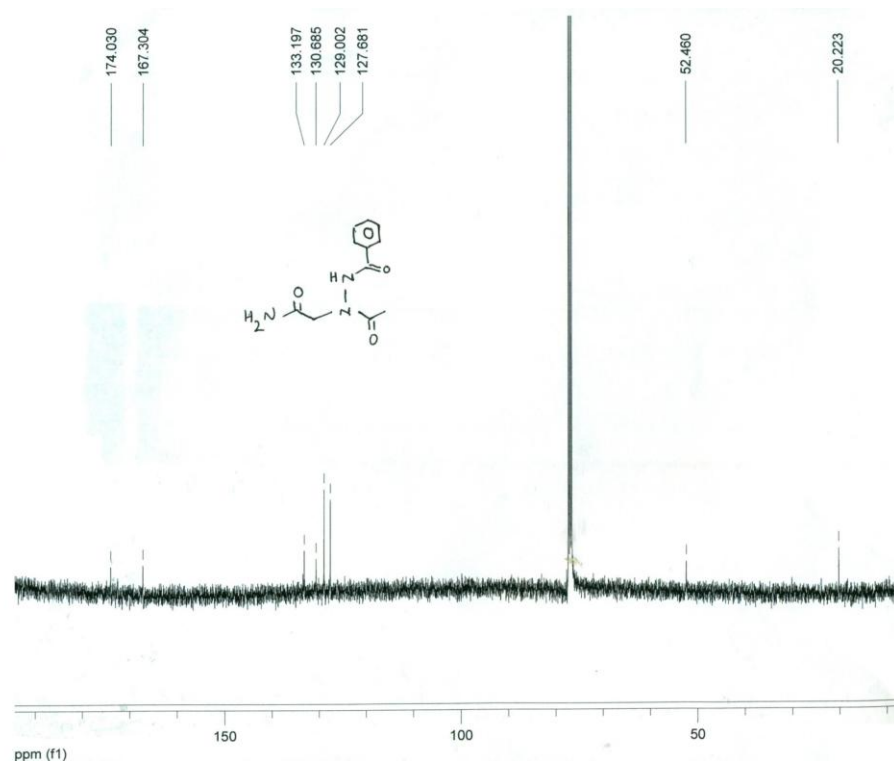


Fig. S6. ¹³C NMR spectra of **1a** in CDCl₃.

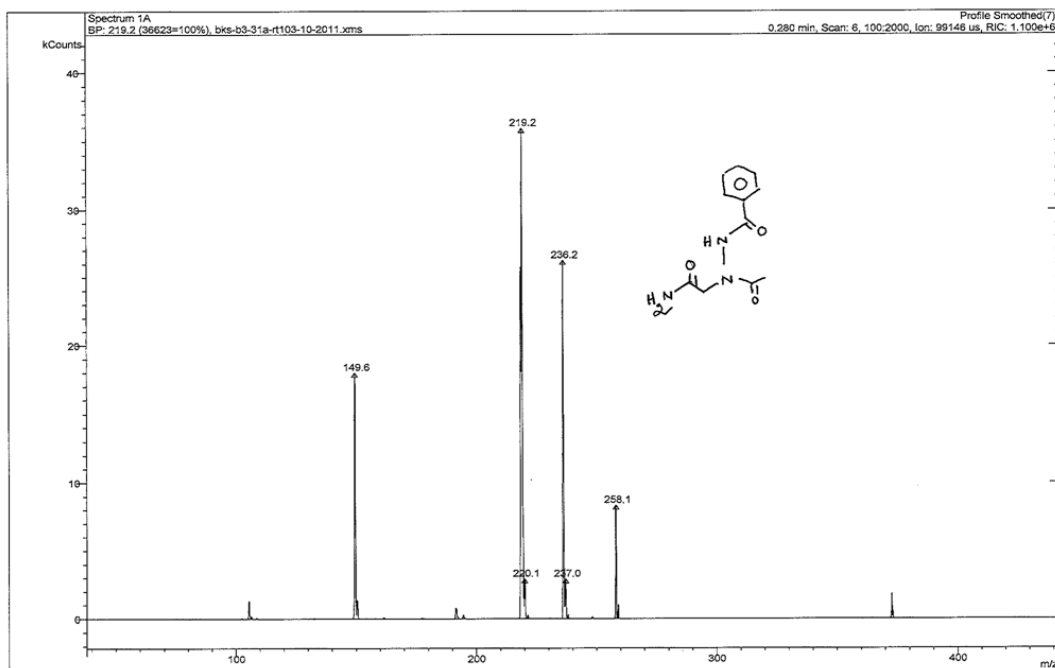


Fig. S7. ESI HRMS of **1a**.

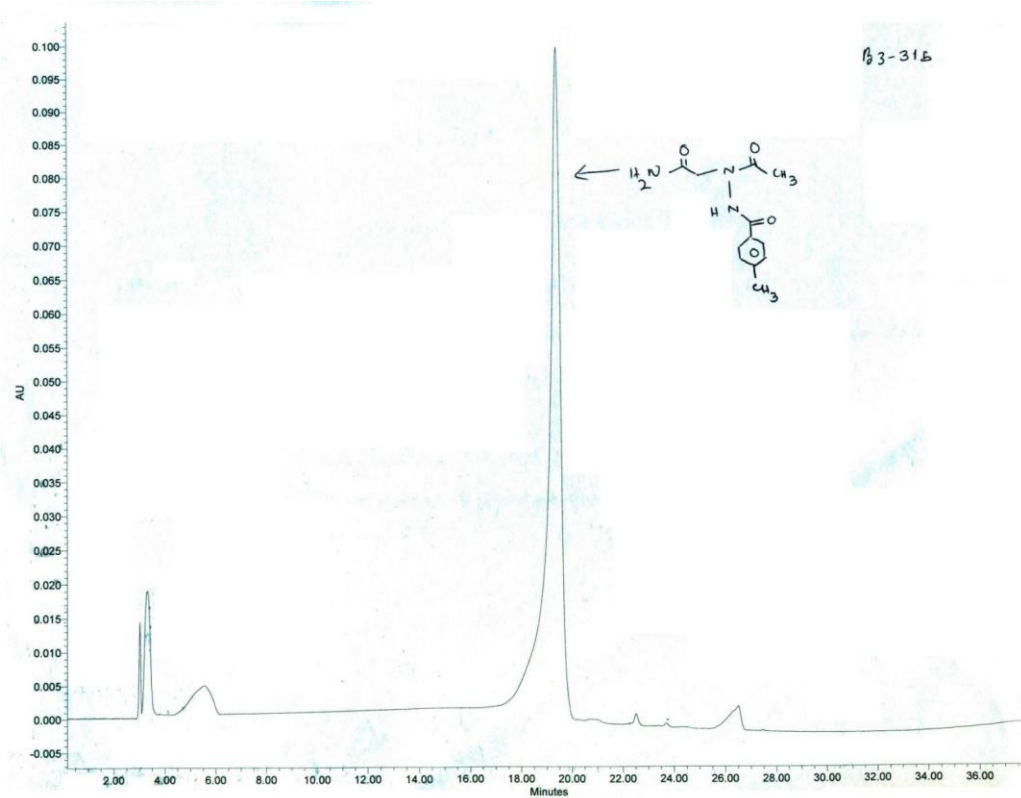


Fig. S8. HPLC chromatogram of crude **1b**.

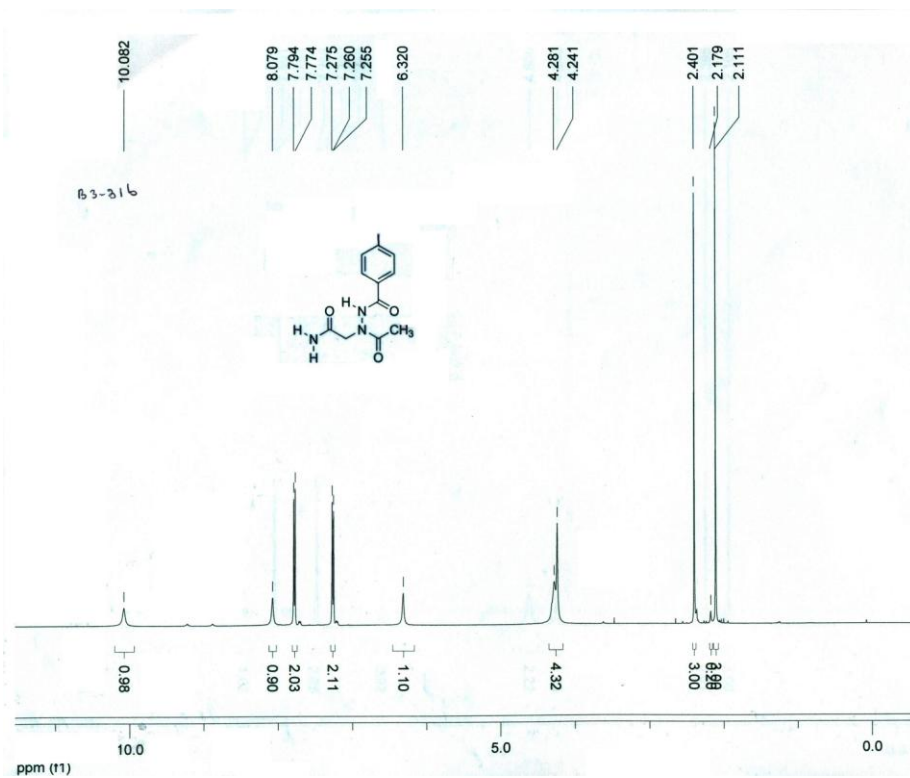


Fig. S9. ^1H NMR spectra of **1b** in CDCl_3 .

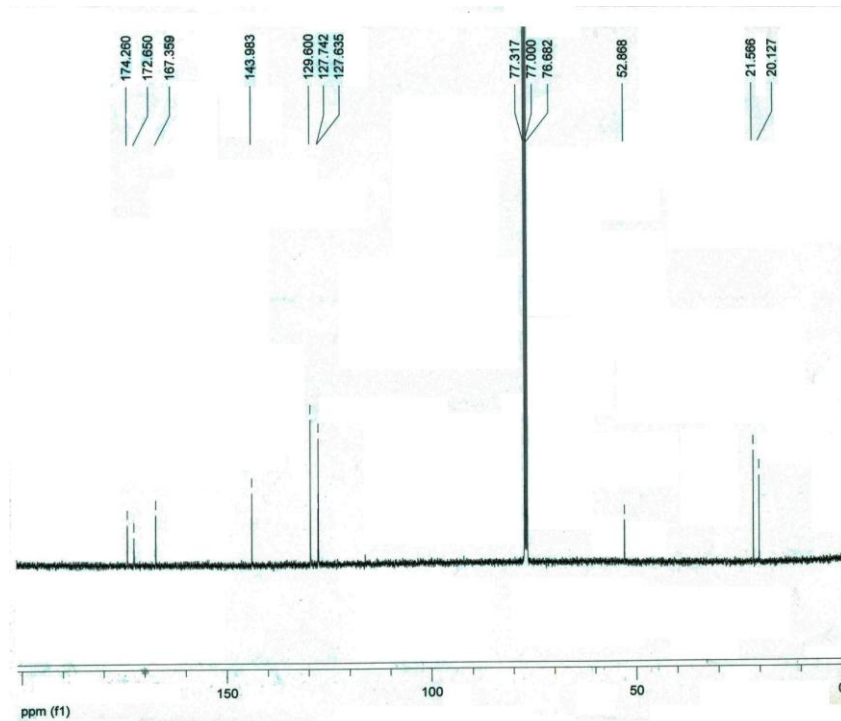


Fig. S10. ^{13}C NMR spectra of **1b** in CDCl_3 .

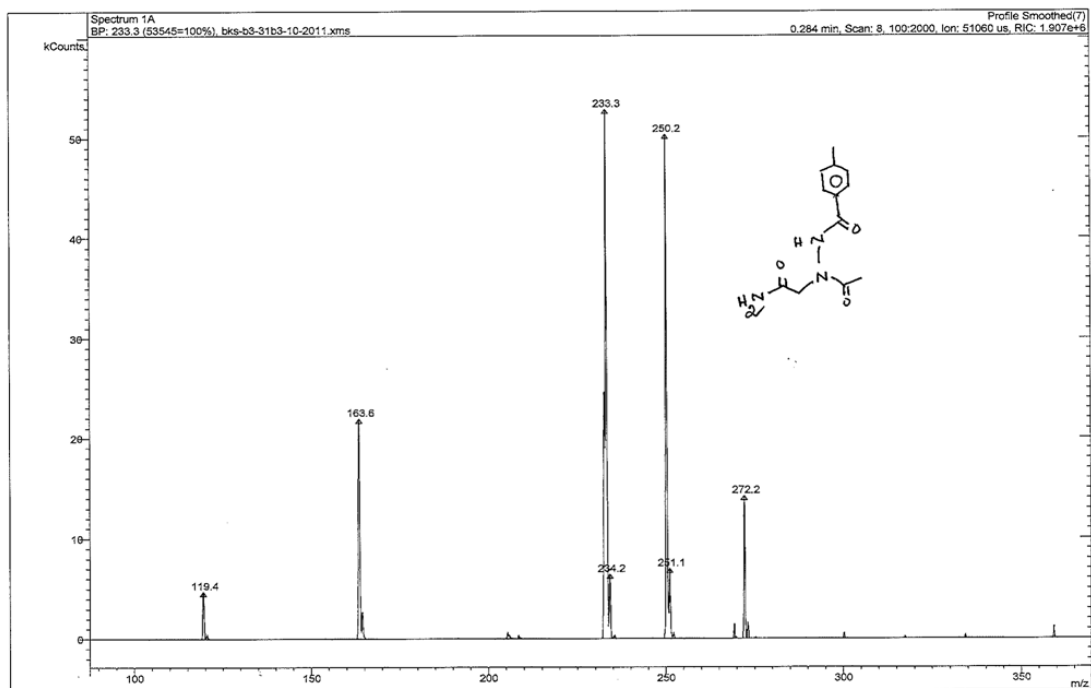


Fig. S11. ESI HRMS of **1b**.

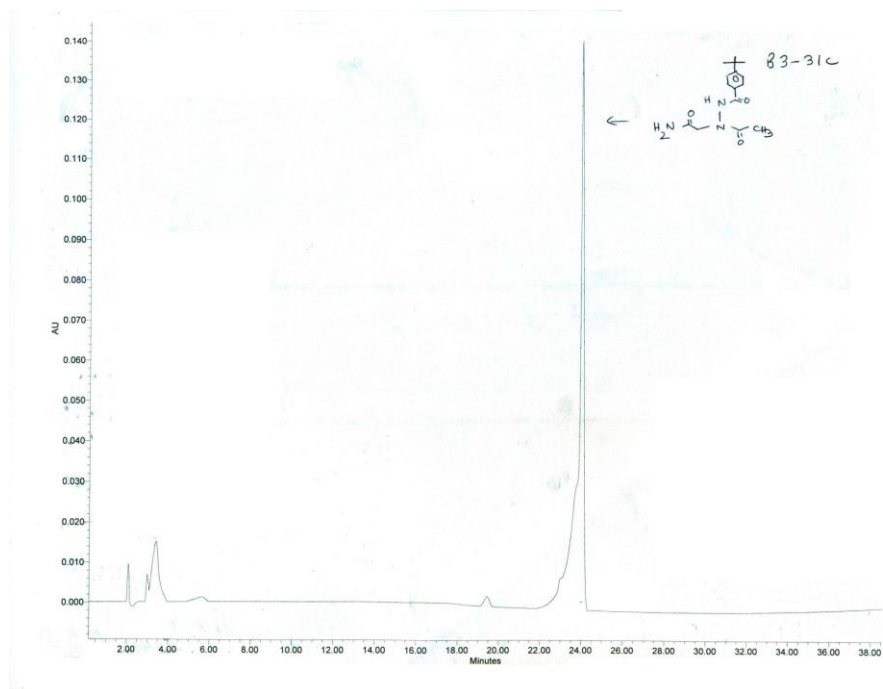


Fig. S12. HPLC chromatogram of crude product **1c**.

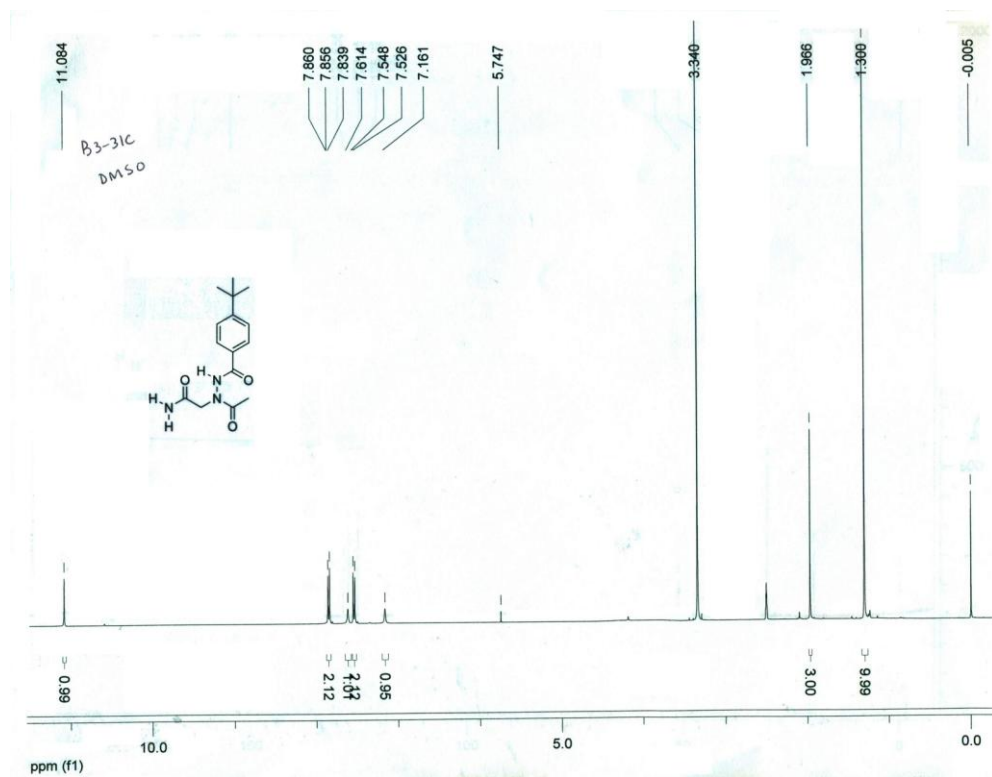


Fig. S13. ^1H NMR spectra of **1c** in DMSO-d_6 .

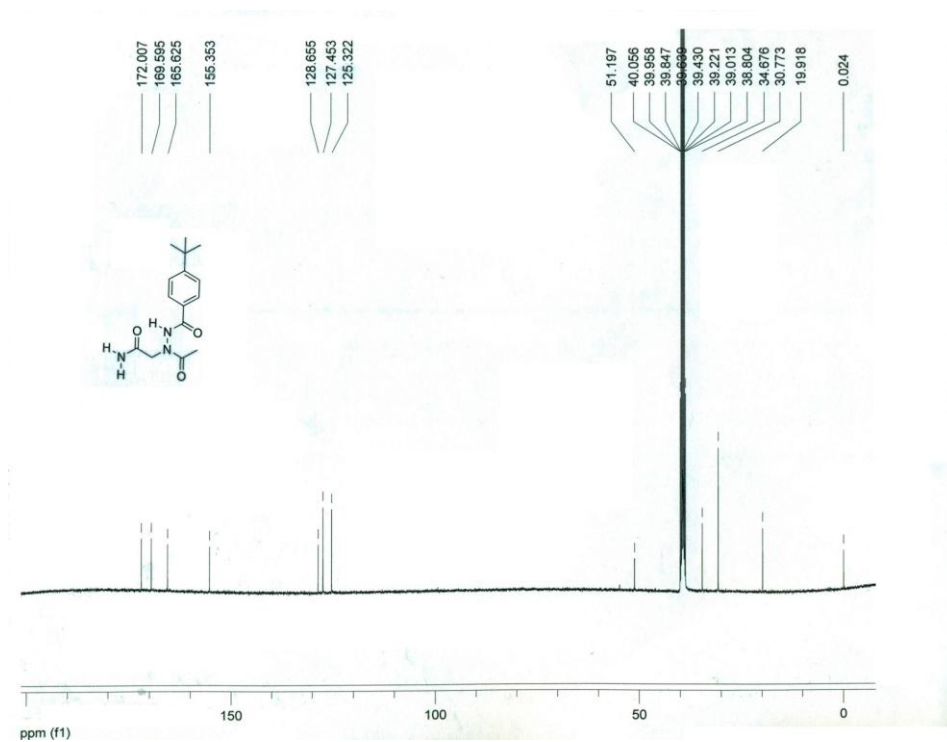


Fig. S14. ¹³C NMR spectra of **1c** in DMSO-d₆.

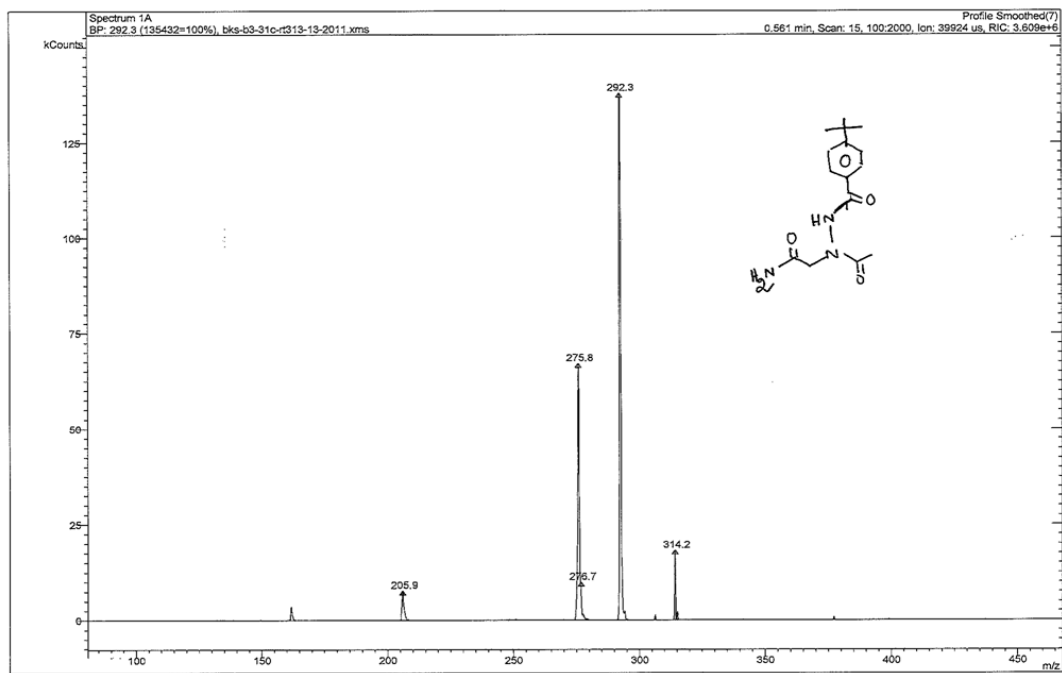


Fig. 15. ESI HRMS of **1c** in in 1:1 CH₃CN:H₂O.

Fig. S17. ^1H NMR spectra of **1d** in DMSO-d_6 .

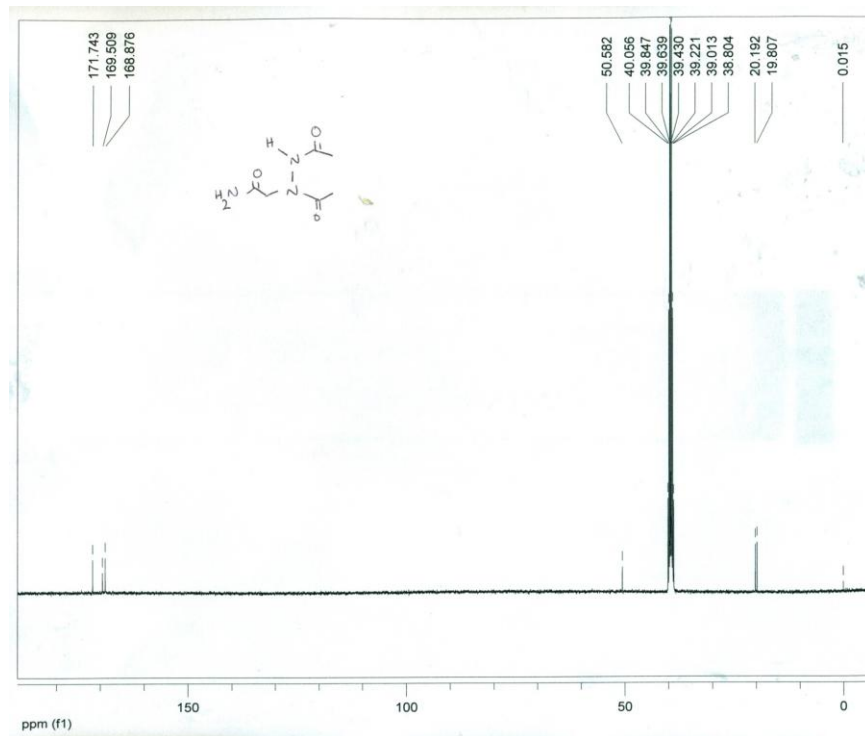


Fig. S18. ^{13}C NMR spectra of **1d** in DMSO-d_6 .

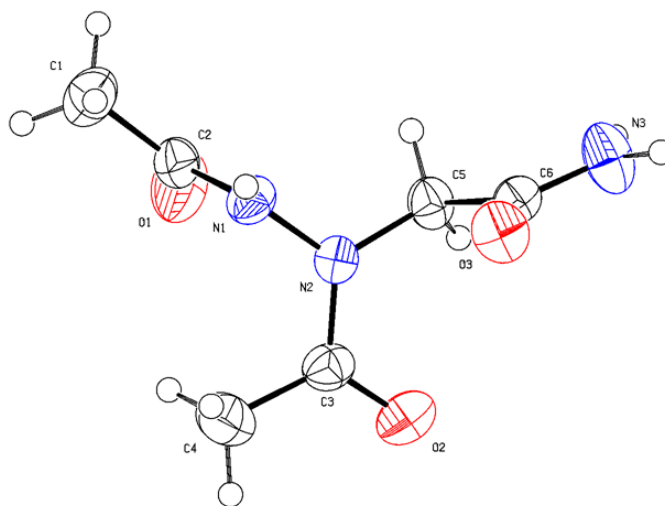


Fig. S19. Single-crystal X-ray structure of **1d** shows the *trans*-amide geometry of both the main chain and side chain amide bonds. $\angle\text{N1-N2-C3-O2} = 179.58^\circ$.

Table S1. Ratio of *cis* and *trans* isomer in **1a-1d**.

Compound	%Cis	%Trans
1a	8	92
1b	5	95
1c	3	97
1d	5	95

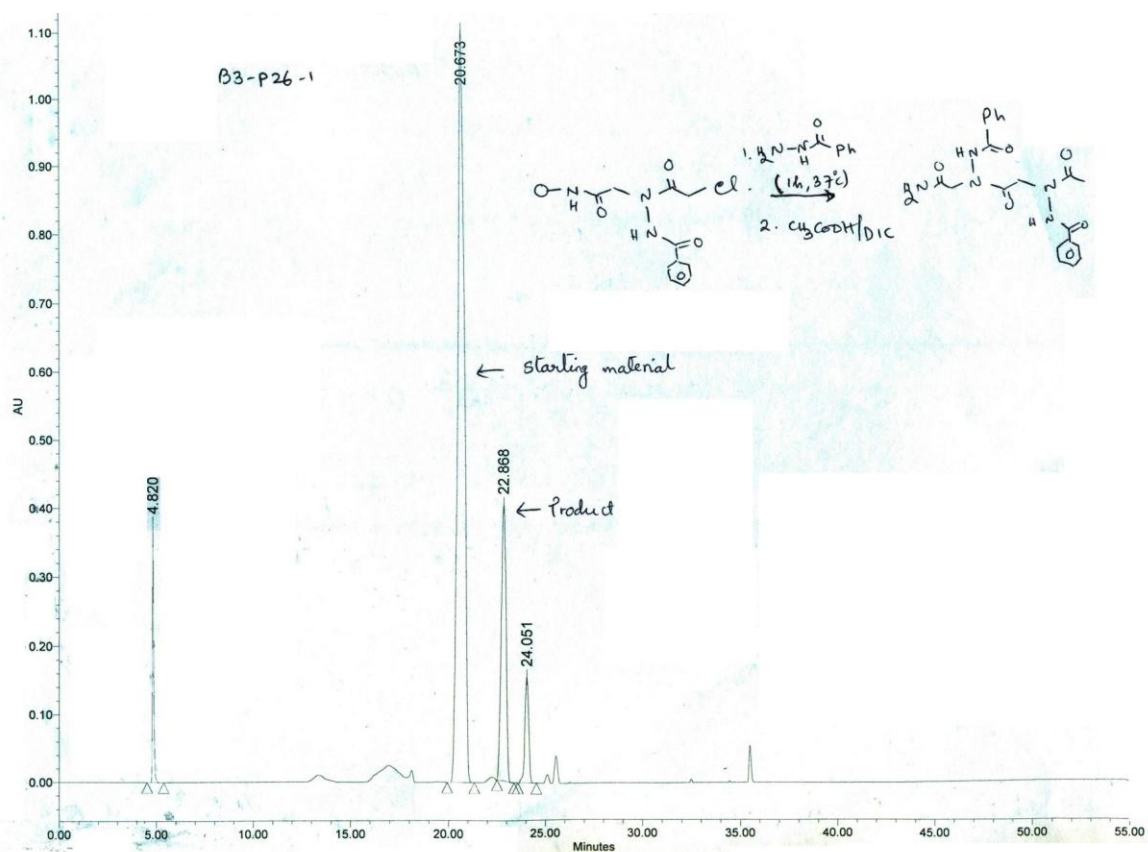


Fig. S20. HPLC chromatogram of crude **2a**, when chloroacetic acid was used for coupling. Gradient of water and acetonitrile was used as eluent.

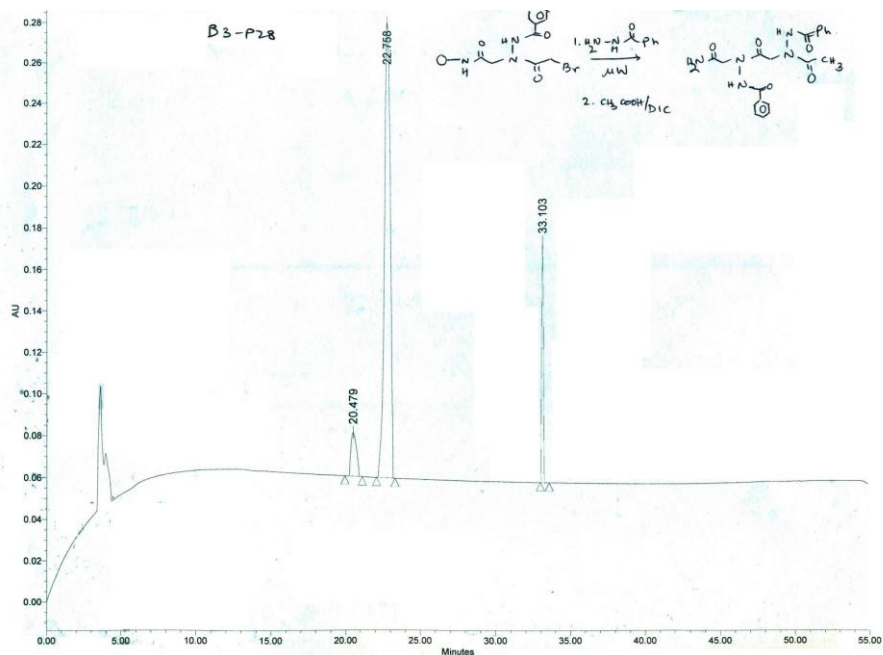


Fig. S21. HPLC chromatogram of crude **2a**. Bromoacetic acid was used and reaction done under microwave condition. Gradient of water and acetonitrile was used as eluent.

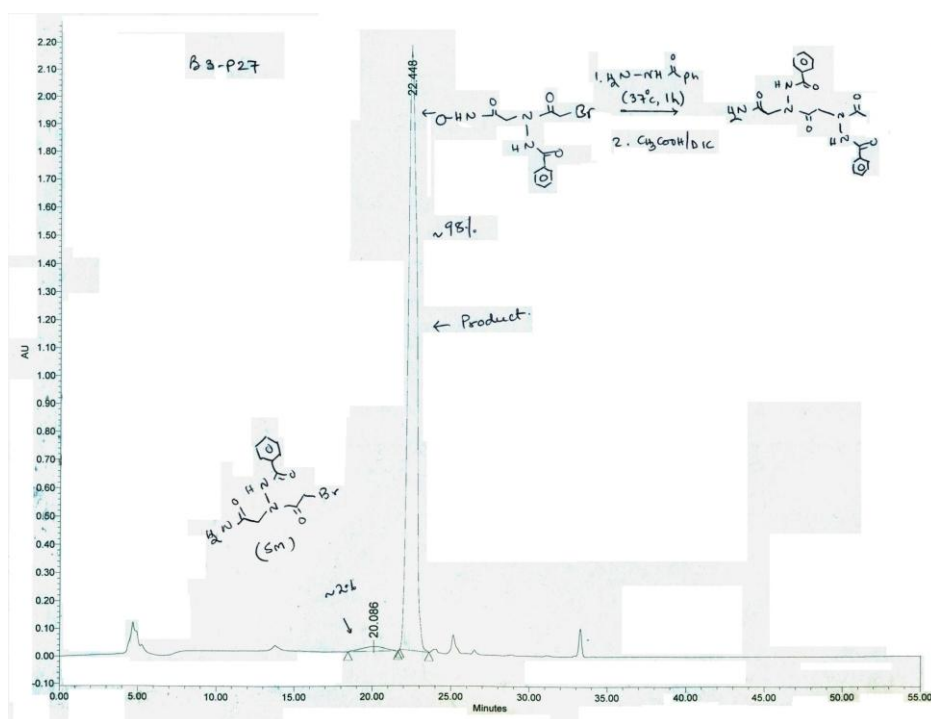


Fig. S22. HPLC chromatogram of crude **2a**. Bromoacetic acid was used and all reactions were done at 37°C . Gradient of water and acetonitrile was used as eluent.

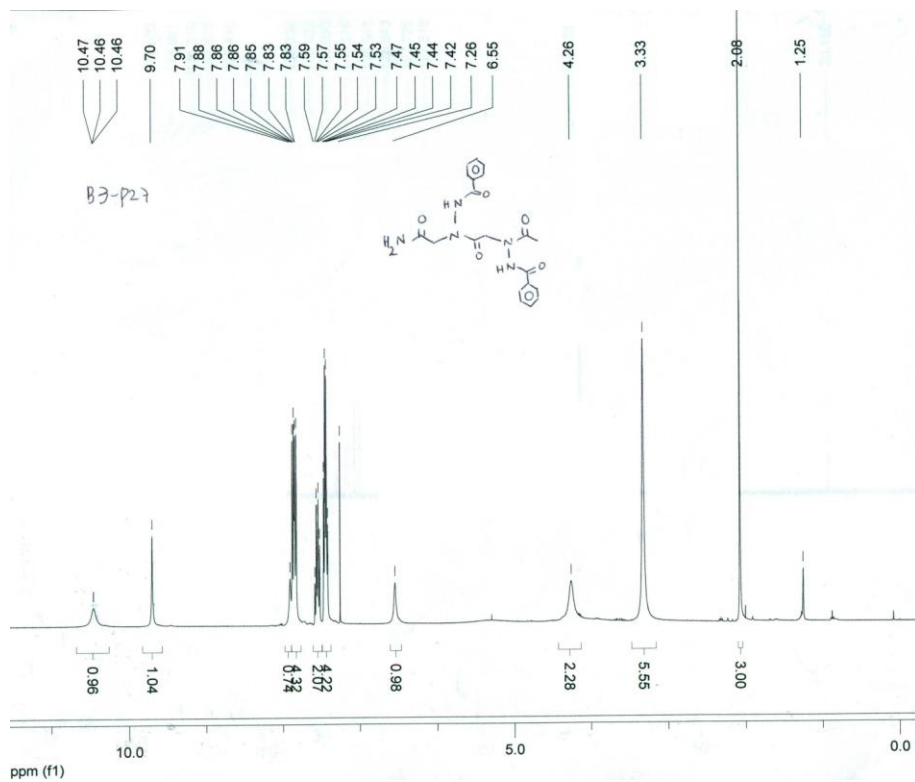


Fig. S23. ^1H NMR spectra of **2a** at 25 °C in CDCl_3 .

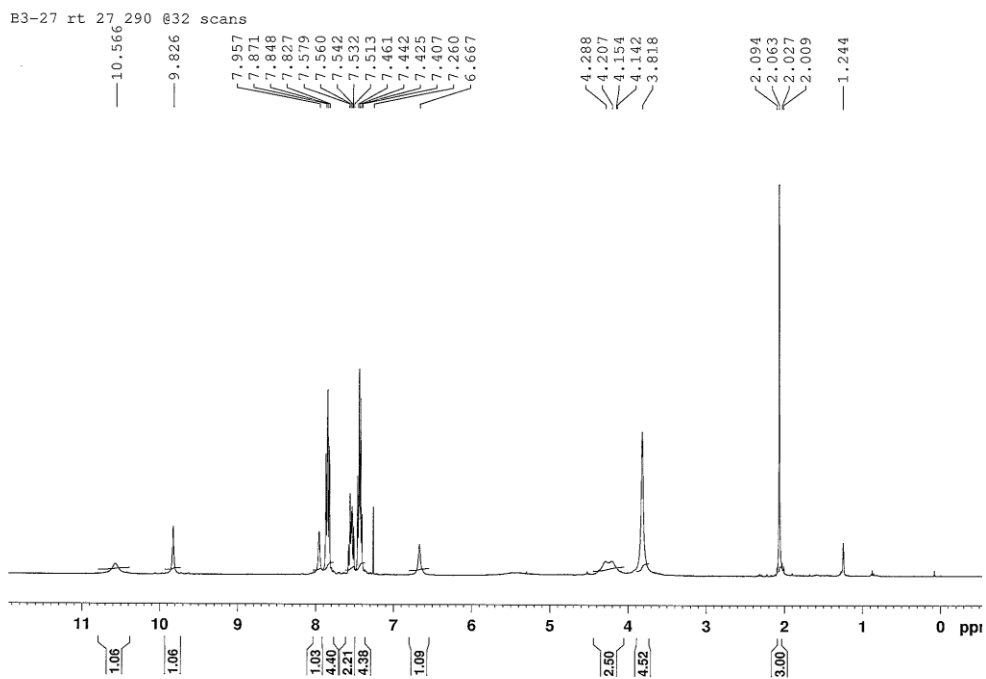


Fig. S24. ^1H NMR spectra of **2a** at 17 °C in CDCl_3 .

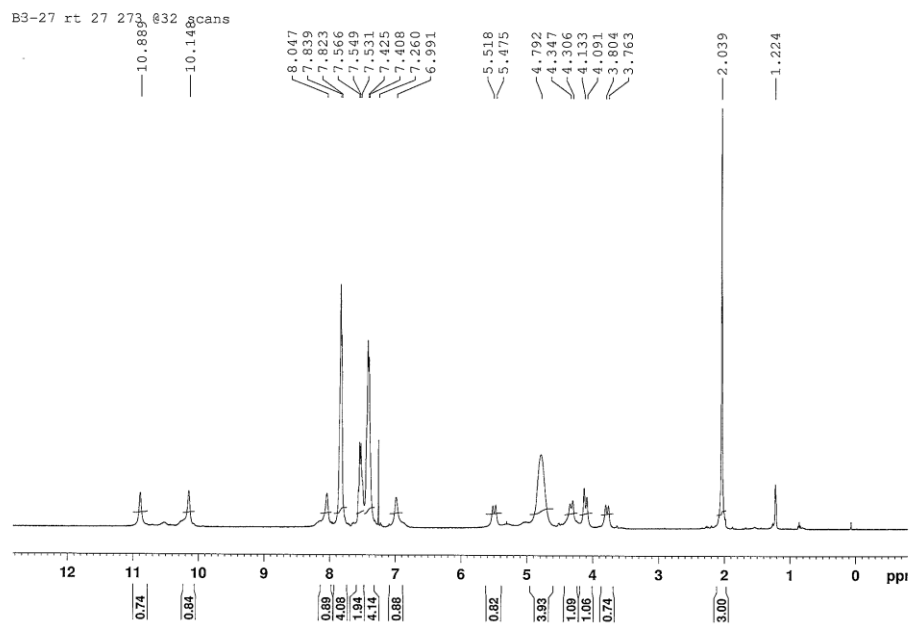


Fig. S25. ^1H NMR spectra of **2a** at 0 °C in CDCl_3 .

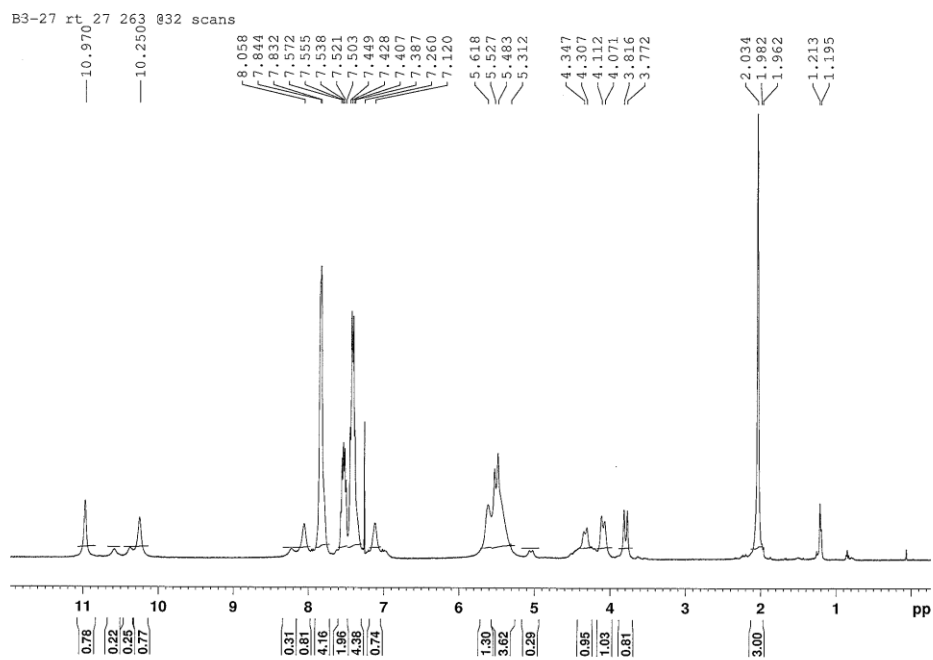


Fig. S26. ^1H NMR spectra of **2a** at -10 °C in CDCl_3 .

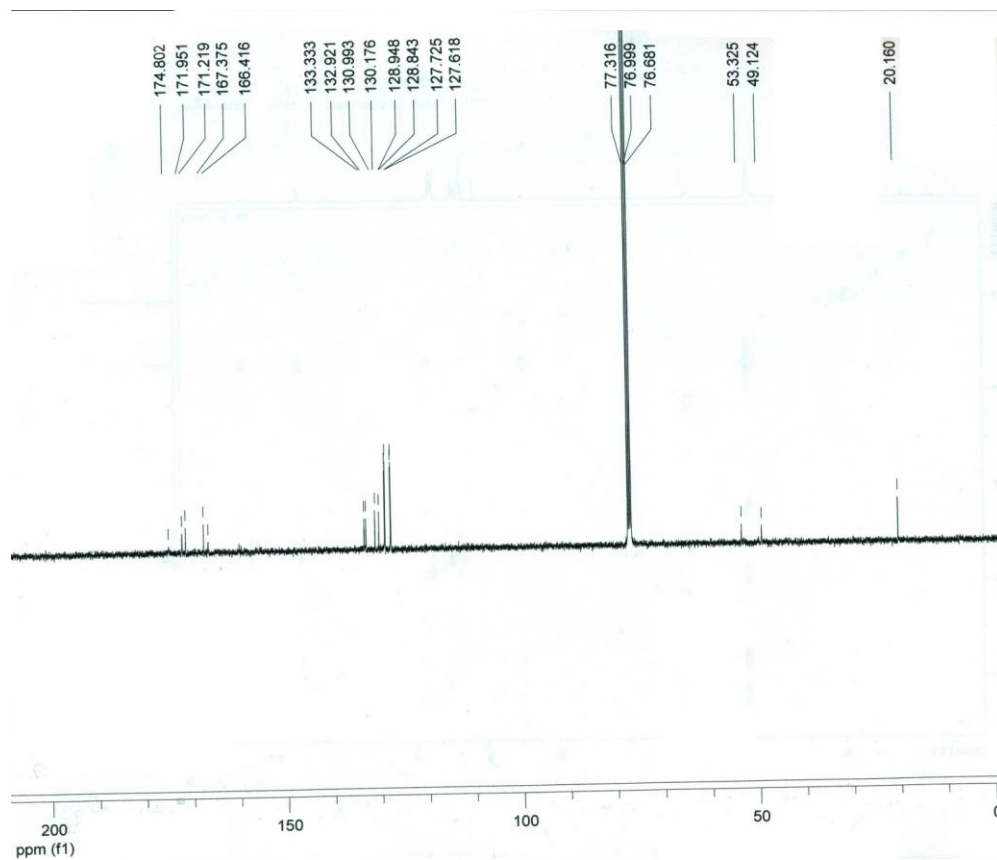


Fig. S27. ^{13}C NMR spectra of **2a** at 25 °C in CDCl_3 .

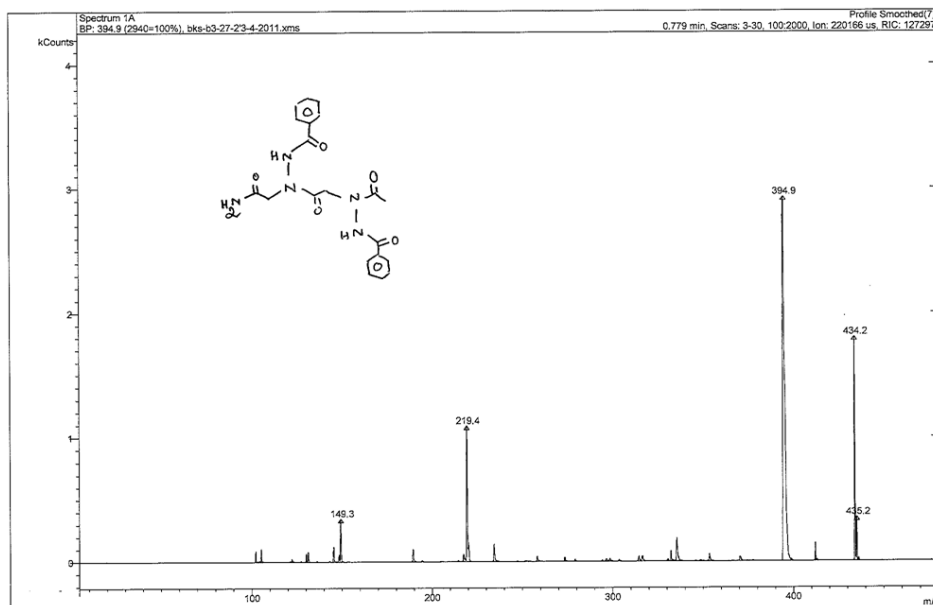


Fig. S28. ESI HRMS of **2a**.

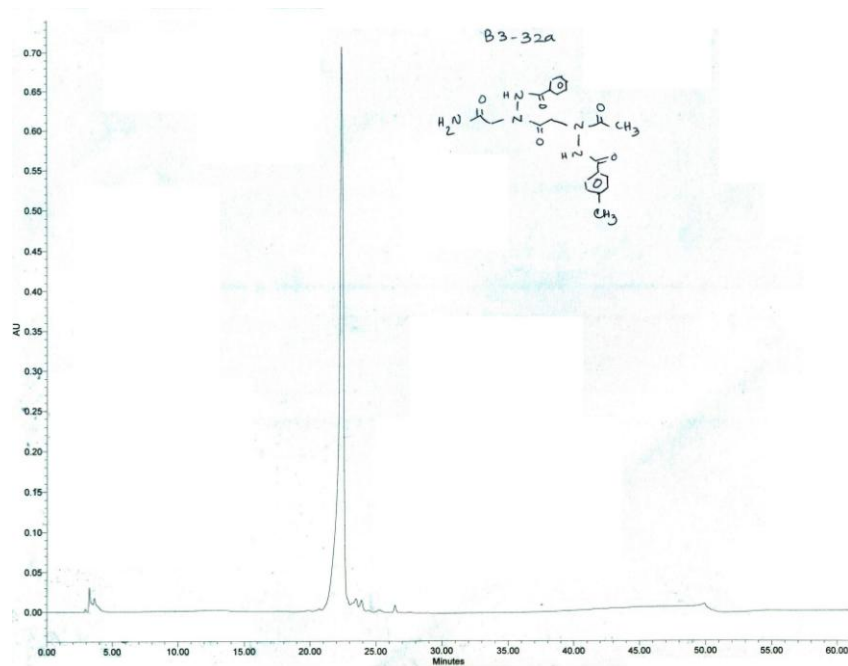


Fig. S29. HPLC chromatogram of crude **2b**.

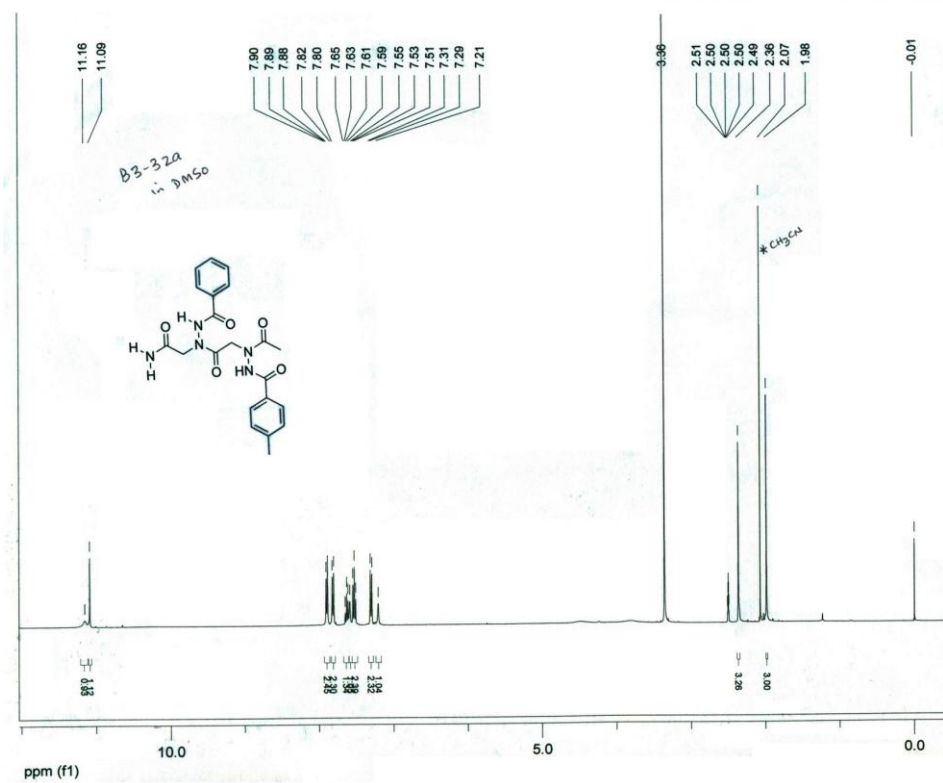


Fig. S30. ^1H NMR spectra of **2b** in DMSO-d_6 .

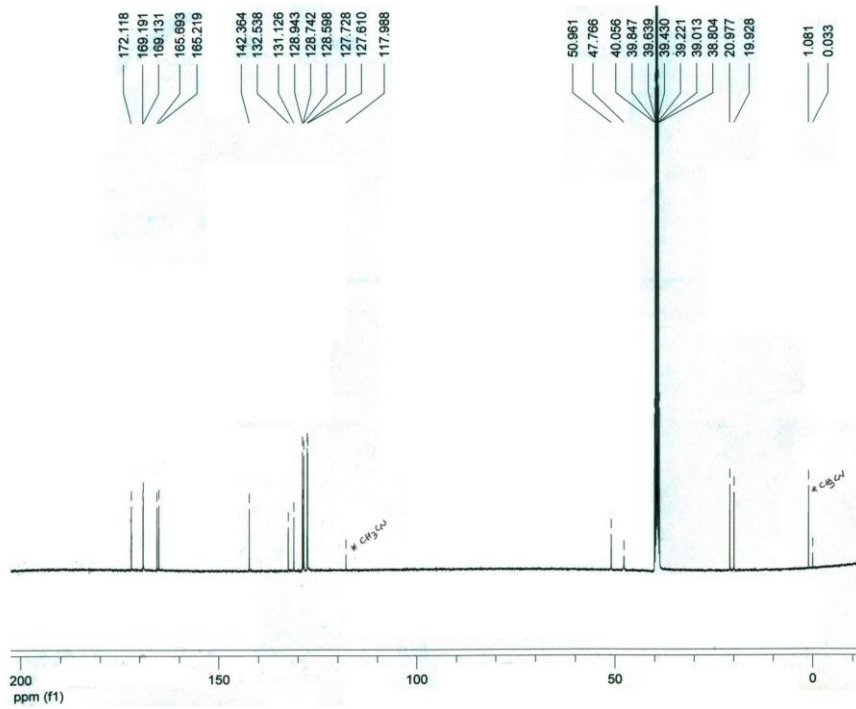


Fig. S31. ^{13}C NMR spectra of **2b** in DMSO-d_6 .

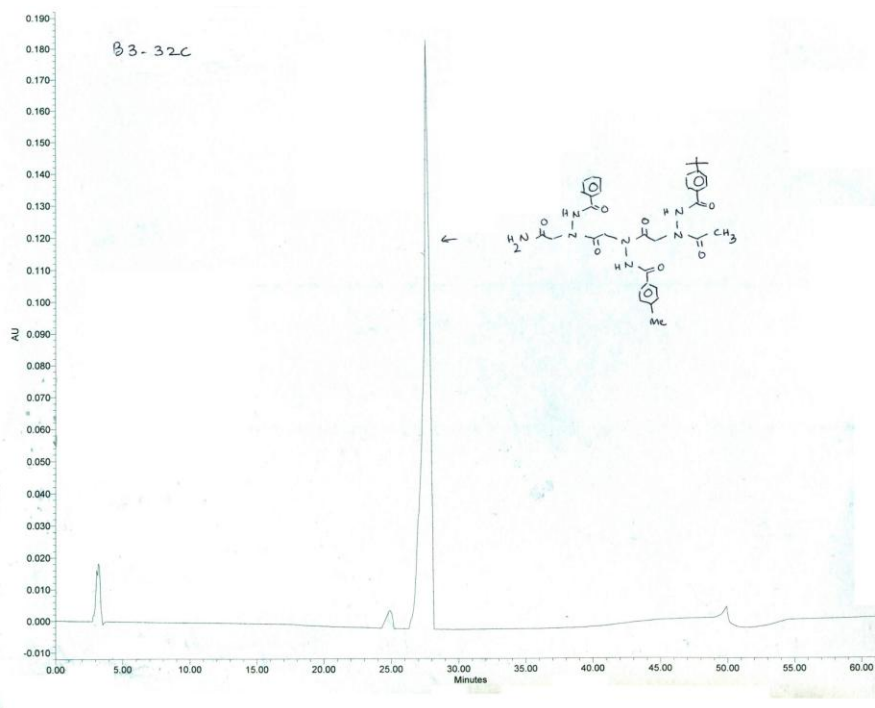


Fig. S32. HPLC chromatogram of crude **3**.

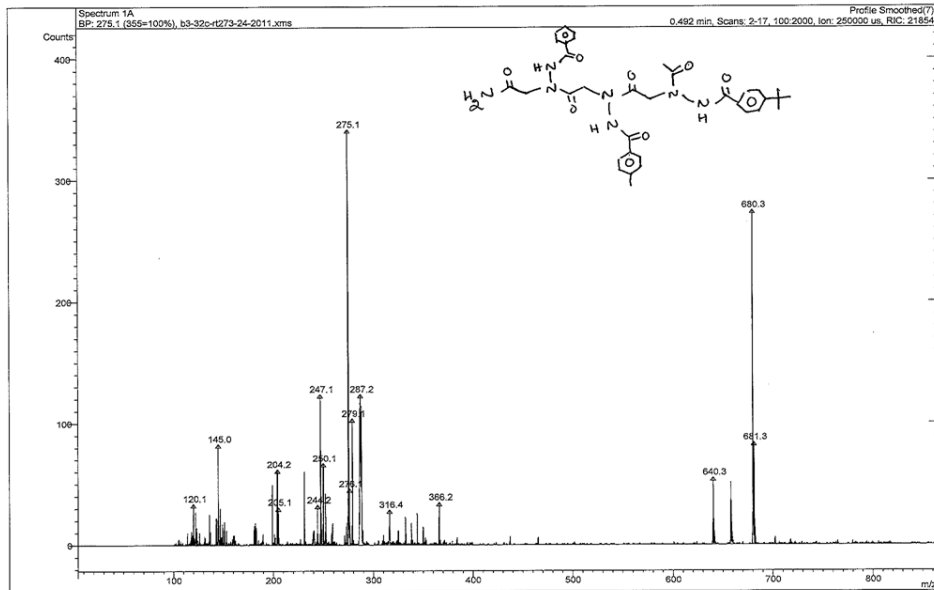


Fig. S33. ESI HRMS of **3**.

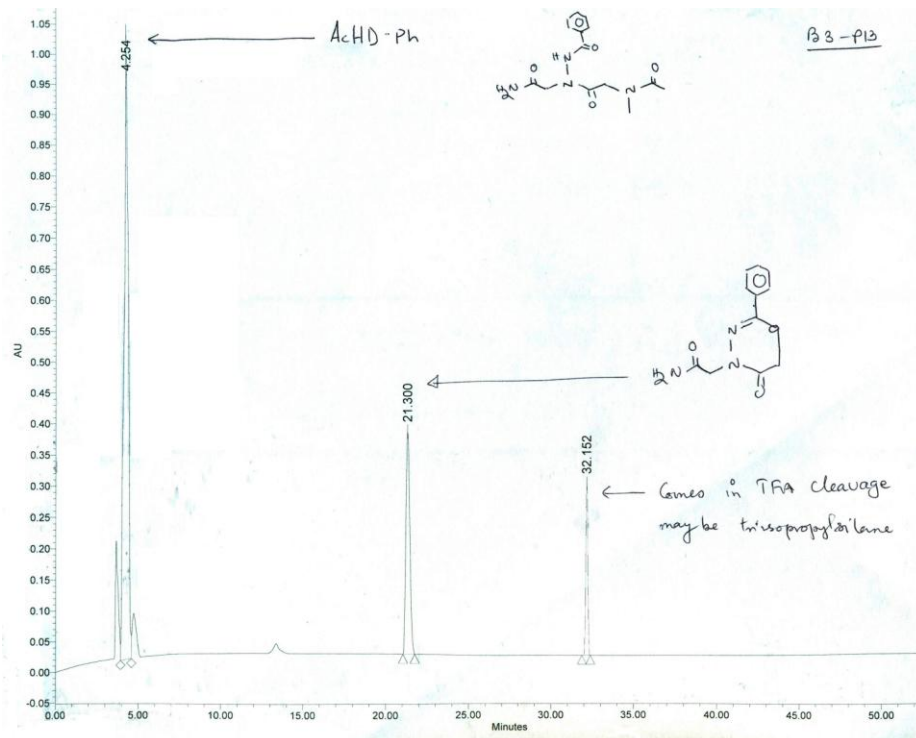


Fig. S34. HPLC chromatogram of crude **4a**.

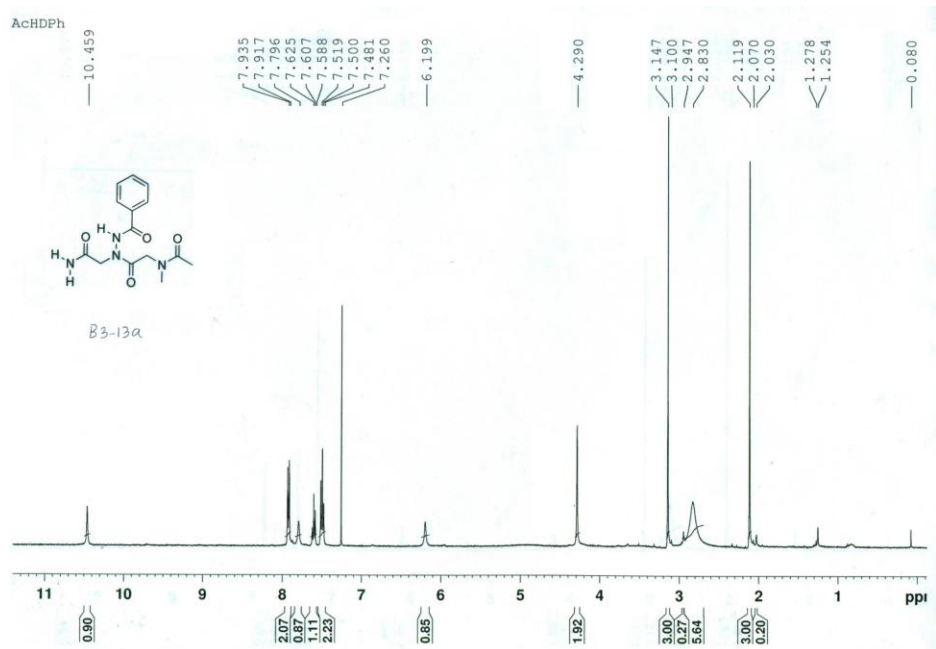


Fig. S35. ^1H NMR spectra of **4a** in CDCl_3 .

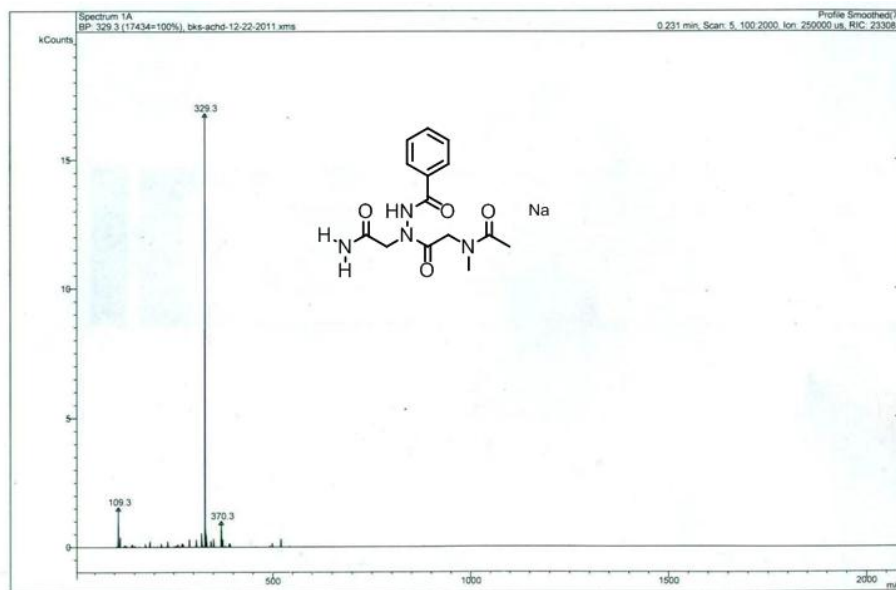


Fig. S36. ESI HRMS of **4a**.

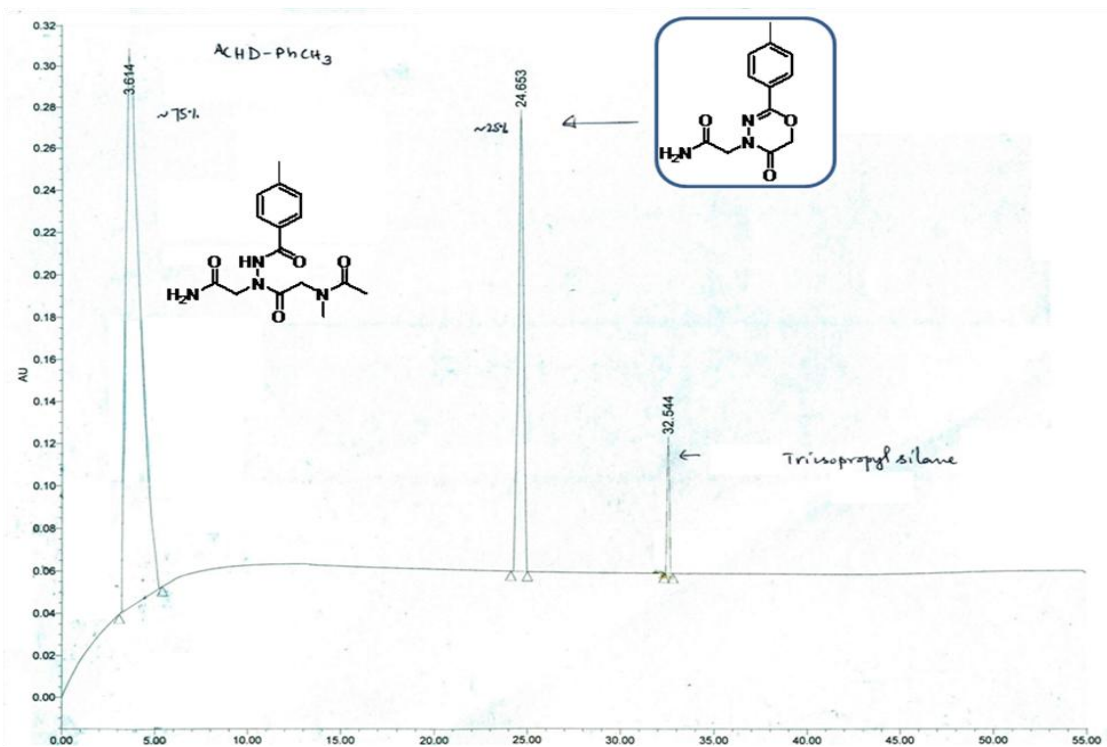


Fig. S37. HPLC chromatogram of crude **4b**.

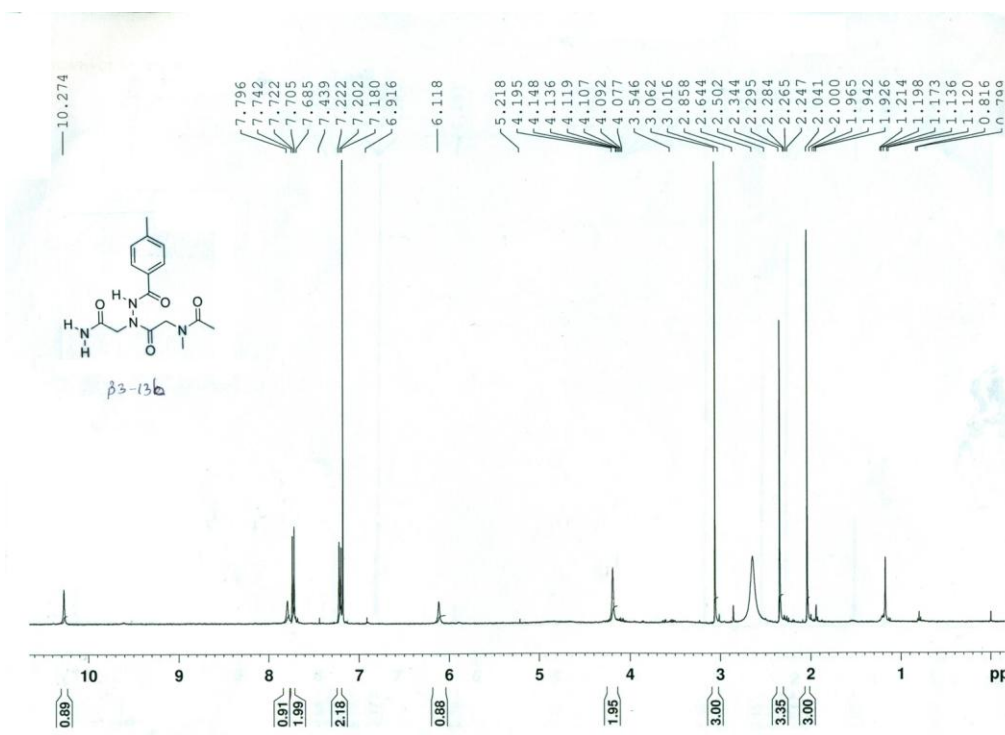


Fig. S38. ¹H NMR spectra of **4b** in CDCl₃.

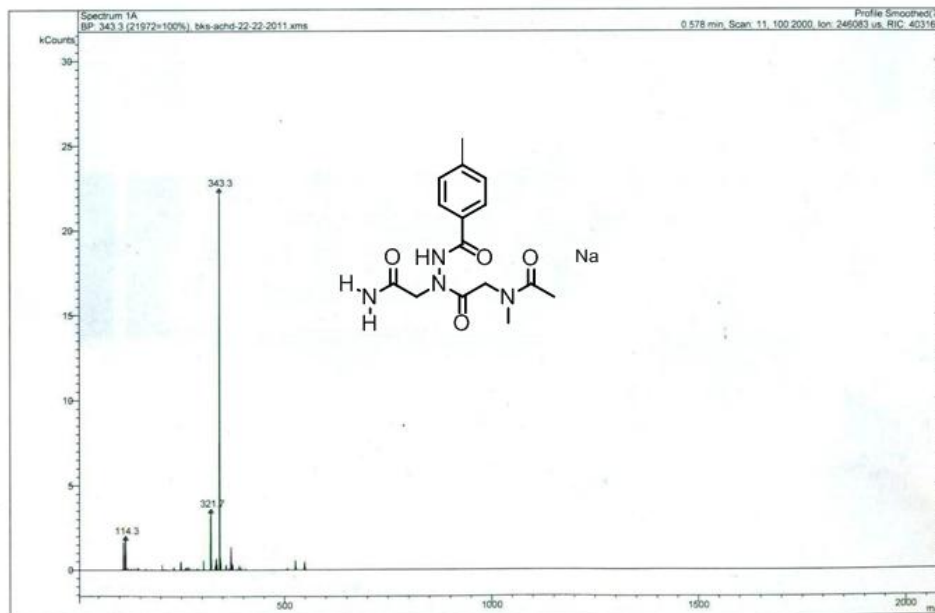


Fig. S39. ESI HRMS of **4b**.

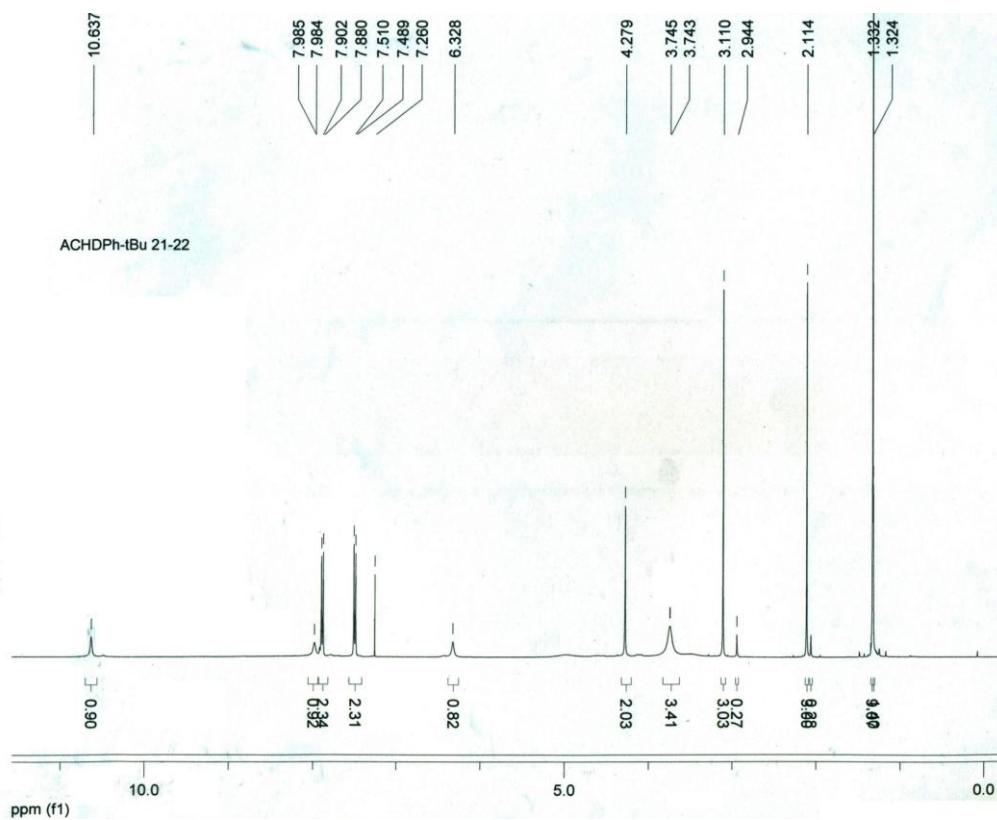


Fig. S40. ^1H NMR spectra of **4c** in CDCl_3 .

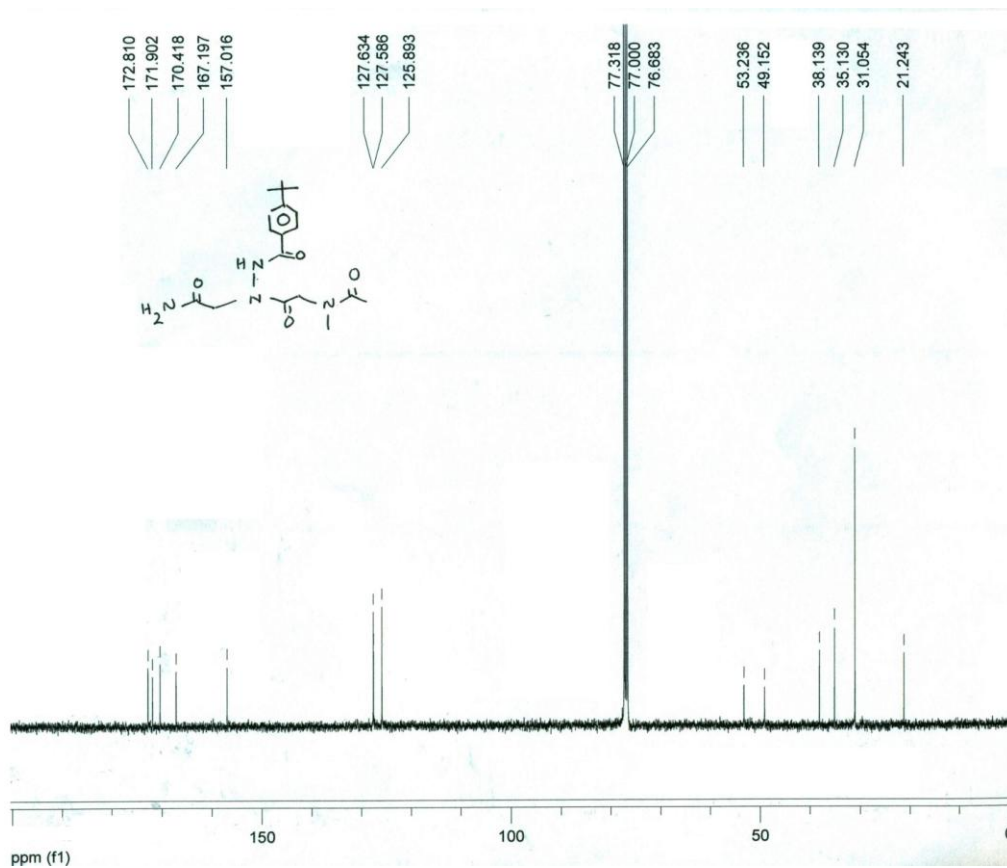


Fig. S41. ^{13}C NMR spectra of **4c** in CDCl_3 .

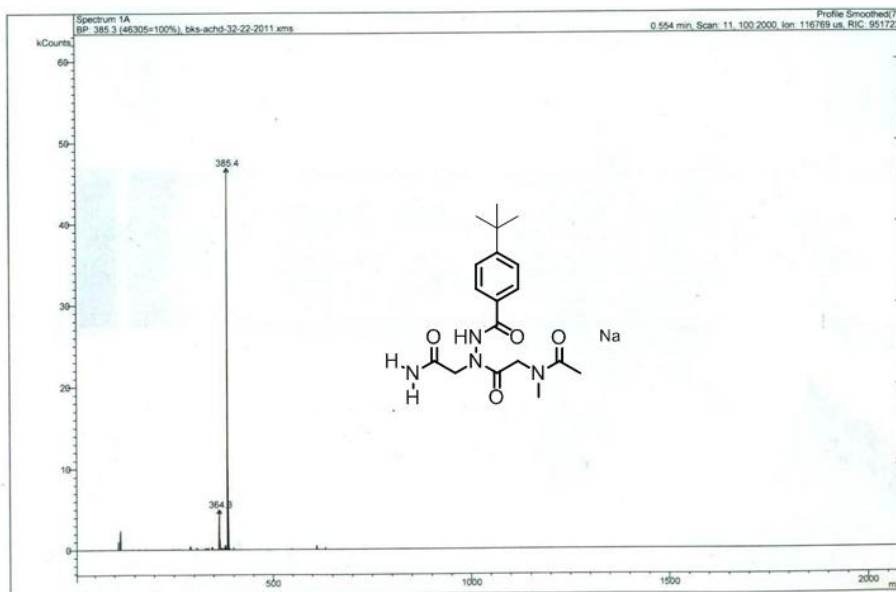


Fig. S42. ESI HRMS of **4b**.

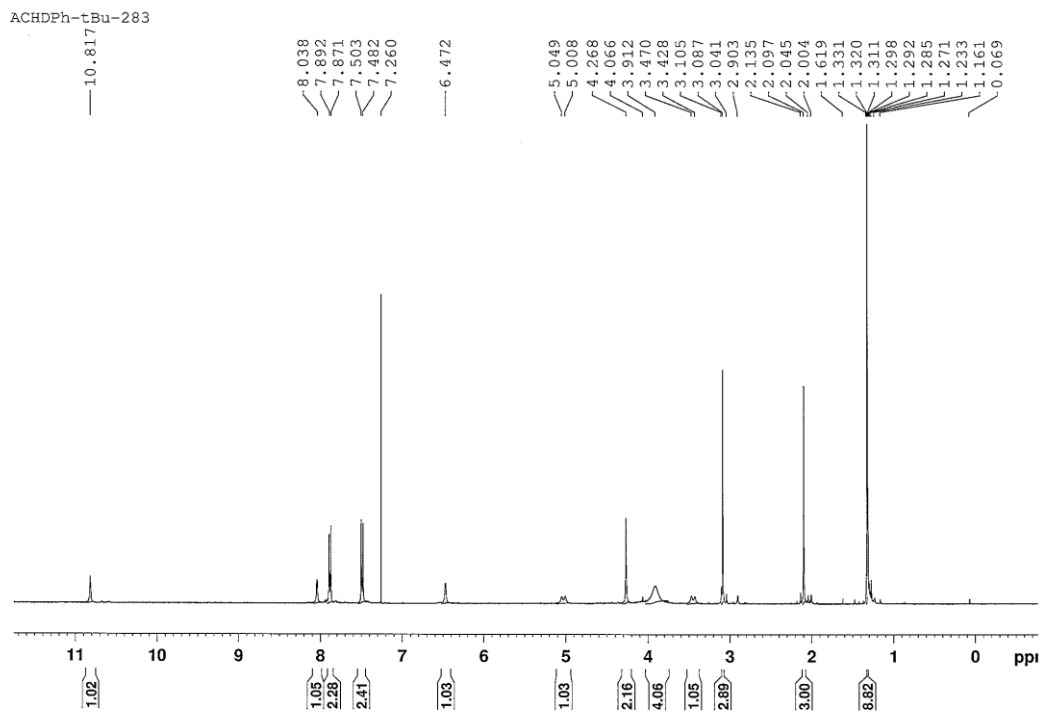


Fig. S43. ^1H NMR spectra of **4c** at 10 °C in CDCl_3 .

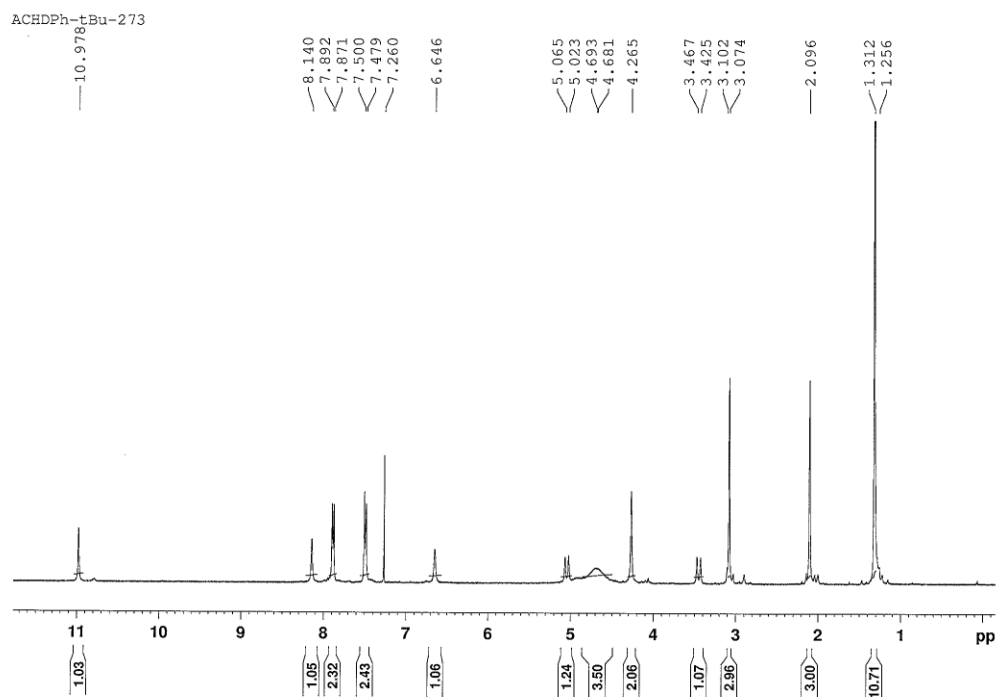


Fig. S44. ^1H NMR spectra of **4c** at 0 °C in CDCl_3 .

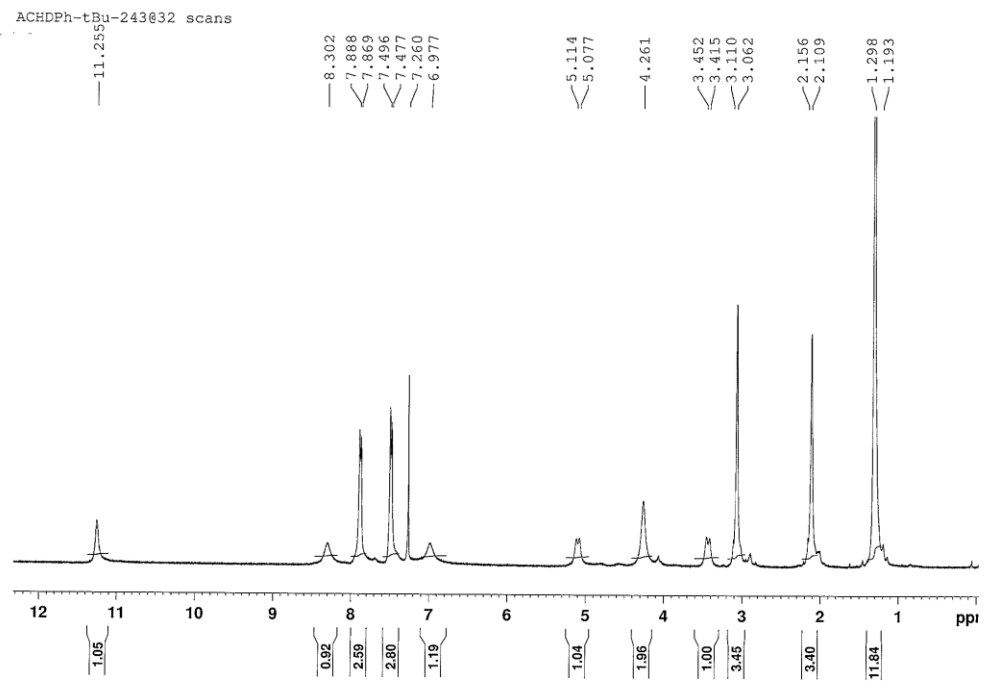


Fig. S45. ^1H NMR spectra of **4c** at -30°C in CDCl_3 .

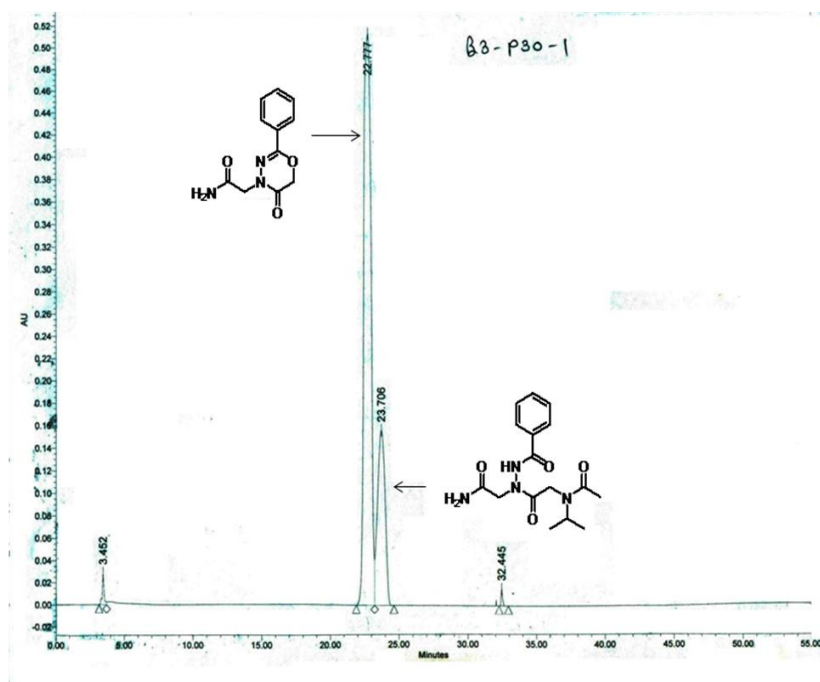


Fig. S46. HPLC chromatogram for crude **6**.

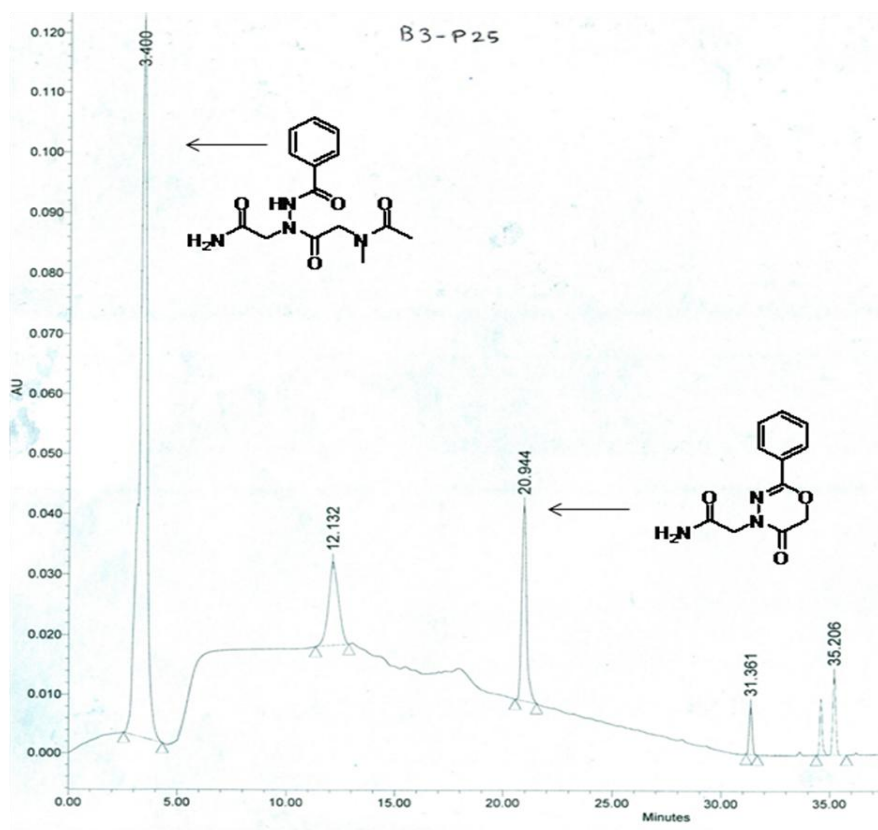


Fig. S47. The HPLC chromatogram of crude **4a** when chloroacetic acid was used as acylating agent.

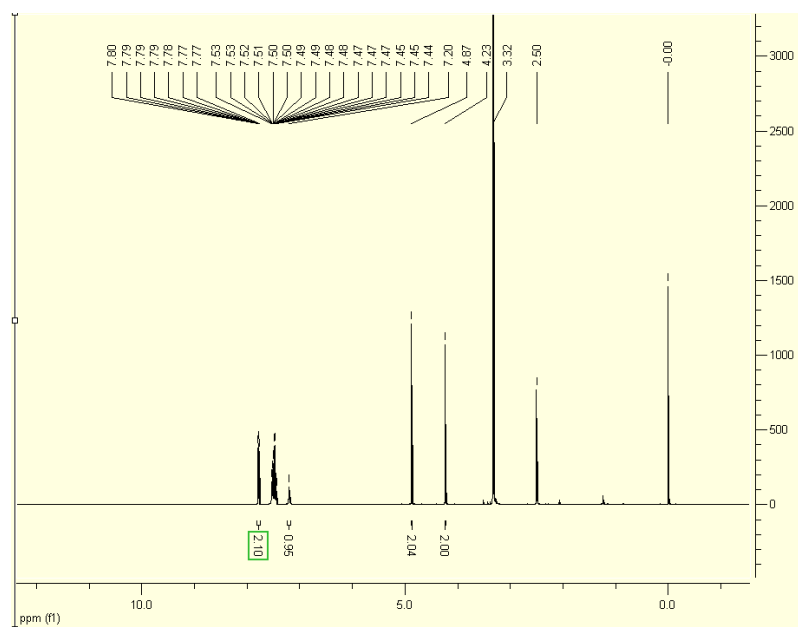


Fig. S48. ^1H NMR spectra of **5a** recorded in DMSO-d_6 .

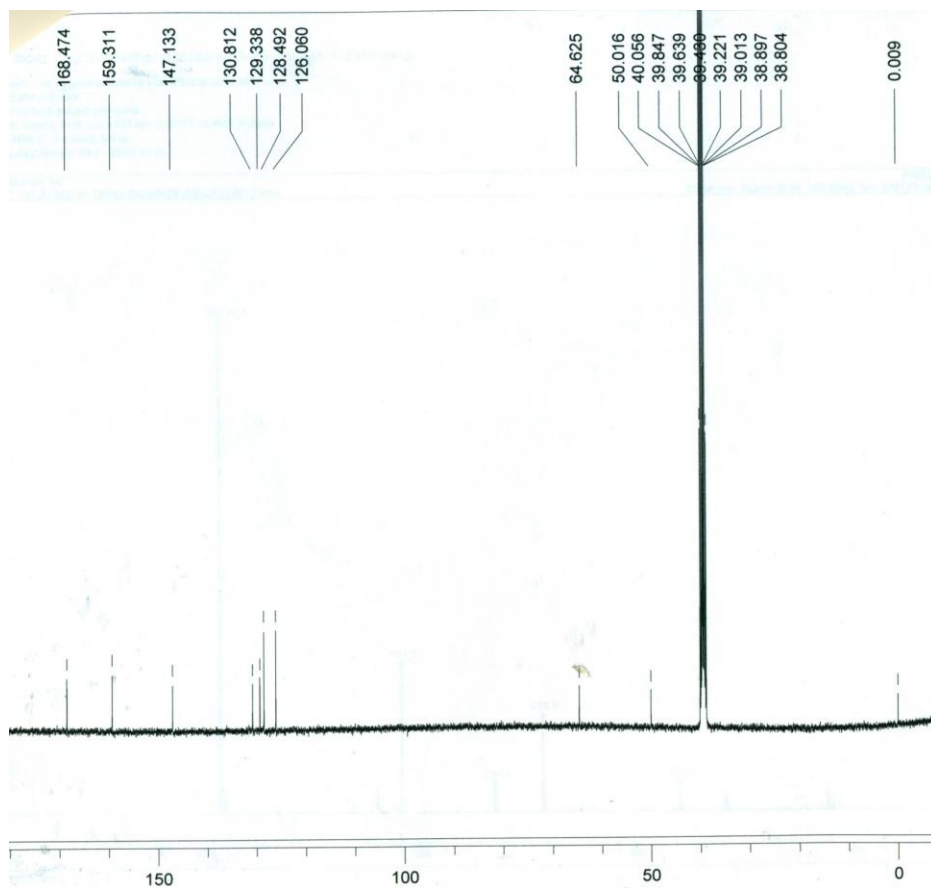


Fig. S49. ^{13}C NMR spectra of **5a** recorded in DMSO-d_6 .

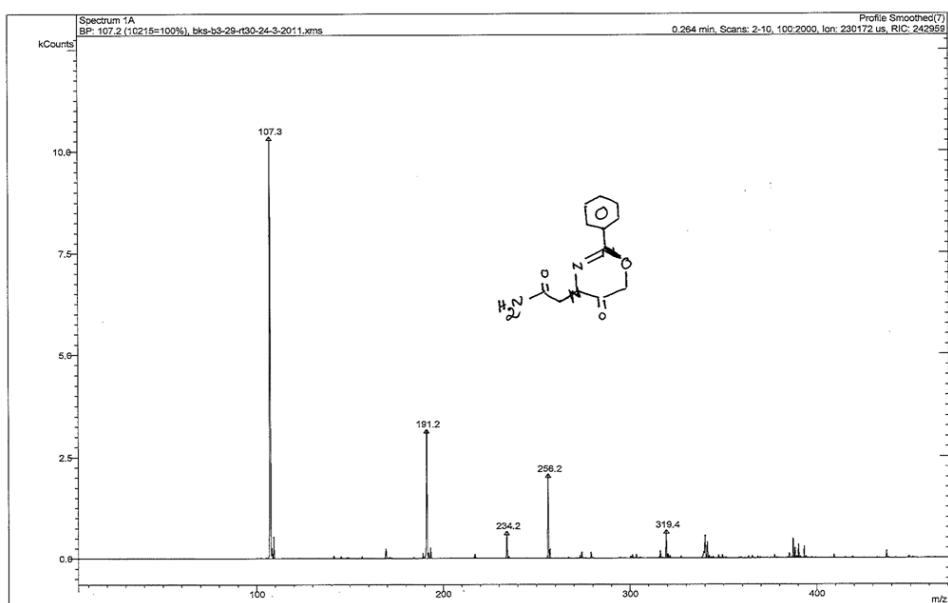


Fig. S50. ESI HRMS of **5a**.

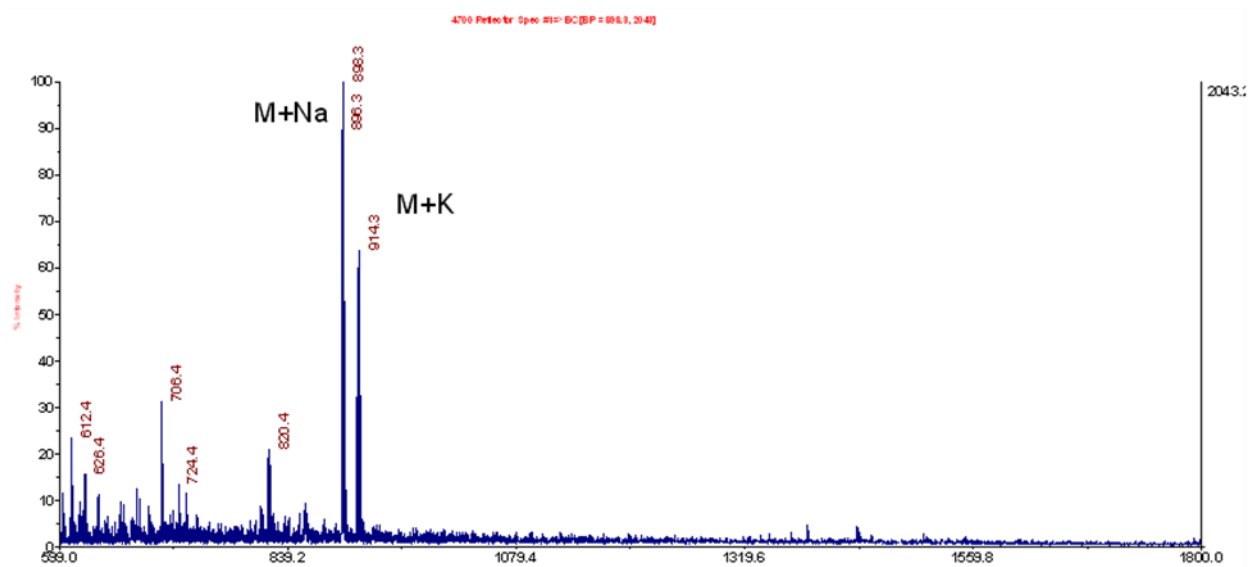
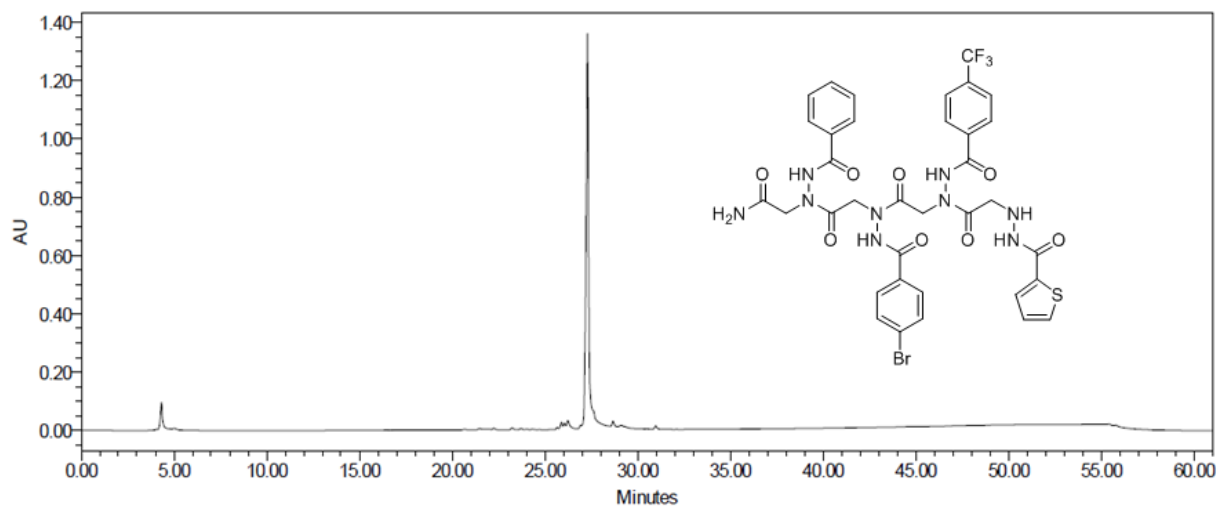


Fig. 51. HPLC chromatogram and MALDI TOF MS spectra of tetramer TM1.

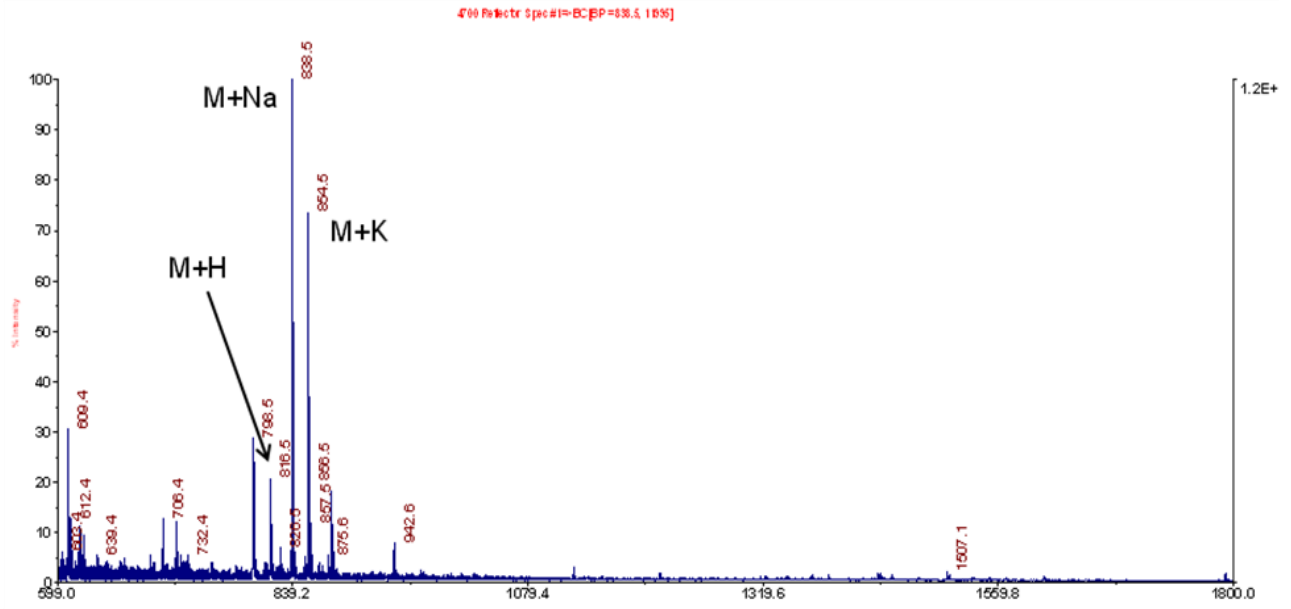
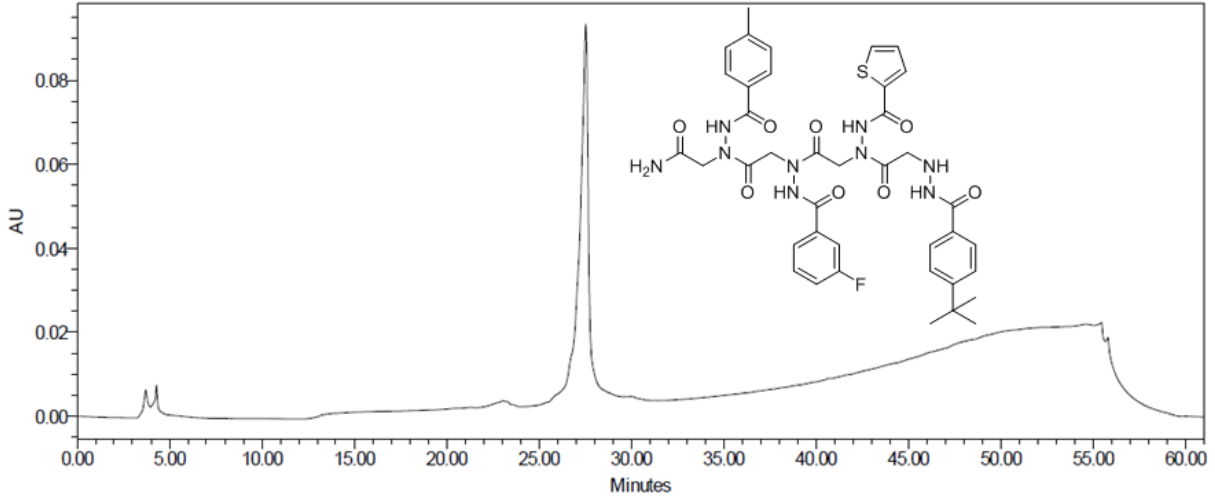


Fig. 52. HPLC chromatogram and MALDI TOF MS spectra of tetramer TM2.

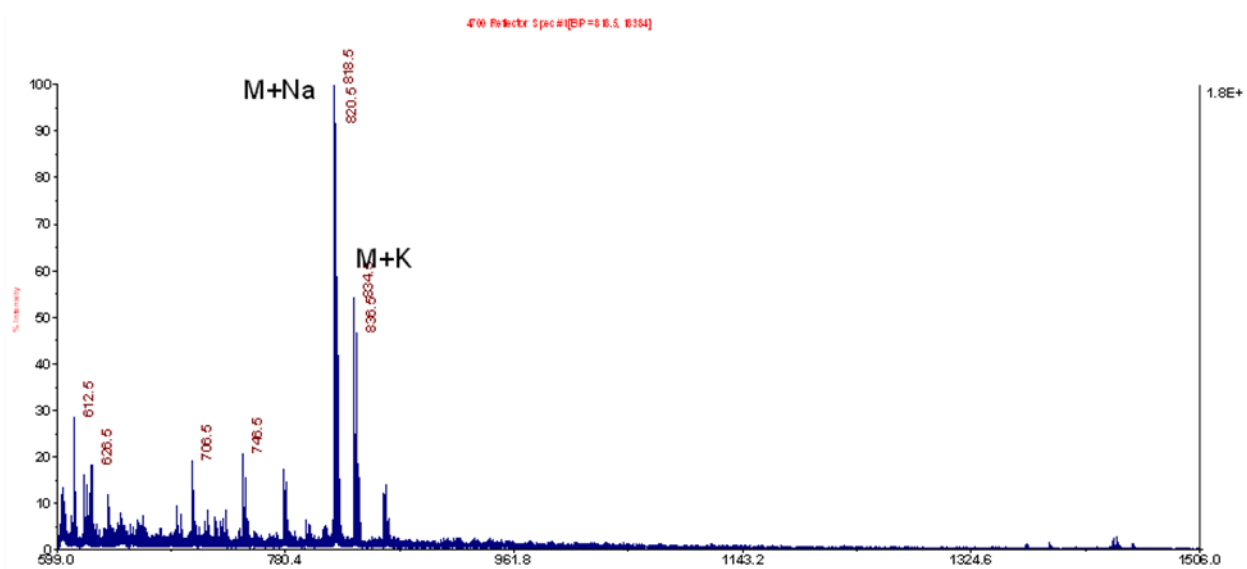
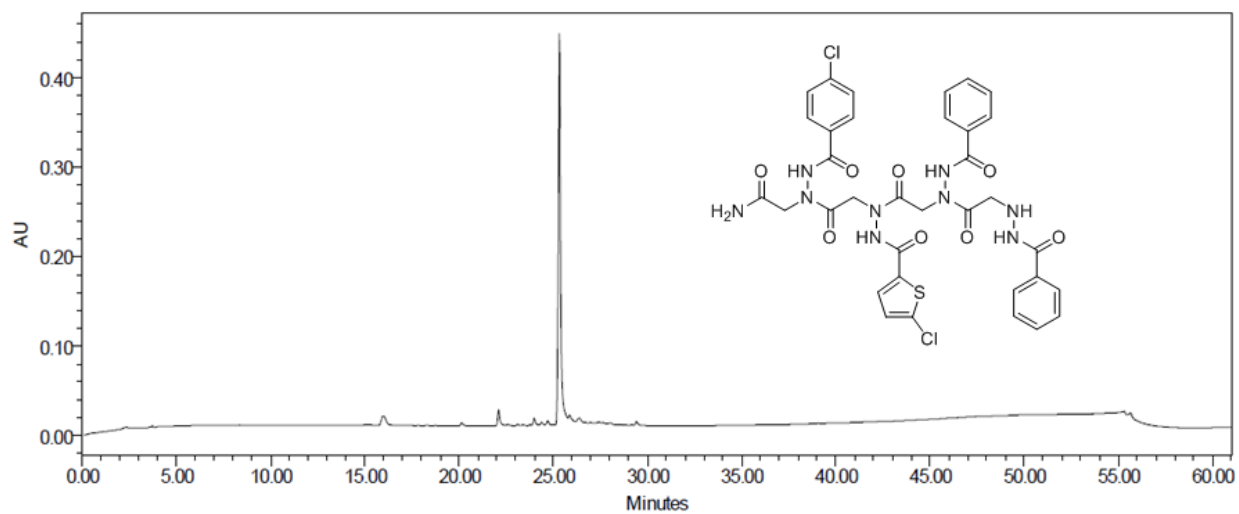


Fig. 53. HPLC chromatogram and MALDI TOF MS spectra of tetramer TM3.

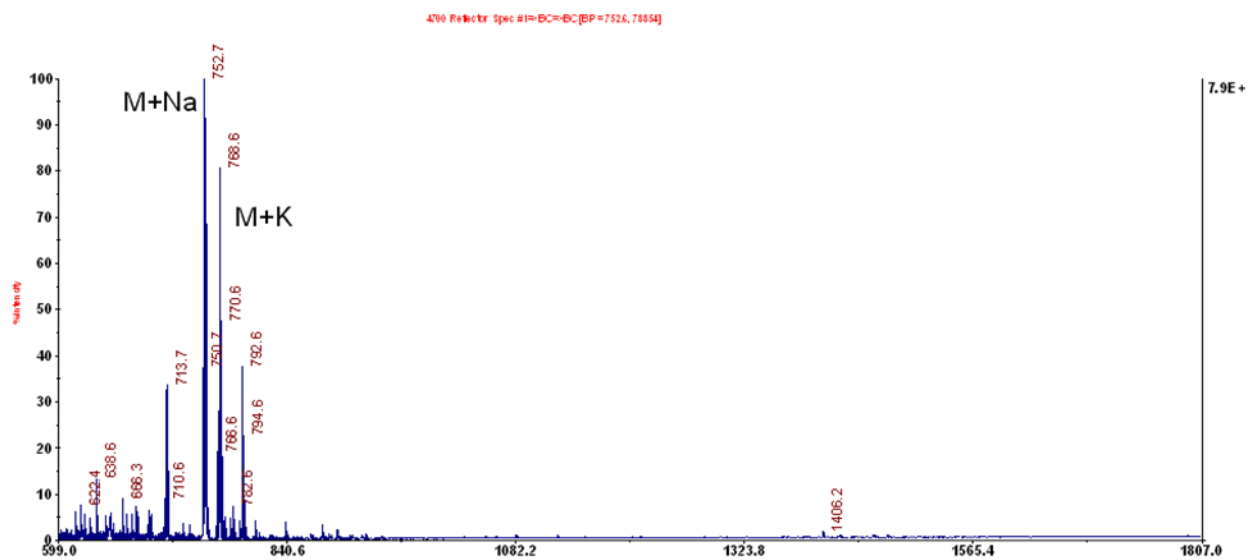
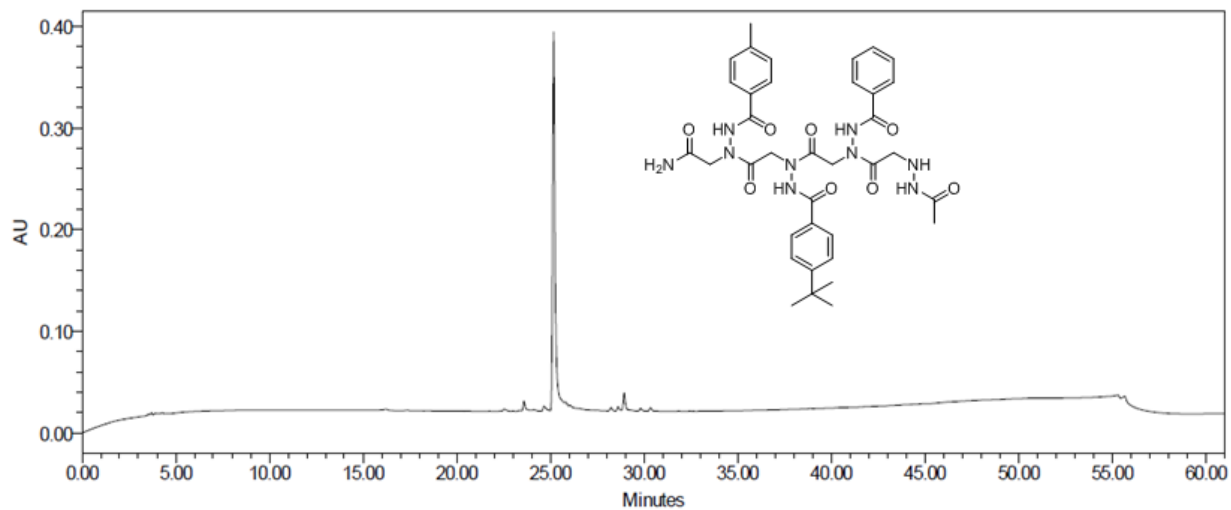


Fig. 54. HPLC chromatogram and MALDI TOF MS spectra of tetramer TM4.

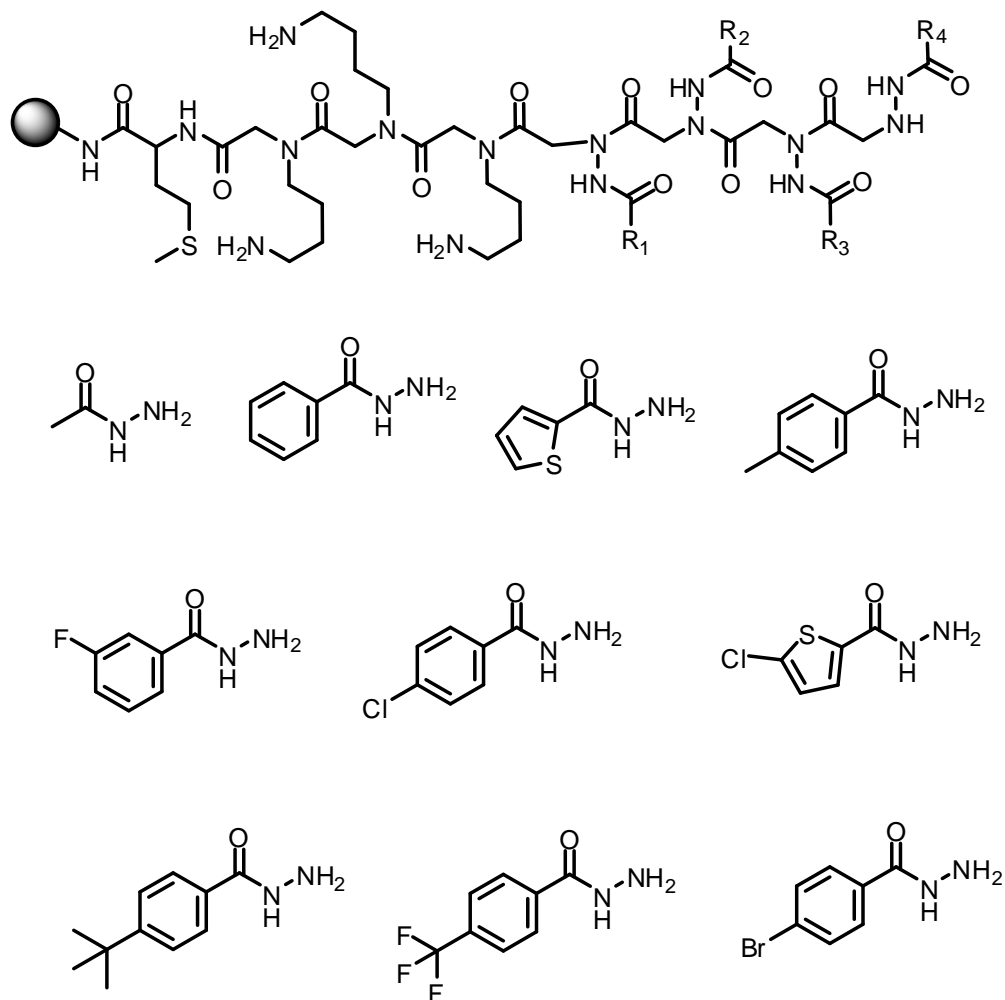
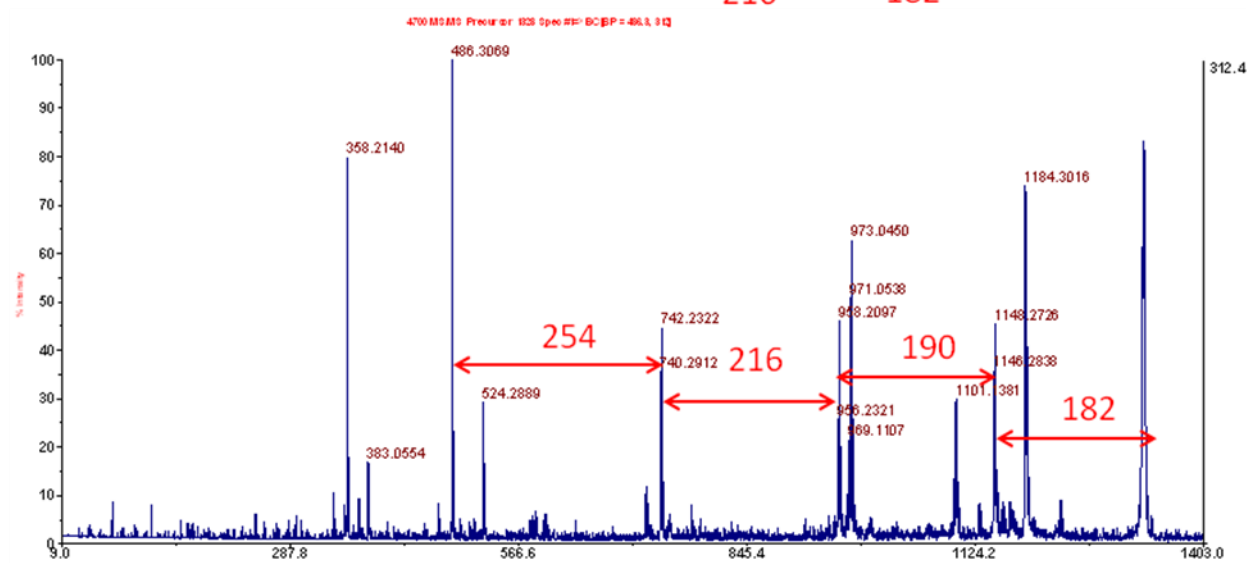
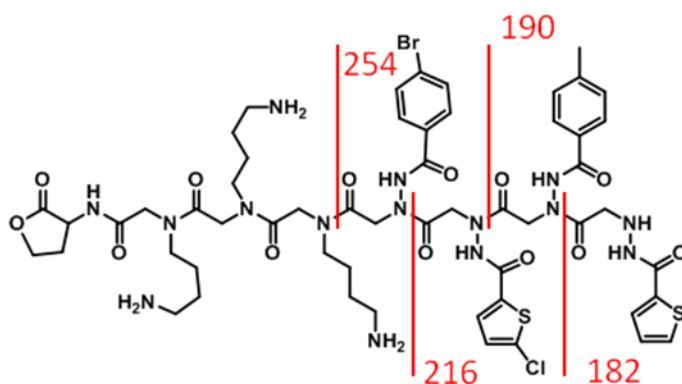
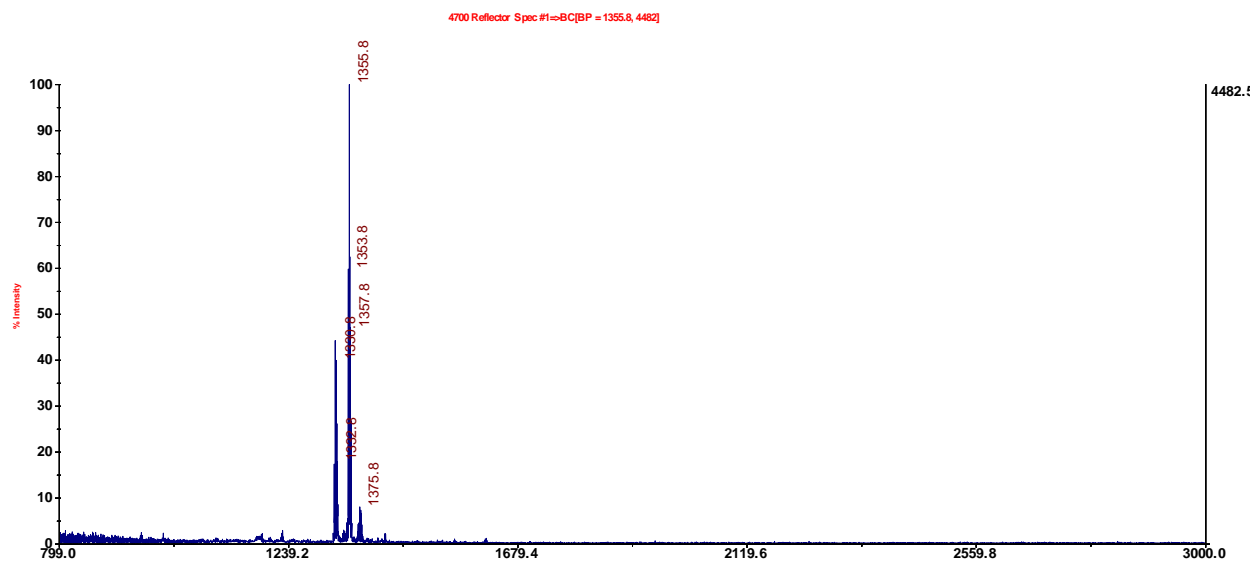
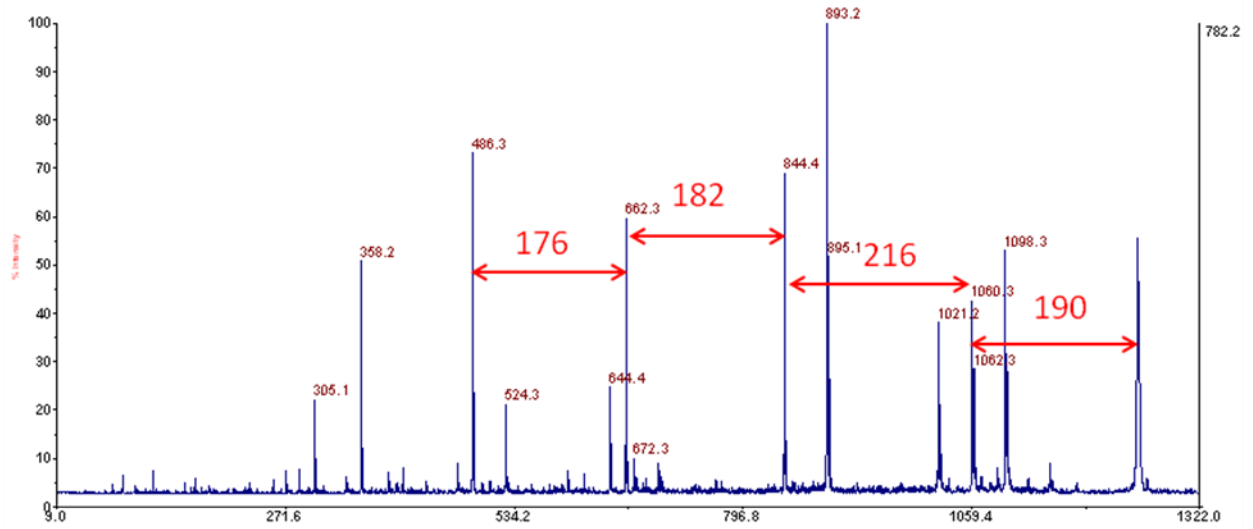
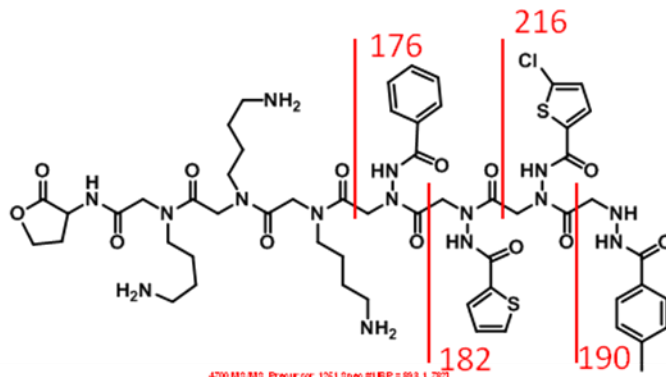
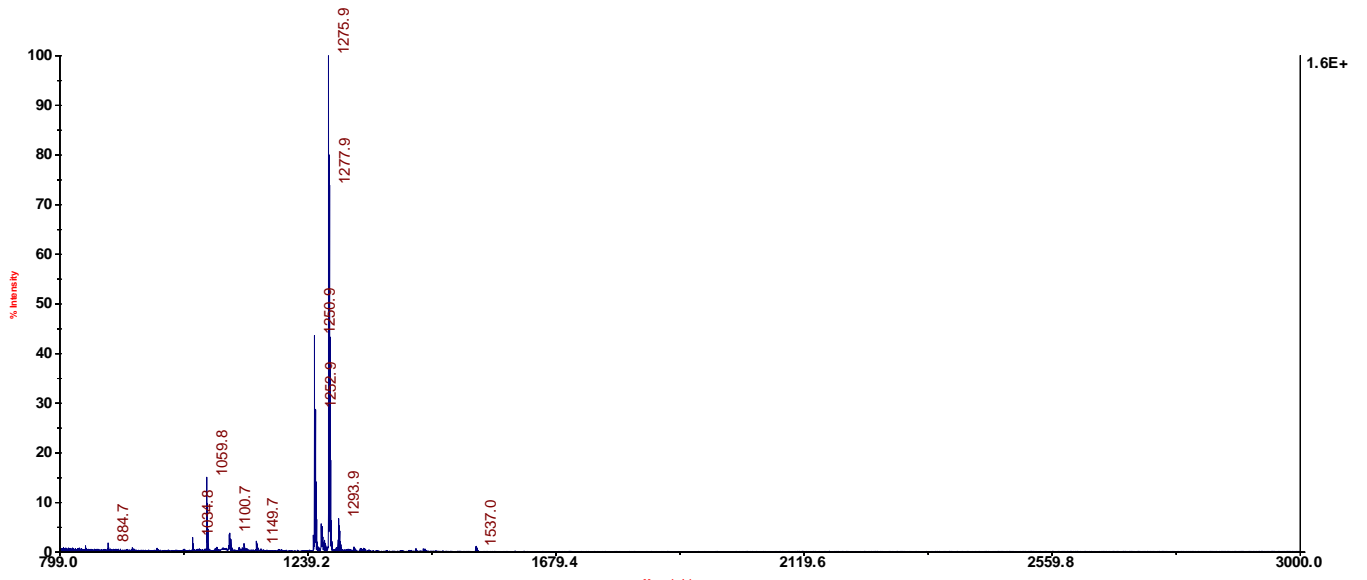


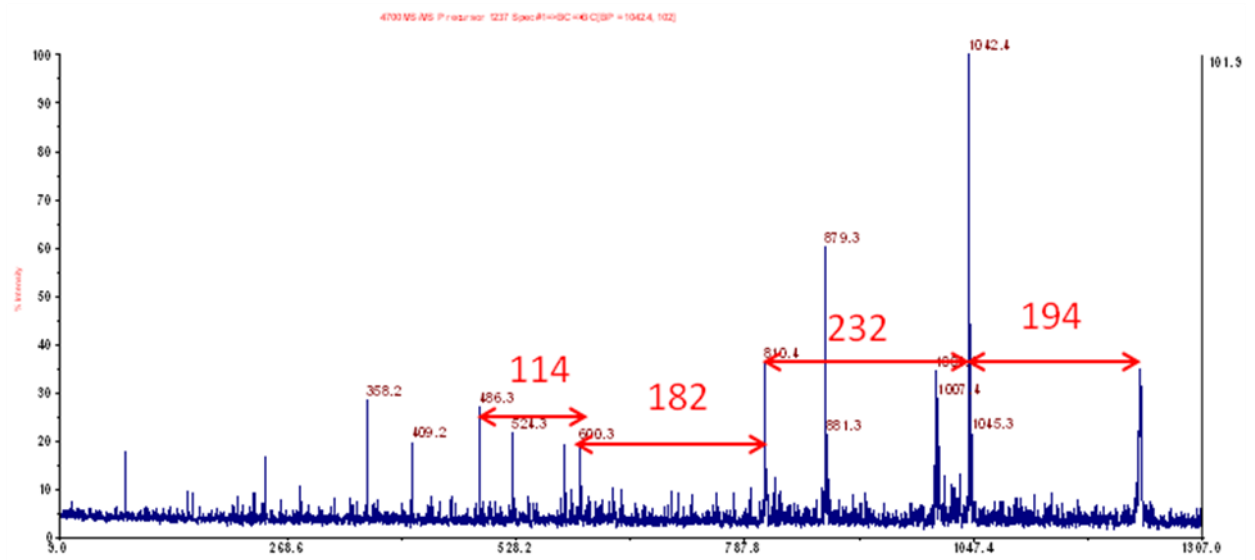
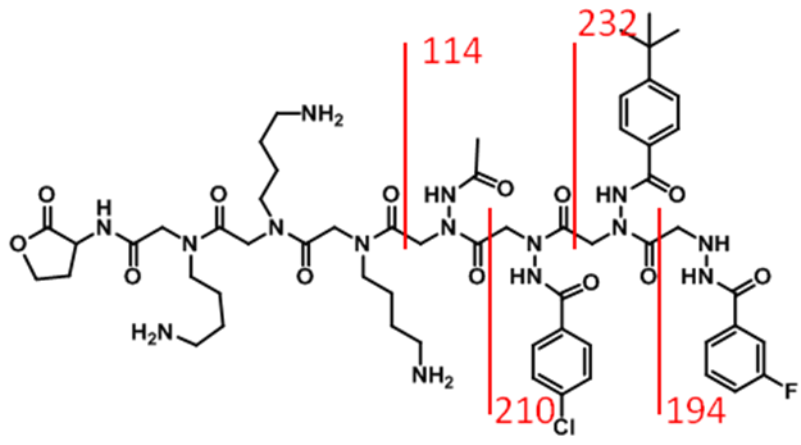
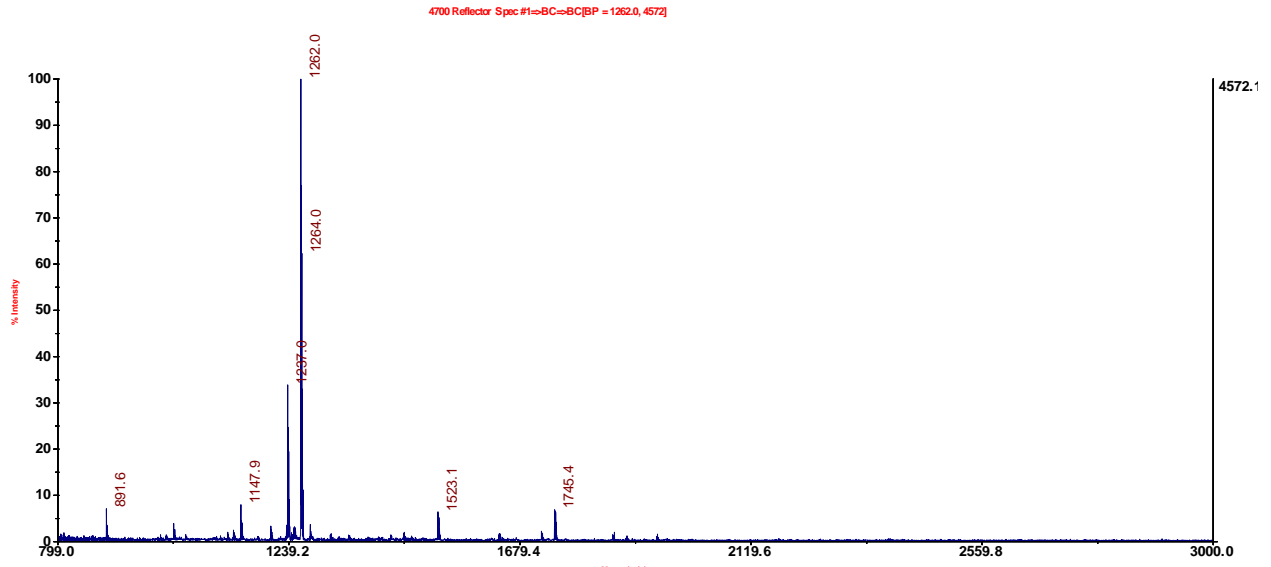
Fig. S55. General structure of tetramer acyl hydrazide library and the chemical structure of the ten different acyl hydrazide monomers used in positions R1-R4.

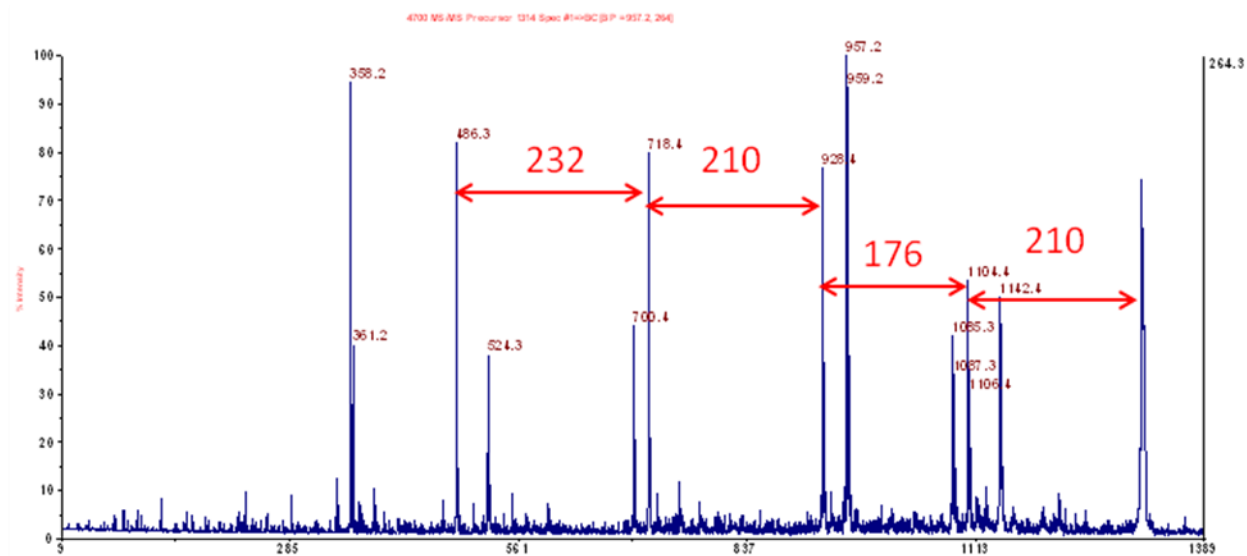
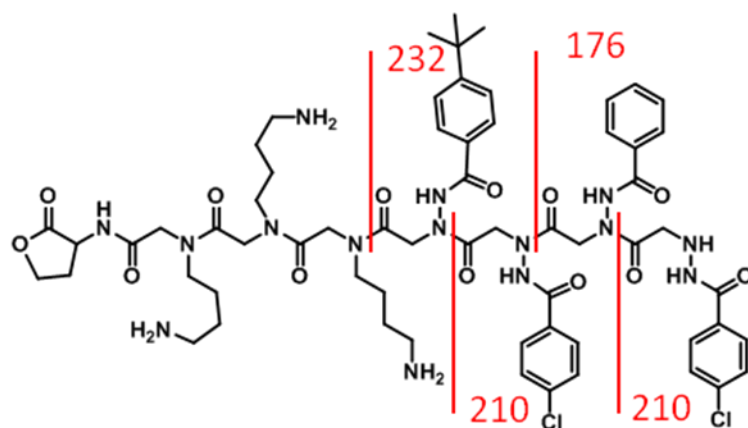
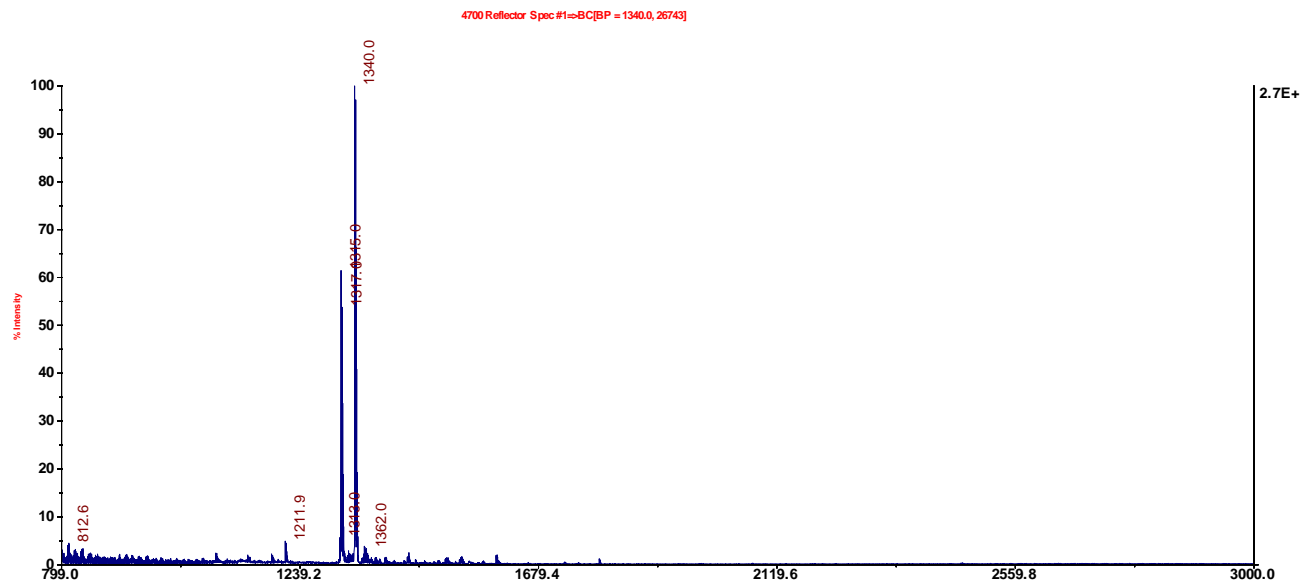
Fig. S56. MALDI TOF MS and MSMS spectra of some compounds of the tetramer acyl hydrazide library obtained after the single bead cleavage by using CNBr.

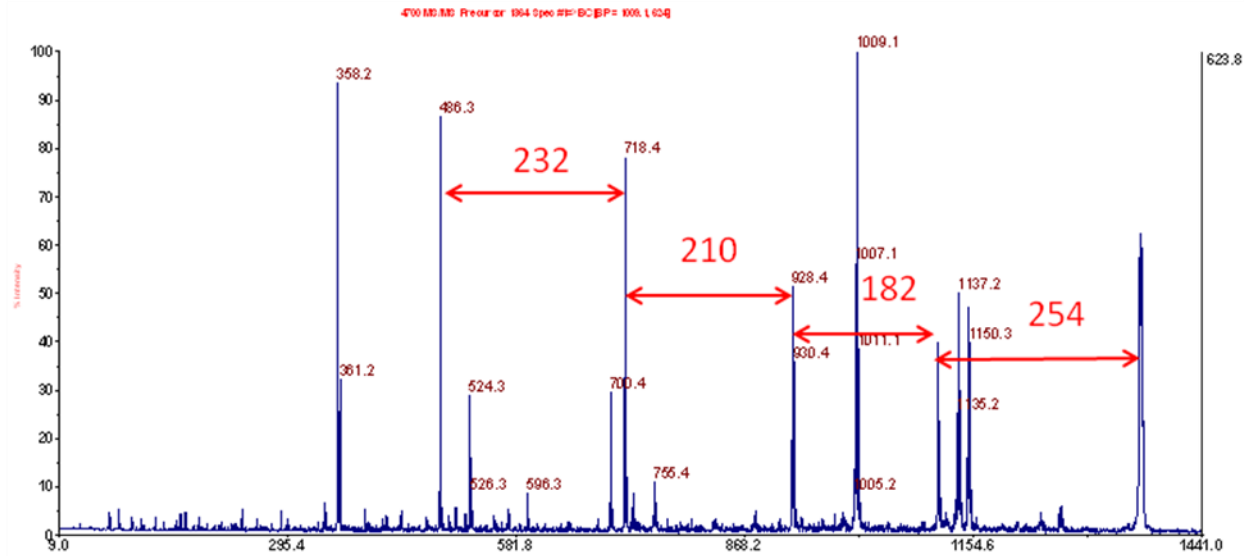
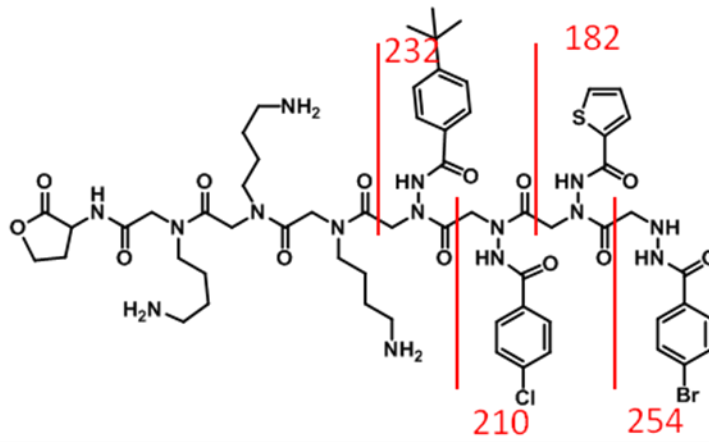
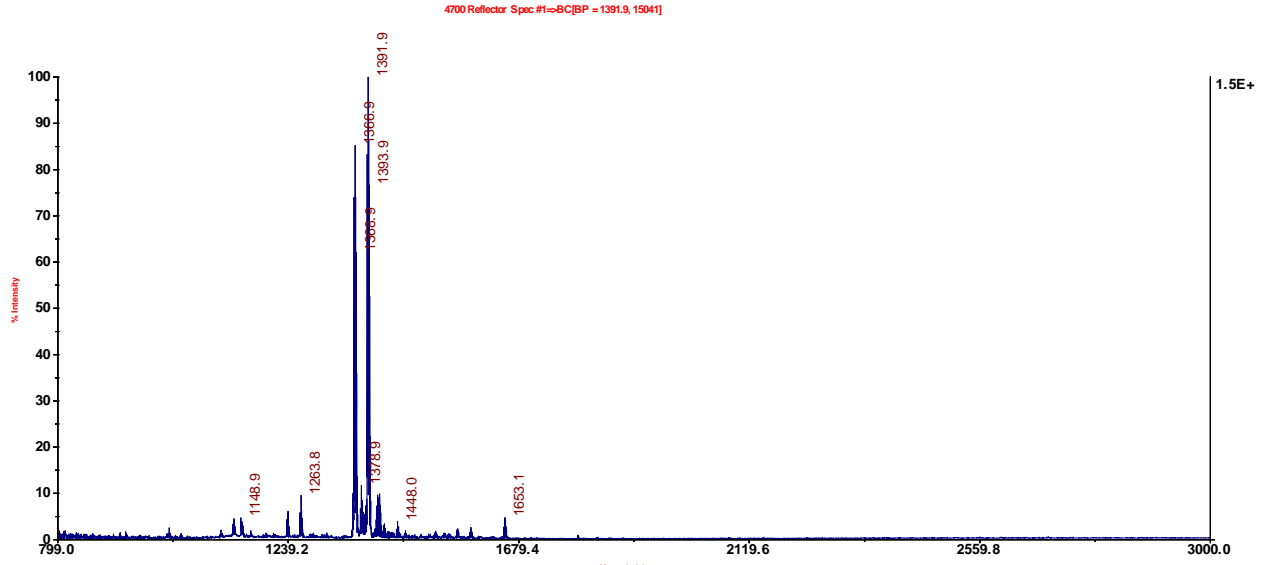


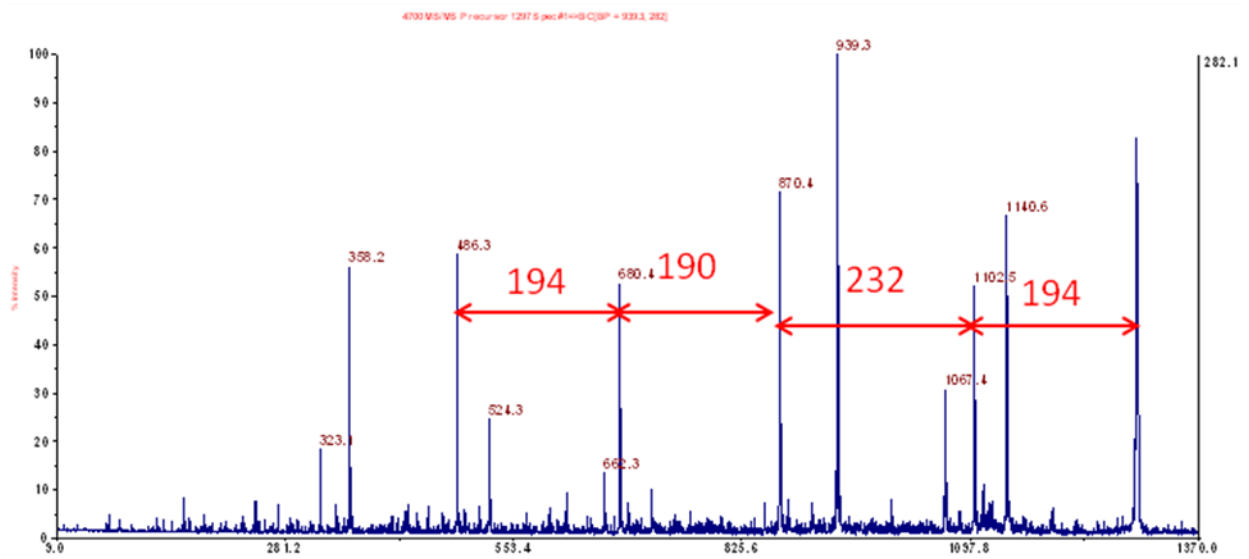
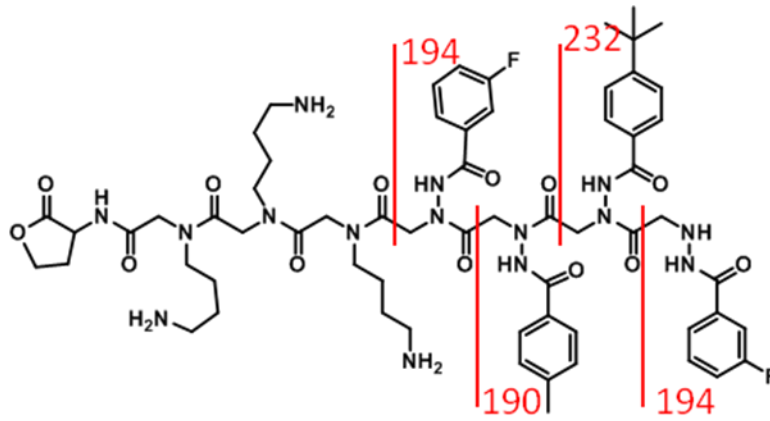
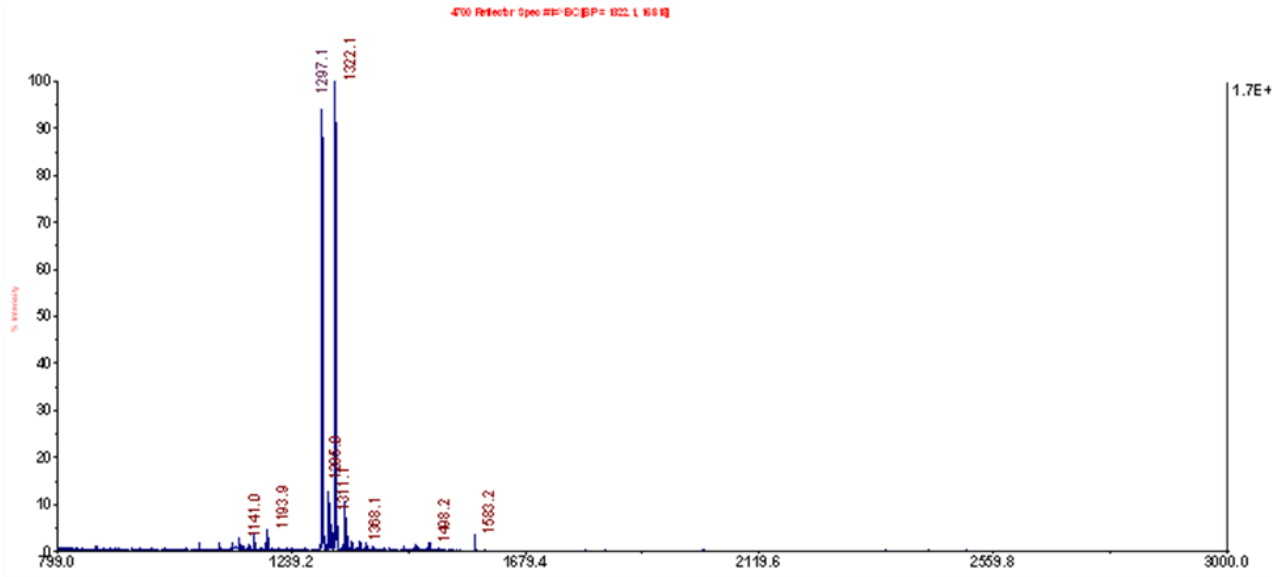
4700 Reflector Spec #1=BC[BP = 1275.9, 16466]

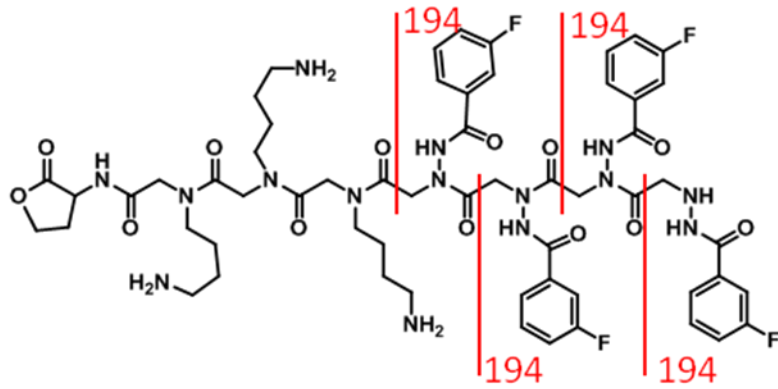
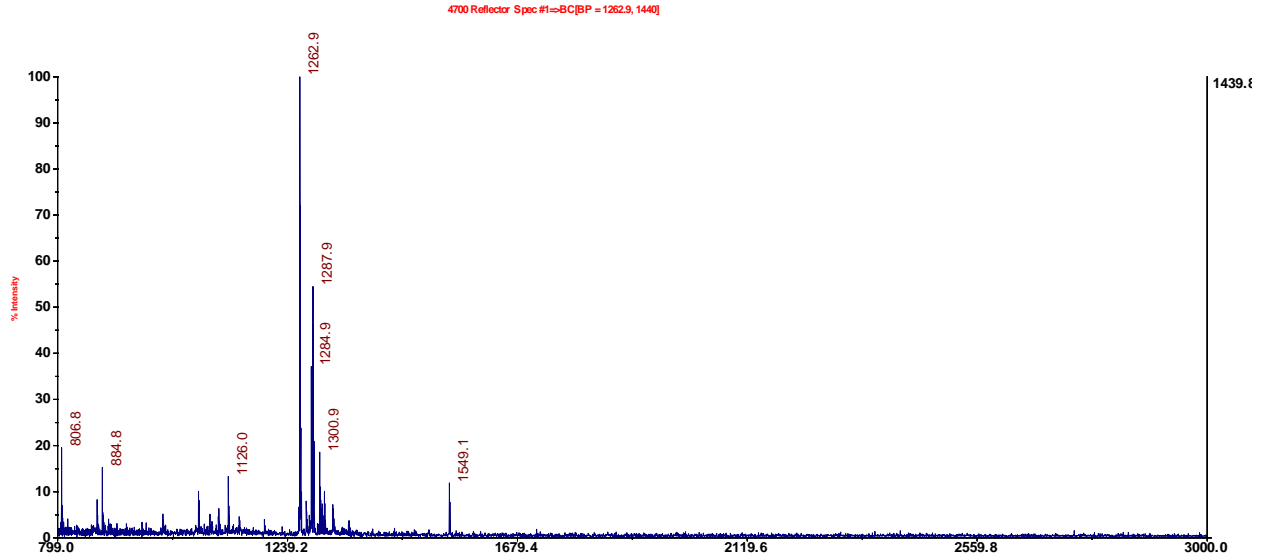




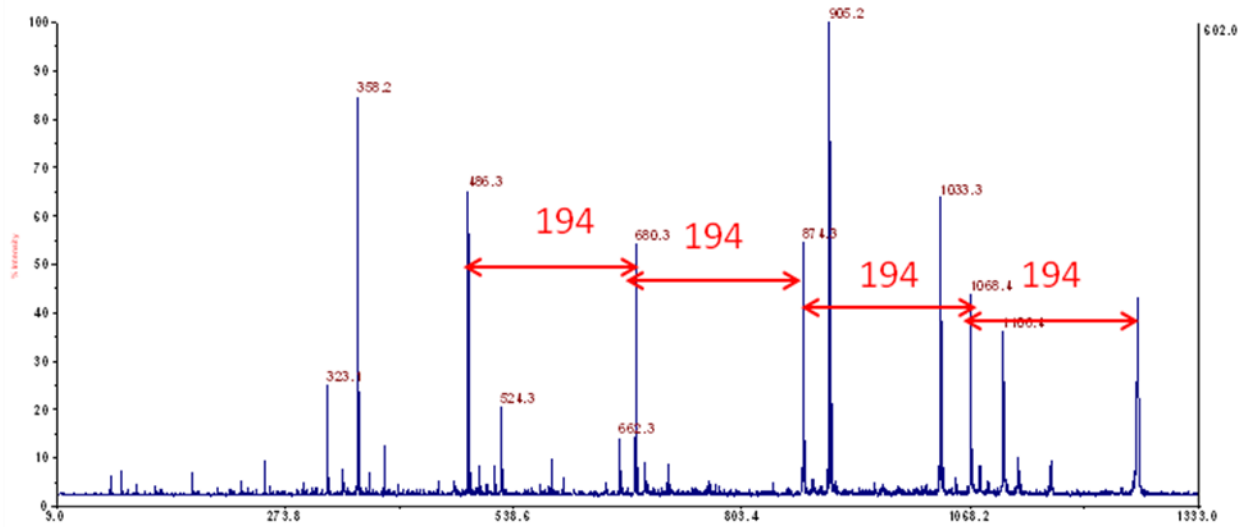


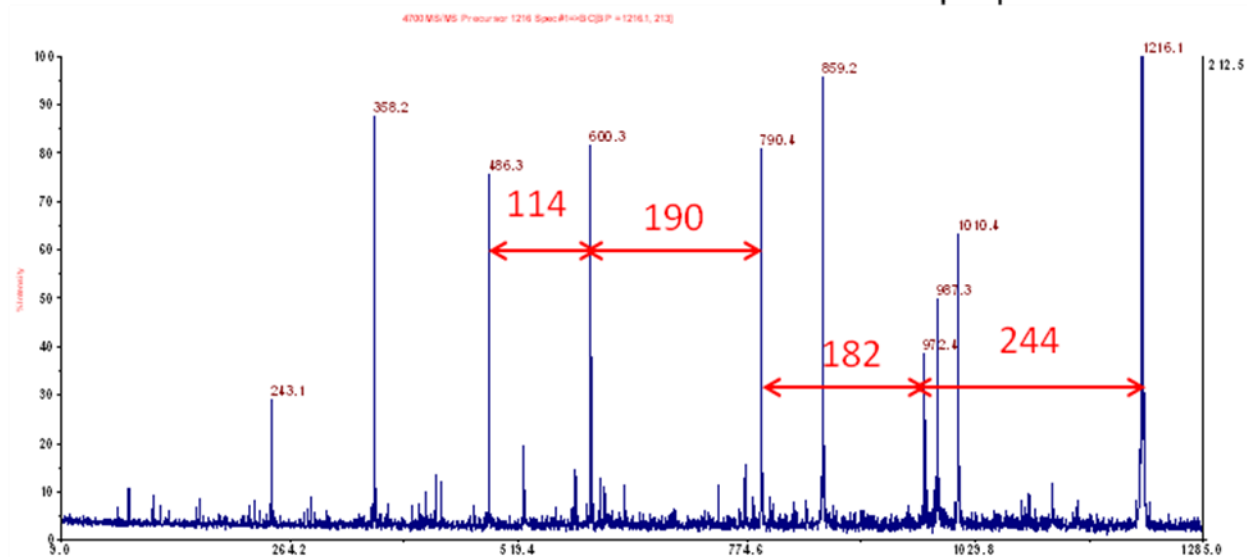
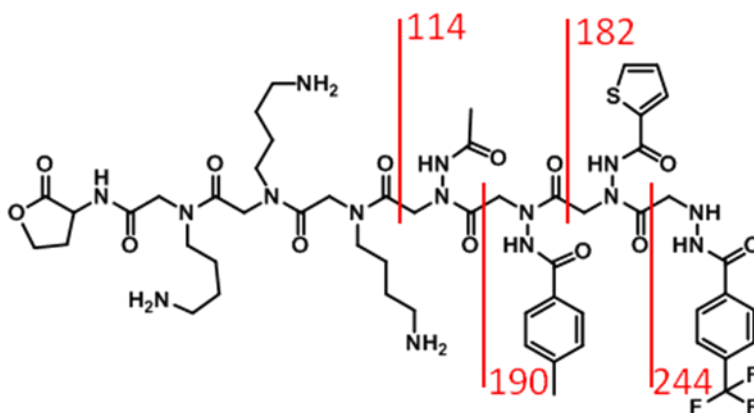
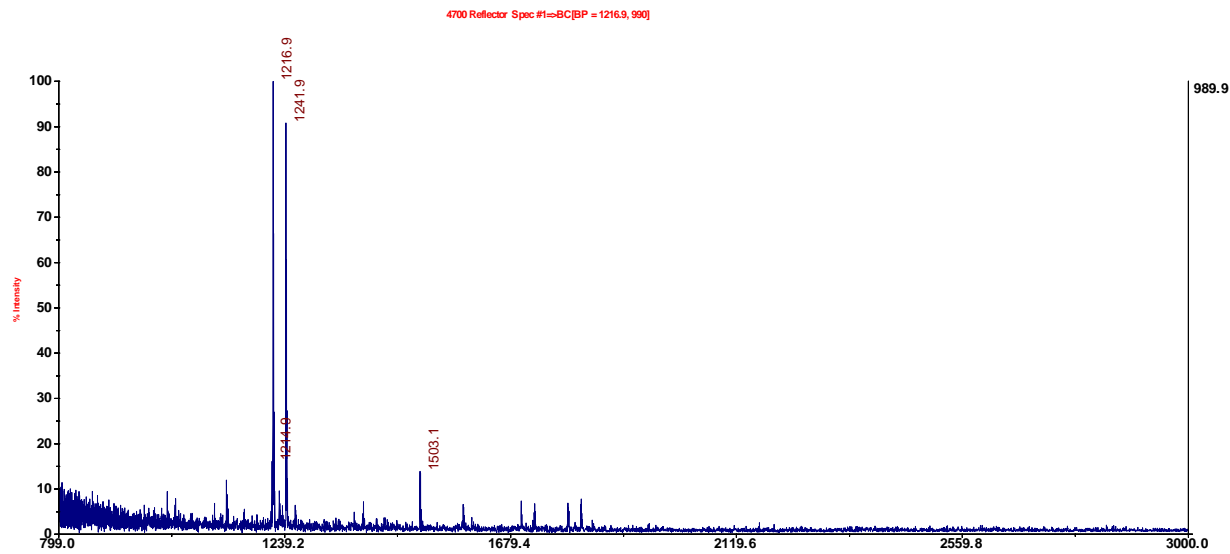


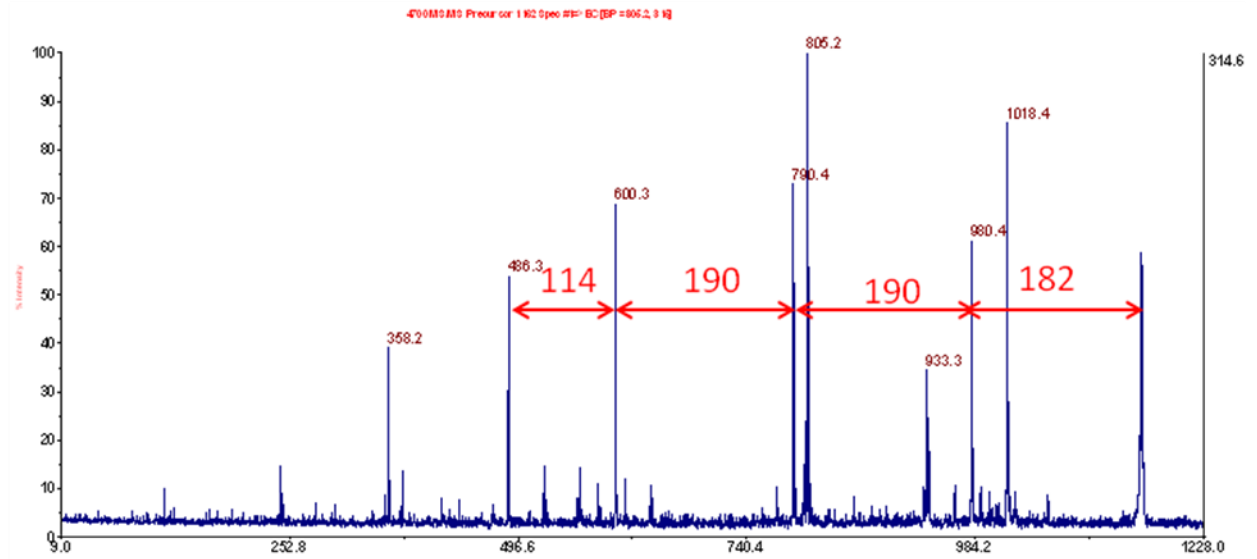
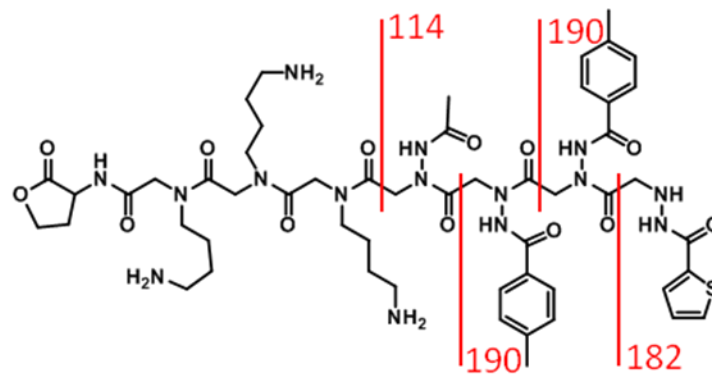
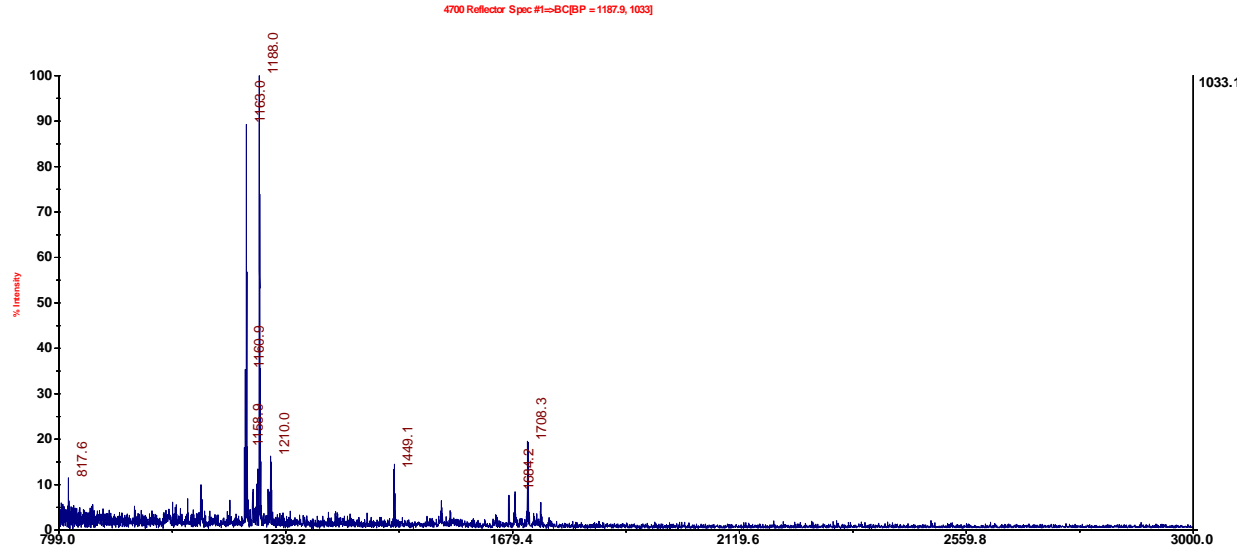


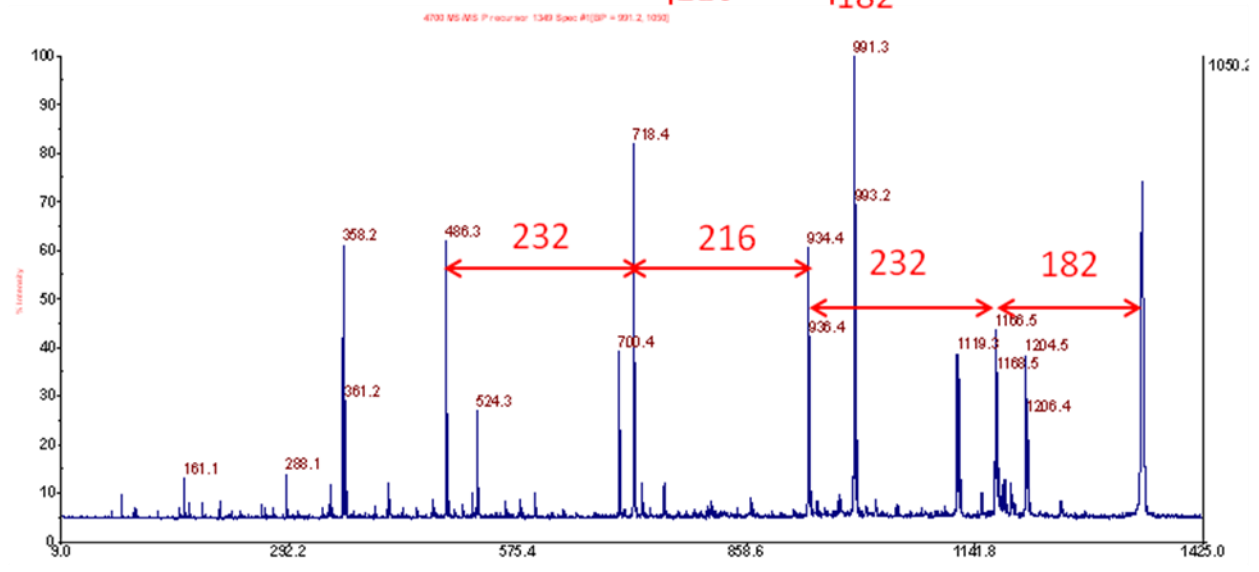
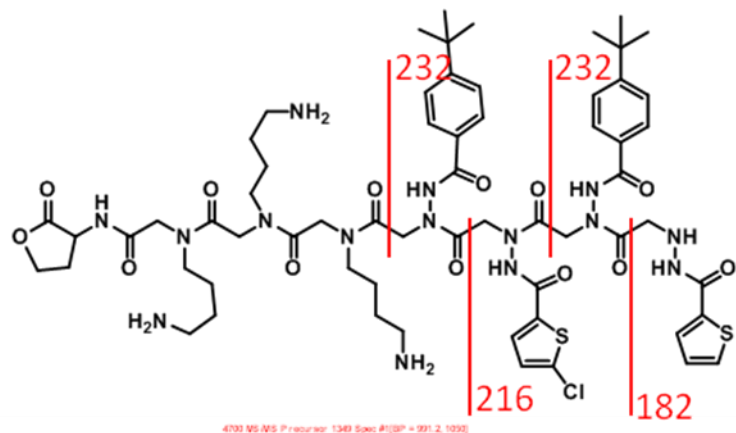
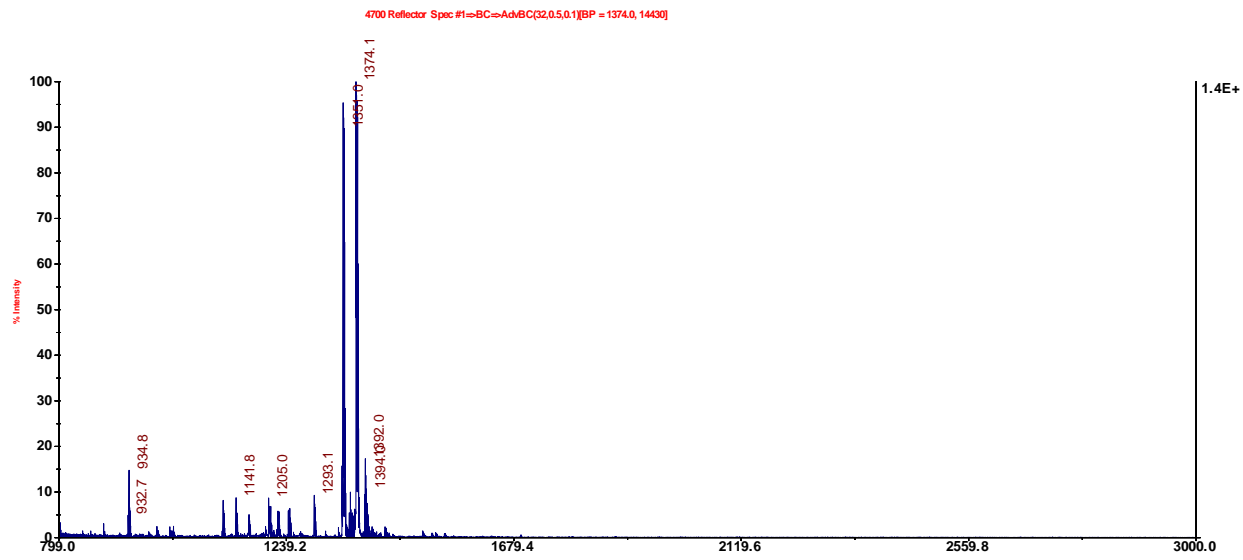


4700 MS MS Precursor 1262 Spec #1(BP = 905.2, 932)

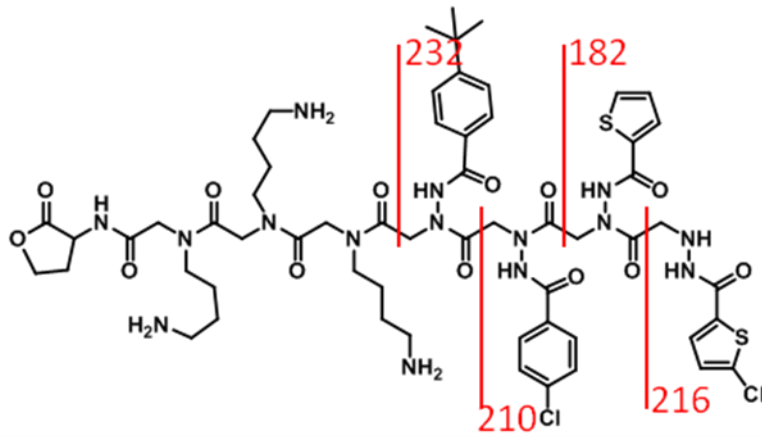
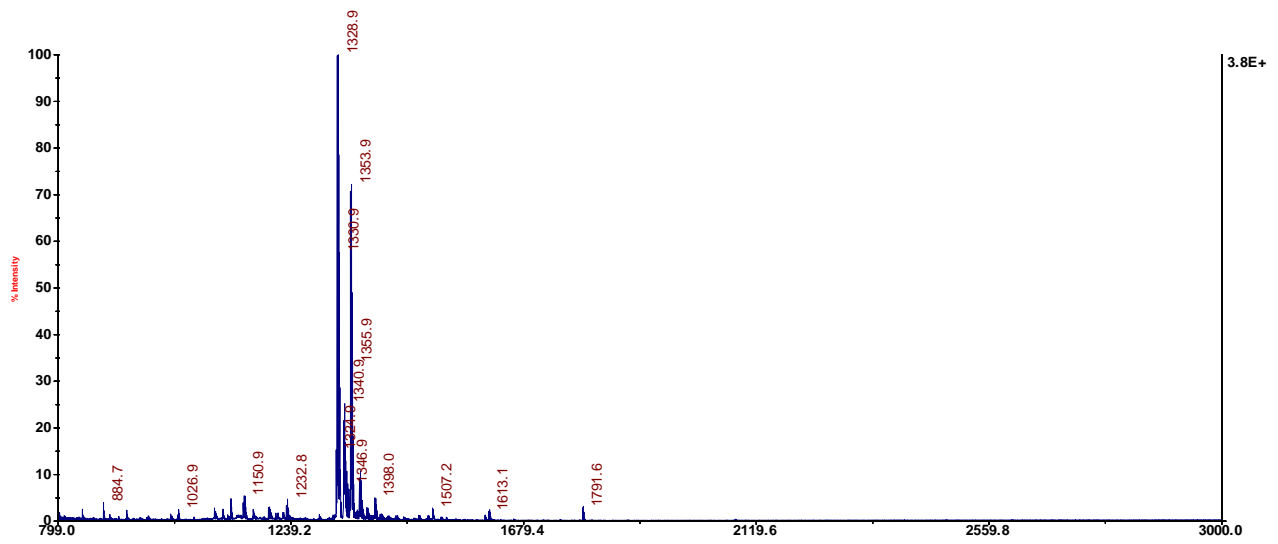




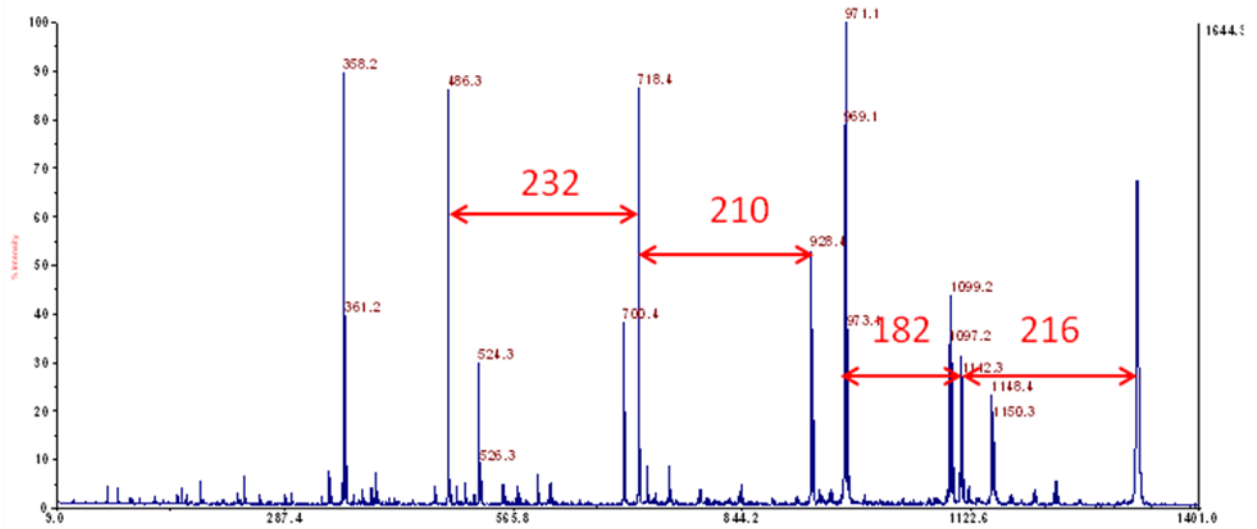


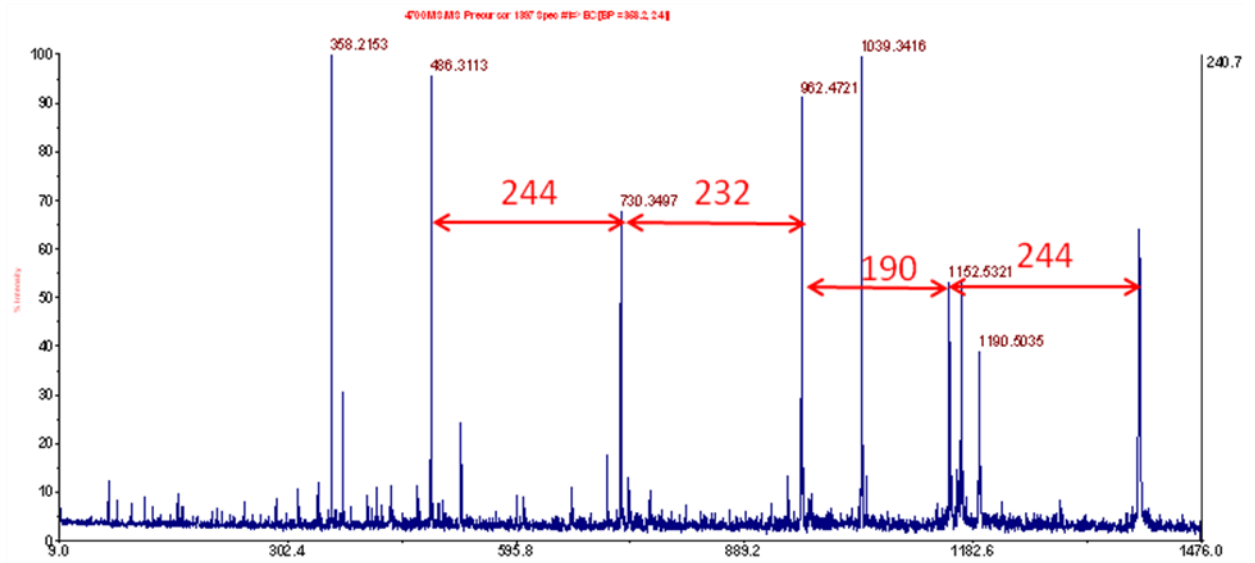
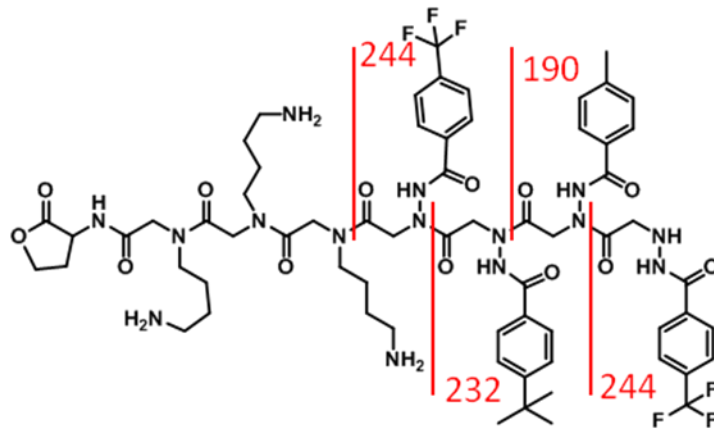
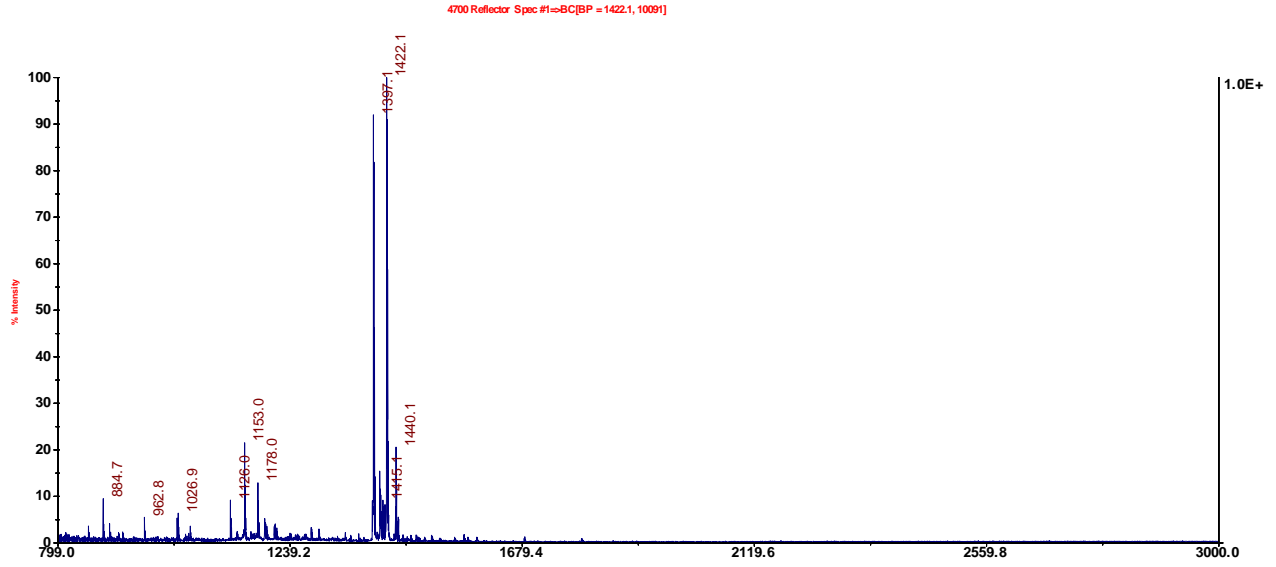


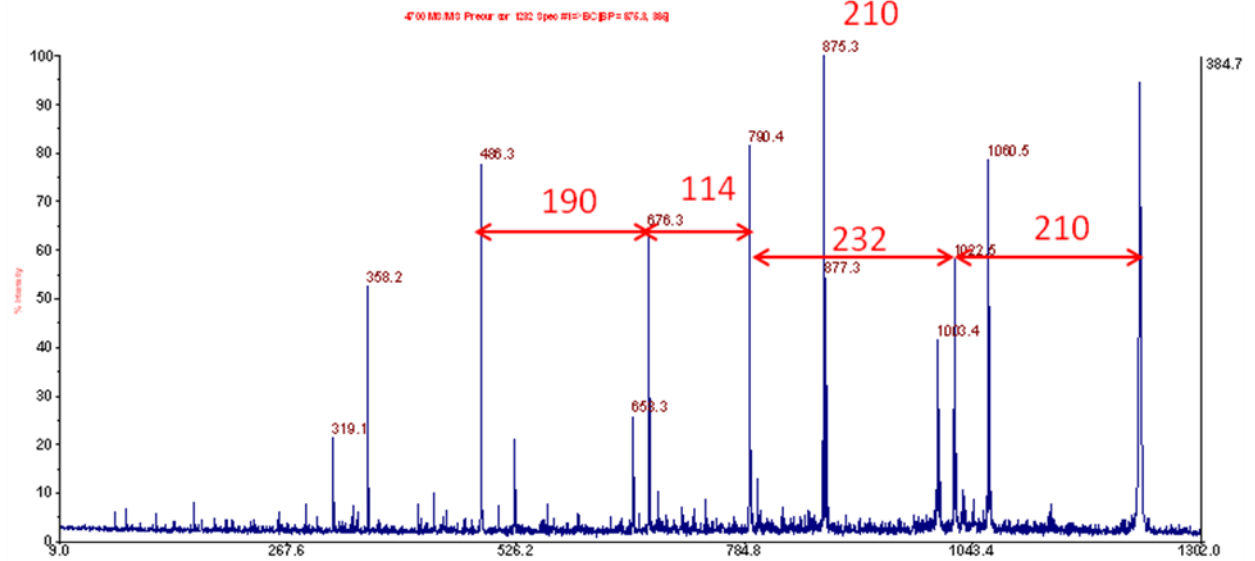
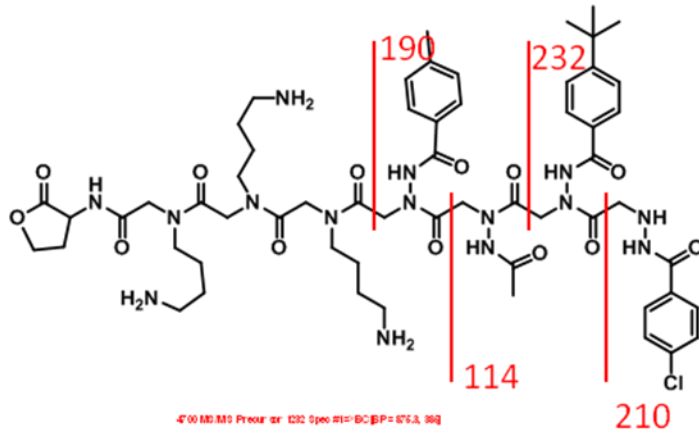
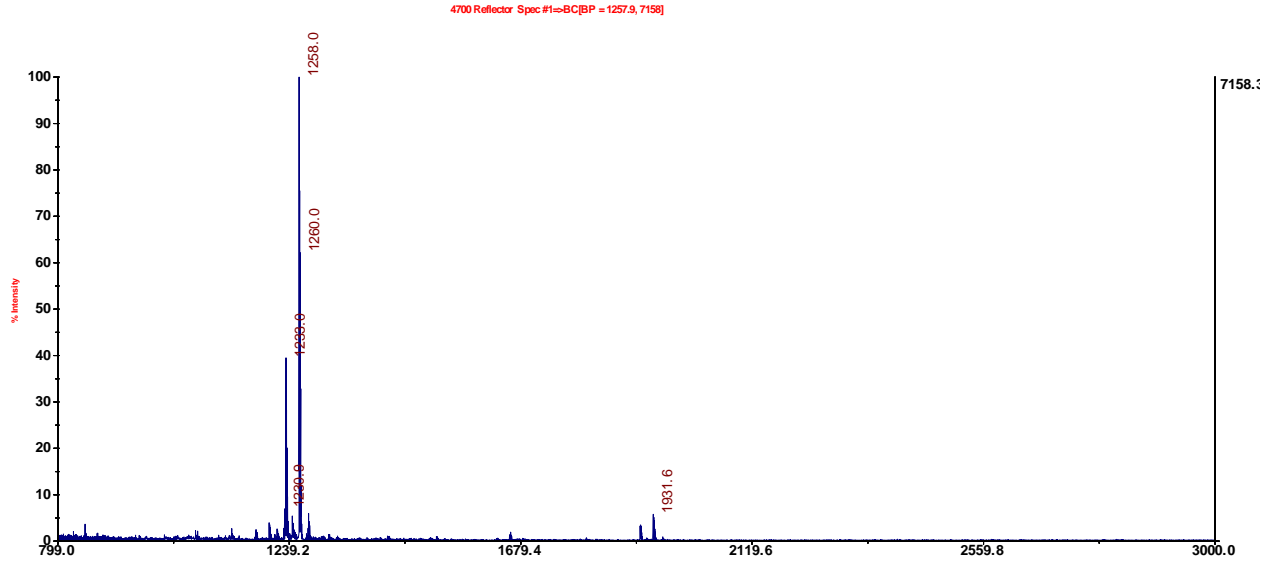
4700 Reflector Spec #1→BC[BP = 1328.9, 38230]

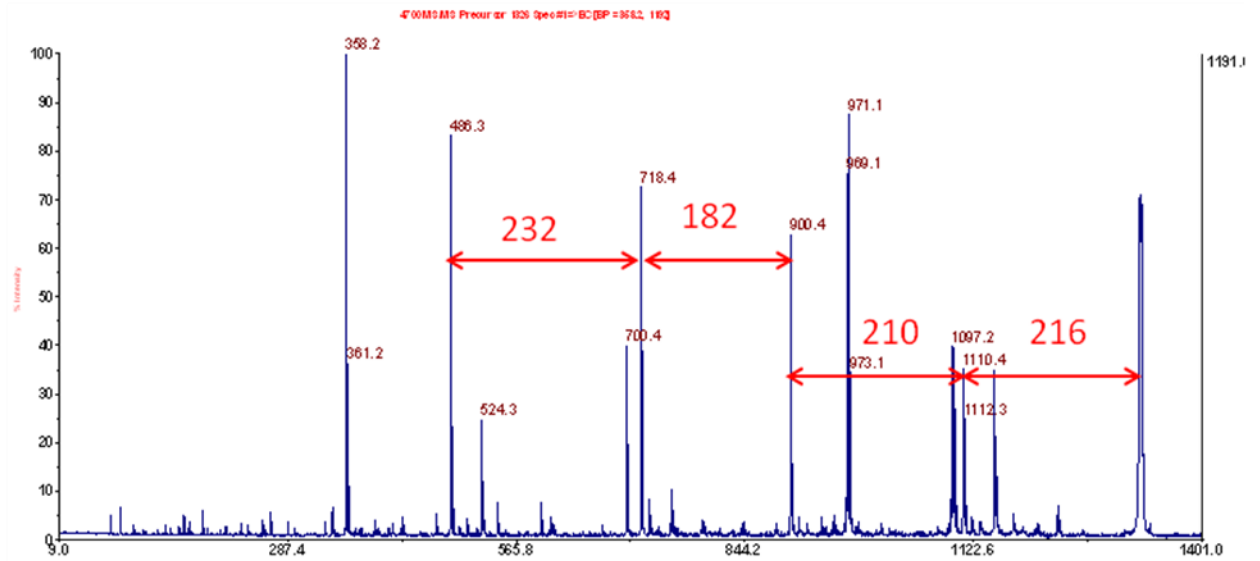
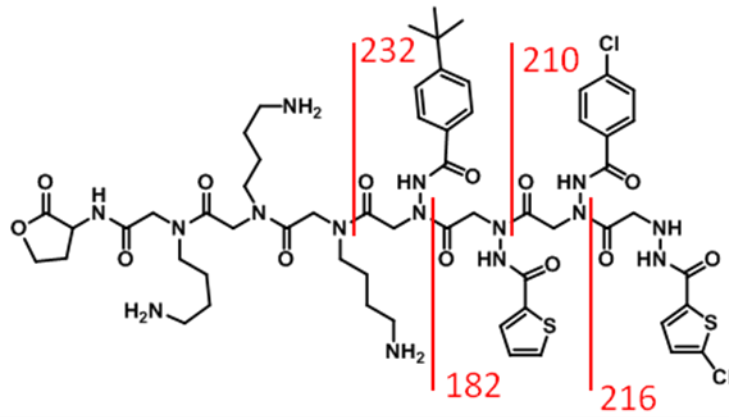
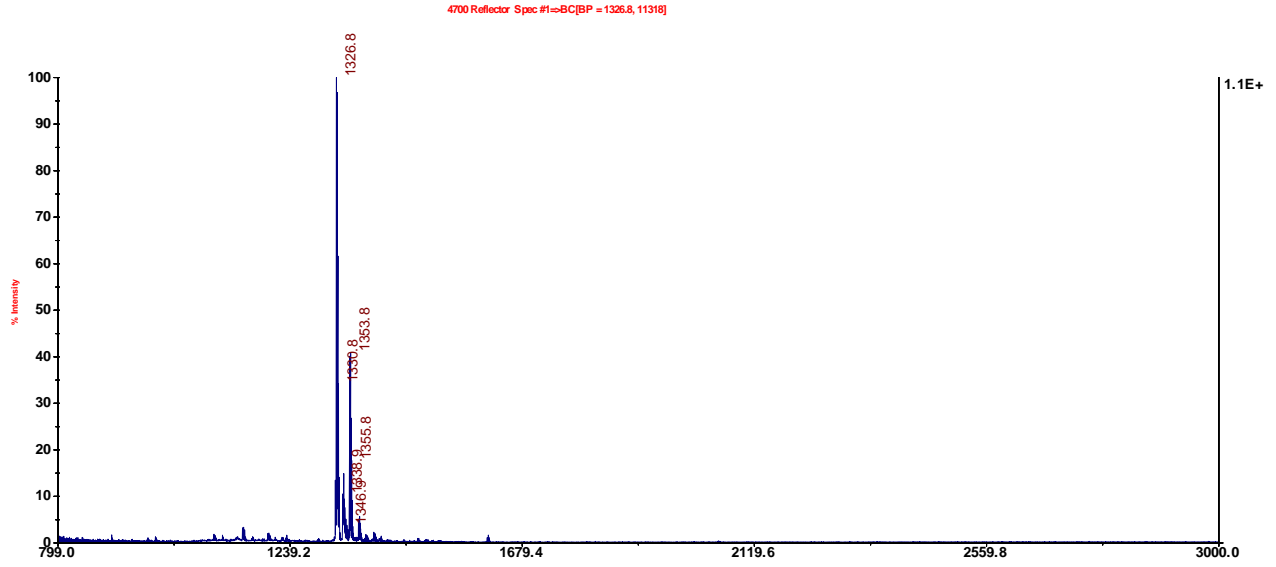


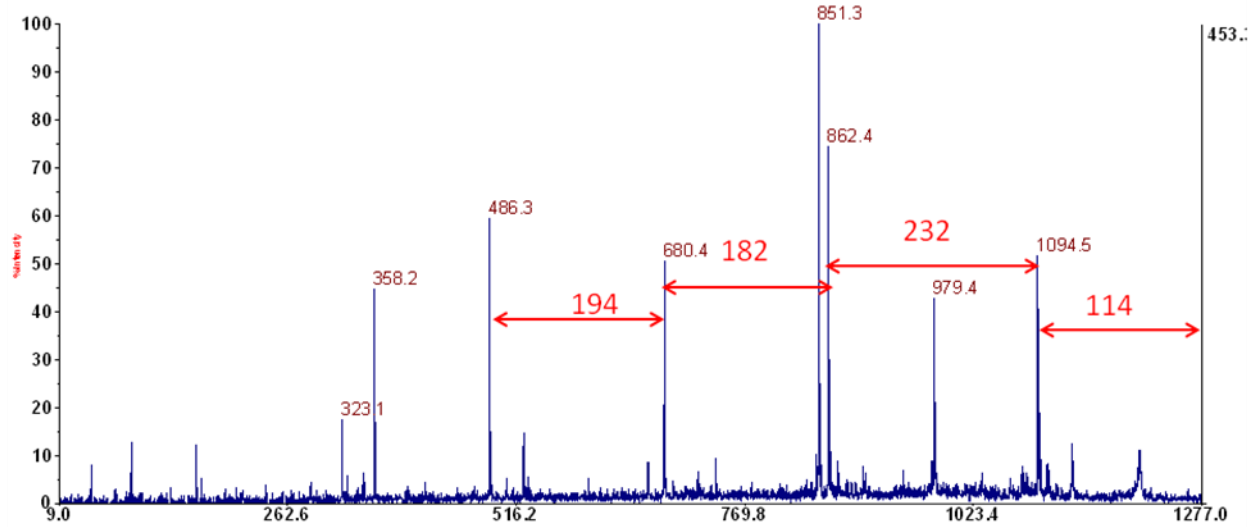
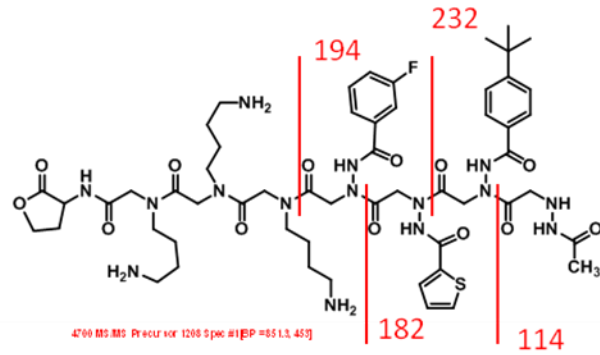
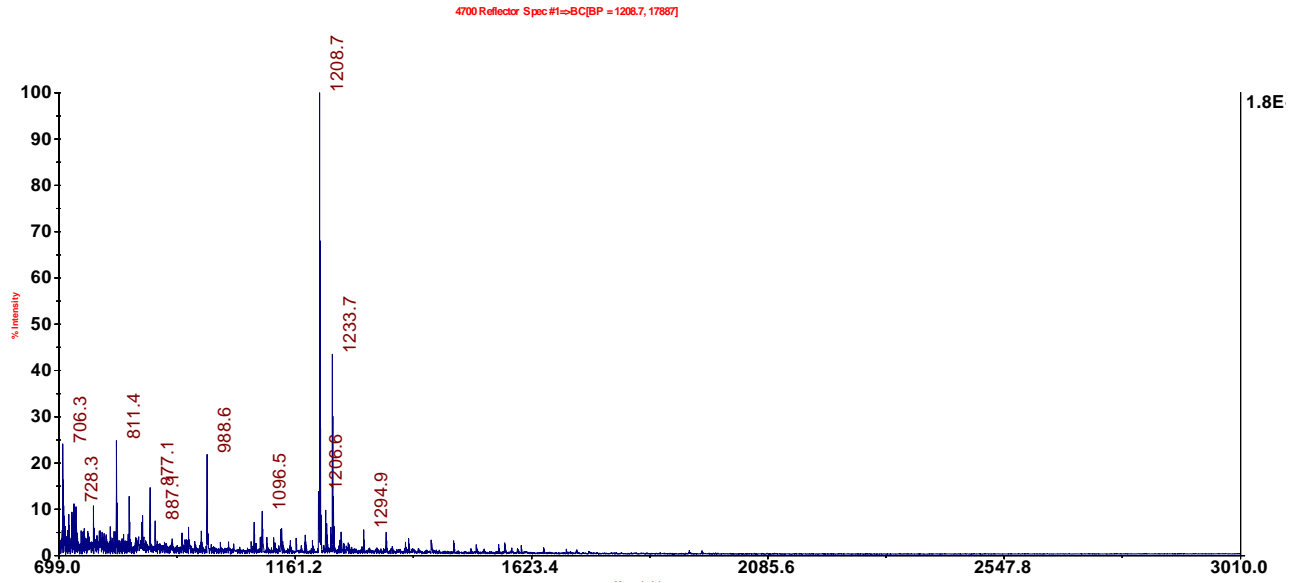
4700 MS/MS Precursor 1328.9 [M+H]⁺ [BP = 971.1, 1644]

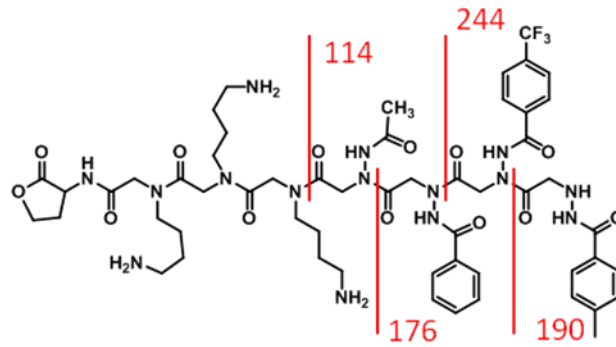
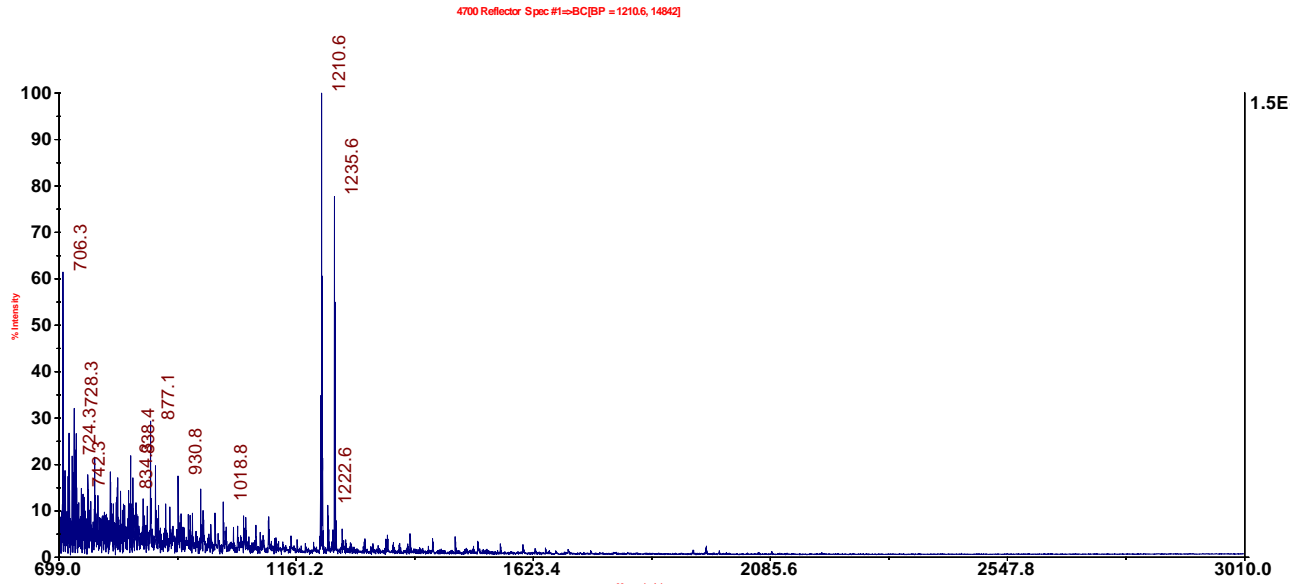




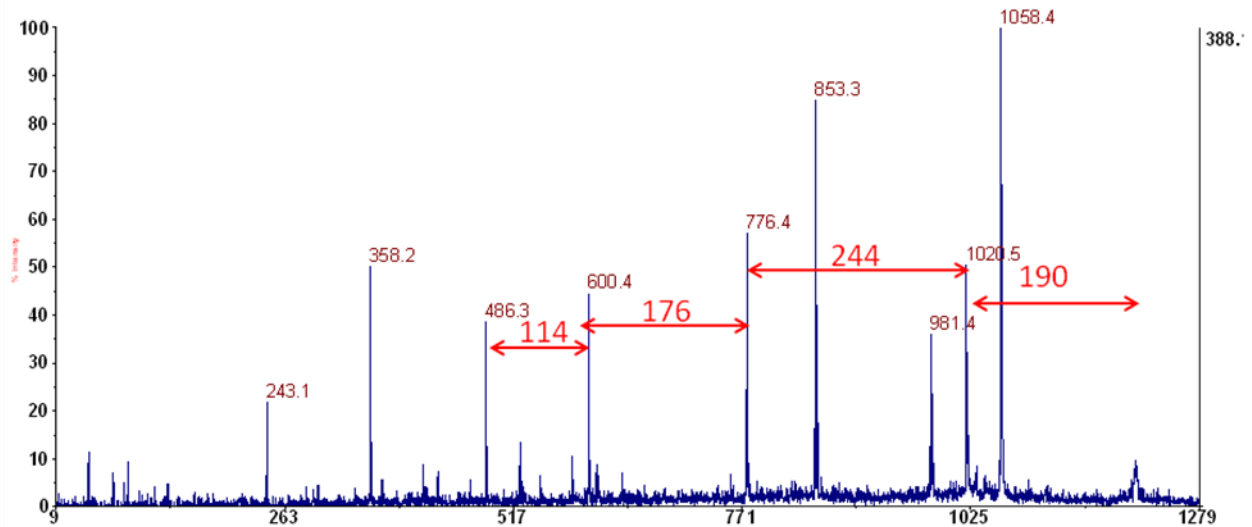


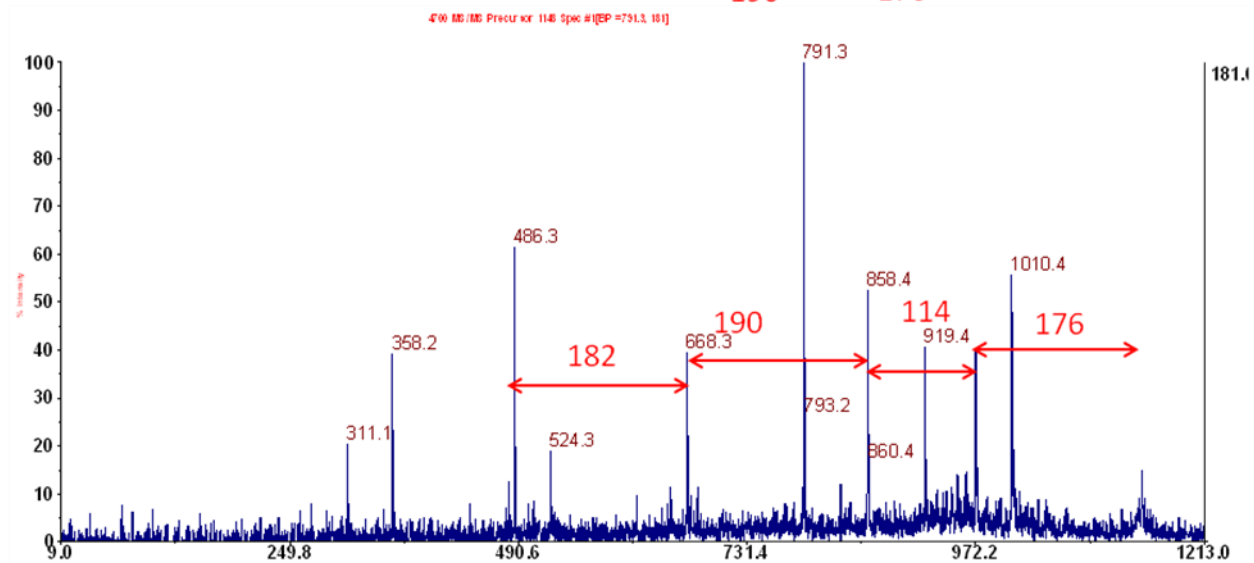
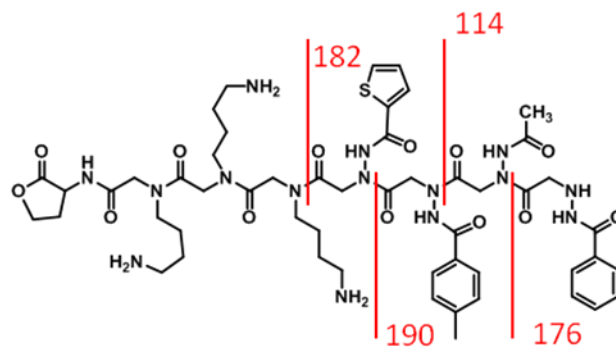
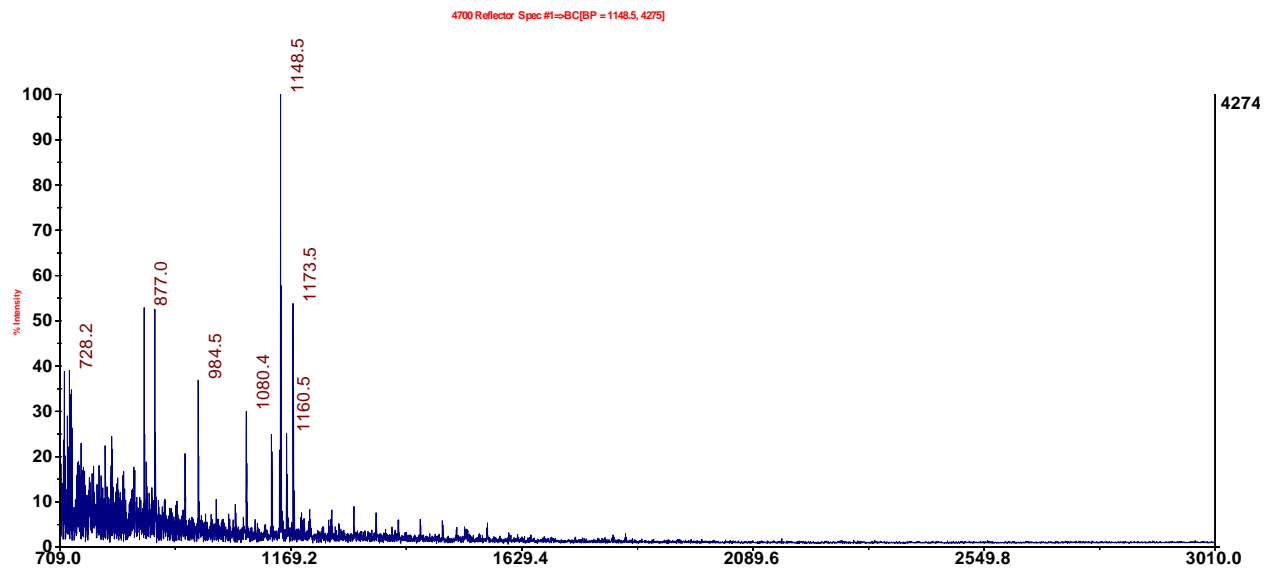






4700 MS/MS Precursor for 1210 Spec #1[BP = 1058.4, 358]





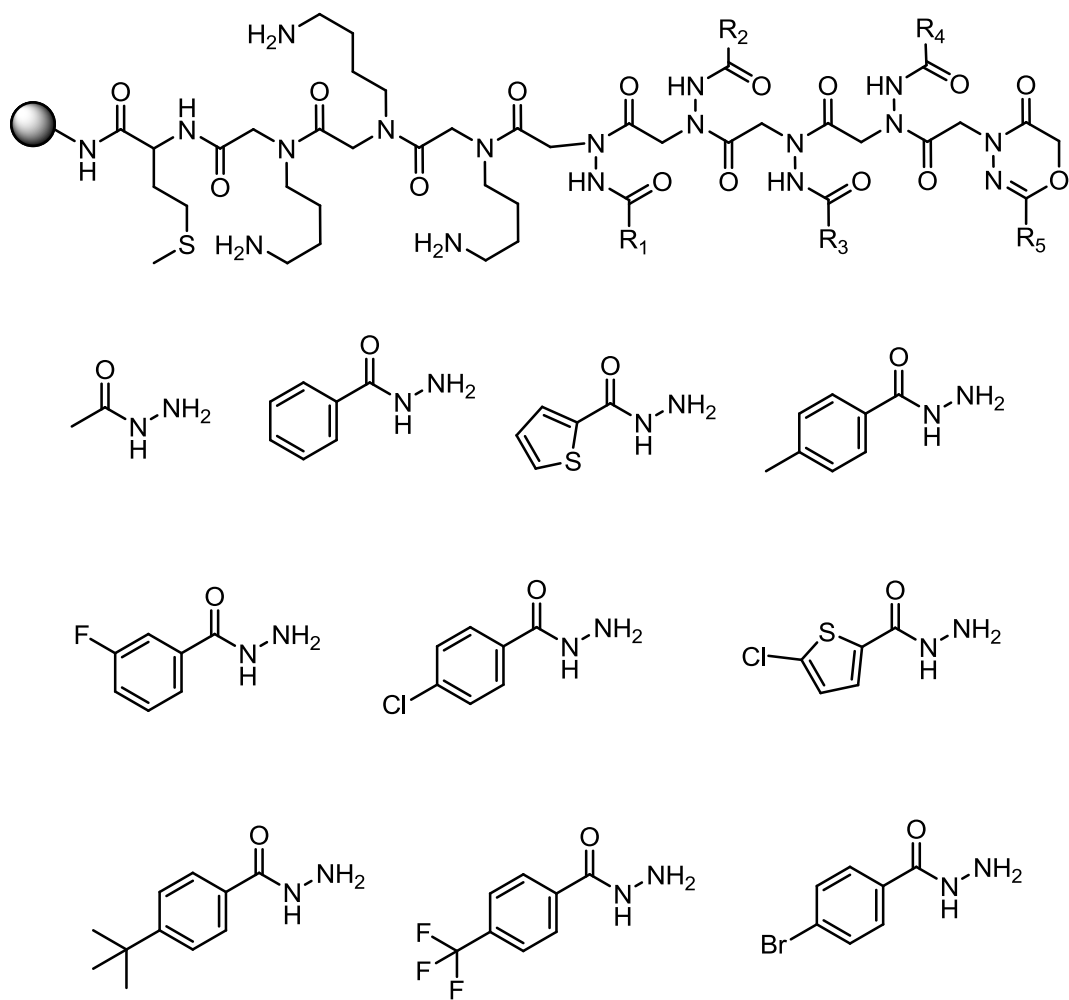
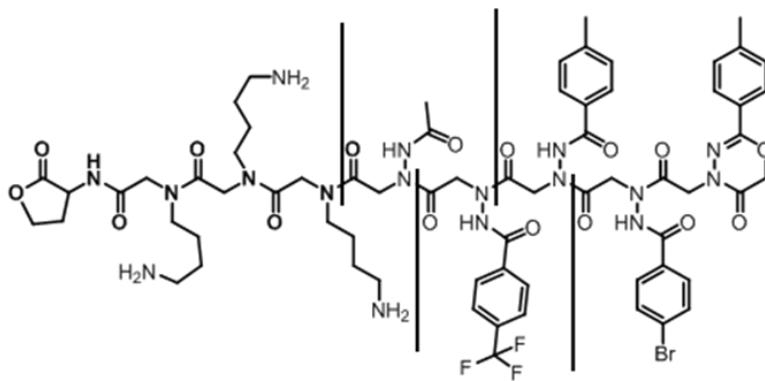
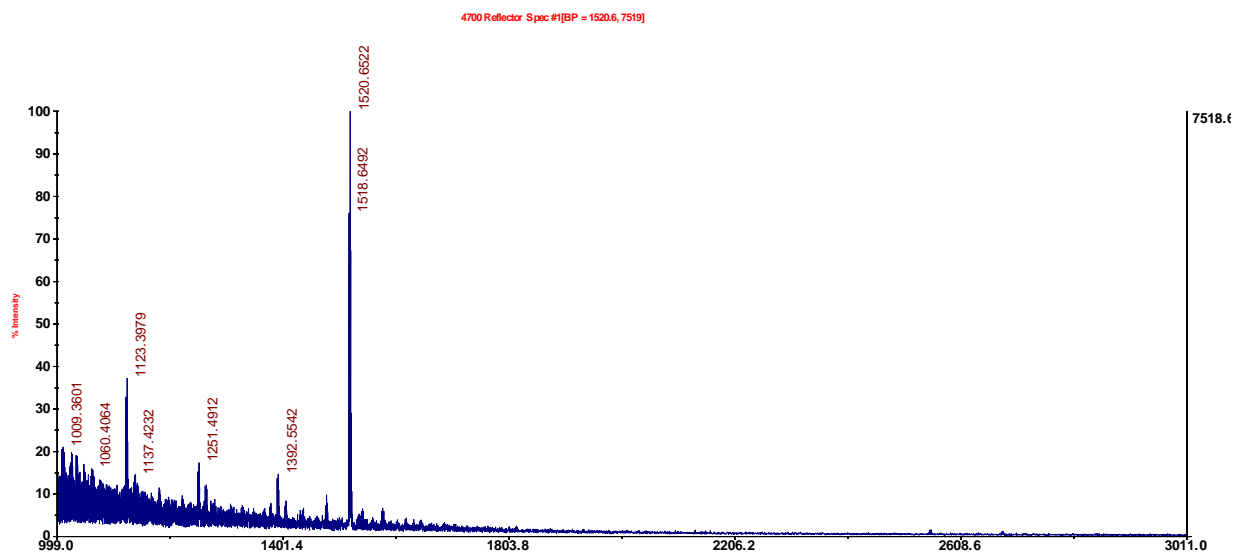
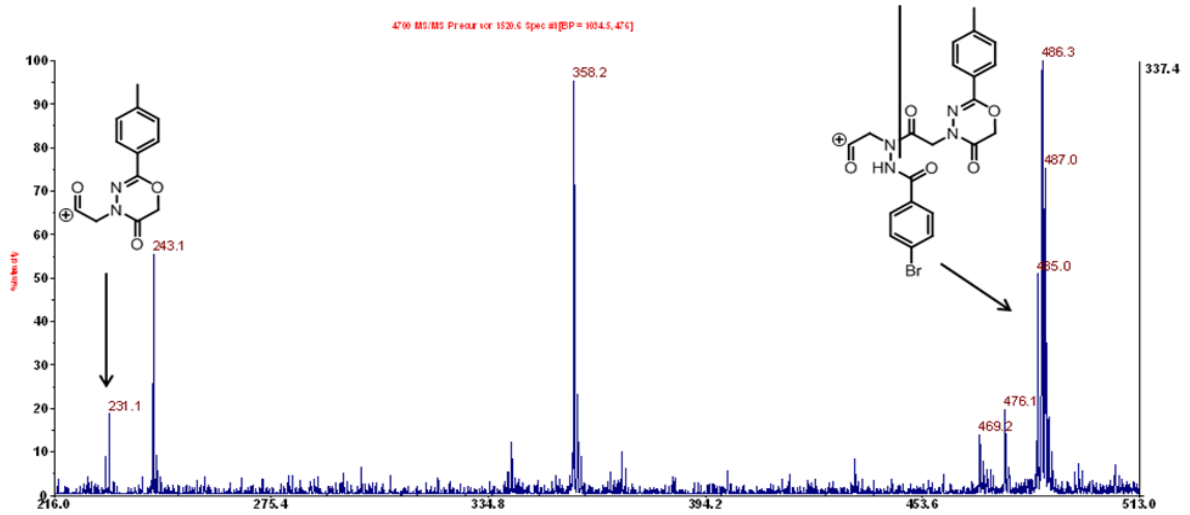
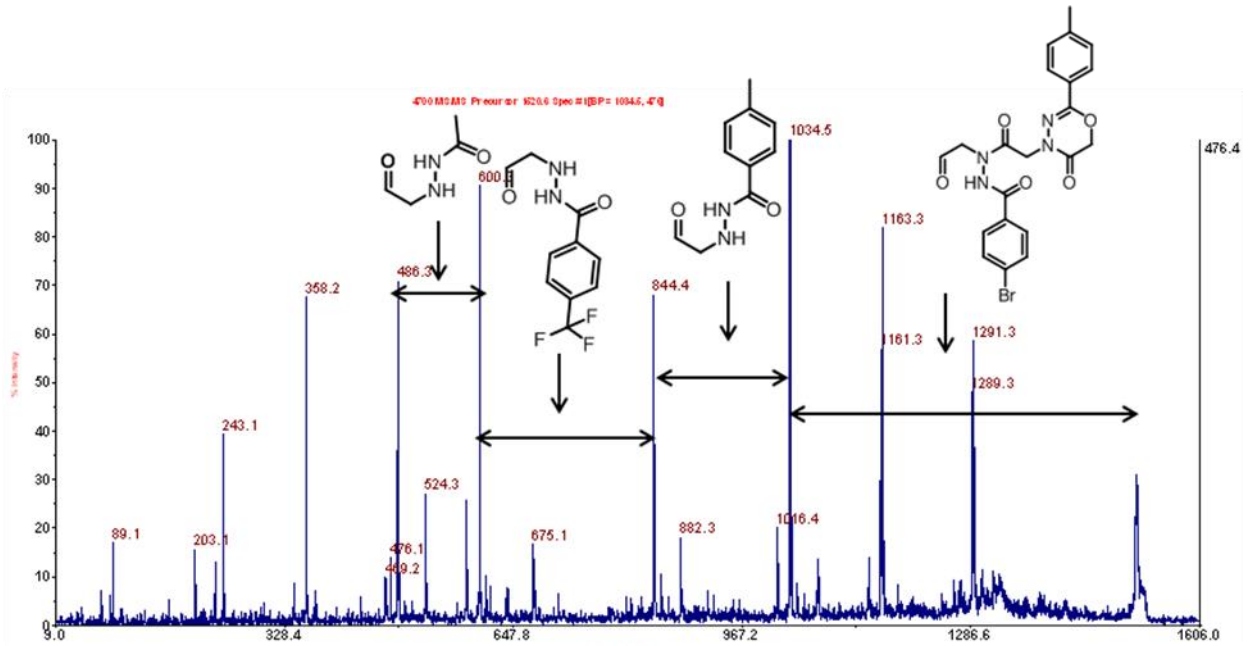
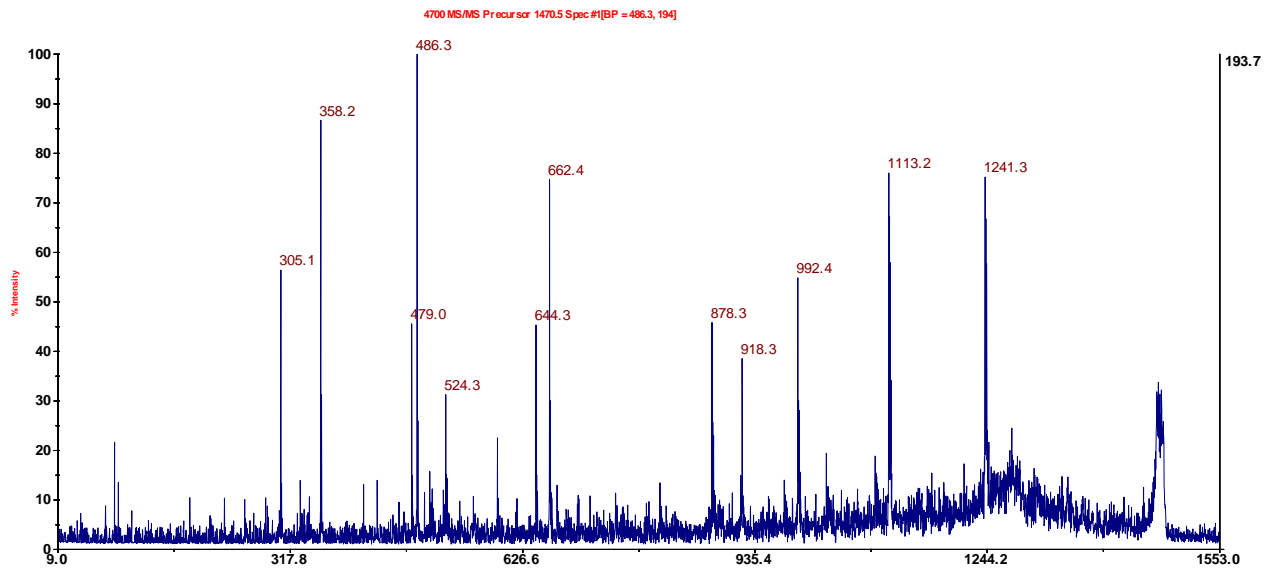
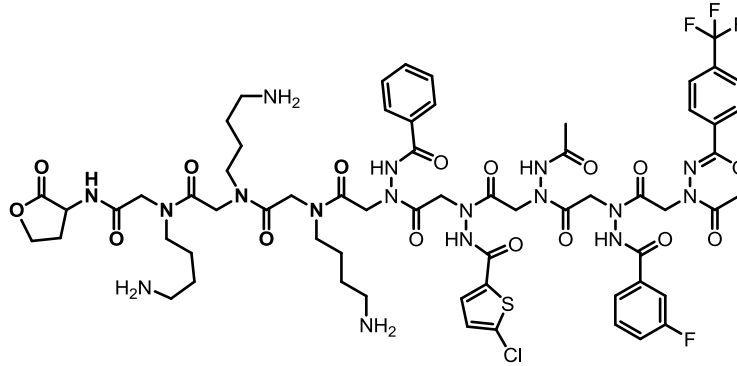
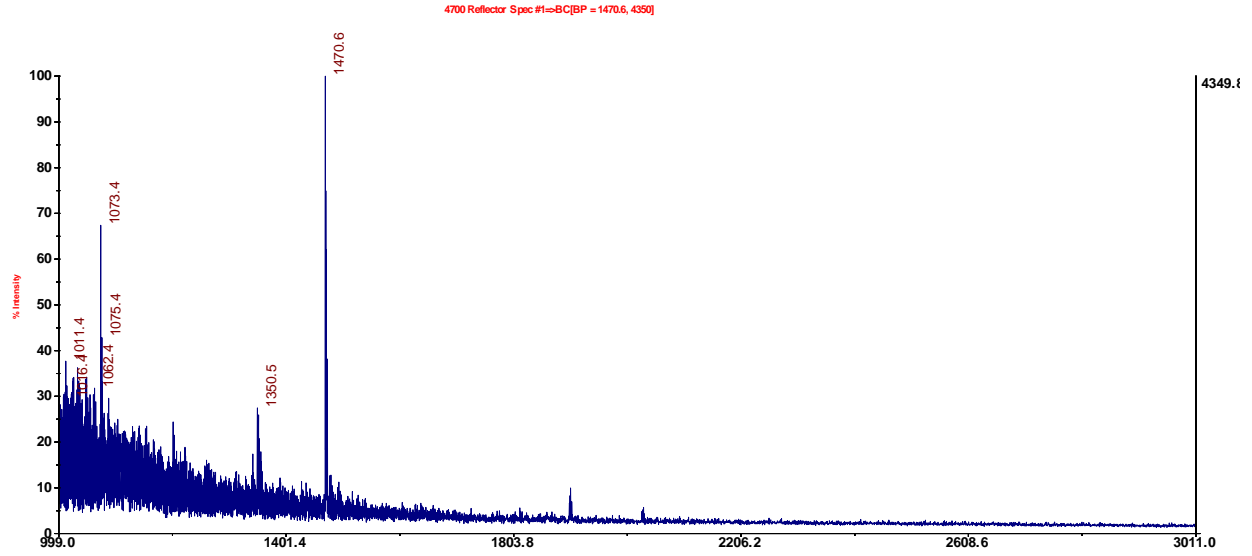


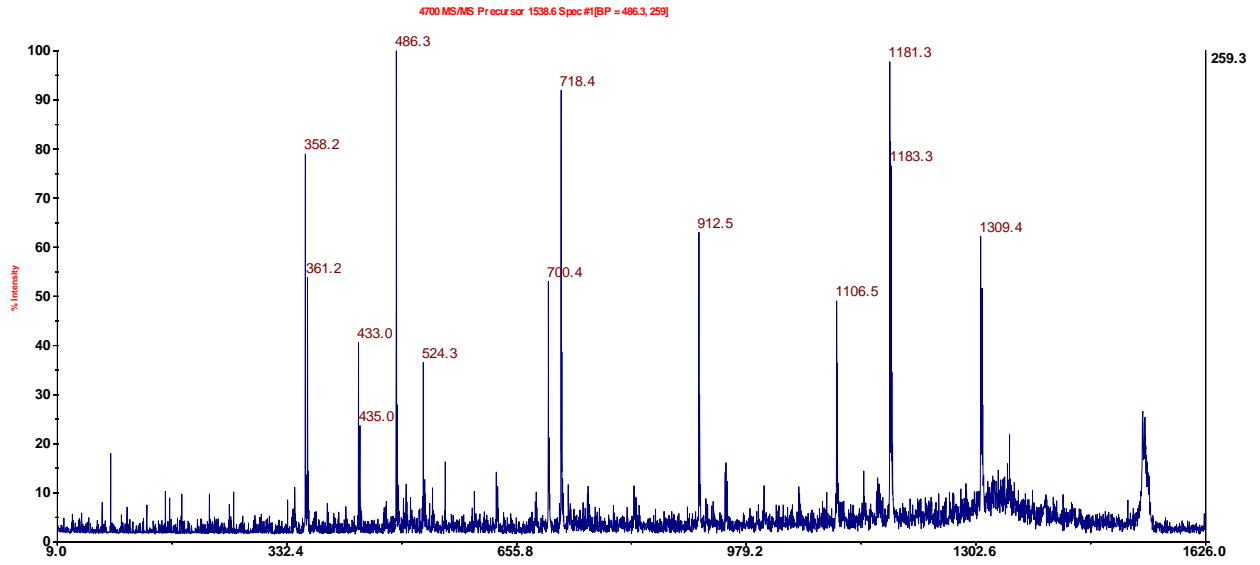
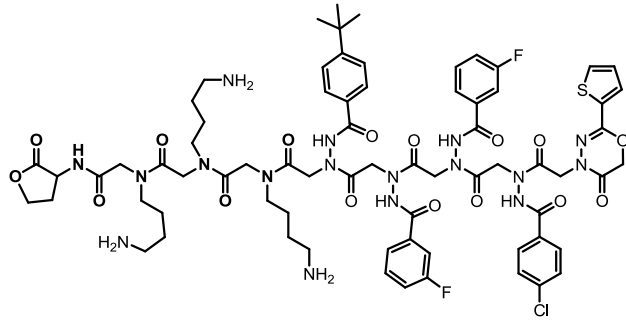
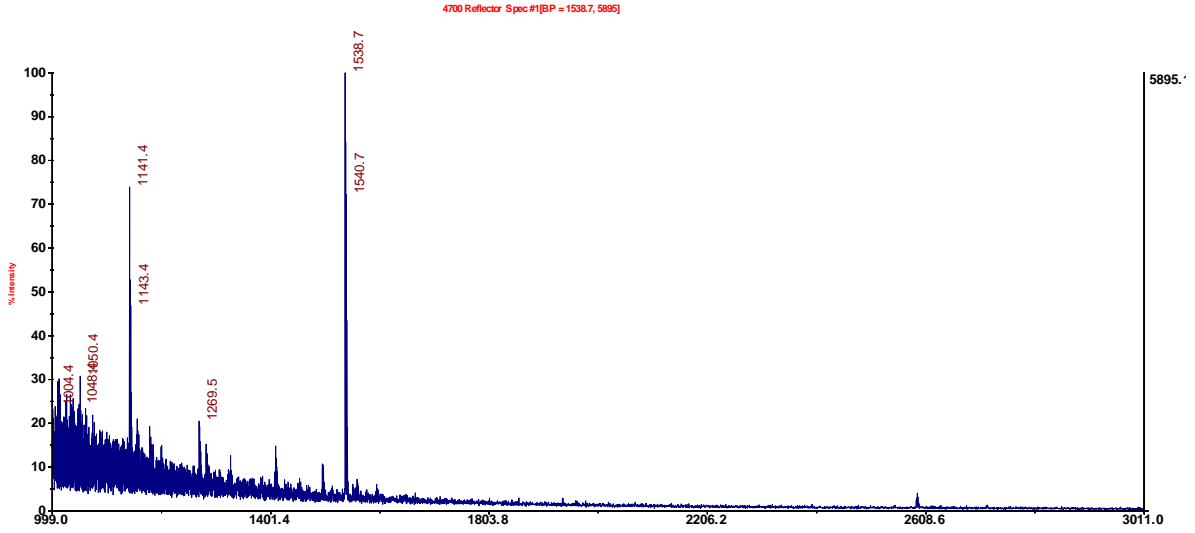
Fig. S57. General structure of the pentamer acyl hydrazide library having ten different 4-substituted 2-aryl-4H-1,3,4-oxadiazin-5(6H)-ones at the 5th position and the chemical structure of the ten acyl hydrazide monomers used in positions R1-R5.

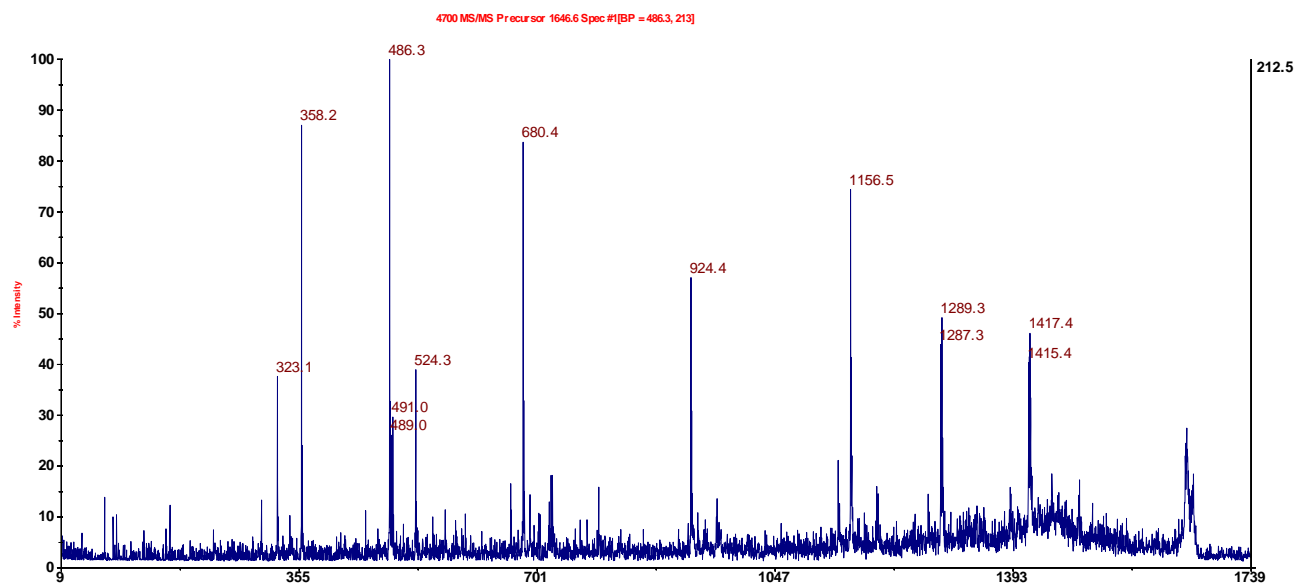
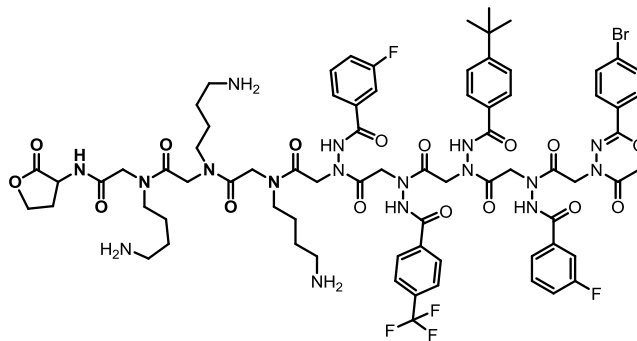
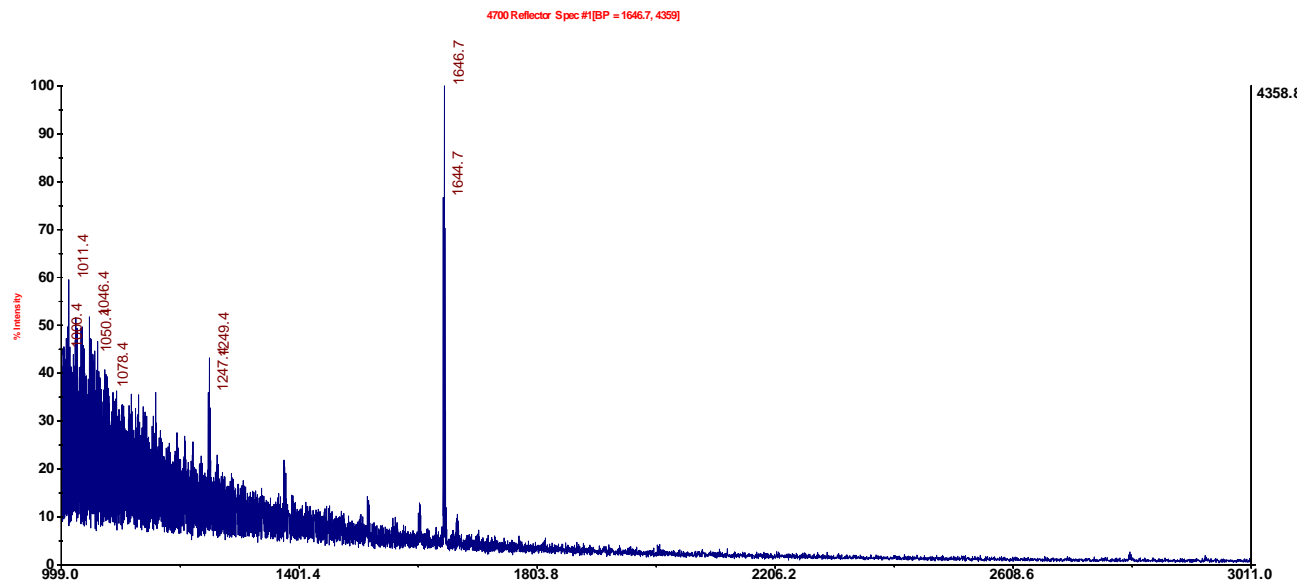
Fig. S58. MALDI TOF MS and MSMS spectra of some compounds of the pentamer library obtained after the single bead cleavage by using CNBr. (Note that the last two residues cleave together during MSMS analysis and these two residues could be identified by looking at the MSMS spectra in the lower mass region as shown below).

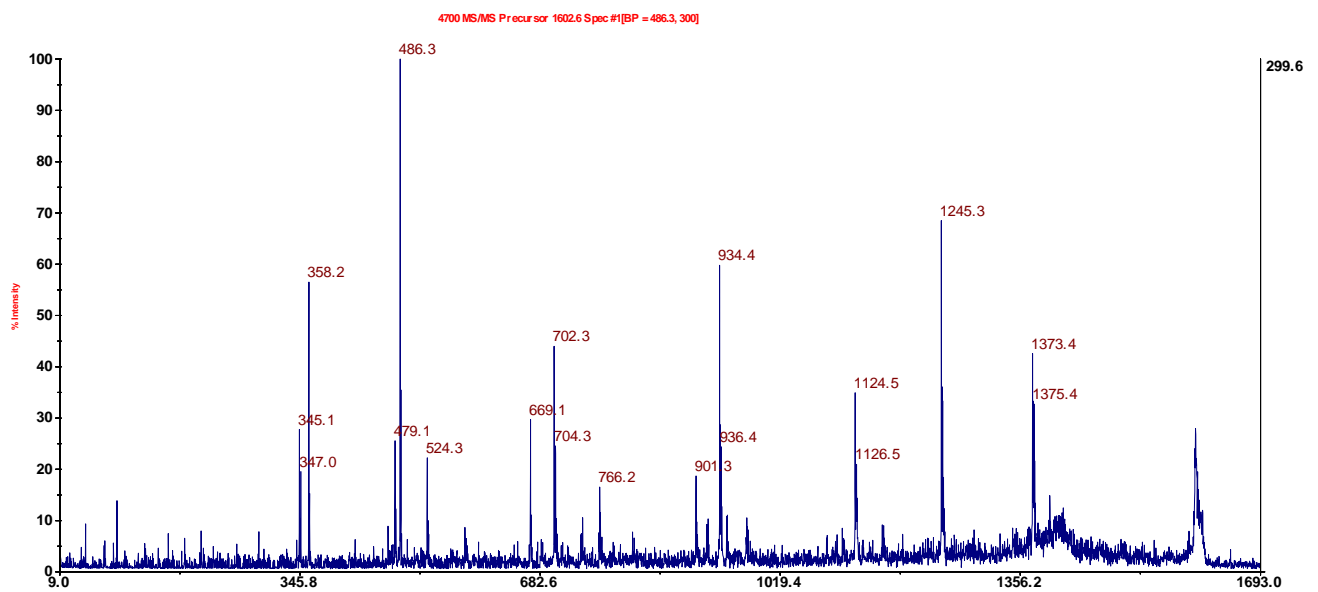
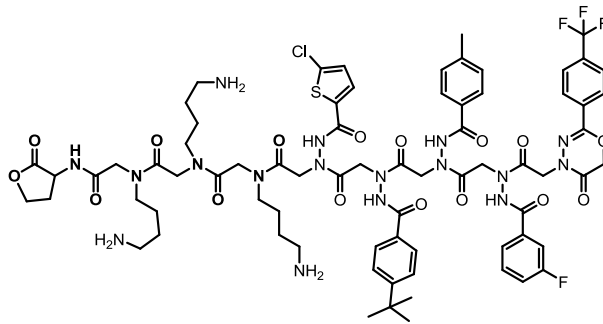
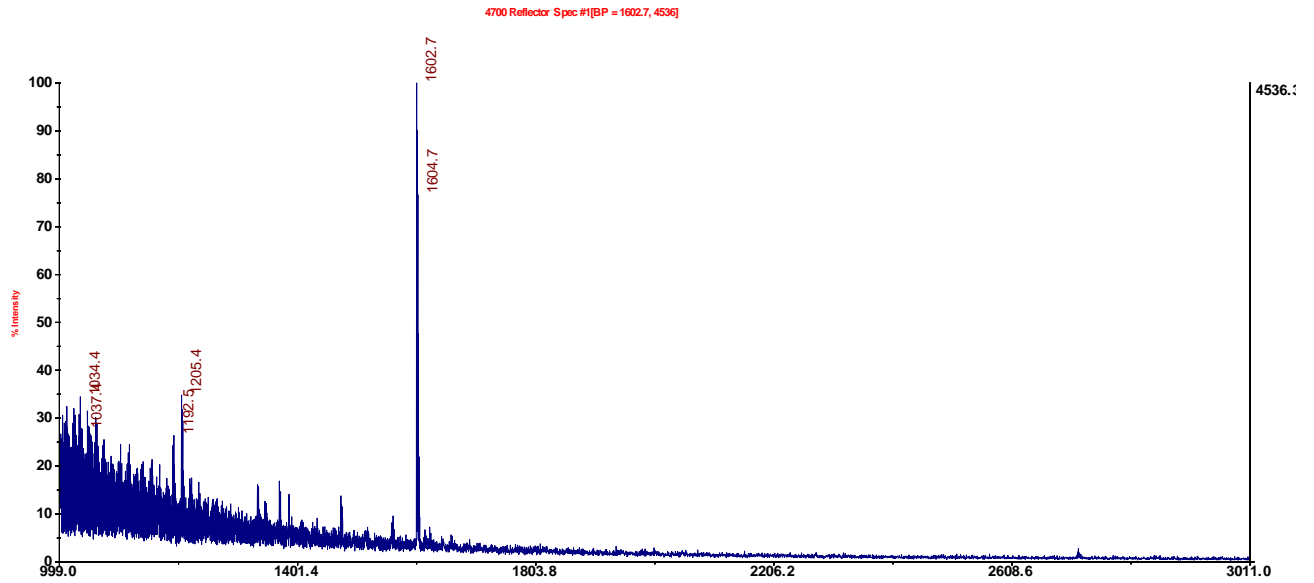












X-ray Crystallography

X-ray crystallographic studies were carried out on a Bruker SMART APEX CCD diffractometer with graphite-monochromatized Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$) controlled by a Pentium-based PC running on the SMART software package.^{S1} A single crystal was mounted onto the end of a nylon loop and data were collected at room temperature. The structure was solved by direct methods and refined using the SHELXTL software package.^{S2} All non-hydrogen atoms were refined anisotropically and hydrogen atoms were assigned idealized locations.

Crystallization of 1d: 20 mg of the compound was dissolved in 2 mL of methanol and 1 mL of dichloromethane. The solvents were allowed to evaporate at room temperature to give light yellow coloured crystals of **1d**.

Crystal data for 1d: C₆H₁₁N₃O₃; M_r = 173.18, Monoclinic P2(1), a = 4.7784(18), b = 10.846(4), c = 8.480(3) Å, $\beta = 97.832(5)$, V = 435.4(3) Å³, Z = 2, $\rho_{\text{calcd}} = 1.321 \text{ g/cm}^3$, MoK α radiation ($\lambda = 0.71073 \text{ \AA}$), T = 296(2) K, Flack Parameter = 0.0(16); 4310 measured data of which 2131 ($R_{\text{int}} = 0.0961$) were unique, $R_1 = 0.0564$, $wR_2 = 0.1328$ ($I > 2\sigma(I)$); $R_1 = 0.0690$, $wR_2 = 0.1440$ (all data).

(S1) SMART, Version 5.05, Bruker AXS, Madison, WI, 1998.

(S2) A. Altomare, G. Cascarano, C. Giacovazzo and A. Gualardi, *J. Appl. Cryst.*, 1993, **26**, 343.