SUPPORTING INFORMATION for

Bolaform Homodiamides of *N*-protected Alanines as Efficient and Versatile Sono- and Thermo-Gelators Offering Phase-selectivity, Optical-transparency and Enzyme-assisted Release

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Experimental section:

Materials and methods: 1,6-diaminohexane, 2,2′-(Ethylenedioxy)bis(ethylamine) and 1,1-Carbonyl di imidazole (CDI) were purchased from Alfa Aesar. 1,8-diaminooctane and phenylacetic acid were obtained from Aldrich. 1-Naphthaleneacetic acid and Benzyl chloroformate were obtained from Spectrochem and were used without any further purification. The organic solvents used in the synthesis were dried or distilled, as required. FTIR spectra were recorded by using IRAffinity-1 Shimadzu. The ^{1}H, ^{13}C, DEPT NMR spectra were recorded by using Bruker Ultra shield (400MHz) spectrometer. Mass spectra were recorded in ESI-MS mode on MicroTOF-Q-II Bruker Daltonics. UV-vis absorption spectra were recorded on PerkinElmer Lambda 25. Elmasonic S30H bath sonicator (37 kHz , 280 W peak power) was used for sonication.

Syntheses:

Dibenzyl((2S,2'S)-(octane-1,8-diylbis(azanediyl))bis(1-oxopropane-2,1-diyl))dicarbamate, CBz8:

White colour solid. (87%) m.p. 174 °C. FTIR (KBr pellet, cm^{-1}): 1690(carbamate, C=O str.), 1653(amide, C=O str.), 1541(N-H bend), 3298(N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.19 (8H, CH_2), 1.28-1.30(6H, CH_3), 1.37(4H, NH-CH2-CH2), 3.09-3.16(4H, NH- CH2), 4.16(2H, C-H), 5.02(Ph-CH_2), 5.53(2H, N-H) 6.32(2H, N-H), 7.26 (10H, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ= 172.3,156, 136.19, 128.55, 128.22, 128.00, 66.97, 50.59, 39.40, 29.19, 28.61, 26.24, 18.70. $C_{30}H_{42}N_4O_6$+Na cacl: 577.2997, found: 577.2992.

Dibenzyl((2S,2'S)-(hexane-1,6-diylbis(azanediyl))bis(1-oxopropane-2,1-diyl))-dicarbamate, CBz6:

White colour solid. (90%) m.p. 153 °C. FTIR (KBr pellet, cm^{-1}): 1689 (carbamate, C=O str.), 1653 (amide, C=O str.), 1541 (N-H bend), 3299 (N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.20(4H, CH_2), 1.26-1.28 (6H, CH_3), 1.37(4H, NH-CH2-CH2), 3.09-3.17(4H, NH- CH2), 4.19(2H, C-H), 5.00(Ph-CH_2), 5.77(2H, N-H) 6.58(2H, N-H), 7.24(10H, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ= 172.84, 156.16, 136.24, 128.52, 128.09, 66.92, 50.69, 38.55, 28.95, 25.26, 18.73. $C_{28}H_{38}N_4O_6$+Na cacl: 549.2684, found: 549.2681.

General procedure for the synthesis of *N*-Protected alanine:

(S)-methyl 2-(2-arylacetamido)propanoate:

L-alanine hydrochloride methyl ester (1 eq, 15 mmol) was taken in dry THF (30 mL) and triethylamine (1.5 eq, 22.5 mmol) was added drop wise to it at 0 °C followed by stirring for 10 min. In a separate flask, arylacetic acid (1.1 eq, 16.5 mmol) and CDI (1.3 eq, 19.5 mmol) were taken in dry THF (15 mL) and stirred for 5 min. This activated acid solution was added drop wise at 0 °C to the basic mixture of L-alanine hydrochloride methyl ester followed by stirring under N_2 atmosphere for 2 h at RT. Reaction was monitored by TLC ($CHCl_3$/MeOH, 9.5:0.5 v/v). THF was removed from the reaction mixture in vacuo and 40 mL of $CHCl_3$ was added to it. The $CHCl_3$ layer was washed with dil HCl and sat. $NaHCO_3$, dried over anhyd. sodium sulphate. $CHCl_3$ was removed by rotary evaporator yielded corresponding ester which was purified by column chromatography (stationary phase was silica 100-200 mesh and eluent phase was $CHCl_3$/MeOH).

(S)-methyl 2-(2-phenylacetamido)propanoate:

White colour solid. (80%) m.p. 54 °C. FTIR (KBr pellet, cm^{-1}):1745 (ester, C=O str.), 1650 (amide, C=O str.), 1530 (N-H bend), 3343 (N-H str.). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.35-1.37 (3H, CH_3), 3.61(2H, Ph-CH_2), 3.73(3H, O-CH_3), 4.60(1H, C-H), 6.01(1H, N-H), 7.29-7.41(5H, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ= 173.38, 170.45, 134.53, 129.40, 129.01, 127.41, 52.45, 48.09, 43.61, 18.36. $C_{12}H_{15}NO_3$+H cacl: 222.1125, found: 222.1132.

(S)-methyl 2-(2-(naphthalen-1-yl)acetamido)propanoate:

White solid. (85%) m.p. 104 °C. FTIR (KBr pellet, cm^{-1}):1741 (ester, C=O str.), 1644 (amide, C=O str.), 1546 (N-H bend), 3301 (N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.24-1.26 (3H, CH_3), 3.64(3H, O-CH_3), 4.02-4.11(2H, Nap-CH_2) 4.58(1H, C-H), 5.93(1H, N-H), 7.45-8.00(7H, Nap-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ= 173.09, 170.44, 133.98, 132.06, 130.80, 128.80, 128.55, 128.31, 126.66, 126.14, 125.65, 123.82, 52.32, 48.07, 41.59, 18.09. $C_{16}H_{17}NO_3$+Na cacl: 294.1101, found: 294.1107.

(S)-2-(2-arylacetamido)propanoic acid:

The respective propionate ester (1 eq, 12.6 mmol) was dissolved in methanol (10 mL) followed by drop-wise addition of 1 N NaOH (1.2 eq, 15.1 mmol) at 0 °C. This mixture was stirred for 3 h at RT. Reaction was monitored by TLC. Methanol was removed by rotary evaporator and reaction mixture was acidified by 1 N HCl to pH 2. The aqueous layer was washed with $CHCl_3$ and the $CHCl_3$ was dried with sodium sulphate, removed by rotary evaporator yielded corresponding acids.

(S)-2-(2-phenylacetamido)propanoic acid:

White colour solid.(86%) m.p. 85 °C. FTIR (KBr pellet, cm^{-1}):2429-3472 (br., O-H str.), 1709 (acid, C=O str.), 1625 (amide, C=O str.), 1556 (N-H bend), 3335(N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.38-1.40 (3H, CH_3), 3.63(2H, Ph-CH_2), 4.57 (1H, C-H), 6.30(1H, N-H), 7.26-7.39(5H, Ar-H), 10.07(1H, O-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ= 175.87, 171.93, 134.07, 129.41, 129.06, 127.54, 48.37, 43.22, 17.93. $C_{11}H_{13}NO_3$+H cacl: 208.0968, found: 208.0977.

(S)-2-(2-(naphthalen-1-yl)acetamido)propanoic acid:

White colour solid. (90%) m.p. 120 °C. FTIR (KBr pellet, cm^{-1}): 2534-3489 (br., O-H str.), 1732 (acid, C=O str.), 1609(amide, C=O str.), 1529 (N-H bend), 3378(N-H str.).^{1}H NMR (400 MHz, NaOD, ppm): δ=1.17-1.19(3H, CH_3), 3.91(2H, Nap-CH_2) 4.00-4.05(1H, C-H), 7.31-87.83(7H, Nap-H). ^{13}C NMR (400 MHz, NaOD) δ= 180.00, 173.46, 133.46, 131.67, 130.99, 128.68, 128.36, 127.98 126.65, 126.13, 125.80, 123.48, 51.03, 39.76, 17.41. $C_{15}H_{15}NO_3$+Na cacl: 280.0944, found: 280.0948.

General procedure for the synthesis of bolaform diamides:

(S)-2-(2-arylacetamido)propanoic acid (1 eq, 10 mmol) and CDI (1.2 eq, 12 mmol) were taken in dry THF (20 mL) and stirred for 5 min. The diamine (1.4 eq, 14 mmol) in THF (7 mL) was added drop wise at 0 °C to the activated acid solution and stirred under N_2 atmosphere for 1.5 h at RT. Reaction was monitored by TLC ($CHCl_3$/MeOH). THF was removed from the reaction mixture by vacuum and 50 mL of $CHCl_3$/isopropanol (9:1) was added to it. The organic layer was washed with dil HCl and sat. $NaHCO_3$, dried with sodium sulphate. Removal of the solvent yielded the crude bolaamphiphile, which was purified by column chromatography (stationary phase was silica 100-200 mesh and eluent phase was $CHCl_3$/MeOH).

(2S,2'S)-N,N'-(octane-1,8-diyl)bis(2-(2-(naphthalen-1-yl)acetamido)propanamide), Nap8:

White colour solid. (79%) m.p. 218 °C. FTIR (KBr pellet, cm^{-1}): 1637(amide, C=O str.), 1541 (N-H bend), 3288(N-H str.).^{1}H NMR (400 MHz, DMSO, ppm): δ=1.19-1.20(6H, CH_3), 1.21(8H, CH_2) 1.34(4H, NH-CH_2-CH_2), 3.01(4H, NH-CH_2), 3.92-3.99(4H, Nap-CH_2), 4.25(2H, C-H), 7.43-7.92(14H, Nap-H), 8.08-8.10(N-H), 8.31-8.33(N-H). ^{13}C NMR (400 MHz, DMSO) δ= 172.38, 170.13, 128.78, 128.18, 127.46, 126.33, 125.99, 124.82, 48.69, 39.99, 38.89, 29.44, 29.09, 26.67, 19.10. $C_{30}H_{42}N_4O_4$+Na cacl: 645.3411, found: 645.3410.

(2S,2'S)-N,N'-(octane-1,8-diyl)bis(2-(2-phenylacetamido)propanamide), Bz8:

White colour solid. (81%) m.p. 189 °C. FTIR (KBr pellet, cm^{-1}): 1642(amide, C=O str.), 1541 (N-H bend), 3288 (N-H str.).^{1}H NMR (400 MHz, DMSO, ppm): δ=1.17-1.19 (6H, CH_3), 1.22(8H, CH_2) 1.36(4H, NH-CH_2-CH_2), 3.02(4H, NH-CH_2), 3.46 (4H, Nap-CH_2), 4.23(2H, C-H), 7.19-7.31(14H, Nap-H), 8.7.81(N-H), 8.18-8.20(N-H). ^{13}C NMR (400MHz, DMSO) δ= 172.40, 170.15, 136.91, 129.48, 128.59, 126.72, 48.62, 42.47, 38.88, 29.45, 29.11, 26.68, 19.08. $C_{30}H_{42}N_4O_4$+Na cacl: 545.3098, found: 545.3096.

(2S,2'S)-N,N'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(2-(2-(naphthalen-1-yl)acetamido)propanamide), OxNap:

White colour solid. (85%) m.p. 164 °C. FTIR (KBr pellet, cm^{-1}): 1638(amide, C=O str.), 1542(N-H bend), 3282(N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.22-1.44(6H, CH_3), 3.20(4H, NH-CH_2), 3.38(4H, O-CH2-CH2-O), 3.46(4H, NH- CH2-CH2), 3.90-3.99(Nap-CH_2), 4.54(2H, C-H) 6.72-6.74(2H, N-H), 7.13(2H, N-H), 7.34-7.94 (14H, Nap-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ= 172.49, 171.05, 133.89, 132.06, 131.01, 128.79,

128.21, 126.49, 125.92, 125.59, 123.69, 70.38, 69.40, 48.84, 41.08, 39.33, 18.21. . $C_{36}H_{42}N_4O_6$+Na cacl: 649.2997, found: 649.2998.

(2S,2'S)-N,N'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(2-(2-phenylacetamido)propanamide), OxBz:

White colour solid. (82%) m.p. 166 °C. FTIR (KBr pellet, cm^{-1}): 1641(amide, C=O str.), 1541(N-H bend), 3284(N-H str.).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ=1.23-1.24(6H, CH_3), 3.23(4H, NH-CH_2), 3.42(4H, O-CH2-CH2-O), 3.46(4H, NH- CH2-CH2), 3.48(Ph-CH_2), 4.45(2H, C-H) 6.62-6.64(2H, N-H), 7.15(2H, N-H), 7.17-7.26 (10H, Ar-H). ^{13}C NMR (400MHz, $CDCl_3$) δ= 172.60, 171.13, 134.67, 129.27, 128.85, 127.25, 70.51, 69.53, 48.85, 43.35, 39.45, 18.34. $C_{28}H_{38}N_4O_6$+H cacl: 527.2864, found: 527.2872.

Gelation by thermal process:

15 mg of compound in was suspended in 1 mL of solvent. This suspension was slowly heated until a homogeneous solution was obtained. This hot sol was allowed to cool undisturbed for a certain duration (from 30 min to 12 h, depending on the gelator and solvent combination) under ambient conditions. Gelation was inferred by the ability of the sample to resist flow upon inversion at the end of this time period.

Gelation by sonication:

The gelator (15 mg) was suspended in 1 mL solvent in a glass vial. This suspension was heated to obtain a clear solution, which was cooled ambiently for 10 min. At the end of this time, the sol was sonicated in a bath sonicator for 60 s. Gel formation was implied if the resultant mass did not fall on inversion on vial. Control experiment, where the sample

vial was immersed in a water bath, was done to ensure that the gelation was not a result of better cooling in the bath.

Measuring minimum gelation concentration (MGC):

(a) For thermal process: 15 mg of the particular compound was taken in glass vial and dissolved in 1 mL of the respective solvent by heating. This solution was left undisturbed for 60 min. If, at the end of this period, the resultant mass was stable to inversion and no solvent flowed, additional 200 µL of the same solvent was added and the process repeated till the gel was unstable to inversion. MGC values were calculated from the maximum volume of solution completely entrapped.

(b) For sonicated sample: 15 mg the gelator was solubilized in the respective solvent by heating. After cooling this sol for 10 min, it was sonicated for 60 s. If the resultant gel was stable to inversion and no solvent flowed from it at the end of this process, additional 200 µL of solvent was added to it and the process repeated till either the flow of excess solvent is observed, or the gel mass was unstable.

Determination of gel-to-sol transition temperature (or gel-melting temperature, T_m):

For all gelator-solvent combinations, gels were prepared (by either thermal process, or by sonication) at 15 mg/mL concentrations in screw-capped glass vials. These vials were attached to a thermometer near its bulb-end. This assembly was immersed in a stirred water bath whose temperature was raised at *ca*. 5˚ C/min. The temperature at which the gel-mass fell down was recorded. This process was triplicated to improve the accuracy of reported values.

UV-vis spectroscopy:

The gel was prepared from **CBz8** by a thermal process in commercial vegetable oil at MGC (6 mg/mL). UV-vis absorption spectrum of this sample was recorded in the region of 400-900 nm against commercial vegetable oil as reference.

Variable temperature NMR (VT-NMR) study:

For VT-NMR study, the **Bz8** organogel was prepared in $CDCl_3$ (15 mg/mL). Spectra were recorded at 5 °C intervals between 25 and 45 °C.

Differential scanning calorimetry: Thermal gels were prepared from **Bz8** and **CBz8** in isopropanol (13 mg/mL). These were subsequently dried *in vacuo* for 12 h to obtain the xerogel. Individual samples (7.5 mg) of these xerogels were taken in separate aluminium pans and crimp-sealed. These pans were heated at the rate of 1 °C/min in the temperature range of 30– 200 °C for **CBz8** and 30 – 220 °C for **Bz8**.

Temperature-dependent UV-vis studies: UV-vis spectra were recorded for **Bz8** (0.2 mg/mL) in water: methanol (6:4 v/v) mixture at 30 °C, 40 °C, 45 °C.

Microscopy:

For SEM, **Nap8** gel was prepared in EtOH:Water (1:1, v/v) mixture and dried in vacuum dessicator for 24 hrs. The xerogel was spread over a double-sided carbon tape on an aluminum stub, and coated with gold vapors for 180 s using JFC-1600 auto fine coater. Images were acquired at 20 kV acceleration voltage and 800x magnification.

For AFM, 10 µL of sol of **Nap8** or **RNap8** (0.2 mg/mL in EtOH:Water (1:1, v/v)) was dropped onto a freshly cleaved mica surface. Sample was air dried for 1 h before AFM imaging. The images were obtained by scanning the mica surface in non-contact mode using NSC 19/AIBN cantilever (Micromash), length=125±5 nm, width=35±3 nm,

thickness =1.0±0.5 nm, tip radius <10 nm, resonant frequency=80 kHz, force constant=0.6 Nm^{-1}. AFM scans were recorded at 256 x 256 pixels resolution and topographic, amplitude and phase images were taken.

Entrapment of Tetracycline hydrochloride (TetH):

Aqueous solution of Tetracycline hydrochloride (TetH, 3 mg in 500 µL distilled water) was heated to 50 °C, and was added completely to the hot clear ethanolic solution of **Nap8** (5 mg in 500 µL of ethanol) to obtain a clear sol. This mixture was allowed to cool undisturbed for 1 h, and the resulting gel was then washed with 1 mL of 10 mM of Tris buffer pH 8.1 containing 5 mM solution of cysteine. The absorbance of the supernatant buffer were measured at 356 nm to obtain the percent entrapment of TetH.

Release of TetH:

1 mL of papain solution (1 mg/mL of papain in 5 mM solution of cysteine in 10 mM of Tris buffer pH 8.1) was added carefully on the top of the gel prepared as given above. This solution was carefully replaced at every 4 h (up to 40 h) with 1 mL of fresh papain solution. The absorbance at 356 nm was recorded for the solution withdrawn. A 1 mL of 5 mM solution of cysteine in 10 mM of Tris buffer pH 8.1 was used for control experiment. Standard curve of TetH in 5 mM cysteine in 10 mM of Tris buffer pH 8.1 was prepared from 1 µg/mL to 15 µg/mL at 356 nm.

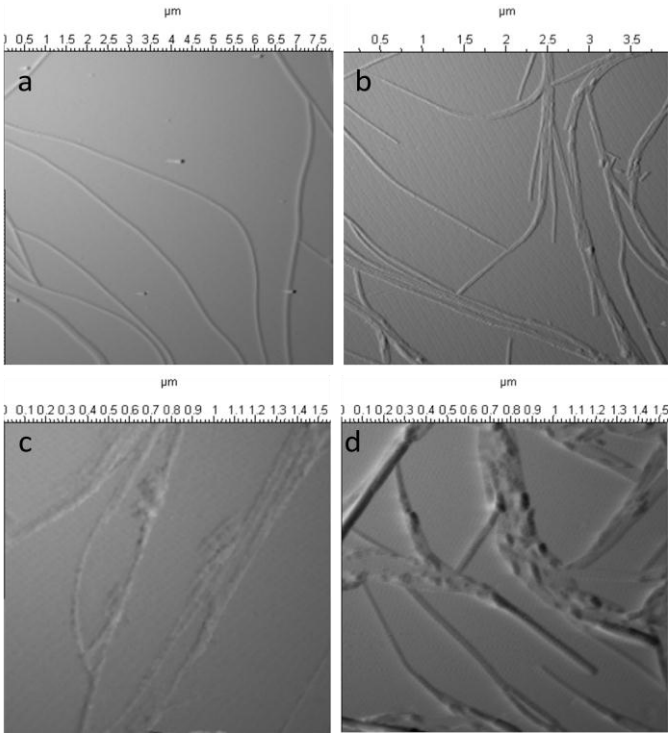

Fig. S1. AFM images of nanofibrils formed by (a) **CBz6**, (b) **CBz8**, (c) **OxNap**, and (d) **Bz8** in isopropanol (gelator concentration = 0.3 mg/mL).

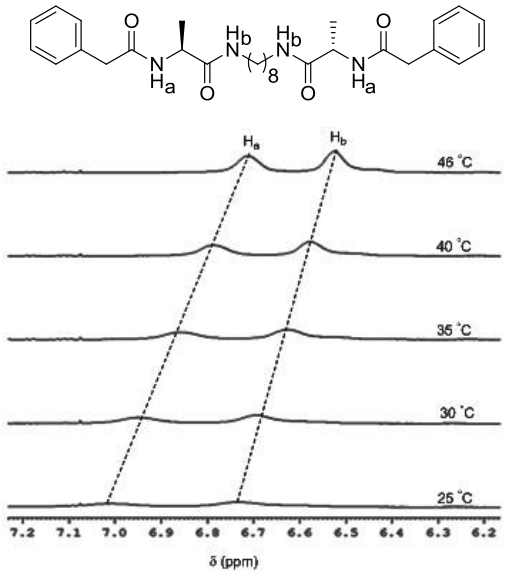

Fig. S2. Variable-temperature ^{1}H NMR of $CDCl_3$ gel of **Bz8**. As the temperature is increased, upfield shifts and increase in intensity of amide resonance peaks were observed, as highlighted by the dashed lines.

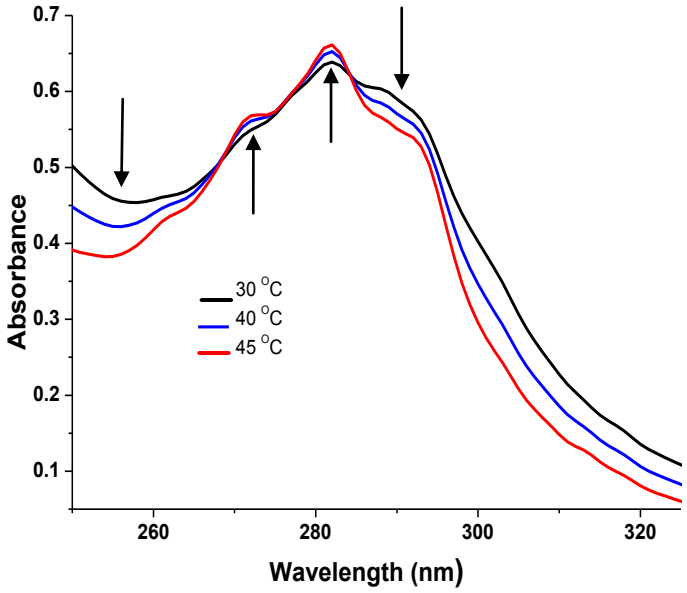

Fig. S3. Temperature-dependent UV-vis spectra of **Bz8** in H_2O:MeOH (6:4 v/v) at 0.2 mg/mL concentration**.** Arrows indicate changes in absorbance on increasing temperature of the solution.

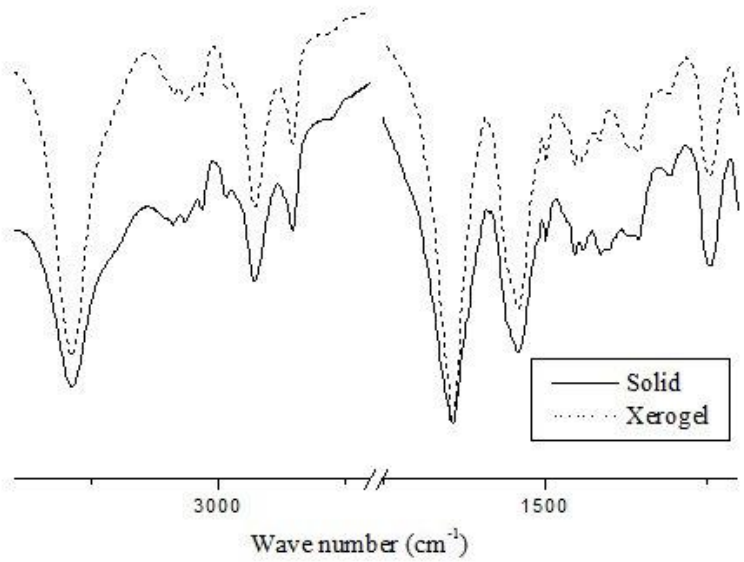

Fig. S4. FTIR spectra for solid **Bz8** (solid line) and xerogel from $CHCl_3$ (dotted line) recorded as KBr pellets indicating β-sheet like assemblies in both the samples.

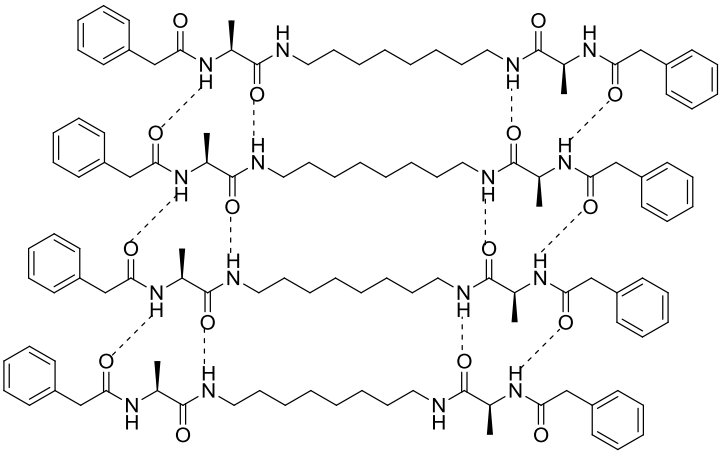

Fig. S5. Schematic model for aggregation of **Bz8** in organic solvents.

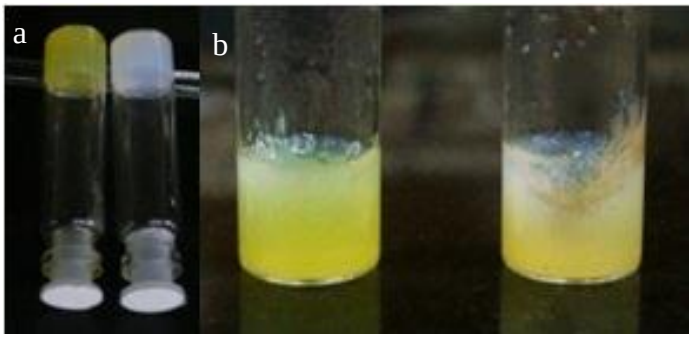

Fig. S6. Entrapment of TetH in ethanol-water gel formed by **Nap8**. (a) With tetracycline (left vial) and without tetracycline (right vial); (b) After 20 h degradation with papain. Vial at left is control sample, and that at right was treated with papain. The gel integrity is maintained in the control vial while disintegration is visible in gel exposed to papain.

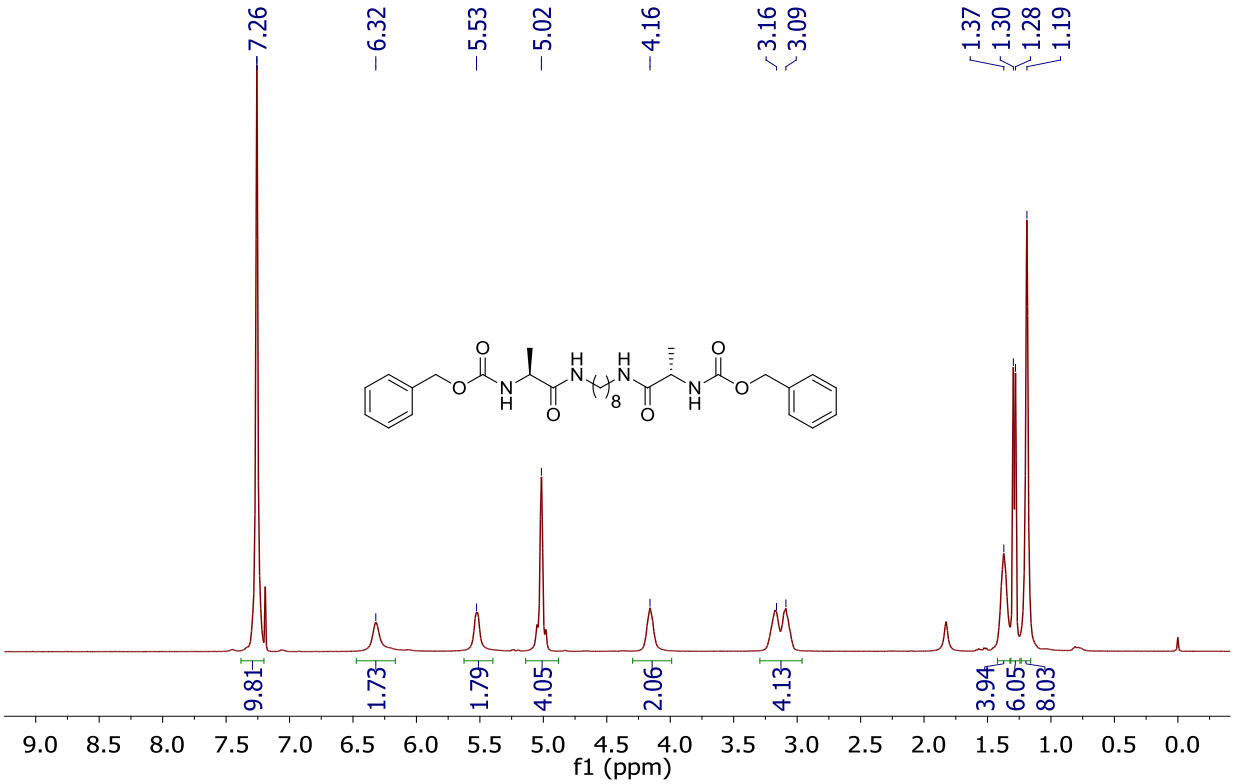

Fig. S7. ^{1}H NMR (400 MHz) spectra of **CBz8** in $CDCl_3$.

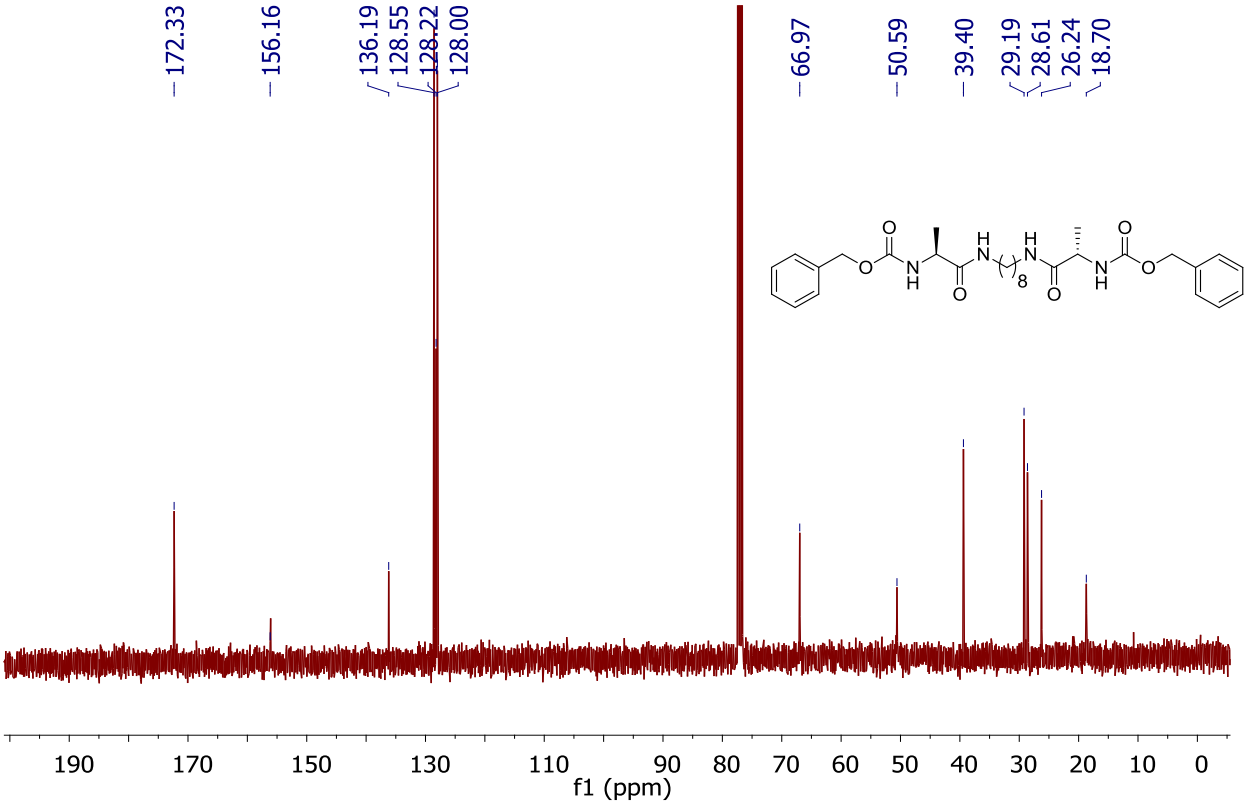

Fig. S8. ^{13}C NMR (100 MHz) spectra of **CBz8** in $CDCl_3$.

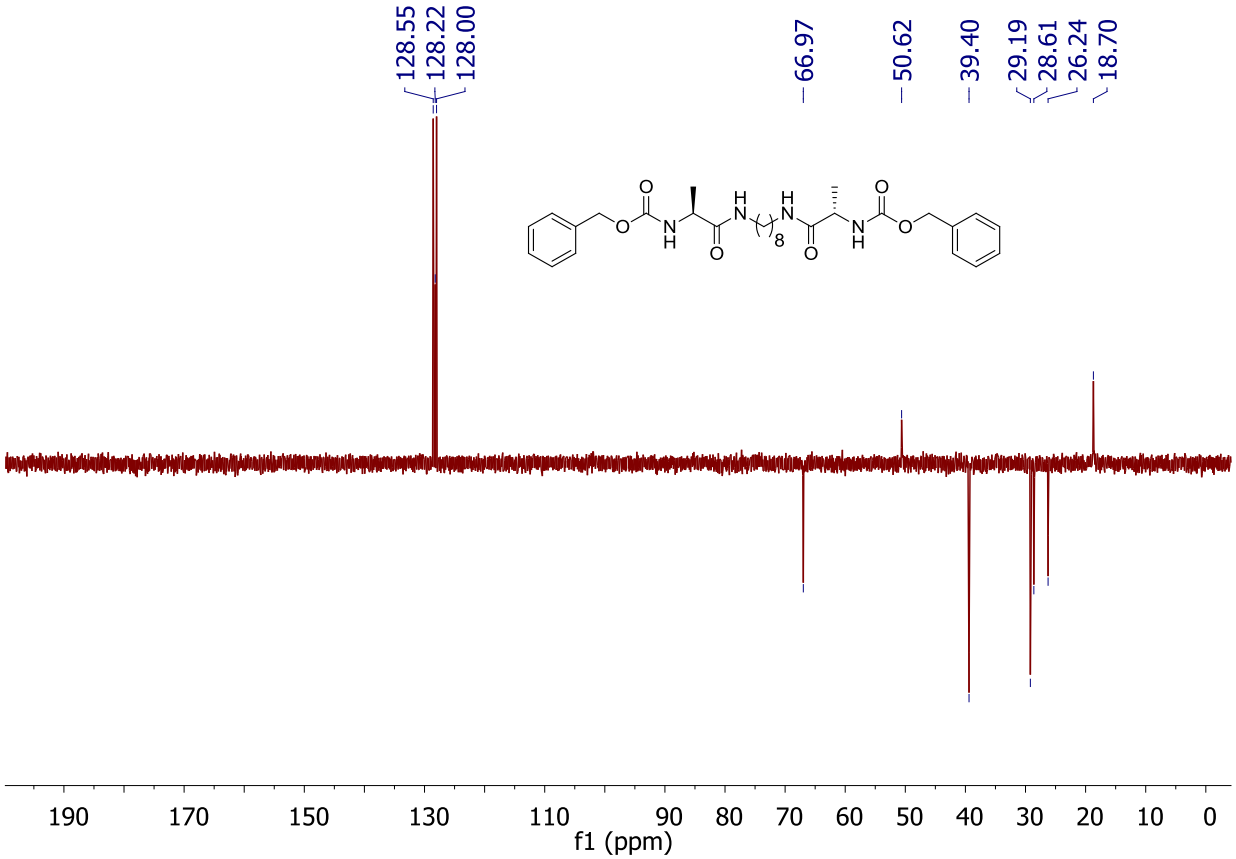

Fig. S9. DEPT (100 MHz) spectra of **CBz8** in $CDCl_3$.

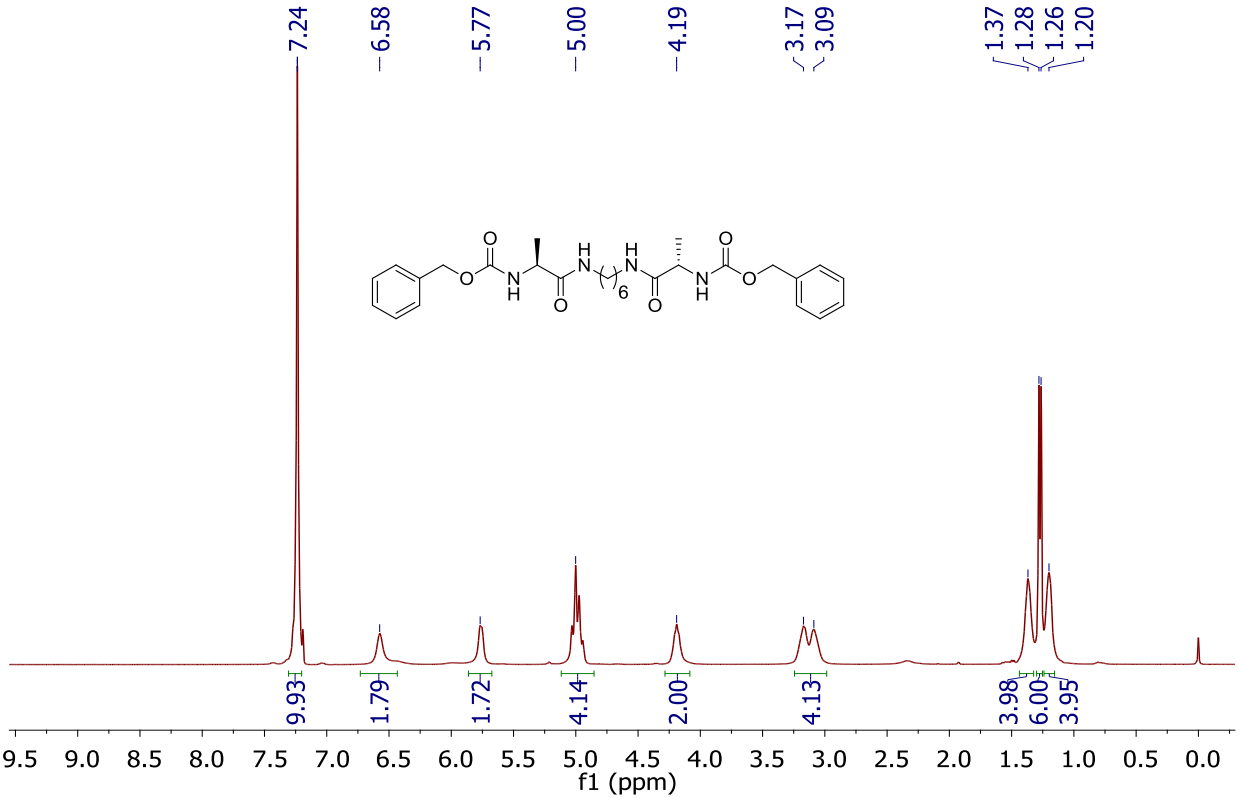

Fig. S10. ^{1}H NMR (400 MHz) spectra of **CBz6** in $CDCl_3$.

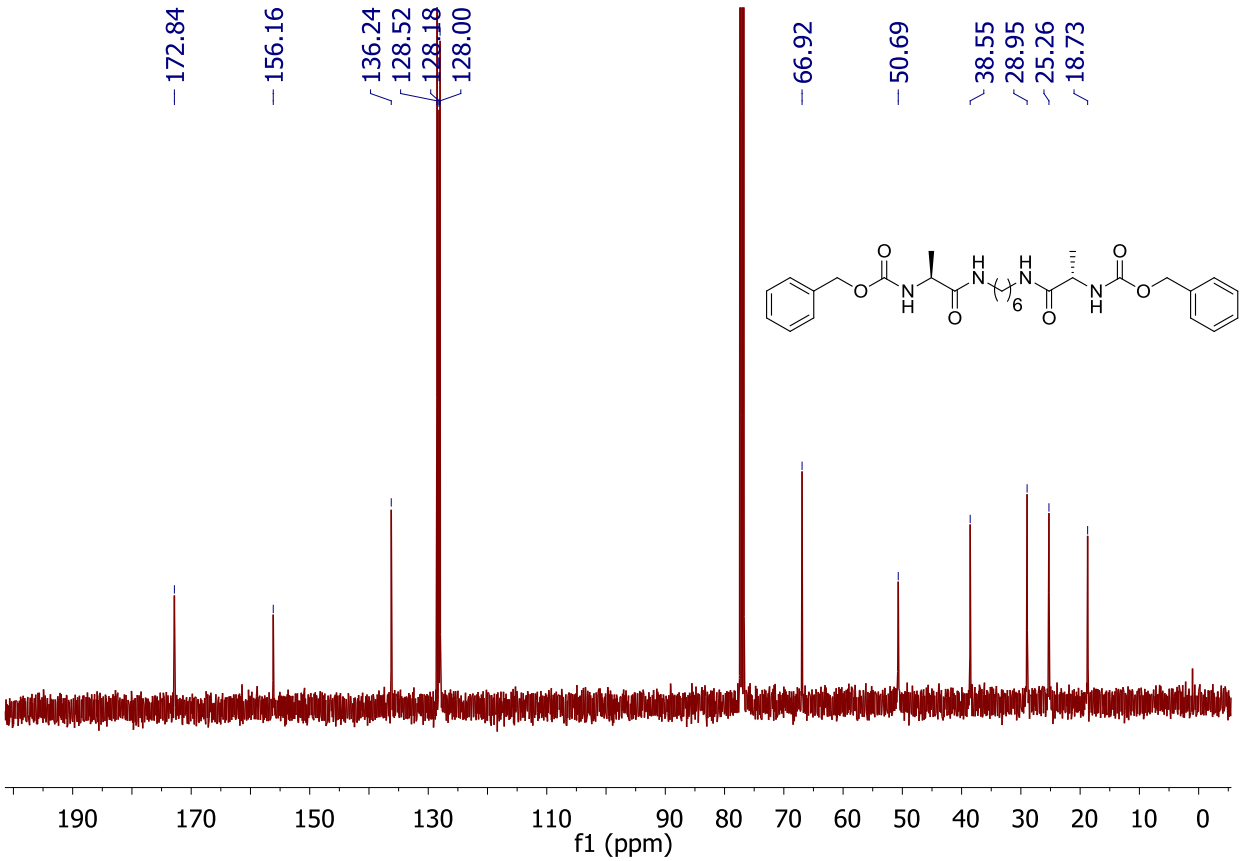

Fig. S11. ^{13}C NMR (100 MHz) spectra of **CBz6** in $CDCl_3$.

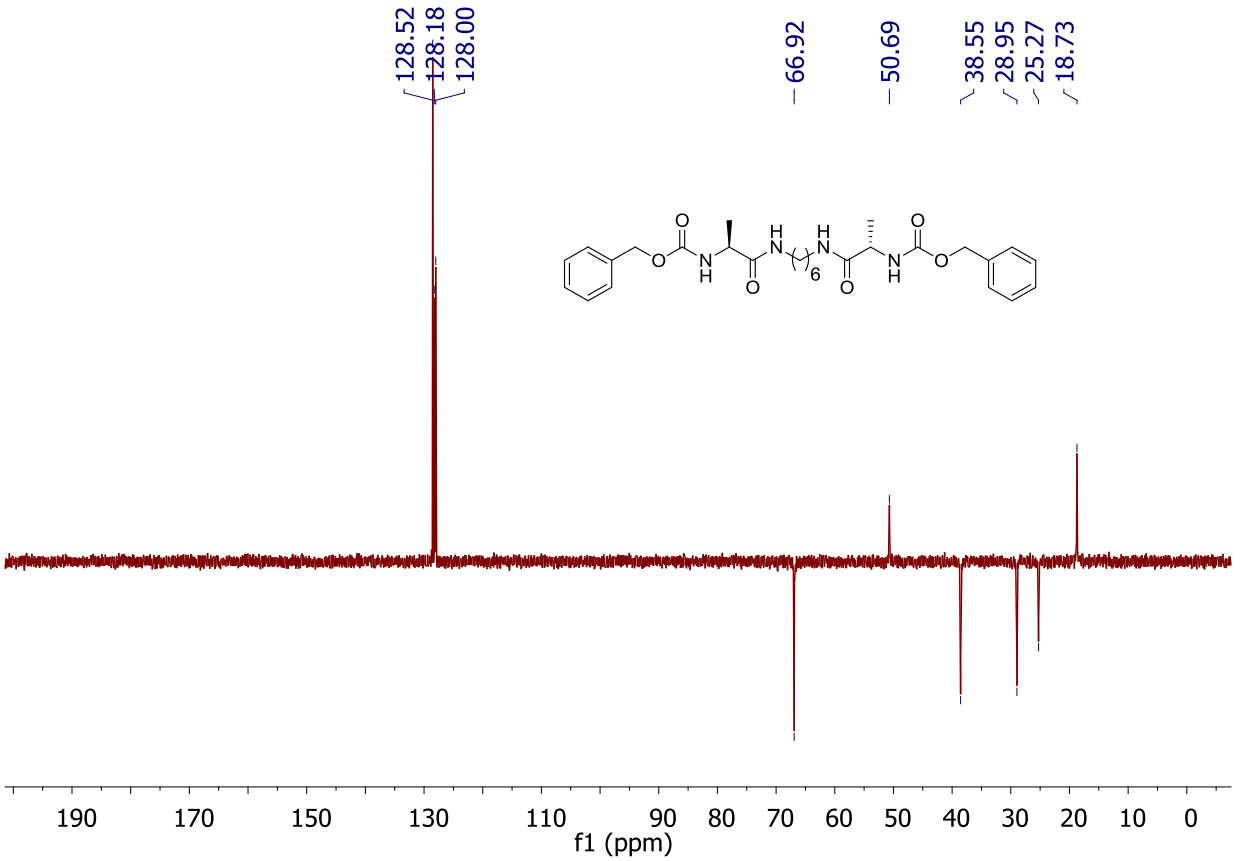

Fig. S12. DEPT (100 MHz) spectra of **CBz6** in $CDCl_3$.

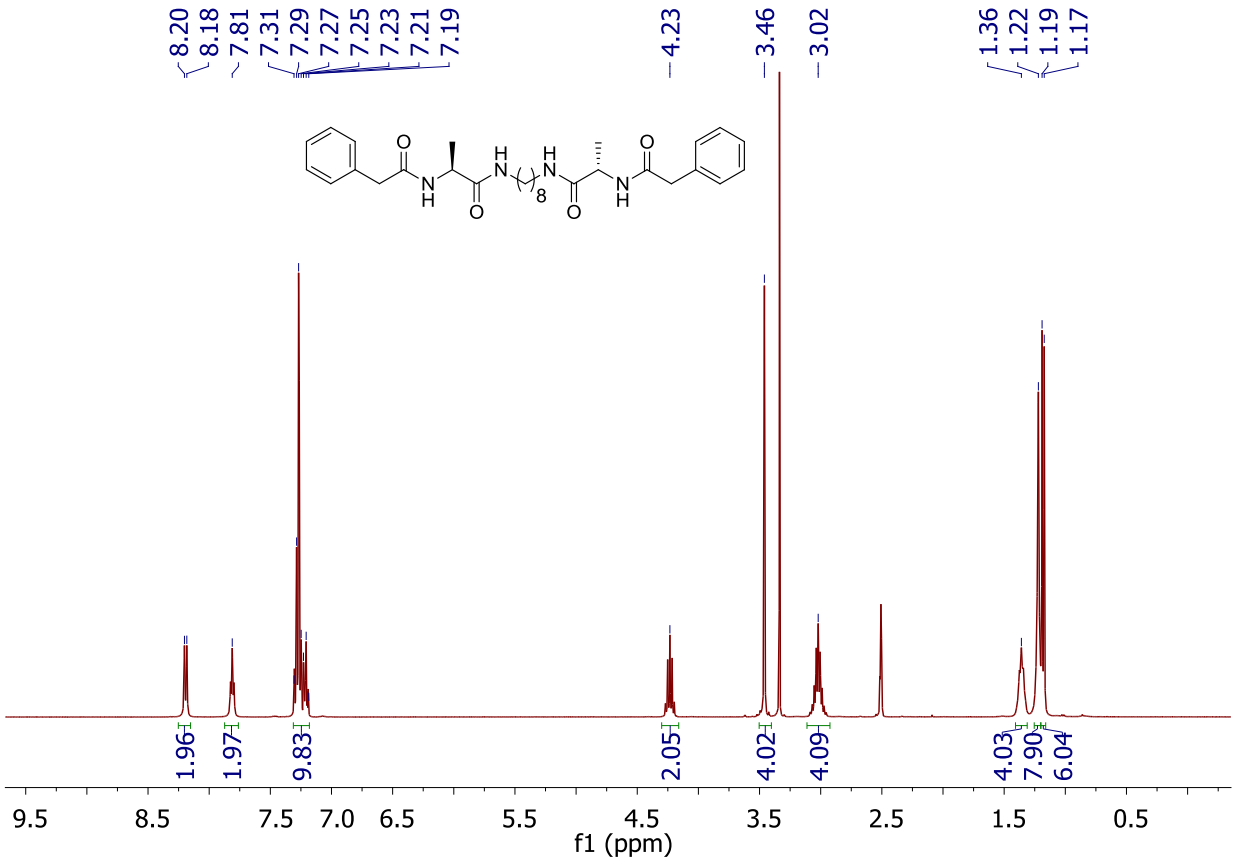

Fig. S13. ^{1}H NMR (400 MHz) spectra of **Bz8** in d^{6}-DMSO**.**

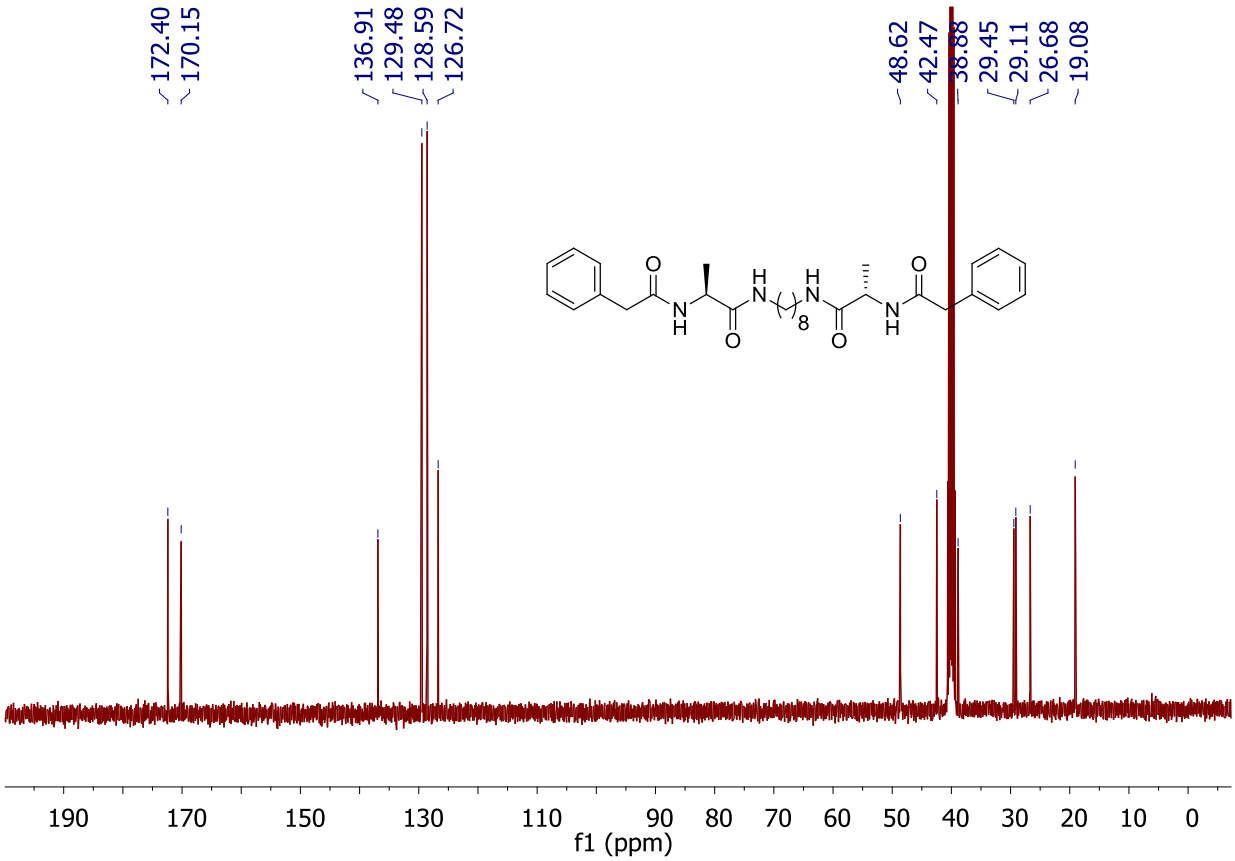

Fig. S14. ^{13}C NMR (100 MHz) spectra of **Bz8** in d^{6}-DMSO**.**

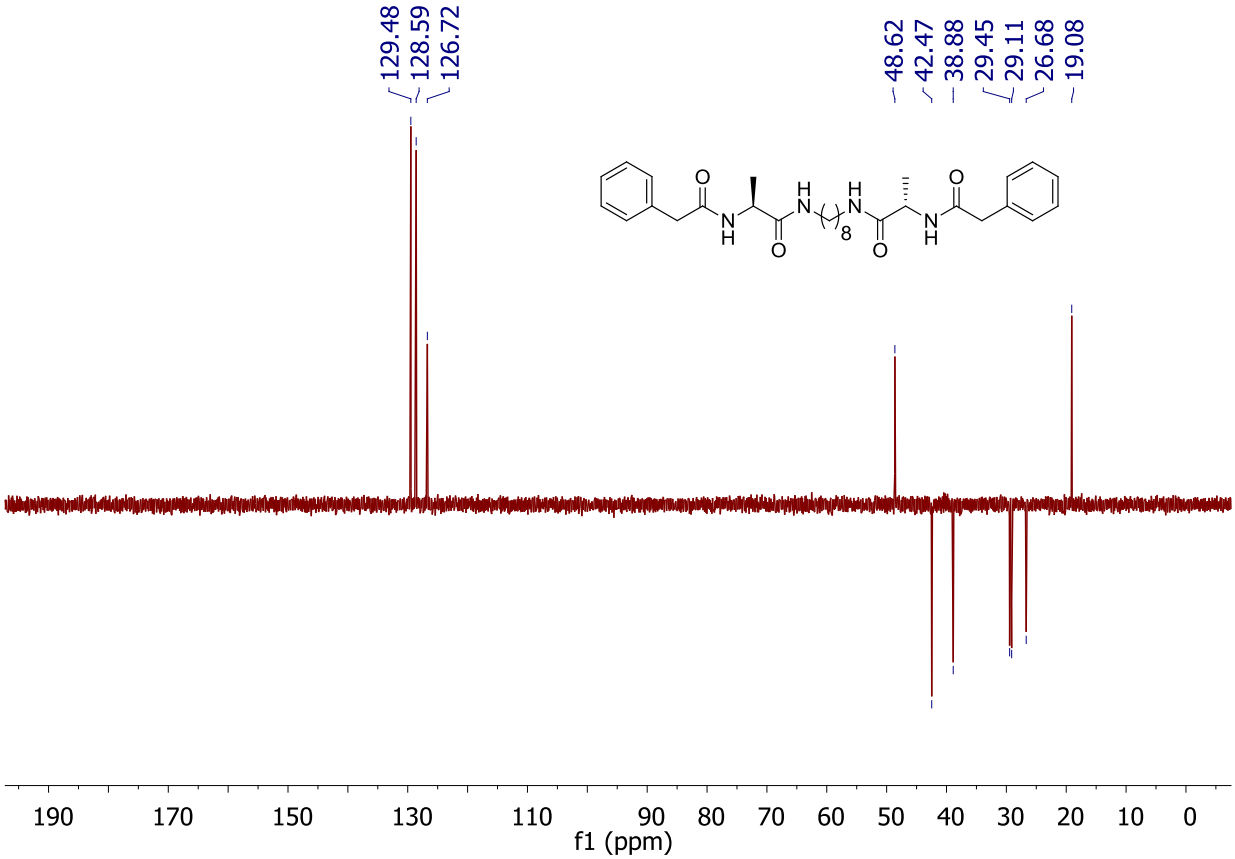

Fig. S15. DEPT (100 MHz) spectra of **Bz8** in d^6-DMSO

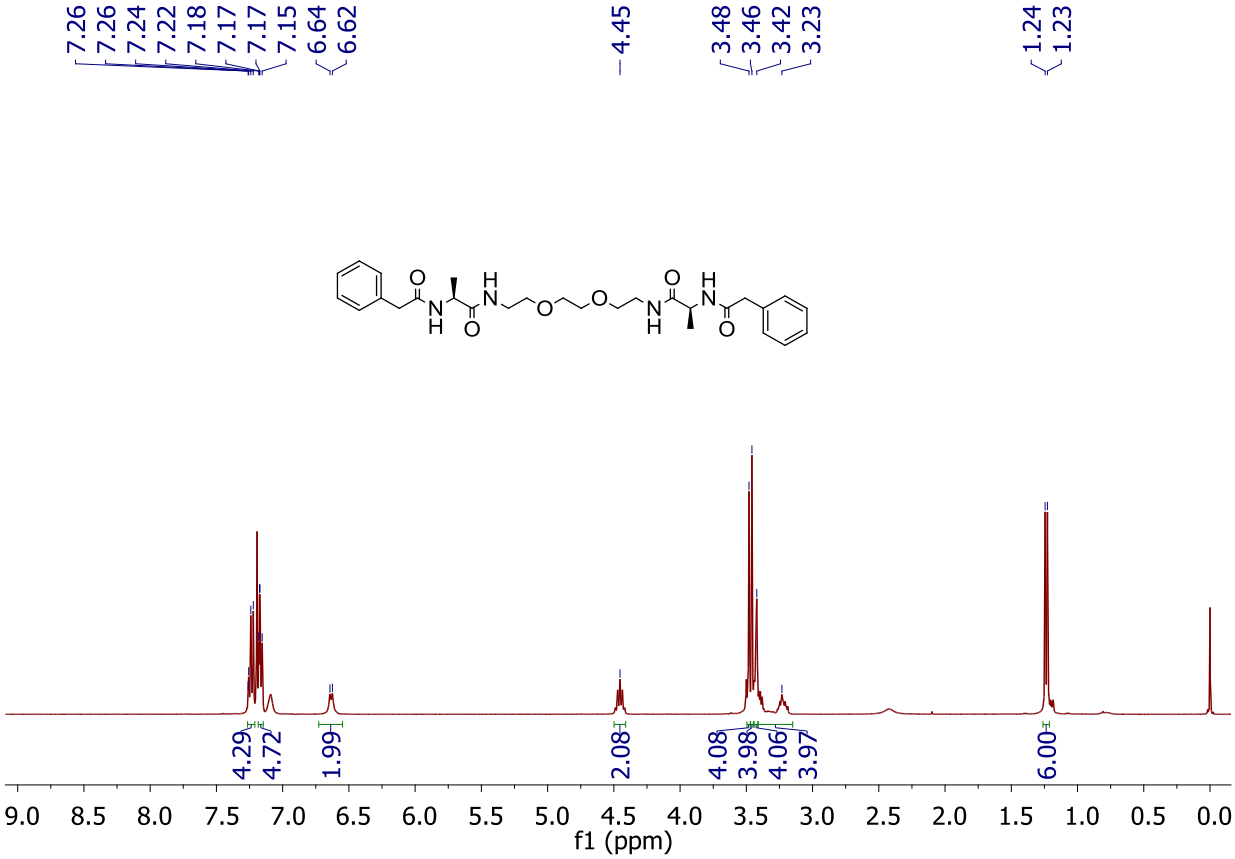

Fig. S16. ^{1}H NMR (400 MHz) spectra of **OxBz** in $CDCl_3$.

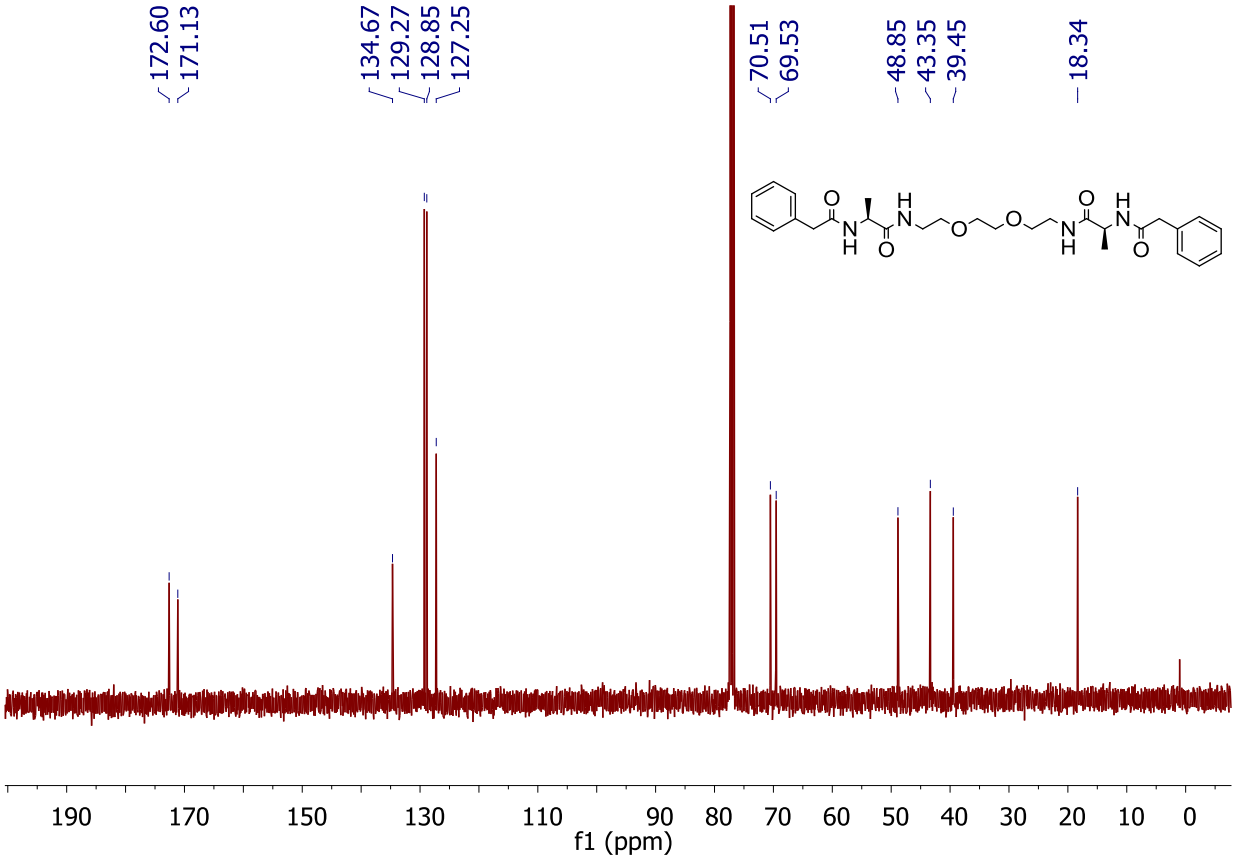

Fig. S17. ^{13}CNMR (100 MHz) spectra of **OxBz** in $CDCl_3$.

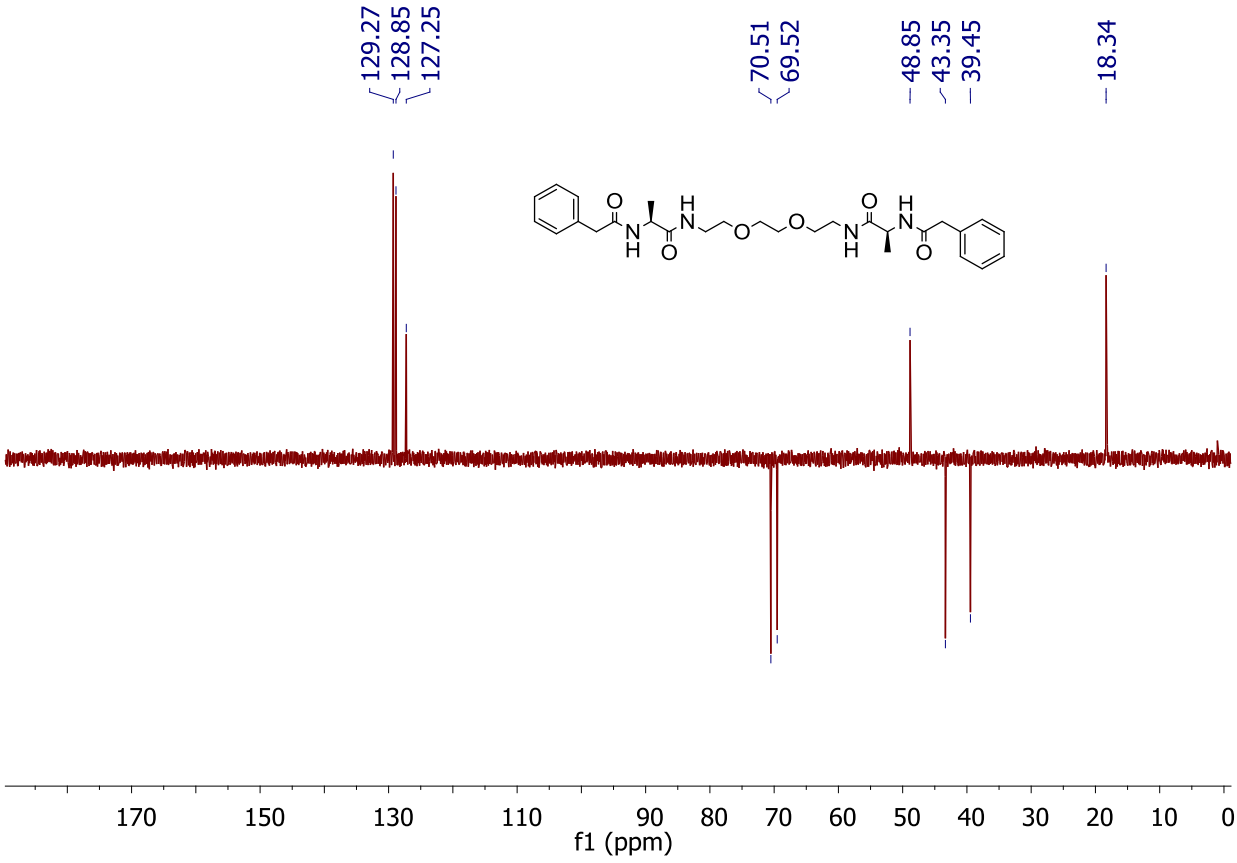

Fig. S18. DEPT (100 MHz) spectra of **OxBz** in $CDCl_3$.

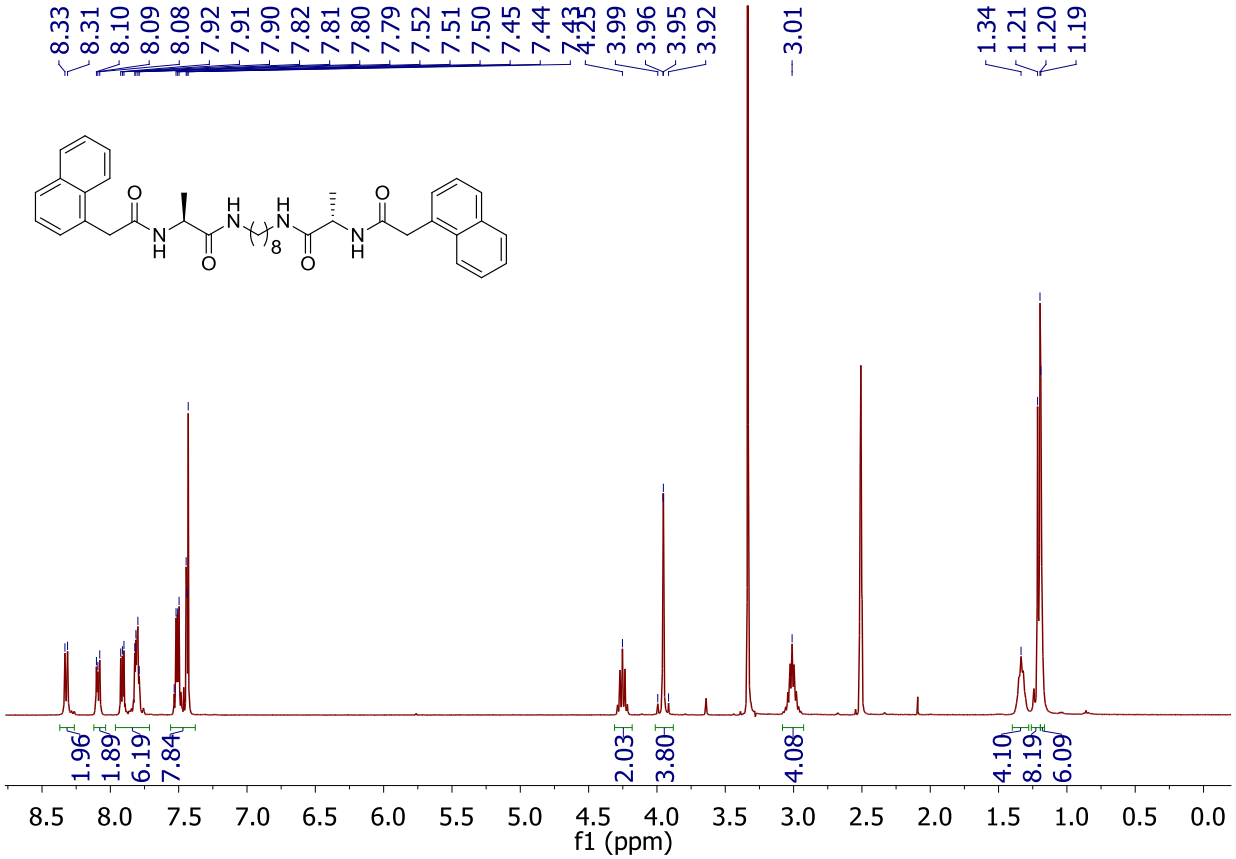

Fig. S19. ^{1}H NMR (400 MHz) spectra of **Nap8** in d^{6}-DMSO.

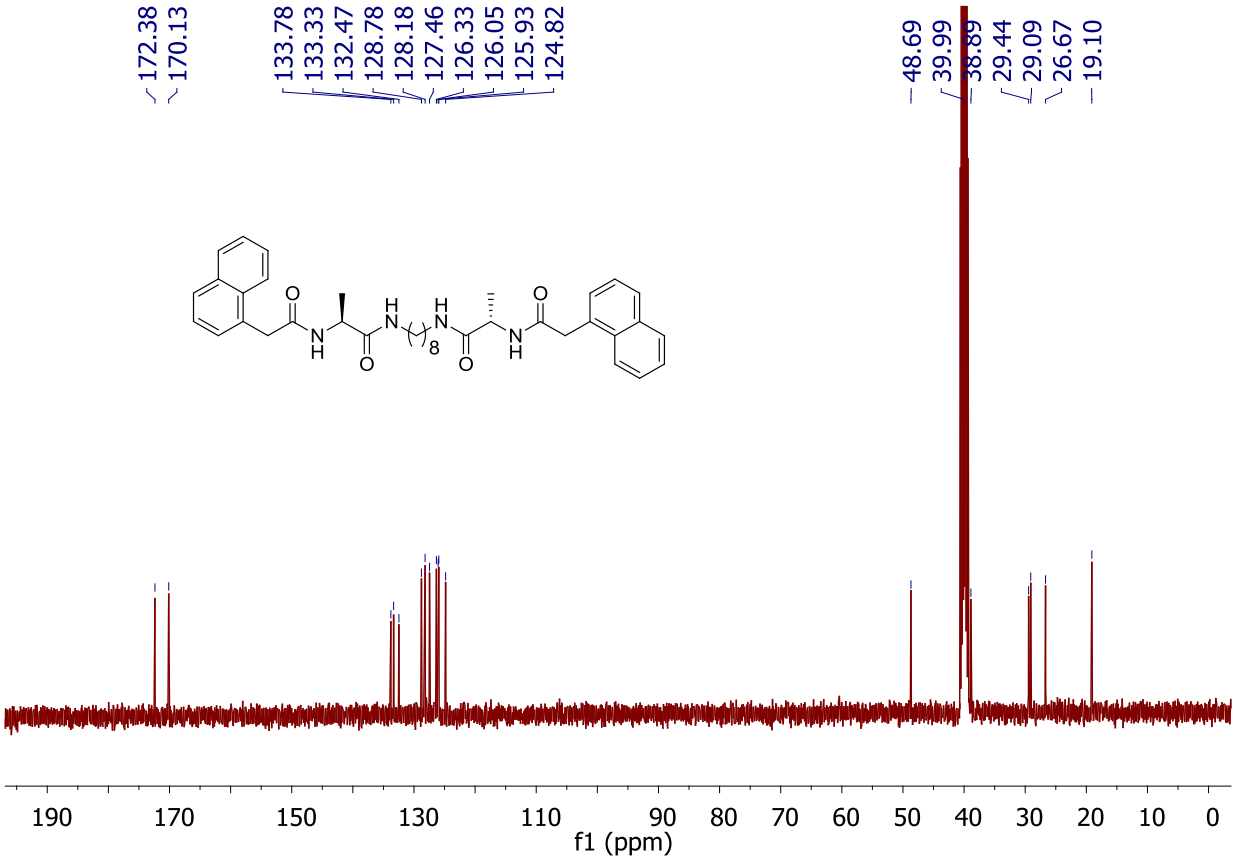

Fig. S20. ^{13}C NMR (100 MHz) spectra of **Nap8** in d^{6}- DMSO.

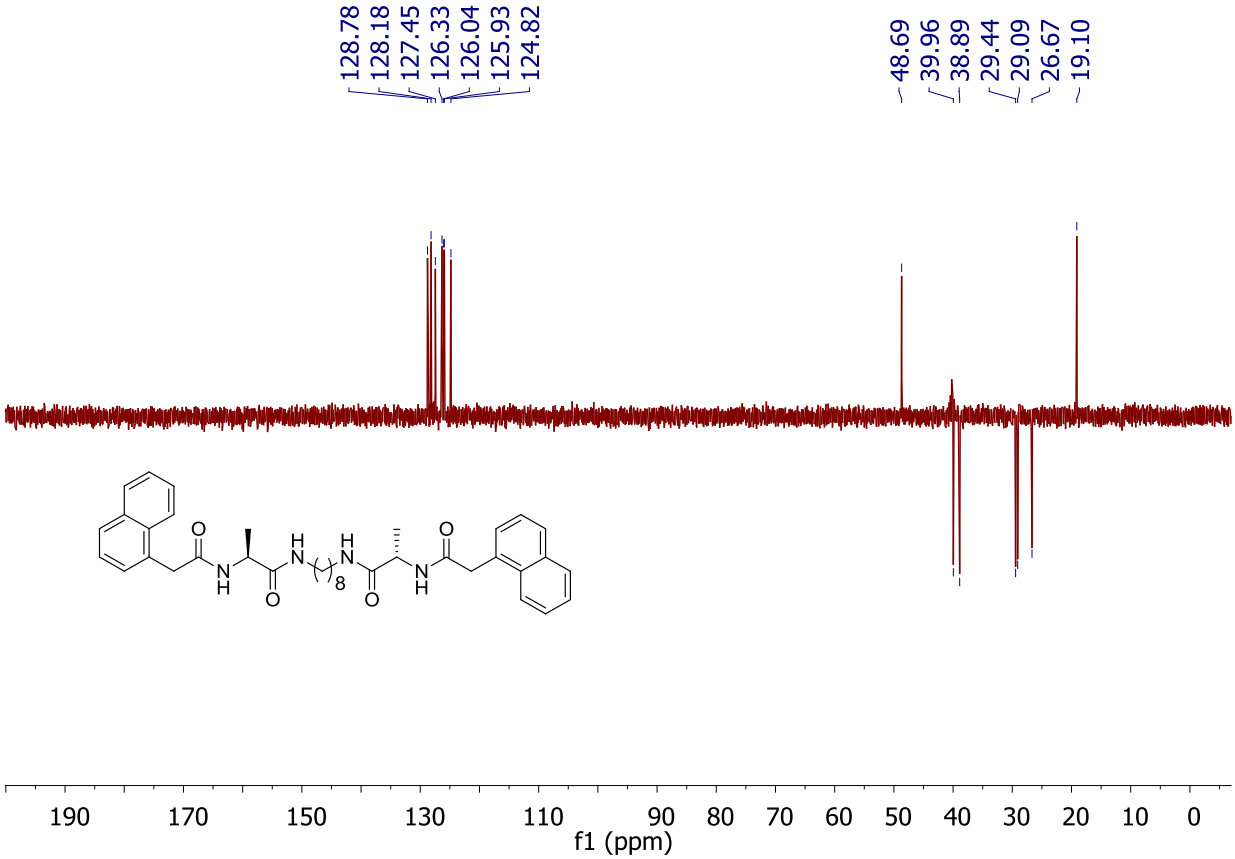

Fig. S21. DEPT (100 MHz) spectra of **Nap8** in d^6-DMSO.

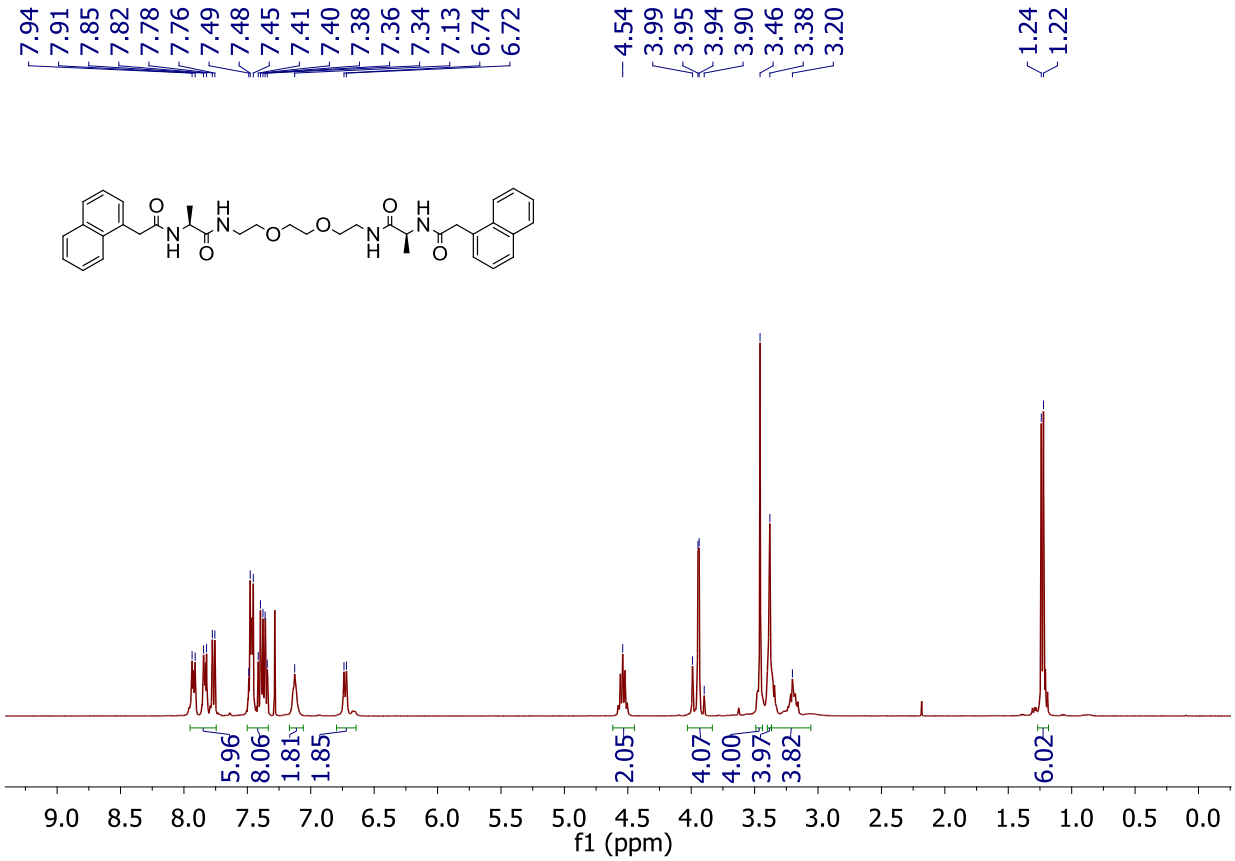

Fig. S22. ^{1}H NMR (400MHz) Spectra of **OxNap** in $CDCl_3$.

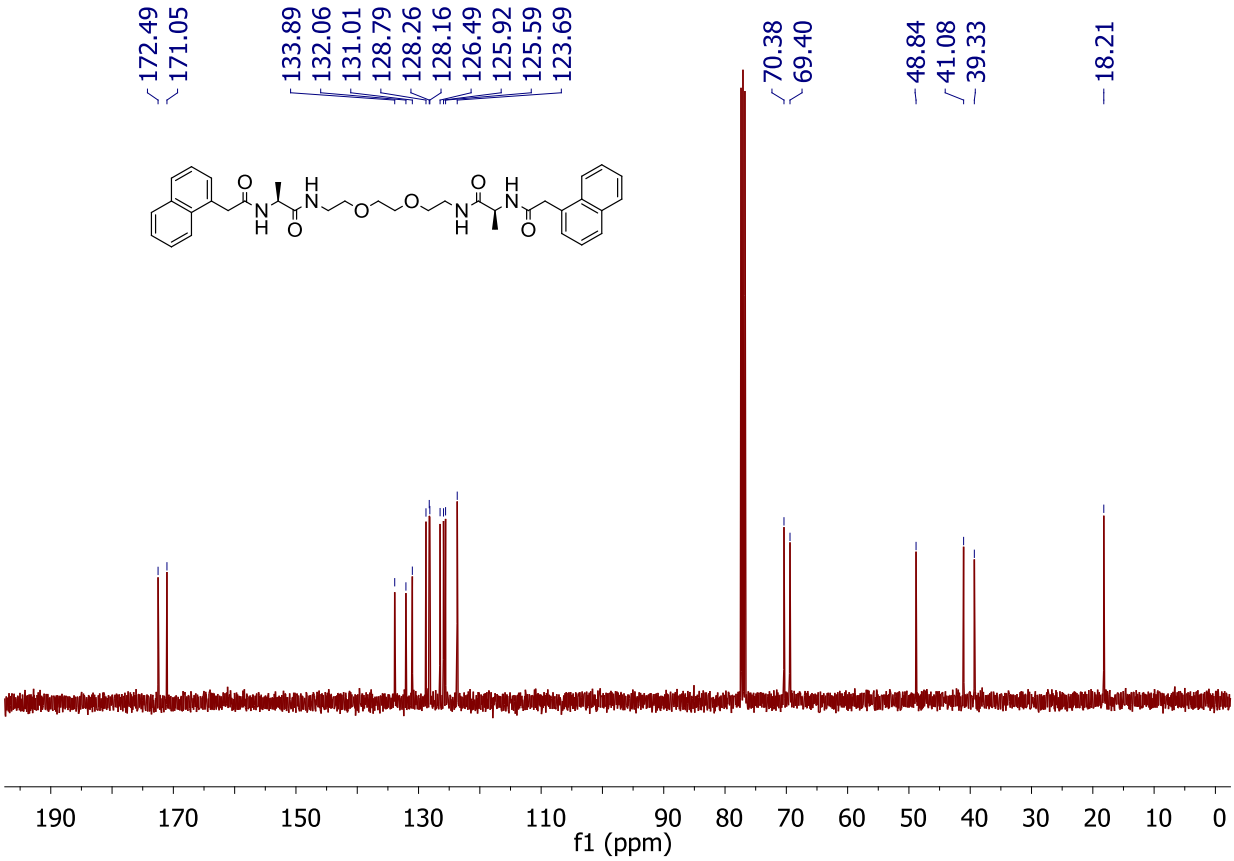

Fig. S23. ^{13}CNMR (100 MHz) spectra of **OxNap** in $CDCl_3$.

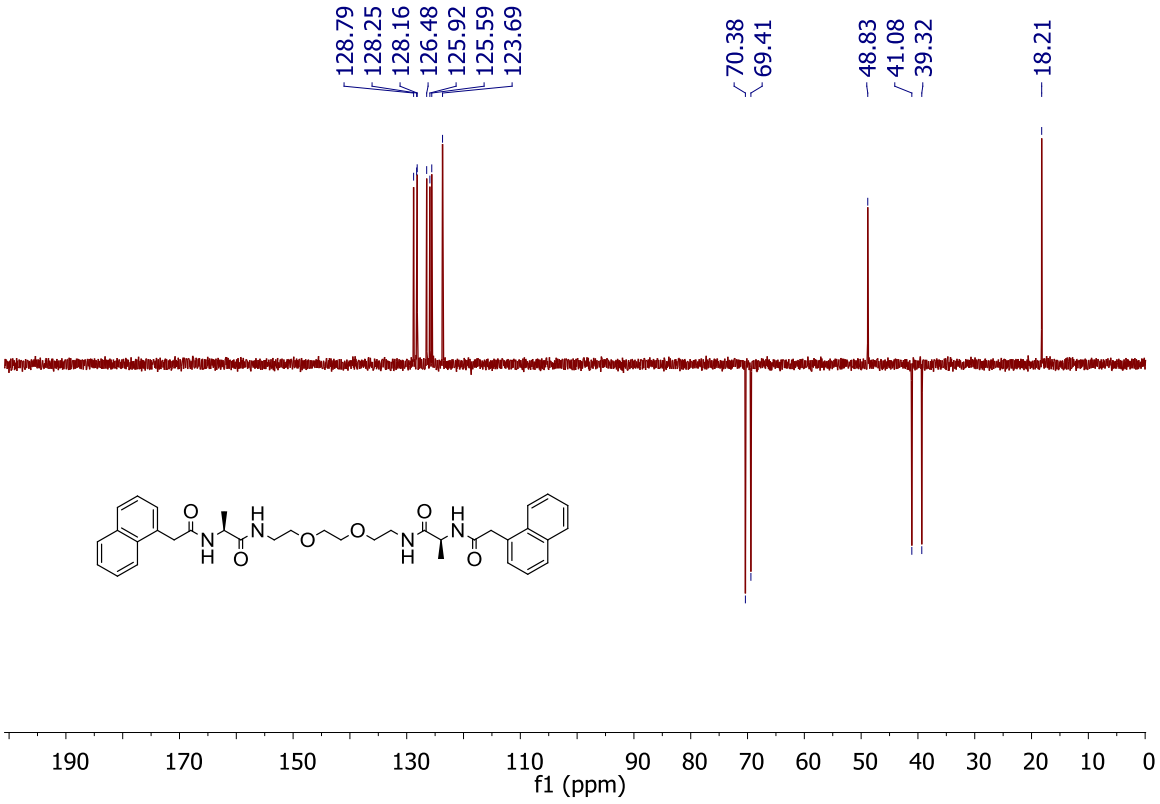

Fig. S24. DEPT (100 MHz) spectra of **OxNap** in $CDCl_3$**.**