

Molecularly Imprinted Cavities Template the Macrocyclization of Tetrapeptide

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Detailed experimental procedure, and ^1H , ^{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)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), triethylamine, *N*-carbobenzyloxy-threonine 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 °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 μ L of solution (Ethylene glycol:H₂O = 1:1), containing tetrapeptide/acrylamide/acrylytyramine/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 °C. 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:H₂O = 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 μ mole) at 110 °C 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 μ mol) and DIEA (320 μ mol) in DMF/DCM (1:3; 500 μ l). The mixture was shaken (250 rpm) at room temperature for 6 h. The fiber was removed and dried with N₂ for 30 min. The product, which was formed on the MIP-cellulose, was extracted using 5 % acetic acid containing *N*-carbobenzyloxy-threanine 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% CH₃CN in 0.05 M, pH 2.4, triethylamine-H₃PO₄ 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 °C with a Dean-Stark trap for 6 h. The fiber was removed and dried with N₂ for 30 min. The MIP-peptide complex was then soaked in DMF/DCM = 1:3 (5 ml) with HATU/HOAt (1.6 μ mole) and DIEA (3.2 μ mole). The mixture was shaken (250

rpm) at room temperature for 6 h. After filtration, the MIP-CTP was washed with CH₃CN to remove HATU/HOAt and extracted three times using 5 % acetic acid in CH₃CN (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/CH₂Cl₂ = 1:99 to 4:96) gave the CTP.

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

¹H-NMR (300 MHz, CDCl₃) δ 3.95 (s, 8H), 6.90 (s, 4H)

¹³C-NMR (75 MHz, CDCl₃) δ 43.4, 174.6.

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

m/e calculated for C₈H₁₃O₄N₄ 229.0937, found 229.0939.

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

[α]²¹_D = -98.1 (c=0.1 in CHCl₃)

¹H-NMR (300 MHz, CDCl₃): δ = 3.09~3.26 (m, 8H);, 4.73 (m, 4H);, 4.93 (s, 4H; NH), 7.03~7.33 (m, 20H).

¹³C-NMR (75 MHz, CDCl₃) 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 C₃₆H₃₇O₄N₄ 589.2815, found 589.2818.

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

[α]²⁰_D = -76.5 (c=0.1 in CHCl₃)

¹H-NMR (300 MHz, CDCl₃) 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)

¹³C-NMR (100 MHz, CDCl₃) 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 C₂₂H₃₇O₄N₄ 421.2815, found 421.2819.

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

[α]²⁰_D = -198.4 (c=0.1 in CHCl₃)

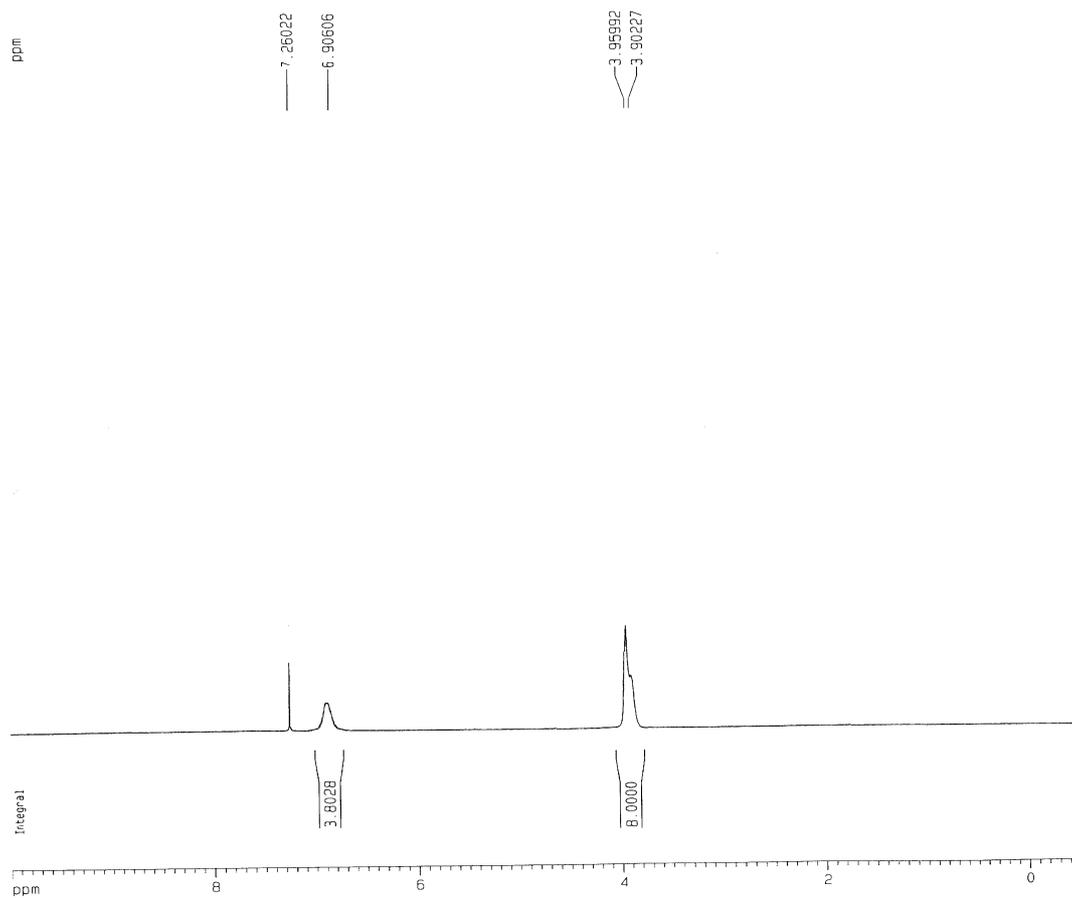
¹H-NMR (300 MHz, CDCl₃) δ 0.94~1.03 (m, 12H), 1.97~2.07 (m, 8H), 2.35~2.38 (m, 2H), 3.47~3.51 (m, 2H), 3.62~3.66 (m, 2H), 4.14~4.16 (m, 2H), 4.41~4.46 (m, 2H). 7.55 (s, 2H).

¹³C-NMR (75 MHz, CDCl₃) δ 17.5, 19.3, 23.8, 28.5, 32.4, 47.6, 57.0, 61.1, 61.3, 172.9, 173.7.

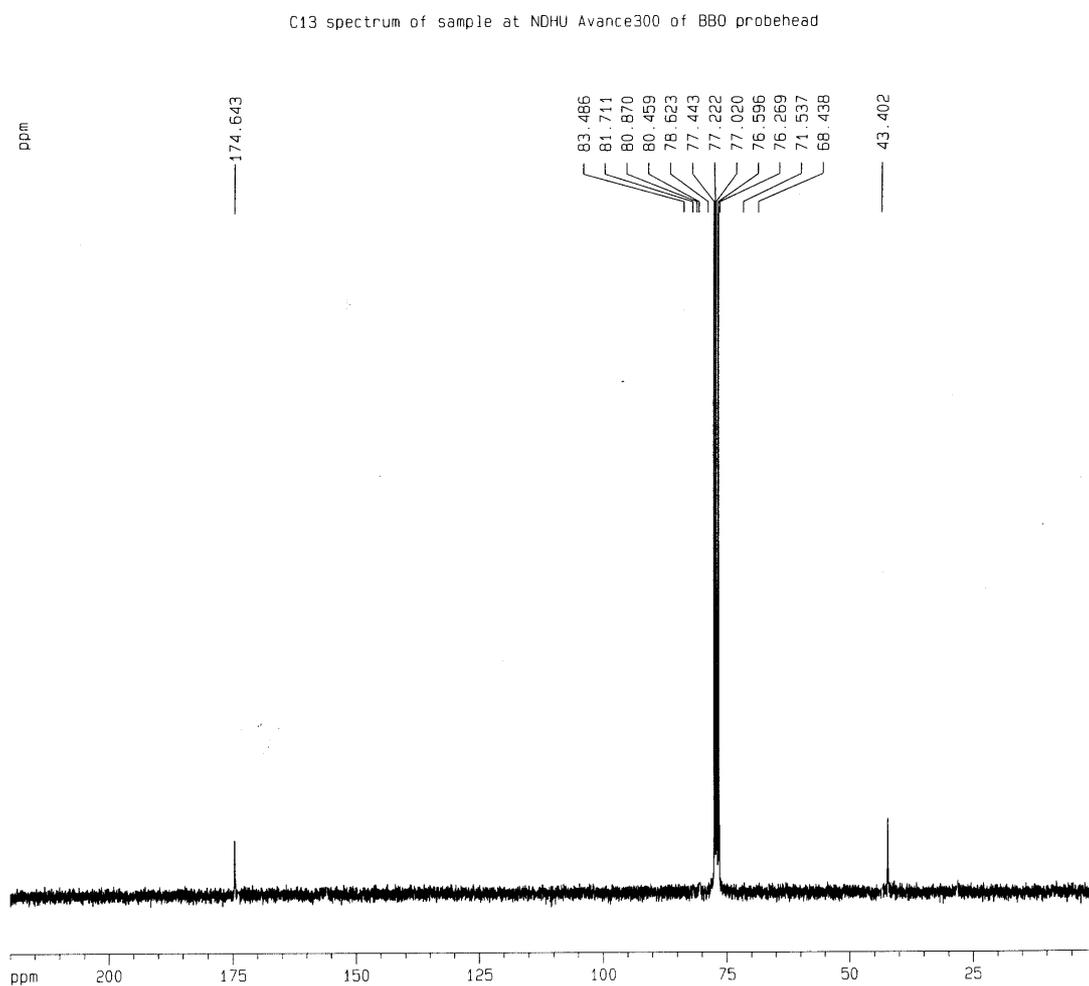
ESI-MS(m/z) : 393(M+H).

m/e calculated for C₂₀H₃₃O₄N₄ 393.2502, found 393.2506.

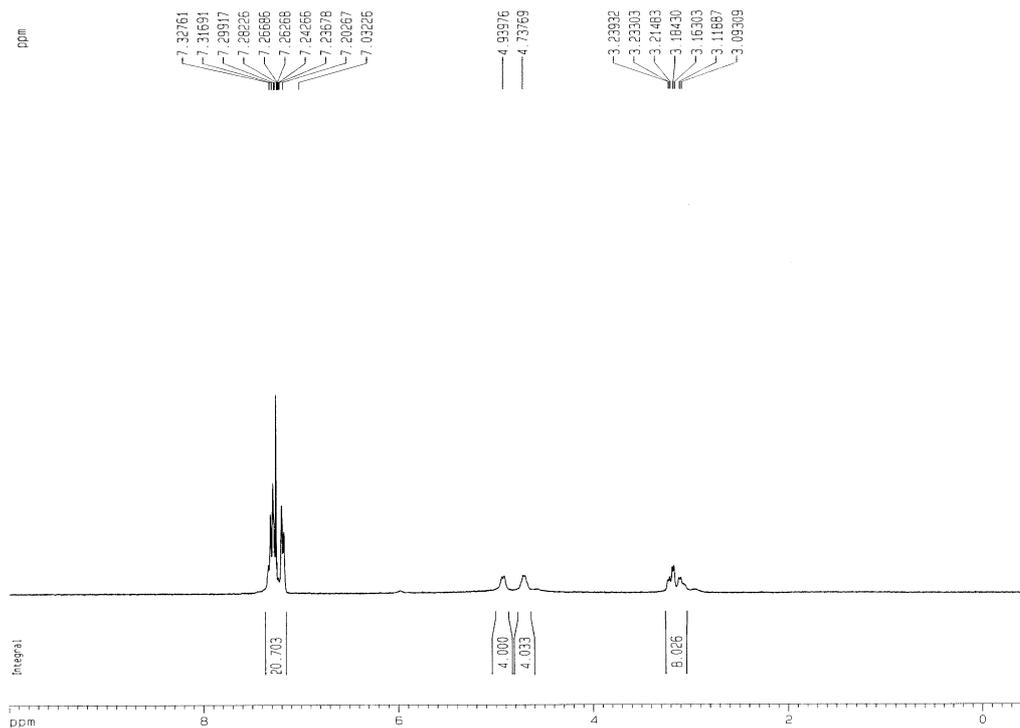
CTPs ^1H and ^{13}C NMR data



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

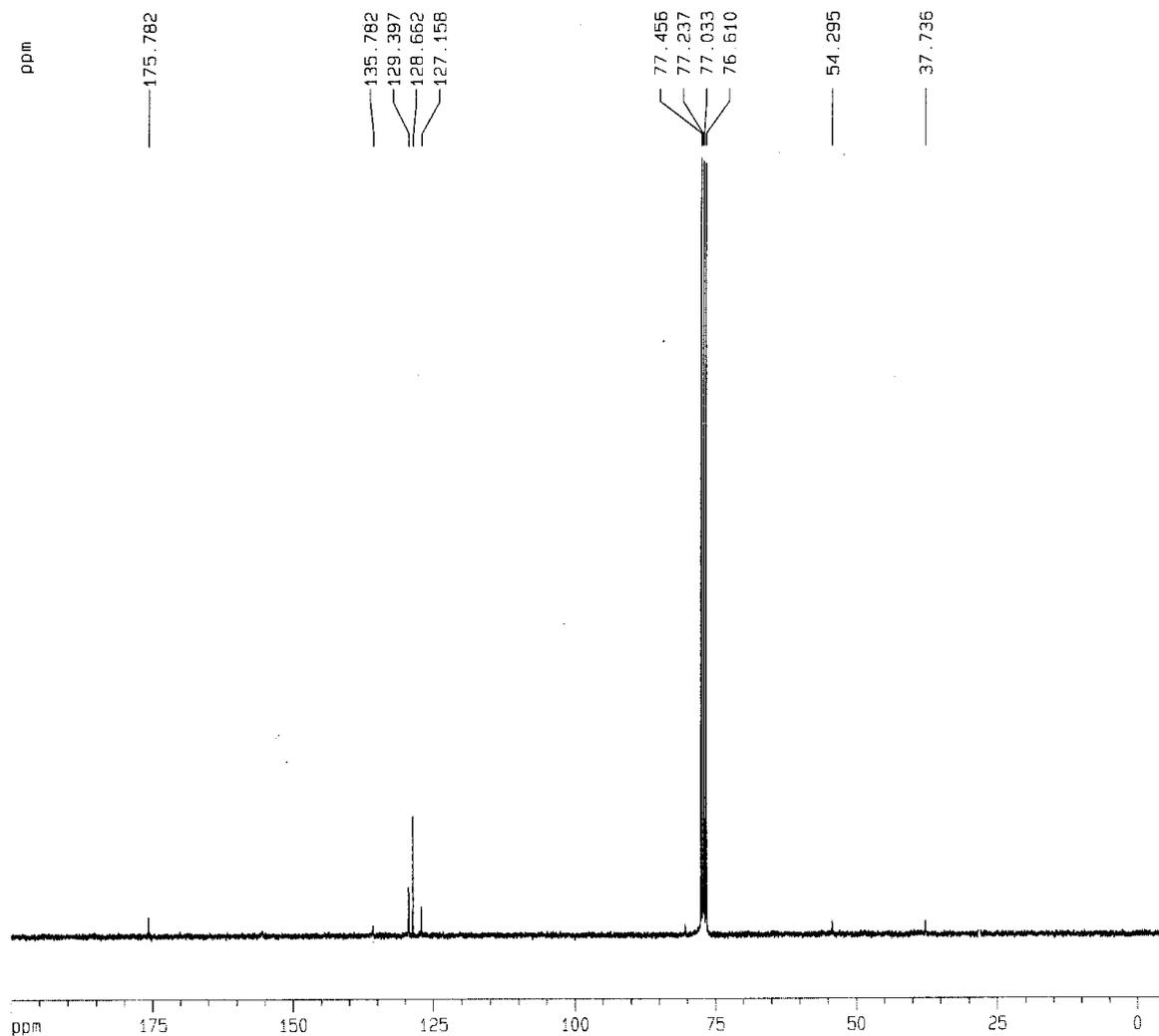


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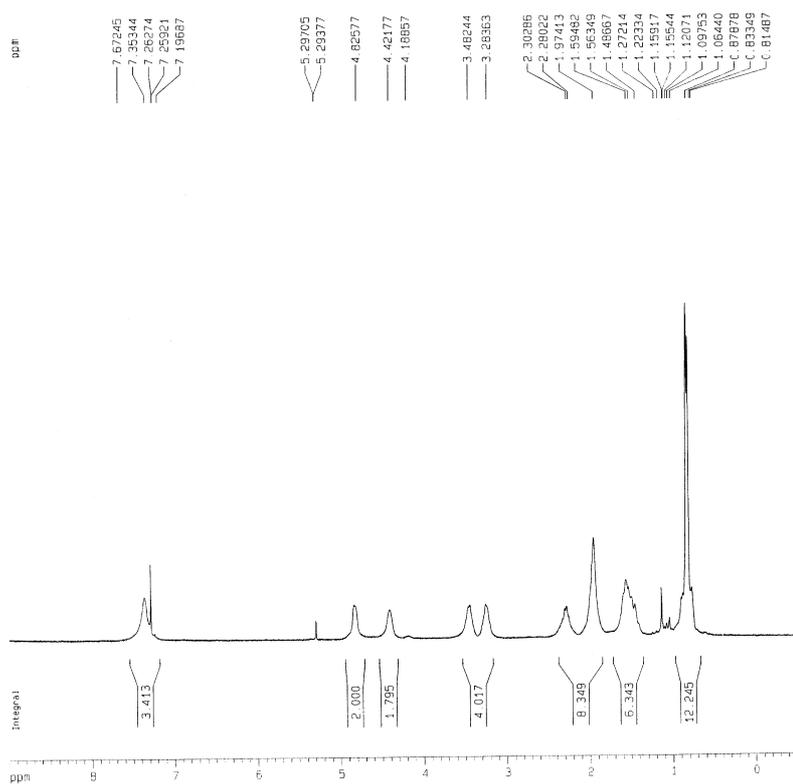


Cyclo-(Phe-Phe-Phe-Phe): $^1\text{H-NMR}$ (300 MHz, CDCl_3)

C13 spectrum of sample at NDHU Avance300 of BBO probehead



Cyclo-(Phe-Phe-Phe-Phe): ¹³C-NMR (75 MHz, CDCl₃)



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 PROCNO 1

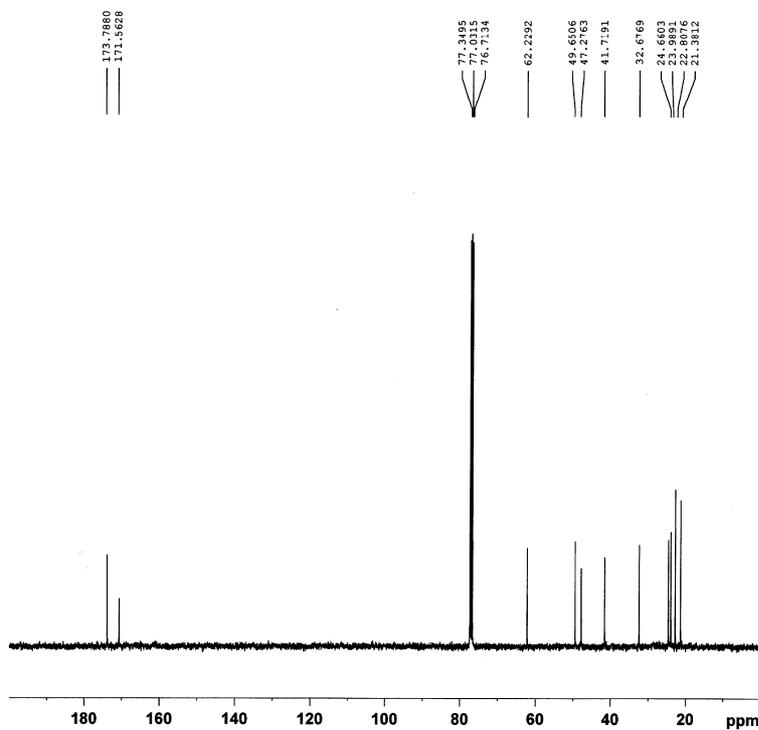
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 FIDRES 0.137219 Hz
 AQ 3.643515 sec
 RG 203.2
 DW 111.200 usec
 DE 6.50 usec
 TE 300.2 K
 D1 1.50000000 sec

***** CHANNEL f1 *****
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 PL1 0.00 dB
 SF01 300.1319506 MHz

F2 - Processing parameters
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 WDW EM
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

1D NMR plot parameters
 CX 20.00 cm
 F1P 9.000 ppm
 F1 2701.17 Hz
 F2P -9.500 ppm
 F2 -150.06 Hz
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 HZCM 142.56175 Hz/cm

Cyclo-(Pro-Leu-Pro-Leu) ¹H-NMR (300 MHz, CDCl₃)



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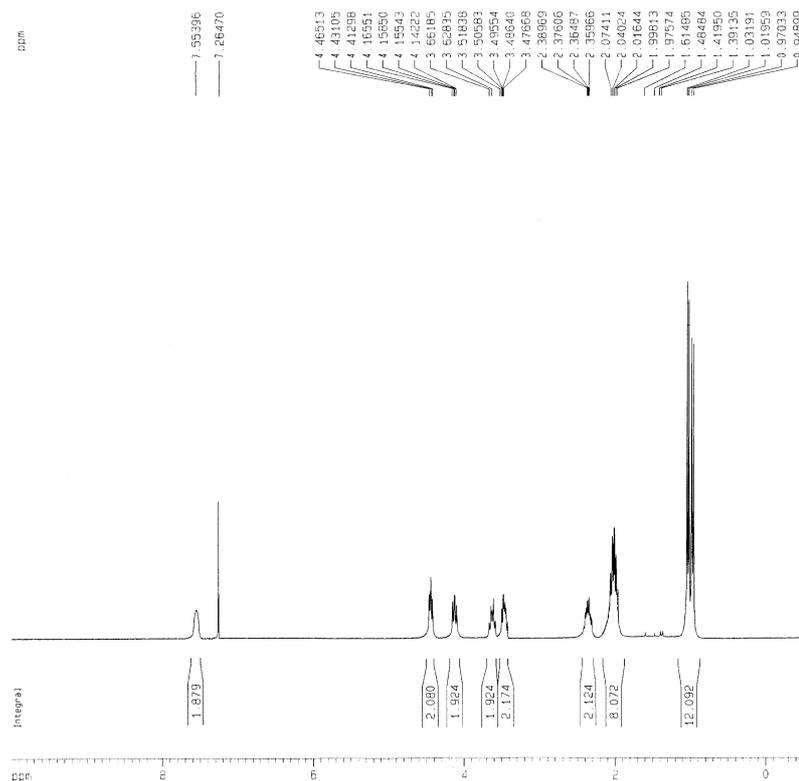
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 NS 1679
 DS 0
 SWH 25510.203 Hz
 FIDRES 0.389255 Hz
 AQ 1.2845556 sec
 RG 2050
 DW 19.600 usec
 DE 7.00 usec
 TE 300.2 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 1.89999998 sec
 TDO 1

***** CHANNEL f1 *****
 NUC1 13C
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 PL1 0.00 dB
 SF01 100.6248425 MHz

***** CHANNEL f2 *****
 CPDPRG2 waltz16
 NUC2 1H
 FCPD2 90.00 usec
 PL2 -3.00 dB
 PL12 14.00 dB
 PL13 17.00 dB
 SF02 400.1316005 MHz

F2 - Processing parameters
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 SF 100.6127690 MHz
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 PC 1.00

Cyclo-(Pro-Leu-Pro-Leu) ¹³C-NMR (100 MHz, CDCl₃)



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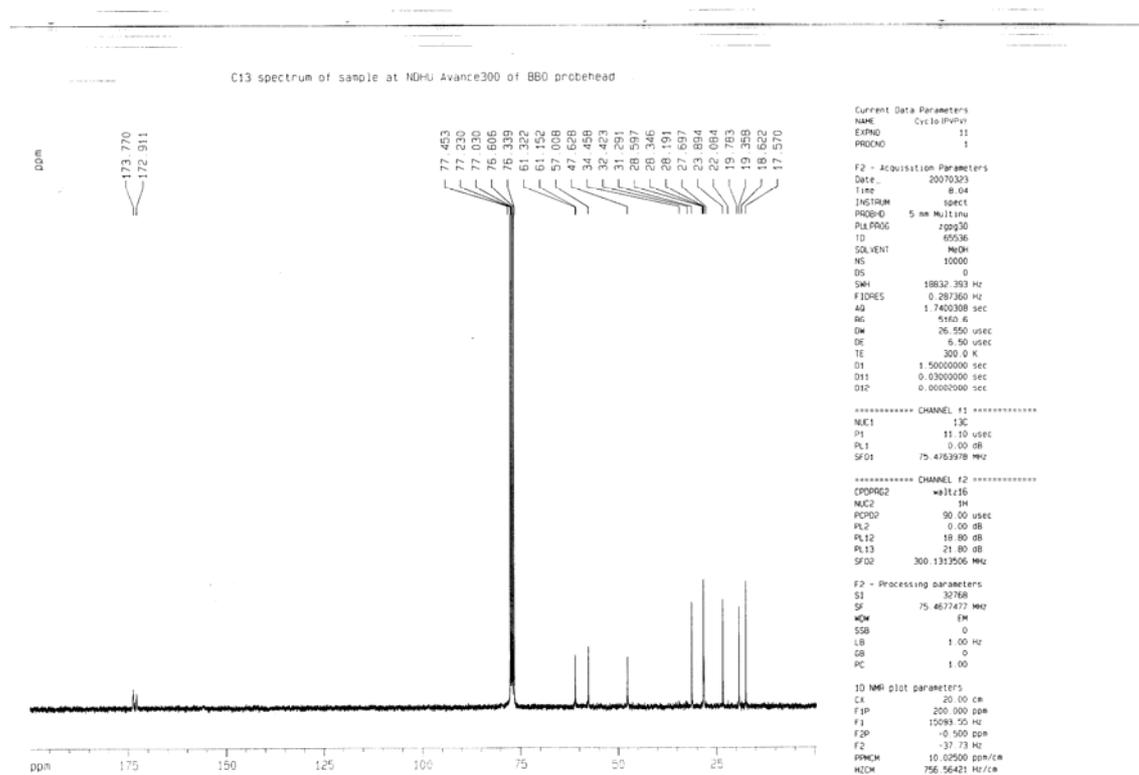
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NS        5
DS        0
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AQ         3.8438515 sec
RG         228.1
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TE        300.0 K
D1        1.5000000 sec

***** CHANNEL f1 *****
NUC1      1H
P1        10.60 usec
PL1       0.00 dB
SFO1     300.1319308 MHz

F2 - Processing parameters
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GB        0
PC        1.00

1D NMR plot parameters
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F1P       10.000 ppm
F1        3001.50 Hz
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F2        -150.00 Hz
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HZCM      157.58525 Hz/cm
    
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Cyclo-(Pro-Val-Pro-Val) ¹H-NMR (300 MHz, CDCl₃)



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Current Data Parameters
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PROCNO   1

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RG         550.6
DW        26.550 usec
DE         6.50 usec
TE        300.0 K
D1        1.5000000 sec
D11       0.0300000 sec
D12       0.0000200 sec

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SFO1     75.4763978 MHz

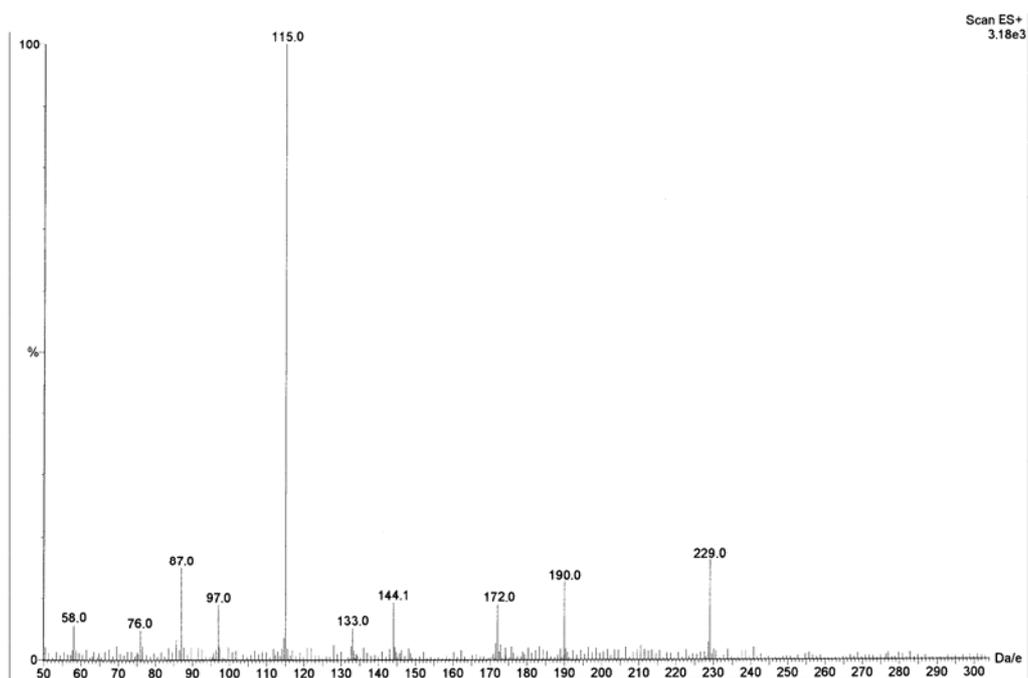
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CPDPRG2  waltz16
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PL2      0.00 dB
PL12     18.80 dB
PL13     21.80 dB
SFO2     300.1313506 MHz

F2 - Processing parameters
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SF        75.4671472 MHz
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PC        1.00

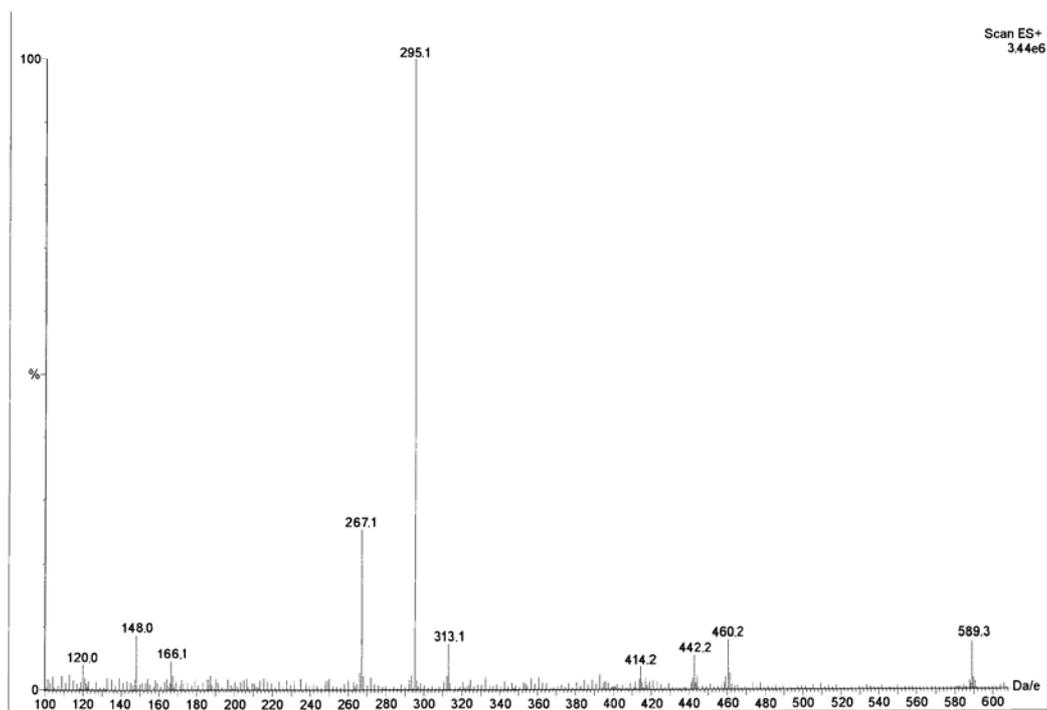
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F3P       -0.500 ppm
F2        -37.73 Hz
PRMCM     10.02500 ppm/cm
HZCM      756.56421 Hz/cm
    
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Cyclo-(Pro-Val-Pro-Val) ¹³C-NMR (75 MHz, CDCl₃)

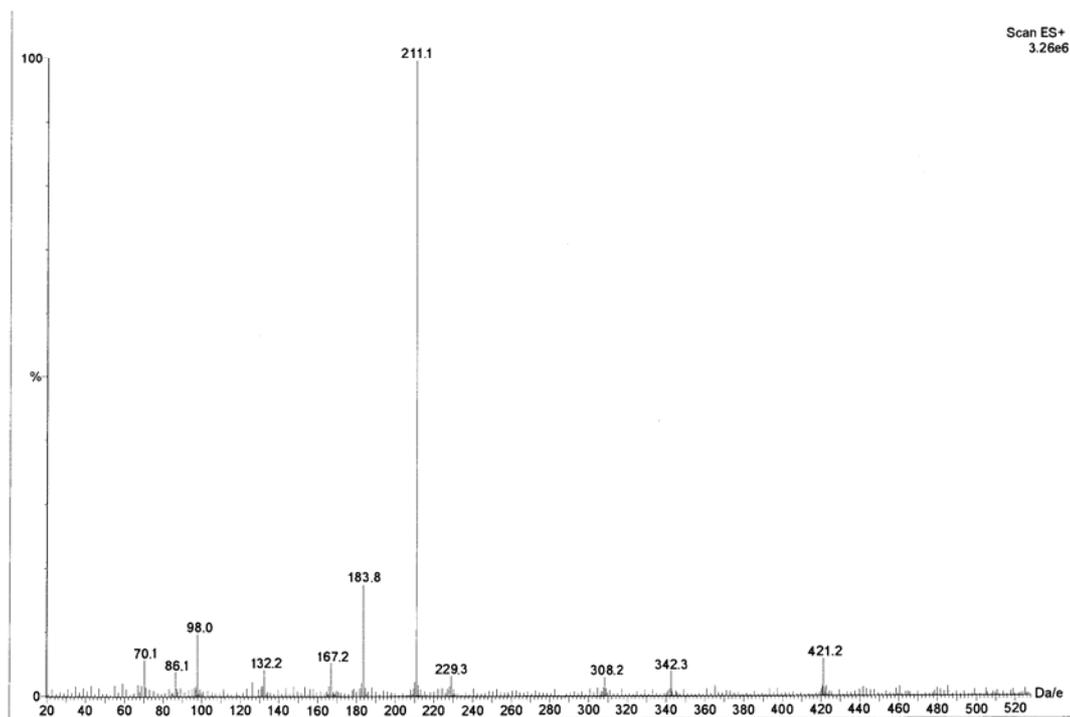
ESI-MS(m/z)



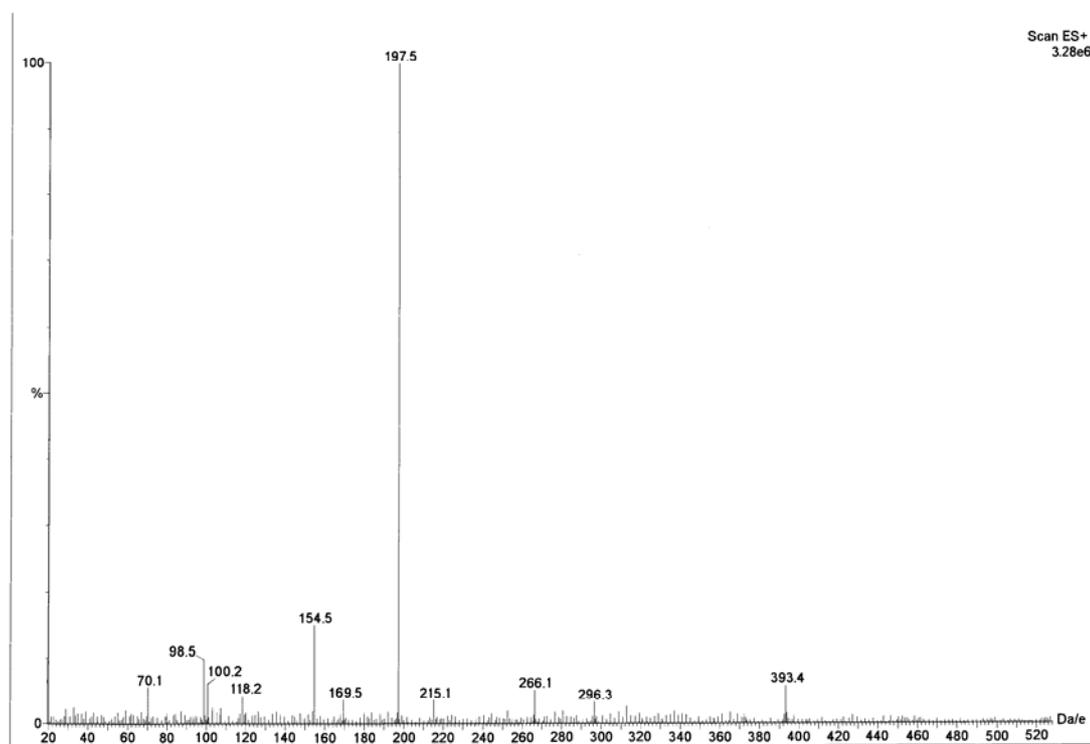
Cyclo-(Gly-Gly-Gly-Gly)



Cyclo-(Phe-Phe-Phe-Phe)



Cyclo-(Pro-Leu-Pro-Leu)



Cyclo-(Pro-Val-Pro-Val)