Supplementary Material (ESI) for Chemical Communications

Molecularly Imprinted Cavities Template the Macrocyclization of Tetrapeptide

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Detailed experimental procedure, and ^{1}H, ^{13}C NMR, and ESI-MS spectroscopic data of all the compounds

Experimental section

Materials:

Acrylamide, 3-methacryloxypropyltrimethoxysilane (MPS), tyramine (TA), 2,2'-azobisisobutyronitrile (AIBN), *N*,*N'*-ethylene bisacrylamide (EBAA), *O*-(7-azabenzotriazol-1-yl)-l,l,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), triethylamine, *N*-carbobenzyloxy-threoine methyl ester, cellulose fiber were obtained from Sigma-Aldrich (St. Louis, MO). DMF, DCM, CH_3CN, DIEA, ethanol and ethylene glycol were purchased from Merck. F-F-F-F, G-G-G-G, and P-P-P-P were purchased from Bachem. P-V-P-V and L-P-L-P were synthesized using solution phase synthesis (2 + 2). *N*-Acryltyramine (ATA) was synthesized from tyramine using acryloyl chloride.

General Procedure for Macrocyclization Using Molecularly Imprinted Polymer.

First stage: ***Construction of turn cavities on cellulose fiber.***

MPS-cellulose fiber formation: The cellulose fibers were Soxhlet-extracted with methanol for 24 h to remove contaminants and air-dried to constant weight. The cellulose fiber (4.5 g) was soaked in MPS (0.48 ml) and NEt_3 (20% molar with respect to MPS) solution (13.44 ml; $EtOH/H_2O$ = 4:1) at room temperature for 2 hours, followed by removing solution and curing in oven at 120 ^{o}C for 2 hour, in order to promote the actual chemical coupling, and then washed with ethanol. The

fibers were allowed to dry at room temperature for 12 hrs. The amount of silane adsorbed was measured by weight and checked with FTIR, as reported previously. [18]

Formation of molecularly imprinted polymer on cellulose fiber: The MPS-cellulose fiber (28.8 mg) was placed into a 4.7 ml vial with 120 μL of solution (Ethylene glycol:H_2O = 1:1), containing tetrapeptide/acrylamide/acryltyramine/EBAA at the 8:1:4:15 molar ratios (26.7 mM concentration of template). The vial was sealed tightly and heated in oven for 25 min at 140 ^oC. Subsequently, the resulting MIP-fiber was washed with 5% acetic acid to remove the template. This was followed by washing with a solution (Ethylene glycol:H_2O = 1:1) and drying.

Second stage: ***Solid phase association of tetrapeptide to their turn cavities***

The resulting MIP-fiber was refluxed with 0.5 mL of toluene containing tetrapeptide (3.2 μmole) at 110 ^oC for 6 hr. After removing the solvent, the complexed fiber was rinsed with distilled water to remove nonspecific bound tetrapeptide.

Third stage: ***Macrocyclization of tetrapeptide in their turn cavities***

The resulting MIP-peptide complex was mixed with HATU/HOAt (160 μmol) and DIEA (320 μmol) in DMF/DCM (1:3; 500 μl). The mixture was shaken (250 rpm) at room temperature for 6 h. The fiber was removed and dried with N_2 for 30 min. The product, which was formed on the MIP-cellulose, was extracted using 5 % acetic acid containing *N*-carbobenzyloxy-threoine methyl ester (1 mM) as the internal standard. After filtration, the filtrate was evaporated in vacuum. The yields of CTPs were determined using HPLC, (RP-18 column, UV 214 nm, mobile phase: 60% CH_3CN in 0.05 M, pH 2.4, triethylamine-H_3PO_4 buffer).

Preparative Procedure for Macrocyclization Using Molecularly Imprinted Cavities.

MIP-fiber (288 mg) was immersed in 5 mL of toluene containing tetrapeptide (6.4 mM) at 110 ^oC with a Dean-Stark trap for 6 h. The fiber was removed and dried with N_2 for 30 min. The MIP-peptide complex was then soaked in DMF/DCM = 1:3 (5 ml) with HATU/HOAt (1.6 μmole) and DIEA (3.2 μmole). The mixture was shaken (250

rpm) at room temperature for 6 h. After filtration, the MIP-CTP was washed with CH_3CN to remove HATU/HOAt and extracted three times using 5 % acetic acid in CH_3CN (10 ml). The MIF-cellulose was reused and the process was repeated 3 times. The extract was combined and the organic solvent was removed under reduced pressure. Purification on silica gel (MeOH/CH_2Cl_2 = 1:99 to 4:96) gave the CTP.

***Cyclo*-(**Gly-Gly-Gly-Gly**).**

^{1}H-NMR (300 MHz, $CDCl_3$) δ3.95 (s, 8H), 6.90 (s, 4H)

^{13}C-NMR (75 MHz, $CDCl_3$) δ 43.4, 174.6.

ESI-MS(m/z) : 229(M+H).

m/e calculated for $C_8H_{13}O_4N_4$ 229.0937, found 229.0939.

***Cyclo*-(**Phe-Phe-Phe-Phe**).**

$[\alpha]^{21}_D$ = −98.1 (*c* = 0.1 in $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.09~3.26 (m, 8H;), 4.73 (m, 4H;), 4.93 (s, 4H; NH), 7.03~7.33 (m, 20H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ 37.7, 54.2, 127.1, 128.6, 129.3, 135.7, 172.7.

ESI-MS(m/z) : 589(M+H).

m/e calculated for $C_{36}H_{37}O_4N_4$ 589.2815, found 589.2818.

***Cyclo*-(**Pro-Leu-Pro-Leu**).**

$[\alpha]^{20}_D$ = −76.5 (*c* = 0.1 in $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.81~0.87 (m, 12H), 1.48~1.59 (m, 6H), 1.97~ 2.30 (m, 8H,), 3.28~3.48 (m, 4H), 4.42 (m, 2H), 4.82 (m, 2H).7.35 (s, 2H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ 21.4, 22.8, 24.0, 24.7, 32.7, 41.7, 47.3, 49.7, 62.2, 171.6, 173.8.

ESI-MS(m/z) : 421(M+H).

m/e calculated for $C_{22}H_{37}O_4N_4$ 421.2815, found 421.2819.

***Cyclo*-(**Pro-Val-Pro-Val**).**

$[\alpha]^{20}_D$ = −198.4 (*c* = 0.1 in $CHCl_3$)

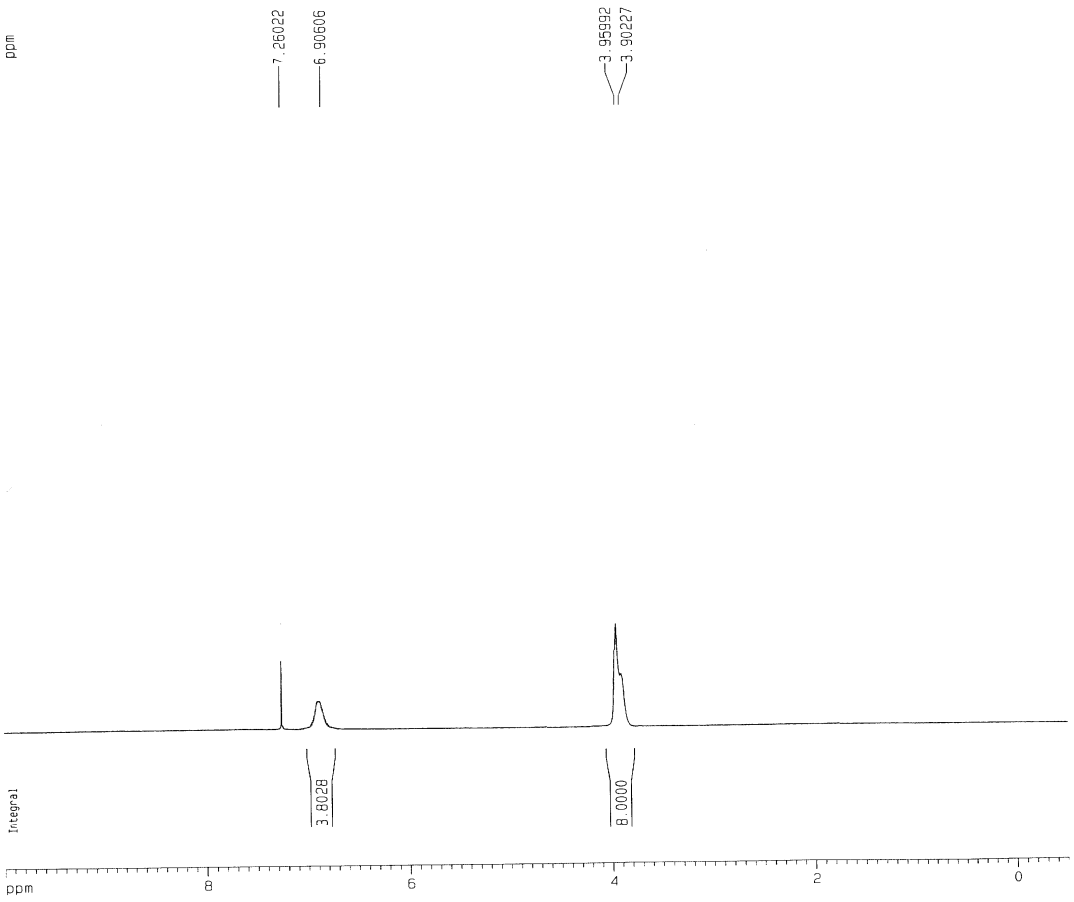
^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.94~1.03 (m, 12H), 1.97~2.07 (m, 8H), 2.35~2.38 (m, 2H), 3.47~3.51 (m, 2H), 3.62~3.66 (m, 2H), 4.14~4.16 (m, 2H), 4.41~4.46 (m, 2H). 7.55 (s, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ 17.5, 19.3, 23.8, 28.5, 32.4, 47.6, 57.0, 61.1, 61.3, 172.9, 173.7.

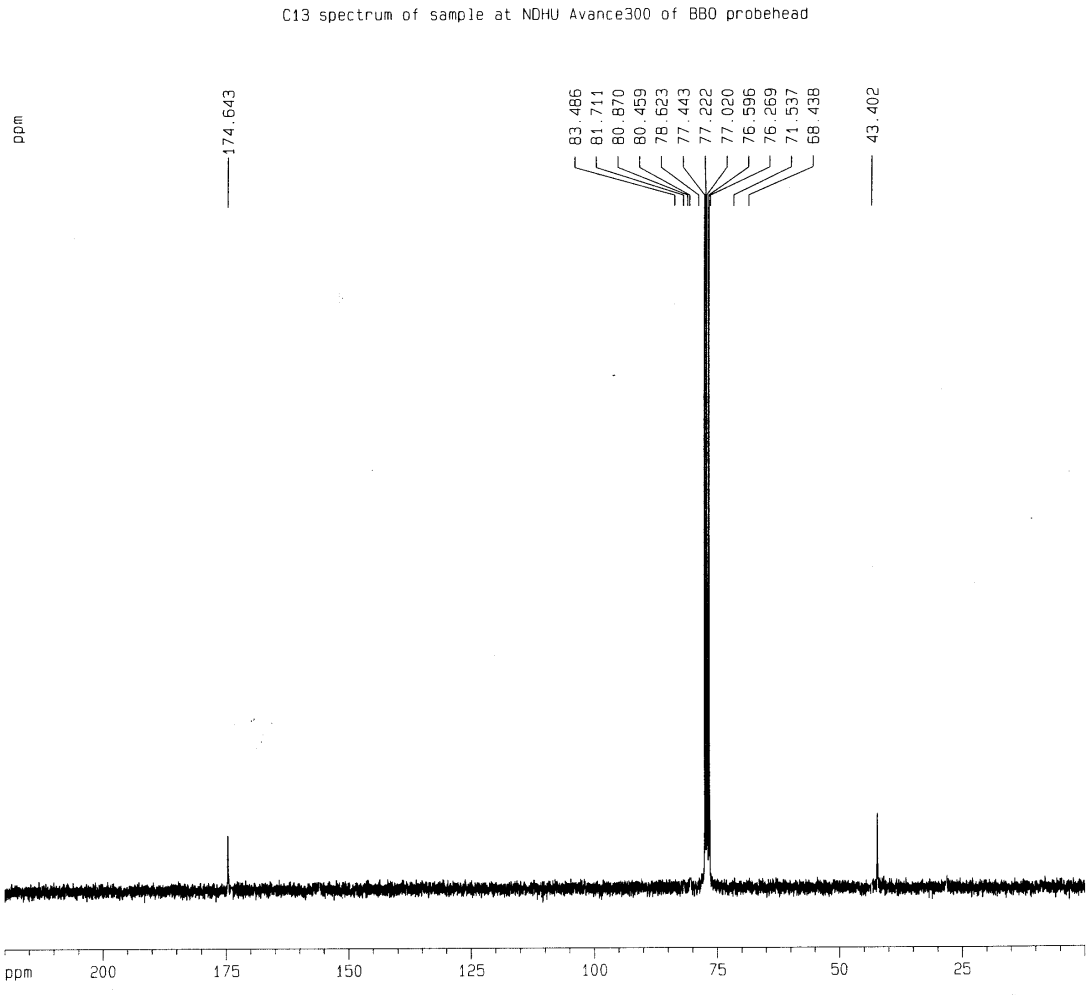
ESI-MS(m/z) : 393(M+H).

m/e calculated for $C_{20}H_{33}O_4N_4$ 393.2502, found 393.2506.

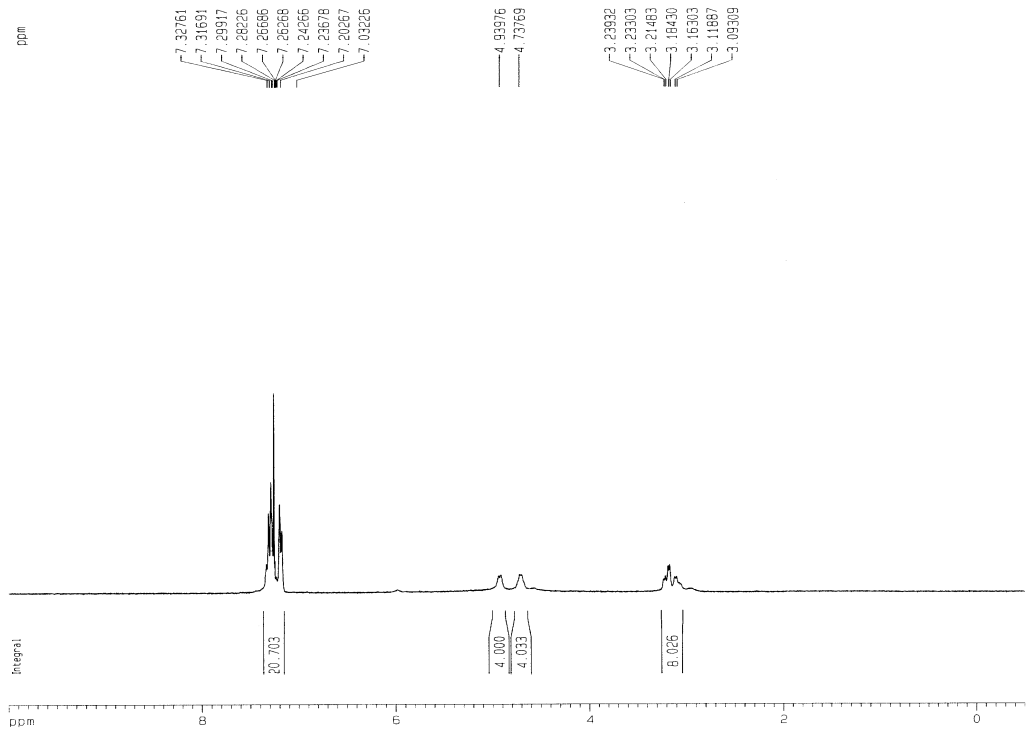
CTPs ^{1}H and ^{13}C NMR data



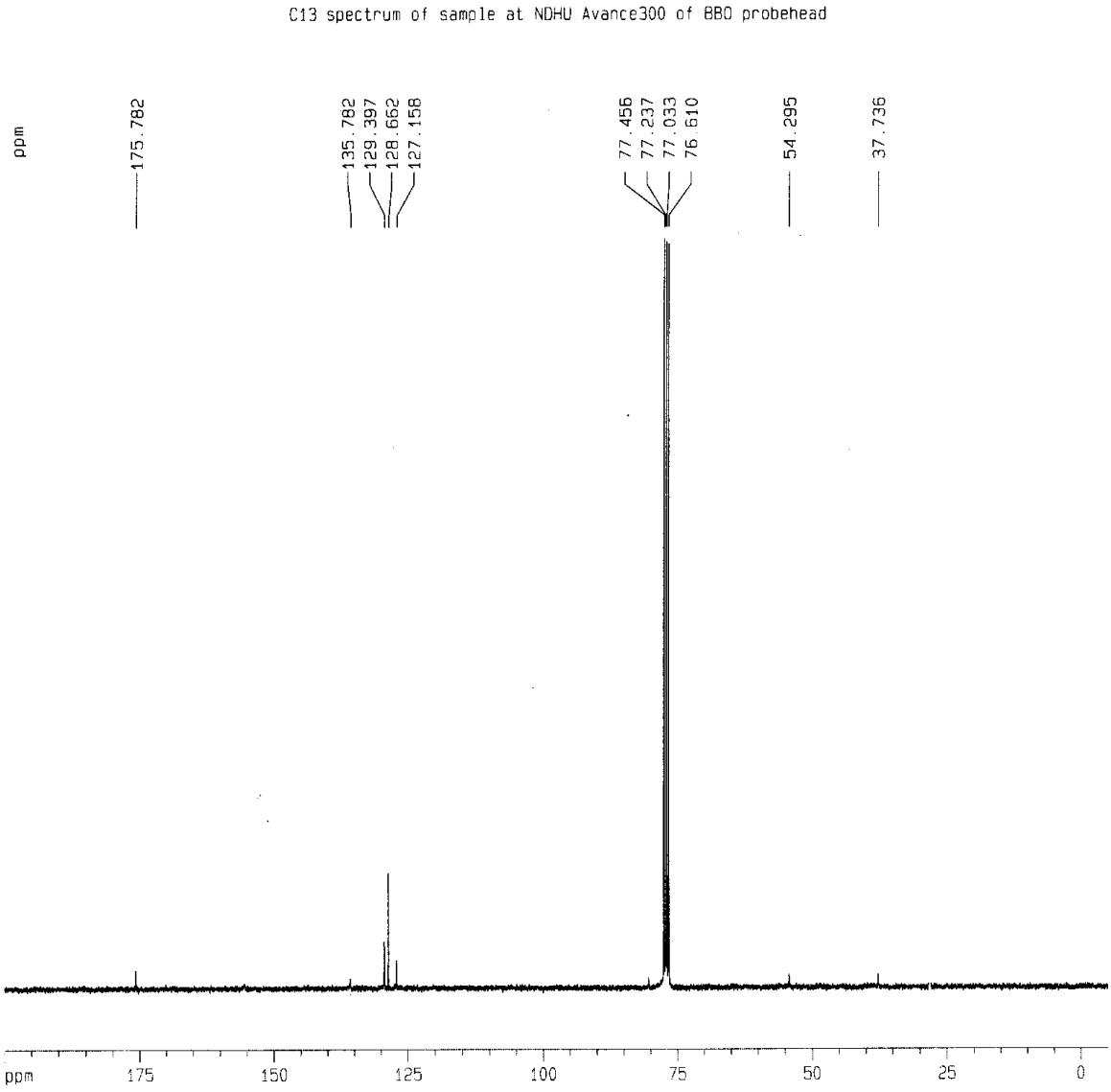
Cyclo-(Gly-Gly-Gly-Gly): ^{1}H-NMR (300 MHz, $CDCl_3$)



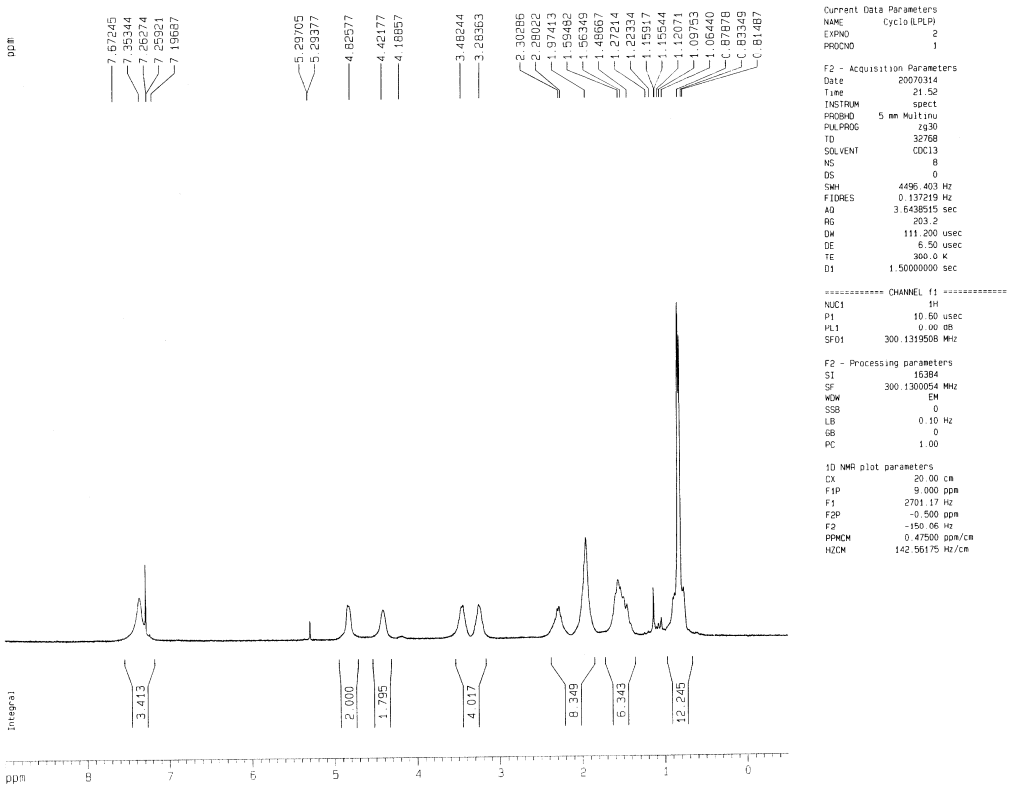
Cyclo-(Gly-Gly-Gly-Gly): ^{13}C-NMR (75 MHz, $CDCl_3$)



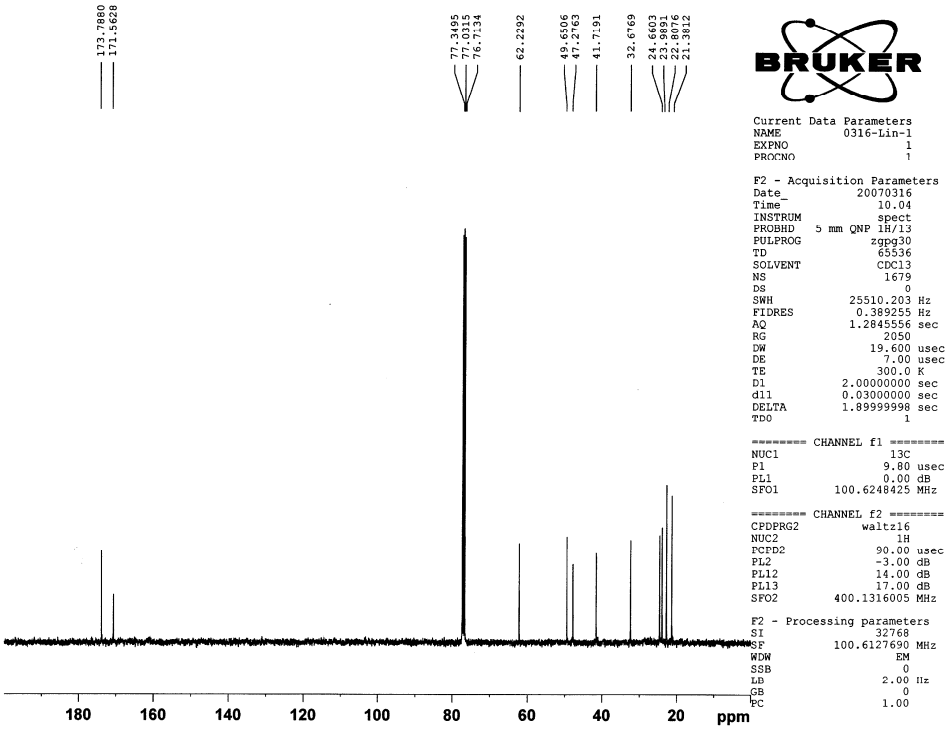
Cyclo-(Phe-Phe-Phe-Phe): ^{1}H-NMR (300 MHz, $CDCl_3$)



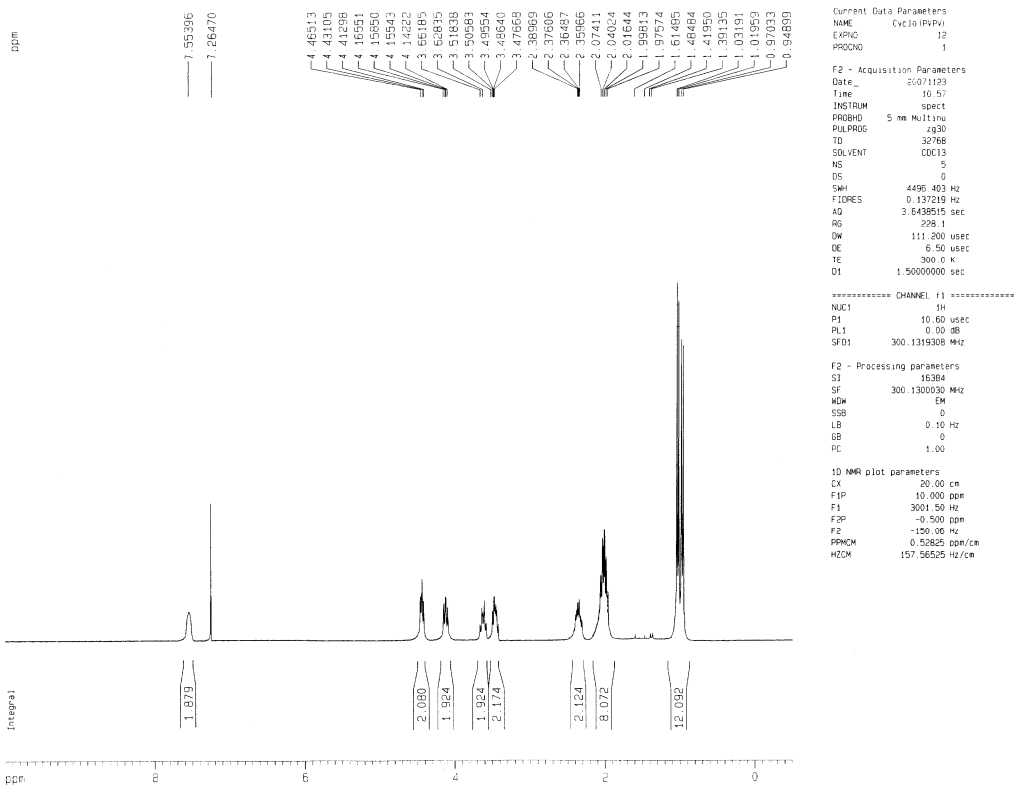
Cyclo-(Phe-Phe-Phe-Phe): ^{13}C-NMR (75 MHz, $CDCl_3$)



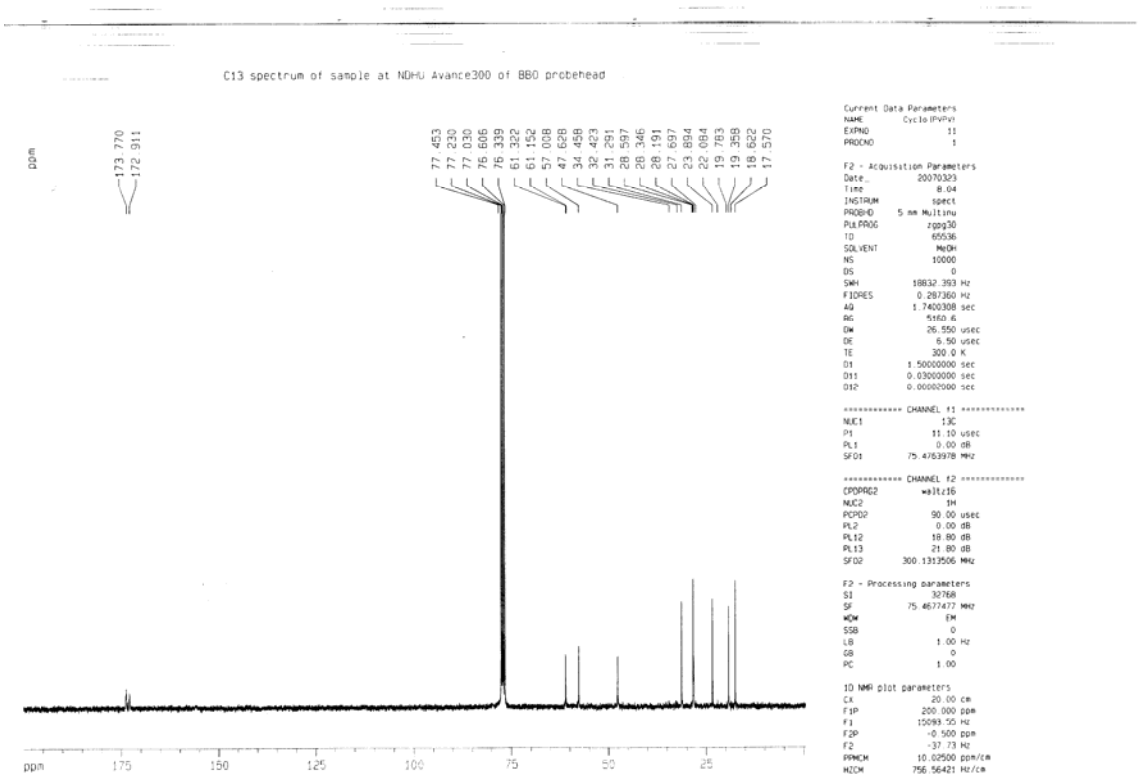
Cyclo-(Pro-Leu-Pro-Leu) ^{1}H-NMR (300 MHz, $CDCl_3$)



Cyclo-(Pro-Leu-Pro-Leu) ^{13}C-NMR (100 MHz, $CDCl_3$)

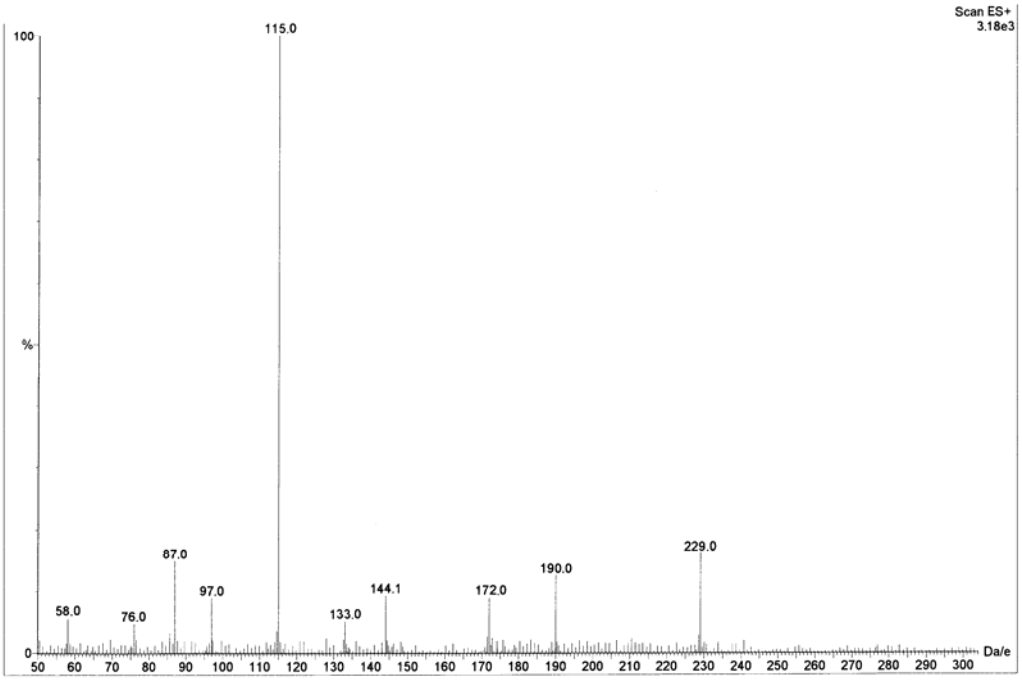


Cyclo-(Pro-Val-Pro-Val) ^{1}H-NMR (300 MHz, $CDCl_3$)

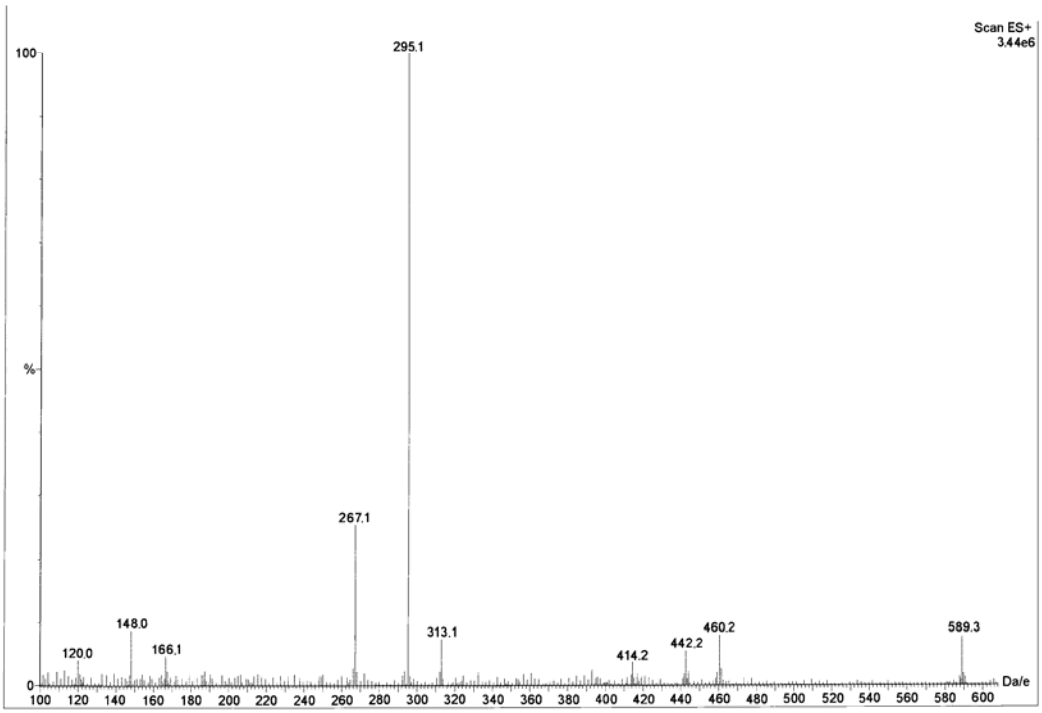


Cyclo-(Pro-Val-Pro-Val) ^{13}C-NMR (75 MHz, $CDCl_3$)

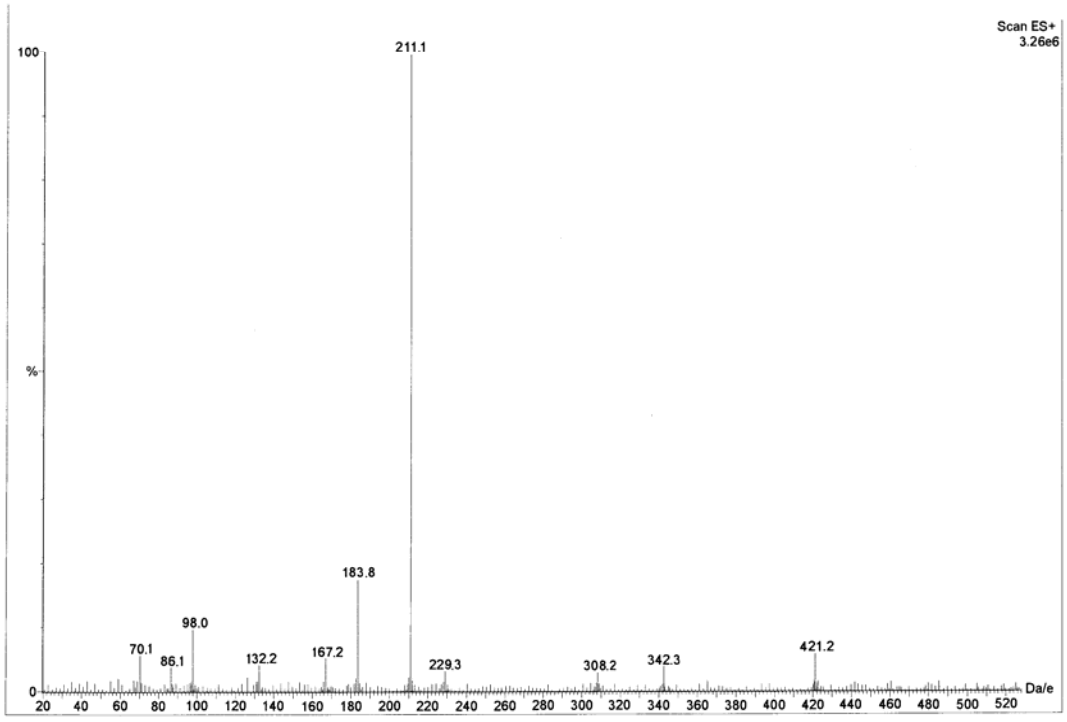
ESI-MS(m/z)



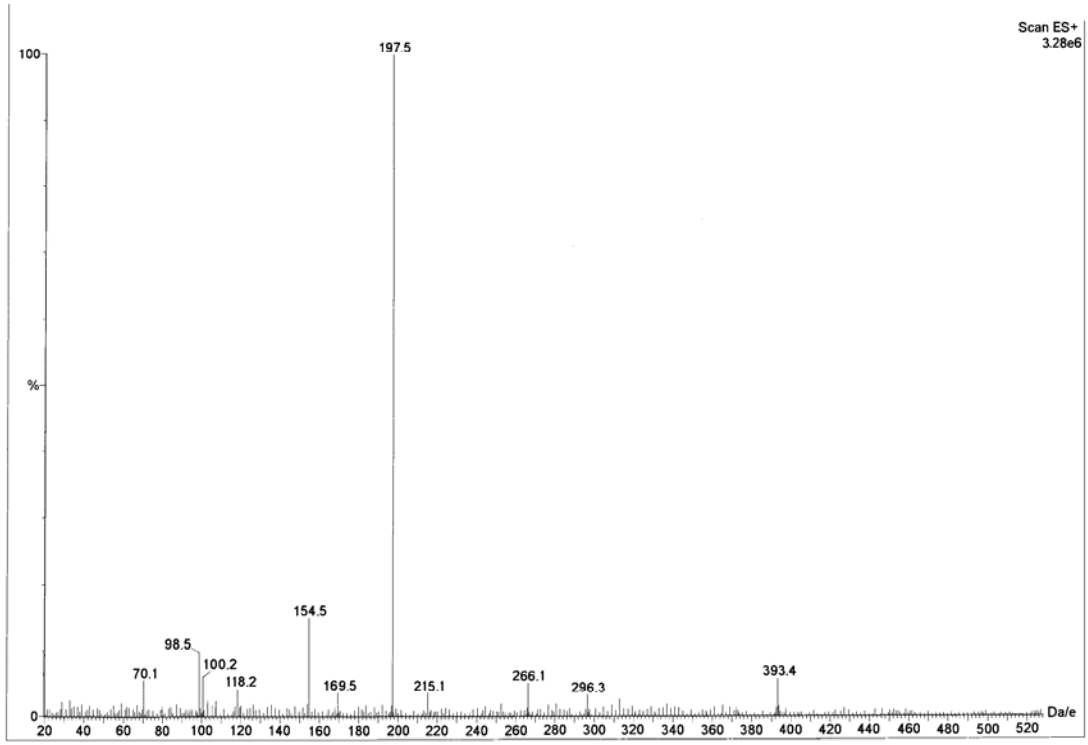
Cyclo-(Gly-Gly-Gly-Gly)



Cyclo-(Phe-Phe-Phe-Phe)



Cyclo-(Pro-Leu-Pro-Leu)



Cyclo-(Pro-Val-Pro-Val)