Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Exploiting self-assembly driven dynamic nanostructured library

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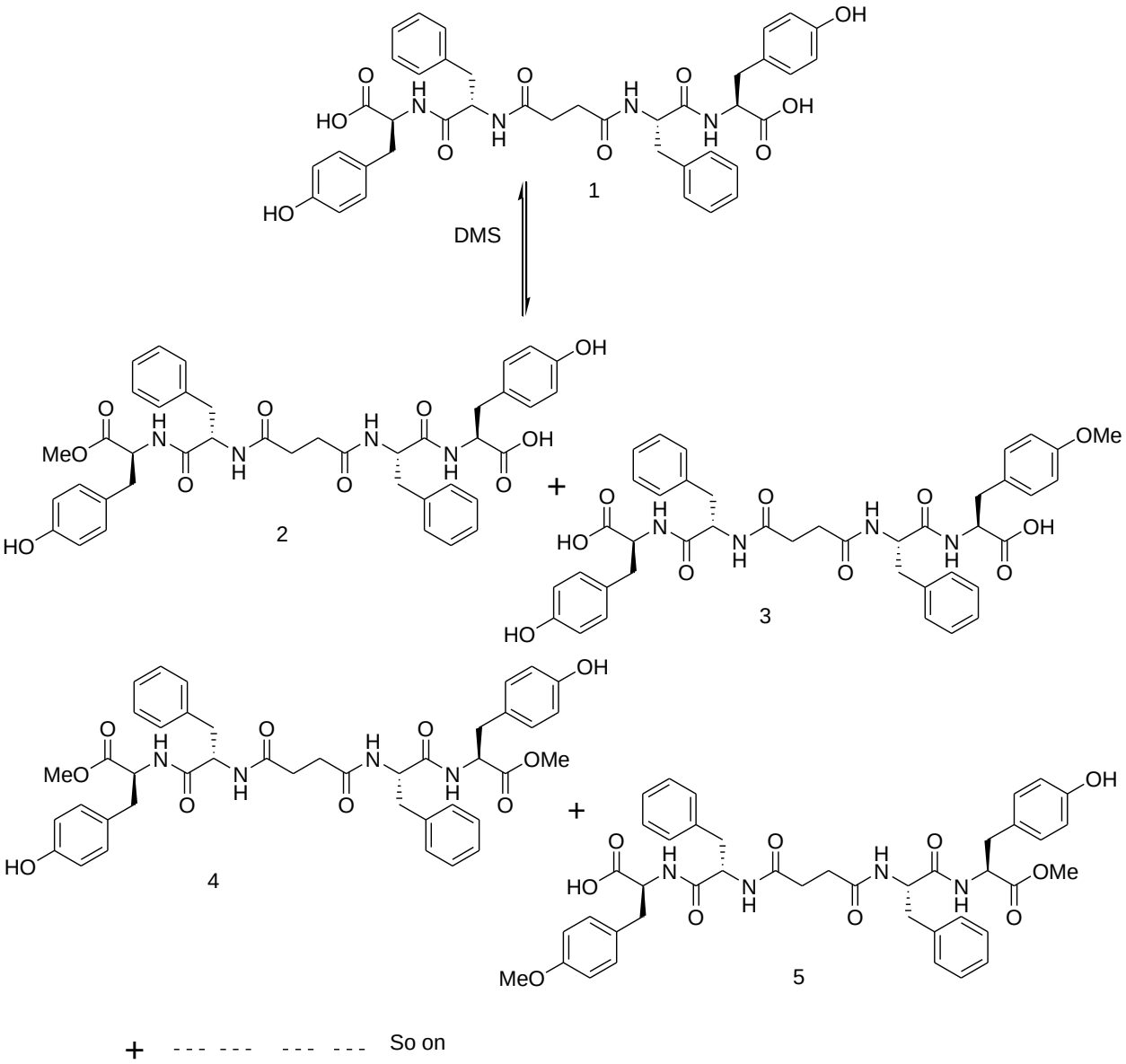
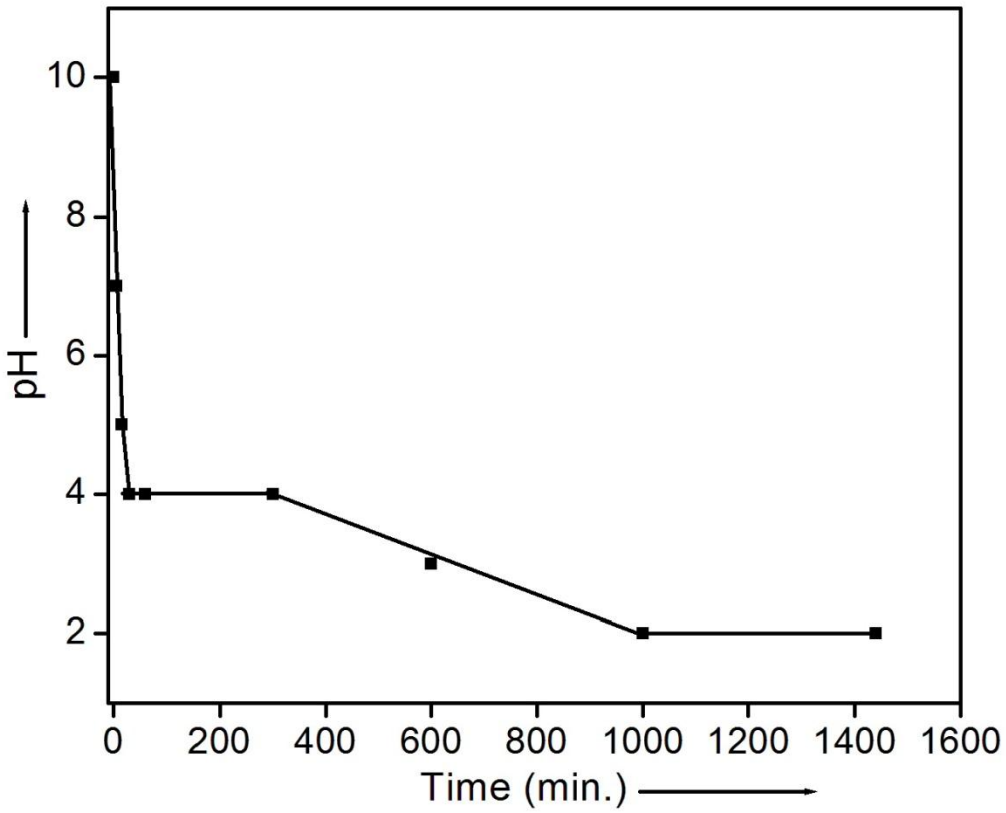
Contents

1. Dynamic Library



ESI Fig. 1. Dynamic library members.

2. Graph of pH Change with Time



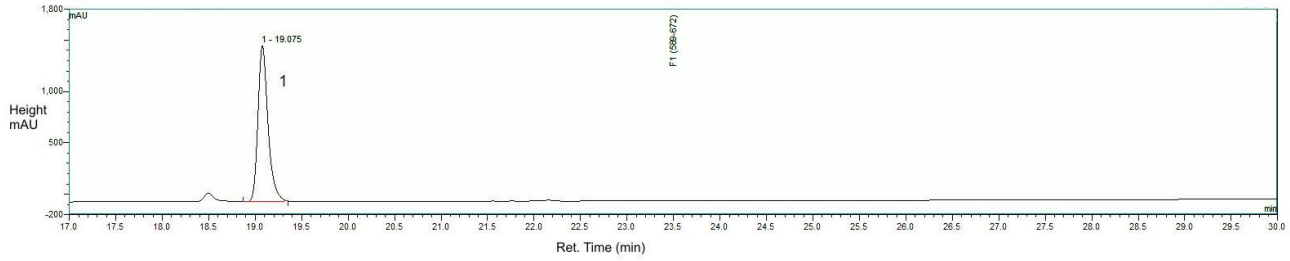
ESI Fig. 2. The plot of decrease in pH with time of the reaction.

3. HPLC Chromatogram

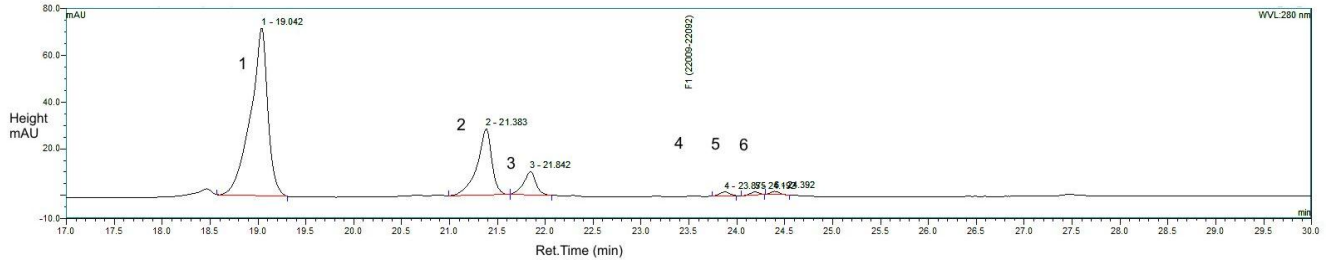
HPLC grade acetonitrile and water were used without further purification. The dynamic reaction mixture was characterized by reverse phase symmetry C18 column (250 x 4.6 cm, 5 µm particle size). UV-Vis absorbance was monitored at 280 nm. Separations were achieved by running the column with acetonitrile-water as eluent at a flow rate of 1 ml min^{-1} at 25 °C. The sample preparation involved mixing 100 µL of gel with acetonitrile–water (900 µL, 50 : 50 mixture). The samples were then filtered through a 0.45 µm syringe filter (Whatman, 150 units, 13 mm diameter, 2.7 mm pore size) prior to injection. A 20 µL of sample was injected into a Dionex Acclaim ® 120 C 18 column of 250 mm length with an internal diameter of 4.6 mm and 5 µm fused silica particles at a flow rate of 1 mL $min^{-}.^{1}$

Time/min	% of H_2O (0.1% TFA)	% of Acetonitrile (0.1% TFA)
0	80	20
4	80	20
35	20	80
40	20	80
42	80	20

(a)



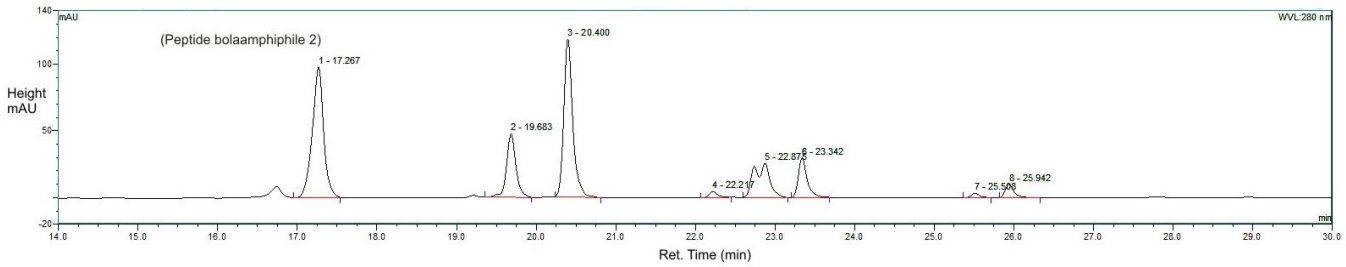
(b)



ESI Fig. 3. HPLC chromatogram of (a) peptide bolaamphiphile **1** and (b) dynamic library of peptide bolaamphiphile **1** with DMS reaction at equilibrium.

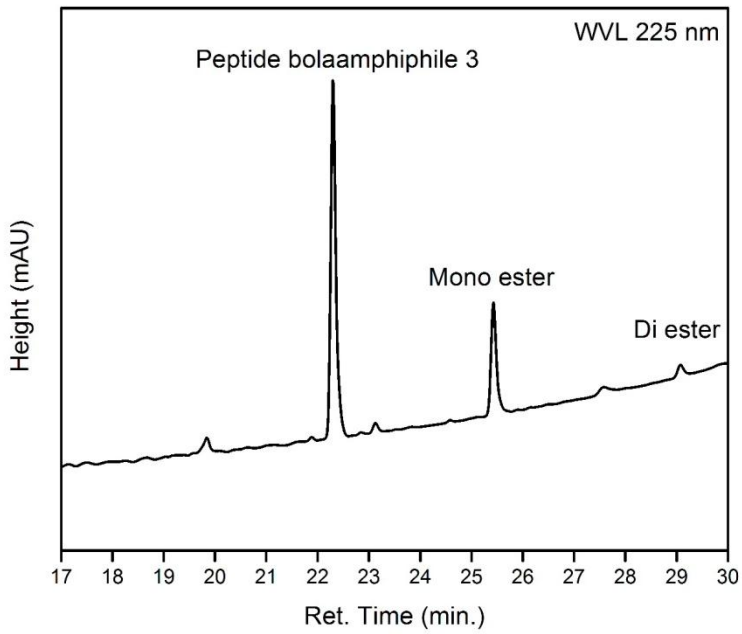
Library members as

1 = di-acid of peptide bolaamphiphile **1**
2 = mono-ester
3 = mono-ether
4 = mono-ester
5 = mono-ester with mono-ether
6 = di-ether

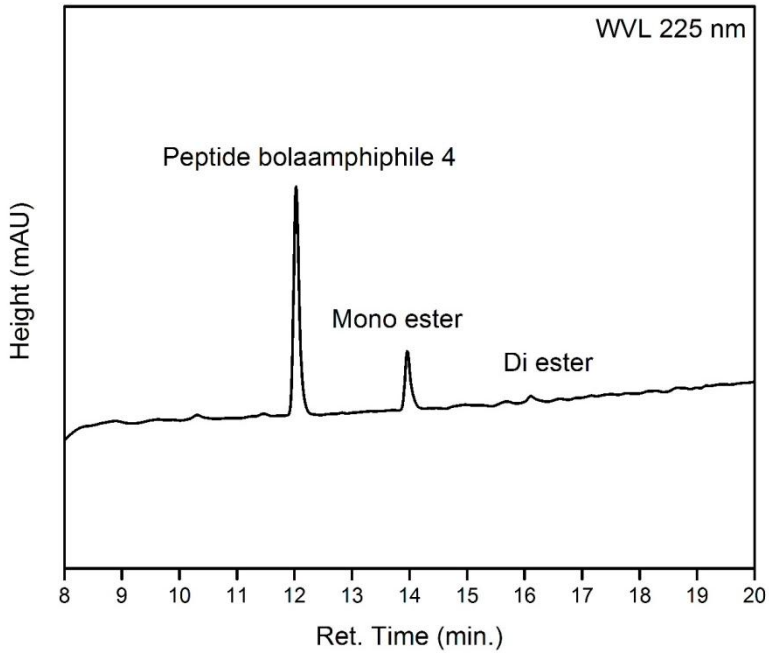


ESI Fig. 4. HPLC chromatogram of a library of peptide bolaamphiphile **2** (HO-Y-L-Suc-L-Y-OH) which gives a mixture of non-selective products.

(a)



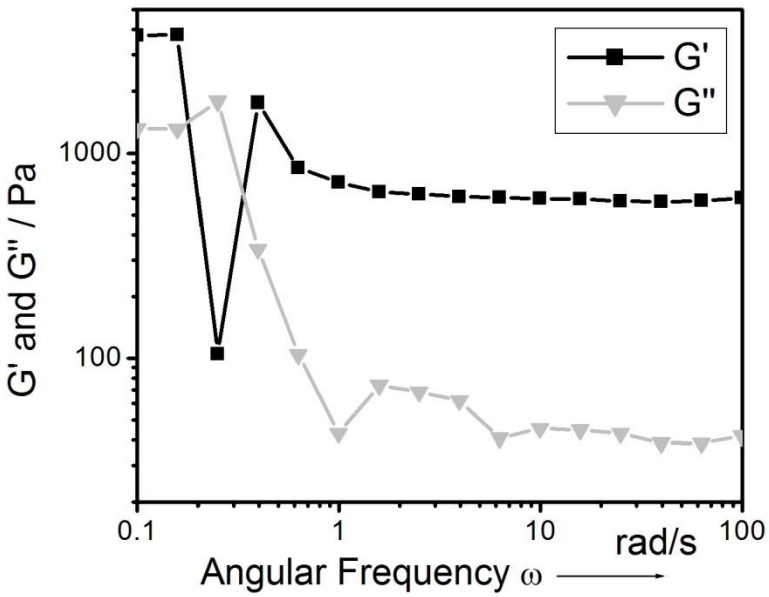
(b)



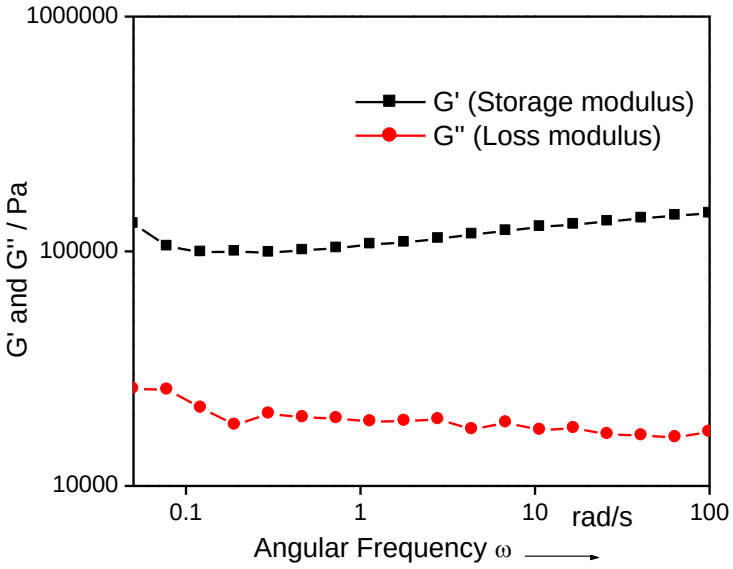
ESI Fig. 5. HPLC chromatogram of library of (a) peptide bolaamphiphile **3** (HO-L-L-Suc-L-L-OH) and (b) peptide bolaamphiphile **4** (HO-G-L-Suc-L-G-OH).

4. Rheological Study

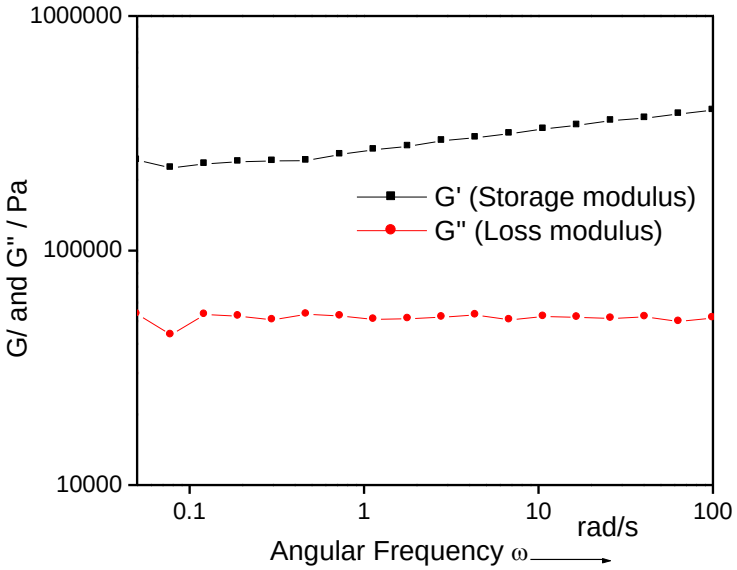
(a)



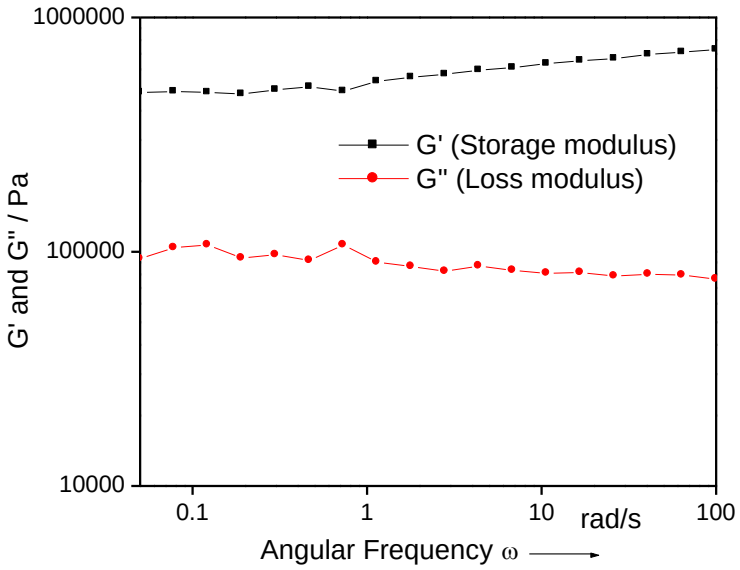
(b)



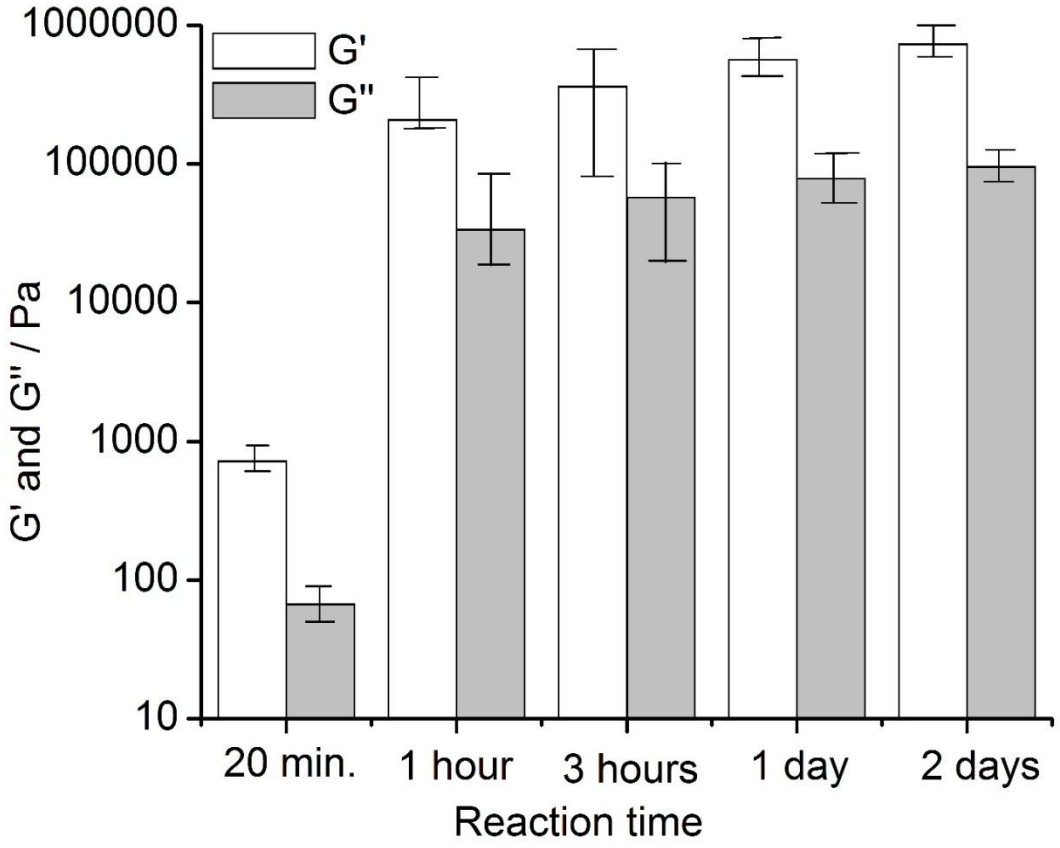
(c)



(d)



ESI Fig. 6. Frequency sweeps of hydrogel **1** of dynamic library at (a) 20 min, (b) 1 hr, (c) 3 hrs, (d) 2 days of course of the reaction.



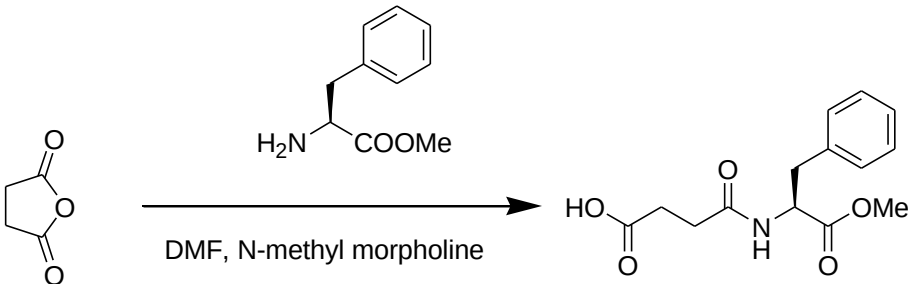
ESI Fig 7. The comparison between the storage modulus (G') and loss modulus (G'') at a particular point of angular frequency (10.6 s^{-1}) with the course of reaction time at constant strain as 0.1%.

5. Synthesis of peptide bolaamphiphiles:

Peptide bolaamphiphiles **1, 2, 3** and **4** employed in this report were synthesized by conventional solution phase methodology. The C-terminus of amino acid was protected as methyl ester. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC-HOBt). The final compounds were purified and fully characterized by FT-IR, ^{1}H NMR and mass spectral studies.

Synthesis of HO-Tyr(4)-Phe(3)-Suc-Phe(1)-Tyr(2)-OH 1

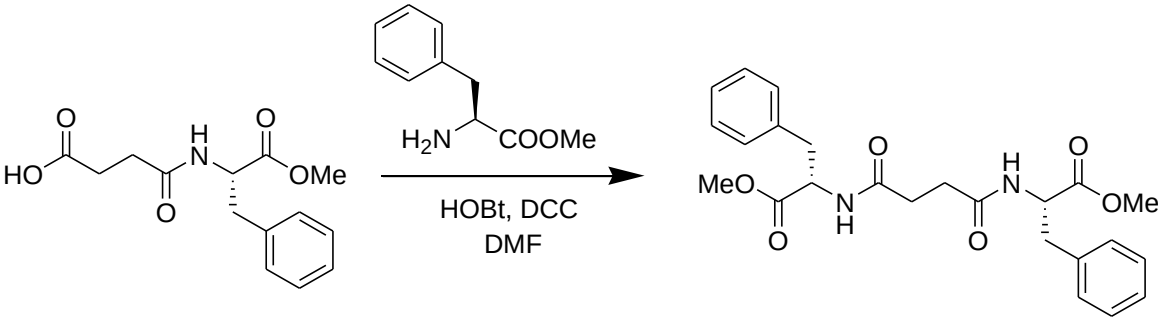
HO-Suc-Phe(1)-OMe 5:



0.5 g (5 mmol) succinic anhydride in 3 ml of DMF were cooled in an ice-water bath and H-Phe-OMe was isolated from 1.07 g (5 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction by ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 0.5 g (5 mmol, 550 µl) N-methyl morpholine. The reaction mixture was stirred for overnight. 50 ml ethyl acetate was added to the reaction mixture and the organic layer was washed with 1M HCl (3 X 50 ml). The ethyl acetate part was dried over anhydrous Na_2SO_4 and filtered. It was evaporated in vacuo to yield **5** as sticky compound. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent.

Yield: 1.12 g (4.01 mmol, 80.2 %); FT-IR (KBr): ῡ = 3307 (s), 3085(m), 1731 (ms), 1652 (s), 1540 (s) cm^{-1} ; ^1H NMR (300 MHz, DMSO-d_6, δ): 8.32 - 8.30 (d, *J* = 7.5 Hz, 1H, NH of Phe(1)), 7.15 - 7.26 (m, 5Hs, aromatic ring protons of Phe(1)), 4.43 - 4.36 (m, 1H, C^{α} H of Phe(1)), 3.62 (s, 3H, COOC$\underline{H}_3$), 2.99 - 2.97 (d, *J* = 5.7 Hz, 2H, C^{β} Hs of Phe(1)), 2.46 - 2.36 (m, 4H, -C$\underline{H}_2$- of Suc); $[\alpha]_D^{20}$ = +11.47 (*c* = 1 in CH_3OH); ESI (*m/z*): 278.0 $[M - H]^+$, M_{calad} = 279.

Meo-Phe(2)-Suc-Phe(1)-OMe 6:

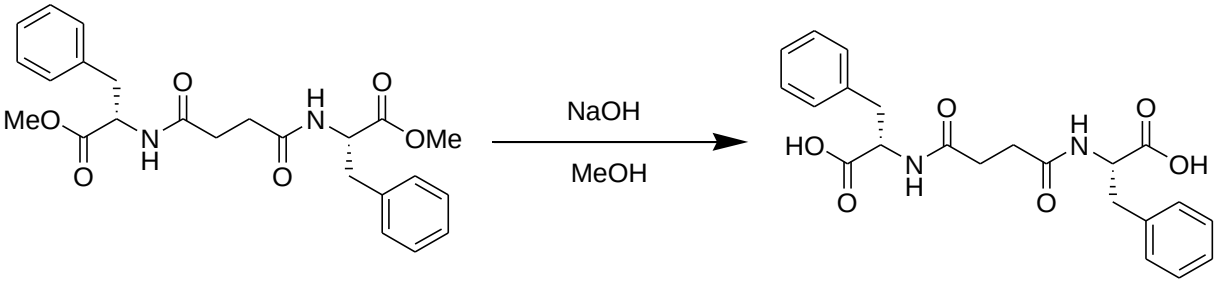


0.97 g (3.5 mmol) of HO-Suc-Phe(1)-OMe **5** in 3 ml of DMF were cooled in an ice–water bath and H-Phe-OMe was isolated from 1.5 g (7 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 0.68 g (3.85 mmol) DCC and 0.520 g (3.85 mmol) of HOBt. The reaction mixture was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **6** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent.

Yield: 1.32 g (3 mmol, 85.7 %); FT - IR (KBr): ῡ = 3304 (m), 1733 (ms), 1680 (s), 1642 (s), 1546 (s), 1496 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 7.33 - 7.24 (m, 10H, ring protons of

Phe(1) and Phe(2)), 6.36 – 6.34 (d, *J* = 7.6 Hz, 2H, NH of Phe(1) and Phe(2)), 4.88 - 4.83 (m, 2H, $C^{\alpha}H$ of Phe(1) and Phe(2)), 3.73 (s, 6H, -COOC$\underline{H}_3$), 3.14 – 3.13 and 3.10 – 3.09 (d, *J* = 6 Hz, *J* = 6 Hz, 4H, $C^{\beta}Hs$ of Phe(1) and Phe(2)), 2.05 – 2.46 (m, 4H,-C$\underline{H}_2$- of Suc); $[\alpha]_D^{20}$ = + 88.58 (*c* = 0.5 in $CHCl_3$); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{24}H_{28}N_2O_6Na$, 463.1845; found 463.1864.

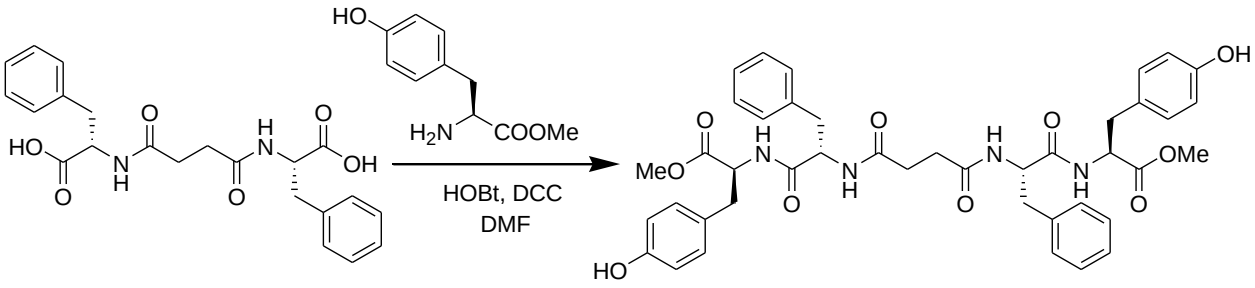
HO-Phe-(2)-Suc-Phe(1)-OH 7:



1.2 g (2.72 mmol) of MeO-Phe(2)-Suc-Phe(1)-OMe **6** in 6 ml MeOH was taken in a round bottom flask (R.B) and 2M NaOH was added dropwise. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo. The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled down under ice water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1 M HCl. It was extracted with ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **7** as a white solid. Yield: 1.1 g (2.67 mmol, 98 %); FT - IR (KBr): ῡ = 3375 (s), 3354 (m), 1712 (s), 1616 (s), 1535 (s), 1496 (s) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ): 8.19 - 8.17 (d, *J* = 8 Hz,2H, NH of Phe(1) and Phe(2)), 7.26 - 7.19 (m, 10H, ring protons of Phe(1)), 4.37 (m, 2H, $C^{\alpha}H$ of Phe(1) and Phe(2)), 3.03 - 3.01 and 2.84 - 2.82 (d, *J* = 8.8 Hz, *J* =

9.6 Hz, 4H, $C^{\beta}Hs$ of Phe(1) and Phe(2)), 2.50 (m, 4H, -C<u>H</u>$_2$- of Suc); $[\alpha]_D^{20}$ = +23.60 (*c* = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{22}H_{24}N_2O_6Na$, 435.1532; found 435.1529.

MeO-Tyr(4)-Phe(3)-Suc-Phe(1)-Tyr(2)-OMe 8:

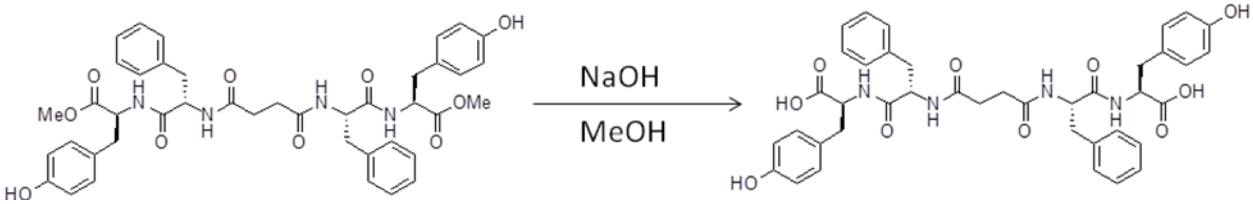


1 g (2.42 mmol) of HO-Phe(2)-Suc-Phe(1)-OH **7** in 3 ml of DMF were cooled in an ice–water bath and H-Tyr-OMe was isolated from 2.24 g (9.7 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 1.09 g (5.32 mmol) DCC and 0.718 g (5.32 mmol) of HOBt. The reaction mixture was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **8** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent.

Yield: 1.55 g (2.03 mmol, 84 %); FT - IR (KBr): ϑ = 3337 (s), 3292 (ms), 1737 (ms), 1616 (s), 1558 (s), 1516 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 8.18 – 8.16 (d, *J* = 7.6 Hz, 2H, NH of Phe(1) and Phe(3)), 8.01 – 7.98 (d, *J* = 8.8 Hz, 2H, NH of Tyr(2) and Tyr(4)), 7.21 – 7.19 (m, 10H, ring protons of Phe(1) and Phe(3)), 7.02 – 7.00 (d, *J* = 8.4, Hz, 4H, ring protons of Tyr(2) and Tyr(4)), 6.66 - 6.64 (d, *J* = 8.4, Hz, 4H of Tyr(2) and Tyr(4)), 4.49 (m, 2H, $C^{\alpha}H$ of Phe(1)

and Phe(3)), 4.34 (m, 2H, $C^{\alpha}Hs$ of Tyr(2) and Tyr(4)), 3.56 (s, 6H, -COOC<u>H</u>$_3$), 3.17 (d, *J* = 8.4 Hz, 4H, $C^{\beta}Hs$ of Phe(1) and Phe(3)), 2.95 (d, *J* = 8.5 Hz, 4H, $C^{\beta}Hs$ of Tyr(2) and Tyr(4)), 2.51 - 2.50 (m, 4H, -C<u>H</u>$_2$- of Suc); $[\alpha]_D^{20}$ = -22.22 (*c* = 0.3 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{42}H_{46}N_4O_{10}Na$, 789.3112; found 789.2974.

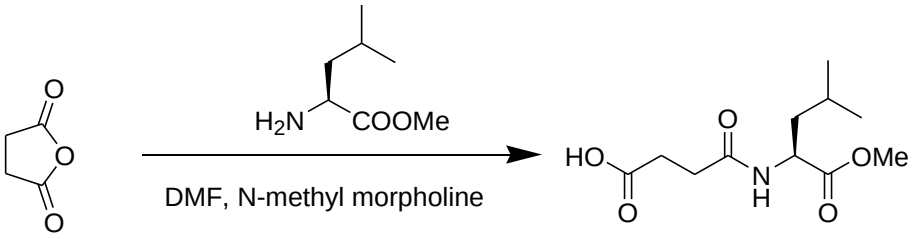
HO-Tyr(4)-Phe(3)-Suc-Phe(1)-Tyr(2)-OH 1:



1.45 g (1.89 mmol) of MeO-Tyr(4)-Phe(3)-Suc-Phe(2)-Tyr(1)-OMe **8** in 10 ml MeOH was taken in a round bottom flask (R.B.) and 2M NaOH was added to it dropwise. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo. The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled under ice water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1M HCl. It was extracted by ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **1** as a white solid. Yield: 1.34 g (1.81 mmol, 96%); FT - IR (KBr): ῡ = 3294 (s), 3278 (ms), 1705 (m), 1640 (s), 1536 (s), 1515 (ms) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 8.19 – 8.17 (d, *J* = 7.6 Hz, 2H, NH of Phe(1) and Phe(3)), 8.02 – 8.00 (d, *J* = 8.8 Hz, 2H, NH of Tyr(2) and Tyr(4)), 7.22 – 7.12 (m, 10H, ring protons of Phe(1) and Phe(3)), 7.02 – 7.00 (d, *J* = 8.4, Hz, 4H, ring protons of Tyr(2) and Tyr(4)), 6.66 - 6.64 (d, *J* = 8.8, Hz, 4H of Tyr(2) and Tyr(4)), 4.49 (m, 2H, $C^{\alpha}H$ of Phe(1) and Phe(3)), 4.34 (m, 2H, $C^{\alpha}Hs$ of Tyr(2) and Tyr(4)), 3.20 (d, *J* = 8.4 Hz, 4H, $C^{\beta}Hs$ of Phe(1) and Phe(3)), 2.92 – 2.89 (d, *J* =

10.8 Hz, 4H, C^{β}Hs of Tyr(2) and Tyr(4)), 2.51 - 2.50 (m, 4H, -C<u>H</u>$_2$- of Suc); ^{13}C NMR (100 MHz, DMSO-d_6, δ_{ppm}): 173.41 and 173.26 (-COOH), 172.48, 171.68, 171.57 and 171.43 (-CONH-), 156.40 (Tyr ring), 138.38, 138.30, 130.62, 130.53, 129.62, 128.41, 127.93, 127.88, 126.64, 115.49, 115.42(aromatic ring) 54.34, 54.13 (C^{α} of Phe and Tyr), 40.57, 40.36, 40.15, 39.73, 39.52, 39.31(C^{β} of Phe and Tyr), 37.87,36.41 (-CH_2- of Suc); $[\alpha]_D^{20}$ = -13.95 (*c* = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{40}H_{42}N_4O_{10}Na$, 761.2799; found 761.2812.

HO- Suc-Leu(1)-OMe 9

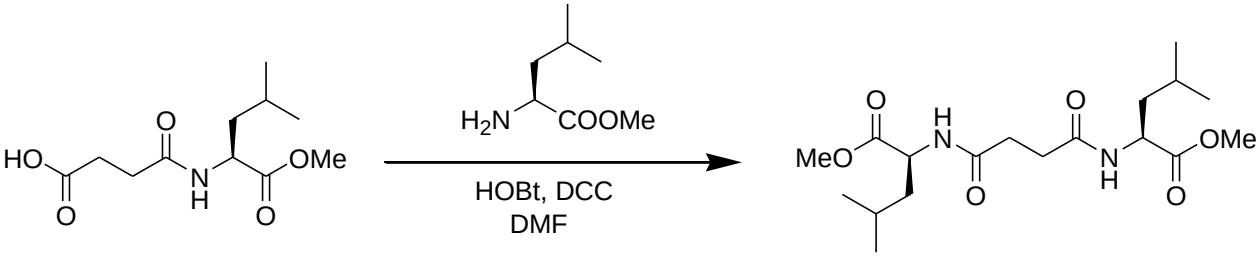


3.021 g (30 mmol) succinic anhydride in 6 ml of DMF were cooled in an ice-water bath and H-Leu-OMe was isolated from 5.43 g (30 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction by ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 3.033 g (30 mmol, 3 ml 300 µl) N-methyl morpholine. The reaction mixture was stirred for overnight. 50 ml ethyl acetate was added to the reaction mixture and the organic layer was washed with 1M HCl (3 X 50 ml.). The ethyl acetate part was dried over anhydrous Na_2SO_4 and was filtered. It was evaporated in vacuo to yield **9** as sticky compound. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent.

Yield: 6.24 g (25.46 mmol, ~85 %); FT - IR (KBr): $\tilde{\upsilon}$ = 3232 (s), 3059 (m), 1731 (ms), 1648 (s), 1558 (s), 1524 (s) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 8.19 - 8.16 (d, *J* = 7.5 Hz, 1H of NH

of Leu(1)), 4.26 - 4.21 (m, 1H, $C^{\alpha}H$ of Leu(1)), 3.52 (s, 3H, $COOC\underline{H}_3$), 2.46 - 2.36 (m, 4H, -$C\underline{H}_2$- of Suc), 1.59 - 1.50 and 1.48 - 1.40 (m, 2H, $C^{\beta}Hs$ of Leu(1), 1H, $C^{\gamma}H$ of Leu(1)), 0.85 - 0.82 (d, J = 6.6 Hz, 6H, $C^{\delta}Hs$ of Leu(1)); $[\alpha]_D^{20}$ = - 6.08 (c = 1 in CH_3OH); ESI (m/z): 244.0 [M - H]$^+$, M_{calcd} = 245.

MeO-Leu(2)-Suc-Leu(1)-OMe 10

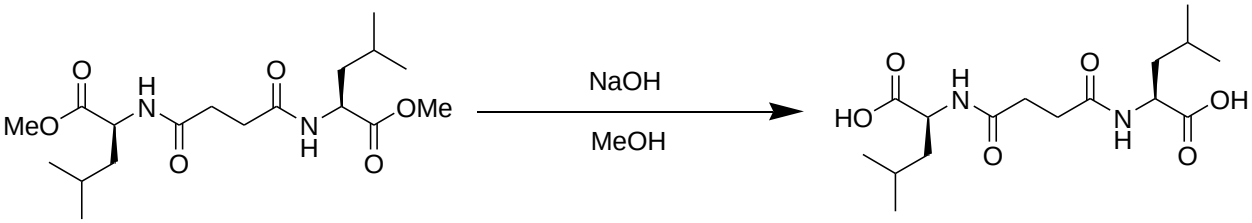


5.49 g (22.5 mmol) of HO-Suc-Leu(1)-OMe **9** in 5 ml of DMF were cooled in an ice–water bath and H-Leu-OMe was isolated from 8.16 g (45 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 5.10 g (24.75 mmol) DCC and 3.33 g (24.75 mmol) of HOBt. The reaction mixture was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **10** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent.

Yield: 7.53 g (20.24 mmol, 90 %); FT - IR (KBr): $\tilde{\upsilon}$ = 3252, 3076 (s), 1745 (ms), 1644 (ms), 1548 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 6.62 – 6.60 (d, J = 8.0 Hz, 2H, NH of Leu(1) and Leu(2)), 4.61 - 4.56 (m, 2H, $C^{\alpha}H$ of Leu(1) and Leu(2)), 3.74 (s, 6H, $COOC\underline{H}_3$), 2.62 - 2.52 (m,

4H, -C<u>H</u>$_2$- of Suc), 1.69 - 1.64 and 1.62 - 1.58 (m, 4H, C$^{\beta}$Hs of Leu(1) and Leu(2), 2H, C$^{\gamma}$Hs of Leu(1) and Leu(2)), 0.96 - 0.94 (d, *J* = 6.4 Hz, 12H, C$^{\delta}$Hs of Leu(1) and Leu(2)); $[\alpha]_D^{20}$ = -35.1 (*c* = 1 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{18}H_{32}N_2O_6Na$, 395.2158; found 395.2180.

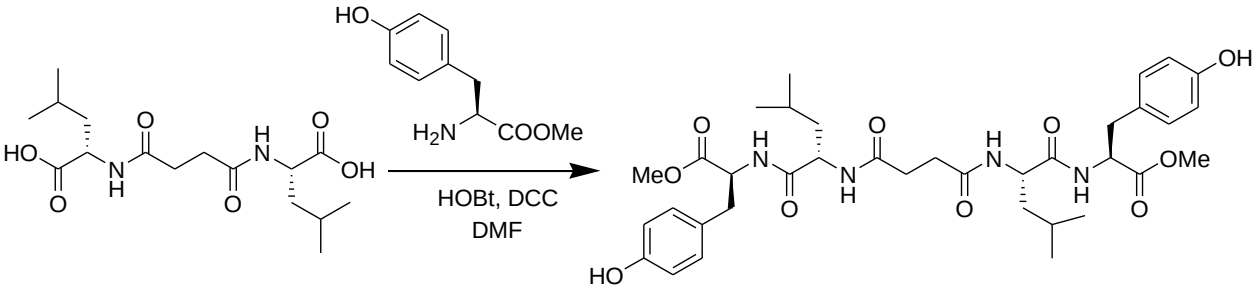
HO-Leu(2)-Suc-Leu(1)-OH 11



6.69 g (18 mmol) of MeO-Leu(2)-Suc-Leu(1)-OMe **10** in 10 ml MeOH was taken in a round bottom flask (R.B) and 2M NaOH was added to it dropwise. The reaction was monitored by thin layer chromatography(TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo. The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled under ice-water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1M HCl. It was extracted with ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **11** as a white solid. Yield: 5.88 g (17.1 mmol, 95 %); FT - IR (KBr): $\tilde{\upsilon}$ = 3338 (s), 1706 (ms), 16173(s), 1568 (s), 1530 (w) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.46 (s, 2H, -COO<u>H</u>), 8.10 – 8.08 (d, *J* = 7.6 Hz, 2H, NH of Leu(1) and Leu(2)), 4.22 - 4.17 (m, 2H, C$^{\alpha}$H of Leu(1) and Leu(2)), 2.40 - 2.30 (m, 4H, -C<u>H</u>$_2$- of Suc), 1.62 - 1.60 and 1.50

- 1.49 (m, 4H, $C^{\beta}Hs$ of Leu(1) and Leu(2), 2H, $C^{\gamma}Hs$ of Leu(1) and Leu(2)), 0.89 - 0.88 and 0.84 – 0.82 (d, J = 6.4 Hz, 12H, $C^{\delta}Hs$ of Leu(1) and Leu(2)); $[\alpha]_D^{20}$ = - 27.8 (c = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{16}H_{28}N_2O_6Na$, 367.1845; found 367.1835.

MeO-Tyr(4)-Leu(3)-Suc-Leu(1)-Tyr(2)-OMe 12

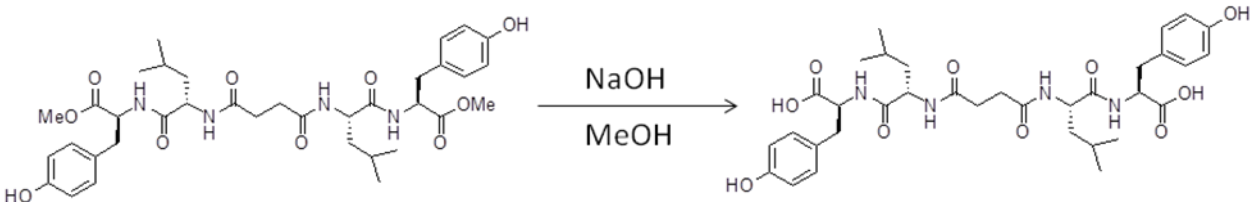


1.72 g (5 mmol) of HO-Leu(2)-Suc-Leu(1)-OH **11** in 3 ml of DMF were cooled in an ice-water bath and H-Tyr-OMe was isolated from 4.63 g (20 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 2.26 g (11 mmol) DCC and 1.48 g (11 mmol) of HOBt. The reaction mixture was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **12** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent to get white solid as product.

Yield: 2.96 g (4.25 mmol, 85 %); FT - IR (KBr): $\tilde{\upsilon}$ = 3309(s), 1745 (ms), 1656 (s), 1547 (s), 1517 (w) cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$, δ): 7.00 – 6.98 (d, J = 8 Hz, 4 H, ring protons of Tyr (1) and Tyr (4)), 6.94 – 6.92 (d, J = 8.4 Hz, 2H, -NH of Leu(2) and Leu(3)), 6.73 – 6.70 (d,

J = 8.8 Hz, 4 H, ring protons of Tyr (1) and Tyr (4)), 6.65 – 6.63 (d, *J* = 8.4 Hz, 2H, -NH of Tyr(1) and Tyr (4)), 4.87 (m, 2H, C^{α}Hs of Tyr (2) and Tyr (4)), 4.47 (m, 2H , C^{α}Hs of Leu (2) and Leu(3)), 3.78 (s, 6H, -COOC$\underline{H}_3$), 2.99 – 2.80 (d, *J* = 8.4 Hz, 4H , C^{β}Hs of Tyr (1) and Tyr (4)), 2.43 -2.42 (m, 4H, -C$\underline{H}_2$- of Suc), 1.62 - 1.61 and 1.51 -1.47(m, 4H, C^{β}Hs of Leu(2) and Leu(3), 2H, C^{γ}Hs of Leu(2) and Leu(3)); 0.96 – 0.94 and 0.91 – 0.89 (d, *J* = 6 Hz, *J* = 6 Hz, 12H, C^{δ}Hs of Leu (2) and Leu(3)); $[\alpha]_D^{20}$ = -26.3 (*c* = 1 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{36}H_{50}N_4O_{10}Na$, 721.3425; found 721.3466.

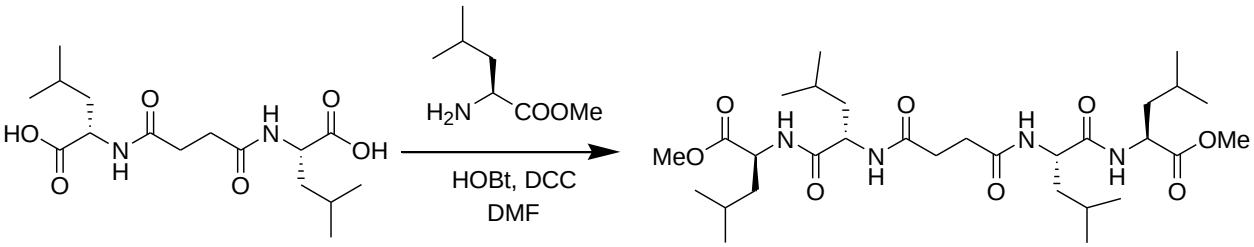
HO-Tyr(4)-Leu(3)-Suc-Leu(1)-Tyr(2)-OH 2



2.79 g (4 mmol) of MeO-Tyr(4)-Leu(3)-Suc-Leu(2)-Tyr(1)-OMe **12** in 150 ml MeOH was taken in a round bottom flask (R.B) and 2M NaOH was added to it dropwise. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo. The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled under ice-water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1M HCl. It was extracted with ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **2** as a white solid. Yield: 2.41 g (3.6 mmol, 90%); FT - IR (KBr): $\tilde{\upsilon}$ = 3293 (bw), 1720 (ms), 1644 (s), 1514 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 8.01 (d, *J* = 8.4 Hz, 2H, -NH of Leu(2) and Leu(3)), 7.94 (d, *J* = 8.4 Hz, 2H, -NH of Tyr(1) and Tyr (4)), 6.98 – 6.96 (d, *J* = 8.4 Hz, 4 H, ring protons of Tyr (1) and Tyr (4)), 6.64 – 6.62 (d, *J* =

8.4 Hz, 4 H, ring protons of Tyr (1) and Tyr (4)), 4.27 (m, 2H, C^{α}Hs of Tyr (2) and Tyr (4)), 4.24 (m, 2H , C^{α}Hs of Leu (2) and Leu(3)), 2.92 – 2.90 and 2.82 – 2.80 (d, *J* = 8.4 Hz, *J* = 8 Hz, 4H , C^{β}Hs of Tyr (1) and Tyr (4)), 2.32 (m, 4H, -C<u>H</u>$_2$- of Suc), 1.55 and 1.40 (m, 4H, C^{β}Hs of Leu(2) and Leu(3), 2H, C^{γ}Hs of Leu(2) and Leu(3)); 0.86 – 0.84 and 0.81 – 0.79 (d, *J* = 6.8 Hz, *J* = 6.8 Hz, 12H, C^{δ}Hs of Leu (2) and Leu(3)); ^{13}C NMR (100MHz , DMSO-d_6 , δ_{ppm}): 173.42 (C=O), 172.33 (C=O), 171.92 (C=O), 171.76 (C=O), 171.68 (C=O), 156.36, 154.28, 130.56, 129.34, 128.02, 115.35, 54.52, 54.37, 51.31, 41.14, 40.57, 40.36, 40.16, 39.95,39.74,39.53,39.32, 36.34, 31.30, 31.10, 30.71, 27.53, 24.75, 24.55, 23.49, 23.32, 22.04, 21.84; $[\alpha]_D^{20}$ = - 26.4 (*c* = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{34}H_{46}N_4O_{10}Na$, 693.3112; found 693.3112.

MeO-Leu(4)-Leu(3)-Suc-Leu(1)-Leu(2)-OMe 13

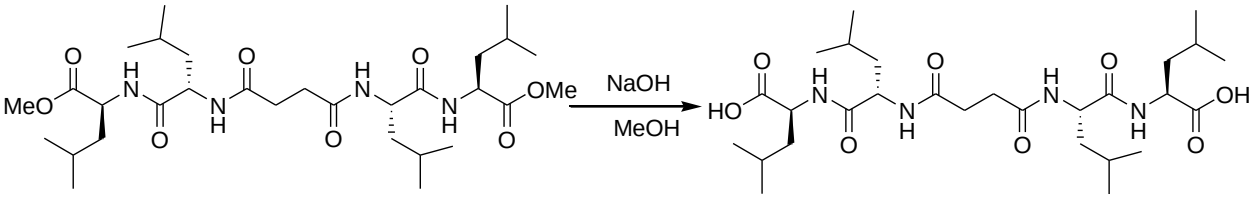


1.72 g (5 mmol) of HO-Leu(2)-Suc-Leu(1)-OH **11** in 3 ml of DMF were cooled in an ice-water bath and H-Leu-OMe was isolated from 3.63 g (20 mmol) of the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 2.26 g (11 mmol) DCC and 1.48 g (11 mmol) of HOBt. The reaction mixture

was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **13** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent to get white solid as product.

Yield: 2.45 g (4.1 mmol, 82 %); FT - IR (KBr): ῡ = 3292(s), 1751 (s), 1640 (s), 1542 (s), 1465 (m) cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$, δ): 6.87 (d, *J* = 8 Hz, 2H, -NH of Leu (1) and Leu (3)), 6.49 (d, *J* = 8 Hz, 2H, -NH of Leu (2) and Leu (4)), 4.54 (m, 2H , $C^{\alpha}Hs$ of Leu (1) and Leu (3)), 4.42 (m, 2H , $C^{\alpha}Hs$ of Leu (2) and Leu (4)), 3.64 (s, 6H, -COOC$\underline{H}_3$), 2.48 (m, 4H, -C$\underline{H}_2$- of Suc), 1.64 – 1.56 and 1.50 -1.46 (m, 8H, $C^{\beta}Hs$ of Leu (1), Leu (2), Leu (3) and Leu (4), 4H, $C^{\gamma}Hs$ of Leu (1), Leu (2), Leu (3) and Leu (4)), 0.85 (broad, 24H, $C^{\delta}Hs$ of Leu (1), Leu (2), Leu (3) and Leu (4)); $[\alpha]_D^{20}$ = -62.2 (*c* = 0.52 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{30}H_{54}N_4O_8Na$, 621.3839; found 621.4281.

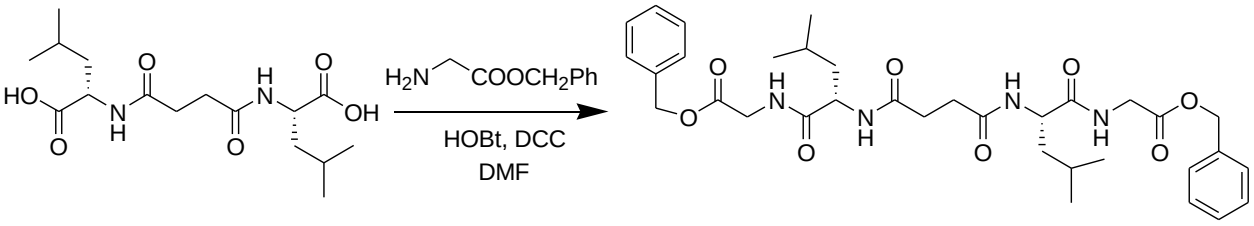
HO-Leu(4)-Leu(3)-Suc-Leu(1)-Leu(2)-OH 3



2.39 g (4 mmol) of MeO-Leu(4)-Leu(3)-Suc-Leu(2)-Leu(1)-OMe **13** in 10 ml MeOH was taken in a round bottom flask (R.B) and 2M NaOH was added to it dropwise. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo.

The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled under ice-water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1M HCl. It was extracted with ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **3** as a white solid. Yield: 2.05 g (3.6 mmol, 90%); FT - IR (KBr): $\tilde{\upsilon}$ = 3303 (bms), 1724 (s), 1646 (s), 1548 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 12.45 (s, 2H, -COOH), 8.11 (d, *J* = 7.6 Hz, 2H, -NH of Leu (1) and Leu (3)), 8.00 (d, *J* = 8.4 Hz, 2H, -NH of Leu (2) and Leu (4)), 4.39 (m, 2H , C^{α}Hs of Leu (1) and Leu (3)), 4.24 (m, 2H , C^{α}Hs of Leu (2) and Leu (4)), 2.41 (m, 4H, -CH_2- of Suc), 1.64 – 1.60 and 1.50 -1.47 (m, 8H, C^{β}Hs of Leu (1), Leu (2), Leu (3) and Leu (4), 4H, C^{γ}Hs of Leu (1), Leu (2), Leu (3) and Leu (4)), 0.96, 0.94, 0.90 and 0.88 (d, *J* = 6 Hz, 24H, C^{δ}Hs of Leu (1), Leu (2), Leu (3) and Leu (4)); ^{13}C NMR (100MHz , DMSO-d_6 , δ_{ppm}): 173.88 (C=O), 172.17 (C=O), 171.19 (C=O), 50.57, 50.13, 40.09, 39.88, 39.67, 39.47, 39.25, 39.04, 38.84, 30.79, 25.18, 23.07, 22.83, 21.61, 21; $[\alpha]_D^{20}$ = - 46.45 (*c* = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{28}H_{50}N_4O_8Na$, 593.3526; found 593.3660.

$PhCH_2O$-Gly(4)-Leu(3)-Suc-Leu(1)-Gly(2)-OCH_2Ph 14

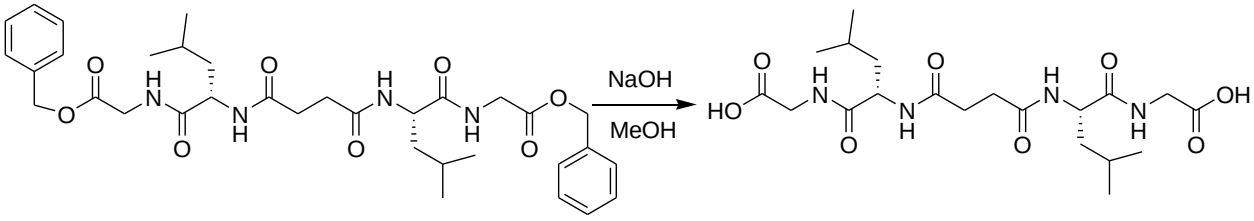


1.72 g (5 mmol) of HO-Leu(2)-Suc-Leu(1)-OH **11** in 5 ml of DMF were cooled in an ice-water bath and H-Gly-OCH_2Ph was isolated from 6.74 g (20 mmol) of the corresponding benzyl ester-p toluene sulfonic acid salt by neutralization and subsequent extraction with ethyl acetate and the

ethyl acetate extract was concentrated to 8 ml. It was then added to the reaction mixture, followed immediately by 2.26 g (11 mmol) DCC and 1.48 g (11 mmol) of HOBt. The reaction mixture was stirred for overnight. The residue was taken up in ethyl acetate (50 ml) and the DCU was filtered off. The organic layer was washed with 1 M HCl (3 × 50 ml), brine (2 × 50 ml), 1 M sodium carbonate (3 × 50 ml), brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **14** as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform–methanol as eluent to get white solid as product.

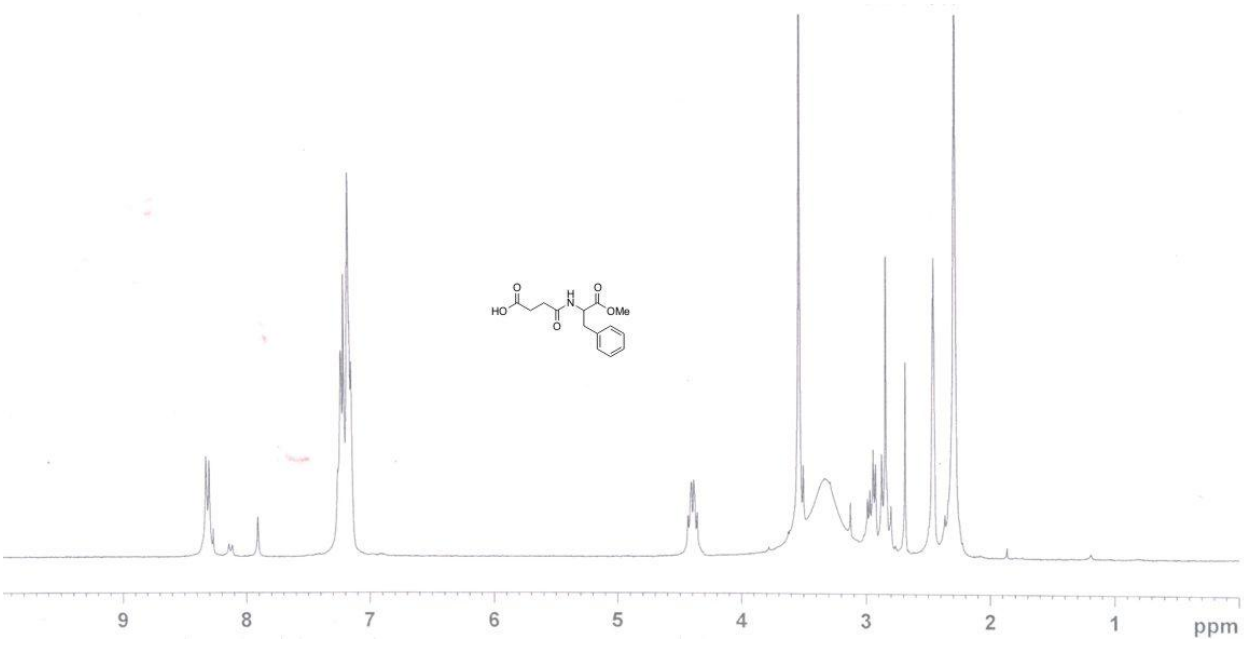
Yield: 2.71 g (4.25 mmol, 85 %); FT - IR (KBr): $\tilde{\upsilon}$ = 3292(s), 1734 (ms), 1636 (s), 1546 (s), 1457 (w) cm^{-1} ; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.37 – 7.32 (m, 10 H, aromatic protons), 7.18 – 7.15 (t, *J* = 5.2 Hz, 2H, -NH of Gly (2) and Gly (4)), 6.74 (d, *J* = 8 Hz, 2H, -NH of Leu (1) and Leu (3)), 5.13 (s, 4H, -COOC$\underline{H}_2$-Ph), 4.49 (m, 2H , C^{α}Hs of Leu (1) and Leu (3)), 4.01 (d, *J* = 5.6 Hz, 4H, C^{α}Hs of Gly (2) and Gly (4)), 2.56 (m, 4H, -C$\underline{H}_2$- of Suc), 1.69 - 1.63 (m, 4H, C^{β}Hs of Leu(1) and Leu(3), 2H, C^{γ}Hs of Leu (1) and Leu (3)); 0.92 – 0.90 and 0.89 – 0.88 (d, *J* = 6.4 Hz, *J* = 6 Hz, 12H, C^{δ}Hs of Leu (1) and Leu(3)); $[\alpha]_D^{20}$ = -46.45 (*c* = 0.31 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{34}H_{46}N_4O_8Na$, 661.3213; found 661.3697.

HO-Gly(4)-Leu(3)-Suc-Leu(1)-Gly(2)-OH 4

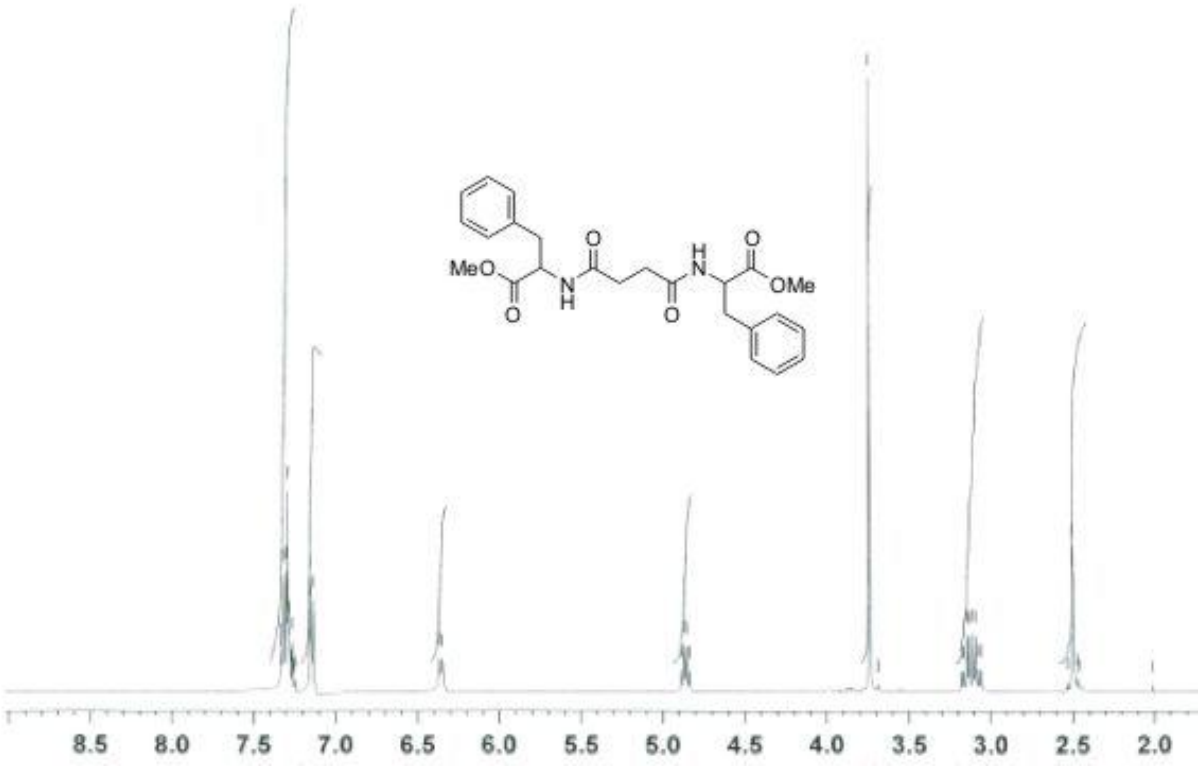


2.23 g (3.5 mmol) of $PhCH_2O$-Gly(4)-Leu(3)-Suc-Leu(1)-Gly(2)-OCH_2Ph **14** in 10 ml MeOH was taken in a round bottom flask (R.B) and 2M NaOH was added to it dropwise. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for overnight. 15 ml of distilled water was added to the reaction mixture and MeOH was removed under vacuo. The aqueous part was washed with diethyl ether (2 x 30 ml). Then it was cooled under ice-water bath for 10 minute and then pH was adjusted to 1 by drop wise addition of 1M HCl. It was extracted with ethyl acetate (3 x 50 ml) and then the ethyl acetate part was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield **4** as a white solid. Yield: 1.44 g (3.15 mmol, 90%); FT - IR (KBr): $\tilde{\upsilon}$ = 3288 (bw), 1733 (ms), 1641 (s), 1546 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 12.41 (s, 2H, -COO<u>H</u>), 8.16 – 8.13 (t, *J* = 5.6 Hz, 2H, -NH of Gly (2) and Gly (4)), 7.99 (d, *J* = 8 Hz, 2H, -NH of Leu (1) and Leu (3)), 4.22 (m, 2H , C^{α}Hs of Leu (1) and Leu (3)), 3.64-3.62 (d, *J* = 5.6 Hz, 4H, C^{α}Hs of Gly (2) and Gly (4)), 2.43 (m, 4H, -C<u>H</u>$_2$- of Suc), 1.53 and 1.39 (m, 4H, C^{β}Hs of Leu (1) and Leu (3), 2H, C^{γ}Hs of Leu(1) and Leu(3)); 0.81 and 0.76 (d, *J* = 6.4 Hz, *J* = 6.4 Hz, 12H, C^{δ}Hs of Leu (1) and Leu (3)); ^{13}C NMR (100MHz , DMSO-d_6 , δ_{ppm}): 172.51 (C=O), 171.55 (C=O), 171.06 (C=O), 50.68, 40.72, 40.55, 40.09, 39.88, 39.67, 39.46, 39.25, 39.04, 38.83, 30.60, 24.07, 23.05, 21.41; $[\alpha]_D^{20}$ = - 36.0 (*c* = 0.5 in CH_3OH); HRMS (ESI, *m/z*): $(M + Na)^+$ Calcd for $C_{20}H_{34}N_4O_8Na$, 481.2274; found 481.2203.

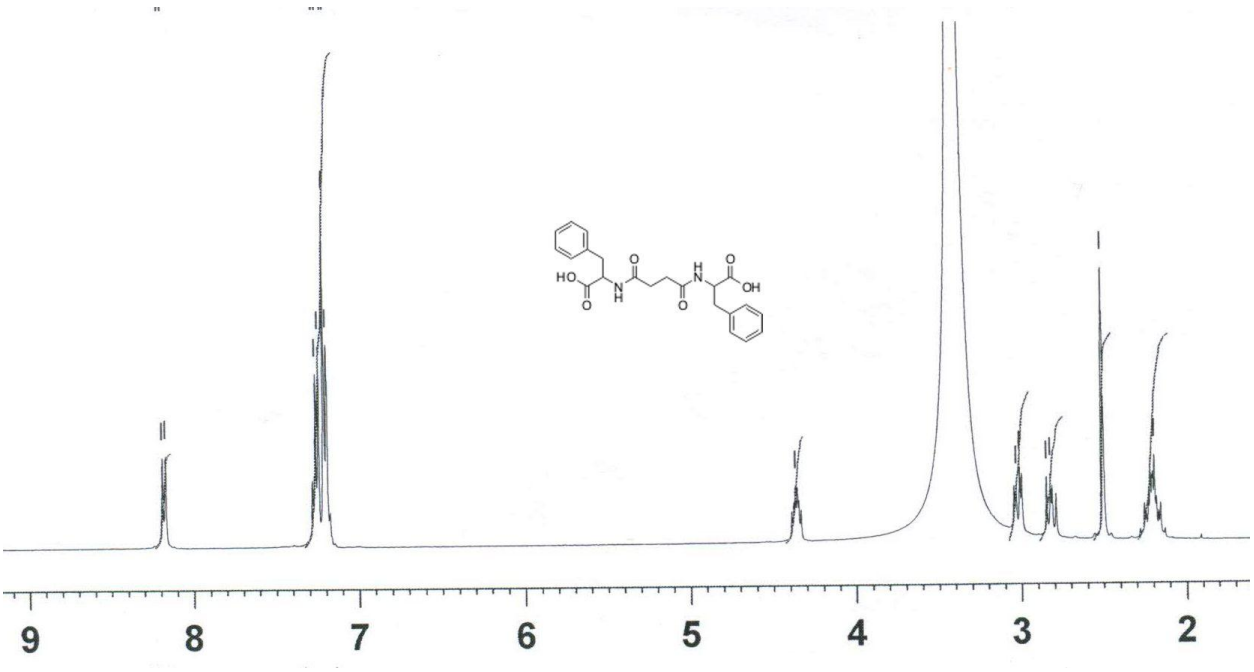
6. Compounds Characterisation



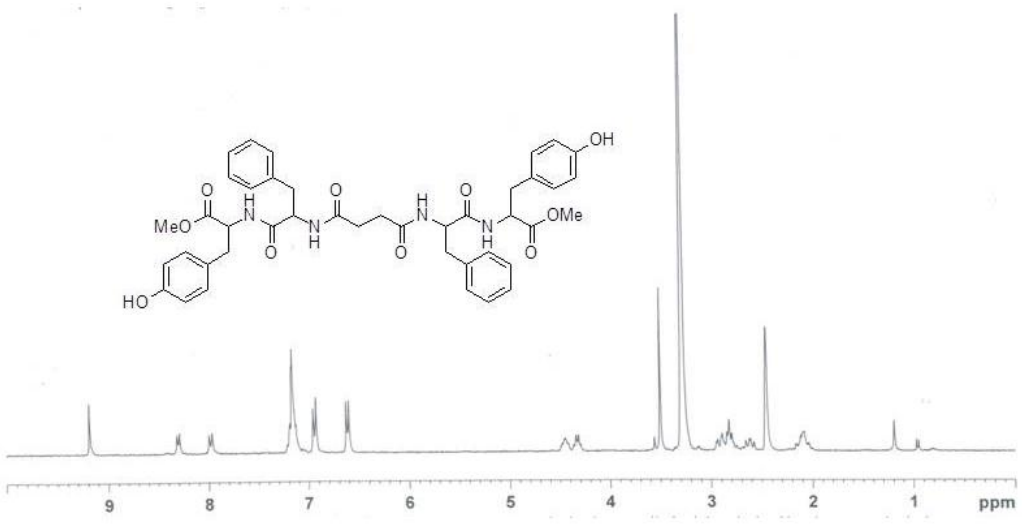
ESI Fig. 8. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of HO-Suc-F-OMe **5**.



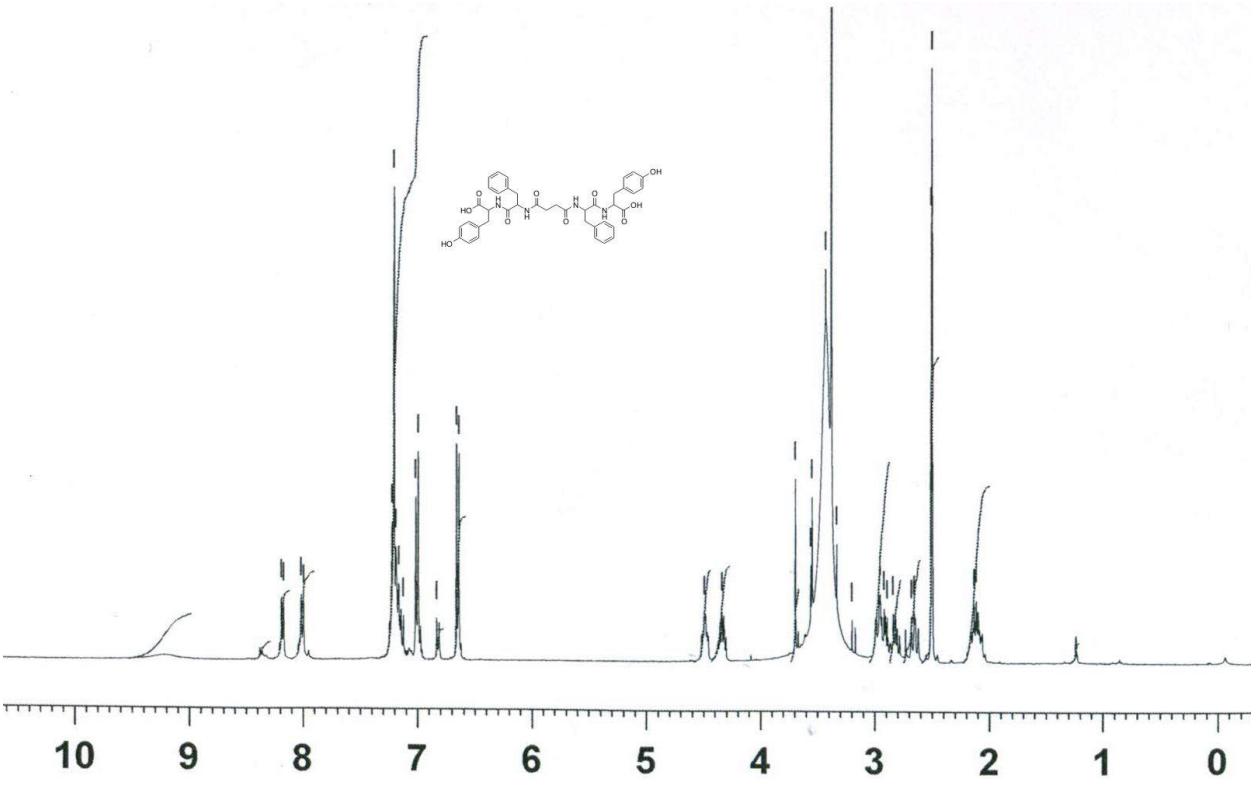
ESI Fig. 9. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of MeO-F-Suc-F-OMe **6**.



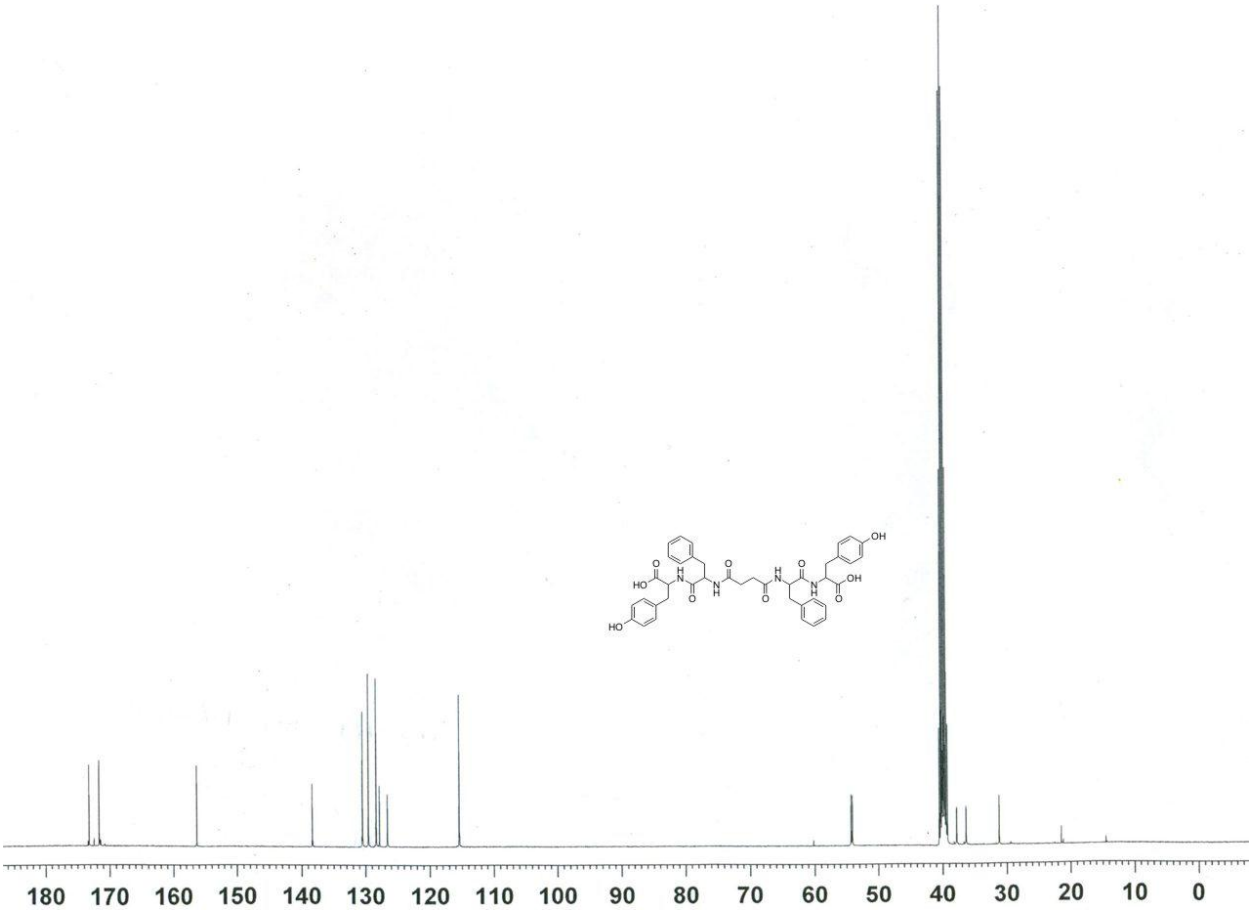
ESI Fig. 10. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-F-Suc-F-OH **7**.



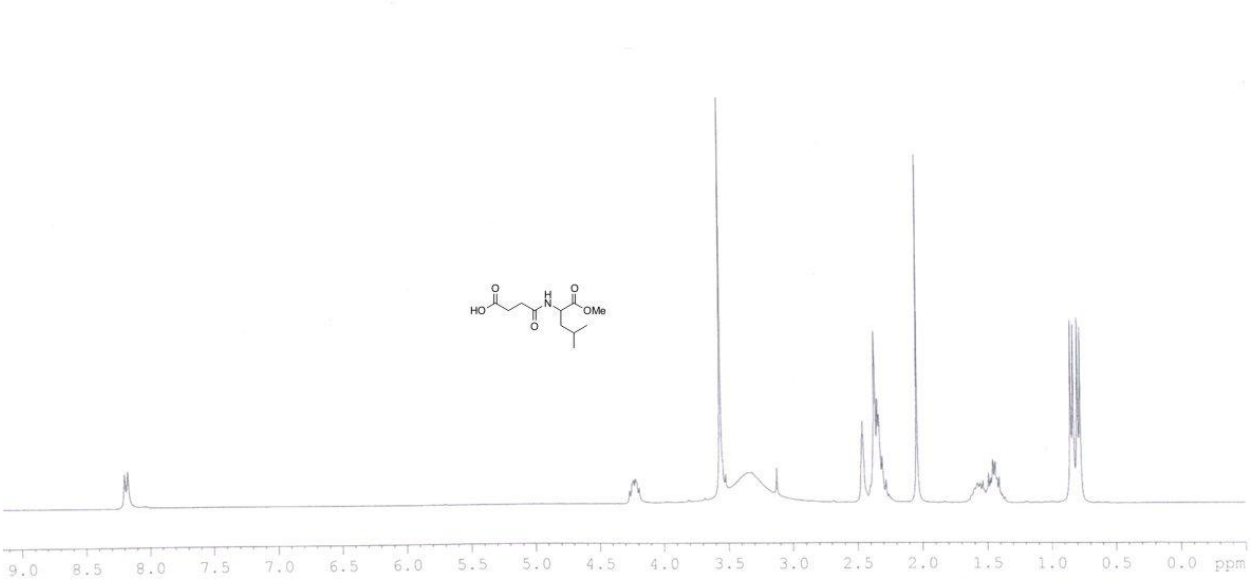
ESI Fig. 11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of MeO-Y-F-Suc-F-Y-OMe **8**.



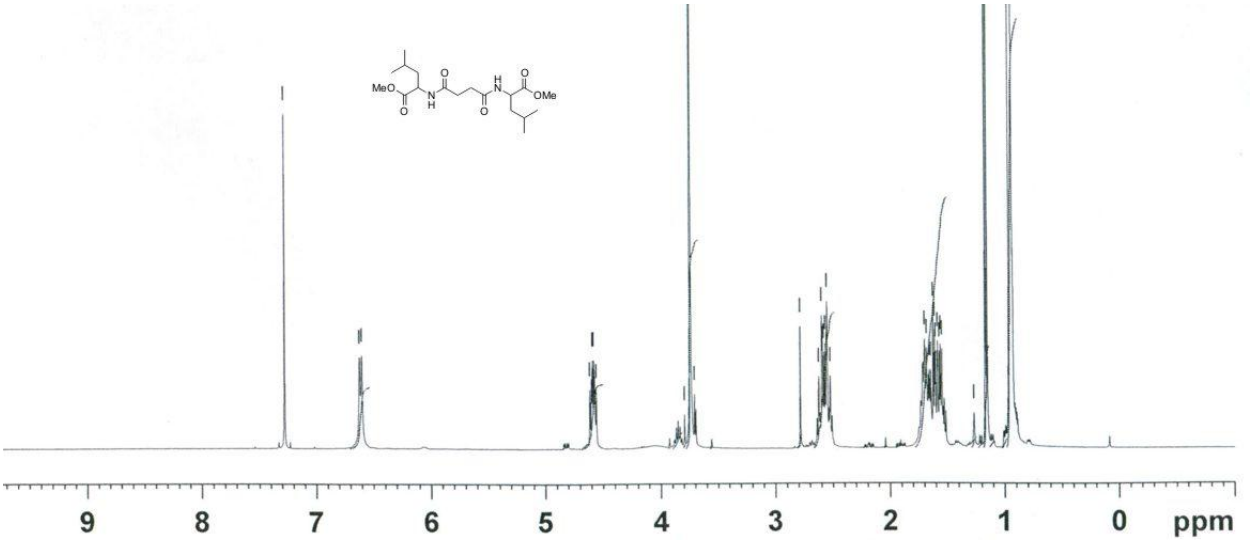
ESI Fig. 12. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-Y-F-Suc-F-Y-OH **1**.



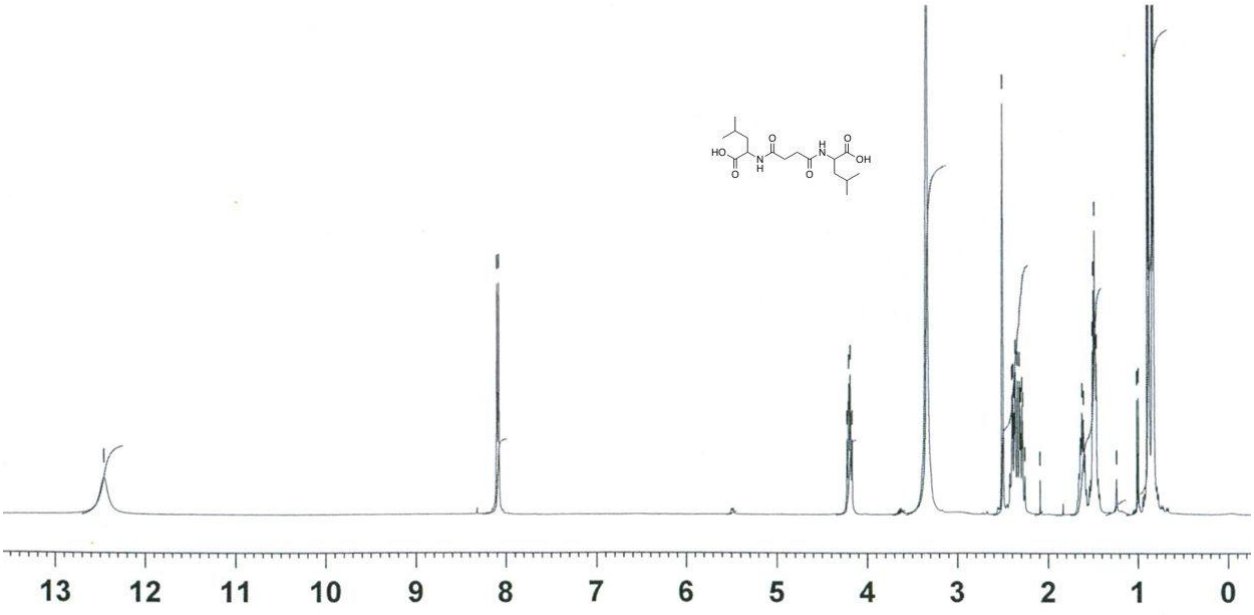
ESI Fig. 13. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of HO-Y-F-Suc-F-Y-OH **1**.



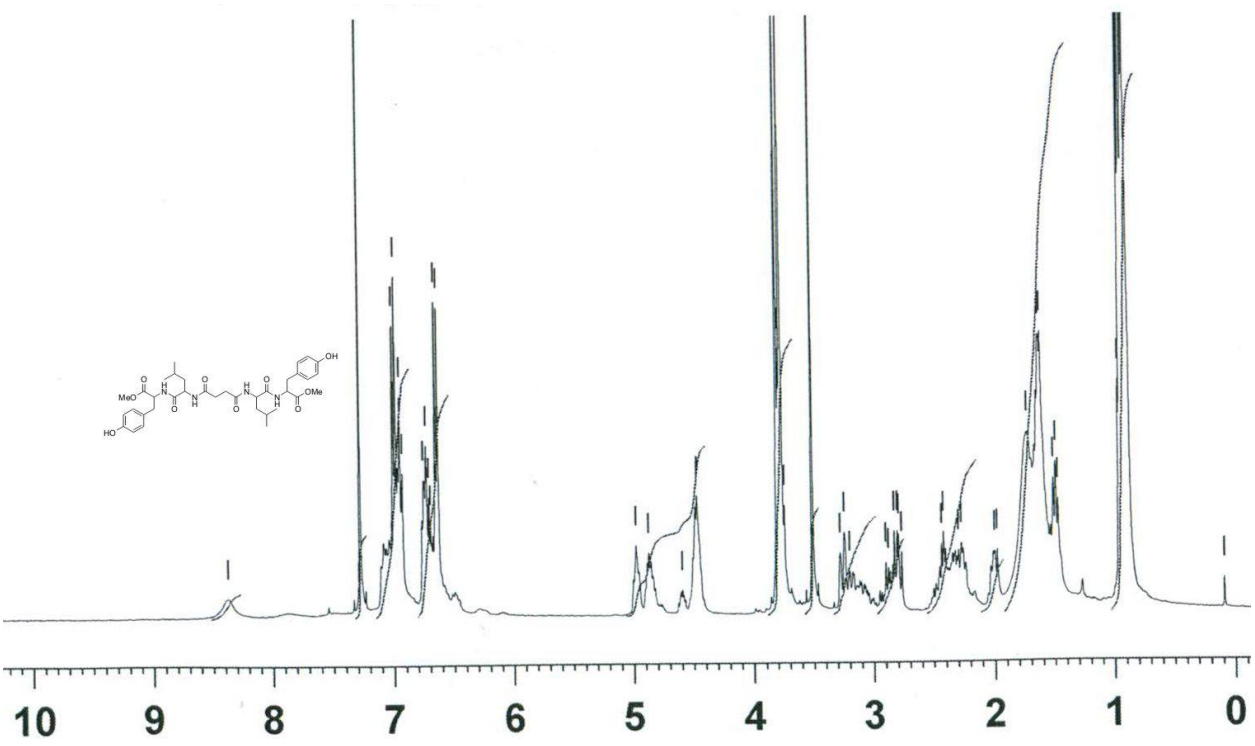
ESI Fig. 14. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of HO-Suc-L-OMe **9**.



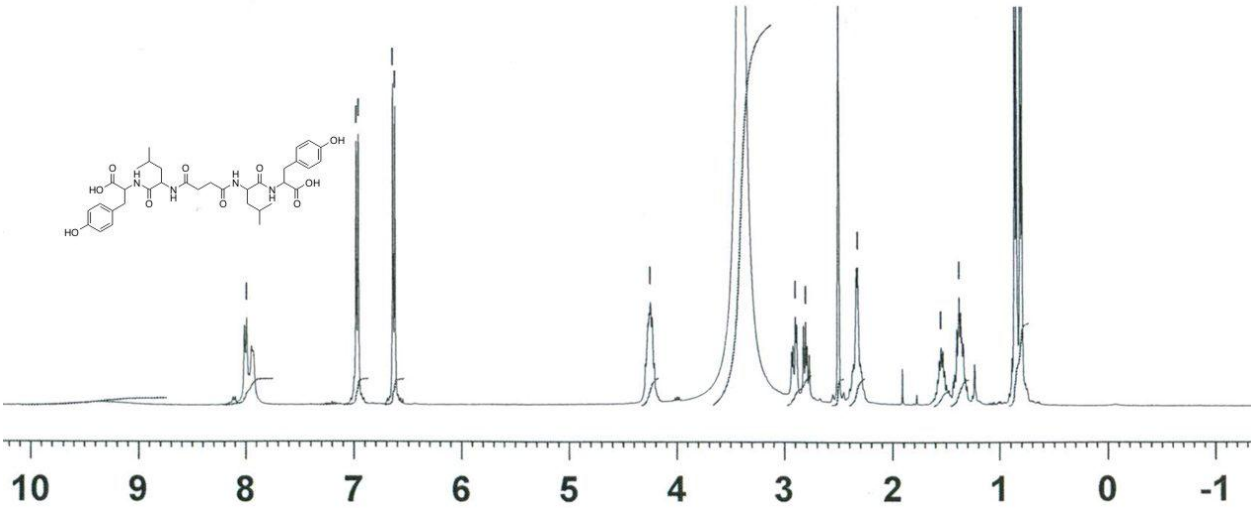
ESI Fig. 15. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of MeO-L-Suc-L-OMe **10**.



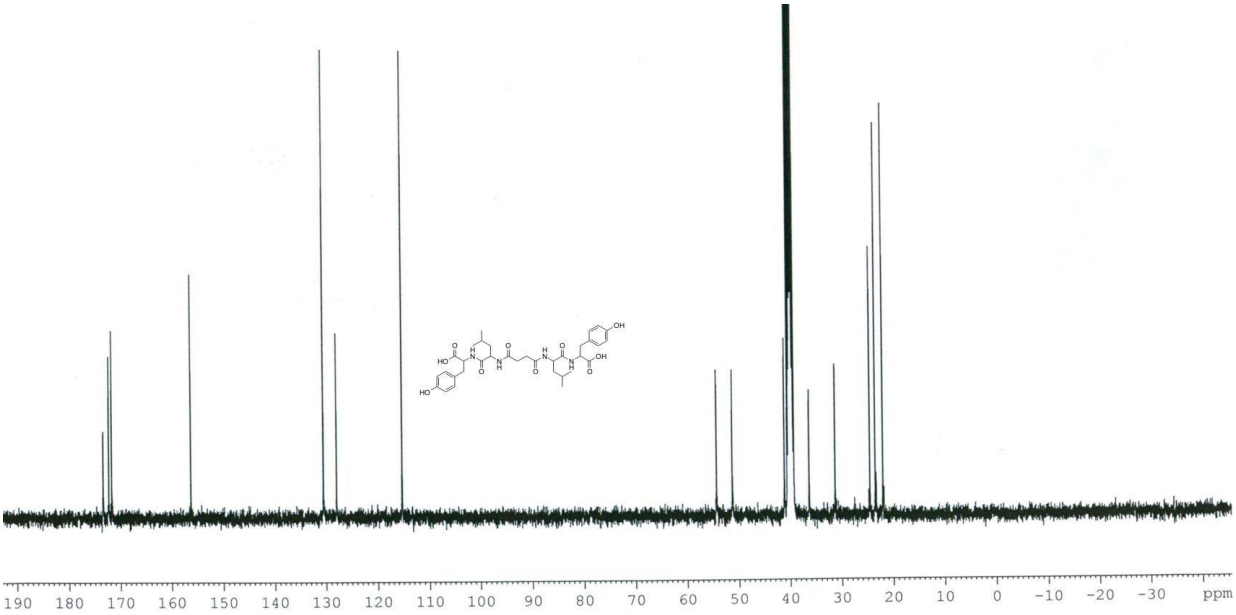
ESI Fig. 16. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-L-Suc-L-OH **11**.



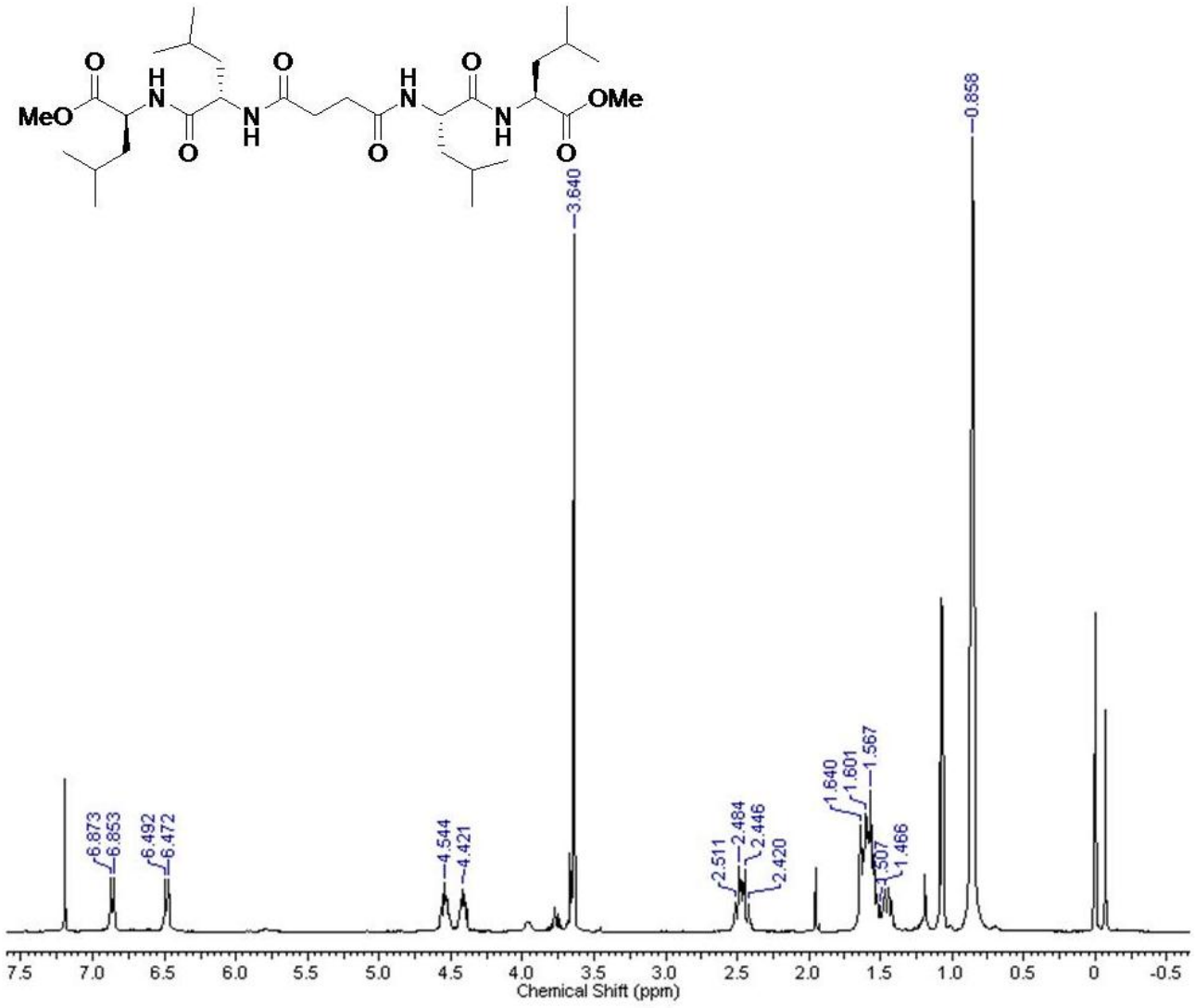
ESI Fig. 17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of MeO-Y-L-Suc-L-Y-OMe **12**.



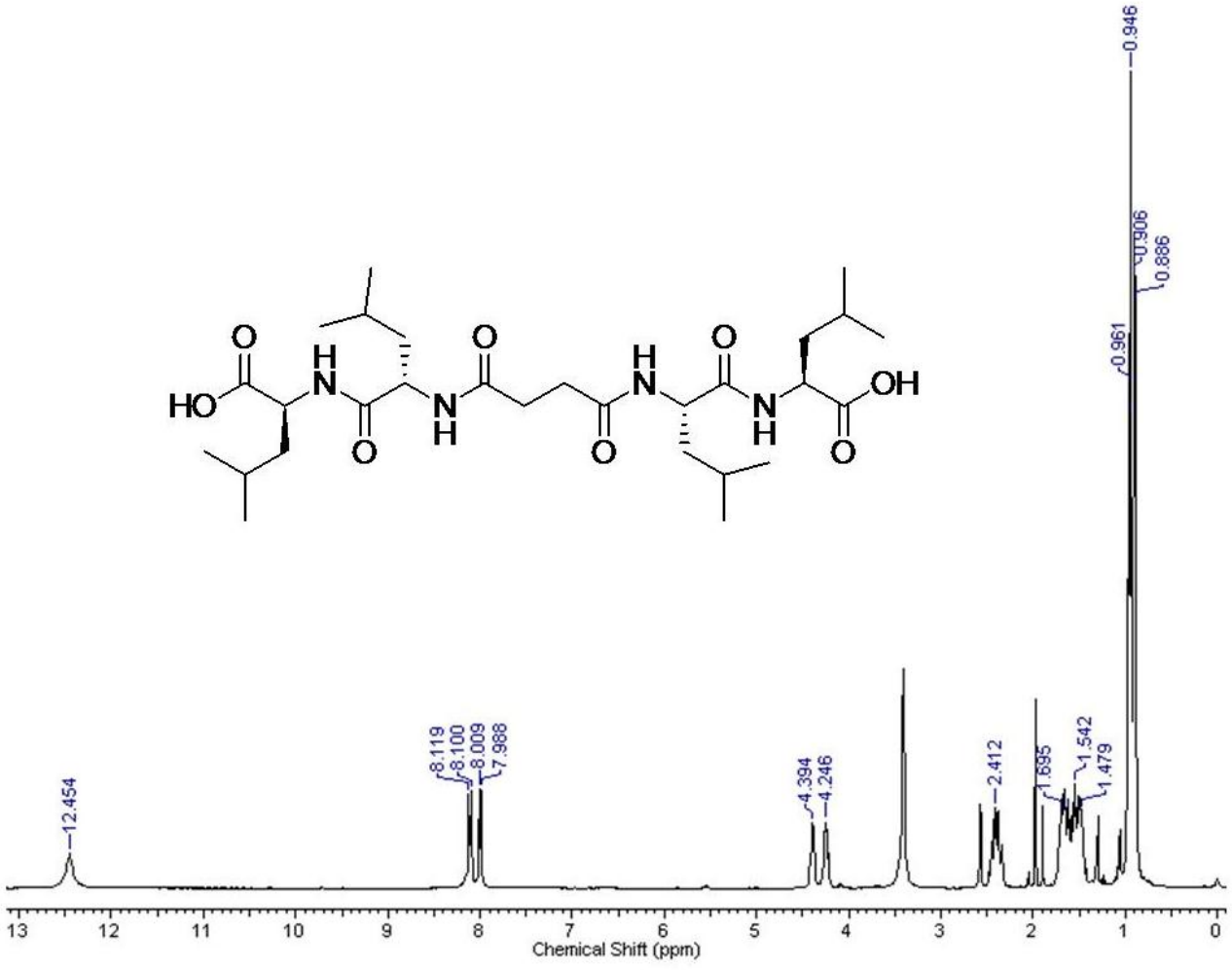
ESI Fig. 18. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-Y-L-Suc-L-Y-OH **2**.



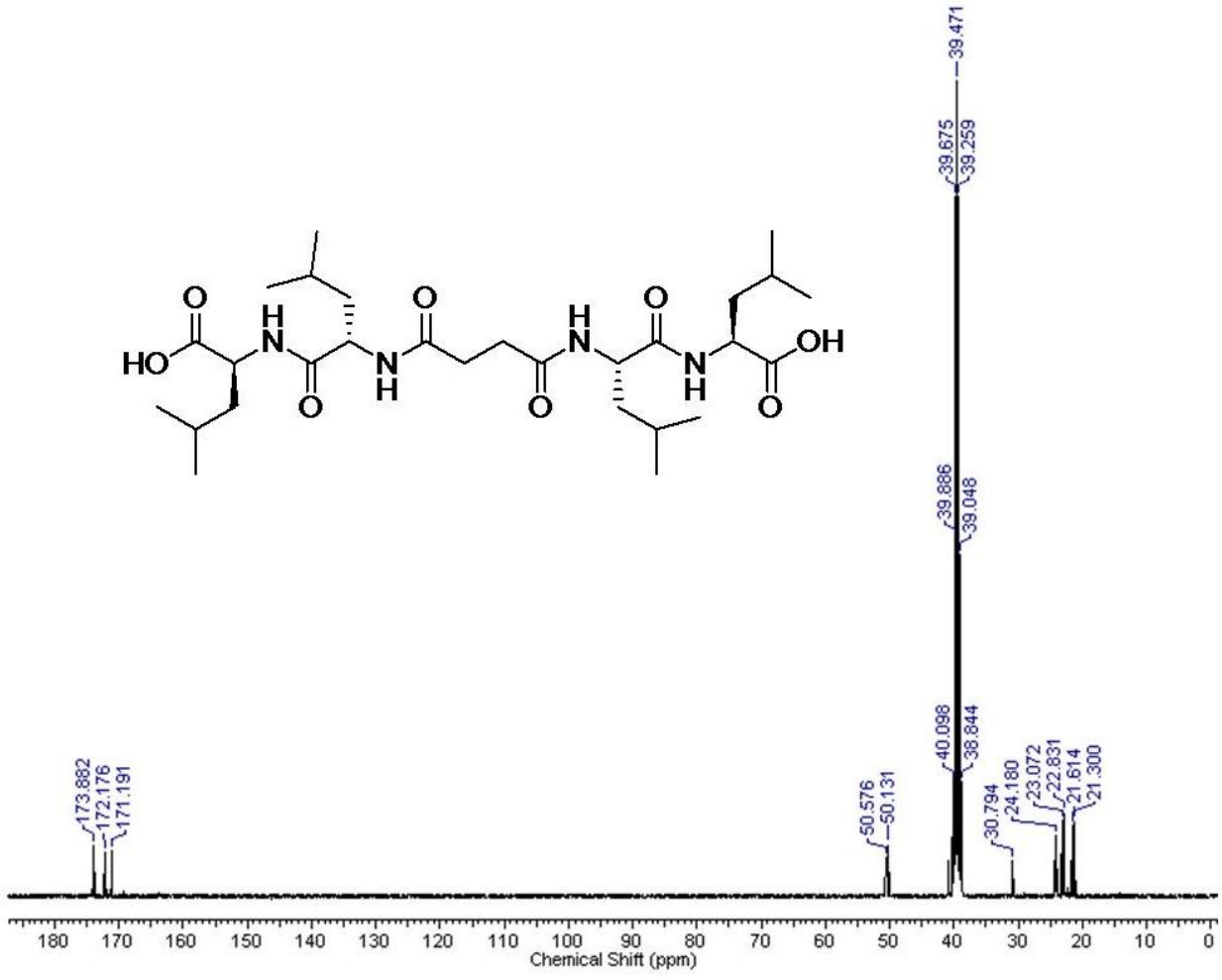
ESI Fig. 19. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of HO-Y-L-Suc-L-Y-OH **2**.



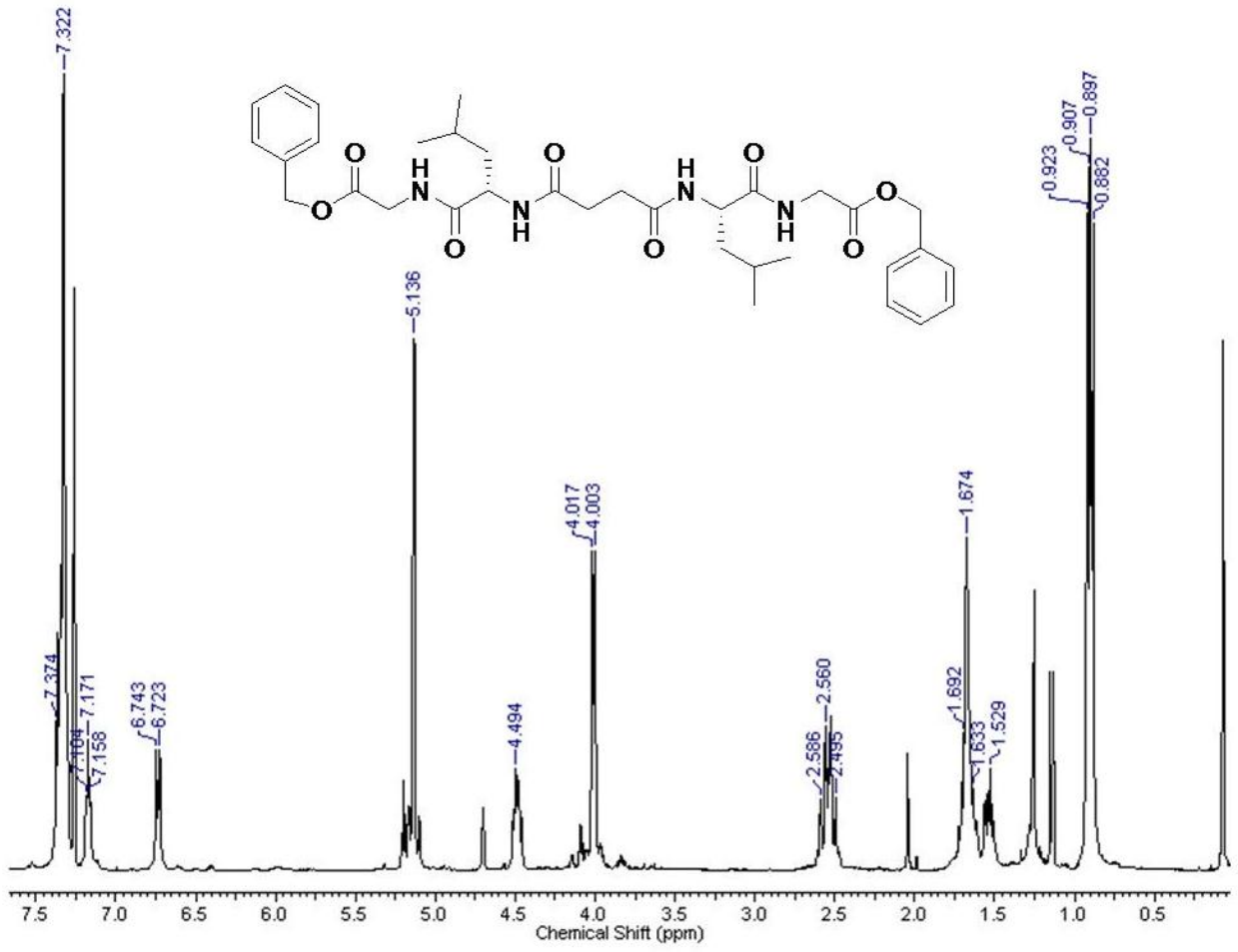
ESI Fig. 20. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of MeO-L-L-Suc-L-L-OMe **13**.



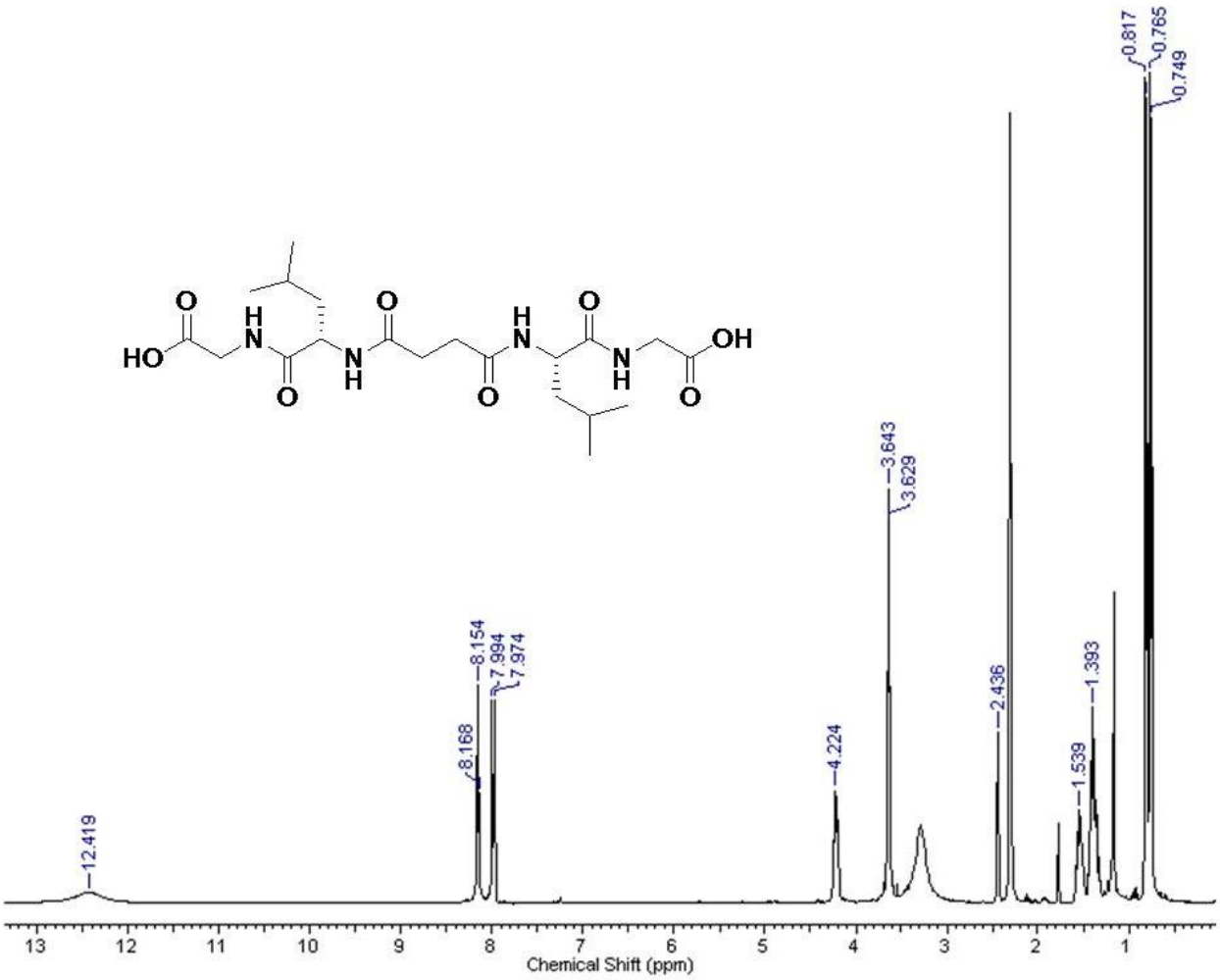
ESI Fig. 21. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-L-L-Suc-L-L-OH **3**.



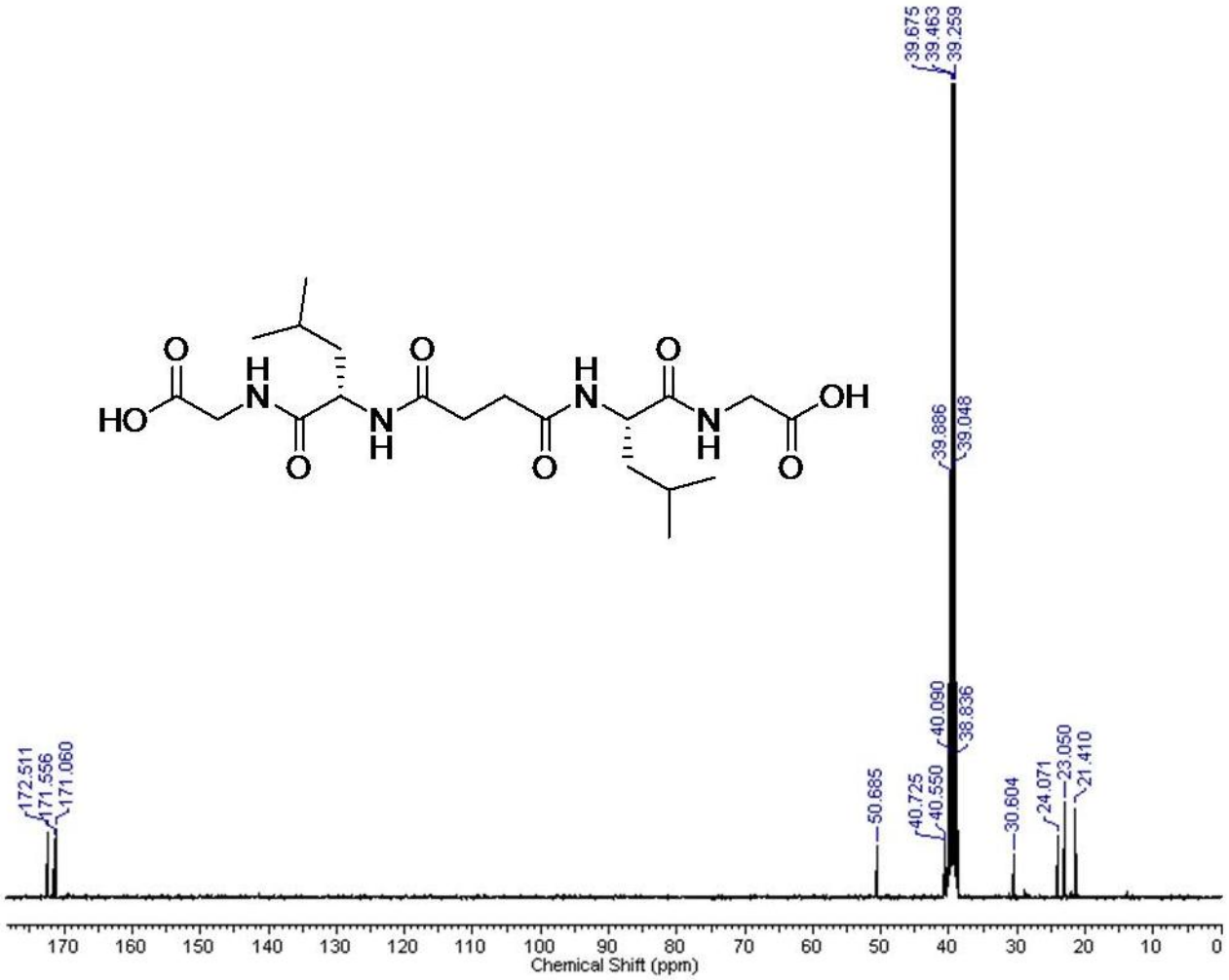
ESI Fig. 22. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of HO-L-L-Suc-L-L-OH **3**.



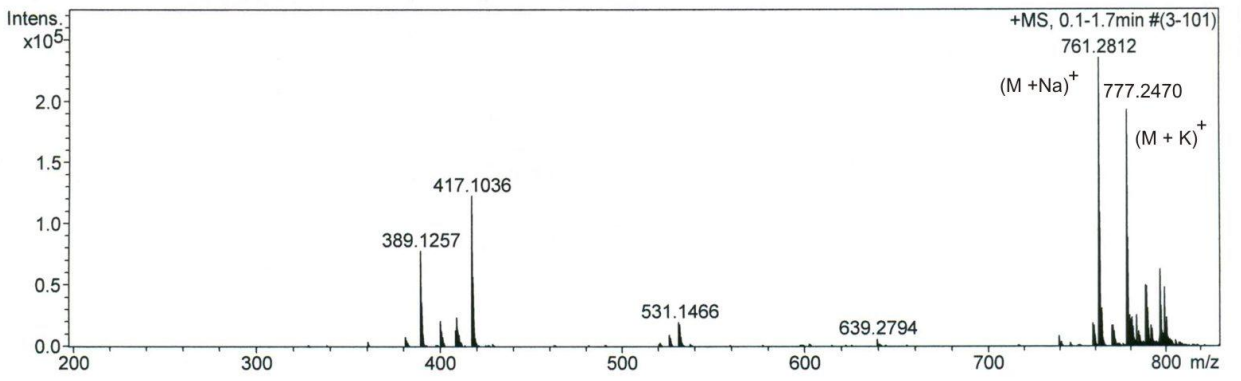
ESI Fig. 23. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of $PhCH_2O$-G-L-Suc-L-G-OCH_2Ph **14**.



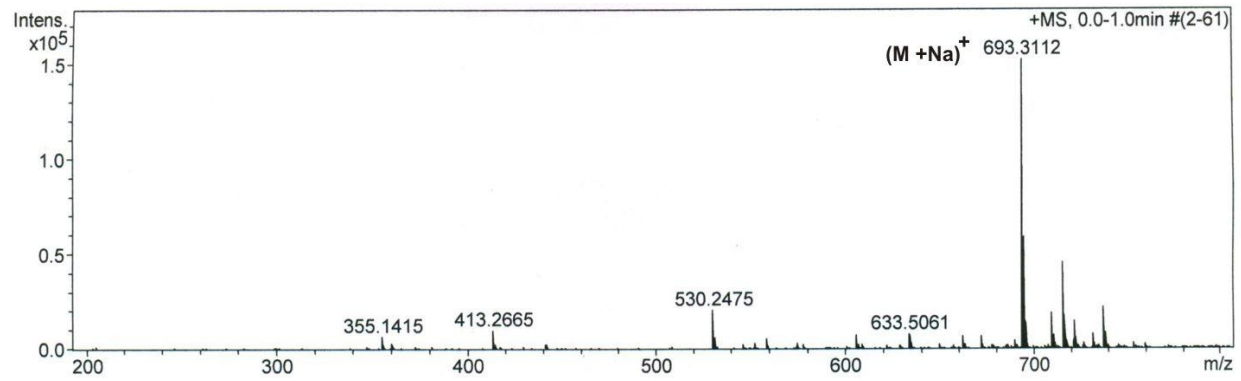
ESI Fig. 24. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of HO-G-L-Suc-L-G-OH **4**.



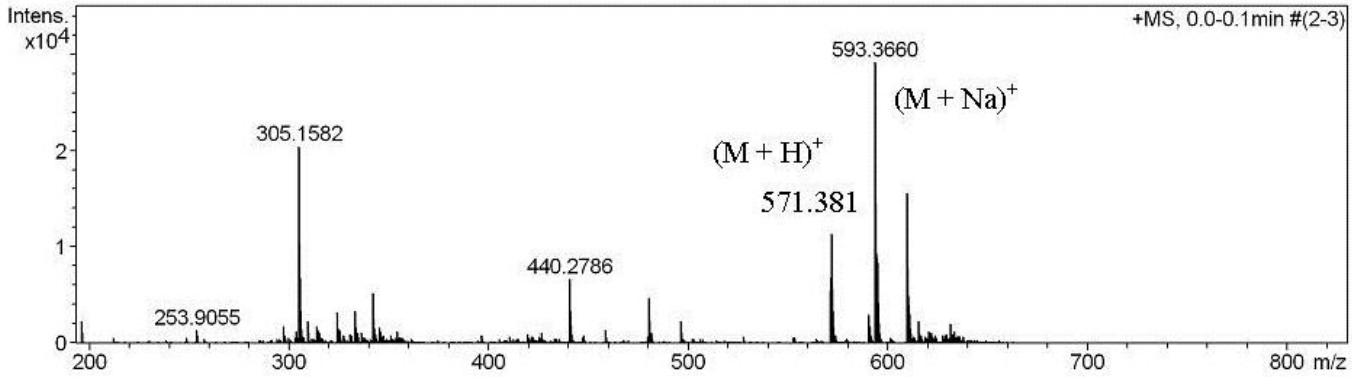
ESI Fig. 25. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of HO-G-L-Suc-L-G-OH **4**.



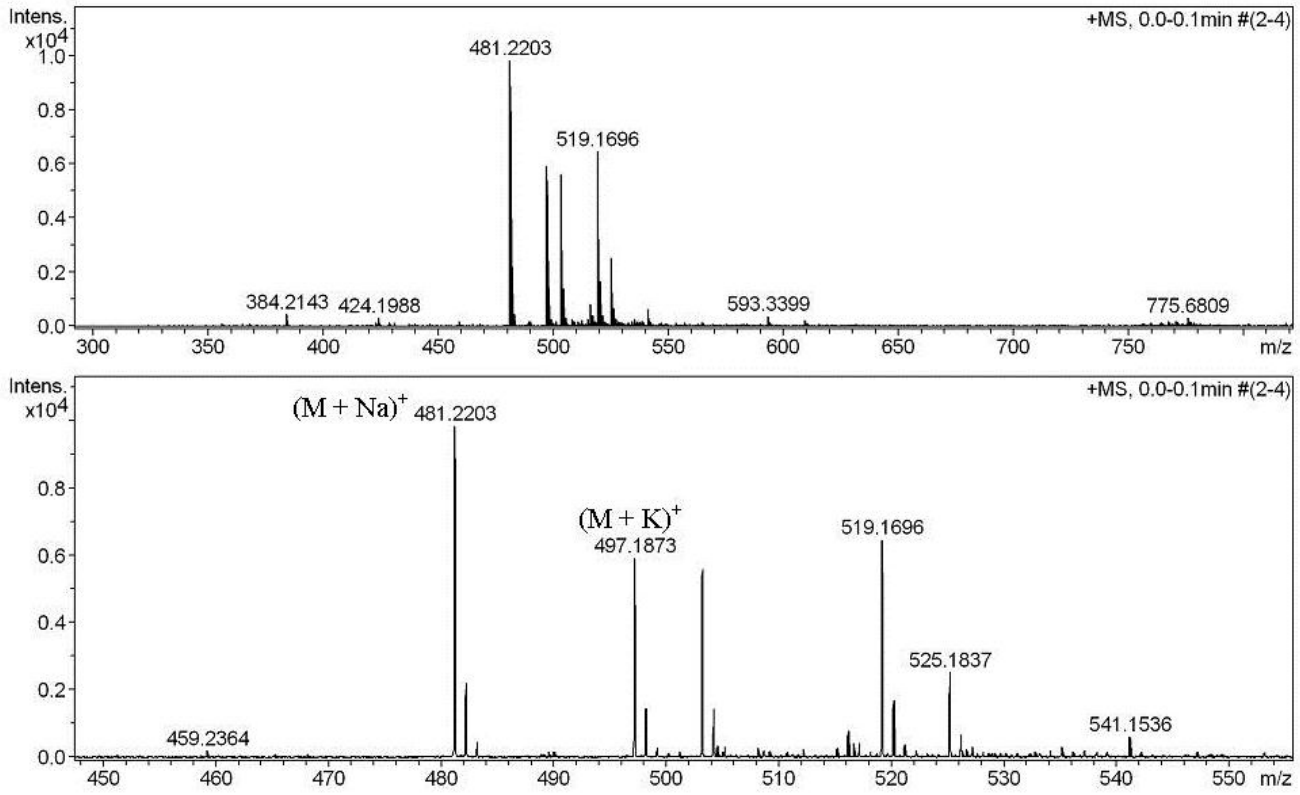
ESI Fig. 26. Mass spectrum of HO-Y-F-Suc-F-Y-OH **1.**



ESI Fig. 27. Mass spectrum of HO-Y-L-Suc-L-Y-OH **2.**



ESI Fig. 28. Mass spectrum of HO-L-L-Suc-L-L-OH **3.**



ESI Fig. 29. Mass spectrum of HO-G-L-Suc-L-G-OH **4.**