Supplemental Information

Solid Phase Syntheses of Peptoid like Arylureido Compounds and Sequencing of Isobars without Molecular Encoding

Kanwal Asif, Greg O'Brien, Scott Goodman, Sujit Suwal *

1. Materials and Instruments:

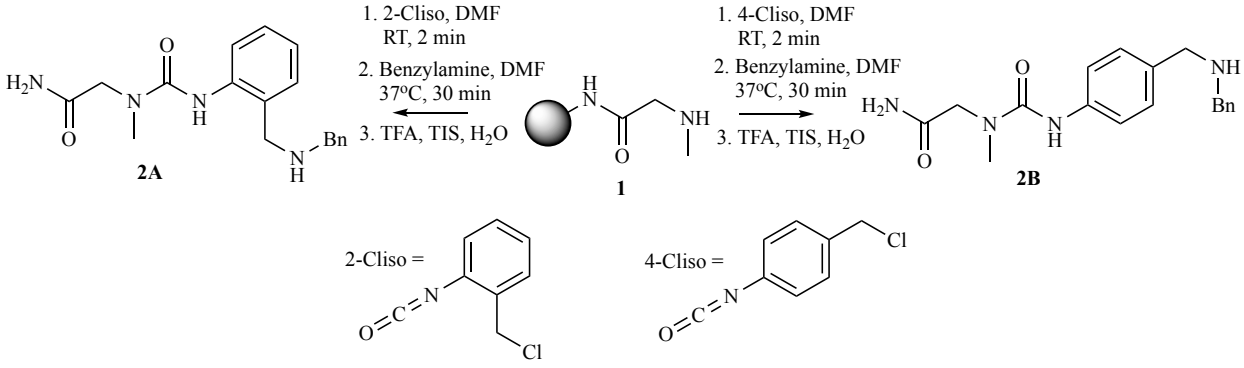
4-Chloromethylphenyl isocyanate and 2-chloromethylphenyl isocyanate were purchased from Alfa Aesar. Rest of the chemicals and reagents were purchased either from Aldrich or Fisher Scientific. Rink amide MBHA resin (0.5 mmol/g) and Tentagel macrobead-NH_2 (160 μg, 0.4 mmol/g) resin were obtained from Chemprep and rapp-polymere, respectively. Disposable fritted columns (5 mL, 50 mL) for peptoid syntheses were bought from Intavis AG. Microwave assisted peptoid syntheses were accomplished utilizing 10% of 1550 W household microwave (GE model JE 1860 BH04). All the isocyanate coupling reactions with secondary amines were carried out at room temperature. HPLC purification of crude peptoids from Rink amide resin after acid cleavage was carried out in Waters 1525 binary HPLC pumps and a 2487 dual absorbance detector, or a 2998 photodiode array detector. Buffer A (H_2O with 5% CH_3CN and 0.1% trifluoroacetic acid (TFA) and buffer B (5m, 250 x 4.6 mm, Alltech) and Apollo C-18 5μ preparative column. Purity of the compounds was assessed by analytical HPLC under UV (214 nm, 254 nm), otherwise mentioned in description of the spectrum. MS and MS/MS were recorded in ESI-MS (Waters) and MALDI -TOF (4800 Proteomics Analyser, Applied Biosystems). Cyano-4-hydroxycinnamic acid (HCCA) was used as MALDI matrices (Aldrich). CNBr-mediated cleavage was carried out at room temperature for 3 hr. ^1H and ^{13}C NMR spectra were recorded in Bruker 400 instrument operating at 400 MHz for ^1H and 100 MHz for ^{13}C NMR using Deuterated-methanol ($DCCl_3$) or Deuterated-Dimethylsulfoxide (DMSO) (Cambridge) as a solvent.

2. General synthetic method for compound 2Aand 2B:

Rink amide MBHA resin (0.5 g, 0.7 mmol) was swelled in N, N'-dimethylformamide (DMF). Fmoc-group was deprotected using 20% piperidine in DMF (2X, 15 mins). The primary amine, thus formed, was coupled with bromoacetic acid (BAA, 2M) activated with N, N'-diisopropyl-carbodiimide (DIC, 3.2 M) in DMF. Subsequently, the bromide was displaced with commercially available methylamine (1M DMF). The beads were washed with DMF (5X), DCM (5X) and finally washed with MeOH (2X). In a separate tube DMF solution of 2-chloromethylphenyl isocyanate (1M, 2Cliso) was prepared and added into the secondary amine. [Note: 4-Chloromethylphenyl isocyanate (4Cliso) is sparingly soluble in DMF. Hence, it is worthwhile filtering the solution before adding into the bead]. The reaction mixture was shaken for 2 min at room temperature. The solution was filtered, and the beads were washed with DMF (5X), methanol (2X) and DCM(5X). In a separate tube, DMF solution of benzyl amine (2M) was prepared and added onto the resin-bound chloride. The reaction mixture was incubated for 2 h at 37°C. The completion of chloride displacement was qualitatively confirmed with chloranil test. Finally, the solid support was washed with DMF, DCM and methanol, and the compound was cleaved using 80% TFA in Acetonitrile along with 15% water and 5% triisopropylsilane. A crude arylureido molecules **2A-B** were obtained after the solvent was evaporated under argon gas and ether precipitation. Compounds **2A-B** were dissolved separately in 50% acetonitrile-water solution and purified using C-18 Reverse Phase preparative HPLC. The retention time for compounds **2A** and

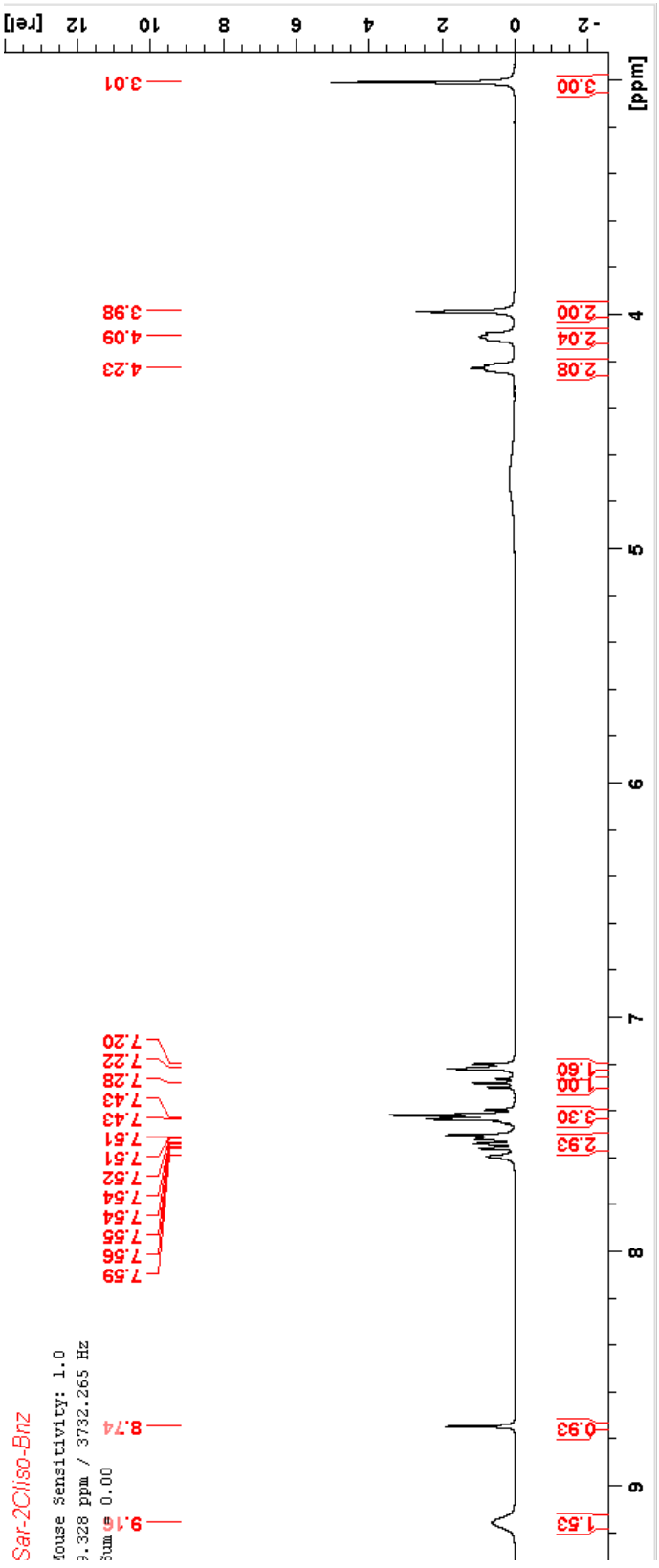
2B were 19 min and 20 min, respectively. Finally, the purified compounds were authenticated separately using different spectroscopic techniques.

Scheme 1: Solid Phase Synthesis of Arylureido Molecules

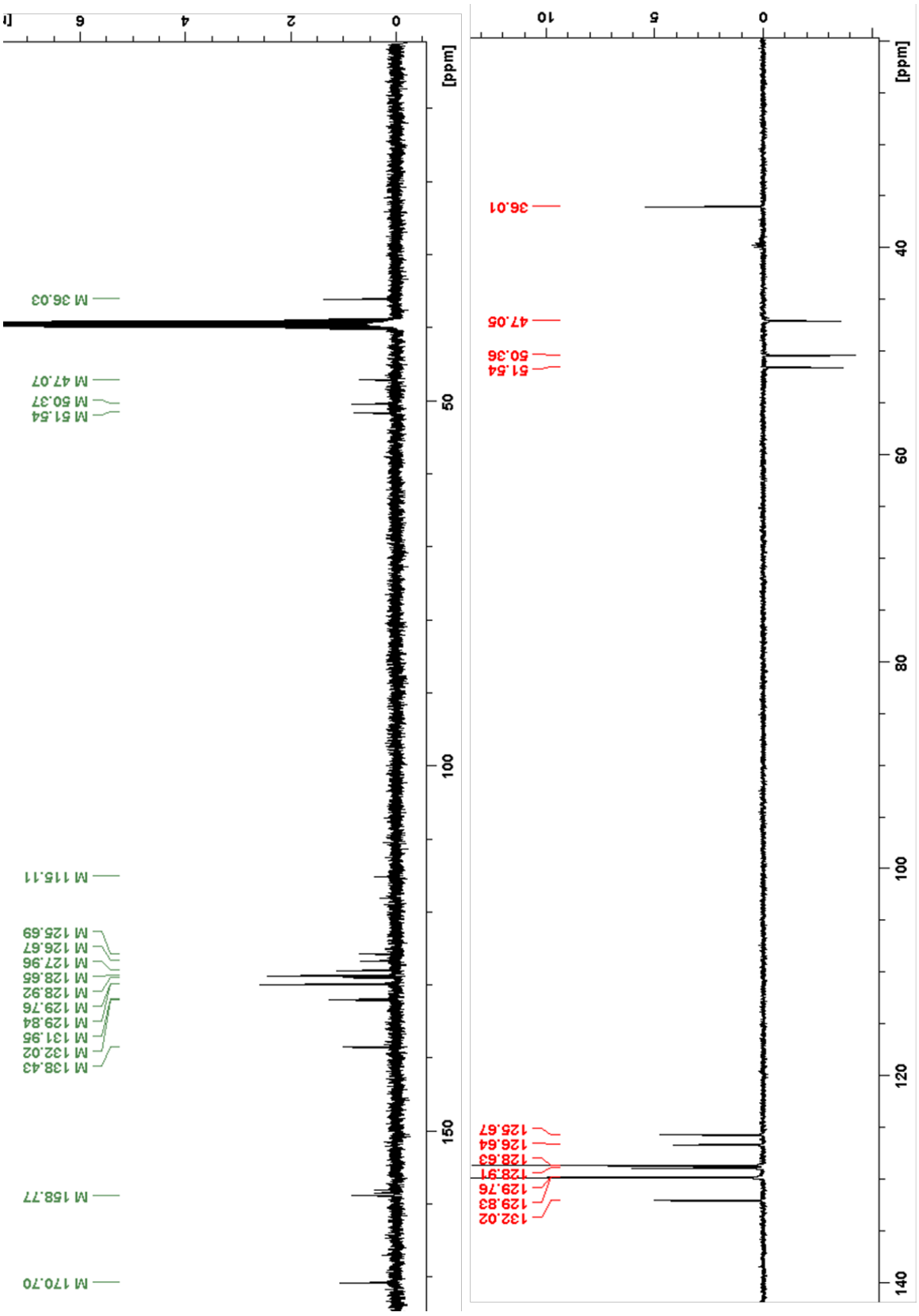


2.1 Spectral data from 2A:

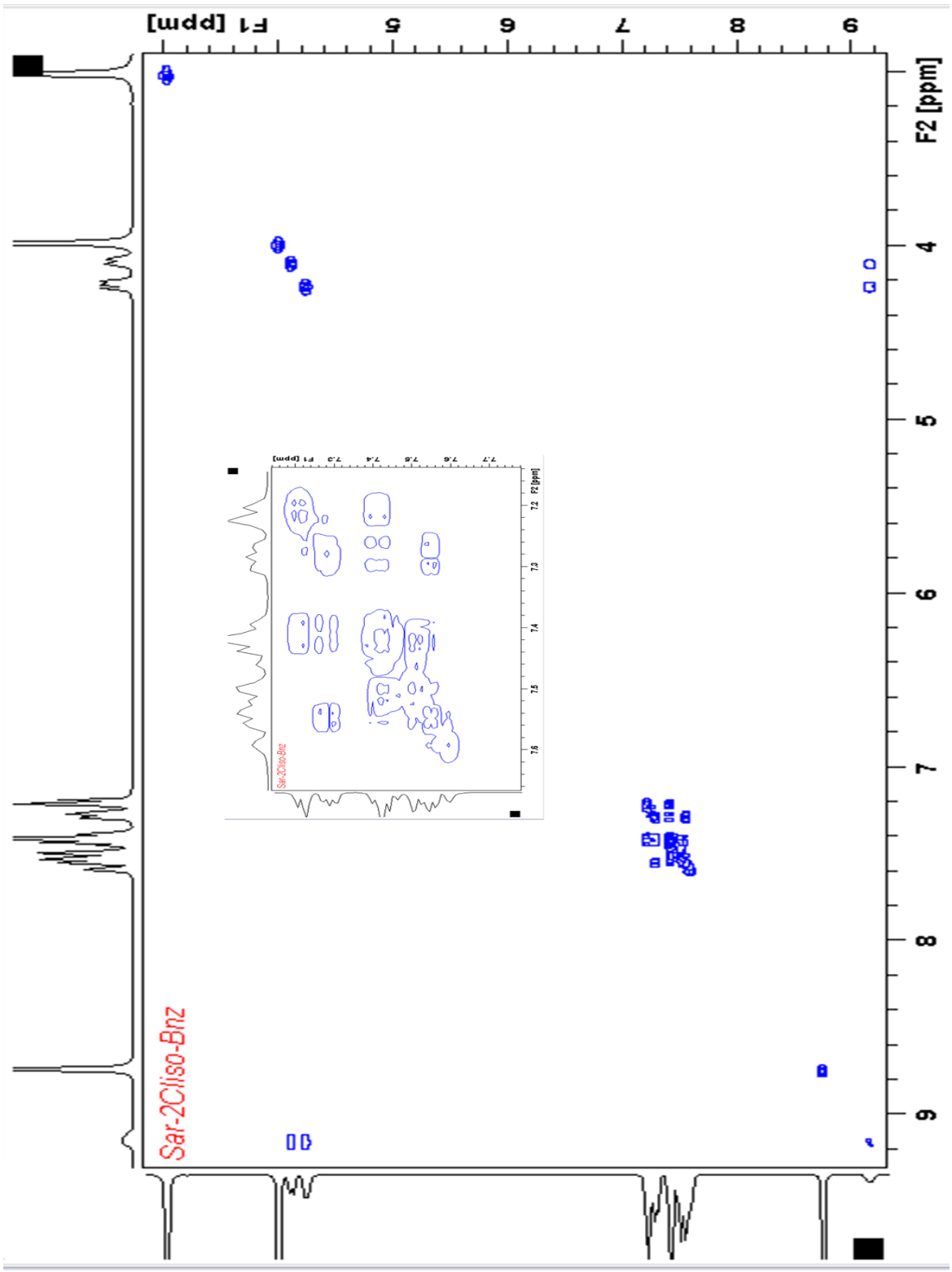
^{1}HNMR (400 MHZ, D_6-DMSO) δ 9.16 (s, 2H), 8.74 (s, 1H), 7.59-7.2 (m, 9H), 4.23 (t, 2H), 4.09 (t, 2H), 3.98 (s, 2H), 3.01 (s, 3H, Me); ^{13}CNMR (100 MHz, DMSO) δ170.7, 158.77, 138.43, 132.02, 131.95, 129.76, 128.92, 128.65, 127.96, 126.67, 125.369, 115.11, 21.54, 50.37, 47.07, 36.03; ESI-MS (Obs. $M+H^+$): 327.2 (327.17 expected).



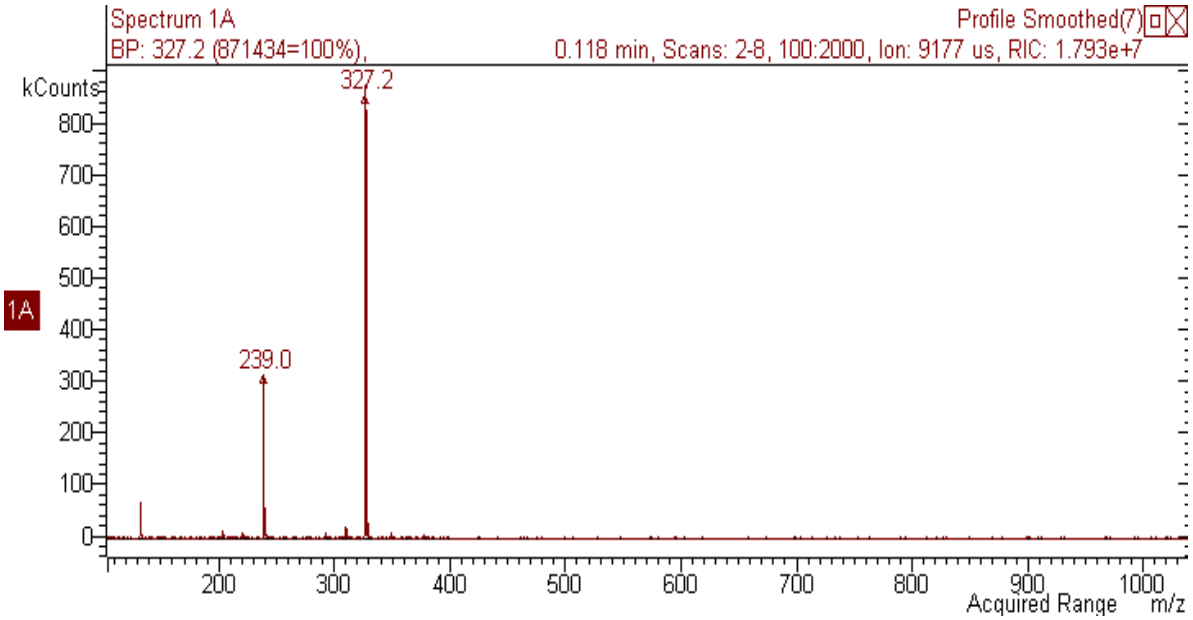
S1: [1]HNMR of a compound **2A** derived from 2-Chloromethylphenyl isocyanate (2-Cliso)



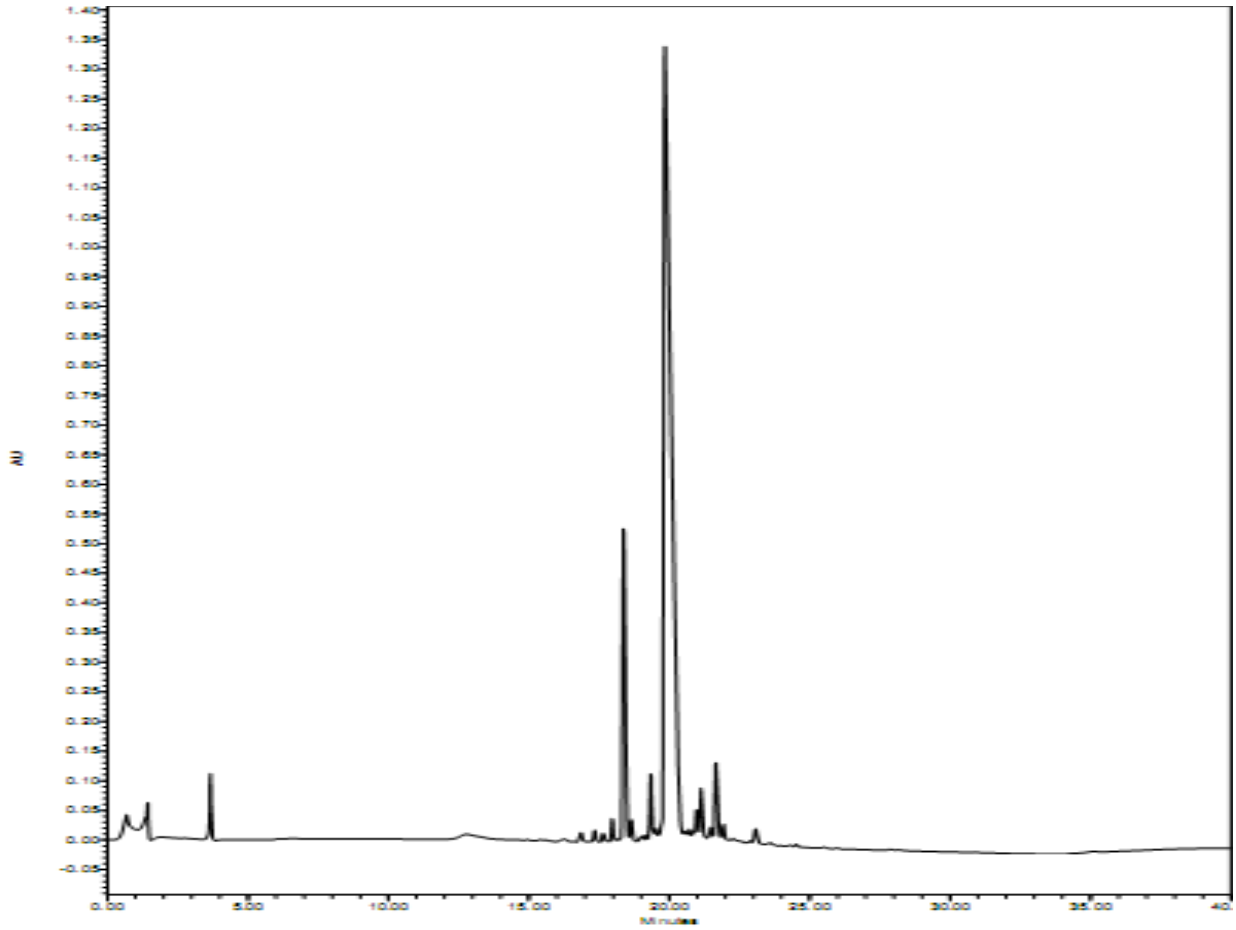
S2: C-13 NMR and DEPT of a compound **2A** derived from 2-Cliso



S3: COSY of a compound **2A** derived from 2-Cliso

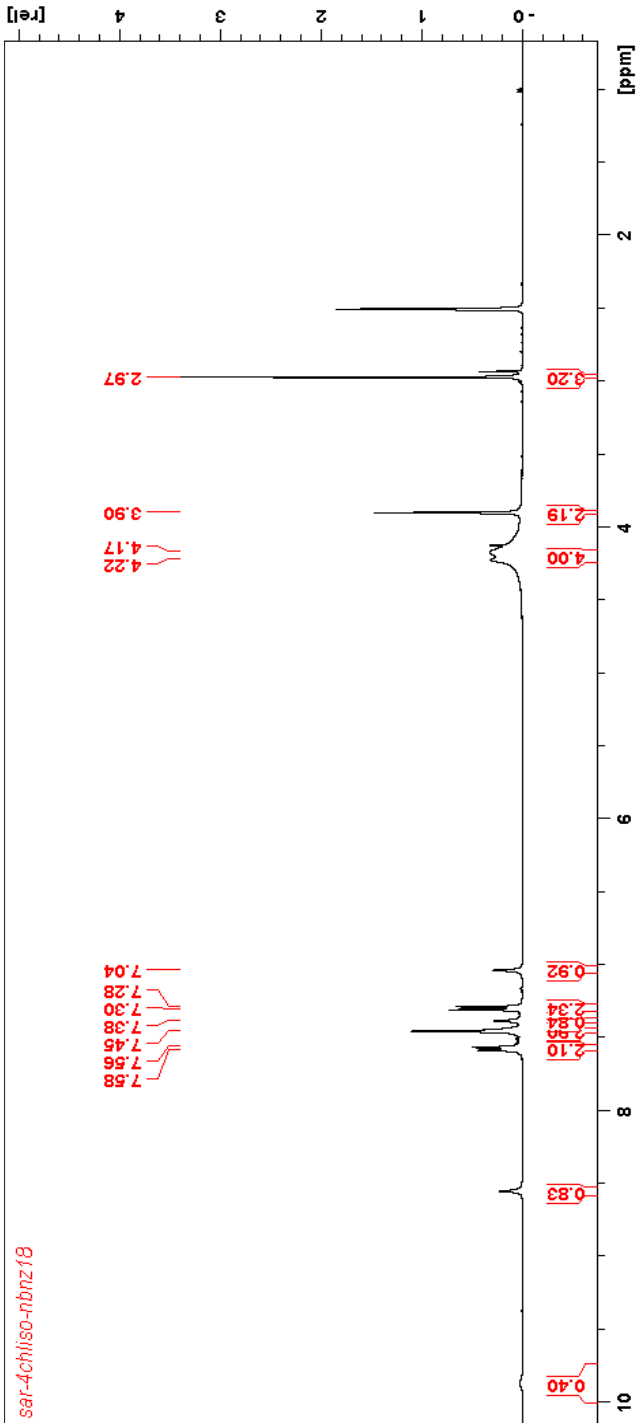


S4: ESI-MS of a compound **2A** derived from 2-Cliso

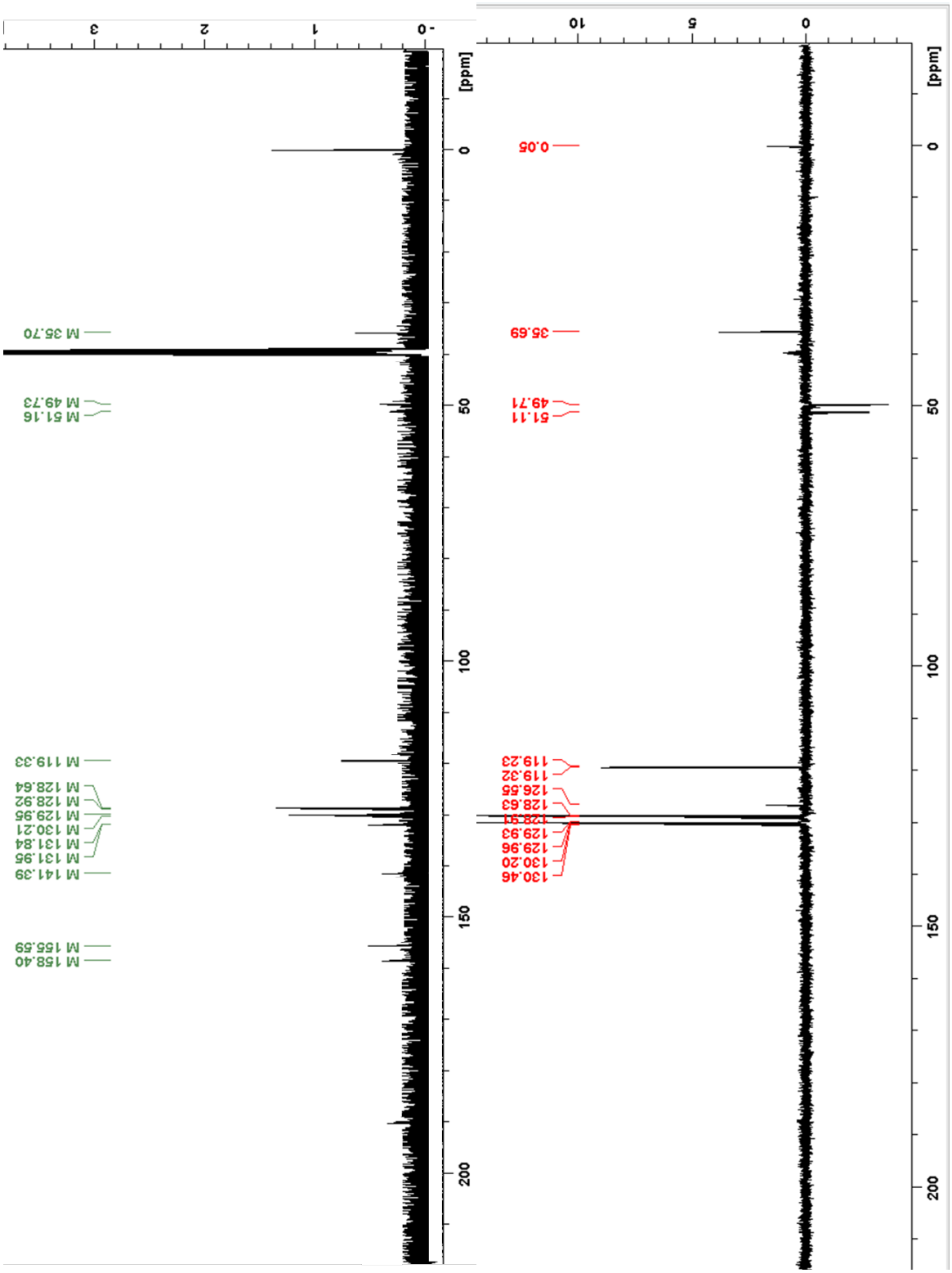


S5: Chromatogram (HPLC) of compound **2A** derived from 2-Cliso (Retention time = 20 min)

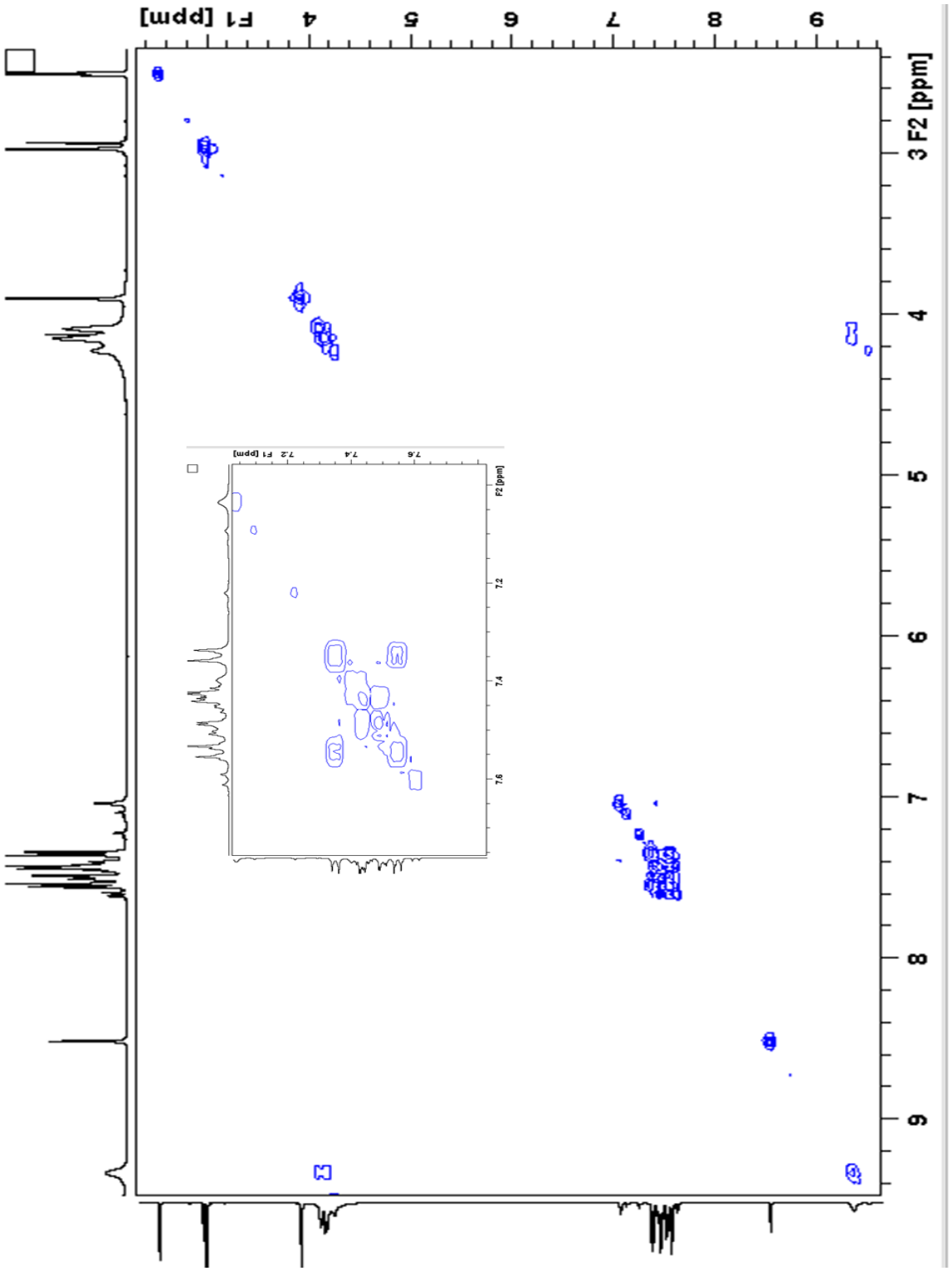
Spectral data from 2B:
^{1}HNMR (400 MHZ, D_6-DMSO) δ9.16 (s, 1H),8.74 (s,1H), 7.58-7.04 (m, 9H), 4.22-4.16 (m, 4H), 3.90 (s, 2H), 2.97 (s, 3H); ^{13}CNMR (100 MHz, DMSO) δ 190, 158.40, 158.59, 141.38, 131.5, 131.84, 130.21, 129.95, 128.92, 128.64, 119.33, 51.16, 49.73, 35.70; ESI-MS(Obs. M+Na^+) 349.1 (349.17 expected).



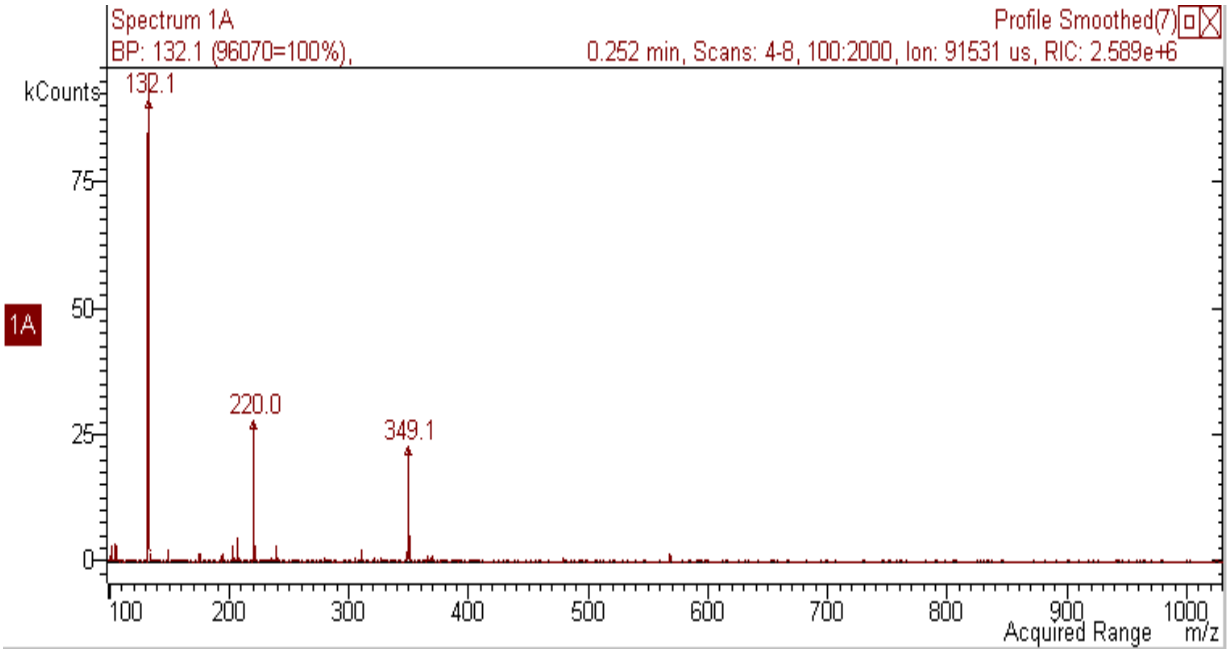
S6: ^{1}HNMR of a compound **2B** derived from 4-Chloromethylphenyl isocyanate (4-Cliso)



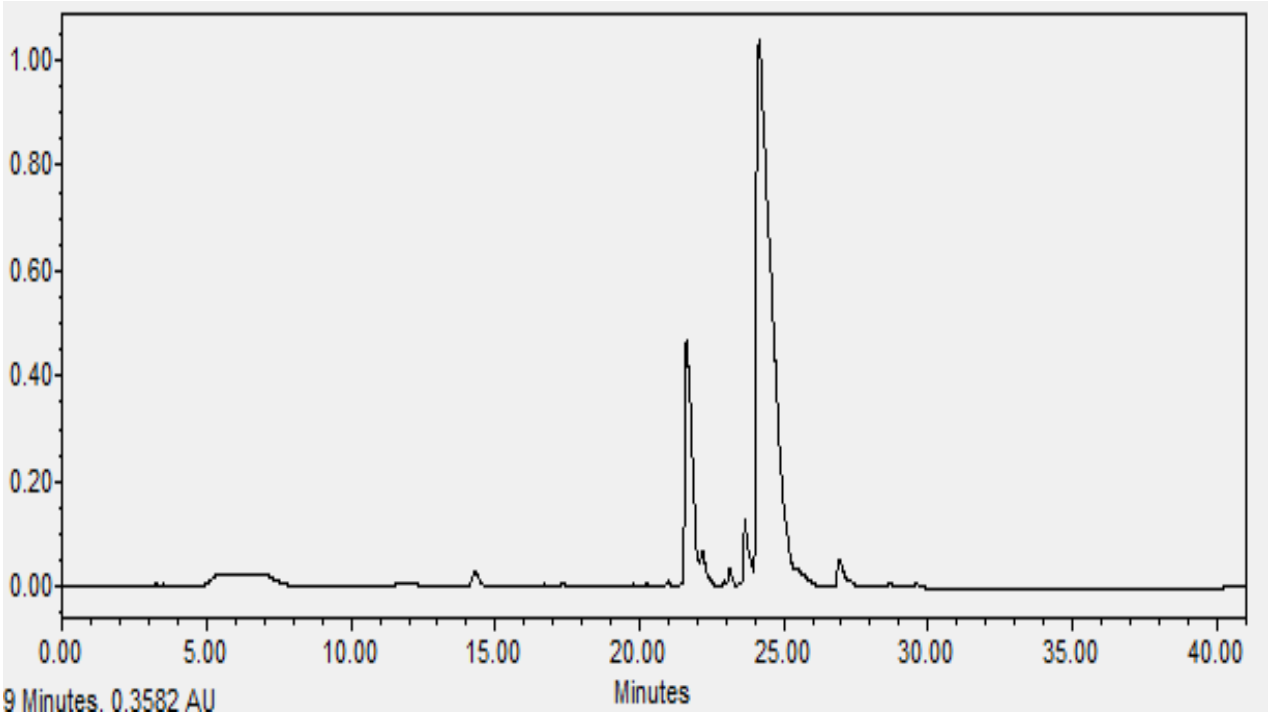
S7: C-13 NMR and DEPT of a compound **2B** derived from 4-Cliso.



S8: COSY of a compound **2B** derived from 4-Cliso.



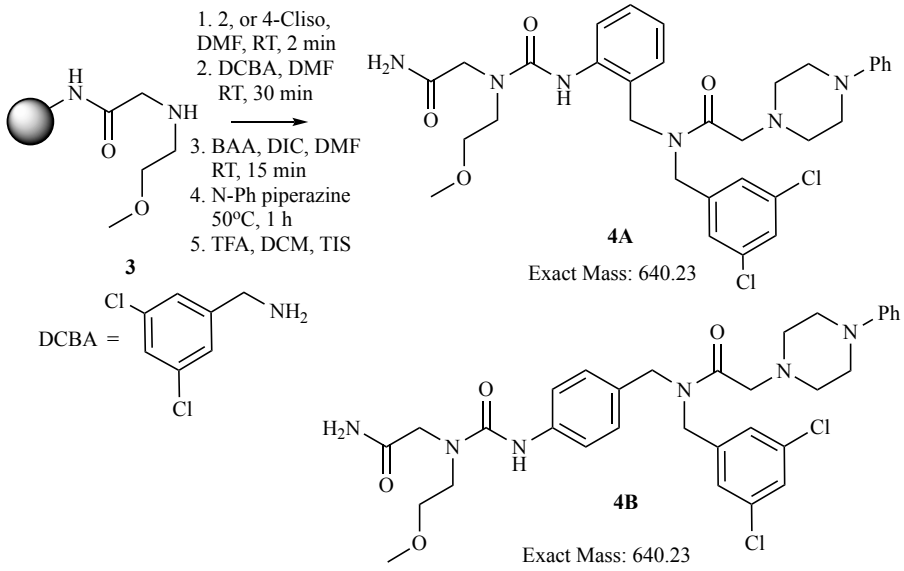
S9: ESI-MS of compound **2B** derived from 4-Cliso



S10: Chromatogram (HPLC) of compound **2B** derived from 4-Cliso (Retention time 24.5 min).

3. Synthesis of compounds 4A and 4B:

For synthetic protocol: Refer general synthetic approach

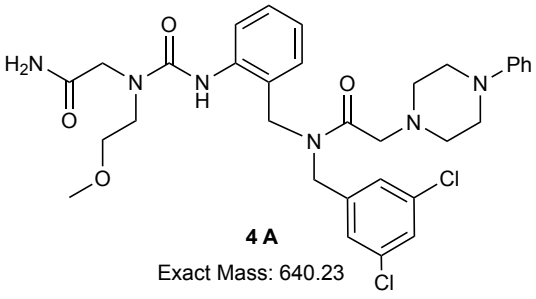


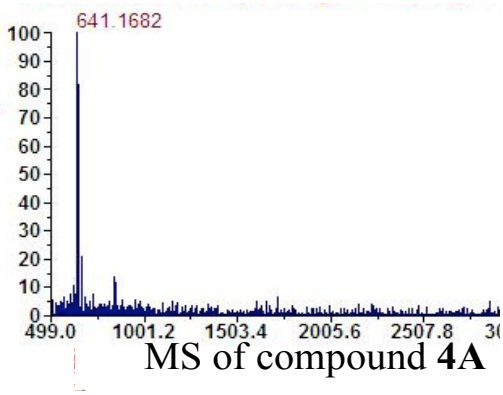
S11: Scheme showing synthesis of mono-ureido trimers, derived from 4-Cliso and 2-Cliso units, utilized to discern fragmentation pattern under MALDI-TOF.

3.1 Study of Fragmentation Pattern on compounds 4A and 4B:

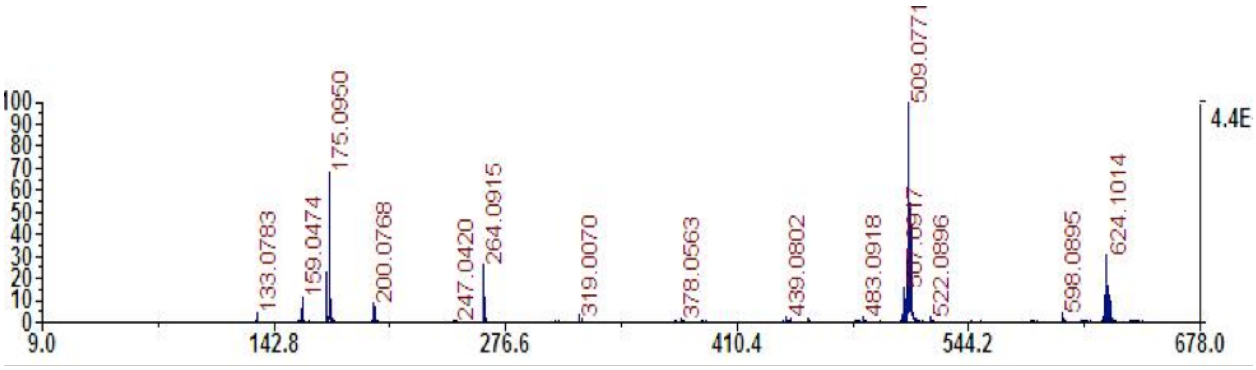
Compound 4A:

The figures, shown below, represent the structure of compound **4A**, its MALDI-TOF MS, following by MS/MS spectrum. Underneath the tandem spectrum is the possible fragmentation pathways that account for the formation of ions representing m/z that are observed in MS/MS. In particular, the tandem MS clearly shows that the arylureido compound **4A** cleaves via SS-1 fragmentation mechanism, generating signature ion at m/z 509.0771. It also shows a characteristic peak at 175.0950 resulted possibly due to a bond cleavage alpha to the piperazine moiety.

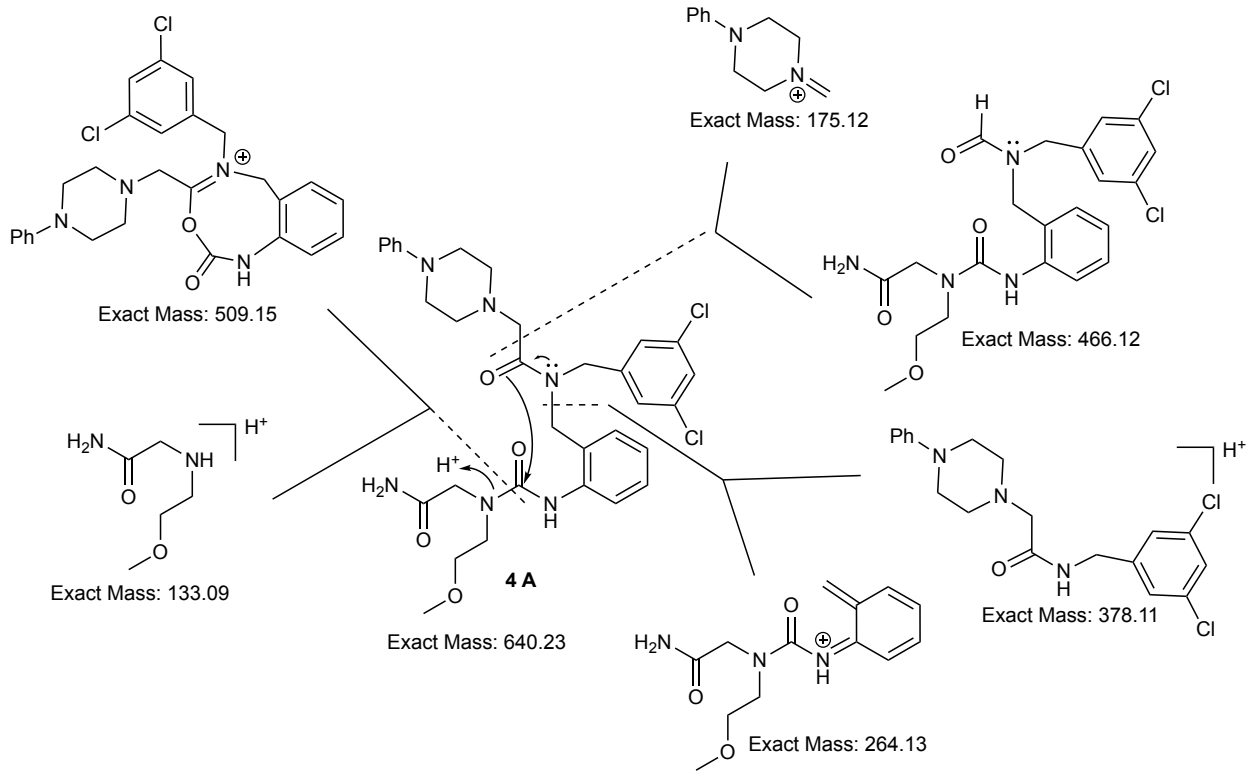




MS of compound **4A**



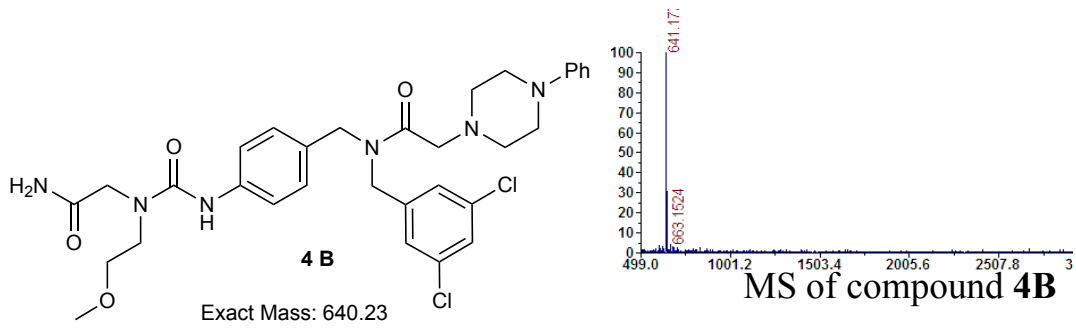
MS/MS of Compound **4A**

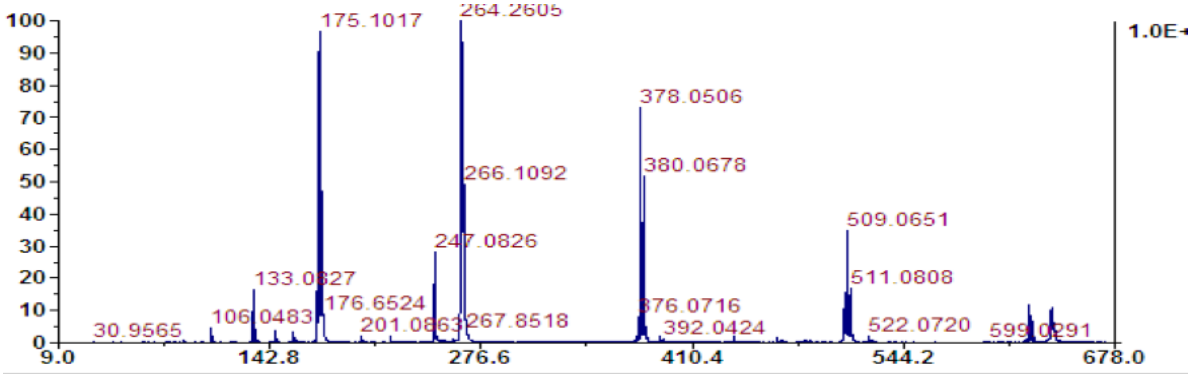


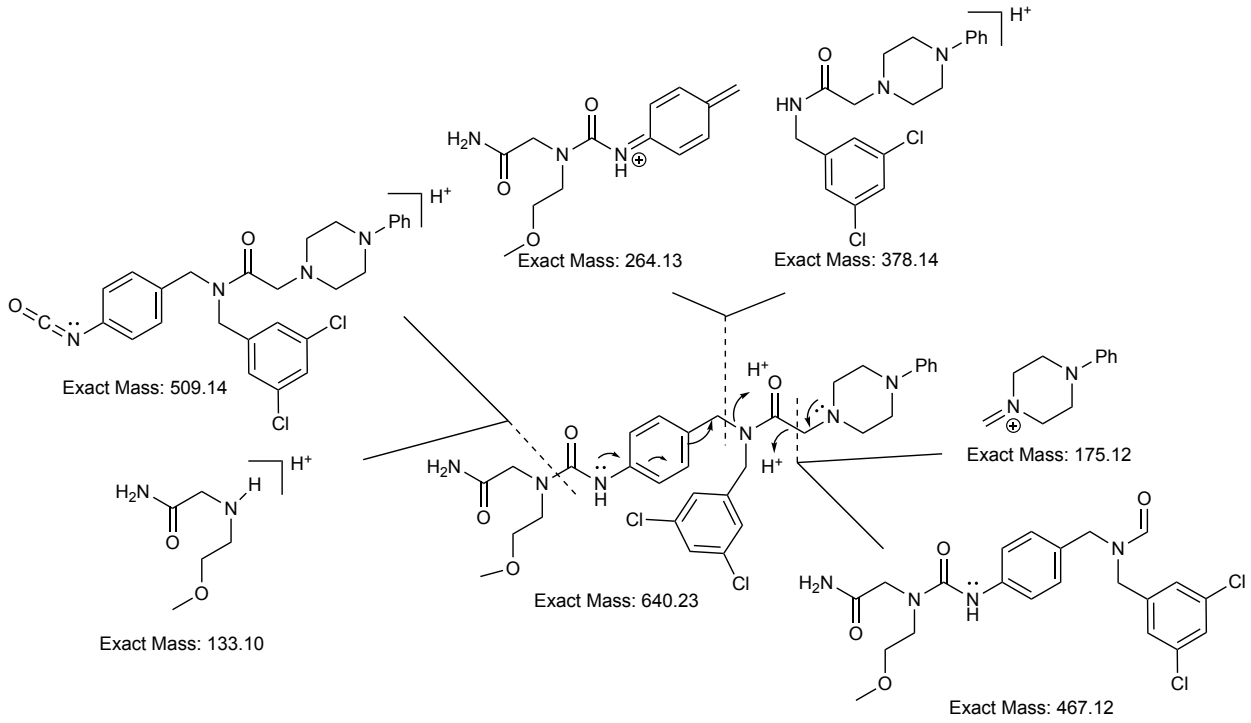
S11.1: Figures showing synthesis of compound **4A**, its MALDI TOF MS and tandem mass followed by possible fragmentation pathways.

Compound 4B:

The figures, shown below, represent the structure of compound **4B**, its MALDI-TOF MS, following by MS/MS spectrum. Underneath the tandem spectrum is the possible fragmentation pathways that account for the formation of ions representing m/z that are observed in MS/MS. In particular, the tandem MS clearly shows that the arylureido compound **4B** cleaves at benzylic position, thus generating signature ion m/z = 264.2605 and 378.05060. Like in compound **4A**, it shows additional signature ions at 175.0950 which possibly resulted via cleavage of the molecule at the alpha carbon to the piperazine moiety, like in compound **4A**.



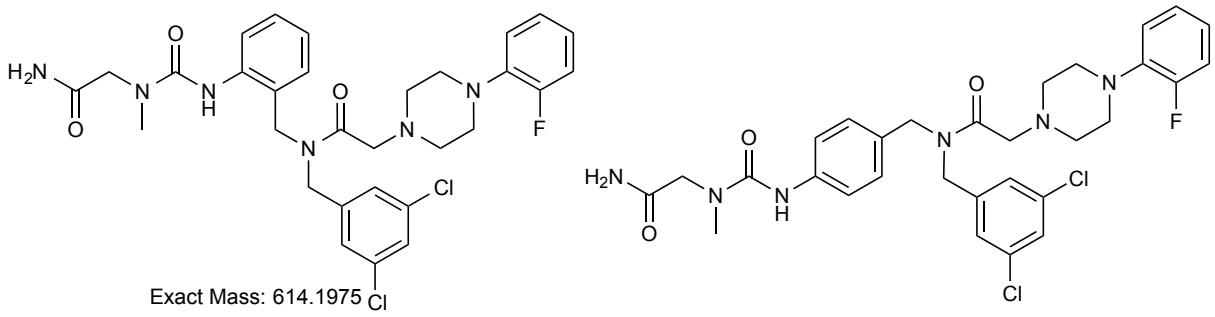




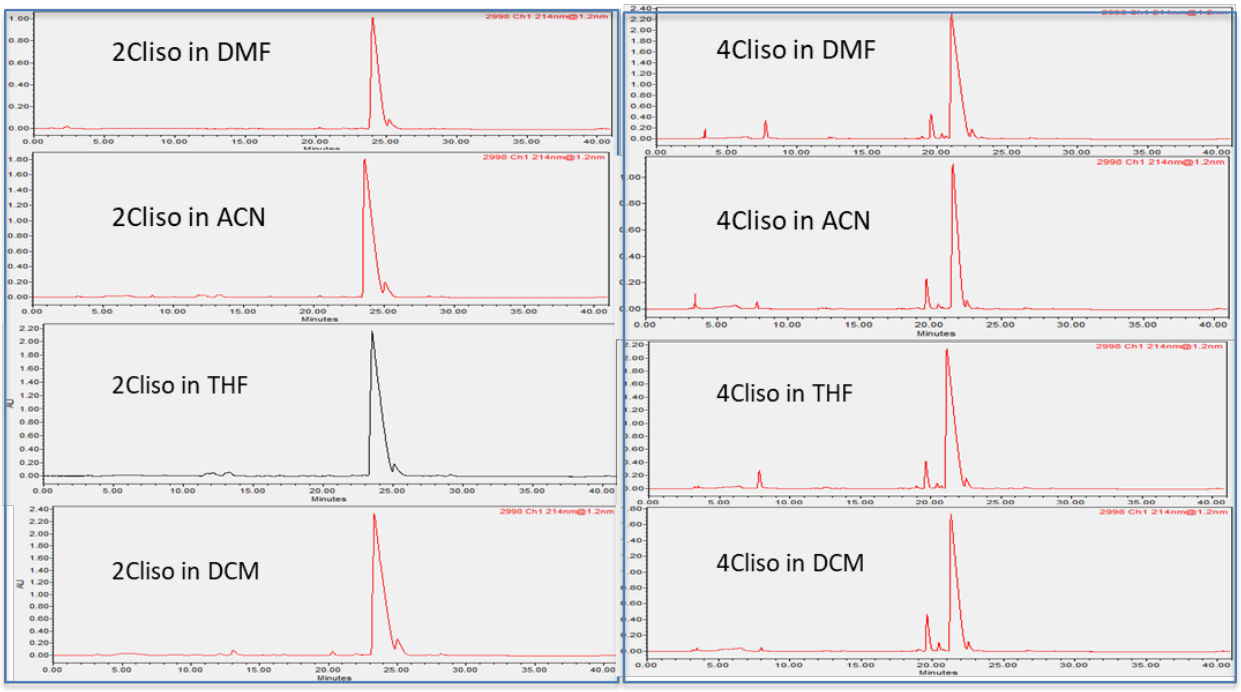
S11.2: Figures showing synthesis of compound **4B**, its MALDI TOF MS and tandem mass followed by possible fragmentation pathways.

4. Choice of Solvent:

Compound **5A** and **5B** were synthesized dissolving 2-Cliso and 4-Cliso isobars in different organic solvent. The compounds were capped with piperazine derivative after chain extension as explained earlier. Resultant compounds were cleaved by 50% TFA and 5% TIS in DCM for 30 min. The purity of the molecules tested using RP-HPLC after ether precipitation.



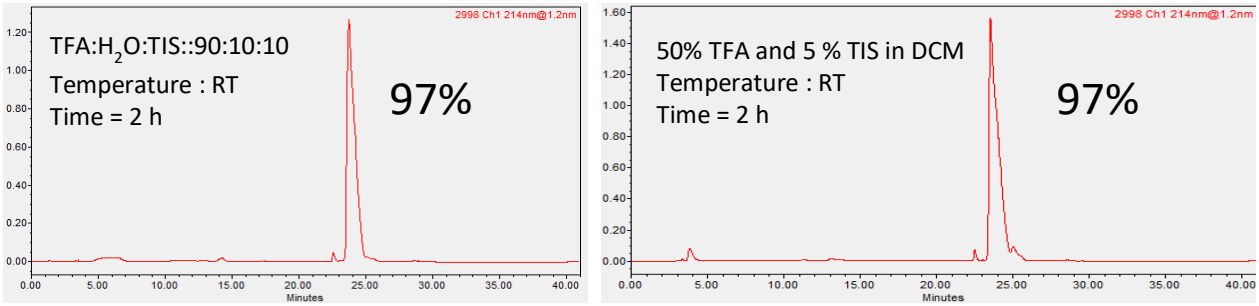
5A **5B**



S12: HPLC chromatograms showing purity of the 2-Cliso and 4-Cliso-derived compounds (shown at the top) synthesized using different organic solvents.

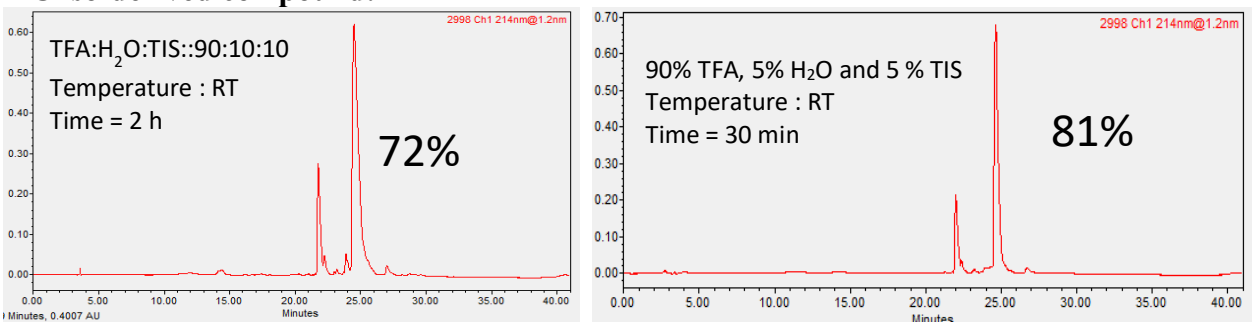
5. Cleavage Condition:

Compound **5A** and **5B** were cleaved under different cleavage condition and duration. Compound 5A tolerated different cleavage cocktail as well as duration, while compound **5B** showed little sensitive to water and cleavage. Cleavage cocktail of 50% TFA and 5% TIS in DCM found to be better for cleaving compound **5B** from the resin. Also, 30 mins incubation under the cleavage cocktail found to be better to obtain high purity compound. Please compare chromatograph shown in S12 vs S14 those are resulted with and without water, respectively.



S13: Comparative HPLC chromatograms showing the purity of **5A** cleaved under different cleavage cocktail.

4-Cliso-derived compound:

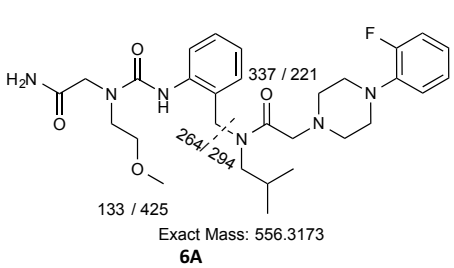


S14: Comparative HPLC chromatograms showing the purity of compound 5B cleaved under different cleavage cocktail.

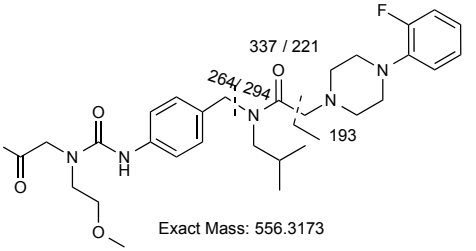
6. Study on Oligomerization:

Following sections illustrates the structures of mono-, di- and tri-ureido molecules obtained by solid phase synthesis of the molecules and their MALDI-TOF MS and tandem mass spectrum along with their purity determined by HPLC. The chromatographs of the molecules showed the purity of the molecules ranges between 90 – 95 %. Most importantly each of the 2-Cliso and 4-Cliso derived molecules undergoes proposed SS-1 and SS-2 fragmentation mechanism.

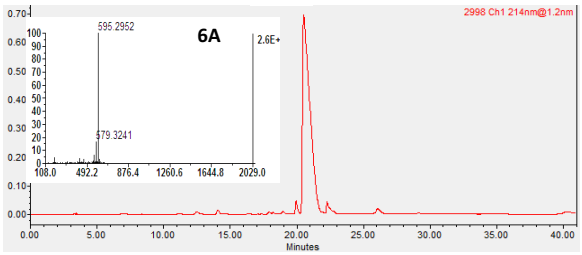
6.1 Mono-ureido compound

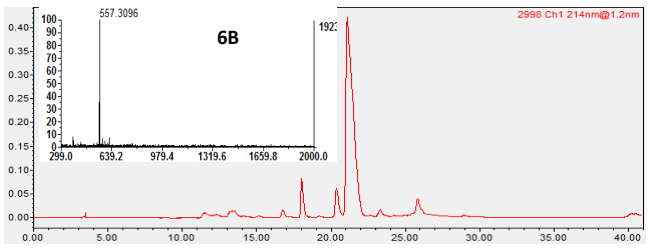


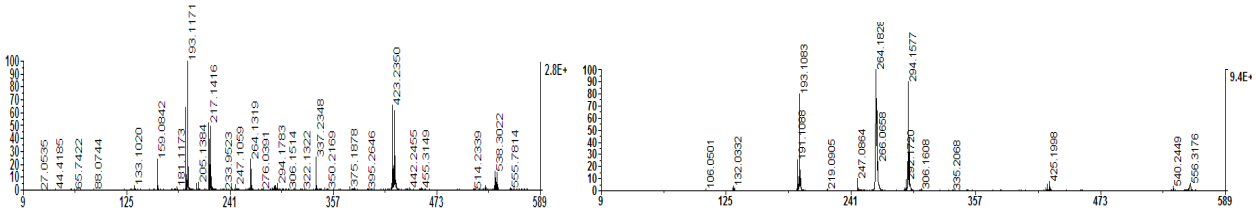
6A



6B

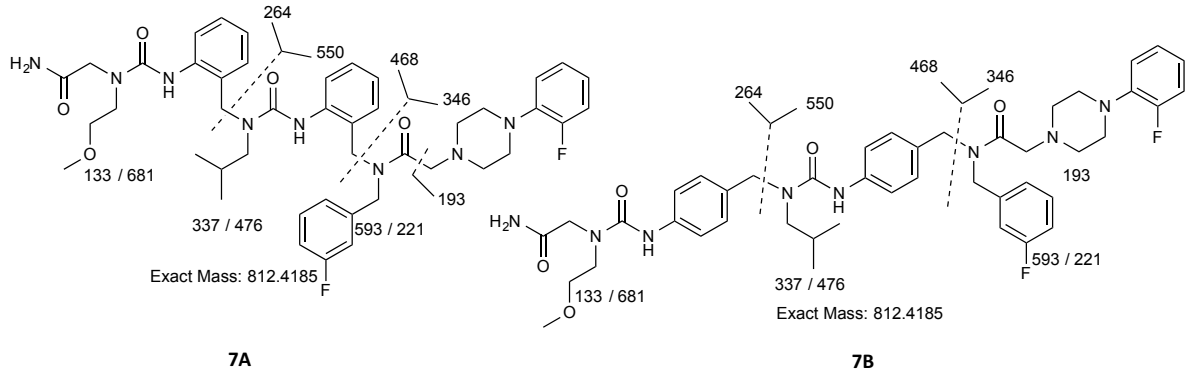


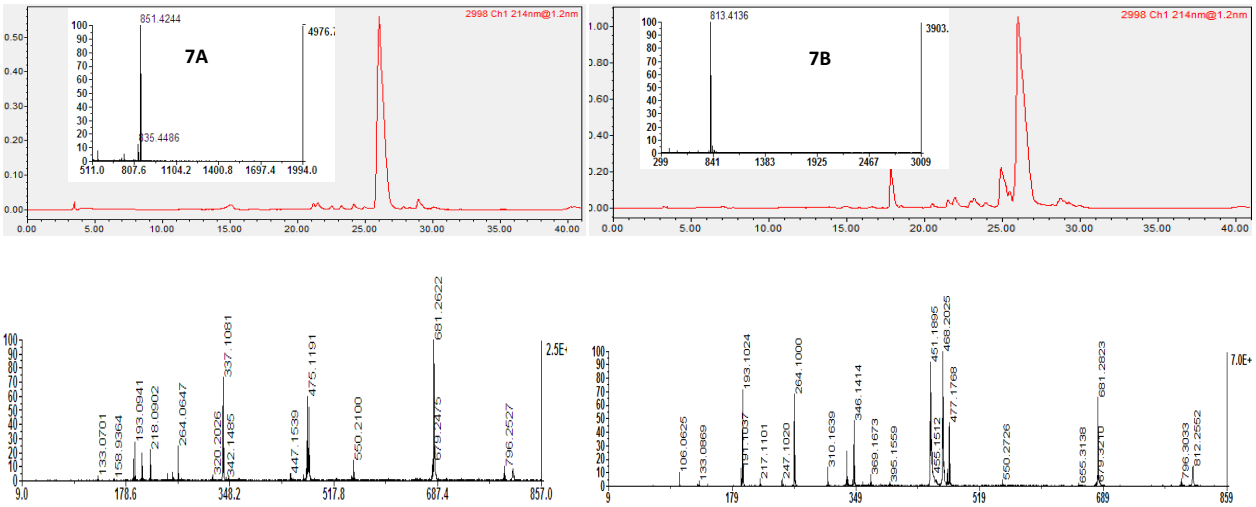




S15: Comparative HPLC chromatograms (middle) of mono-ureido isobars (top, m/z=556.31), and their fragmentation pattern (bottom).

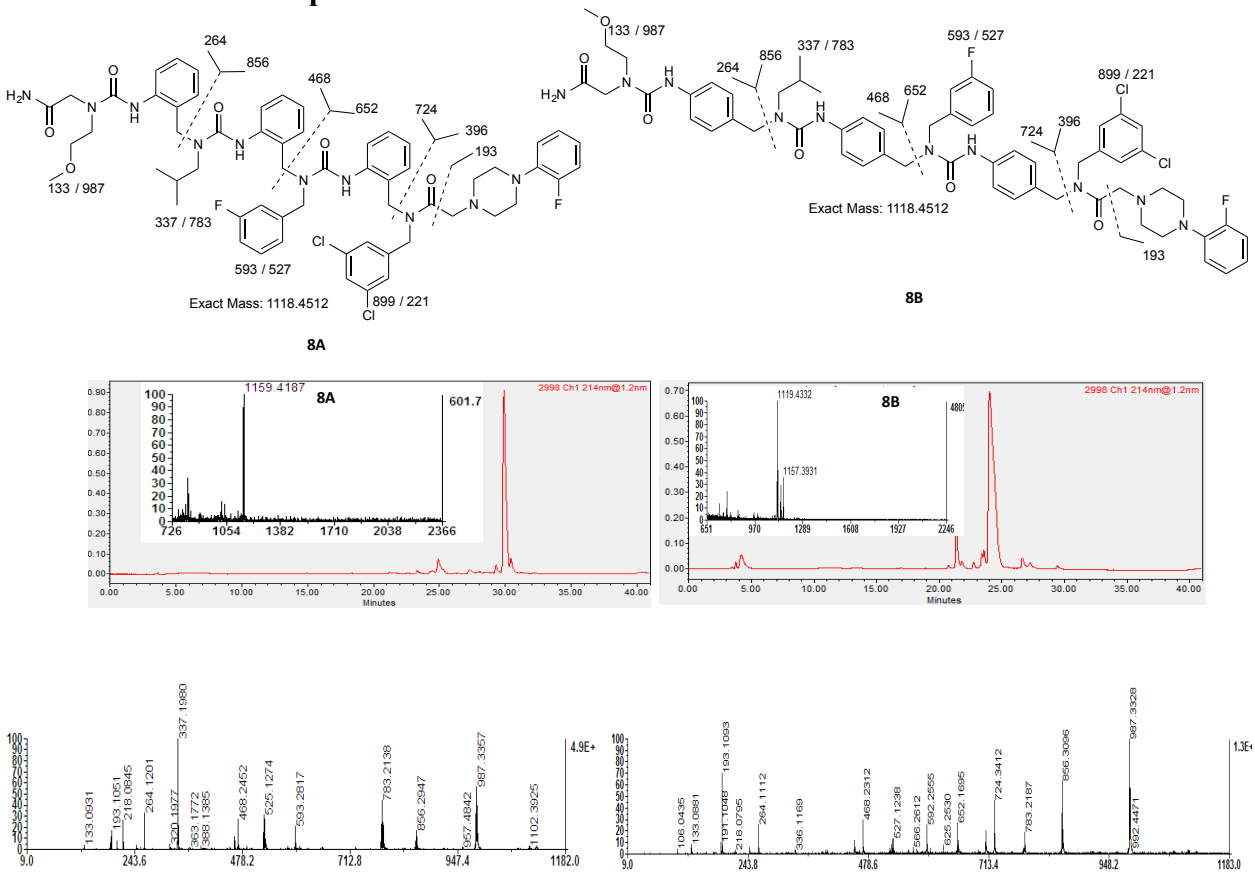
6.2 Di-ureido compound





S16: Comparative HPLC chromatograms (middle) of mono-ureido isobars (top, m/z=812.41), and their fragmentation pattern (bottom).).

6.3 Tri-ureido compound



S17: Comparative HPLC chromatograms (middle) of tri-ureido isobars (top, m/z=1118.45), and their fragmentation pattern (bottom).

Library 1:

Tentagel MB NH_2 (0.5 g, initial loading 0.4 mmol/g) was soaked in DMF for 30 min, RT. The beads were coupled with fmoc-methionine (5X) activated with N, N, N′, N′-Tetramethyl-O-(1Hbenzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (5X), N-Hydroxybenzotriazole (HOBt) (8X) and Diisopropyl ethylamine (DIEA) (10x) in DMF. Mixture was incubated 3 h at room temperature. Fmoc-group was deprotected using 20% piperidine in DMF. Resultant primary amine was treated with BAA/DIC and the bromide was displaced with furfurylamine under microwave condition. The resins were split and incubated with BAA/DIC. The bromides were separately displaced using nine different amines. Resins were pooled and further split into two different portions. Each portion was separately incubated with isobaric isocyanates (2M DMF) at room temperature to install arylureido backbone. The resins pooled and split into nine portions. Each portion were separately treated with different primary amines at 50°C for 30 mins to obtain secondary amine. Finally, after another round of split and pool, the resins was treated with BAA/DIC, followed by N-substituted piperazine, as shown in the following figure Library-1.

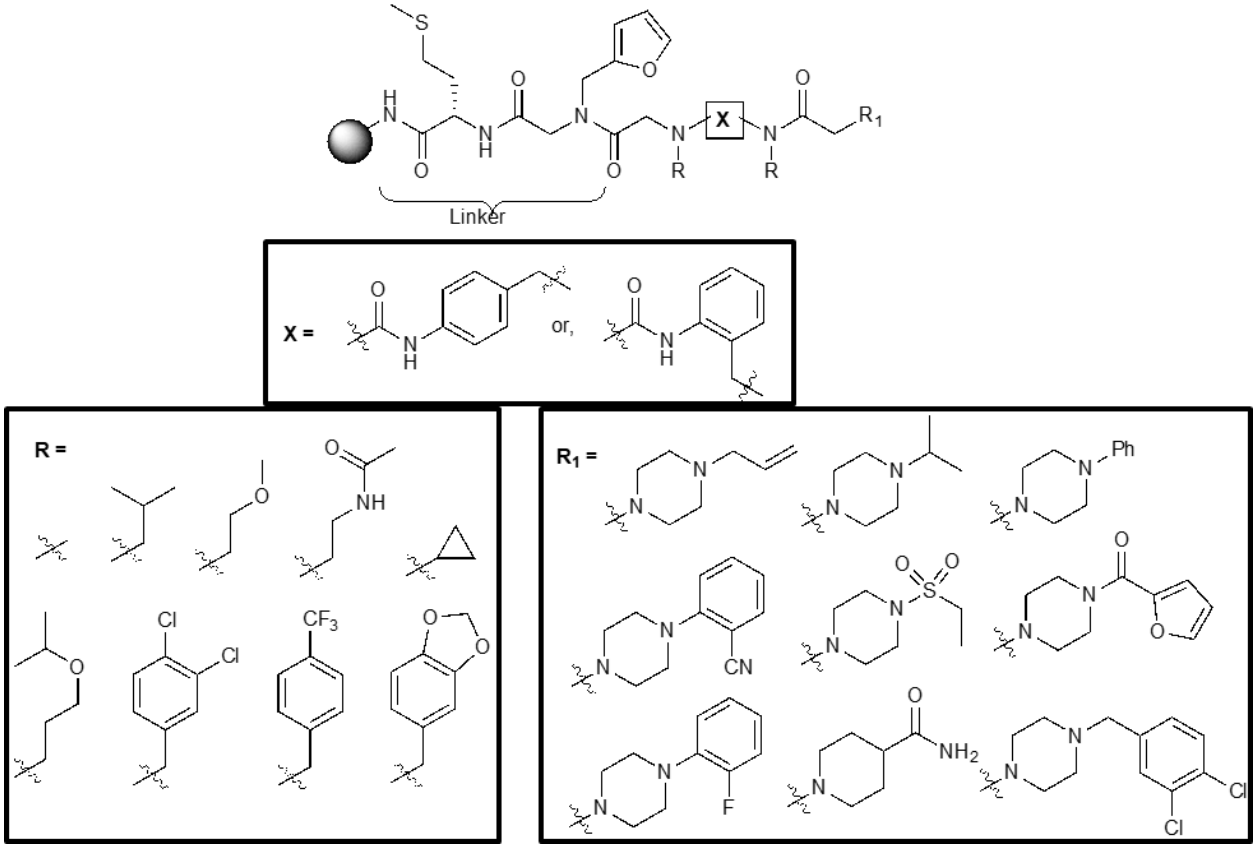
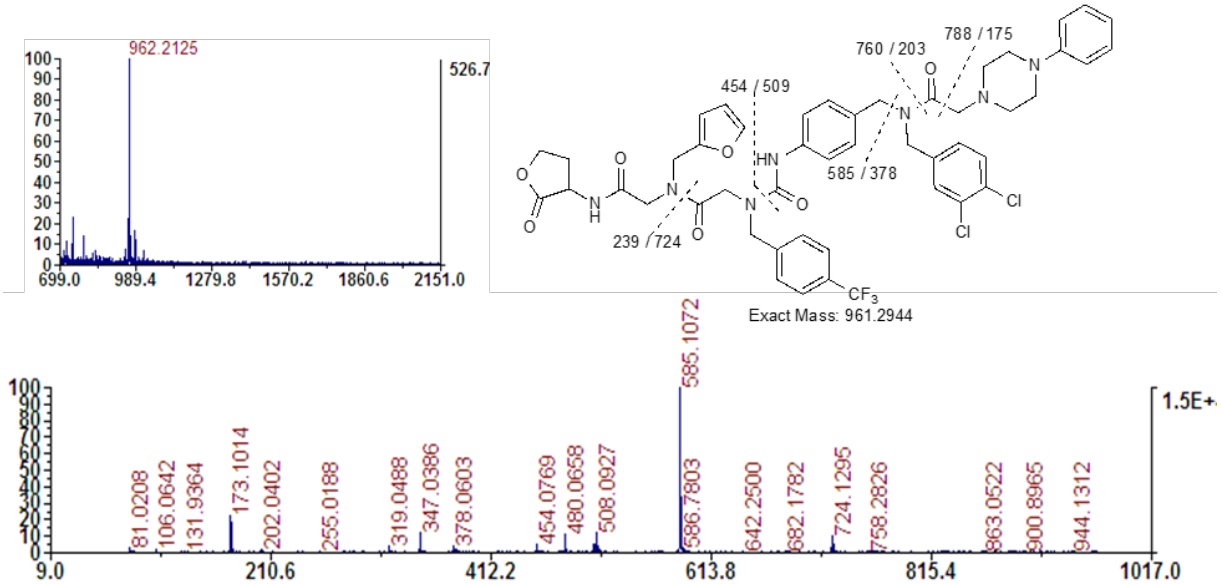
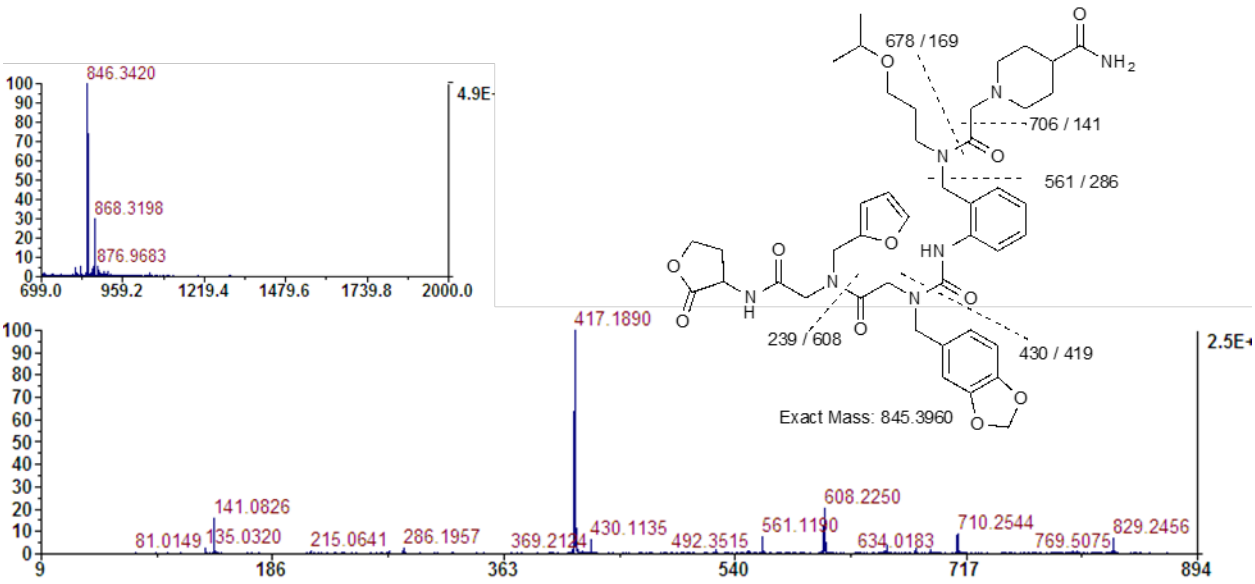


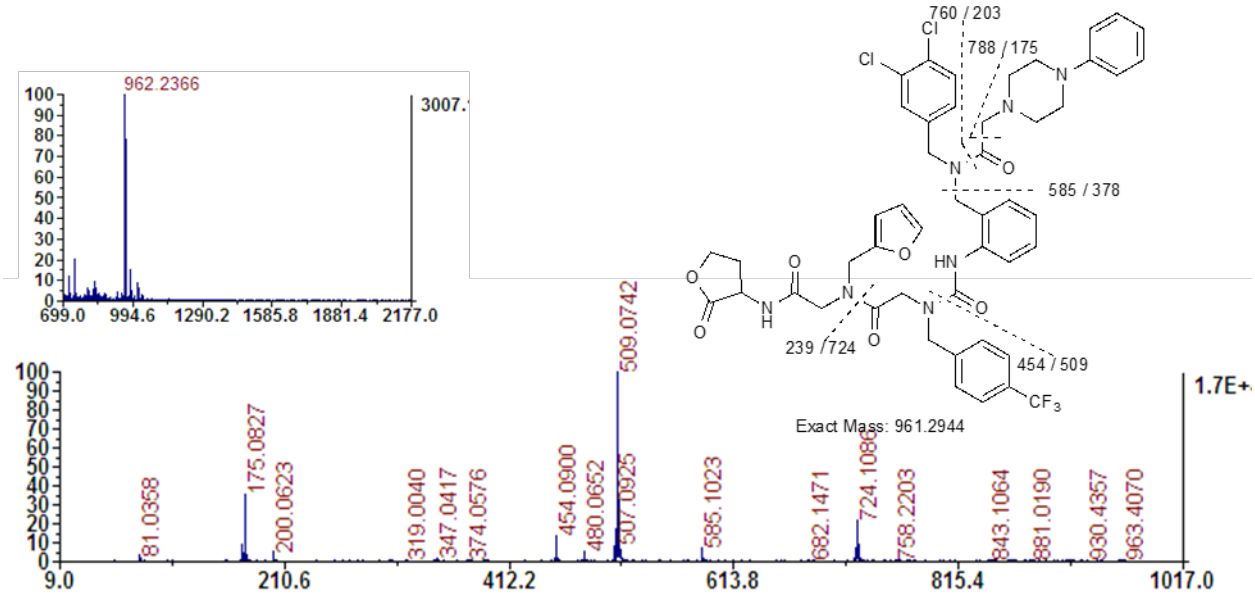
Figure showing design of **Library 1**.



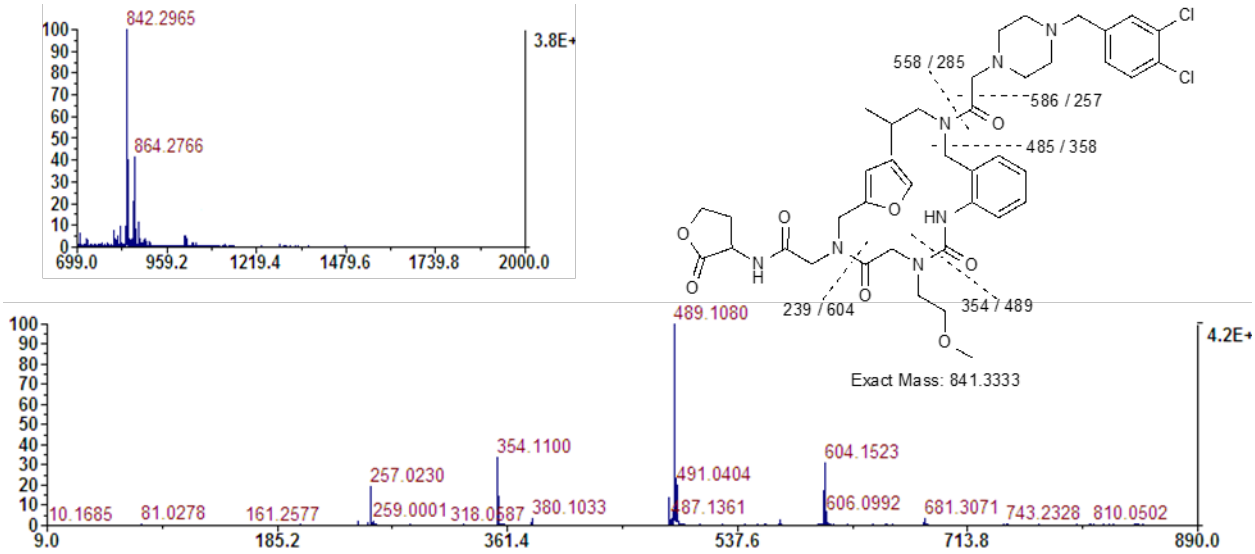
S18: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



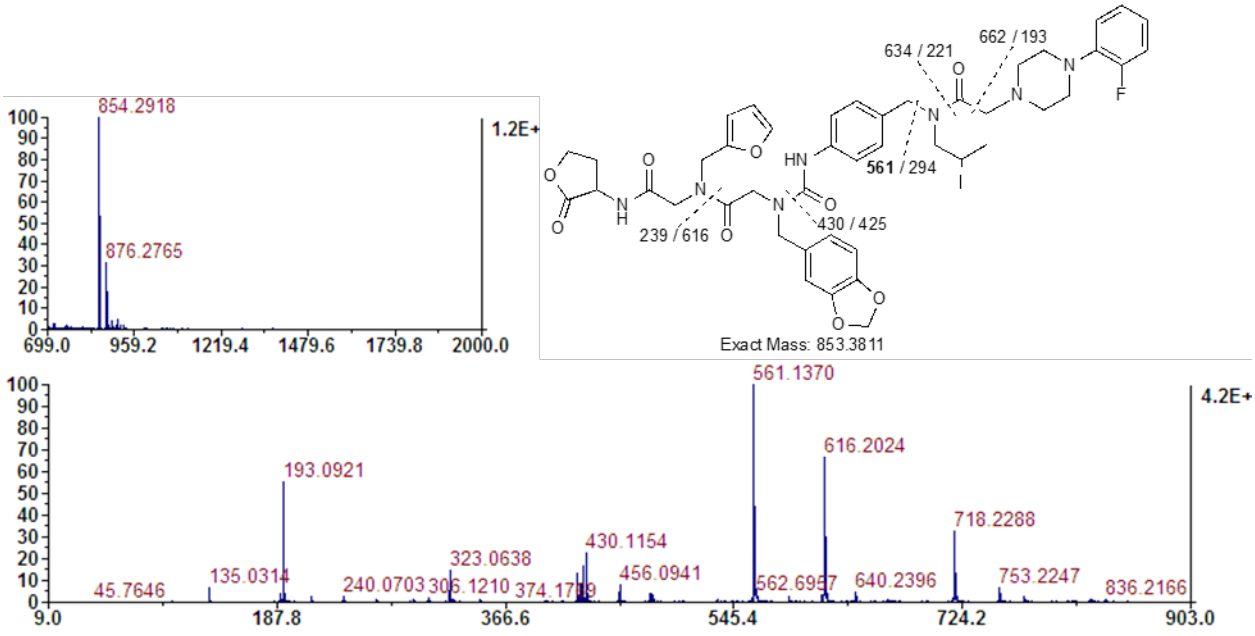
S19: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



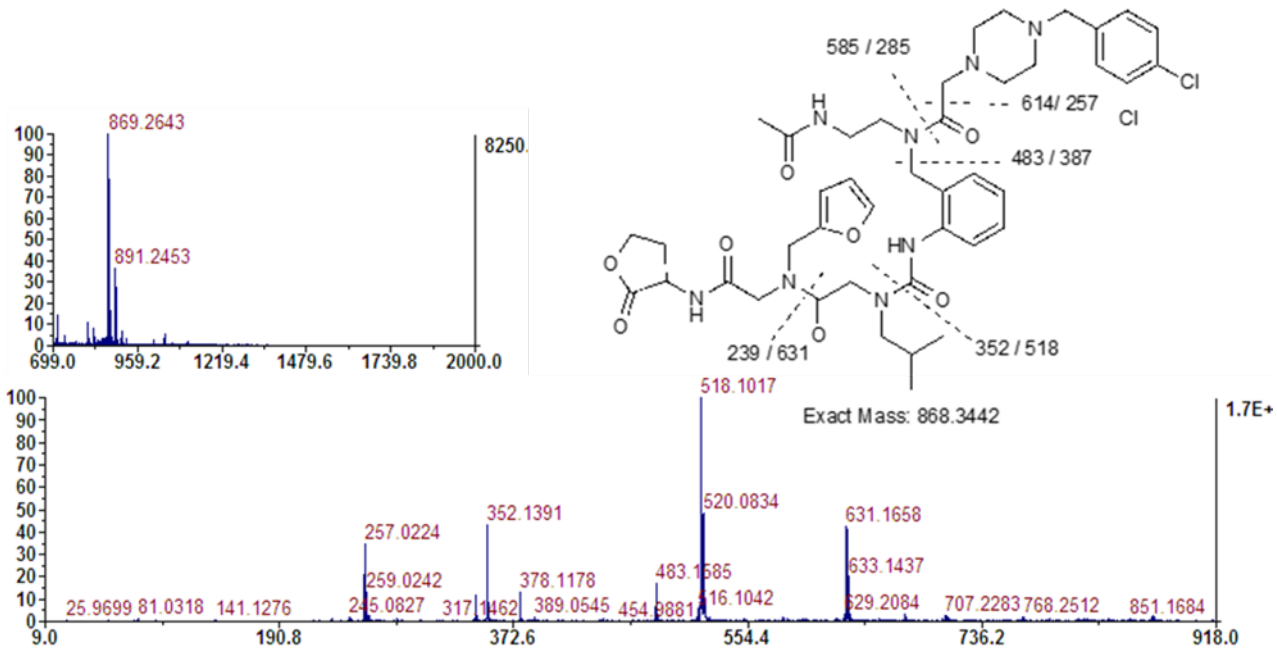
S20: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



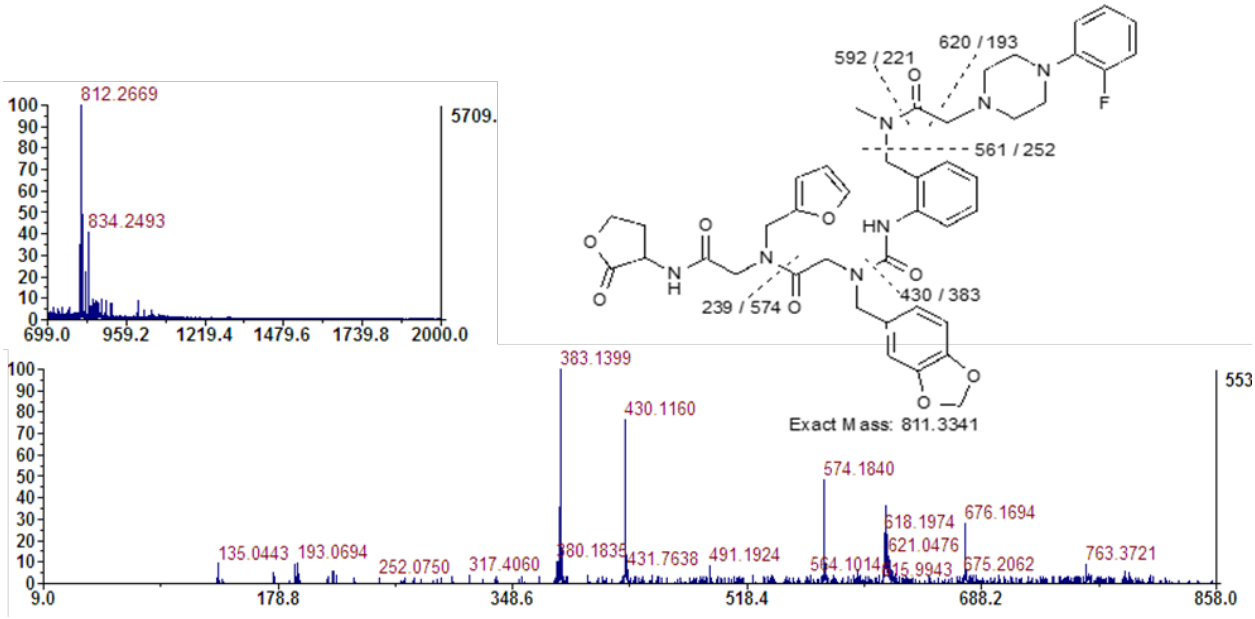
S21: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



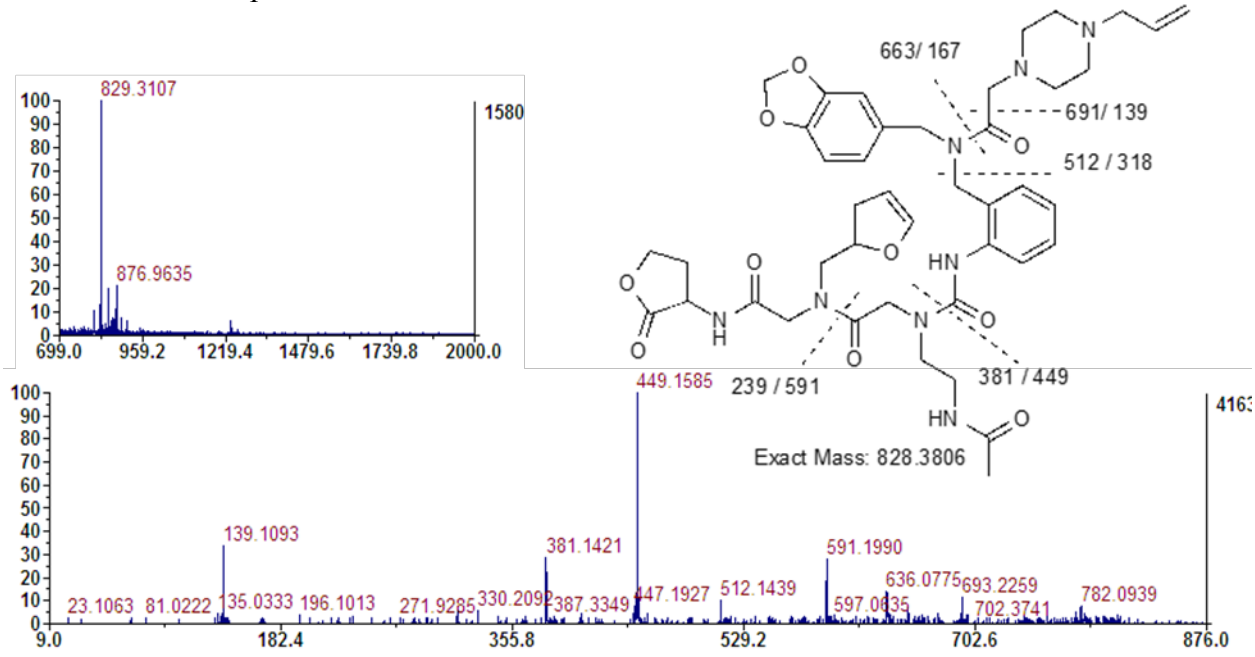
S22: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



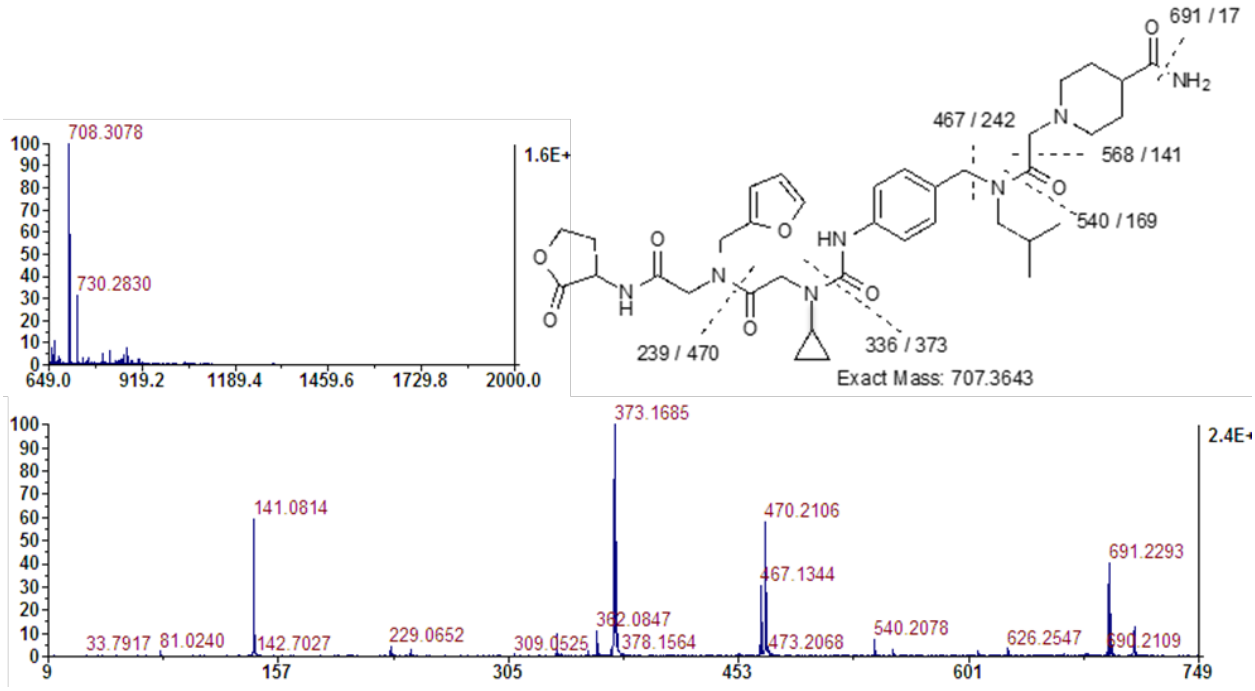
S23: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



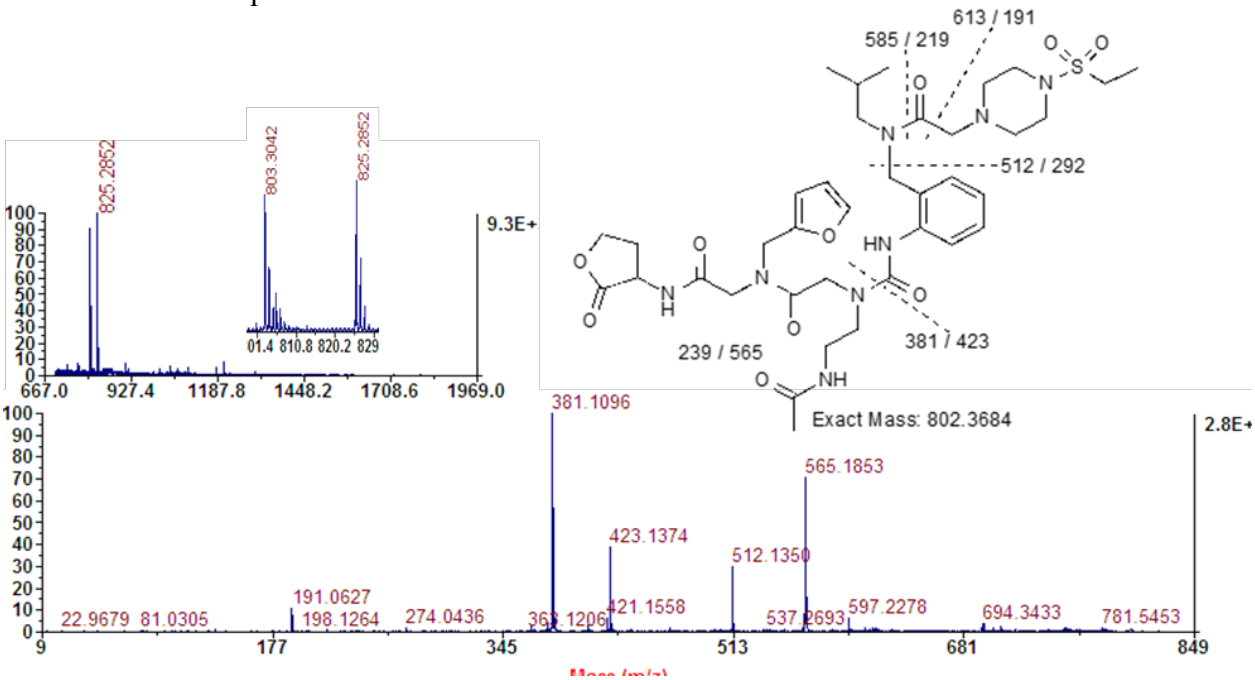
S24: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



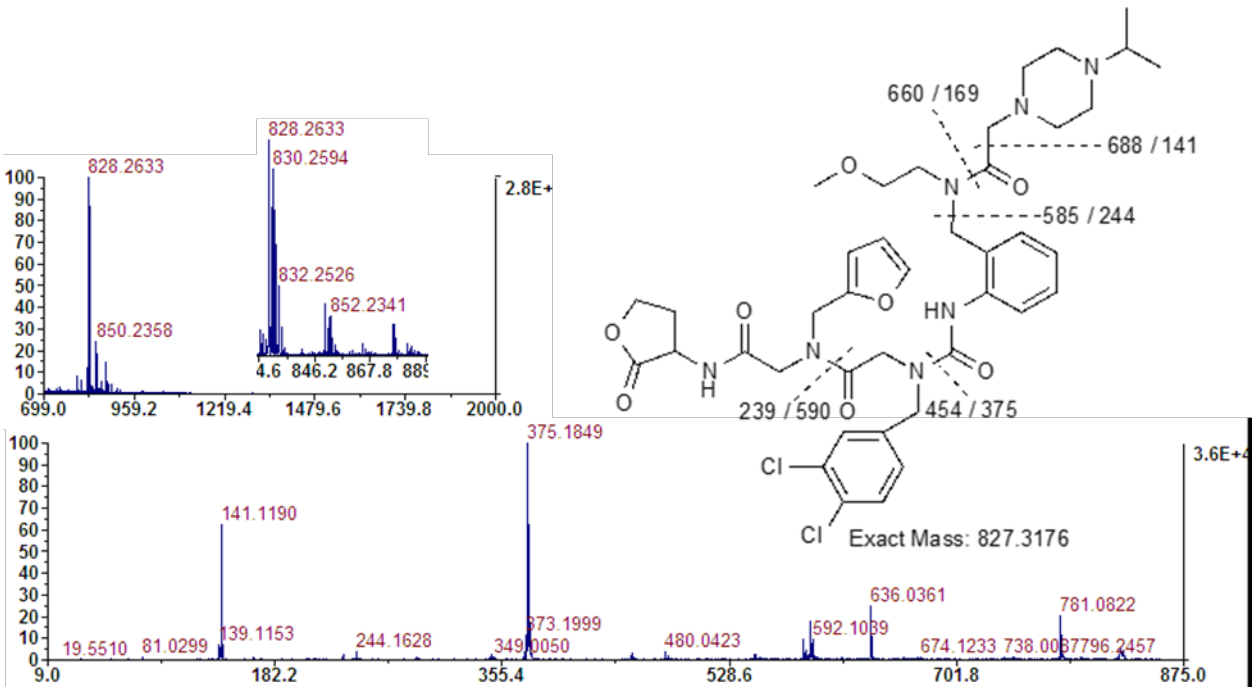
S25: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



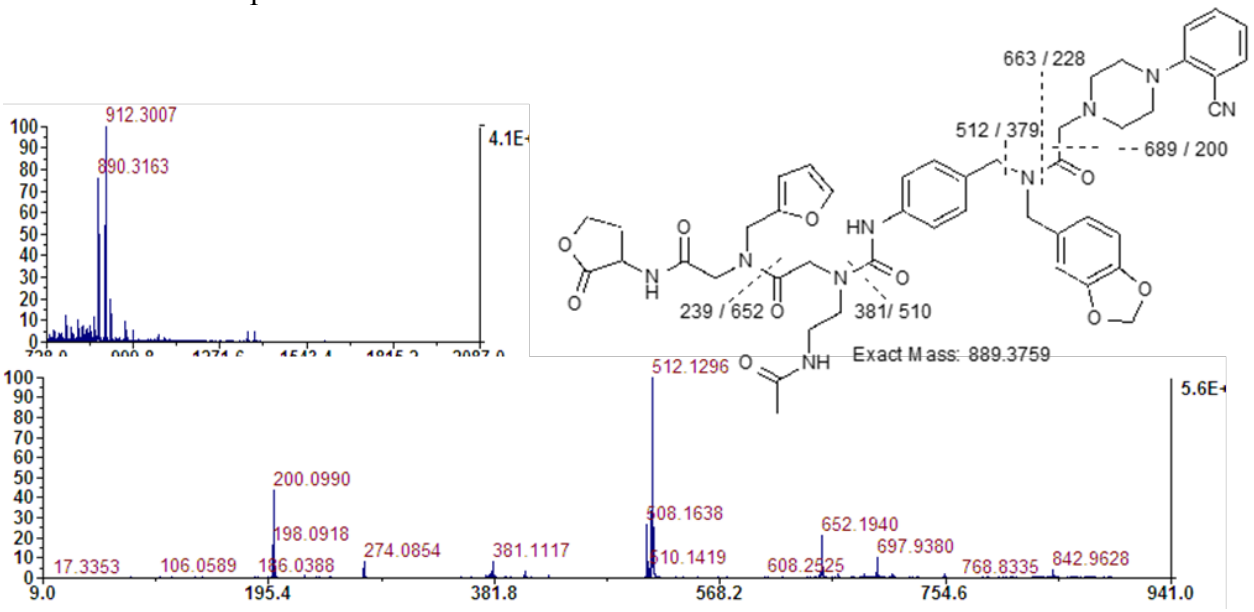
S26: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



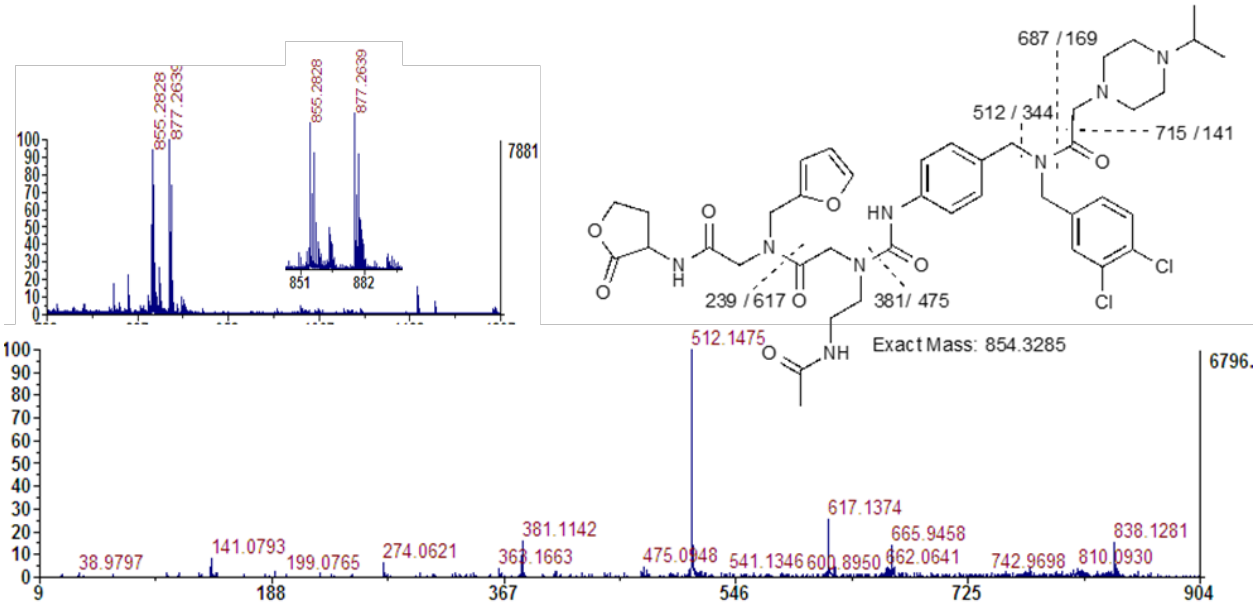
S27: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



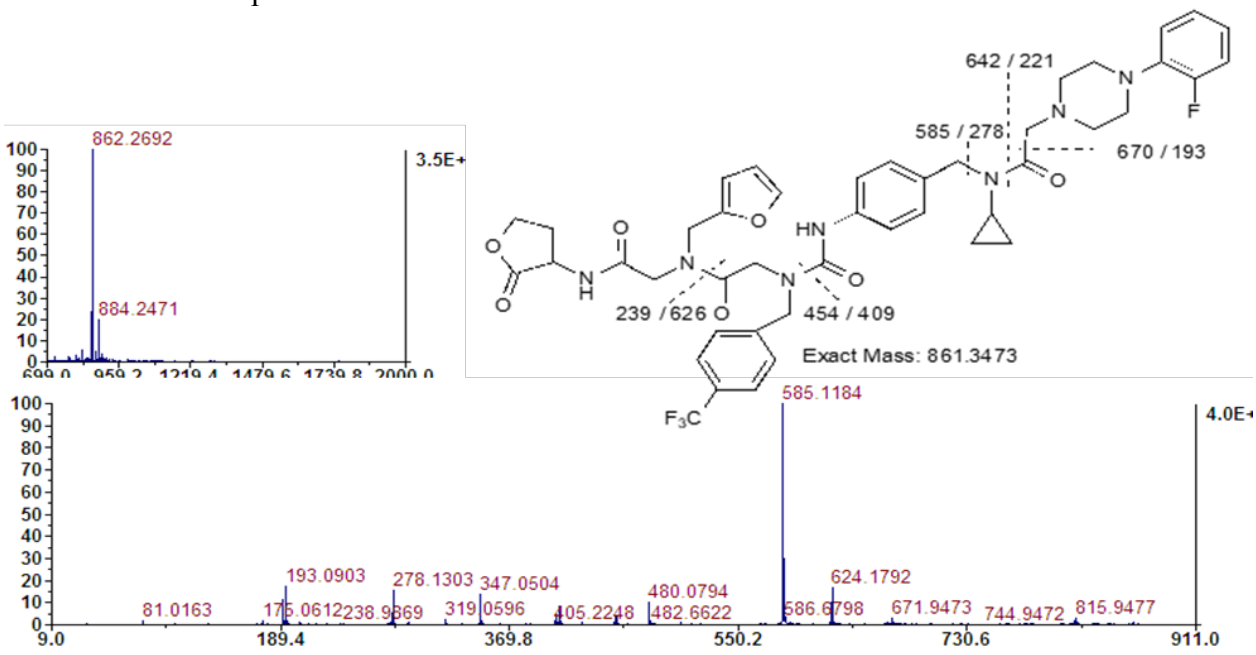
S28: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



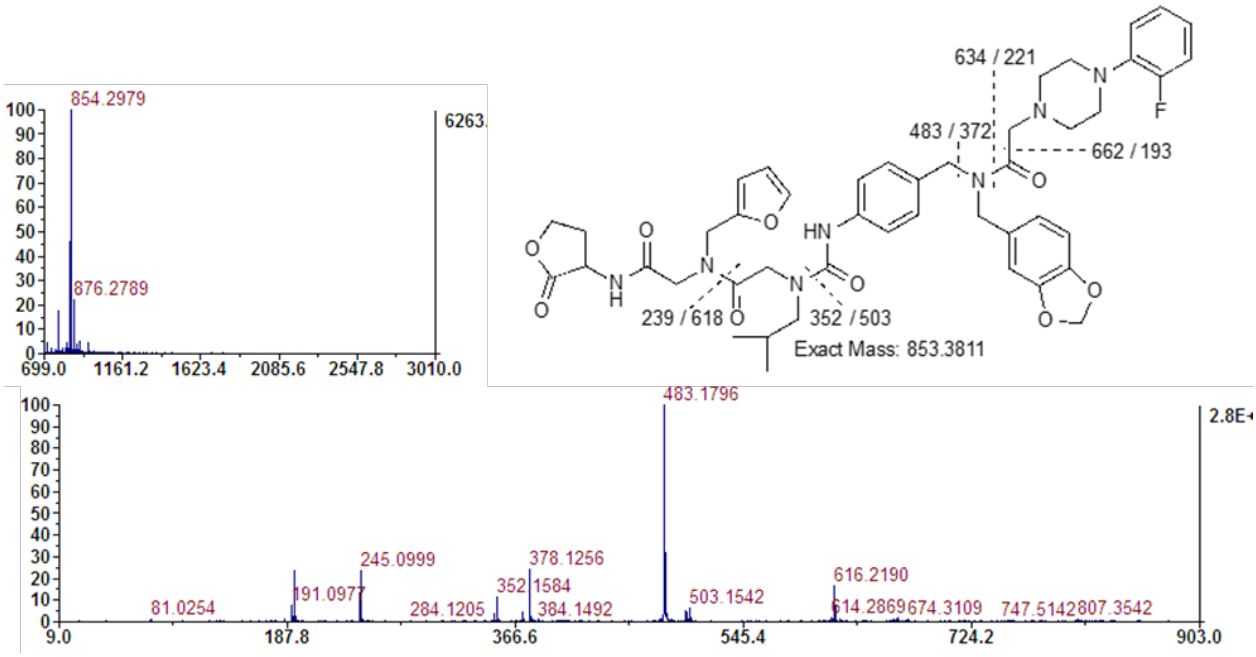
S29: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



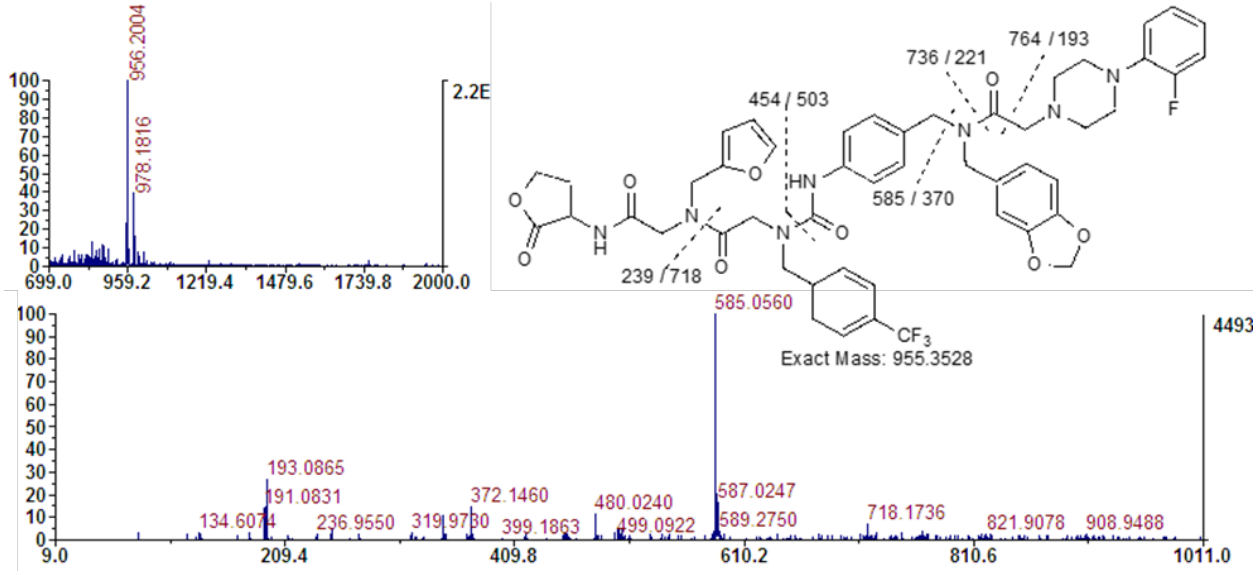
S30: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



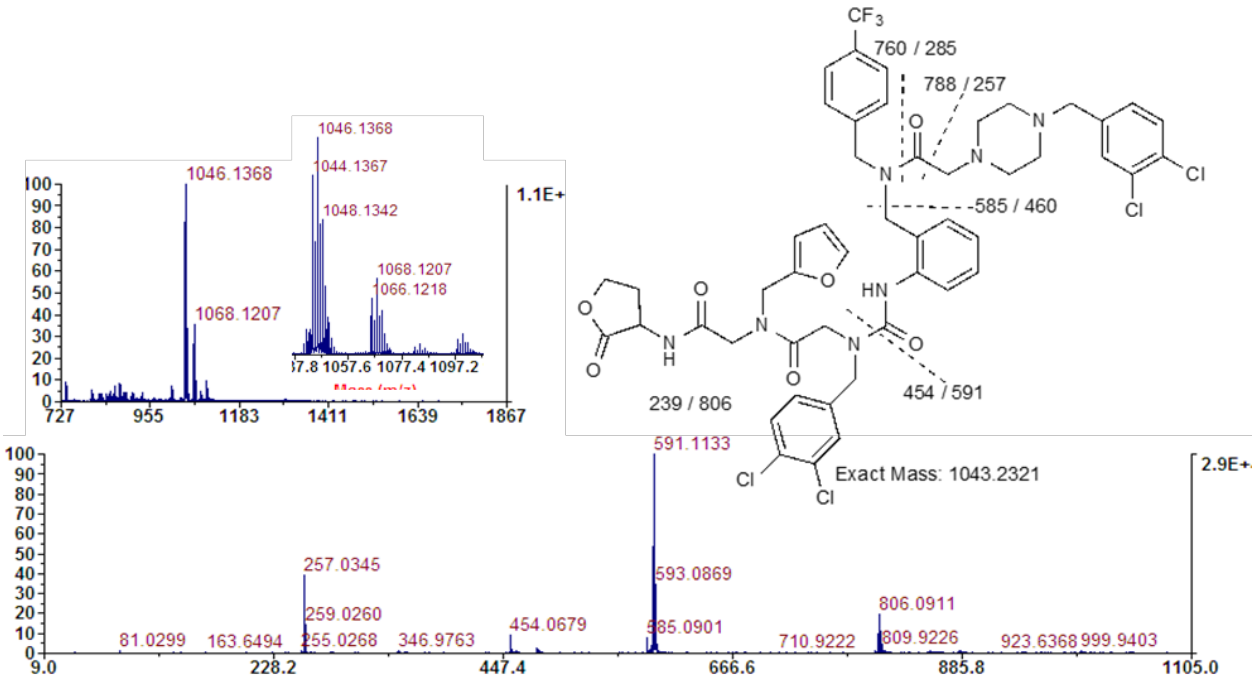
S31: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



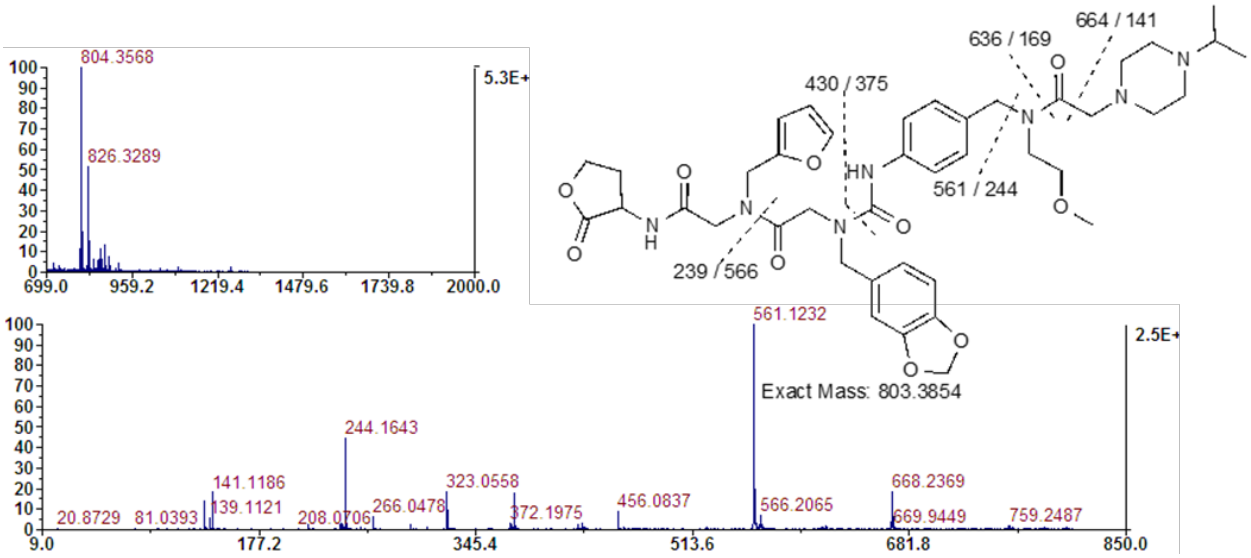
S32: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



S33: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.

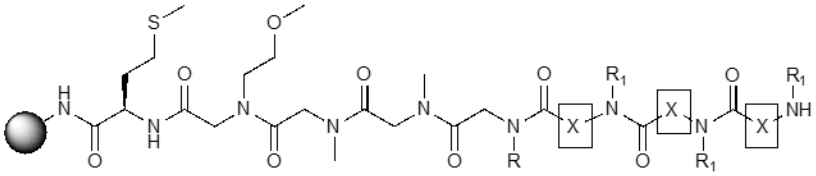


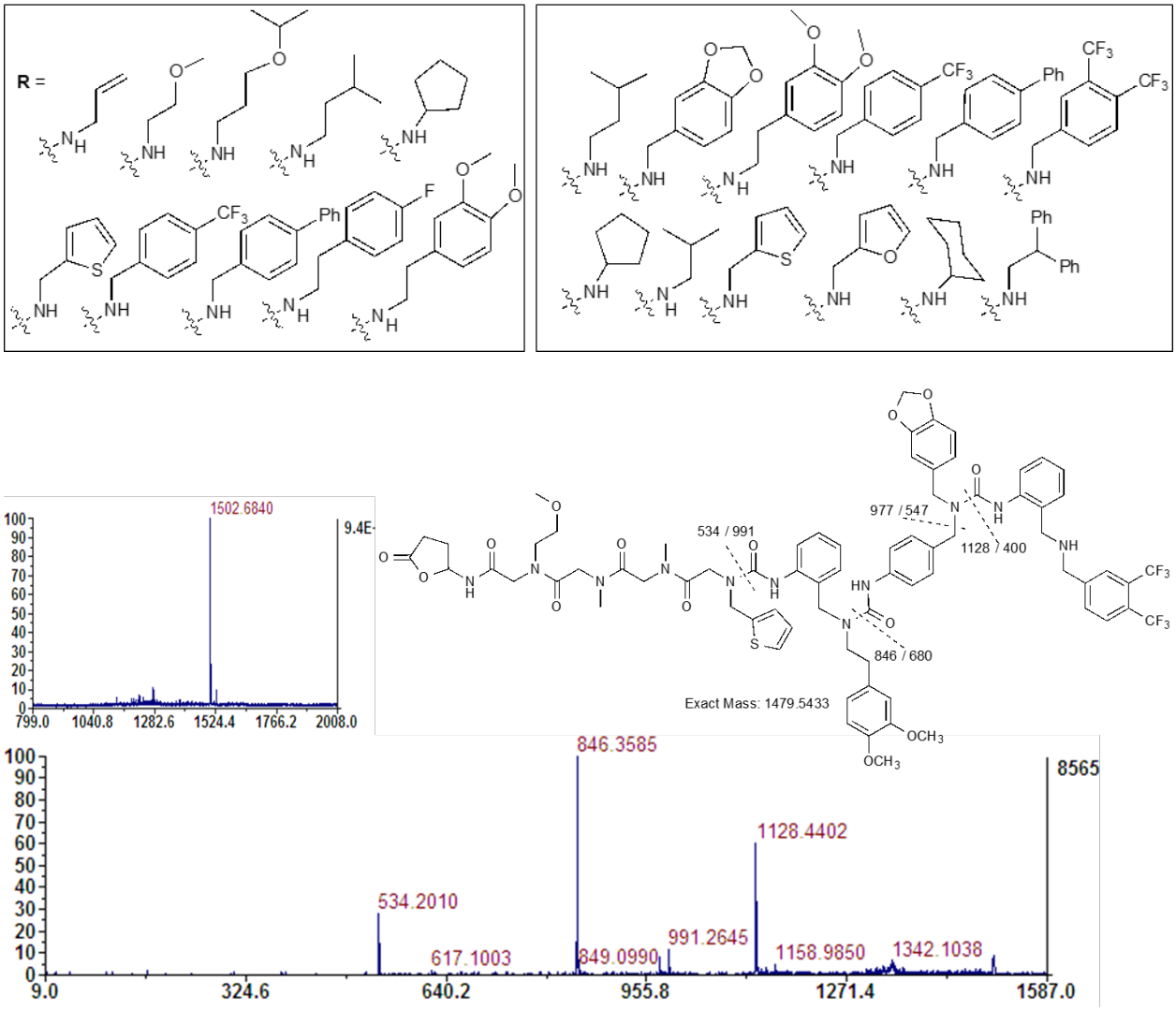
S34: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



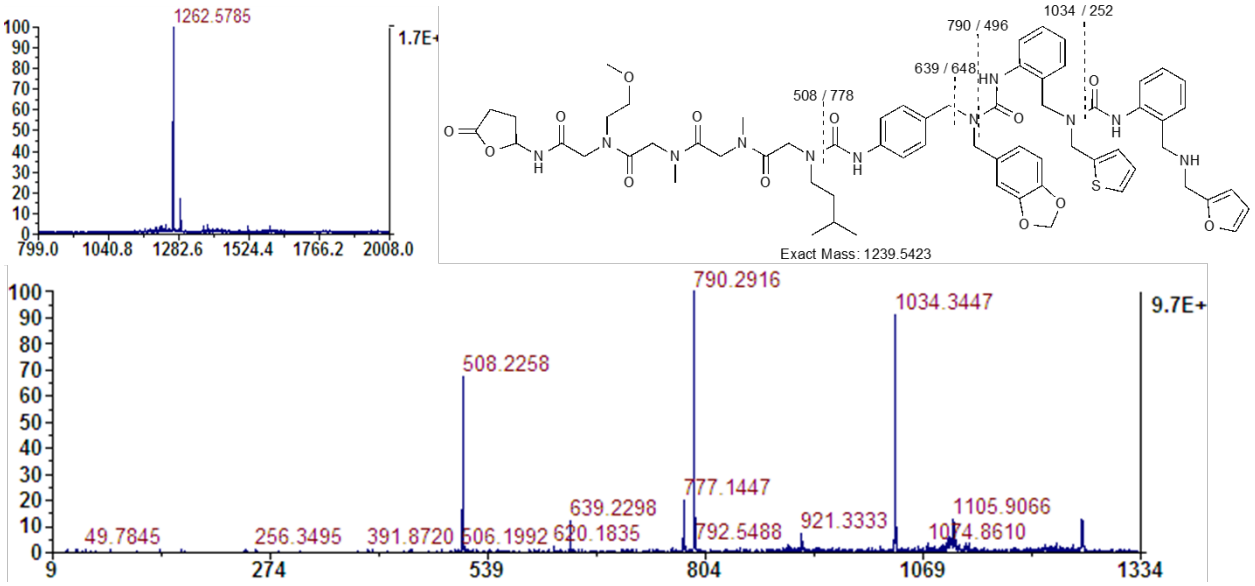
S35: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.

Library 2:
For synthetic protocol: Refer to Library 1.

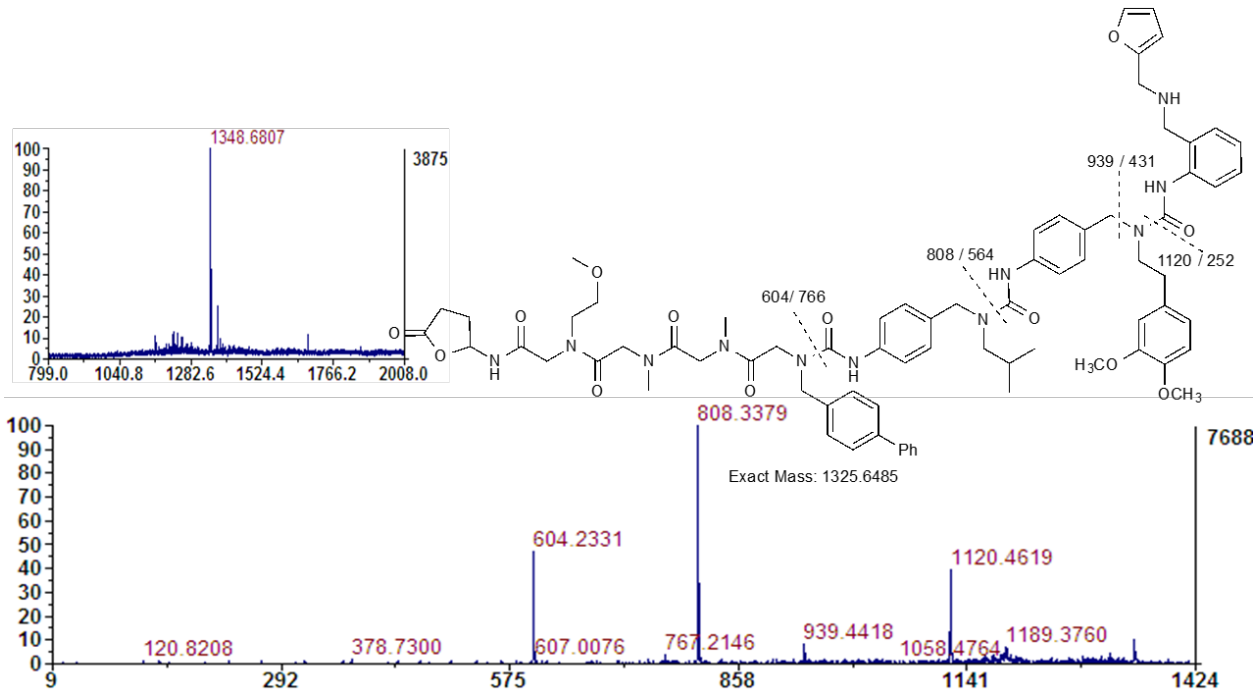




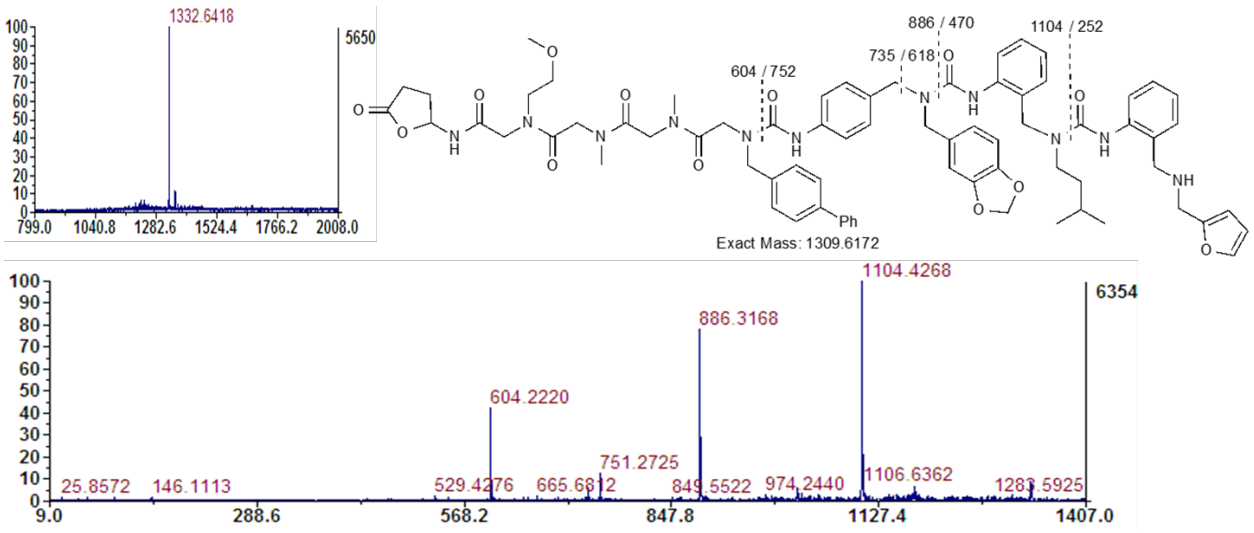
S36: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



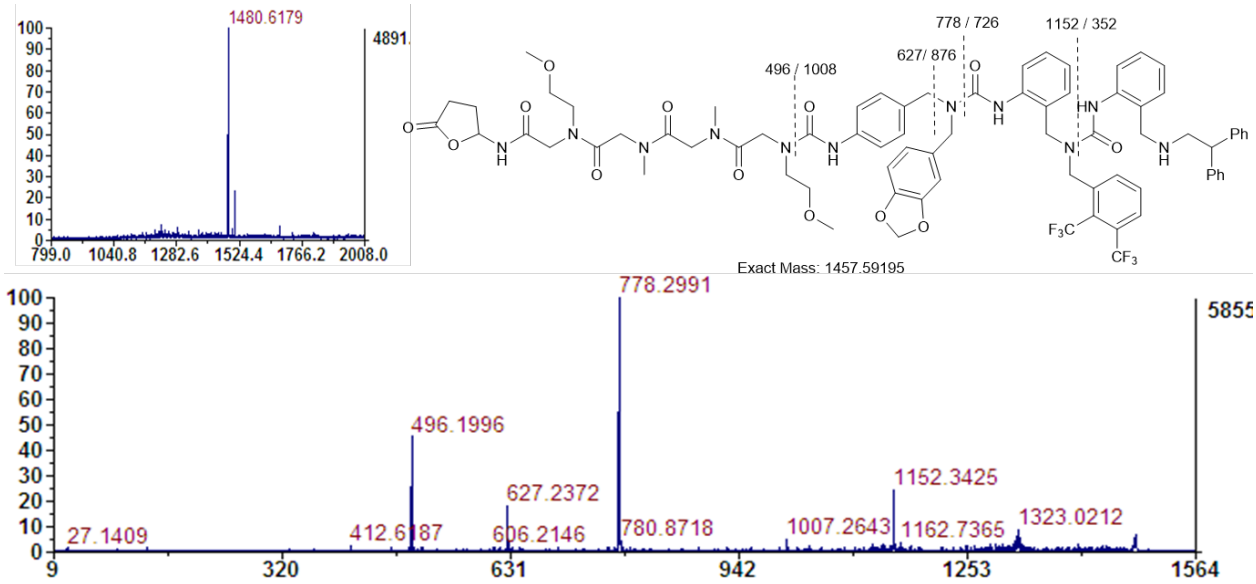
S37: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



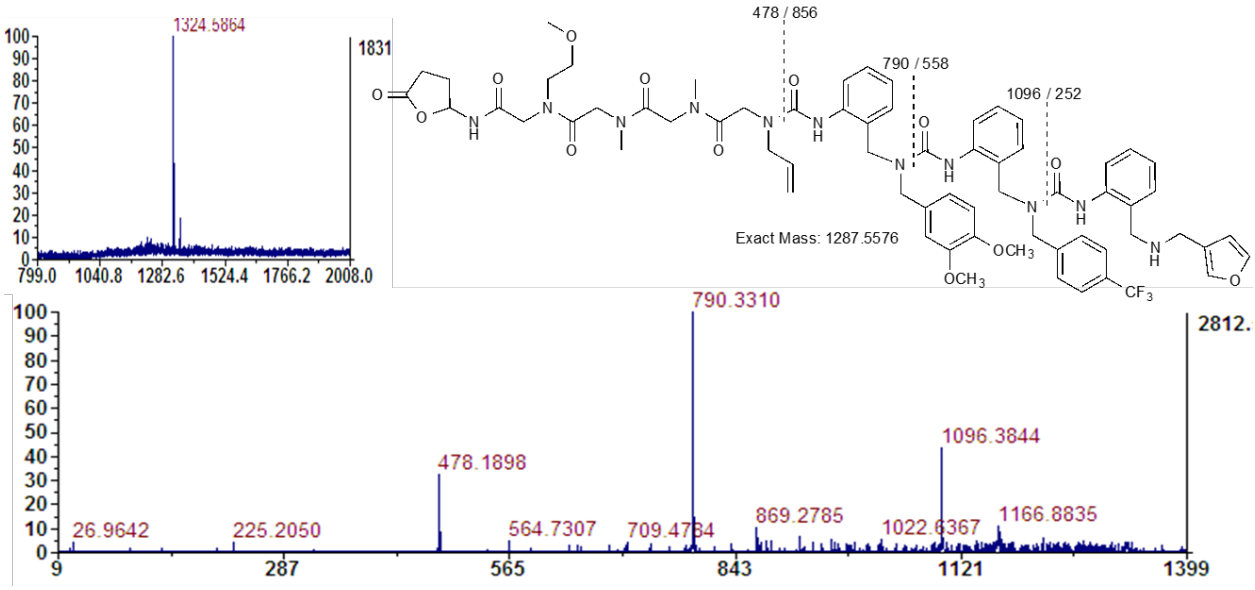
S38: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



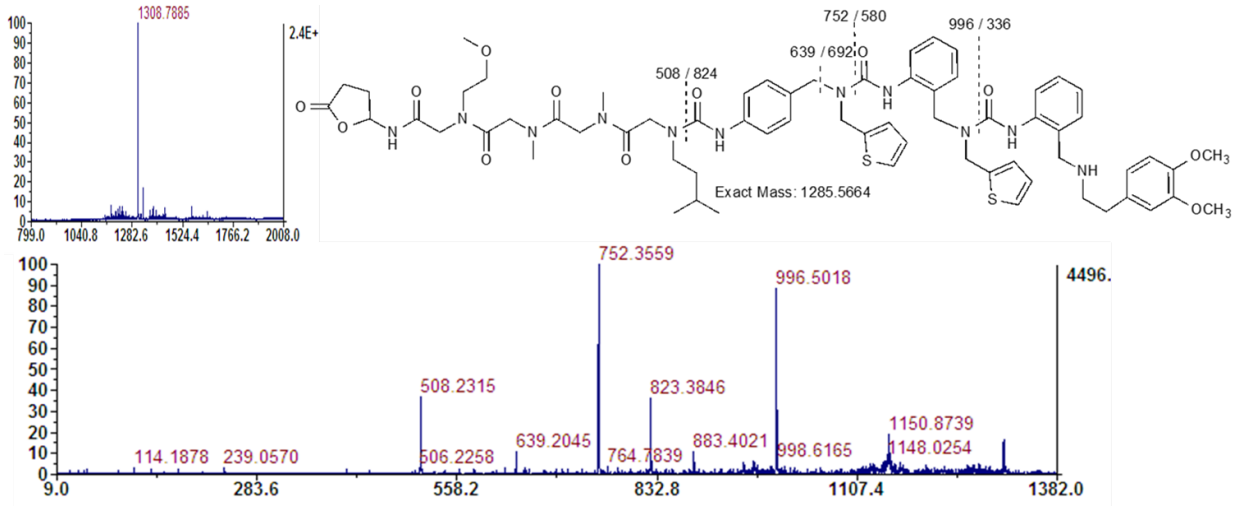
S39: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



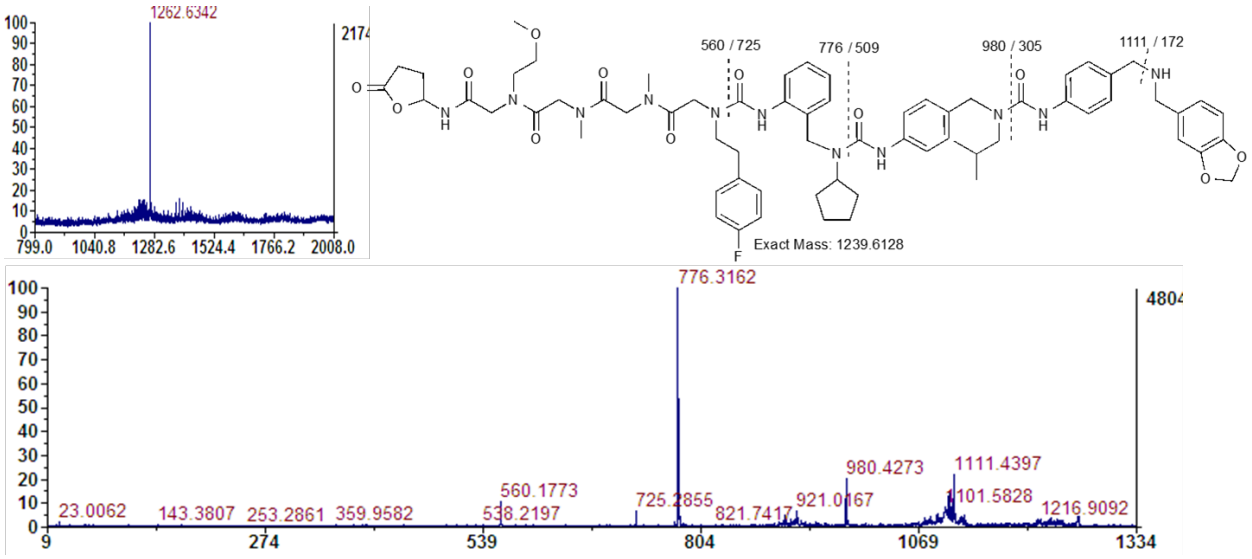
S40: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



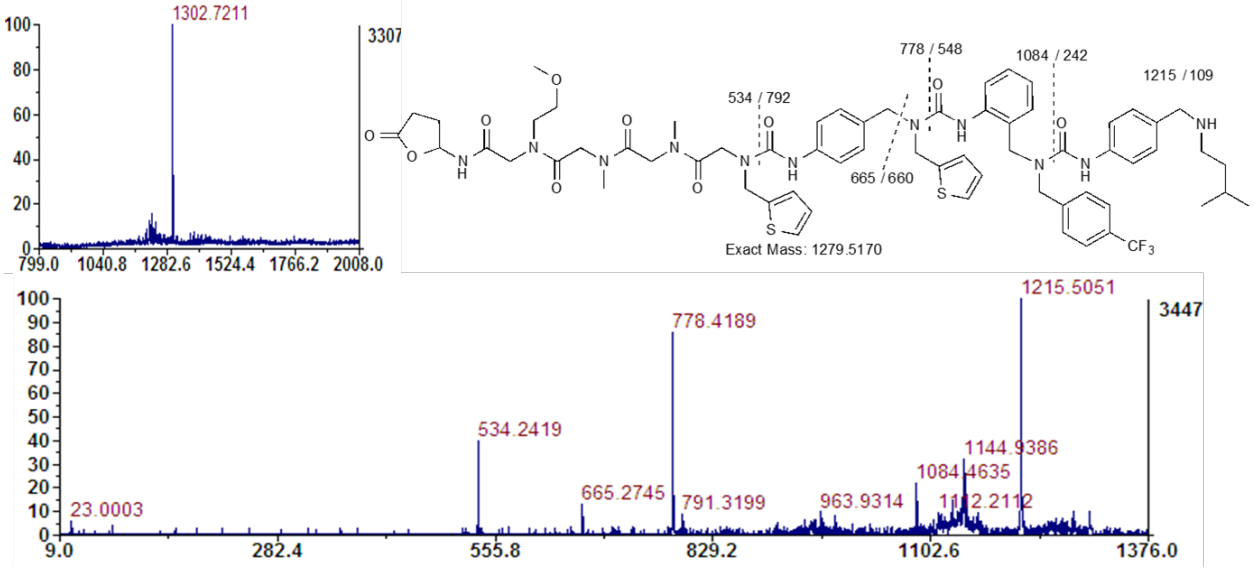
S41: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



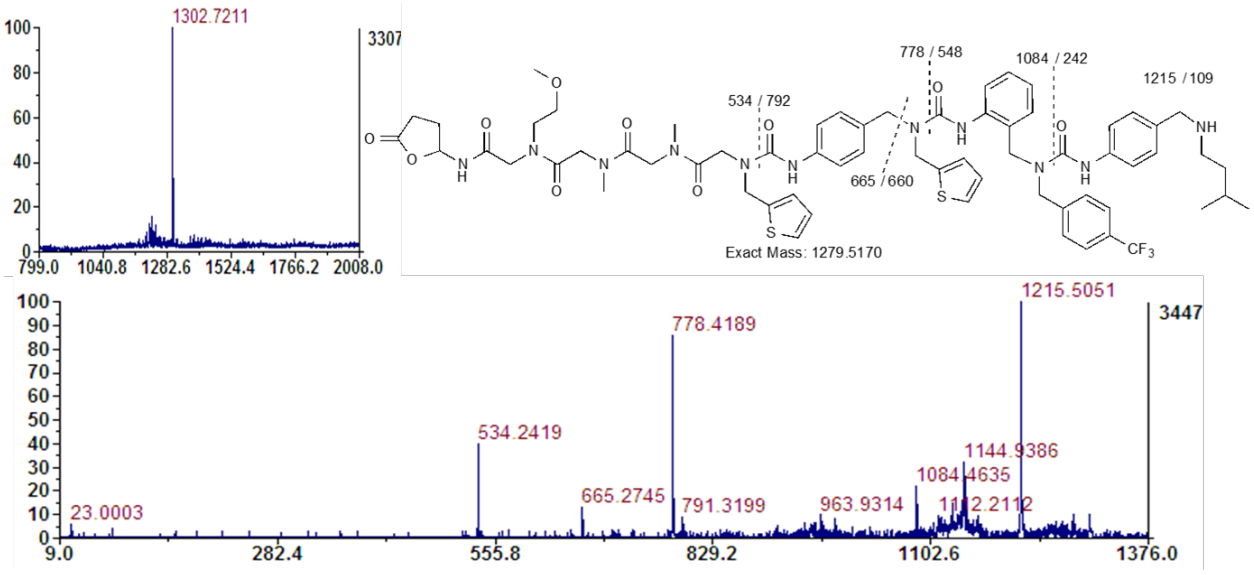
S42: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



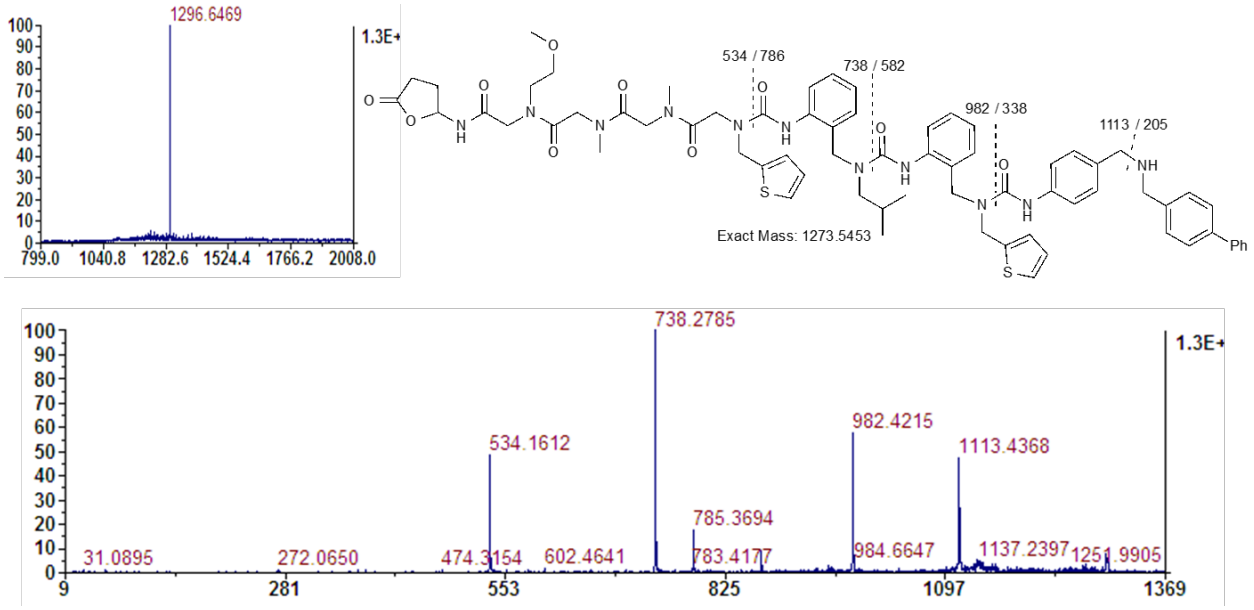
S43: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



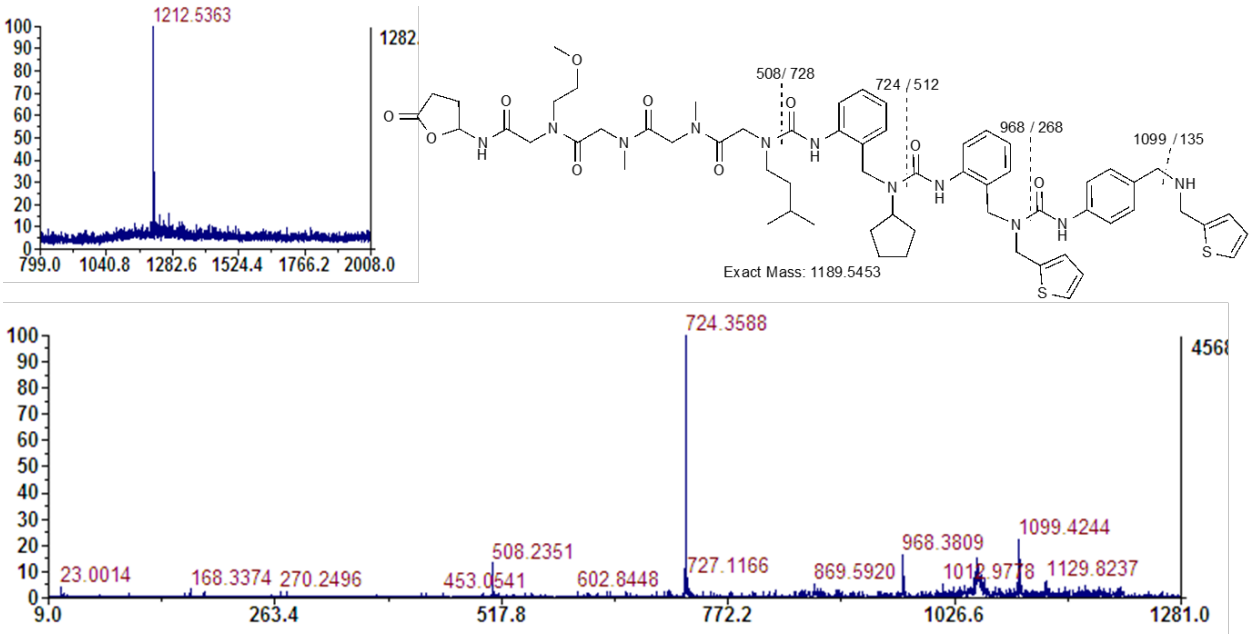
S44: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



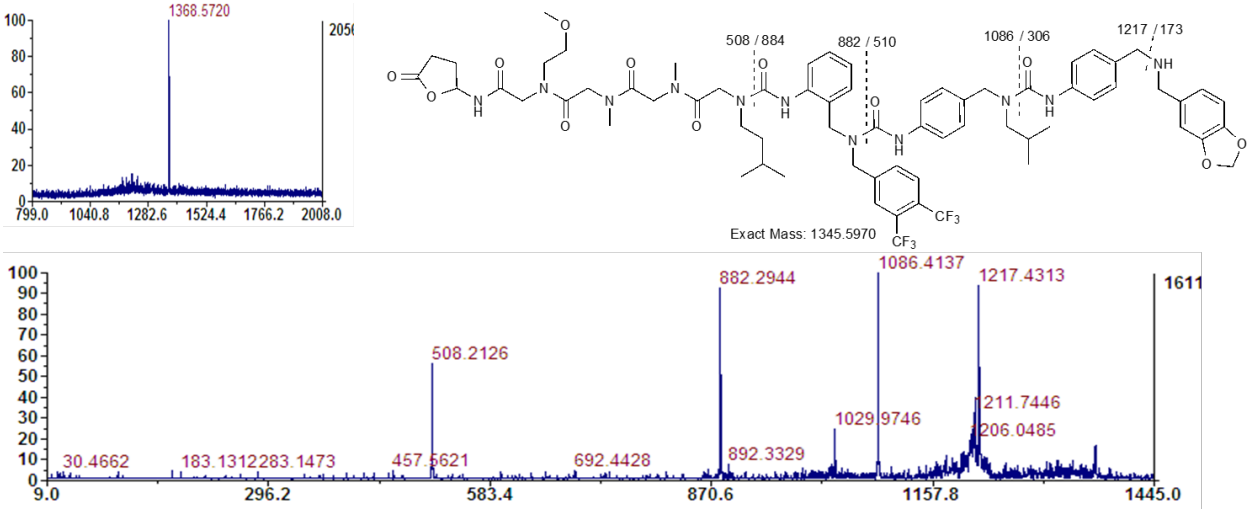
S45: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



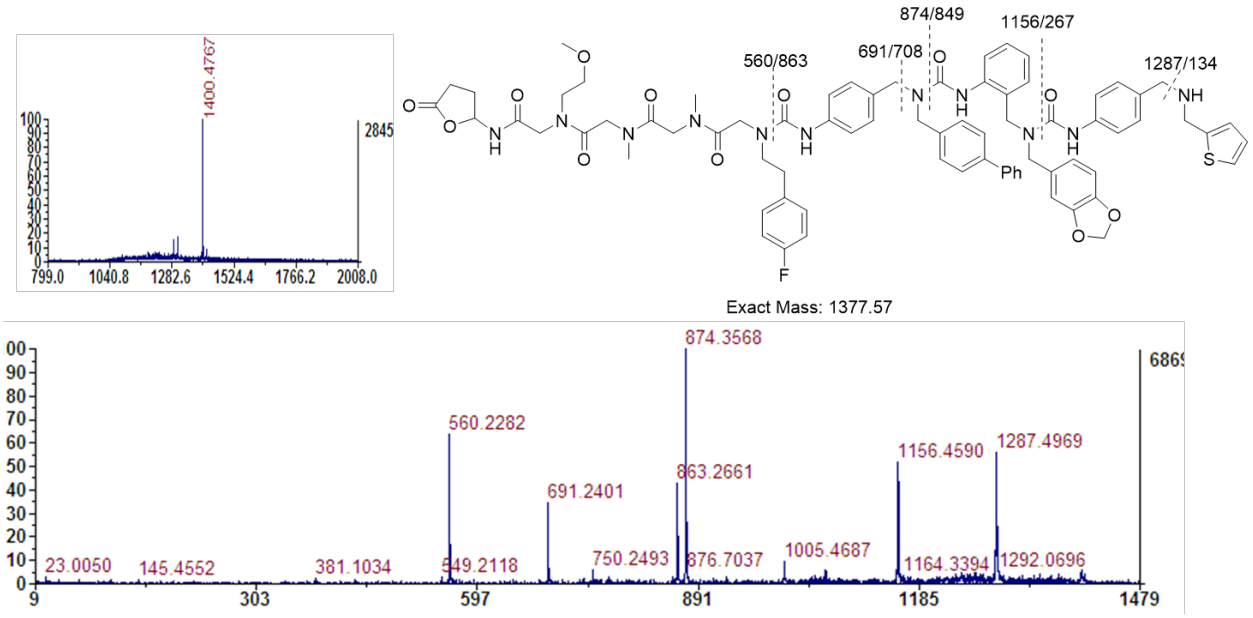
S46: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



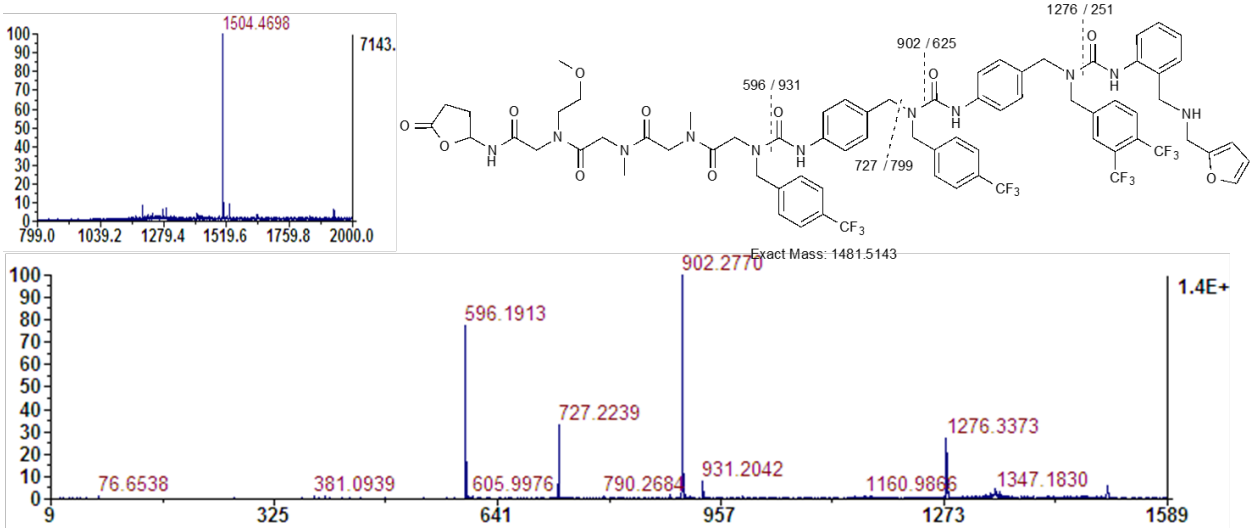
S47: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



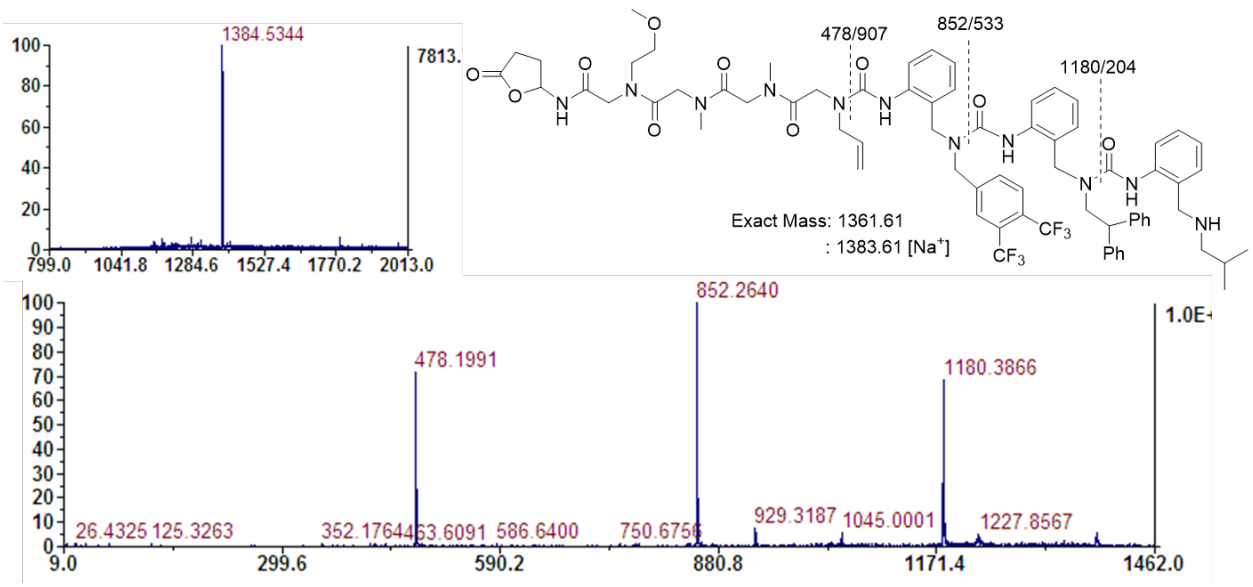
S48: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



S49: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



S50: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.



S51: TOP: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-MS/MS of the compound.