Electronic Supplementary Information

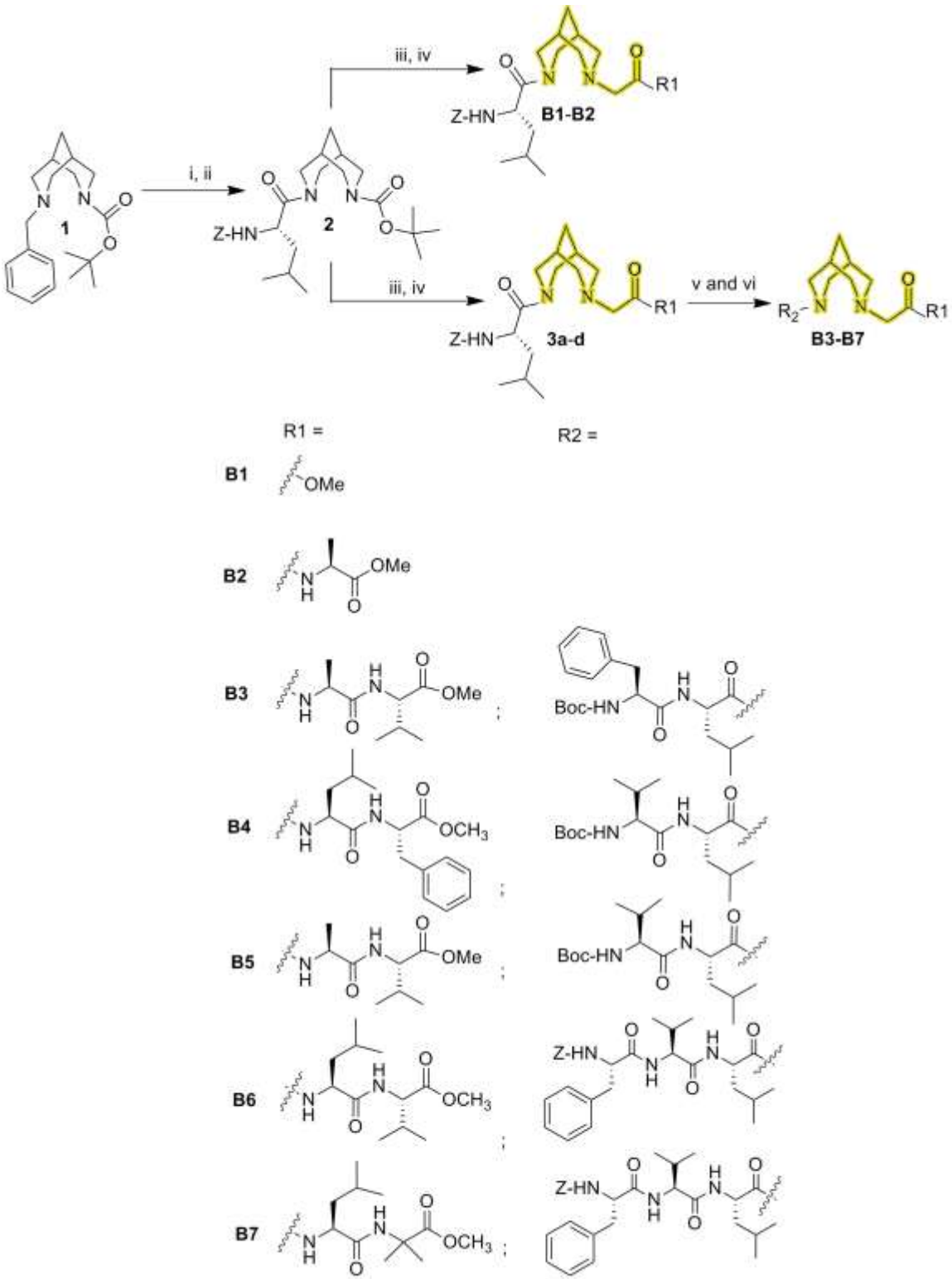
Bispidine as a helix inducing scaffold: Examples of helically folded linear peptides

V. Haridas,* Sandhya Sadanandan, M. V. S Gopalakrishna, M. B. Bijesh, Ram. P. Verma, Srinivas Chinthalapalli, Ashutosh Shandilya
Department of Chemistry, Indian Institute of Technology Delhi, New Delhi 110016, India.

General Experimental Section	S2
Synthetic schemes	S3
Preparation of bispidine-based compounds	S4-S19
NMR and Mass spectral data	S20-S36
Table S1 for major to minor conformer ratio	S37
Conformational analysis of **B3**	S38-S41
Solution FT-IR data	S42
Molecular modeling studies	S43-S45
NMR analysis of **B1**-**B2**	S46
MD simulated structure of **B3**	S47
CD spectra of **B1**-**B7**	S48-S51
Conformational analysis of **B6**	S52-S55
X-ray structure analysis of **B3**	S56-S58
HPLC analysis of **B6**	S59
Reference	S60

General Experimental Section

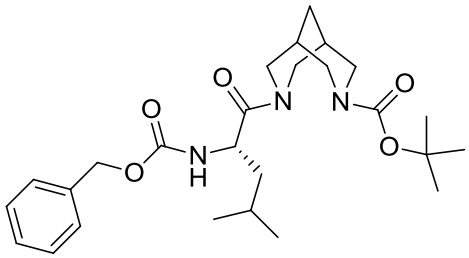
All amino acids used were of L-configuration. Unless otherwise stated, all reagents were used without further purification. All solvents employed in the reactions were distilled or dried from appropriate drying agent prior to use. Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100-200 mesh) columns, which were generally made from slurry in hexane, hexane/ethyl acetate or chloroform. Purification and Purity checking were done by analytical cum preparatory HPLC (Schimdazu-LC-6AD) using phenomenex C-18 column and acetonitrile/water as the solvent system. Melting points were recorded in a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured with a Rudolph Research Analytical Autopol® V Polarimeter; concentrations are given in grams/100mL. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets and also in chloroform. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Brucker-DPX-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer using tetramethylsilane (^{1}H) as an internal standard. Coupling constants are in Hz and the ^{1}H NMR data are reported as s (singlet), d (doublet), br (broad), br d (broad doublet), t (triplet), q (quartet), m (multiplet). HRMS were recorded with micrOTOF-Q II using ESI-technique. Circular Dichroism (CD) spectra were recorded on AVIV Model 410 spectrometer and Jasco-J815 spectropolarimeter equipped with a temperature controller using 1 mm length cell. The samples were prepared in acetonitrile, methanol, methanol/TFE with a concentration of 100-500μM. The spectra were recorded with a scan speed of 1nm/min and averaged over 3-5 scans. The baseline is substracted from the sample solutions. The melting experiments were carried out between 10-55^{o}C with an averaging time 30s and equillibrium time 2min. The CD data are reported as mean residual ellipticity (MRE). MRE was calculated using the equation $\theta/10 \times C \times l \times n$, where θ is the raw data in milli degree. C is concentration in moles/litre, l is path length of the cell in cm and n is the number of peptide bonds. MRE gives a value with units of degree cm^2 $dmol^{-1}$. The calculations were done with n =1 for **B1**, n = 2 for **B2**, n = 4 for **B3** and **B4**, n=5 for **B6** and **B7**. X-ray diffraction analysis was carried out on a BRUKER AXS SMART-APEX diffractometer with a CCD area detector (Mo Kα = 0.71073Å, monochromator: graphite). Frames were collected at T = 298 by ω,ϕ and 2θ-rotation at 20 s per frame with SMART. The measured intensities were reduced to F^2 and corrected for absorption with SADABS. Structure solution, refinement, and data output were carried out with the SHELXTL (version 6.12) package. Non-hydrogen atoms were refined anisotropically. C-H hydrogen atoms were placed in geometrically calculated positions by using a riding model. Image was created with the Diamond 3.2 program. The Amber v.10 package was used to prepare ligand files as well as for the Molecular Dynamics (MD) simulations. MD simulations were performed on a 320 processors SUN Microsystems clusters at Supercomputing Facility (SCFBio) at IIT Delhi.



(i) Pd/C, EtOH (ii) Z-Leu-OH, N-hydroxysuccinimide, DCC; (iii) 25% TFA in DCM; (iv) R1-Br, NEt_3; (v) Pd/C, H_2 , MeOH; vi) R2-OH, N-hydroxysuccinimide, DCC, NEt_3.

Scheme S1: Synthesis of bispidine anchored peptides

Preparation of Bisp (Boc) (Leu-Z) 2



To a solution of compound **1** (0.98g, 0.31mmol) in 15mL ethanol, added 0.125g of Pd(10 wt. %) on activated carbon. Purged hydrogen gas through the reaction mixture for 4h. TLC showed complete consumption of starting materials. Filtered the reaction mixture through the sintered funnel and evaporated the solvent under vacuum to yield 0.70g of the product Bisp NH (Boc) as pale yellow oil. The product was used as such for the next step without purification.

Yield: Quantitative.

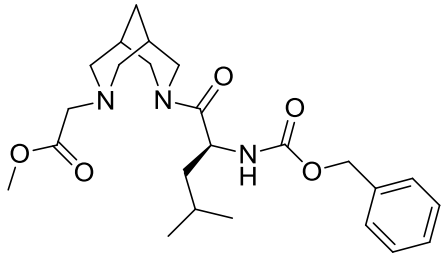
To an ice cold and well stirred solution of Z- Leu-OH (0.99g, 3.10mmol) in dry dichloromethane (60 mL), was added sequentially N-hydroxysuccinimide (0.356g , 3.10mmol), DCC (0.639g, 3.10mmol), Bisp NH (Boc) (0.70g, 3.10mmol) mixed with NEt_3(0.9mL, 6.20mmol), the reaction mixture was left stirred for 24h. The reaction mixture was filtered through the sintered funnel, the filtrate was diluted wash sequentially with 0.2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120 EtOAc: Hexane, 4:6), gave 1.10g of the pure product **2**.

Appearance: Colourless viscous liquid.

Yield: 75%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.83-1.01 (4ds, 6H, J=6.3Hz), 1.31 and 1.36 (s, 9H), 1.77-1.98 (m, 5H), 2.63 (br s, 1H), 2.79-2.96 (m, 3H), 3.22 (d, 1H, J=11.1Hz), 3.37 (d, 1H, J=12Hz), 3.80 (d, 1H, J=12Hz), 3.96 (d, 1H, J=12.6Hz), 4.14 (m, 1H), 4.40 (d, 1H, J=13.5Hz), 4.57-4.69 (m, 1H), 5.03 and 5.08 (s, 2H), 5.57 and 5.95 (d, 1H, J=8.1Hz), 7.29 (s, 5H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 23.3, 24.7, 27.3, 27.7, 27.9, 28.1, 28.4, 42.7, 46.7, 49.2, 66.6, 79.6, 127.8,

128.1, 128.4, 136.4, 154.6, 156.3, 170.5, 172.6. IR (KBr): 3408, 3288, 2926, 2863, 1816, 1785, 1690, 1638, 1506, 1433, 1361, 1315, 1241, 1172, 1131, 1048 cm^{-1}. HRMS: Calcd for $C_{26}H_{39}N_3NaO_5$ m/z 496.2782 found m/z 496.2778. $[\alpha]_D$ -33.64 (*c* 0.11, MeOH).

Preparation of Bisp (Gly-OMe) (Leu-Z) B1



To an ice cold and well stirred solution of **2** (0.80g, 1.69mmol) in dry dichloromethane (4mL), added trifluoroacetic acid (TFA) (1.94mL, 25.35mmol), the reaction mixture was stirred for 4h. The reaction mixture was subjected to vacuum to remove dichloromethane and TFA. The product was washed with saturated $NaHCO_3$ solution then extracted with ethylacetate. EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to yield 0.63g of the product as colourless sticky compound Bisp (Leu-Z) NH.

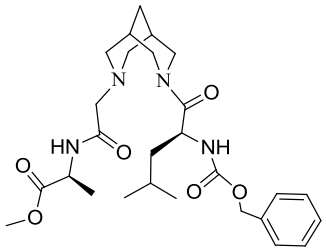
Yield: Quantitative.

To a well stirred solution of deprotected compound Bisp (Leu-Z) NH (0.48g, 1.29mmol) in dry acetonitrile (50mL), was added methyl 2-bromoacetate (0.25g, 1.65mmol) followed by addition of diisopropylethylamine (DIPEA) (0.2mL, 1.29mmol). The reaction mixture was left stirred for overnight. Reaction mixture was evaporated and dissolved in chloroform and washed sequentially with 2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120mesh, EtOAc:Hexane, 4:6), gave 0.38g of the pure product as yellow liquid compound **B1**.

Yield: 65%. ^1H NMR ($CDCl_3$, 300MHz): δ 0.90-1.05 (4ds, 6H, J=6.3Hz), 1.60-2.0 (m, 8H), 2.60-3.12 (m, 5H), 3.33 (d, 1H, J=13.2Hz), 3.60 and 3.65 (s+s, 3H), 3.85 (d, 1H, J=12.6Hz),

4.00 (d, 1H, J=13.2Hz), 4.71 (m, 2H), 5.11 (m, 2H), 5.95 (d, 1H, J=8.4Hz), 7.34 (s, 5H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 22.2, 24.5, 29.0, 31.6, 42.5, 46.8, 49.3, 49.9, 51.2, 56.9, 58.6, 66.6, 127.9, 128.0, 128.1, 128.3, 128.4, 136.5, 156.3, 170.5, 171.1. IR (KBr): 3297, 2951, 2864, 1715, 1638, 1529, 1450, 1230, 1119, 1046 cm^{-1}. HRMS: calcd m/z $C_{24}H_{36}N_3O_5$ 446.2649 found m/z 446.2647. $[\alpha]_D$ -11.46 (*c* 0.096, MeOH).

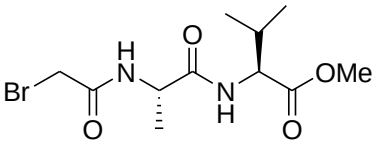
Preparation of Bisp (Gly-Ala-OMe)(Leu-Z) B2



To a well stirred solution of Bisp (Leu-Z) NH (0.19g, 0.5mmol) in dry acetonitrile (40mL), added dipeptide (Br-Gly-Ala-OMe) (0.132g, 0.59mmol) followed by addition of DIPEA (0.08mL, 0.49mmol). The reaction mixture was left stirred for overnight. Reaction mixture was evoperated and dissolved in chloroform and washed sequentially with 2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated the product. Column chromatography over silica gel (60-120 mesh, EtOAc:Hexane, 5:5), gave 0.19g of the pure product as colourless sticky compound **B2**.

Yield: 74.2%. ^{1}H NMR (DMSO-*d6*, 300MHz): δ 0.89 (br d, 6H), 1.28 and 1.38 (br ds, 6H), 1.46-1.61 (m, 2H), 1.74-2.08 (m, 4H), 2.2-2.4 (m, 1H), 2.76-2.91 (m, 2H), 3.20 (d, 2H, J=10.5Hz), 3.60 and 3.64 (s, 3H), 3.88 (s, 1H), 4.1 (s, 1H), 4.27-4.40 (m, 2H), 4.53 (m, 1H), 4.97 (br s, 2H), 7.33 (s, 5H), 7.58 (d, 1H, J=8.1Hz), 8.79 (br d, 1H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 17.5, 18.0, 18.1, 22.4, 22.8, 24.4, 28.6, 42.3, 46.0, 48.0, 48.3, 48.6, 52.3, 52.6, 58.0, 58.2, 62.4, 66.6, 128.0, 128.4, 136.3, 156.1, 165.1, 165.5, 169.2, 172.0, 172.7, 172.7. IR (KBr): 3326, 2951, 2866, 2809, 1743, 1711, 1670, 1631, 1528, 1457, 1327, 1216, 1160, 1117, 1053 cm^{-1}. HRMS: calcd for $C_{27}H_{41}N_4O_6$ m/z 517.3021 found m/z 517.3030. $[\alpha]_D$ -15.19 (c 0.08, MeOH).

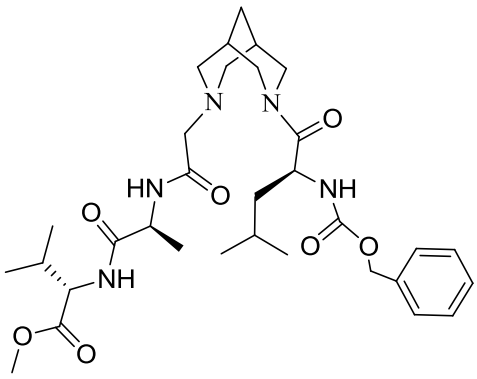
Preparation of Br-CH_2-CO-Ala-Val-OMe



To a well stirred solution of bromoacetic acid (0.99g, 7.13mmol) in dry dichloromethane (50mL), added sequentially N-hydroxysuccinimide (0.81g, 7.13mmol), DCC (1.47g, 7.13mmol) dipeptide (NH_2-Ala-Val-OMe) (1.2g, 5.94mmol). The reaction mixture was left stirred for 24h. Reaction mixture was evoporated and dissolved in chloroform and washed sequentially with 2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120mesh, EtOAc: Hexane, 4:6), yielded 0.98g of the product.

Yield: 51%. Mp: 118-120^{o}C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d+d, 6H, J=7.8Hz), 1.43 (d, 3H, J=6.9Hz), 2.21 (m, 1H), 3.76 (s, 3H), 3.88 (s, 2H), 4.55 (m, 2H), 6.53 (d, 1H, NH, J=8.4Hz), 7.11 (d, 1H, J=6.9Hz). ^{13}C NMR ($CDCl_3$, 75MHz): δ 17.8, 18.5, 18.9, 28.5, 30.9, 49.3, 52.2, 57.5, 165.9, 172.0, 172.2. IR (KBr): 3289, 3057, 2970, 2929, 2852, 1741, 1644, 1549, 1444, 1390, 1316, 1212, 1162, 1088, 1004, 950, 893 cm^{-1}. HRMS: calcd for $C_{11}H_{19}BrN_2NaO_4$ 345.0420 found m/z 345.0425 and 347.0425. $[\alpha]_D$ -61.0 (*c* 0.30, MeOH).

Preparation of Bisp (Gly-Ala-Val-OMe) (Leu-Z) 3a

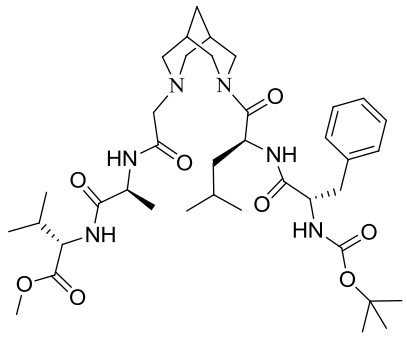


To a well stirred solution of Bisp (Leu-Z) NH (0.63g, 1.69mmol) in dry acetonitrile (50mL), added tripeptide (Br-CH_2-Ala-Val-OMe) (0.65g, 2.03mmol) followed by addition of DIPEA (0.3mL, 1.69mmol). The reaction mixture was left stirred for overnight. Reaction mixture was evoperated and dissolved in chloroform and washed sequentially with 2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The

organic layer was dried over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120mesh, EtOAc: Hexane, 4:6), gave 0.55g of the pure product as colourless sticky compound **3a**.

Yield: 53%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.89 (br d, 12H), 1.26 (br s, 1H), 1.46-1.61 (m, 6H), 1.78 (d, 1H, J=12.9Hz), 1.90 (br s, 2H), 2.04-2.22 (m, 3H), 2.33 (d, 1H, J=16.5Hz), 2.71-2.83 (m, 3H), 3.07 (d, 1H, J=9.9Hz), 3.36 (d, 1H, J=13.2 Hz), 3.70 (s, 3H), 4.50-4.66 (m, 3H), 4.80-4.91 (m, 2H), 5.02 (s, 1H), 5.07 (s, 1H), 6.76 (d, 1H, J=8.1Hz), 7.22-7.35 (m, 5H+1H), 7.70 (d, 1H, J=8.4Hz). ^{13}C NMR: δ 17.9, 18.1, 18.8, 22.5, 22.7, 24.6, 29.0, 29.9, 31.4, 32.6, 42.1, 46.2, 48.3, 49.1, 50.4, 52.1, 57.1, 58.1, 58.3, 62.8, 66.4, 127.9, 128.1, 128.5, 136.7, 156.4, 169.7, 172.5, 172.7, 172.9. IR (KBr): 3425, 2928, 2865, 1741, 1635, 1535, 1457, 1375, 1316, 1247, 1160, 1117, 1049 cm^{-1}. HRMS: calcd for $C_{32}H_{50}N_5O_7$ m/z 616.3705, obtained m/z 616.3710. $[\alpha]_D$ -51.0 (*c* 0.10, MeOH).

Preparation of Bisp (Gly-Ala-Val-OMe) (Leu-Phe-Boc) B3



To a solution of compound **3a** (0.62g, 1.00mmol) in 15mL ethanol, added 0.04g of Pd (10 wt. %) on activated carbon and kept under hydrogen atmosphere for 4h. TLC showed complete consumption of starting material. Filtered the reaction mixture through the sintered funnel and evaporated the solvent under vacuum to yield 0.489g of the product as pale yellow oily compound. The product was used as such for the next step without purification.

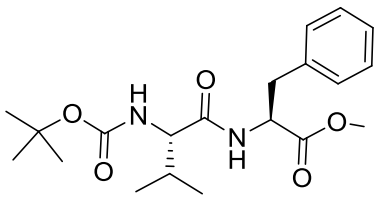
Yield: Quantitative.

To an ice cold and well stirred solution of Boc-Phe-OH (0.32g, 1.21mmol) in dry CH_2Cl_2 (60mL), added sequentially N-hydroxysuccinimide (0.14g, 1.21mmol), dicyclohexylcarbodimide

(0.25g, 1.21mmol), deprotected **3a** (0.489g, 1.01mmol) mixed with NEt_3 (0.16mL, 1.20mmol), the reaction mixture was left stirred for 24h. The reaction mixture was filtered through the sintered funnel, the filtrate was diluted wash sequentially with 0.2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120mesh, EtOAc: Hexane, 8:2), gave 0.43g of the pure product **B3**.

Yield: 59%. Mp: 165-167^oC. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.91 (d+d, 12H), 1.32 (s, 9H), 1.40 (br s, 3H), 1.48-1.58 (m, 5H), 1.70 (br s, 3H), 1.80 (s, 1H), 1.95 (d, 1H, J=14.4Hz), 2.04-2.20 (m, 2H), 2.37 (d, 1H, J=11.1Hz), 2.87(m, 2H), 3.08 (m, 3H), 3.41 (d, 1H, J=12Hz), 3.71 (s, 3H), 4.40-4.62 (m, 2H), 4.62-4.84 (m, 2H), 5.17 (m, 1H), 5.40 (d, 1H, 9.3Hz), 6.88 (d, 1H, J=7.8Hz), 7.1-7.32 (m 5H), 7.69 (d, 1H, J=9.6Hz), 7.84 (d, 1H, J=9Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.1, 18.5, 18.8, 22.4, 22.8, 24.3, 28.1, 28.9, 29.9, 31.5, 32.3, 38.2, 46.1, 46.9, 48.9, 50.7, 52.1, 55.7, 57.2, 58.1, 58.4, 63.0, 126.4, 128.2, 129.4, 137.3, 155.0, 169.6, 171.3, 172.1, 173.0, 173.4. IR (KBr): 3405, 3349, 3275, 3075, 2926, 2863, 2813, 1742, 1710, 1675, 1642, 1623, 1510, 1459, 1361, 1249, 1176 cm^{-1}. HRMS: calcd for $C_{38}H_{60}N_6O_8Na$ m/z 751.4365, obtained m/z 751.4366. $[\alpha]_D$ -53.0 (*c* 0.10, MeOH).

Preparation of Boc-Val-Phe-OMe

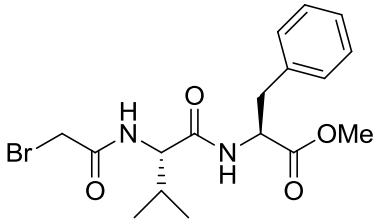


To an ice-cooled solution of Boc-Val-OH (2.86g, 13.17mmol) in dry dichloromethane (150mL) was added N-hydroxysuccinimide (2.3g, 19.76mmol), DCC (4.07g, 19.4mmol) and stirred for 20 min. Added $HCl.NH_2$-Phe-OCH_3 (3.4g, 15.8mmol) and NEt_3 (5.5mL, 39.5mmol) then the reaction mixture was stirred for 24h. Dicyclohexylurea was removed by filtration and the filtrate was washed sequentially with 0.2N H_2SO_4, saturated $NaHCO_3$ and water. The organic layer was

collected, dried over anhydrous Na_2SO_4 and eveporated. Chromatographic purification over silica gel (60-120mesh, EtOAc:Hexane, 7:3) yielded 2.9g of the pure product.

Yield: 58%. Mp: 167- 170^{o}C. ^{1}H NMR ($CDCl_3$, 300MHz): δ 0.91 (d+d, 6H, J=6.9Hz), 1.45 (s, 9H), 2.09 (m, 1H), 3.12 (br d, 2H), 3.71 (s, 3H), 3.90 (m, 1H), 4.87 (m, 1H), 5.02 (br d, 1H), 6.33 (br d, 1H), 7.11 (d, 2H, J=7.2Hz), 7.27 (m, 3H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 17.6, 19.1, 28.2, 30.8, 37.9, 52.2, 53.0, 59.8, 79.8, 127.1, 128.5, 129.2, 135.6, 155.7, 171.2, 171.6. IR (KBr): 3282, 3062, 2928, 2858, 1738, 1640, 1545, 1444, 1223 cm^{-1}. HRMS calcd for $C_{20}H_{30}N_2NaO_5$ m/z 401.2047 found m/z 401.2050.

Preparation of Br-CH_2-CO-Val-Phe-OCH_3



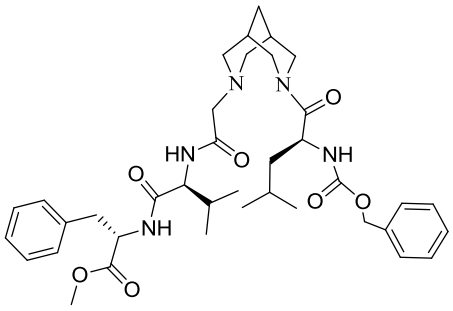
To an ice-cooled solution of Boc-Val-Phe-OMe (2.3g, 6.09mmol) in dry CH_2Cl_2 was added TFA (7.0mL, 91.33mmol). The reaction mixture was stirred for 4h. TFA was evaporated under high vacuum, redissolved in ethylacetate and washed with 5% Na_2CO_3 solution. Organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated to yield 1.83g of NH_2-Val-Phe-OCH_3.

Yield: 98%.

NH_2-Val-Phe-OCH_3 (1.69g, 6.08mmol) was added as a solution in dry dichloromethane to a well stirred solution of bromoacetic acid (1.0g, 7.29mmol), N-hydroxysuccinimide (0.839g, 7.29mmol) and DCC (1.5g, 7.2mmol). The reaction mixture was left stirred for 24h. The precipitated dicyclohexylurea was removed by filtration and the filtrate was washed sequentially with 0.2N H_2SO_4, saturated $NaHCO_3$ and water. The organic layer was collected, dried over anhydrous Na_2SO_4 and removed the solvent to give the product.

Appearance: Yellow Viscous Liquid. Yield: 87%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d+d, 6H, J=6.3Hz), 2.12 (m, 1H), 3.16 (m, 2H), 3.75 (s, 3H), 3.87 (s, 2H), 4.22 (m, 1H), 4.89 (m, 1H), 6.21 (d, 1H, J=7.8Hz), 6.94 (d, 1H, J=7.8Hz), 7.11 (d, 1H, J=6Hz), 7.32 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$,75MHz):δ 17.9, 19.0, 28.8, 29.7, 31.1, 37.8, 52.4, 53.1, 58.9, 127.3, 128.7, 129.2, 135.4, 165.5, 169.9, 171.5 ppm. IR (KBr): 3285, 3070, 2962, 2856, 1743, 1648, 1546, 1442, 1383, 1307, 1267, 1209, 1151, 1024 cm^{-1}. HRMS: calcd for $C_{17}H_{23}BrN_2O_4Na$ m/z 421.0733 obtained m/z 421.0740 and 423.0719. $[\alpha]_D$ -45.0 (*c* 0.1, MeOH).

Preparation of 3b

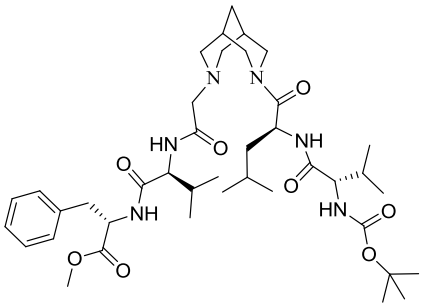


To an ice-cooled solution of Bisp (Leu-Z) NH (0.55g, 1.47mmol) in dry acetonitrile (75mL) was added diisopropylethylamine (0.25mL, 1.47mmol), and Br-CH_2-CO-Val-Phe-OCH_3 (0.585g, 1.47mmol). The reaction mixture was stirred for 12h, evaporated the solvent, re dissolved the reaction mixture in dichloromethane and was washed sequentially with 0.2N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected, dried over anhydruous Na_2SO_4 and evaporated. Chromatographic purification over silica gel (200-400mesh, EtOAc:Hexane, 1:1) to yield 0.59g of the pure product.

Yield: 58%. ^{1}H NMR ($CDCl_3$, 300MHz): δ 0.94 (br d+d, 12H), 1.52-2.30 (m, 8H), 2.48 (m, 2H), 2.66-3.45 (m, 9H), 3.64 and 3.67 (s, 3H), 4.05 (m, 1H), 4.59 (m, 1H), 4.93 (m, 4H), 5.14 (s, 1H), 6.65 (br d, 1H), 7.09-7.36 (br m, 10H), 7.69 (br d, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.8, 21.6, 22.7, 24.6, 29.1, 29.9, 32.5, 37.7, 42.4, 46.1, 48.1, 50.4, 52.1, 53.3, 58.4, 58.7, 60.6, 63.2, 66.4, 126.8, 127.0, 127.9, 128.5, 129.5, 135.7, 136.6, 156.4, 169.9, 171.7, 171.8, 172.4. IR (KBr): 3285, 3023, 2955, 2917, 2868, 2809, 1744, 1709, 1634, 1526, 1456, 1377, 1322, 1250,

1219, 1116 cm^{-1}. HRMS: calcd for $C_{38}H_{54}N_5O_7H$ m/z 692.4018, obtained m/z 692.4006. $[\alpha]_D$ -48.15 (*c* 0.108, MeOH).

Preparation of B4



To a solution of compound **3b** (0.32g, 0.46mmol) in 10mL ethanol, added 0.032g of Pd (10 wt. %) on activated carbon and kept under hydrogen atmosphere for 4h. TLC showed complete consumption of starting material. Filtered the reaction mixture through the sintered funnel and evaporated the solvent under vacuum to yield 0.26g of the product. The product was used as such for the next step without purification.

Yield: Quantitative.

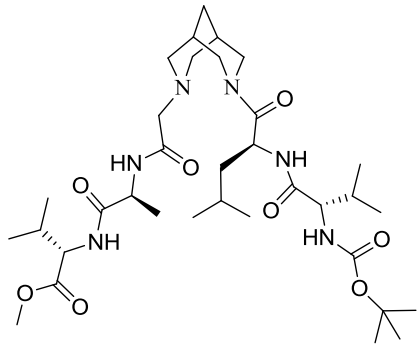
To an ice-cooled solution of Boc-Val-OH (0.12g, 0.55mmol) in dry dichloromethane (70mL) added N-hydroxysuccinimide (0.064g, 0.56mmol), DCC (0.115g, 0.56mmol), and the reaction mixture was stirred for 20min. Bisp (Leu-NH) (Gly-Val-Phe-OCH_3) (0.26g, 0.46mmol) and NEt_3 (0.13mL, 0.93mL) was added. The reaction mixture was stirred for 24h, dicyclohexylurea was removed by filtration and the filtrate was washed sequentially with 0.2N H_2SO_4, saturated $NaHCO_3$ and water. The organic layer was collected, dried over anhydrous Na_2SO_4 and removed the solvent under low pressure. Chromatographic purification over silicagel (60-120mesh, EtOAc:Hexane, 3:2) yielded 0.22g of the pure product.

Appearance: Colourless viscous liquid

Yield: 63%. ^1H NMR ($CDCl_3$, 300MHz): δ 0.88-1.04 (br d+d, 18H), 1.41 and 1.44 (s, 9H), 1.50-2.20 (m, 7H), 2.31 (m, 1H), 2.42 (d, 1H, J=10.8Hz), 2.60 (m, 2H), 2.75-3.35 (m, 5H), 3.44 (d, 1H, J=12.6Hz), 3.66 and 3.69 (s, 3H), 3.96 (m, 1H), 4.11-4.24 (m, 2H), 4.39 (d, 1H, J=12.3Hz),

4.76 (d, 1H, J=13.5Hz), 4.87 (d, 1H, J=13.5 Hz), 5.07 (d, 1H, J=9Hz), 5.17 (m, 2H), 6.79 (d, 1H, J=8.4 Hz), 7.19-7.30 (m, 5H), 7.69 (d, 1H, J=9.6Hz), 8.09 (d, 1H, J=8.7Hz). ^{13}C NMR ($CDCl_3$, 75MHz): δ 17.4, 19.6, 20.0, 21.7, 22.5, 22.8, 23.8, 24.7, 28.4, 28.7, 29.9, 30.4, 30.8, 31.8, 31.9, 38.4, 43.3, 46.1, 47.0, 50.9, 52.3, 53.0, 58.6, 59.2, 63.1, 79.4, 127.2, 128.7, 129.4, 135.6, 155.5, 169.8, 171.3, 171.8, 172.0, 172.6. IR (KBr): 3305, 2961, 2921, 2860, 2803, 1745, 1712, 1636, 1507, 1460, 1367, 1216, 1167, 1103 cm^{-1}. HRMS: calcd for $C_{40}H_{65}N_6O_8$ m/z 757.4858 obtained m/z 757.4877. $[\alpha]_D$ -50.51 (*c* 0.10, MeOH).

Preparation of Bisp (Gly-Ala-Val-OMe) (Leu-Val-Boc) B5



To a solution of compound **3a** (0.180g, 0.29mmol) in 5mL ethanol, added 0.04g of Pd (10 wt. %) on activated carbon and kept under hydrogen atmosphere for 4h. TLC showed complete consumption of starting material. Filtered the reaction mixture through the sintered funnel and evaporated the solvent under vacuum to yield 0.139g of the product as pale yellow oily compound. The product was used as such for the next step without purification.

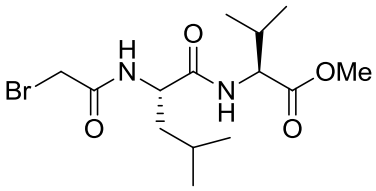
Yield: Quantitative.

To an ice cold and well stirred solution of Boc-Val-OH (0.087g, 0.40mmol) in dry CH_2Cl_2 (20mL), added sequentially N-hydroxysuccinimide (0.046g, 0.40mmol), dicyclohexylcarbodimide (0.082g, 0.40mmol), deprotected **3a** (0.139g, 0.288mmol) mixed with NEt_3 (0.05mL, 0.40mmol), the reaction mixture was left stirred for 24h. The reaction mixture was filtered through the sintered funnel, the filtrate was diluted wash sequentially with 0.2N H_2SO_4, saturated aqueous $NaHCO_3$ solution and finally with water. The organic layer was dried

over anhydrous Na_2SO_4 and evaporated. Column chromatography over silica gel (60-120mesh, EtOAc: Hexane, 8:2), gave 0.089g of the pure product **B5**.

Yield: 45%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.91 (br d, 18H), 1.26 (s, 3H), 1.39 (br s, 9H), 1.54 (m, 5H), 1.79 (m, 1H), 2.02 (m, 2H), 2.18 (m, 1H), 2.52 (d, 1H, J=9.9Hz), 2.8-2.96 (m, 3H), 3.10 (m, 2H), 3.40 (d, 1H, J=12.6Hz), 3.77 (s, 3H), 3.85-4.05 (br d ,1H), 4.16 (m, 2H), 4.44 (d, 1H, J=12Hz), 4.61 (br s, 1H), 4.74 (d, 1H, J=11.4Hz), 5.13 (m, 1H), 5.44 (d, 1H, J=9.3Hz), 7.08 (d, 1H, J=8.7Hz), 7.86 (d, 1H, J=8.4Hz), 7.99 (d, 1H, J=8.7Hz). IR (neat): 3313, 2960, 2867, 1714, 1632, 1514, 1461, 1367, 1212, 1162 cm^{-1}. HRMS: calcd for $C_{34}H_{61}N_6O_8$ m/z 681.4545, found m/z 681.4541. $[\alpha]_D$ -35.87 (*c* 0.18, MeOH).

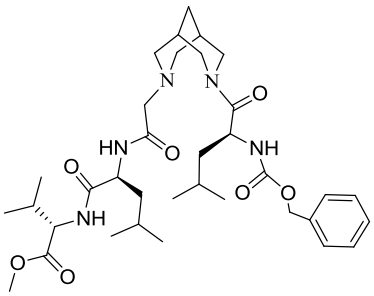
Preparation of Br-CH_2-CO-Leu-Val-OMe



To an ice-cooled solution of bromoacetic acid (0.11mL, 1.53mmol) in 70mL dry dichloromethane, was added N-hydroxysuccinimide (0.20g, 1.74mmol), DCC (0.36g, 1.75mmol) and left stirred for 10min. To this solution was added NH_2-Leu-Val-OMe (0.42g, 1.72mmol) in 20mL dichloromethane and left stirred overnight. Filtered the reaction mixture, washed the filtrate with saturated aqueous $NaHCO_3$ solution and then with water. The organic layer was dried over anhydrous Na_2SO_4, and evaporated to yield 0.52g of the compound.

Yield: 93%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (d+d, 12H), 1.62 (m, 3H), 2.16 (m, 1H), 3.75 (s, 3H), 3.87 (s, 2H), 4.49 (m, 1H), 4.62 (m, 1H), 7.07 (d, 1H, J=8.7Hz), 7.45 (d, 1H, J=8.4Hz). IR (KBr): 3273 (br), 3074, 2962, 2879, 1748, 1651, 1555, 1442, 1380, 1312, 1264, 1211, 1159, 1024 cm^{-1}. HRMS: calcd for $C_{14}H_{25}N_2O_4BrNa$ m/z 387.0895, found m/z 387.0897 and 389.0872.

Preparation of 3c



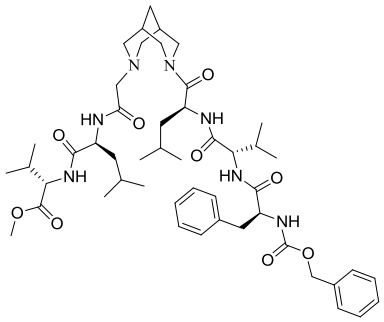
To an ice-cooled solution of **2** (0.31g, 0.65mmol) in dry dichloromethane (1mL) was added TFA (2mL) and stirred at RT for 2h. The reaction mixture was subjected to vacuum to remove CH_2Cl_2 and TFA to afford 0.24g of the compound Bisp (Leu-Z) NH.

Yield: Quantitative.

Compound Bisp (Leu-Z) NH (0.24g, 0.65mmol) was dissolved in dry acetonitrile (40mL), cooled to 0°C. To this solution was added NEt_3 (0.11mL, 0.78mmol) and Br-CH_2-CO-Leu-Val-OMe (0.29g, 0.79mmol). The reaction mixture was stirred overnight at RT. Evaporated the reaction mixture and then dissolved it in 80mL of dichloromethane. Washed it with 2N H_2SO_4, then with water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4. Filtered and evaporated to yield 0.376g of the compound **3c**.

Yield: 88%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80-1.18 (m, 18H), 1.52-1.84 (m, 7H), 1.84-1.95 (m, 2H), 2.00-2.25 (m, 4H), 2.30-2.40 (br d, 1H), 2.60-2.88 (m, 3H), 3.06 (d, 1H, J=10.2Hz), 3.34 (d, 1H, J=12.9Hz), 3.75 (s, 3H), 4.50-4.65 (m, 3H), 4.78-4.95 (m, 2H), 5.05 (m, 2H), 6.63 (d, 1H, J=8.7Hz), 7.20-7.40 (m, 6H), 7.62 (d, 1H, J=9.0Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.9, 18.8, 21.8, 22.5, 22.7, 23.0, 24.6, 25.1, 29.1, 30.0, 31.3, 40.6, 42.1, 46.1, 48.3, 50.3, 52.0, 57.1, 58.2, 58.6, 62.9, 66.3, 127.9, 128.1, 128.4, 136.8, 156.4, 169.8, 172.3, 172.6, 172.7. IR (KBr): 3298 (br), 2956, 2868, 2807, 1742, 1709, 1638, 1538, 1459, 1376, 1255, 1220, 1157 cm^{-1}. HRMS: calcd for $C_{35}H_{55}N_5O_7Na$ m/z 680.3999, found m/z 680.3970. $[\alpha]_D$ -49.4 (*c* 0.09, MeOH).

Preparation of B6



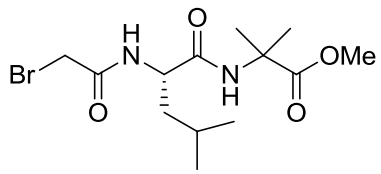
To a solution of Bisp (Gly-Leu-Val-OMe) (Leu-Z) **3c** (0.25g, 0.38mmol) in 30mL of dry ethanol was added 30% of the Pd, 10 wt. % on activated carbon, and H_2 was bubbled through the reaction mixture for 4h. The solution was filtered and evaporated to yield 0.20g of the compound.

Yield: 99%.

To an ice-cooled solution of Z-Phe-Val-OH (0.18g, 0.45mmol) in 80mL of dry dichloromethane, was added N-hydroxysuccinimide (0.05g, 0.45mmol) and DCC (0.09g, 0.45mmol) and stirred for 10min. Bisp (Gly-Leu-Val-OMe) (Leu-NH_2) (0.20g, 0.38mmol) and NEt_3 (0.06mL, 0.64mmol) were added. The reaction mixture was left stirred overnight, filtered, washed the filtrate with 2N H_2SO_4, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield 0.42g of the compound **B6**.

Yield: 80%. ^{1}H NMR (300MHz, DMSO-d_6): δ 0.85 (m, 24H), 1.32-1.68 (br m, 6H), 1.70-1.92 (br m, 2H), 1.96-2.1 (m, 2H), 2.27 (br s, 2H), 2.77 (m, 2H), 2.94 (m, 2H), 3.18-3.46 (br, 3H), 3.62 (s, 3H), 3.77 (br, 2H), 4.08-4.26 (m, 4H), 4.34 (m, 2H), 4.56 (m, 1H), 4.70 (br s, 1H), 4.95 (s, 2H), 7.12-7.36 (m, 10H), 7.56 (d, 1H, J=9Hz), 7.91 (d, 1H, J=9Hz), 8.19 (br s, 1H), 8.39 (d, 1H, J=9Hz), 8.66 (br, 1H). IR (KBr): 3299, 3035, 2960, 2873, 1714, 1650, 1537, 1457, 1381, 1215 cm^{-1}. HRMS: calcd for $C_{49}H_{74}N_7O_9$ m/z 904.5548, found m/z 904.5548. $[\alpha]_D$ -27.4 (*c* 0.38, MeOH).

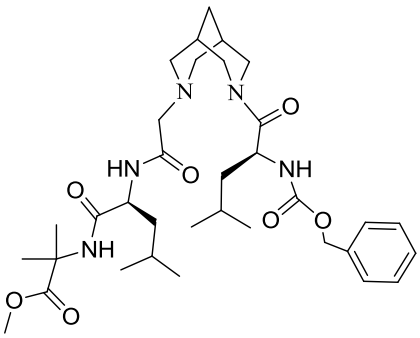
Preparation of Br-CH_2-CO-Leu-Aib-OMe



To an ice-cooled solution of bromoacetic acid (0.25mL 3.42mmol) in 70mL dry dichloromethane, was added N-hydroxysuccinimide (0.39g, 3.42mmol), DCC (0.71g, 3.43mmol) and left stirred for 10 min. To this solution was added H_2N-Leu-Aib-OMe (0.79g, 3.43mmol) in 20mL dry dichloromethane and left stirred overnight. Filtered the reaction mixture, washed the filtrate with saturated aqueous $NaHCO_3$ solution and then with water. The organic layer was dried over anhydrous Na_2SO_4, and evaporated to yield 1.05g of the compound.

Yield: 87%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (m, 6H), 1.53 (s, 6H), 1.63-1.77 (br m, 3H), 3.73 (s, 3H), 3.87 (s, 2H), 4.45 (m, 1H), 6.87 (br d, 1H), 7.18 (br d, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.7, 22.4, 24.2, 24.4, 28.1, 40.6, 51.8, 52.1, 56.0, 165.8, 170.6, 174.1. IR (KBr): 3268, 3074, 2957, 1746, 1651, 1554, 1465, 1438, 1193, 1151 cm^{-1}. $[\alpha]_D$ -46.6 (*c* 0.12, MeOH). HRMS: calcd for $C_{13}H_{23}N_2O_4BrNa$ m/z 373.0739, found m/z 373.0735.

Preparation of 3d



To an ice-cooled solution of Bisp (Boc) (Leu-Z) **2** (0.53g, 1.11mmol) in dry dichloromethane (1mL) was added TFA (2mL) and stirred at RT for 2 h. The reaction mixture was subjected to vacuum to remove dichloromethane and TFA to afford 0.41g of the compound.

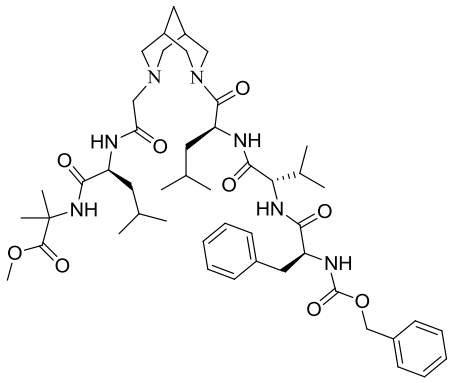
Yield: Quantitative.

Compound Bisp (Leu-Z) NH (0.414g, 1.11mmol) was dissolved in dry acetonitrile (40mL), cooled to 0°C. To this solution was added NEt_3 (0.21mL, 1.52mmol) and Br-CH_2-Leu-Aib-OMe (0.43g, 1.22mmol). The reaction mixture was stirred overnight at RT. Evaporated the reaction

mixture and then dissolved it in 80mL of dichloromethane. Washed it with 2N H_2SO_4, then with water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4. Filtered and evaporated to yield 0.65g of the compound **3d**.

Yield: 91%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88-1.09 (d+d, 12H), 1.45 (s+s, 6H), 1.46-1.82 (m, 6H), 1.86 (br m, 2H), 2.00-2.22 (m, 4H), 2.25-2.43 (m, 2H), 2.63-2.84 (m, 2H), 3.04 (d, 1H, J=9.9Hz), 3.33 (d, 1H, J=12.9Hz), 3.67 (s, 3H), 4.47-4.61 (m, 2H), 4.69-4.90 (m, 2H), 5.02 (d, 2H, J=4.5Hz), 6.60 (s, 1H), 7.29 (s, 5H), 7.42 (d, 1H, J=9.9Hz), 7.55 (d, 1H, J=9.0Hz). ^{13}C NMR ($CDCl_3$, 75MHz): δ 21.7, 22.5, 22.9, 23.1, 24.1, 24.4, 24.7, 25.1, 29.1, 29.9, 32.5, 40.1, 41.8, 46.0, 48.1, 50.1, 51.3, 52.4, 56.5, 58.3, 58.6, 63.0, 66.3, 127.9, 128.2, 128.4, 136.6, 156.3, 169.9, 171.8, 172.1, 174.9. IR (KBr): 3295, 3062, 2953, 2867, 2807, 1743, 1709, 1637, 1543, 1459, 1384, 1257, 1154, 1157 cm^{-1}. $[\alpha]_D$ -45.2 (*c* 0.14, MeOH). HRMS: calcd for $C_{34}H_{54}N_5O_7$ m/z 644.4023, found m/z 644.4040.

Preparation of B7



To a solution of Bisp (Gly-Leu-Aib-OMe) (Leu-Z) **3d** (0.35g, 0.54mmol) in 30mL of dry ethanol was added 30% of the Pd, 10 wt. % on activated carbon, and H_2 was bubbled through the reaction mixture for 4h. The solution was filtered and evaporated to yield 0.28g of the compound.

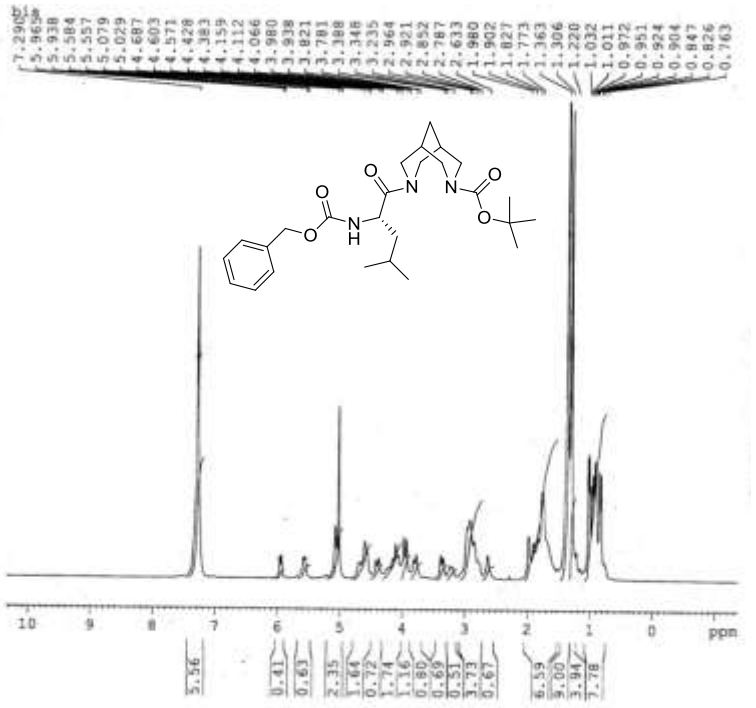
Yield: 99%.

To an ice-cooled solution of Z-Phe-Val-OH (0.26g, 0.65mmol) in 80mL of dry dichloromethane, was added N-hydroxysuccinimide (0.75g, 0.65mmol) and DCC (0.13g, 0.65mmol) and stirred for 10min. Bisp (Gly-Leu-Aib-OMe) (Leu-NH_2) (0.27g, 0.54mmol) and NEt_3 (0.09mL, 0.64mmol) were added. The reaction mixture was left stirred overnight, filtered, washed the

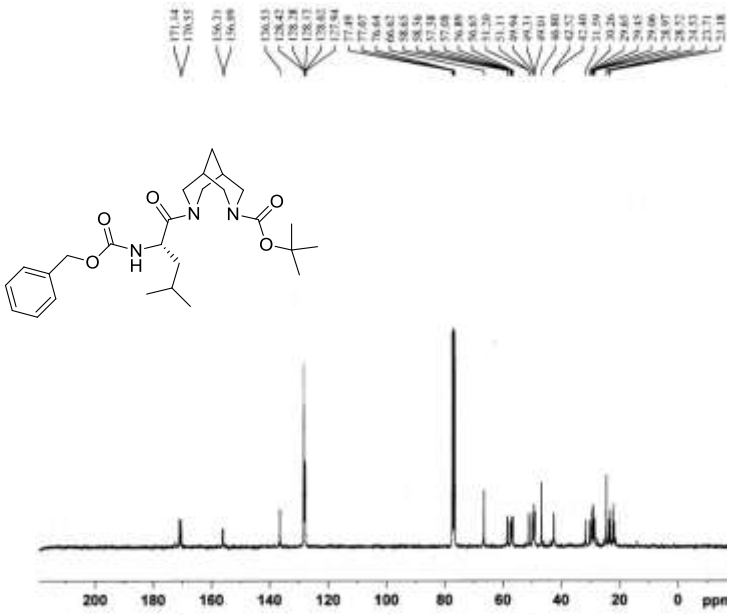
filtrate with 2N H_2SO_4, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield 0.42g of the compound **B7**.

Yield: 87%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.73-1.08 (m, 18H), 1.44-1.85 (m, 13H), 1.91-2.06 (m, 2H), 2.10-2.26 (m, 2H), 2.42-2.60 (m, 1H), 2.67 (s, 2H), 2.80-2.91 (m, 1H), 2.98-3.25 (m, 3H), 3.38-3.54 (m, 1H), 3.73 (s, 3H), 4.24 (d, 1H, J=10.2Hz), 4.40-4.65 (m, 3H), 4.69 (d, 1H, J=13.5Hz), 5.05 (s, 4H), 5.40-5.60 (br d, 1H), 6.68-6.90 (br s+s, 2H), 7.10-7.40 (m, 11H), 7.62 (d, 1H, J=7.8Hz), 8.33 (d, 1H, J=6.6Hz). IR (KBr): 3301 (br), 3063, 2958, 2871, 1714, 1645, 1536, 1459, 1388, 1219, 1153, 1081 cm^{-1}. HRMS: calcd for $C_{48}H_{72}N_7O_9$ m/z 890.5392, found m/z 890.5399. $[\alpha]_D$ -30.9 (*c* 0.11, MeOH).

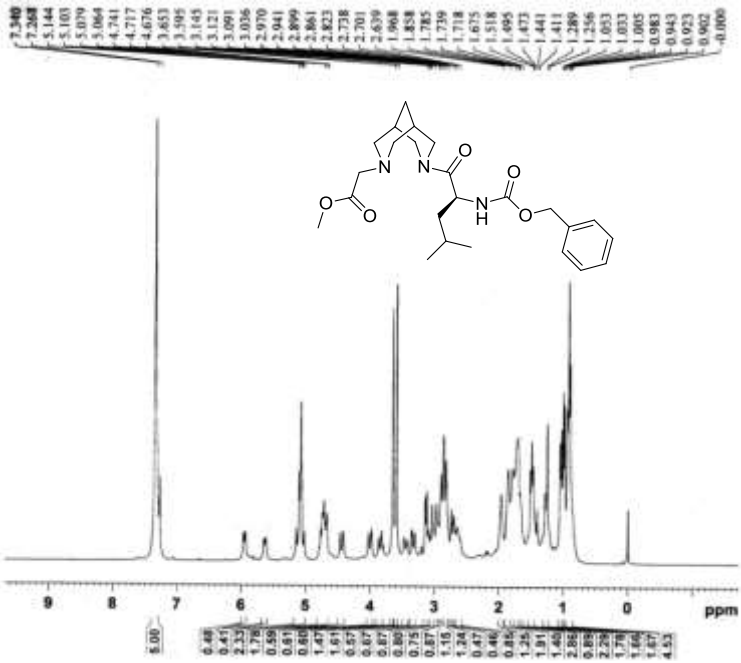
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **2**



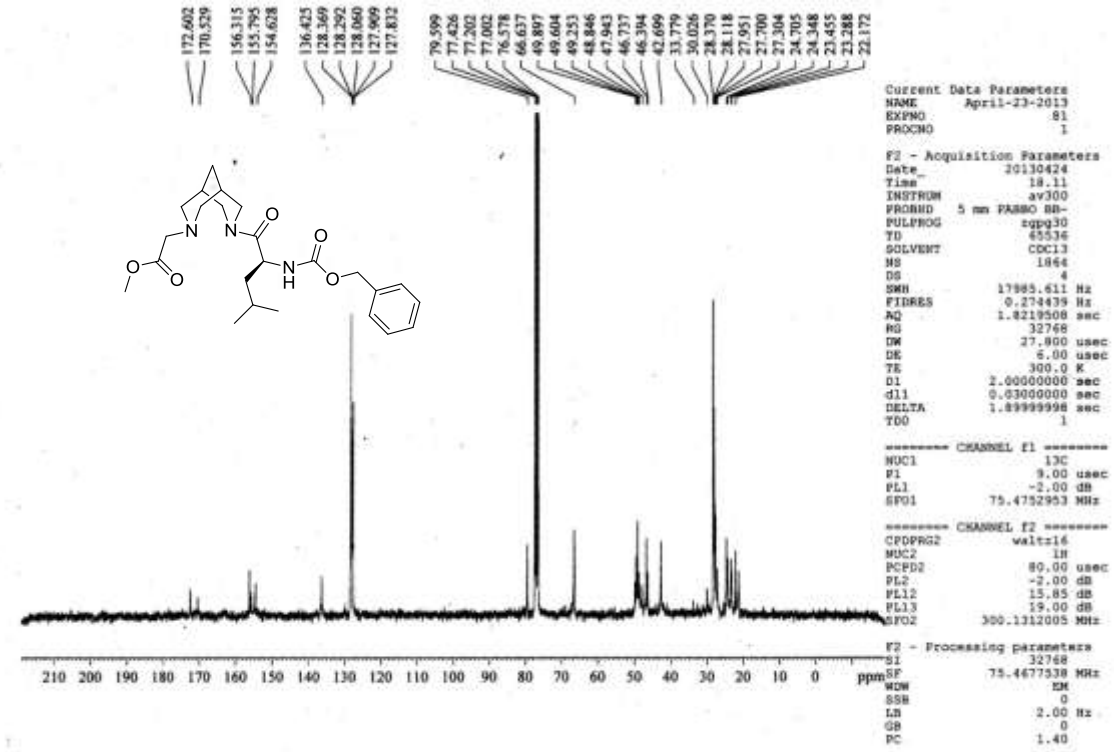
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **2**



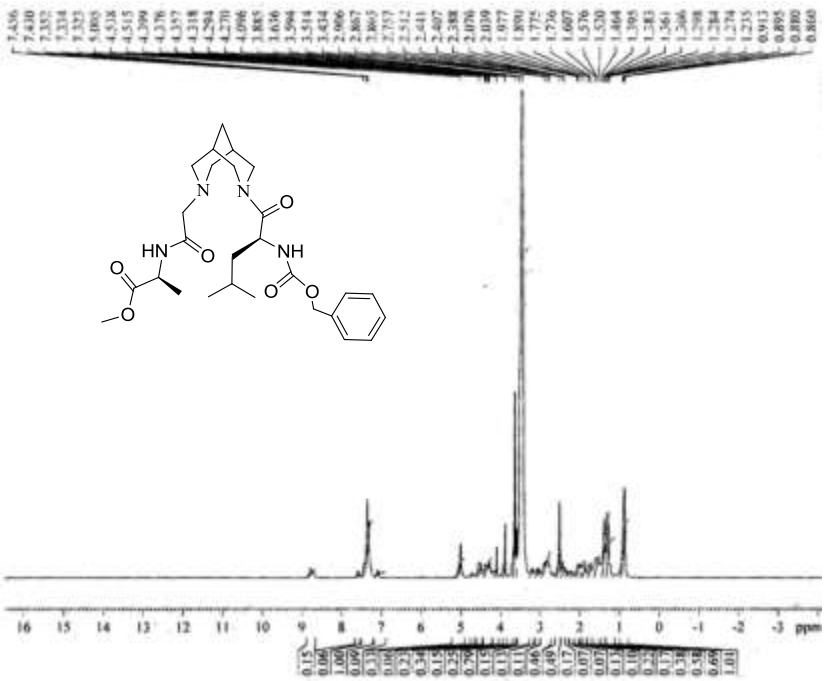
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **B1**



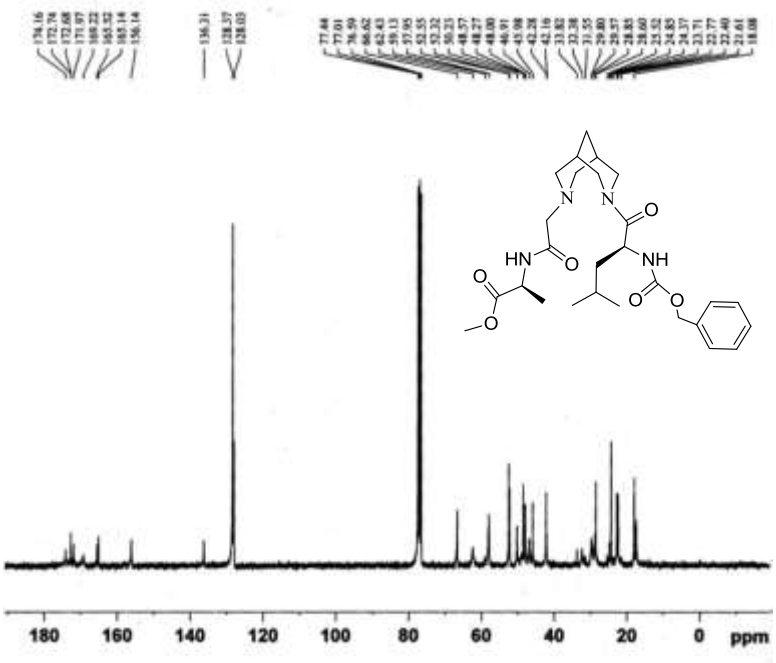
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **B1**



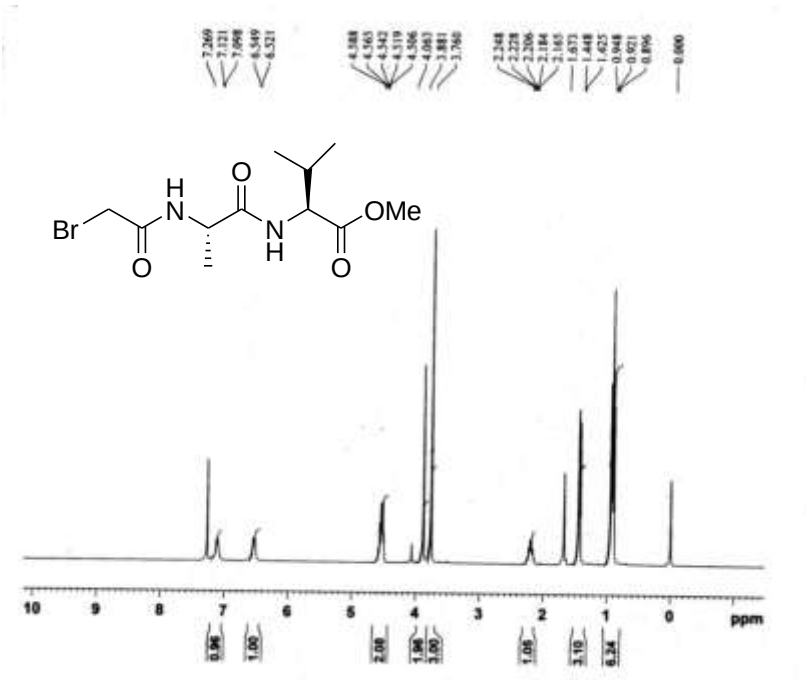
^{1}H NMR (DMSO-*d6*, 300 MHz) of compound **B2**



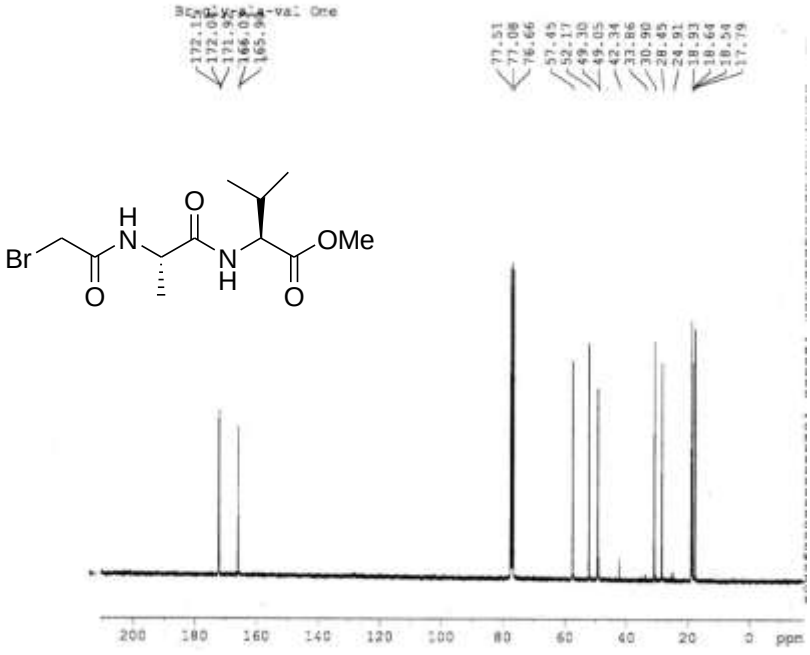
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **B2**



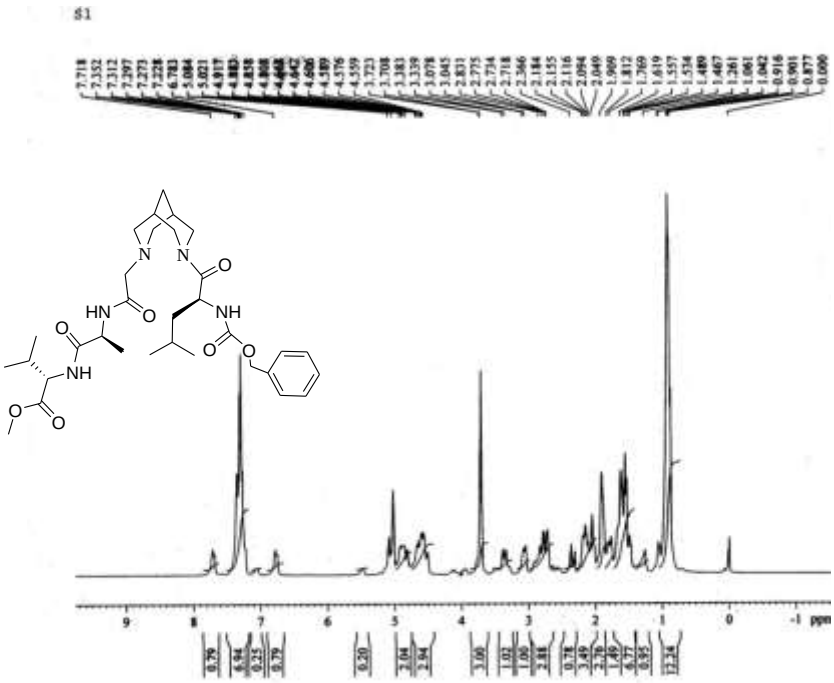
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **Br-Gly-Ala-Val OMe**



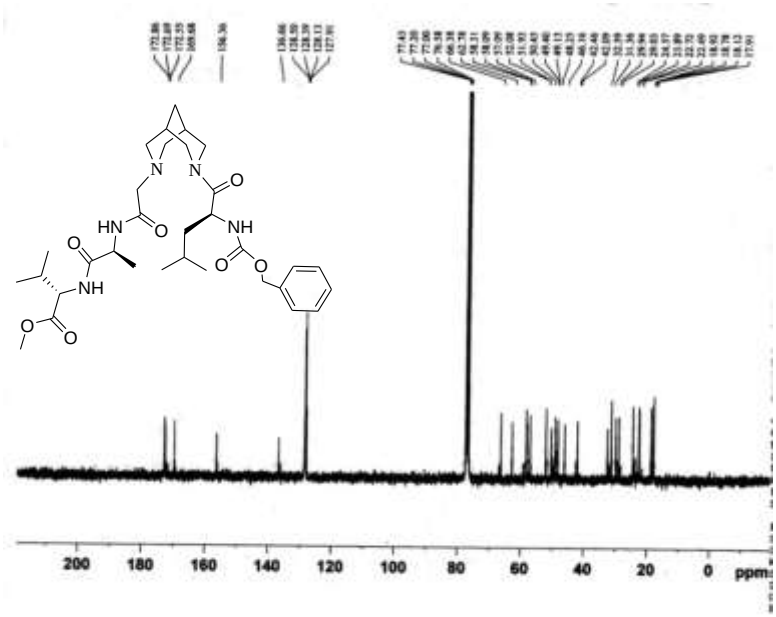
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **Br-Gly-Ala-Val OMe**



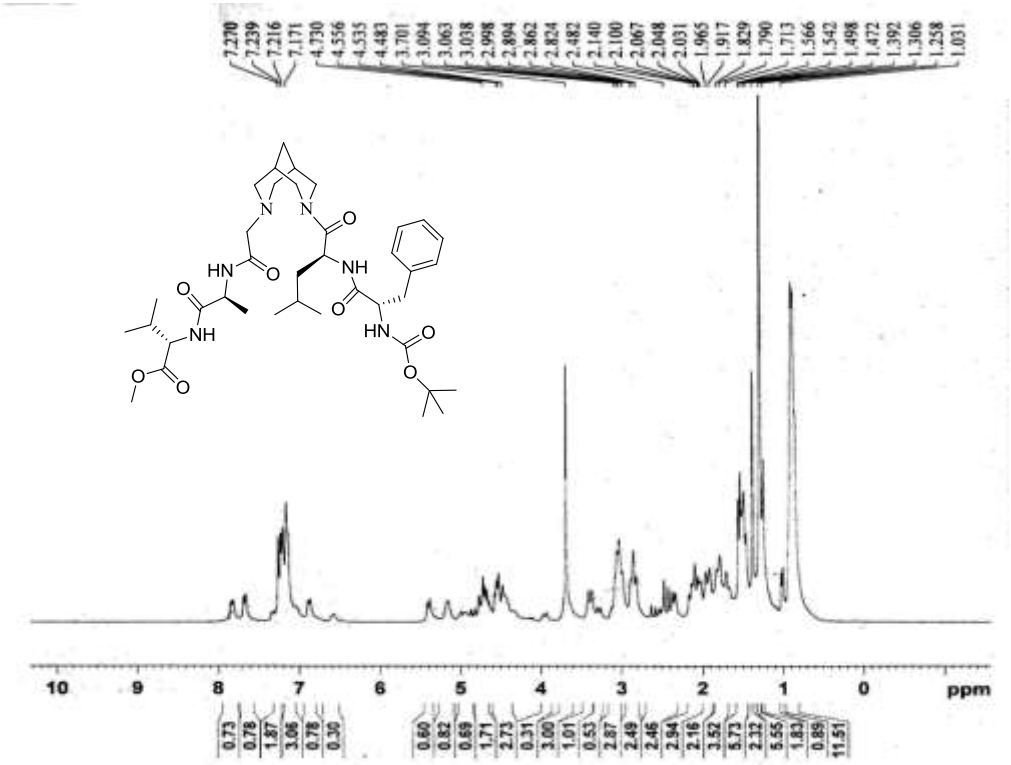
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **3a**



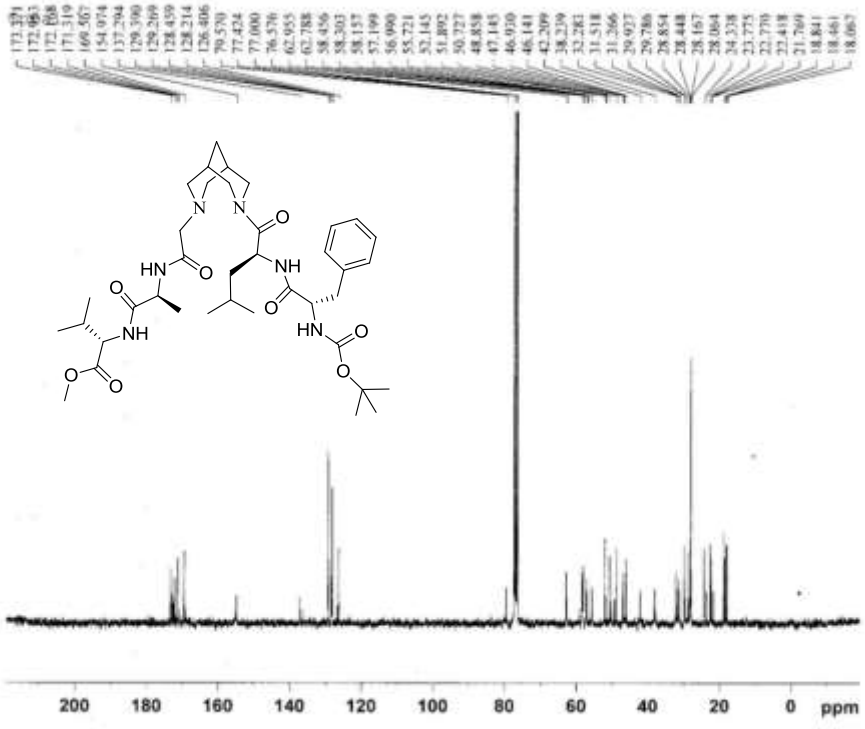
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **3a**



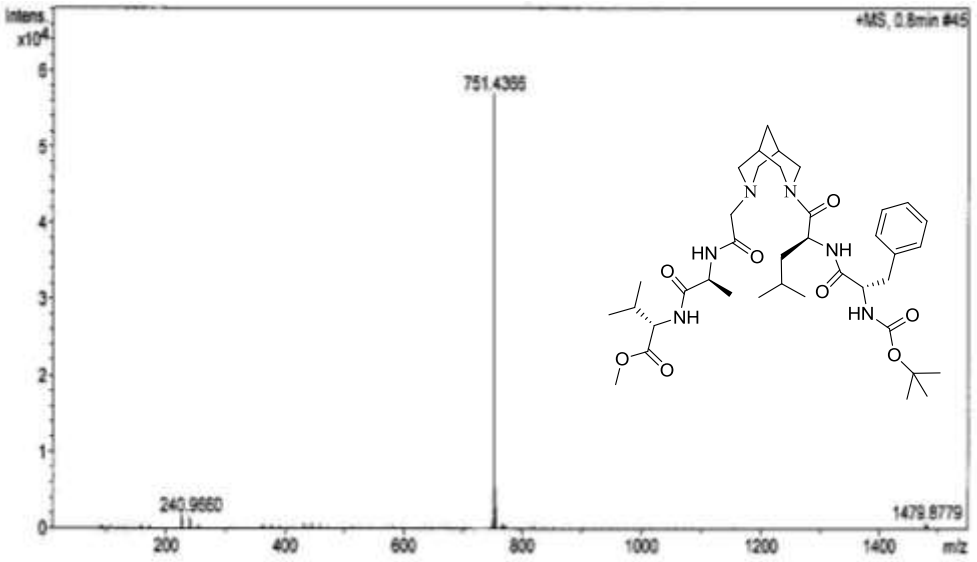
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **B3**



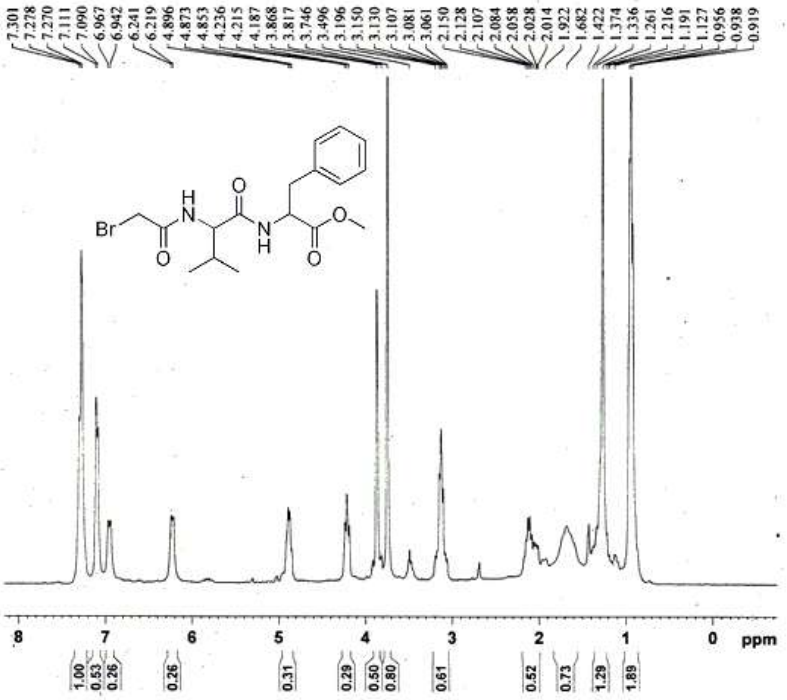
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **B3**



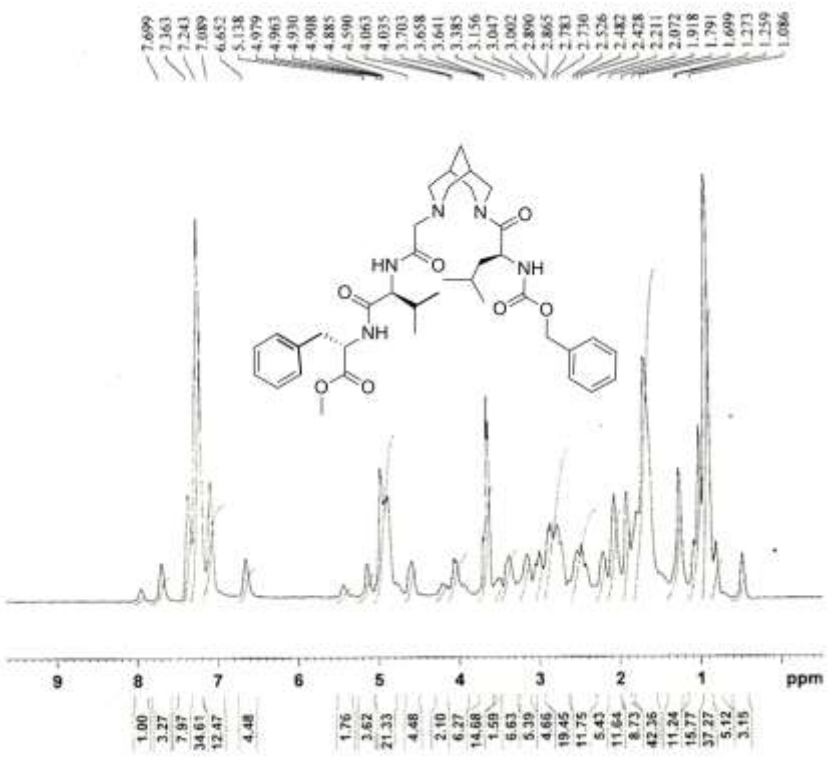
HRMS of compound **B3**



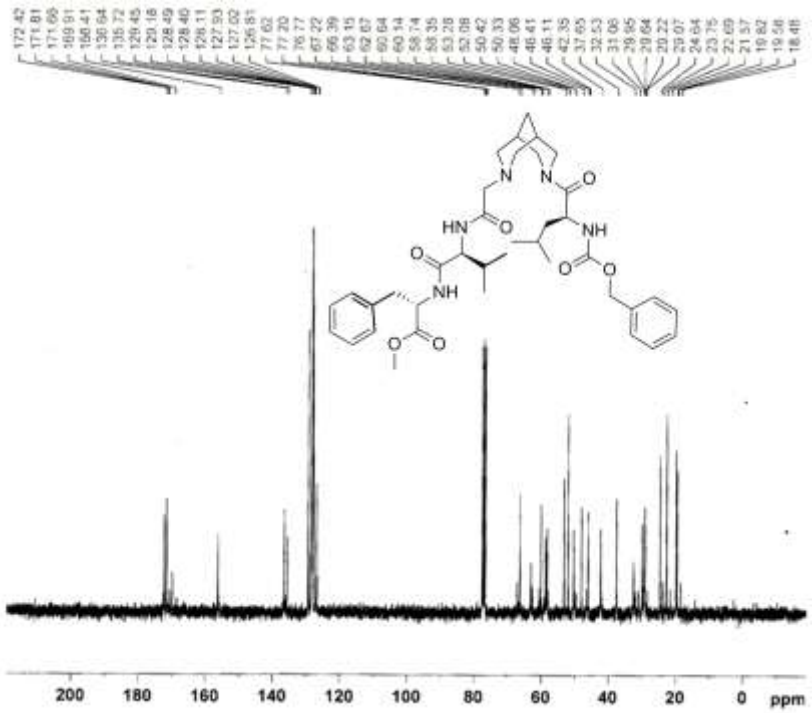
^{1}H NMR (300 MHz, $CDCl_3$) of **Br-CH_2-CO-NH-Val-Phe-OCH_3**



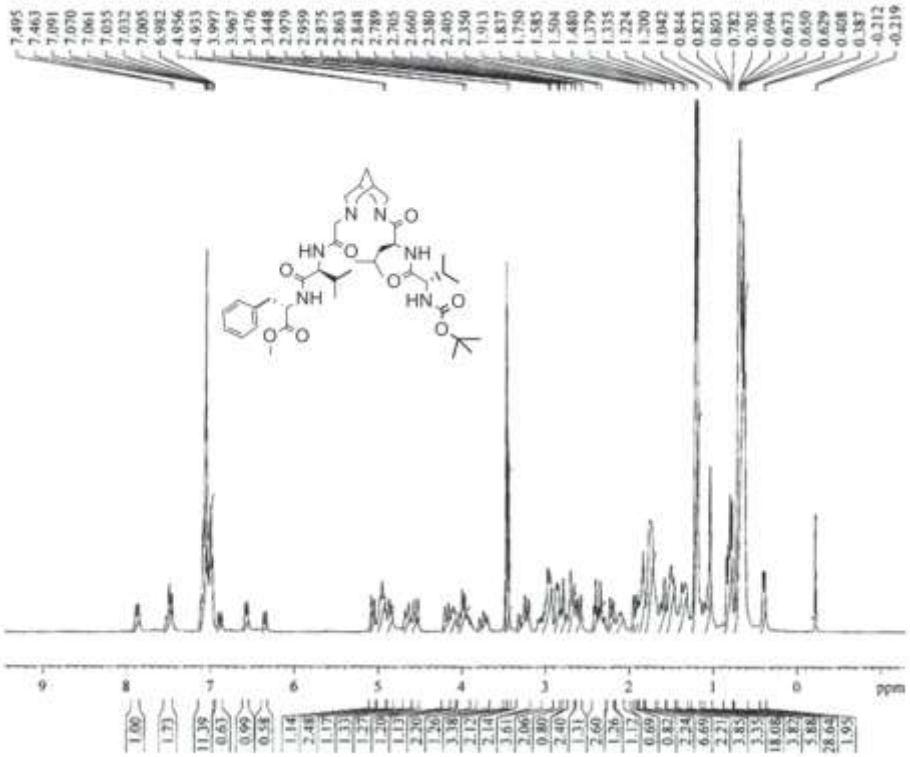
^{1}H NMR (300 MHz, $CDCl_3$) of compound **3b**



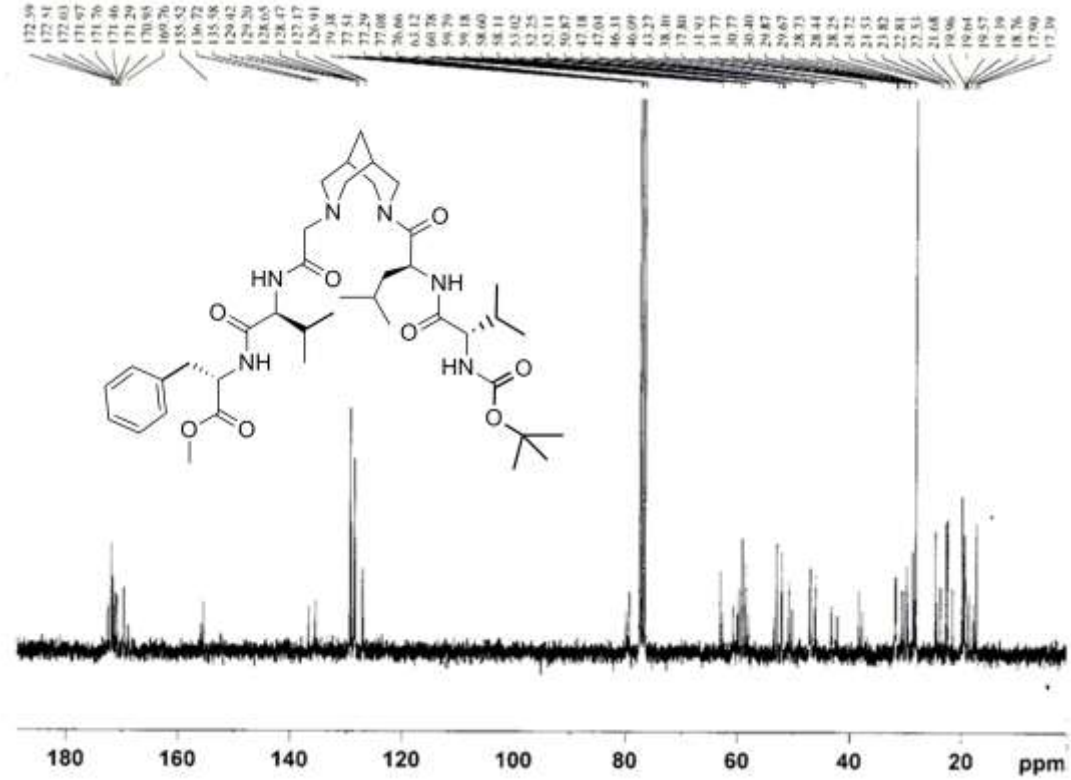
^{13}C NMR (75MHz, $CDCl_3$) of compound **3b**



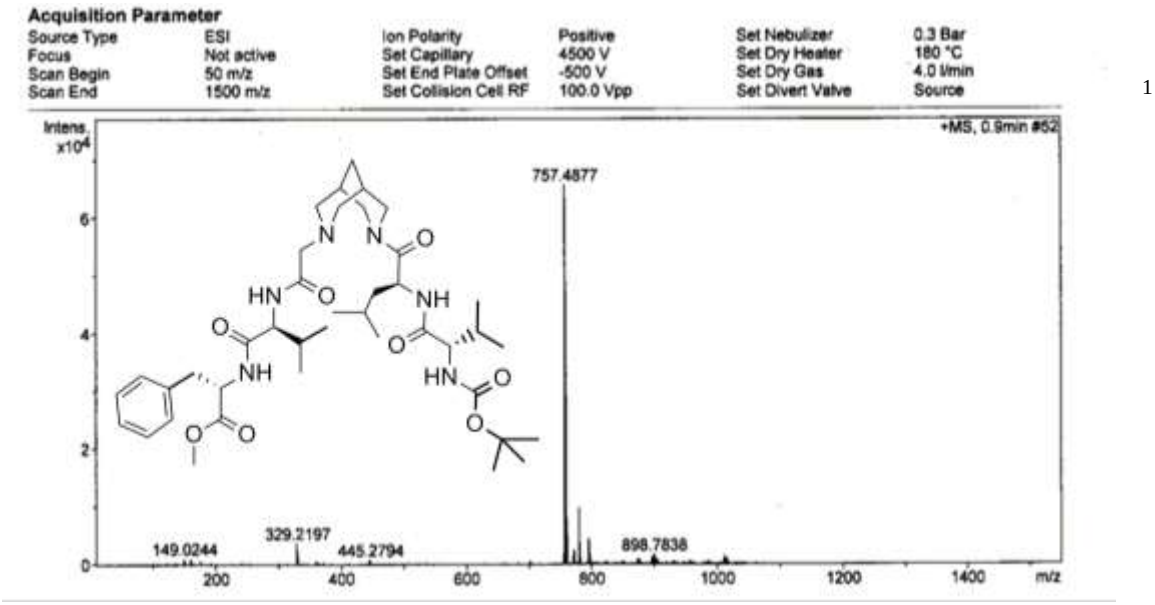
^{1}H NMR($CDCl_3$, 300MHz) of compound **B4**



^{13}C NMR (75MHz, $CDCl_3$) of compound **B4**

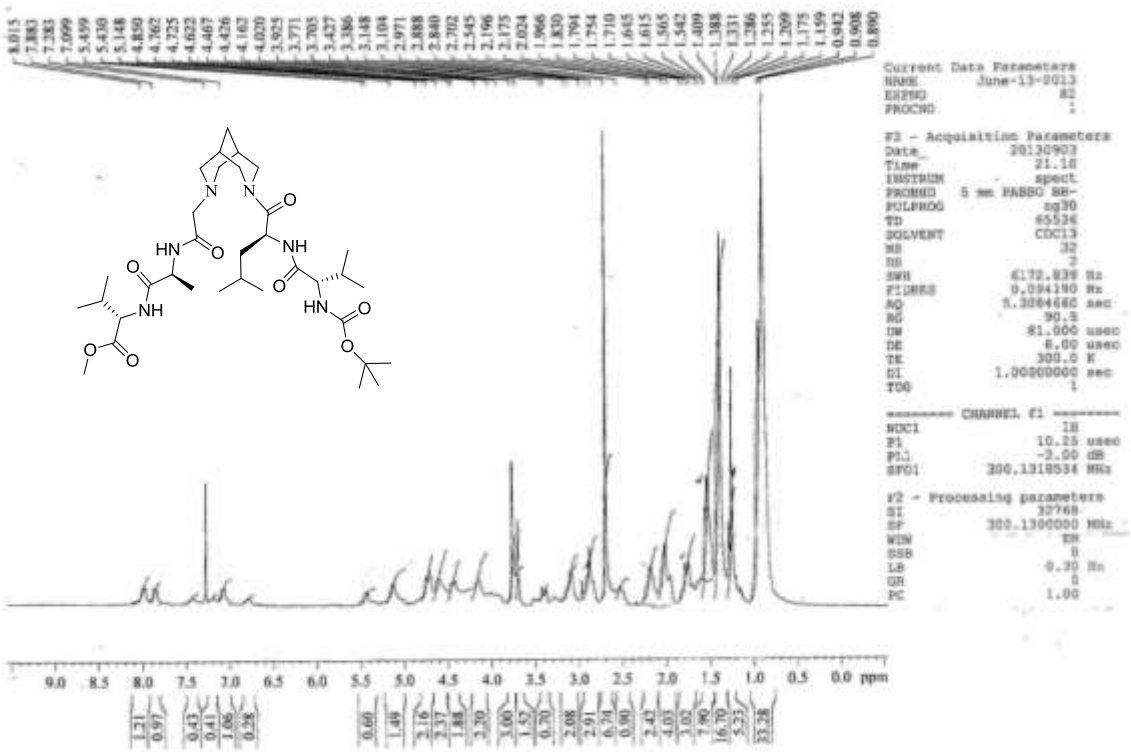


HRMS spectrum of **B4**

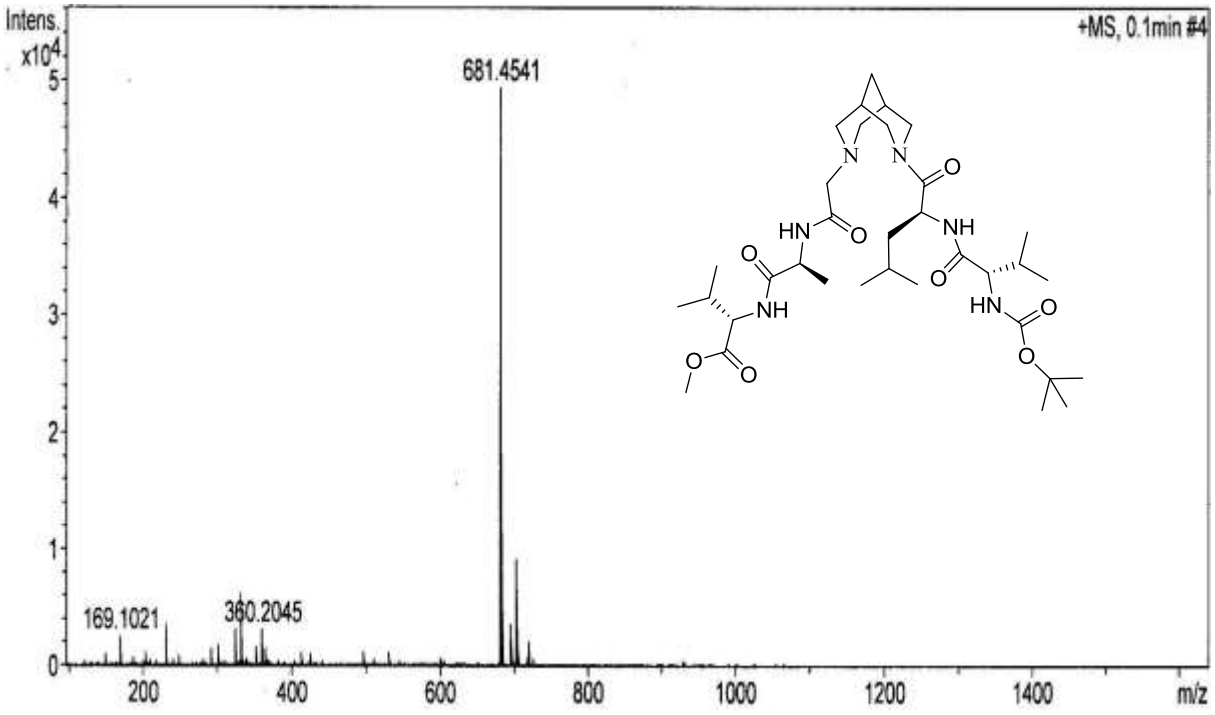


1

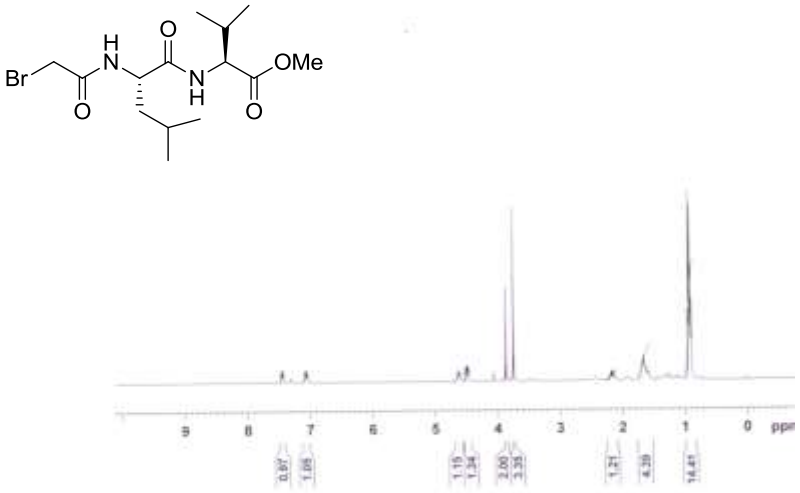
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **B5**



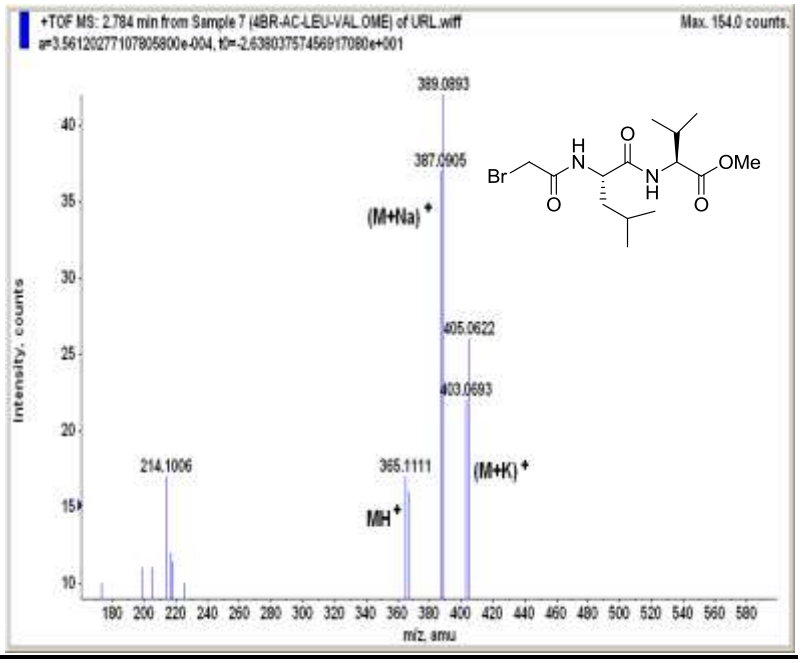
HRMS of the compound **B5**



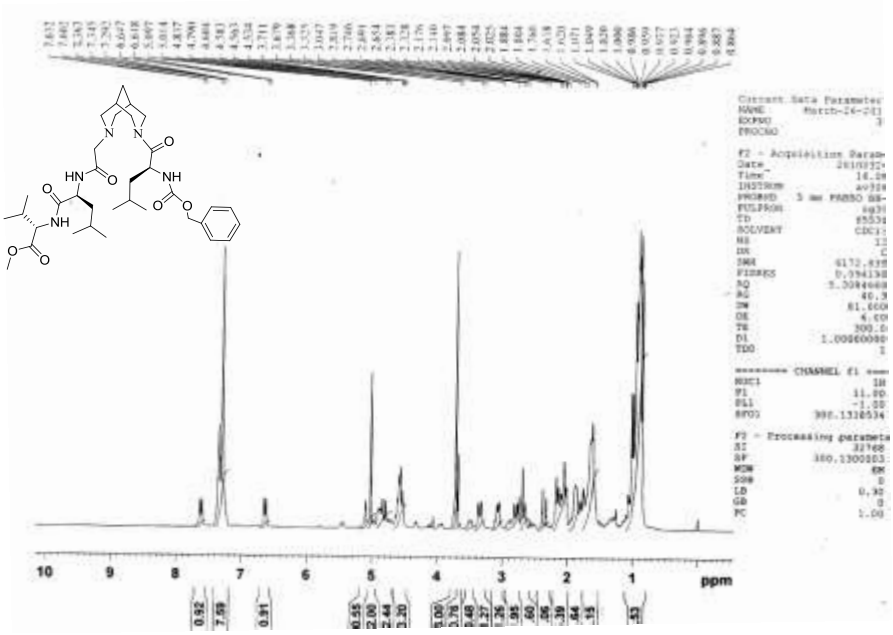
^{1}H NMR (300 MHz, $CDCl_3$) of **Br-CH_2-CO-Leu-Val-OMe**



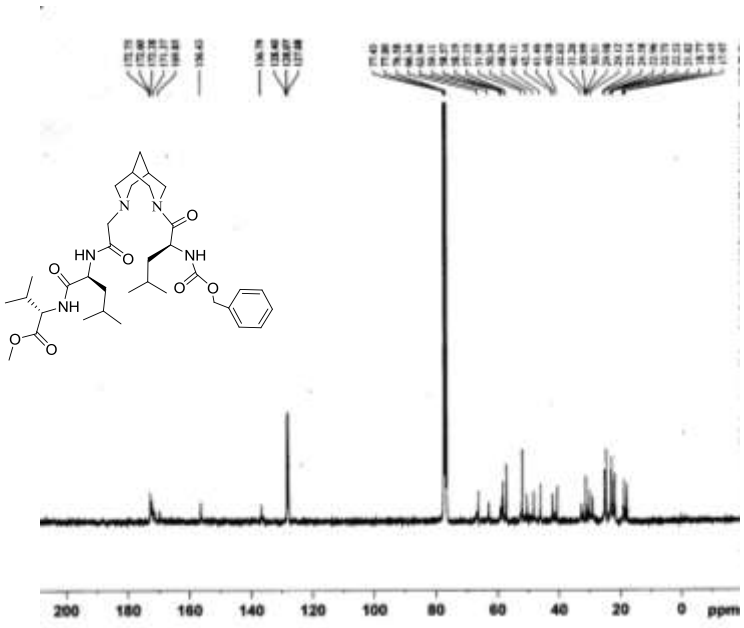
HRMS of **Br-CH_2-CO-Leu-Val-OMe**



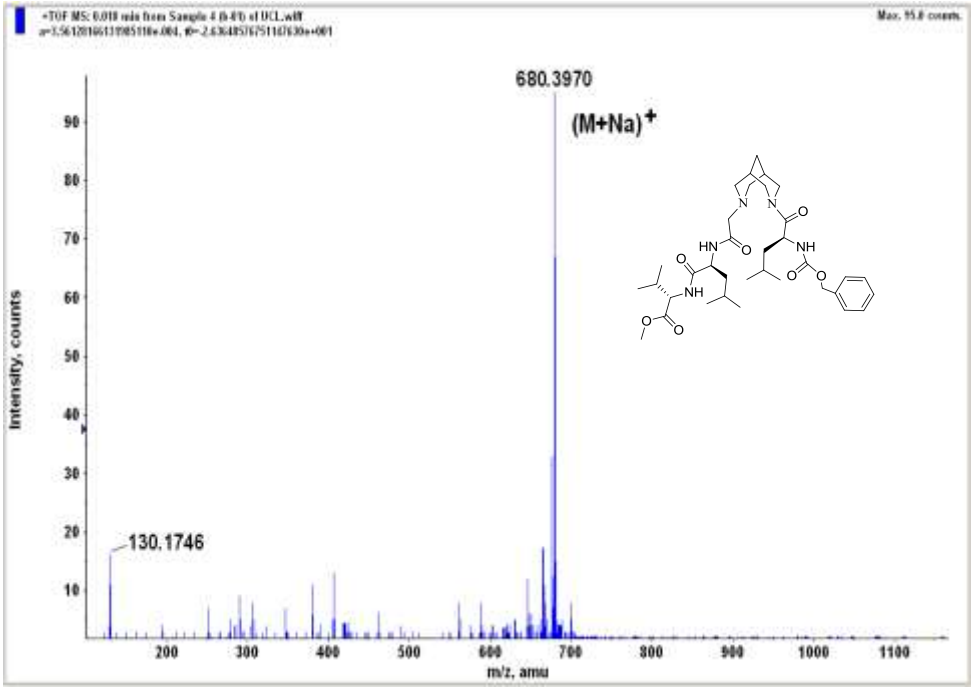
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **3c**



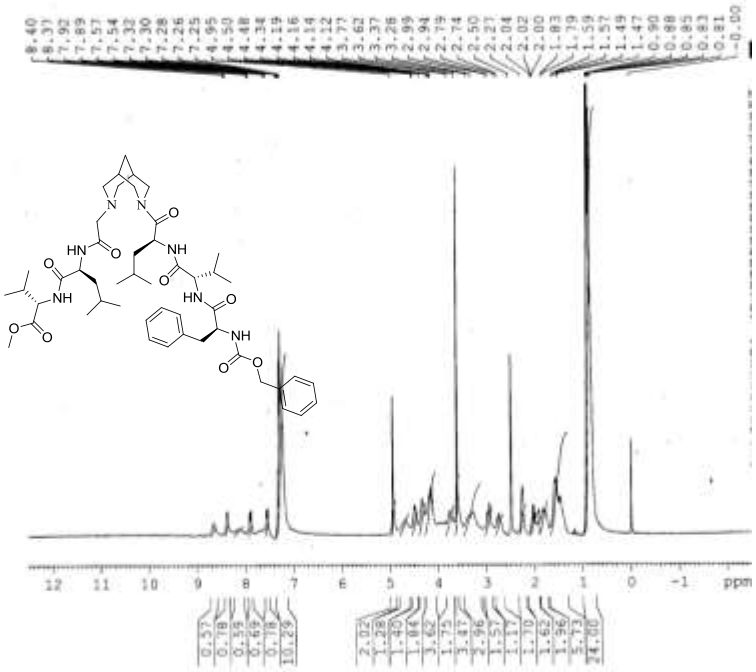
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **3c**



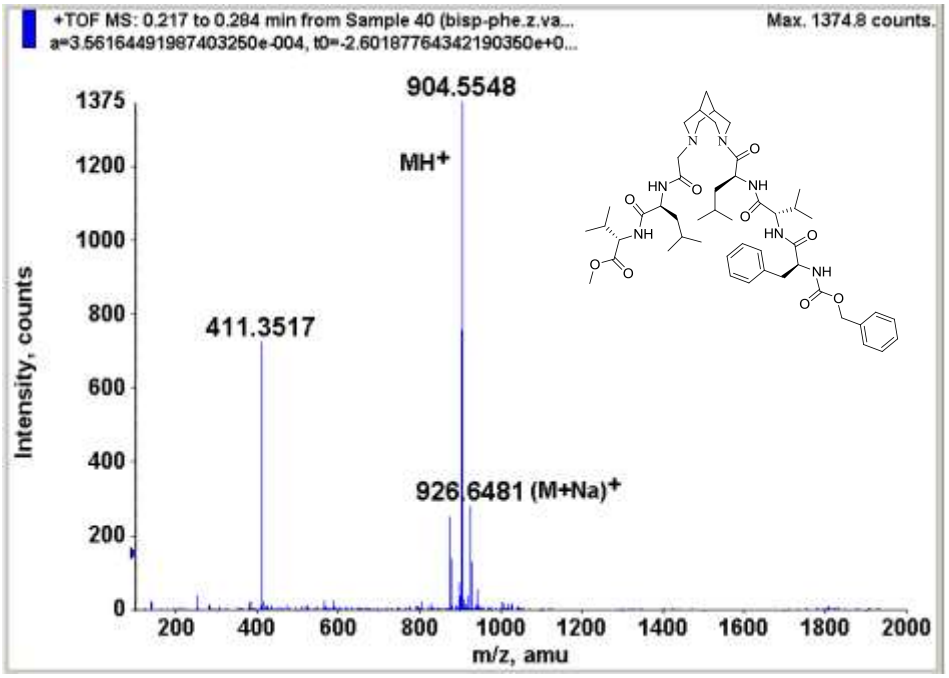
HRMS of compound **3c**



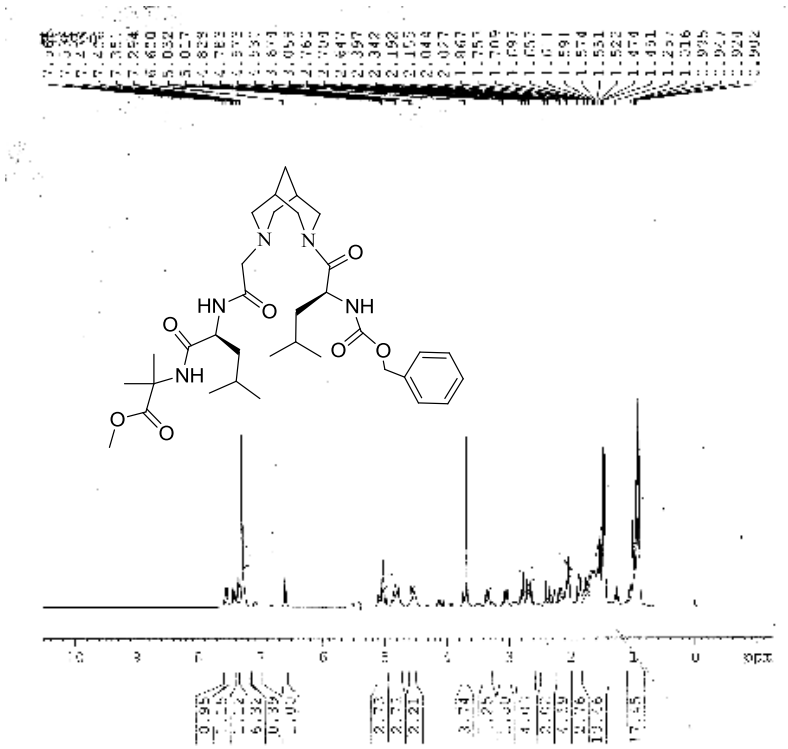
^{1}H NMR (DMSO-d_6, 300 MHz) of compound **B6**



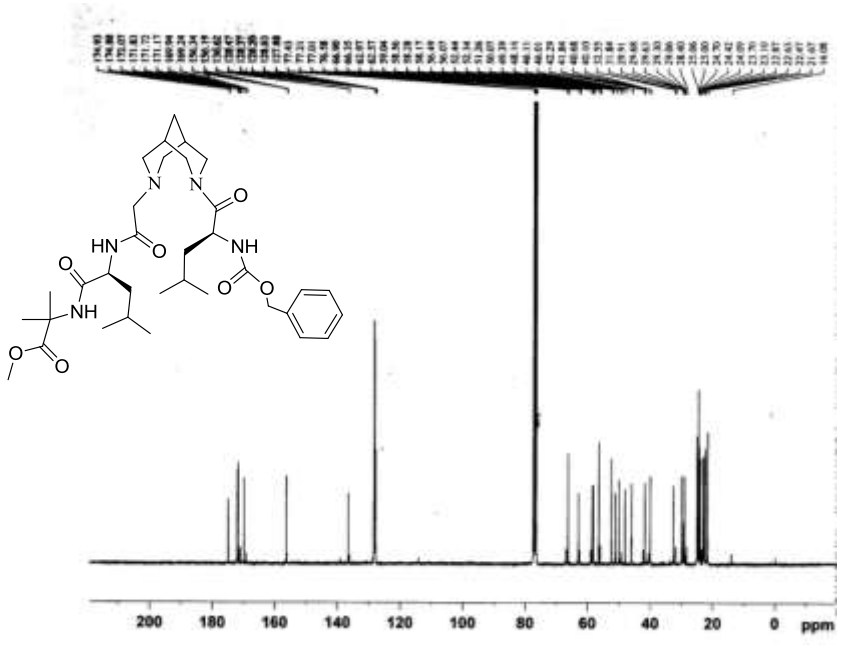
HRMS of Compound **B6**



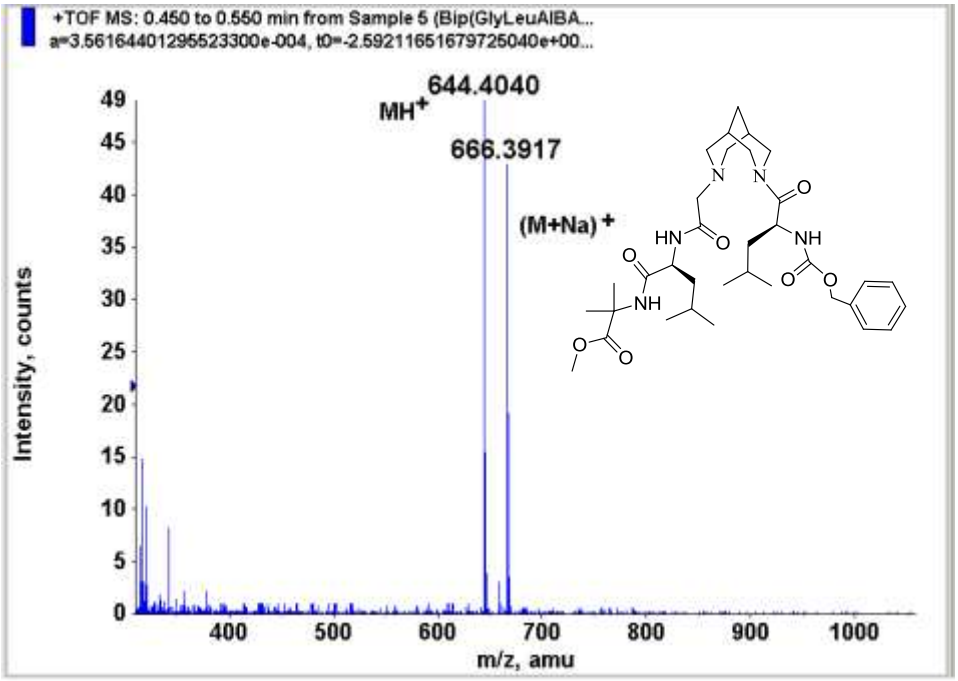
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **3d**



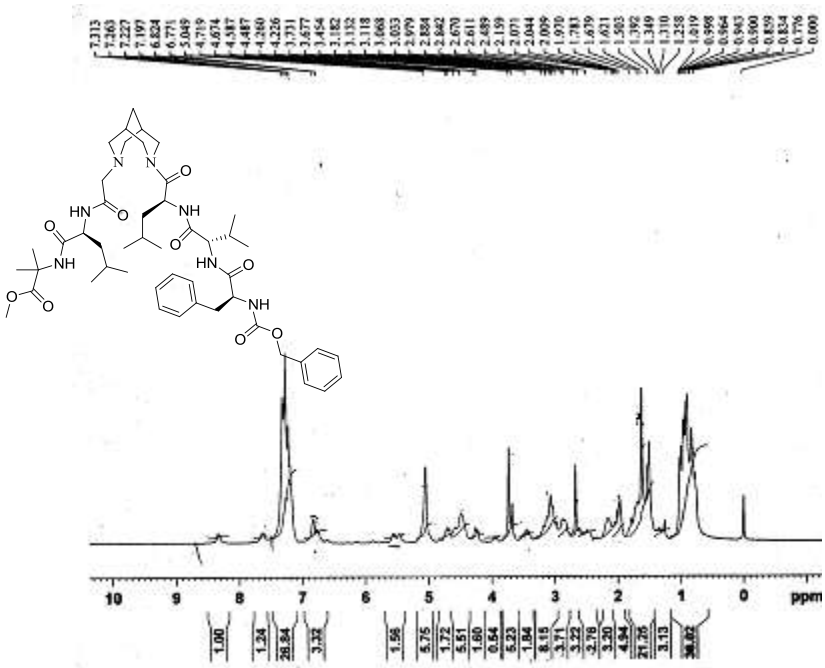
^{13}C NMR ($CDCl_3$, 75 MHz) of compound **3d**



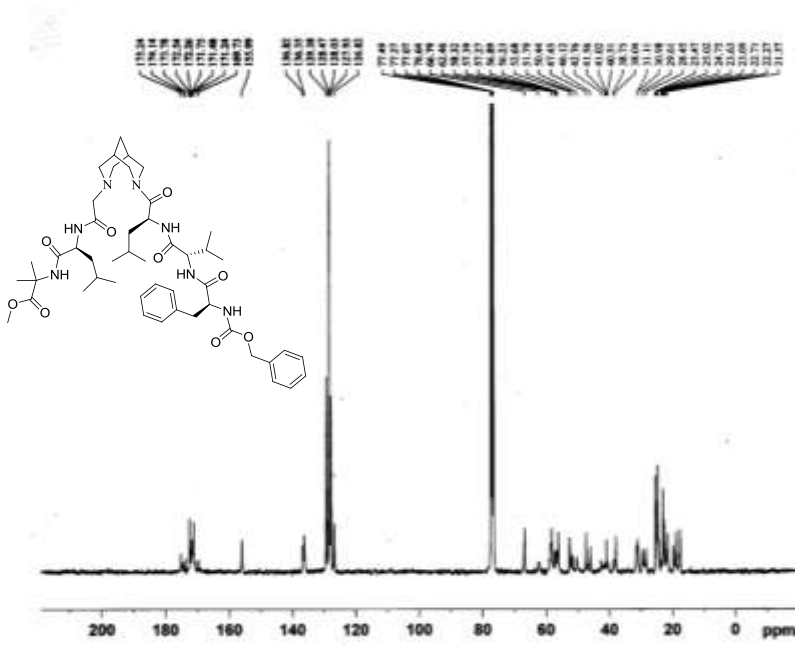
HRMS of compound **3d**



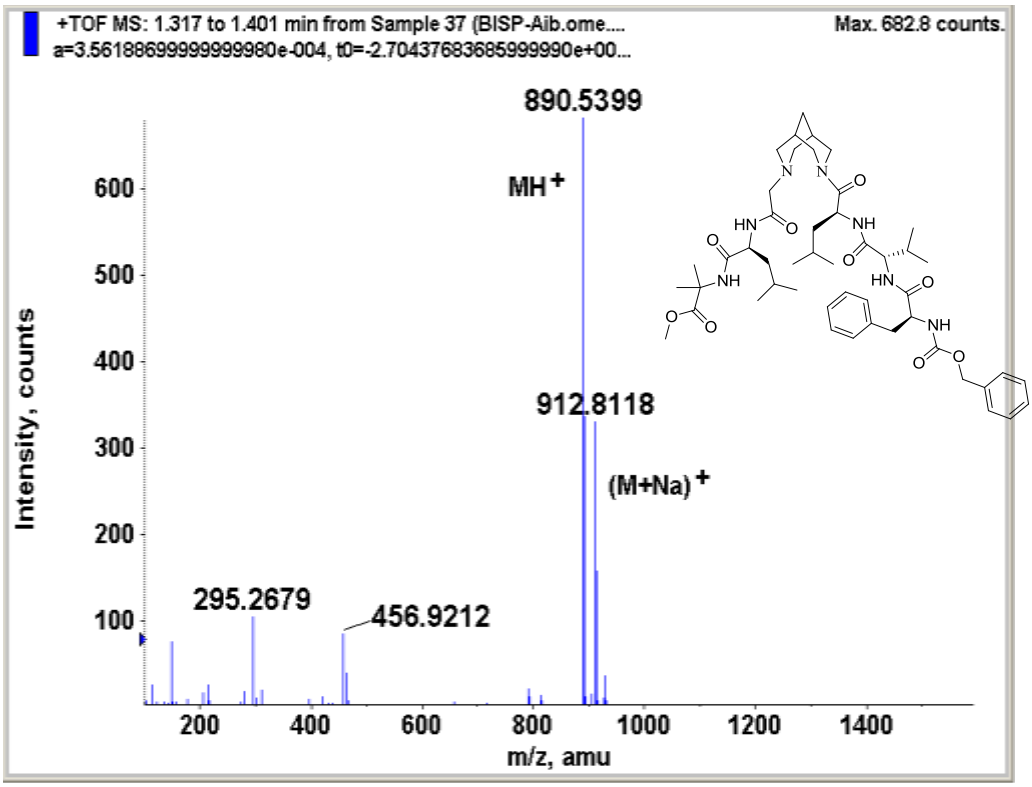
^{1}H NMR ($CDCl_3$, 300 MHz) of compound **B7**



^{13}C NMR ($CDCl_3$, 75 MHz) of compound **B7**



HRMS of Compound **B7**



Compound No	Ratio of major to minor conformers
B1	1: 0.85
B2	1:0.60
3a	1:0.30
B3	1:0.38
3b	1:0.31
3c	1:0.25
B5	Negligible amount of minor form

Table S1: Table showing the ratio of major to minor conformers of **B1**-**B7** in $CDCl_3$ based on ^1H NMR studies

2D NMR spectroscopy

COSY, ROESY, TOCSY and HSQC were recorded on a Bruker Avance 300 at 25^{o}C. ROESY spectrum of **B3** was recorded by collecting 1024 complex data points in the t_2 domain by averaging 32 scans and 256 increments in the t_1 domain with a mixing time 600ms. TOCSY spectrum was recorded by collecting 1024 complex data points in the t_2 domain by averaging 16 scans and 256 increments in the t_1 domain with a mixing time 80ms. All the data were processed and analyzed using Bruker TOPSPIN 2.1 program.

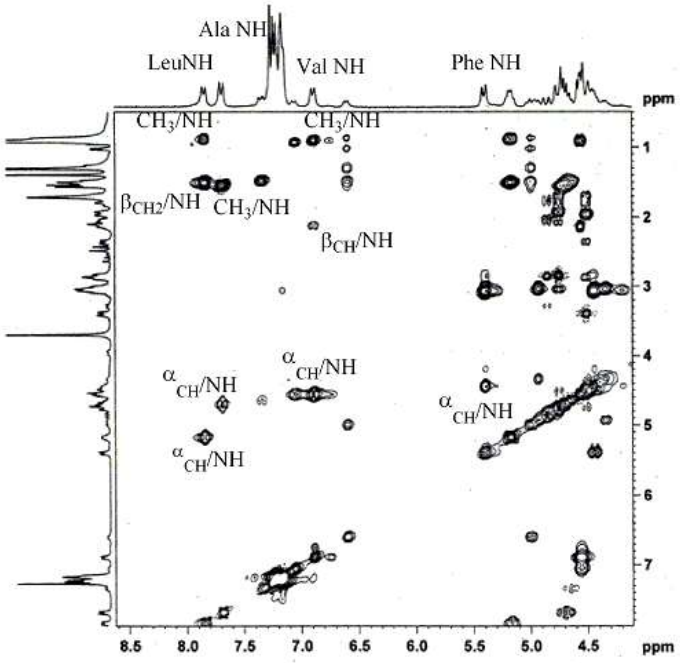


Figure S1: The expanded amide NH region of the TOCSY spectrum of **B3** in $CDCl_3$

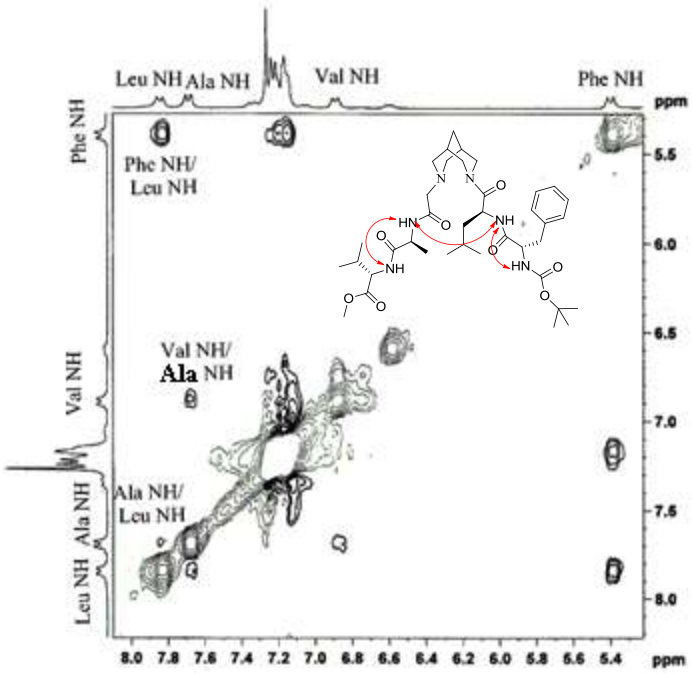


Figure S2: ROESY (300 MHz, $CDCl_3$) spectrum of **B3.** NOEs between Phe NH/Leu NH, Val NH/Ala NH, and Ala NH/Leu NH are marked in the spectrum.

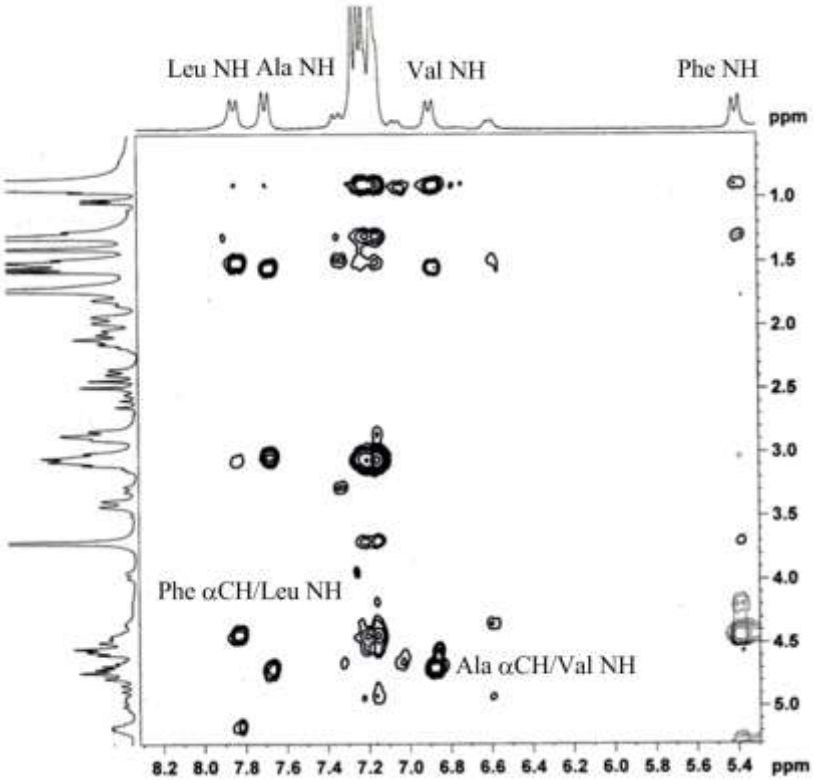


Figure S3: ROESY (300 MHz, $CDCl_3$) spectrum of **B3,** ROEs between α CH/ NH are marked in the spectrum

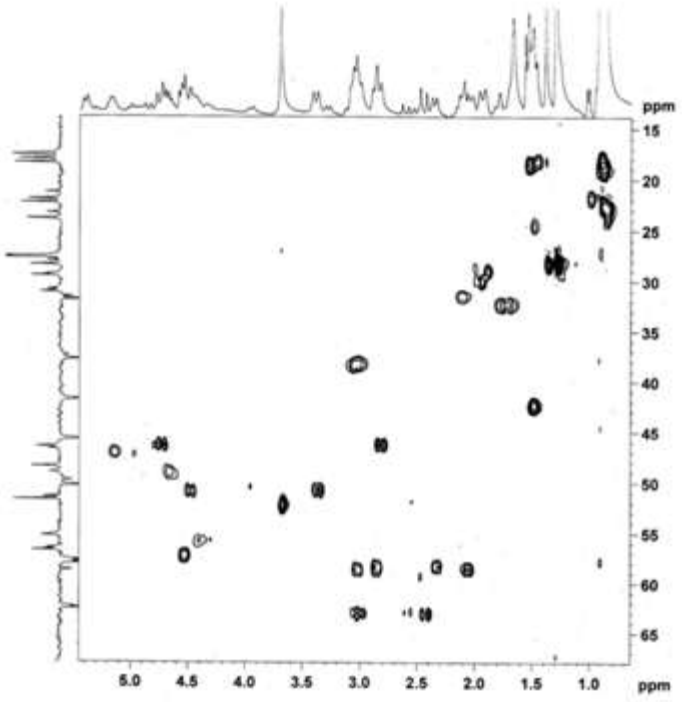
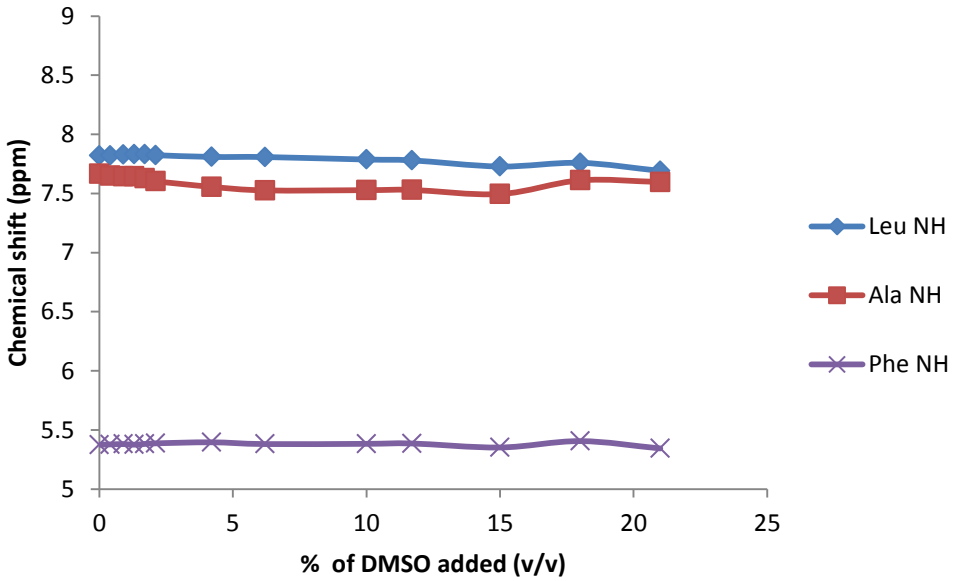
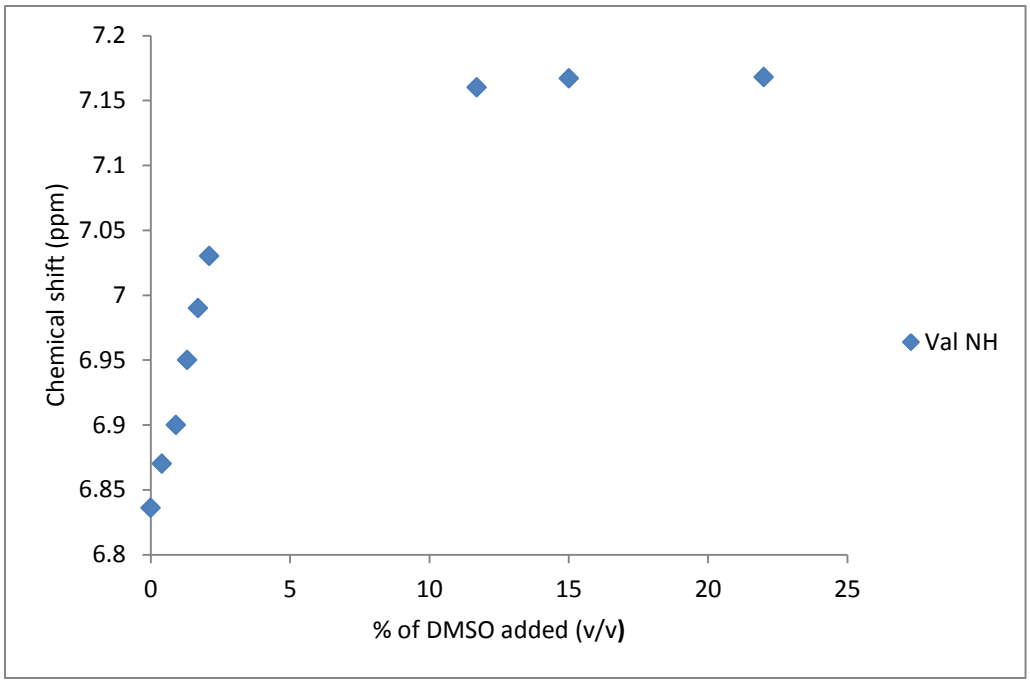
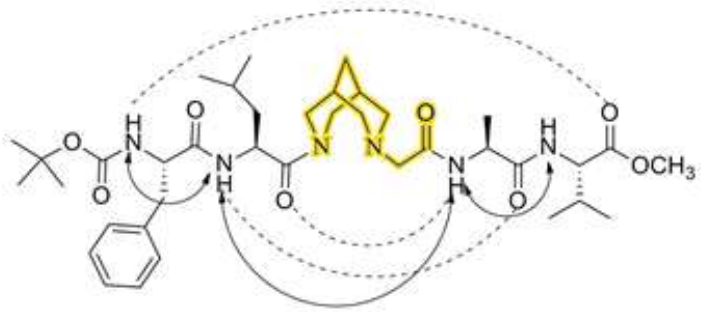


Figure S4: HSQC (300MHz, $CDCl_3$) of **B3**





a



b

Figure S5: a) Chemical shift of NHs versus % of DMSO added (v/v) to a solution of **B3** in $CDCl_3$. b) Observed NOEs in **B3** are represented in double headed arrows, and H-bonds are represented by the dotted lines. Hydrogen bonding donor acceptors are identified from combined NMR and FTIR studies.

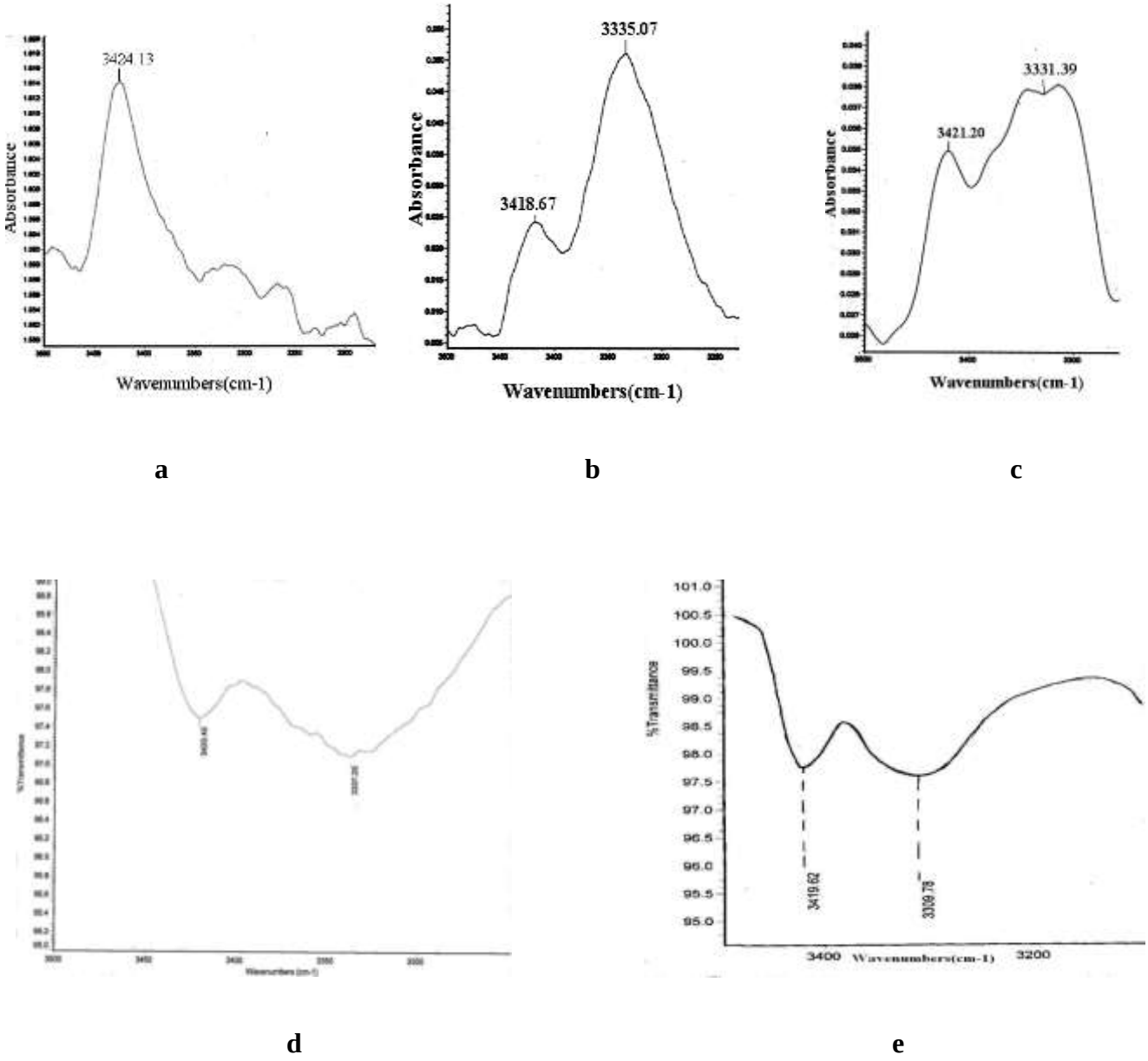
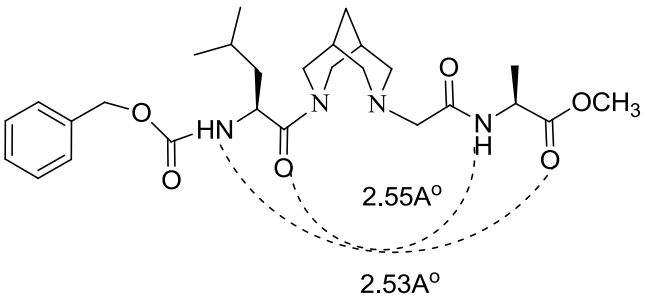
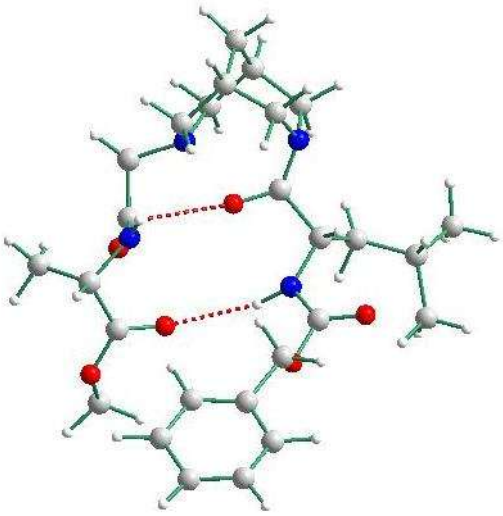
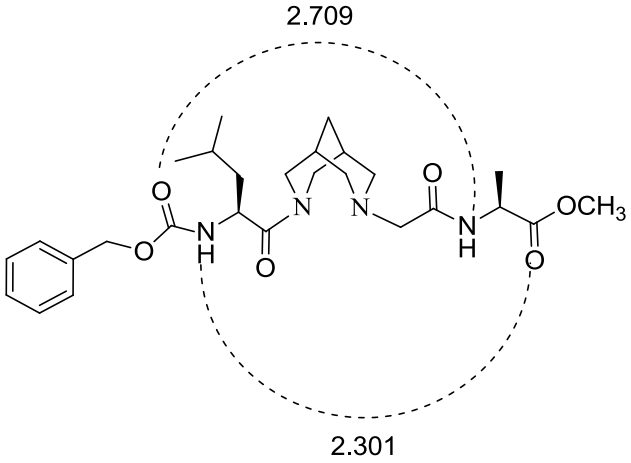
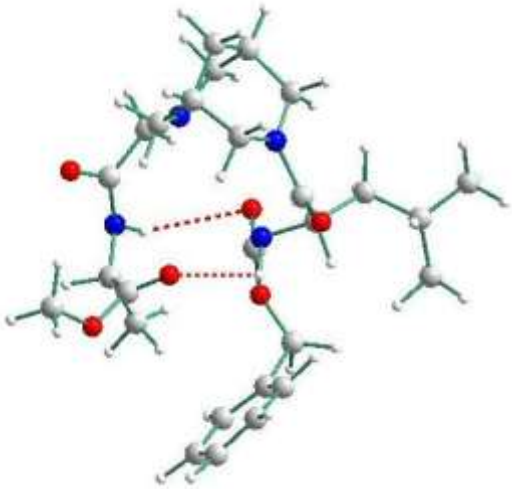


Figure S6: The NH strtetching region in the solution FT-IR spectrum of a) **B1**, b) **B2**, c) **B3**, d) **B6** and e) **B7**. Spectra presented are of 1 mM solution of the compounds in chloroform after subtraction of the spectrum of pure chloroform.

Protocol for Molecular modelling

The energy minimization and MD simulations were carried out with the aid of the SANDER module of the Amber 10. Compounds were solvated in an octahedron box of $CHCl_3$/MeOH at a 10Å distance between the compound surface and the box boundary. The"antechamber" module of Amber 10 was used for obtaing partial atomic charges for all the compounds. Bad steric contacts were removed by 1000 step minimization using the steepest descent algorithm followed by a 2000 step minimization using conjugate gradient Topology and parameter files for the compounds were prepared using "gaff" force field model.[1] The molecules were equillibrated with a fixed configuration of the solvent molecules and the system was heated from T=10 to 300K in sixty intervals of 5ps each. The entire system was then equilibrated at 300K for 300ps before 10ns MD simulation at room temperature. A periodic boundary condition in the NPT ensemble at T=298.15K with Berendsen temperature coupling and a constant pressure P=1atm with isotropic molecule-based scaling were employed for the MD simulations. A time step of 2fs and a nonbond-interaction cutoff radius of 12Å were used. The Particle Mesh Ewald (PME) method was used to treat long-range electrostatic interactions. The coordinates of the trajectory was sampled every 5ps for analysis of the energy stabilization.

No	Possible H-bonding and H-bond distances calculated from MD for **B2**	Strcuture after MD simulation
B2a		 Etot = -187.3865 Kcal/mol
B2b		 Etot = -78.6724 Kcal/mol

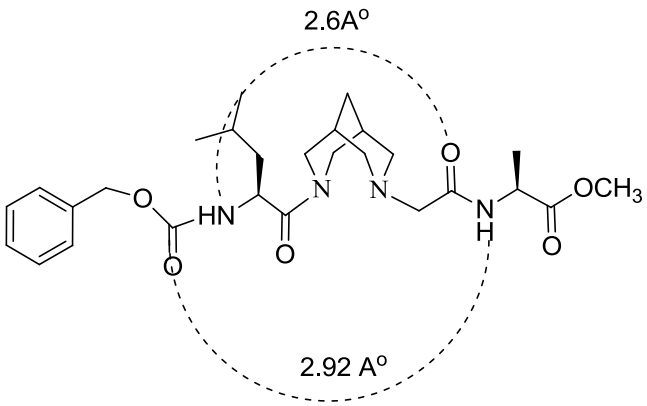
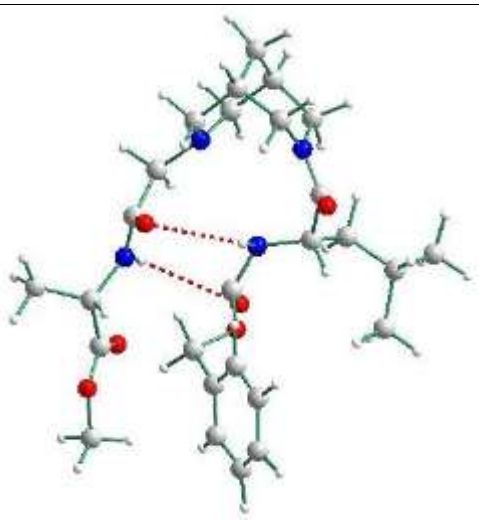
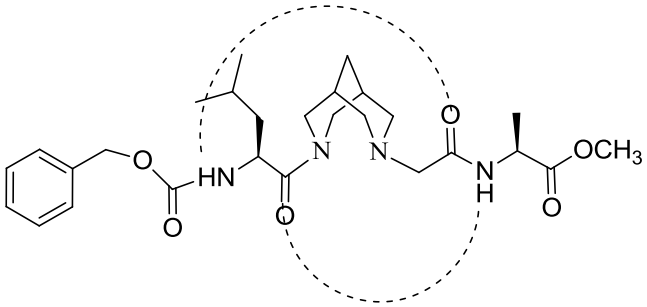
B2c		 Etot = -120.6647 Kcal/mol
B2d		Energetically not favoured

Table S2: Possible H-bonding pattern and H-bond distances calculated from MD for **B2**

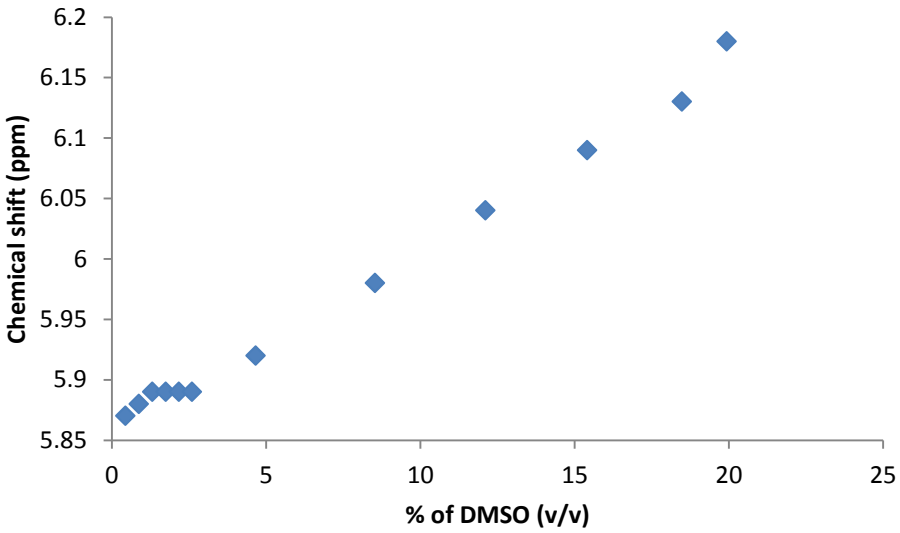


Figure S7: Chemical shift of Leu NH versus % of DMSO added (v/v) to a solution of **B1** in $CDCl_3$

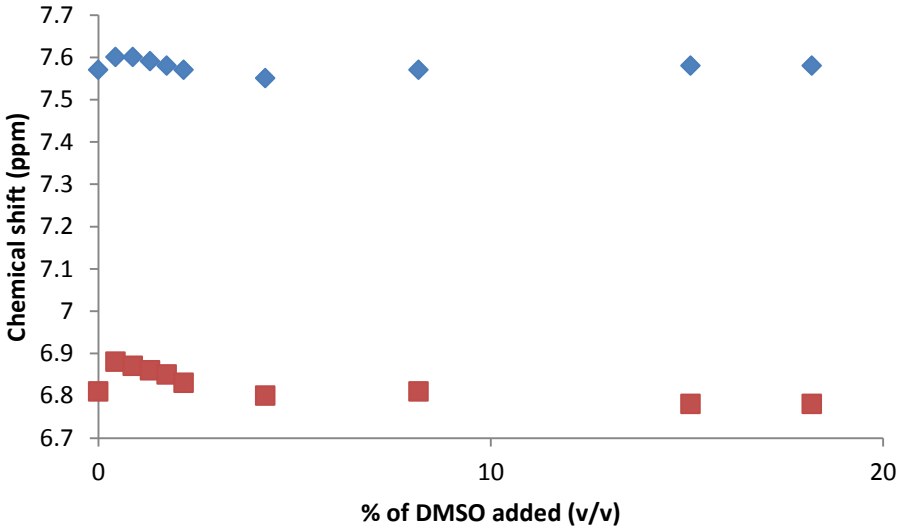
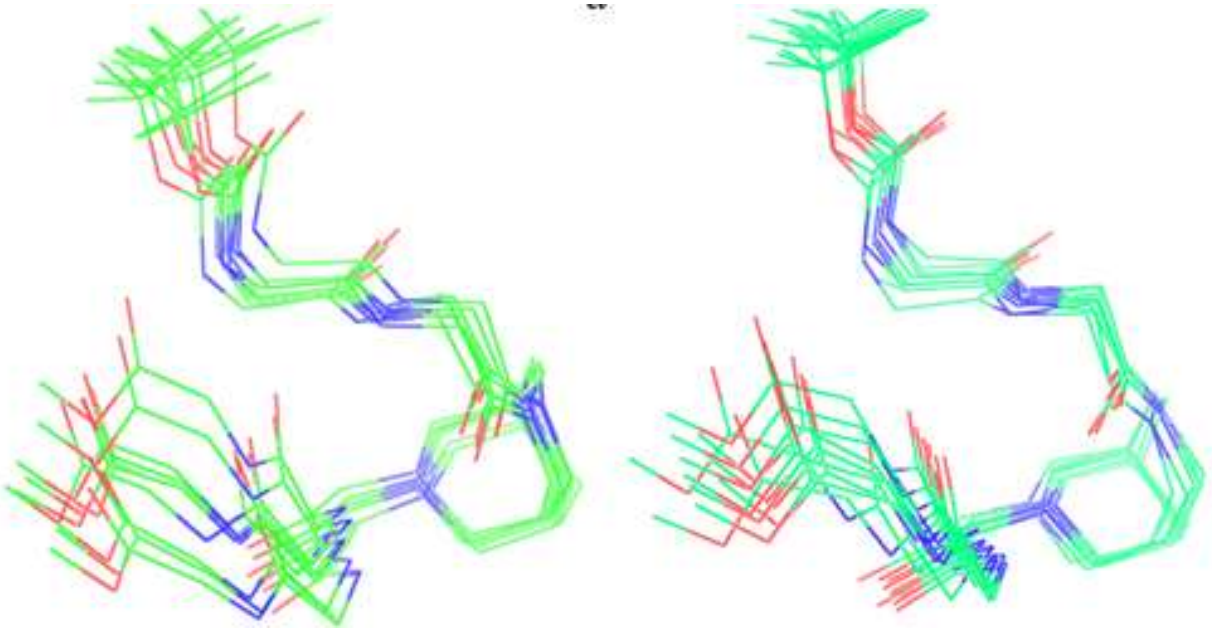
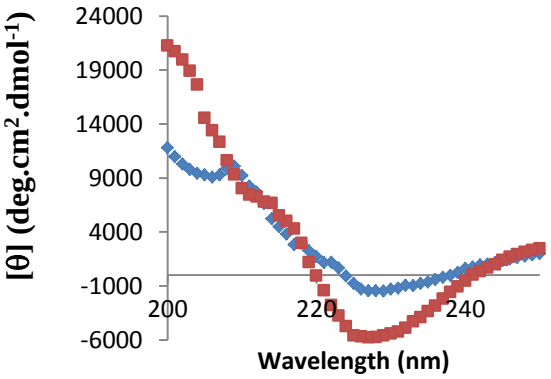


Figure S8: Chemical shift of NHs versus % of DMSO added (v/v) to a solution of **B2** in $CDCl_3$

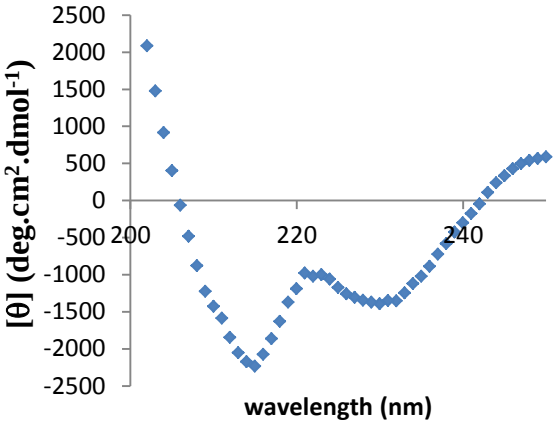


a b

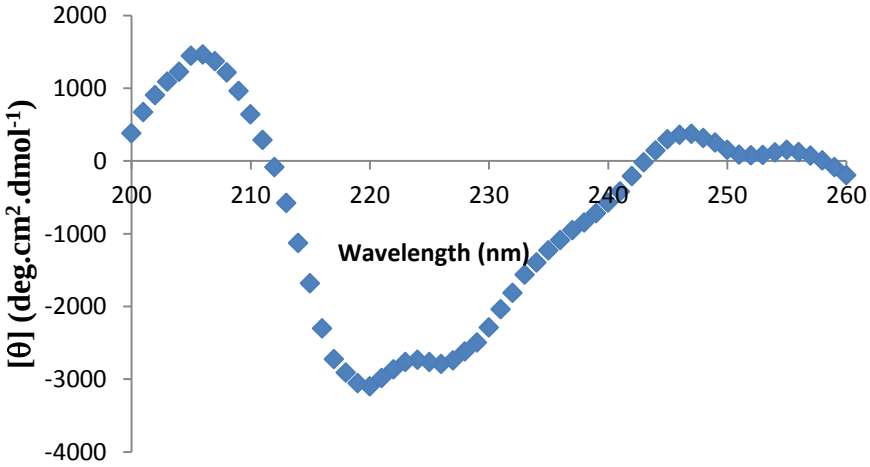
Figure S9: Overlap of ten minimum energy structures of **B3** obtained after performing MD simulation in a) methanol b) chloroform.


24000
19000
14000
9000
4000
-1000
-6000
200
220
240
[θ] (deg.cm2.dmol-1)
Wavelength (nm)

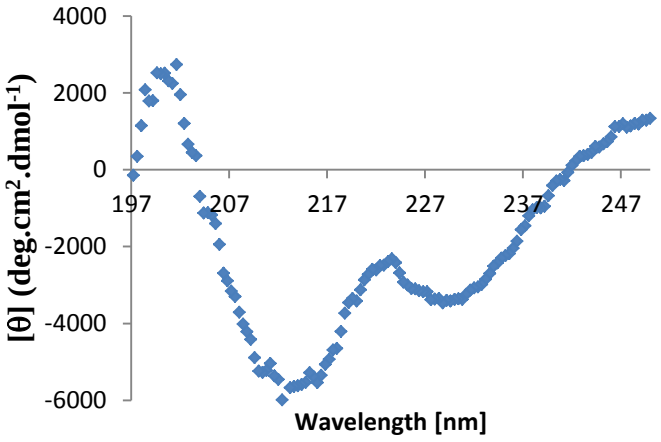
a

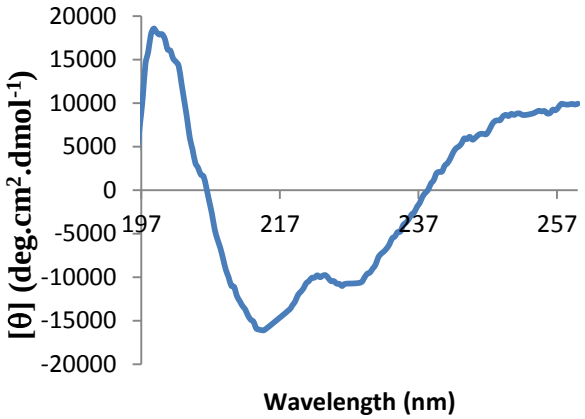

2500
2000
1500
1000
500
0
-500
-1000
-1500
-2000
-2500
200
220
240
[θ] (deg.cm2.dmol-1)
wavelength (nm)

b


2000
1000
0
-1000
-2000
-3000
-4000
200
210
220
230
240
250
260
[θ] (deg.cm2.dmol-1)
Wavelength (nm)

c





d **e**

Figure S10: CD spectrum of a) **B1**, **B2** b) **B3** c) **B5** d) **B6 e) B7** (250-500 μm) in methanol. The molar ellipticity remained constant between 250-500uM concentration range suggesting reduced aggregation effects.

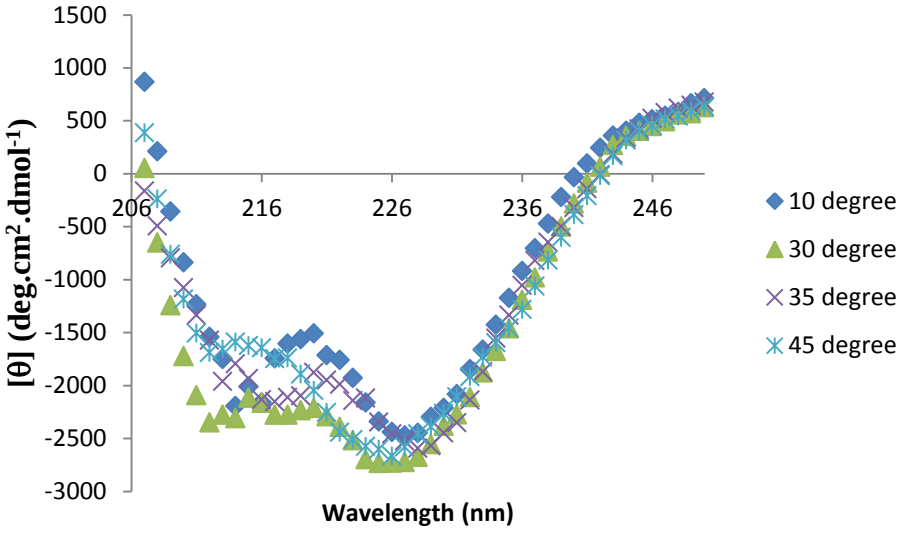


Figure S11: CD spectra showing little change in helicity of **B4** with temperature

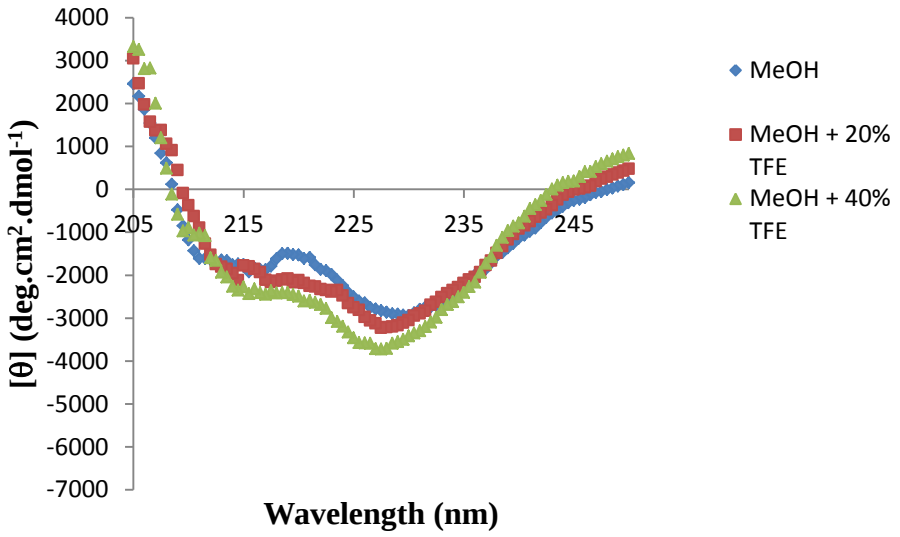
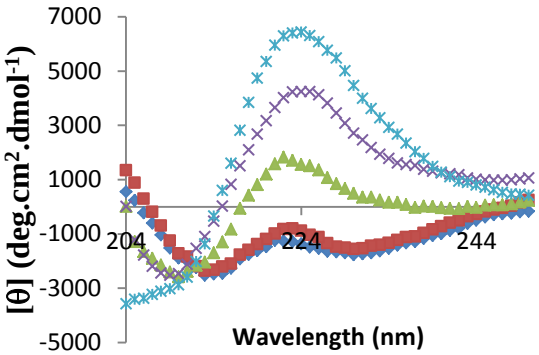
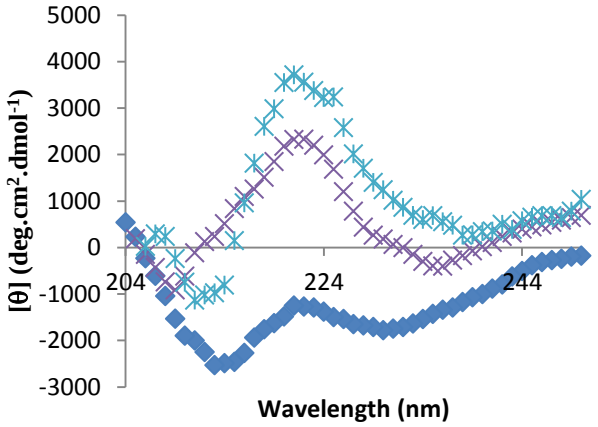


Figure S12: CD spectra showing little change in helicity of **B3** with TFE addition

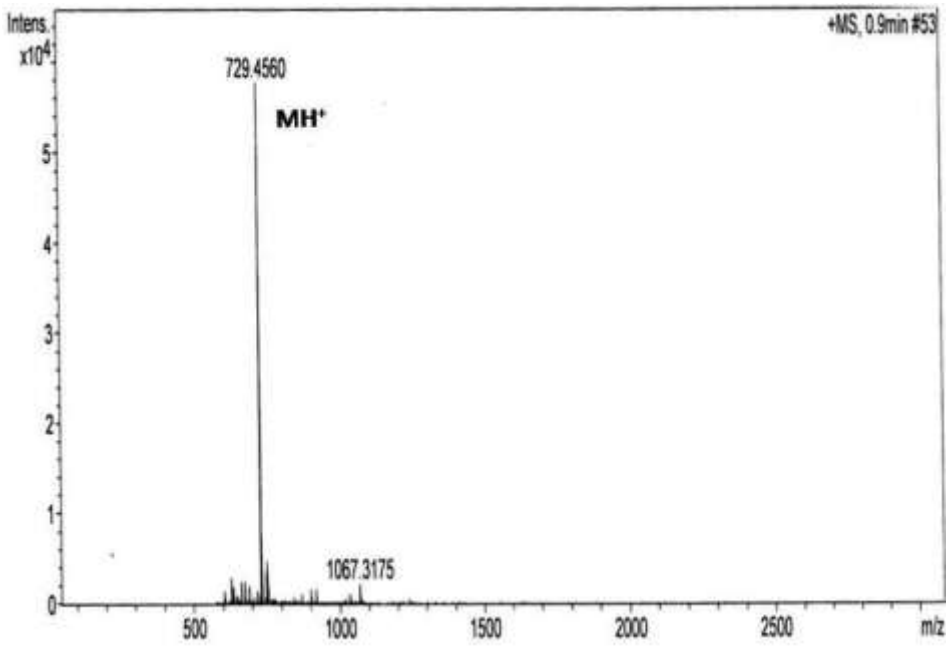
a)



b)



c)



d)

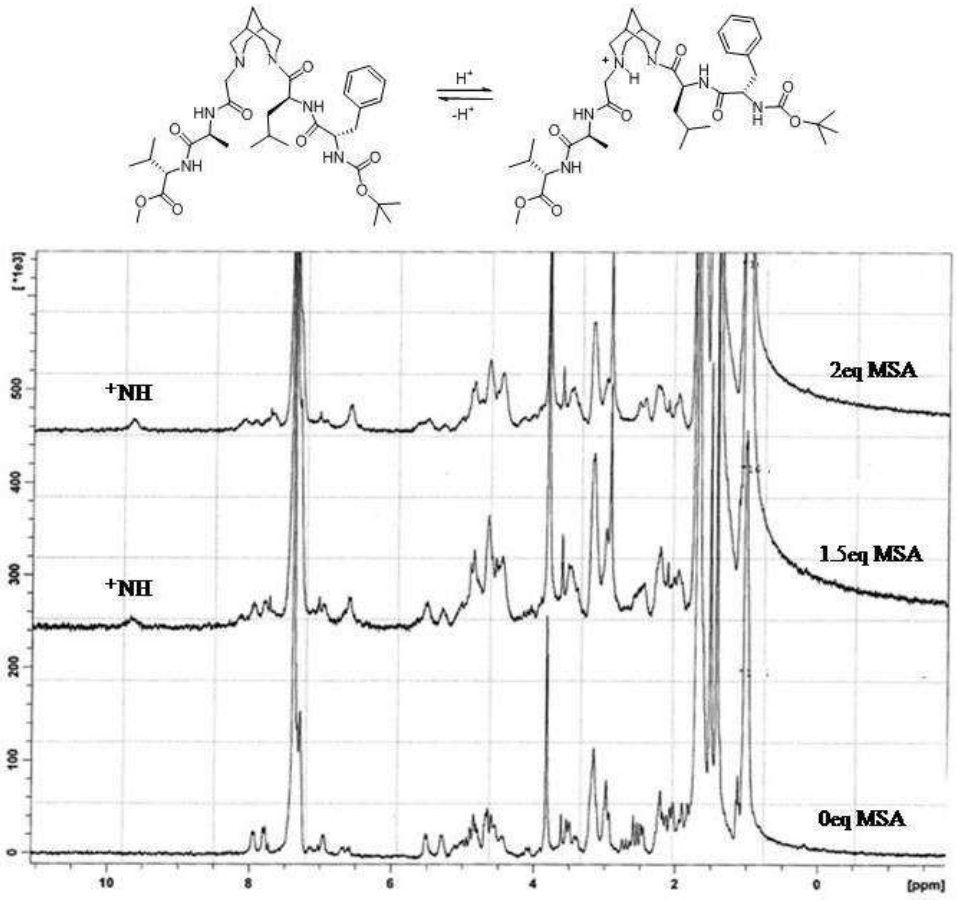


Figure S13: a) Changes in CD spectrum of **B3** with addition of ◊ (0.0), □ (0.5), Δ (1.0), x (1.5), * (2) equivalents trifluromethanesulfonic acid. b) Changes in CD spectrum of **B3** with addition of □ (0.0), x (1.0), * (2) equivalents methanesulfonic acid (MSA). c) HRMS of **B3** recorded after the addition of 2 equivalents of trifluromethanesulfonic acid. The data was collected after keeping the methanolic solution of **B3** containing 2eqvts. of trifluoromethanesulfonic acid for ~2hr d) Protonation equillibrium of **B3** and ^{1}HNMR showing the protonated species after the addition of 1.5, 2 equivalents of MSA.

Conformational analysis of B6

The ROESY spectrum of **B6** showed cross-peaks between NH (*i*)-C□H (*i+1*) and between Phe-NH and Val-NH ($d_{NN}(i, i+1)$), typical of a helical conformation (Figures S12-17). Solution FT-IR also supported an intramolecular hydrogen bonded conformation (Figure S6). The carbonyl stretch around 1666 cm^{-1} was consistent with a helical conformation.

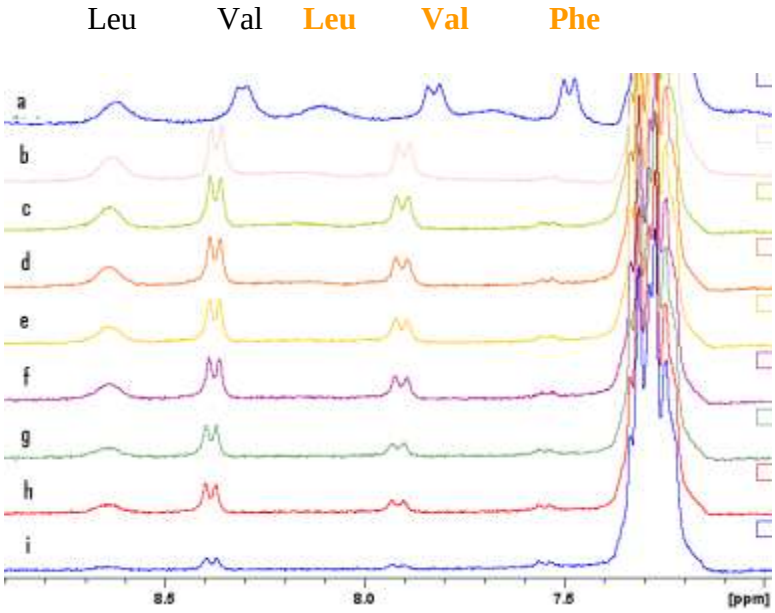


Figure S14: ^{1}H NMR spectra of **B6** in DMSO-d_6 was taken before and after adding D_2O. Spectra were recorded at different intervals (a) **B6** in DMSO-d_6, after addition of D_2O (b) after 10 min. (c) 40 min. (d) 80 min. (e) 133 min. (f) 156 min. (g) 193 min. (h) 343 min. (i) 403 min. (j) 17 hrs

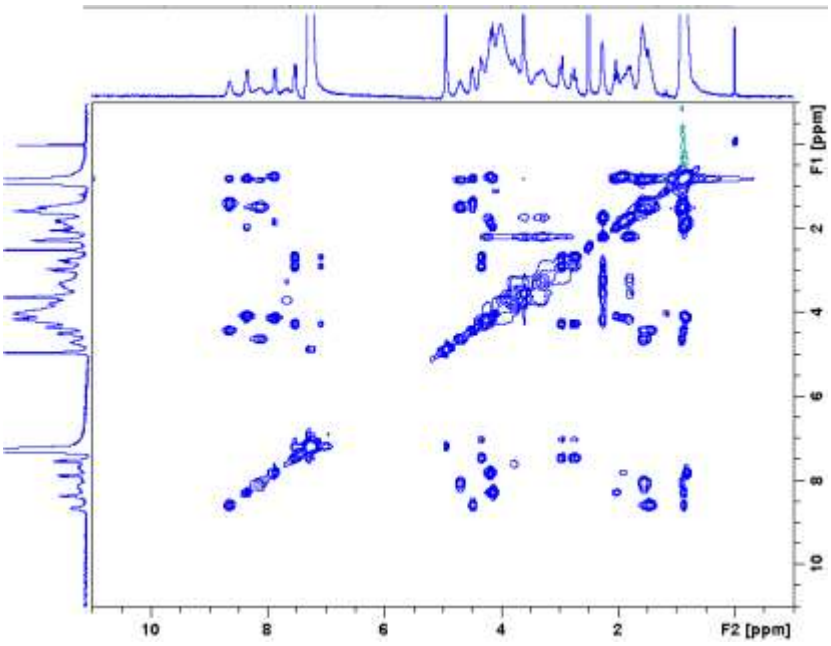


Figure S15: TOCY (300 MHz, DMSO-d_6) spectrum of **B6**

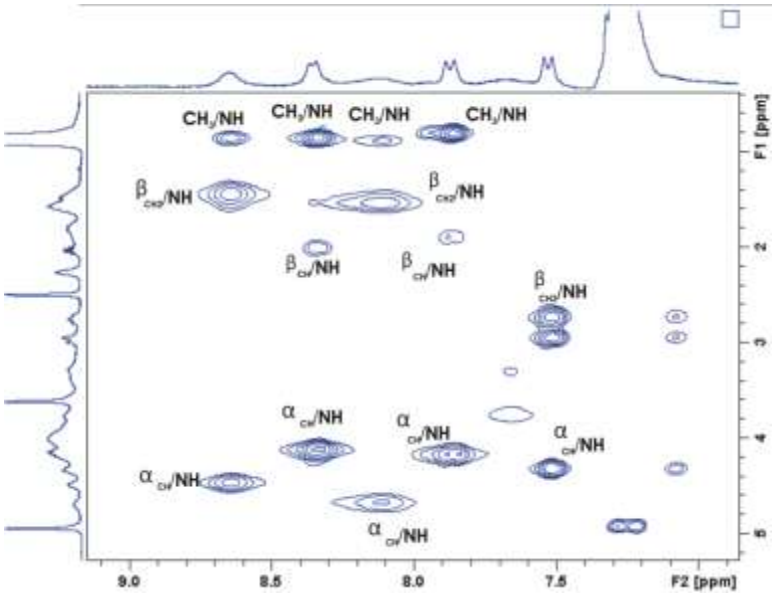


Figure S16: The expanded amide NH region of the TOCSY spectrum of **B6** in DMSO-d_6

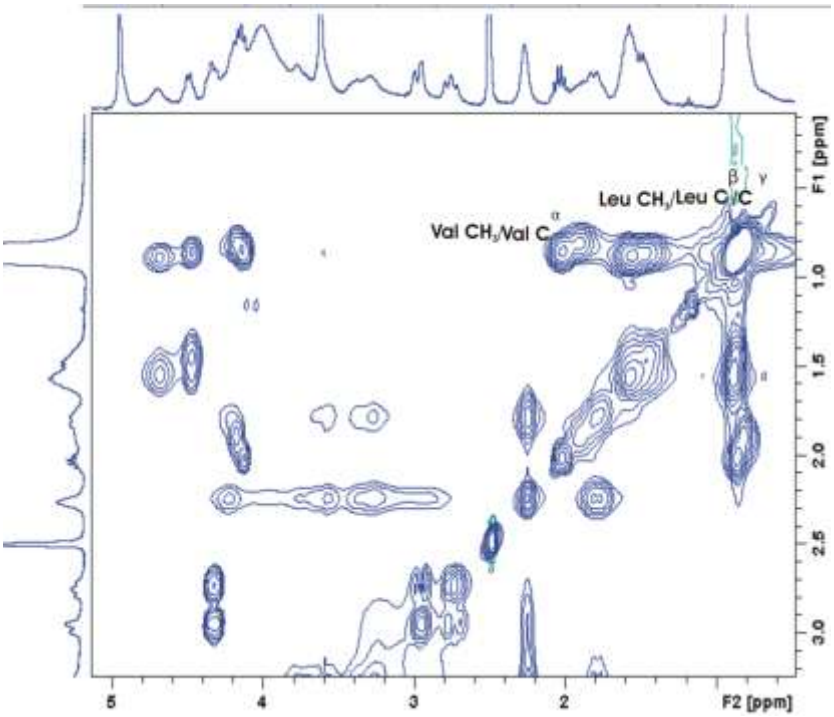


Figure S17: The expanded region of TOCSY spectrum of **B6** in DMSO-d_6

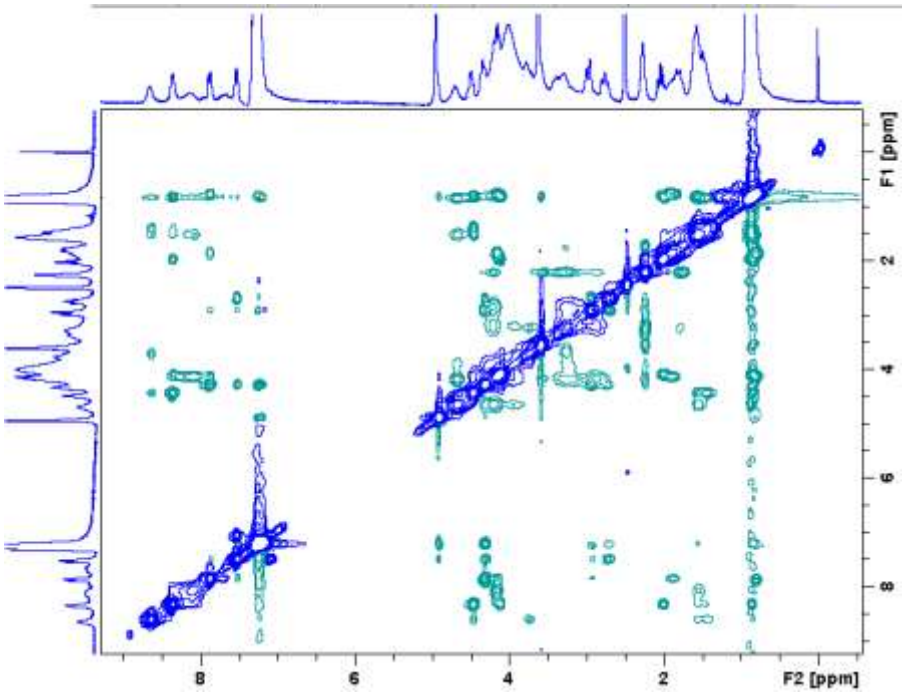


Figure S18: ROESY (300 MHz, DMSO-d_6) spectrum of **B6**.

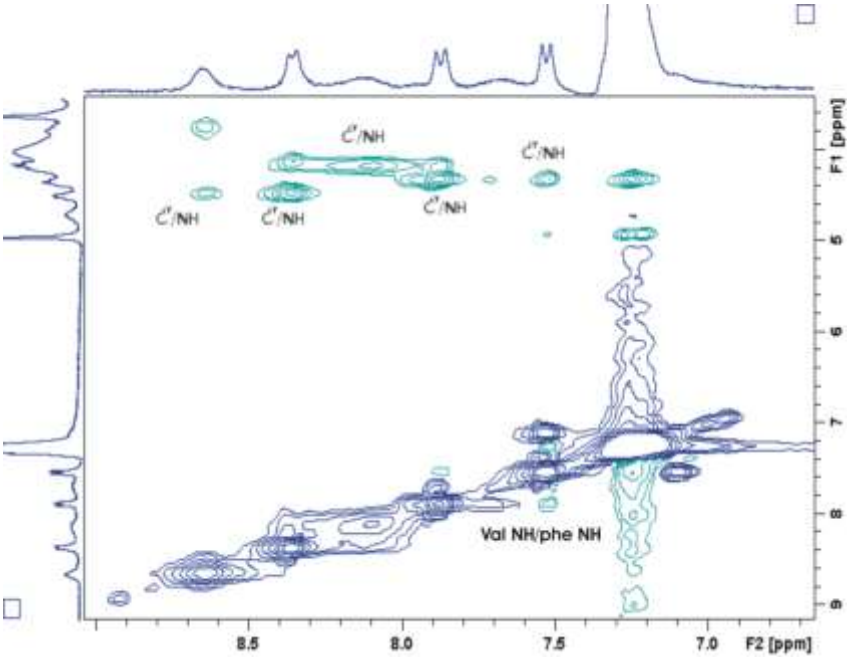


Figure S19: ROESY (300 MHz, DMSO-d_6) spectrum of **B6**. NOEs between Phe NH/Val NH and NH/C$^{\alpha}$H are marked in the spectrum.

Table S3. Crystal data and structure refinement for **B3** (CCDC 946097)

Empirical formula	C_{38} H_{60} N_6 O_8
Formula weight	728.92
Temperature	273(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 13.848(3) Å α= 90°.
b = 9.570(2) Å	β= 94.215(5)°.
c = 15.768(4) Å	γ = 90°.
Volume	2084.0(8) $Å^3$
Z	2
Density (calculated)	1.162 Mg/m^3
Absorption coefficient	0.082 mm^{-1}
F(000)	788
Crystal size	0.20 x 0.11 x 0.07 mm^3
Theta range for data collection	1.89 to 25.00°.
Index ranges	-16<=h<=16, -11<=k<=11, -18<=l<=18
Reflections collected	20052
Independent reflections	7293 [R(int) = 0.0619]
Completeness to theta = 25.00°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.942 and 0.839
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7293 / 7 / 469
Goodness-of-fit on F^2	1.097
Final R indices [I>2sigma(I)]	R1 = 0.0945, wR2 = 0.2053
R indices (all data)	R1 = 0.1256, wR2 = 0.2225
Absolute structure parameter	-1(2)
Largest diff. peak and hole	0.429 and -0.243 e.

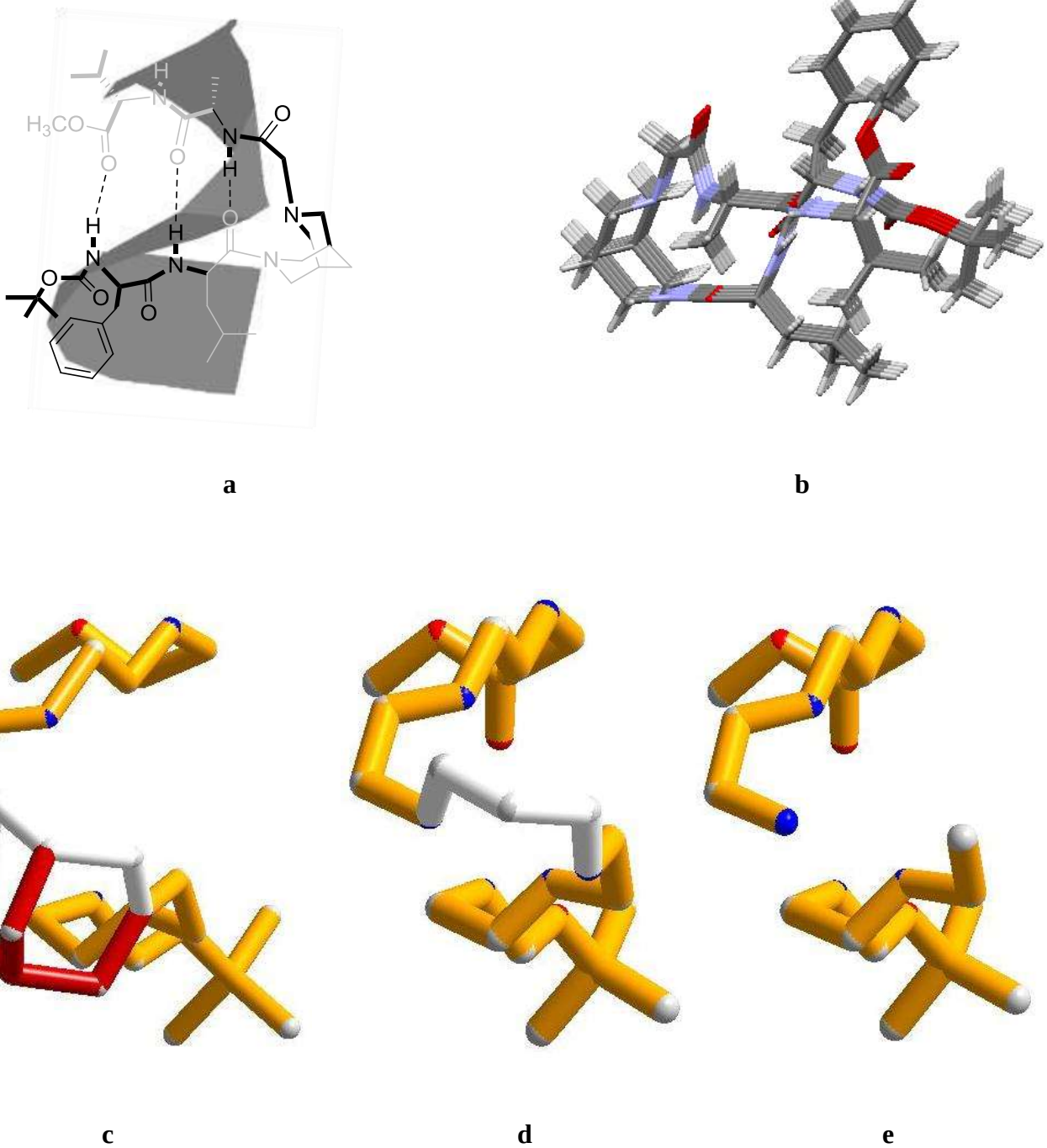


Figure S20. X-ray crystal structure of **B3** a) ribbon diagram based on Xray structure and the chemical structure of **B3** indicating hydrogen bonds b) view along b axis c) helical orientation of the peptide backbone, the hydrogen atoms and side chains were removed for clarity bispidine structure is coloured in red and white d) peptide backbone of **B3** keeping the 1, 3 diaminopropane unit (-NH-CH_2-CH_2-CH_2-NH-), other part of bispidine structure is removed for clarity. e) Peptide backbone of **B3** without bispidine unit, the two peptide fragments adopt right handed helical conformation.

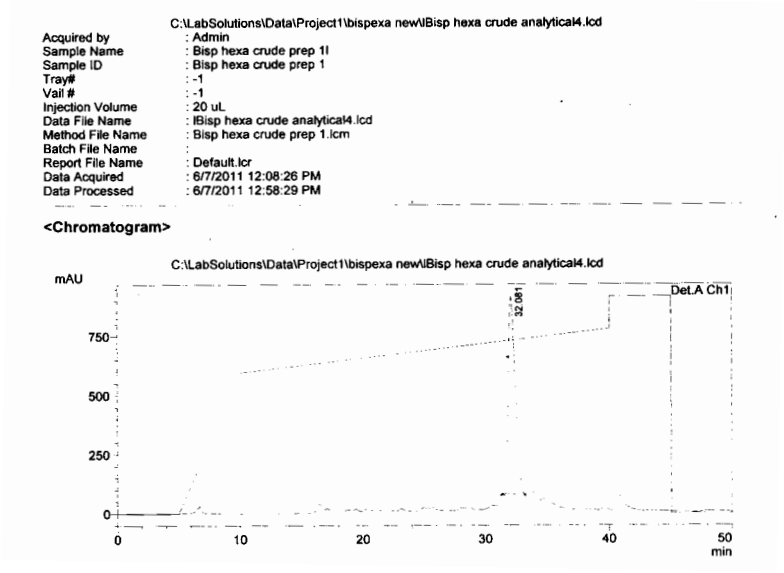
Residue	Angle	Distance O···HN	Distance N···O
Leu CO-NH Ala	146.10^{o}	2.338Å	3.089Å
Ala CO-HN Leu	163.91^{o}	2.072Å	2.908Å
Ala-CO- HN-Phe	161.34^{o}	2.396Å	3.22Å

Table S4 : Hydrogen bond parameters of **B3** as observed in the X-ray structure.

Residues	ϕ	Ψ
Ala	-96.14	179.74
Val*	57.60	46.77
Leu	-115.97	105.22
Phe	61.47	61.65

Table S5 : Dihedral angeles derived from Xray crystal structure of **B3.** * Val is a terminal residue with an ester linkage

a)



b)

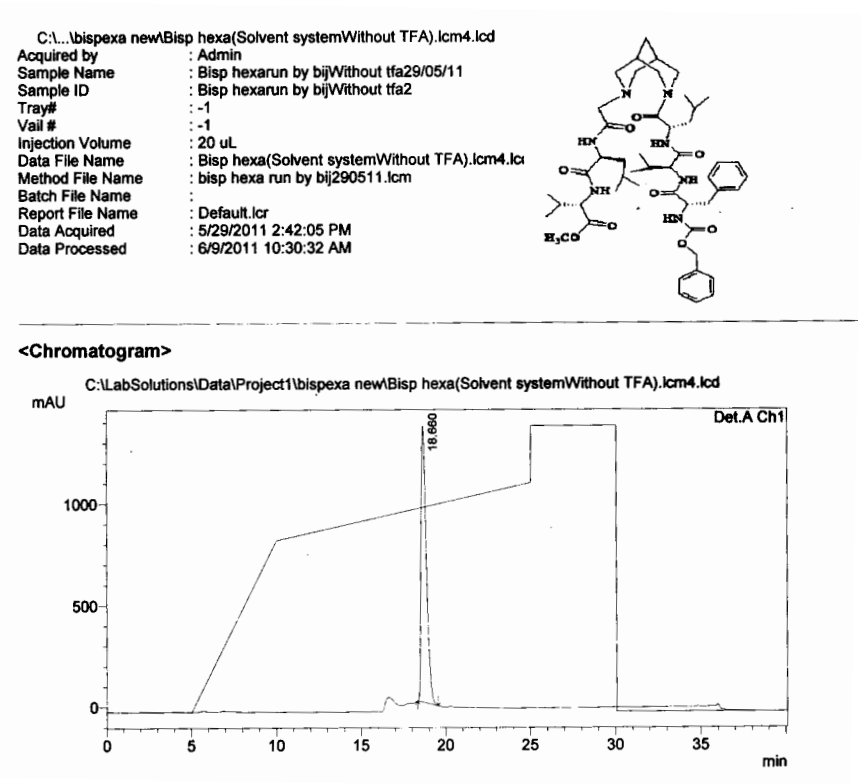


Figure S21: HPLC chromatograms of **B6** (a) Preparative HPLC after silica gel column chromatography (b) Analytical HPLC after purification by preparatory HPLC.

References

1. W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell and P. A. Kollman, *J. Am. Chem. Soc.* 1996, **118**, 2309-2309.