SUPPLEMENTARY INFORMATION

Photo-degradation of amyloid β by a designed fullerene-sugar hybrid

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General Methods for Chemical Synthesis.

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL ECA-500 (500 MHz) spectrometer using trimethylsilane as the internal standard, unless otherwise noted. ESI-TOF Mass spectra were measured on a Waters LCT premier XE. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) and Silica Gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.), respectively. Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

Synthesis of 5.

To a solution of **4**[1] (503 mg, 1.09 mmol) in dry THF (22.0 mL) was added Pd-C (107 mg)

and diglycolic anhydride (146 mg, 1.25 mmol) at 25 °C. The reaction mixture was hydrogenolyzed for 2 h under H_2 gas atmosphere. The reaction mixture was filtered through cerite. The filtrate was poured into water (50 mL), and the resulting mixture was extracted with $CHCl_3$ (50 mL × 3). The combined organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (50 g, $CHCl_3$:MeOH:AcOH = 5:1:0.1) to give **5** (549 mg) as a yellow syrup in 91% yield: *Rf* 0.48 ($CHCl_3$:MeOH: AcOH = 5:1:0.1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (1H, t, br-s), 5.26 (1H, dd, $J_{2,3}$ = 8.9 Hz, $J_{3,4}$ = 9.5 Hz), 5.09 (1H, dd, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.8 Hz), 5.00 (1H, dd, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 8.7 Hz), 4.64 (1H, d, $J_{1,2}$ = 7.7 Hz), 4.29-4.15 (6H, m, H-6), 3.95 (1H, dt, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 4.0 Hz), 3.78-3.71 (2H, m), 3.64-3.45 (6H, m), 2.09 (3H, s), 2.05 (3H, s), 2.03 (3H, s), 2.02 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.04, 170.56, 169.91, 169.61, 100.85, 72.82, 71.87, 71.48, 70.24, 69.73, 69.26, 68.89, 68.52, 62.08, 39.03, 20.84, 20.81, 20.72, 20.68; HRMS (ESI-TOF) *m/z* 550.1764 (550.1772 calcd for $C_{22}H_{32}NO_{15}$, $[M-H]^-$).

Synthesis of 6.

To a solution of fullerene **3**[2] (54.1 mg, 54.4 μmol) in dry CH_2Cl_2 (13.5 mL) was added trifluoromethanesulfonic acid (135 μL) at 25 °C. After stirring for 30 min, the reaction mixture was centrifuged and then the resulting precipitate washed with diethyl ether (20 mL × 3) to give a dark brown solid. To a suspension of the solid in dry CH_2Cl_2 (10.8 mL) were added **4** (30.0 mg, 54.4 μmol), EDC (15.6 mg, 0.816 mmol) and DIEA (20.6 μl, 0.120 mmol) at 25 °C. After stirring for 6 h, the mixture was poured into water (50 mL), and the resulting mixture was extracted with $CHCl_3$ (50 mL × 3). The combined organic

layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (50 g, $CHCl_3$:MeOH:AcOH = 5:1:0.1) to give **6** (26.7 mg) as a dark brown solid in 38% yield: *Rf* 0.48 ($CHCl_3$:MeOH:CH_3COOH = 5:1:0.1); ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ7.41 (1H, t, J = 5.2 Hz), 5.51 (2H, br-s), 5.38 (2H, br-s), 5.24 (1H, dd, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 9.4 Hz), 5.10 (1H, dd, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.8 Hz), 5.02 (1H, dd, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.4 Hz), 4.75 (2H, s), 4.66 (1H, d, $J_{1,2}$ = 7.7 Hz), 4.32 (2H, s), 4.28 (1H, dd, $J_{5,6}$ = 4.3 Hz, $J_{6,6}$ = 12.2 Hz), 4.15 (1H, dd, $J_{5,6}$ = 4.3 Hz, $J_{6,6}$ = 12.2 Hz), 3.97 (1H, dt, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 4.3 Hz), 3.80-3.35 (2H, m), 3.67 (6H, m), 2.09 (3H, s), 2.07 (3H, s), 2.03 (3H, s), 2.01 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ170.77, 170.40, 169.57, 169.49, 169.07, 167.51, 153.35, 152.69, 147.54, 146.49, 146.30, 145.74, 145.62, 145.51, 144.64, 144.52, 143.27, 142.86, 142.25, 142.07, 140.44, 140.30, 136.19, 135.77, 100.97, 72.89, 71.90, 71.45, 70.95, 70.60, 70.20, 69.94, 69.06, 68.57, 58.06, 57.45, 38.93, 20.88, 20.83, 20.76, 20.73; HRMS (ESI-TOF) *m/z* 1297.2206 (1297.2245 calcd for $C_{84}H_{37}N_2O_{14}$, $[M+H]^+$).

Synthesis of 1.

To a solution of **6** (43.8 mg, 37.7 μmol) in dry CH_2Cl_2:MeOH = 2:1 (24.6 mL) was added NaOMe (34.2 μl, 0.169 mmol) at 0 °C. After stirring for 4 h, the reaction mixture was neutralized by addition of AmberLite CG-50. The resulting mixture was filtered, and then the filtrate was concentrated in *vacuo*. The resulting precipitation was washed with $CHCl_3$ and MeOH to give **1** (18.0 mg) as a dark brown solid in 47% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ8.04 (1H, t, J = 5.7 Hz, -NHCO-), 5.53 (2H, br-s, -N(CH_2)$_2$-), 5.47 (2H, br-s), 5.01 (1H, d, J = 5.1 Hz), 4.94 (1H, d, J = 4.9 Hz), 4.90 (1H, d, J = 5.2 Hz), 4.74 (2H,

s), 4.50 (1H, t, J = 6.0 Hz), 4.17 (2H, s), 4.16 (1H, d, J = 8.9 Hz), 3.87 (1H, m), 3.66 (1H, m), 3.62-3.41 (6H, m), 3.17-3.01 (3H, m), 2.96 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.38, 168.43, 147.31, 146.39, 146.34, 146.09, 145.45, 145.30, 144.56, 144.56, 142.66, 142.33, 142.15, 141.90, 139.89, 136.18, 136.11, 103.55, 79.72, 77.42, 77.26, 73.93, 70.76, 70.57, 70.33, 70.05, 68.34, 39.89; HRMS (ESI-TOF) *m/z* 1151.1610 (1151.1642 calcd for $C_{76}H_{28}N_2O_{10}Na$, $[M+Na]^+$).

Synthesis of 8.

To a solution of commercially available **7** (206 mg, 1.14 mmol) in dry DMF (1.0 mL) were added TBDPSCl (314 mg, 1.14 mmol) and imidazole (398 mg, 5.71 mmol) at 25 °C. After stirring for 1 h, the mixture was poured into water (20 mL), and the resulting mixture was extracted with hexane (20 mL × 3). The combined organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (30 g, hexane:EtOAc = 2:1) to give **8** (416 mg) as a colorless syrup in 86% yield: *Rf* 0.65 (hexane:EtOAc = 2:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73-7.66 (4H, m), 7.43-7.34 (6H, m), 3.82 (2H, t, J = 4.9 Hz), 3.67-3.57 (8H, m), 3.35 (2H, t, J = 4.3 Hz), 1.06 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.73, 133.81, 129.74, 127.75, 72.65, 71.51, 70.96, 70.88, 63.58, 50.81, 42.83, 26.94, 19.32; HRMS (ESI-TOF) *m/z* 436.2027 (436.2032 calcd for $C_{22}H_{31}N_3O_3NaSi$, $[M+Na]^+$).

Synthesis of 9.

To a solution of **8** (181 mg, 0.438 mmol) in dry THF (7.2 mL) was added Pd-C (36.2 mg) and diglycolic anhydride (67.8 mg, 0.592 mmol) at 25 °C. The reaction mixture was hydrogenolyzed for 2 h under H_2 gas atmosphere. The reaction mixture was filtered

through cerite. The filtrate was poured into water (20 mL), and the resulting mixture was extracted with $CHCl_3$ (20 mL × 3). The combined organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (20 g, $CHCl_3$:MeOH:AcOH = 5:1:0.1) to give **9** (137 mg) as a colorless syrup in 60% yield: *Rf* 0.33 ($CHCl_3$:MeOH:AcOH = 5:1:0.1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73-7.64 (4H, m), 7.44-7.35 (6H, m), 4.25 (2H, d, *J* = 6.0 Hz), 4.12 (2H, s), 3.86-3.49 (10H, m), 1.05 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.27, 135.57, 133.44, 129.72, 127.69, 72.36, 70.82, 70.18, 69.59, 69.30, 38.99, 26.80, 19.16; HRMS (ESI-TOF) *m/z* 502.2257 (502.2261 calcd for $C_{26}H_{36}NO_7Si$, $[M-H]^+$).

Synthesis of 10.

To a solution of fullerene **3** (304 mg, 0.305 mmol) in dry CH_2Cl_2 (75.0 mL) was added trifluoromethanesulfonic acid (750 μL) at 25 °C. After stirring for 30 min, the reaction mixture was centrifuged and then the resulting precipitate washed with diethyl ether (50 mL × 3) to give a dark brown solid. To a suspension of the solid in dry CH_2Cl_2 (40.0 mL) were added **9** (238 mg, 0.458 mmol), EDC (117 mg, 0.611 mmol) and DIEA (79.8 mg, 0.611 mmol) at 25 °C. After stirring for 3 h, the mixture was poured into water (20 mL), and the resulting mixture was extracted with $CHCl_3$ (20 mL × 3). The combined organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (100 g, toluene:acetone = 3:1) to give **10** (70.0 mg) as a dark brown solid in 18% yield: *Rf* 0.49 (toluene:acetone = 3:1) ; ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 7.70-7.66 (4H, m),

7.44-7.35 (6H, m), 7.32 (1H, br-s), 5.47 (2H, br-s), 5.31 (2H, br-s), 4.64 (2H, br-s), 4.28 (2H, s), 3.82 (2H, t, J = 5.5 Hz), 3.79-3.50 (10H, m), 1.05 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.85, 167.24, 153.32, 152.65, 147.52, 146.49, 146.28, 145.72, 145.62, 145.50, 145.35, 145.31, 144.63, 144.51, 143.25, 142.84, 142.25, 142.05, 135.69, 133.73, 129.78, 127.78. 72.56, 71.30, 70.78, 70.67, 70.49, 69.84, 63.56, 58.12, 57.35, 38.90, 31.02, 29.79, 26.93, 19.30; HRMS (ESI-TOF) *m/z* 1249.2690 (1249.2734 calcd for $C_{88}H_{41}N_2O_6Si$, $[M+H]^+$).

Synthesis of 2.

To a solution of **10** (10.9 mg, 8.61 μmol) in dry THF (2.0 mL) was added HF/pyridine (50.0 μl) at 25 °C. After stirring for 3 h, the mixture was poured into water (10 mL), and the resulting mixture was extracted with $CHCl_3$ (10 mL × 3). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The residue was subjected to silica-gel column chromatography (50 g, $CHCl_3$:MeOH = 20:1) to give **2** (7.2 mg) as a dark brown solid in 83% yield: *Rf* 0.30 ($CHCl_3$:MeOH = 20:1) ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (1H, br-s), 5.51 (2H, br-s), 5.35 (2H, br-s), 4.72 (2H, s), 4.30 (2H, s), 3.78 (2H, t, J = 4.3 Hz), 3.70-3.63 (8H, m), 3.59 (2H, t, J = 5.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.05, 167.47, 146.49, 146.30, 145.75, 145.63, 145.50, 144.60, 144.54, 143.27, 142.85, 142.25, 142.07, 140.42, 140.35, 72.69, 71.47, 70.45, 70.38, 69.90, 61.88, 58.03, 57.32, 38.91, 29.79, 14.21; HRMS (ESI-TOF) *m/z* 1033.1382 (1033.1376 calcd for $C_{72}H_{22}N_2O_6Na$, $[M+Na]^+$).

Thioflavin T (Th T) binding assay.

Inhibition of amyloid β aggregation was measured using a Thioflavin T (ThT) binding

assay.[3] a) Without photo-irradiation: Th T binding assay was performed with $A\beta_{42}$ monomer (40 μM) and each fullerene hybrid in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM, 25 μL) containing 150 mM NaCl at 37 °C in an atmosphere of 5% CO_2 for 24 h. Fullerene hybrids were tested over a broad concentration range (100 nM-300 μM) using 8-10 concentration points. After the incubation period, a solution of 6.67 μM Th T in water (75 μL) was added to each sample. Fluorescence was measured on Safire (TECAN) micro plate reader with excitation and emission wavelengths at 430 nm and 491 nm, respectively. Data were analyzed using GraphPad Prism to obtain IC_{50} values using log(compound) vs. normalized response-variable slope. b) With photo-irradiation: Th T assay was performed with $A\beta_{42}$ monomer (40 μM) and fullerene hybrid **1** in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM, 25 μL) containing 150 mM NaCl at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample, and an additional incubation of the mixture at 37 °C in an atmosphere of 5% CO_2 for 22 h. Fullerene hybrid **1** were tested over a broad concentration range (10 nM-300 μM) using 8-10 concentration points. After the incubation period, a solution of 6.67 μM Th T in water (75 μL) was added to the sample. Fluorescence was measured and data were analyzed by the same ways mentioned above. $A\beta_{42}$ monomer was prepared according to the literature[4] using $A\beta_{42}$ purchased from American Peptide Company Inc. at purity levels of > 95% and hexafluoro-2-propanol (HFIP).

Inhibition of $A\beta_{42}$ aggregation from $A\beta_{42}$ monomer with or without photo-irradiation.

$A\beta_{42}$ monomer (5.0 μM), which was prepared as mentioned above, was incubated with

fullerene hybrid **1** (50-1.5 μM) in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM, 10 μL) containing 150 mM NaCl at 25 °C for 2 h with or without irradiation using a UV lamp (365 nm, 100 W) placed 10 cm from the mixture, and the mixture was further incubated at 25 °C for 14 h.

Photo-degradation of $A\beta_{42}$ monomer and oligomers with photo-irradiation.

To a mixture of $A\beta_{42}$ monomer and oligomers (5.0 μM/ $A\beta_{42}$ monomer), which was prepared by incubation of $A\beta_{42}$ monomer in 20 mM Tris-HCl buffer (pH 8.0, 8 μL) containing 150 mM NaCl at 25 °C for 14 h,[4] was added a solution of fullerene hybrid **1** (50-1.5 μM) in 50% DMF/Tris-HCl buffer (pH8.0, 20 mM, 2 μL) containing 150 mM NaCl. The mixture was then incubated at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample.

Polyacrylamide gel electrophoreses and immunoblotting.

Electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol); 4.8 μL of buffer was added to samples. Gels (10%) were run by applying 64 V for 2 h. $A\beta_{42}$ monomer and oligomers were transferred at 200 mA for 2 h onto Amersham Hybond ECL Nitrocellulose Membrane (GE Healthcare). Nonspecific binding sites were blocked for 4 h by immersing the membrane in a blocking solution, Tris-buffered saline with Tween 20 (TBST): 10 mM Tris-HCl, (pH 8.0) containing 150 mM NaCl, 0.1% Tween 20 (vol/vol), and 5% (wt/vol) nonfat dry milk. After a short wash in TBST, the membrane was incubated in a 1:2500 dilution of a primary antibody 6E10 (Covance Research Products) in TBST for 14 h at 4 °C followed by 30 min of washing with TBST. The bound antibody

was then detected with horseradish peroxidase-conjugated secondary antibody Anti-mouse lgG (GE healthcare) diluted at 1:1500 in TBST by incubation with it for 2 h at 25 °C. After having been washed for 30 min in TBST, the immunocomplexes were detected by using ECL reagent, Immobilon Western (Millipore, Billerica, MA). Exposure to RX-U films (Fuji Film, Kanagawa, Japan) was carried out for 10 s to 2 min.

MALDI TOF MS analysis.

Two microliters of sample was mixed with 18 μL of 3,5-dimethoxy-4-hydroxycinnamic acid (in 50:50 0.1% TFA in water: acetonitrile) matrices. Analyses by MALDI TOF MS were performed in the positive ion mode on a Ultra flex (Bruker).

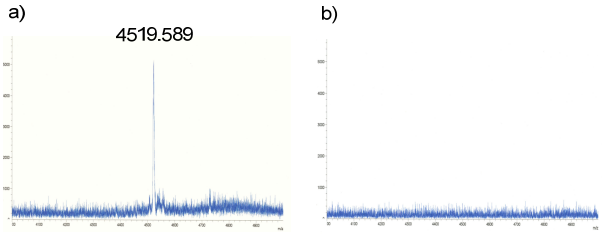


Fig. S1 MALDI-TOF MS profile of $A\beta_{42}$ monomer obtained by photo-degradation using fullerene hybrid **1**. $A\beta_{42}$ monomer (5.0 μM) was incubated with **1** (50 μM) in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM) at 25 °C for 2 h with (b) or without (a) photo-irradiation using a UV lamp (365 nm, 100 W) placed at 10 cm from the mixture. The resulting products were analyzed by MALDI-TOF MS (matrix: 3,5-dimethoxy-4-hydroxycinnamic acid). A peak was observed at m/z 4519.6 indicating $A\beta_{42}$ monomer in (a); the peak was not observed in (b).

Transmission Electron Microscope (TEM) analysis.

To determine whether filaments had formed, 3 μL of sample solution was applied to

150-mesh copper grids coated with Formvar/carbon film (Okenshoji) for 30 s. Excess solution on the other side of grids was absorbed with filter paper, grids were stained with one drop of 1% ammonium heptamolybdate for 60 s, and staining solution was absorbed with filter paper again. After air drying for overnight, grids were examined with an electron microscope TECNAI Sprit (FEI Company).[5]

Electron Paramagnetic Resonance (EPR) Spin Trapping Method.

Electron Paramagnetic Resonance (EPR) experiments were carried out with a E-500 CW/EPR (Bruker) and recorded under the following conditions: temperature 298 K, microwave power 16 mW, field modulation 0.1 mT at 100 kHz, scan time 2 min. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was used as a spin-trapping agent. To a 500 μM fullerene hybrid solution (80 μL) in DMF, 5 mM DETAPAC (40 μL), 50 mM NADH (40 μL) in 20 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl, DMPO (11.1 μL), and 20 mM Tris-HCl buffer (pH 8.0, 28.9 μL) containing 150 mM NaCl were added and mixed well under an aerobic condition.[6] The mixed solution was collected in a flat cell, irradiated with UV lamp (365 nm, 100 W) at a distance of 40 cm, and subjected immediately to EPR measurement.

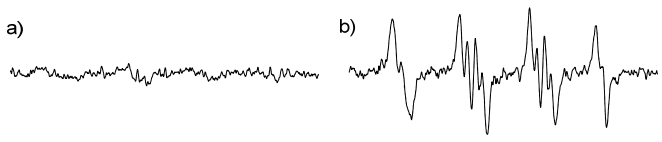


Fig. S2 EPR spectrum of DMPO adducts with superoxide and hydroxy radicals generated in an aqueous solution of **1** (200 μM) in the presence of DMPO (500 mM) and NADH (10

mM) with (b) or without (a) photo-irradiation using a UV lamp (365 nm, 100 W) placed at 40 cm from the mixture. Irradiation time: 60 s. Experimental conditions: temperature 25 °C, microwave power 16 mW, field modulation 0.1 mT at 100 kHz.

References

1 S. Zoltan, S. Lajos and L. Andras, *Acta Chimica Hungarica,* 1989, **126**, 259.

2 M. Maggini, G. Scorrano and M. Prato, *J. Am. Chem. Soc.,* 1993, **115**, 9798.

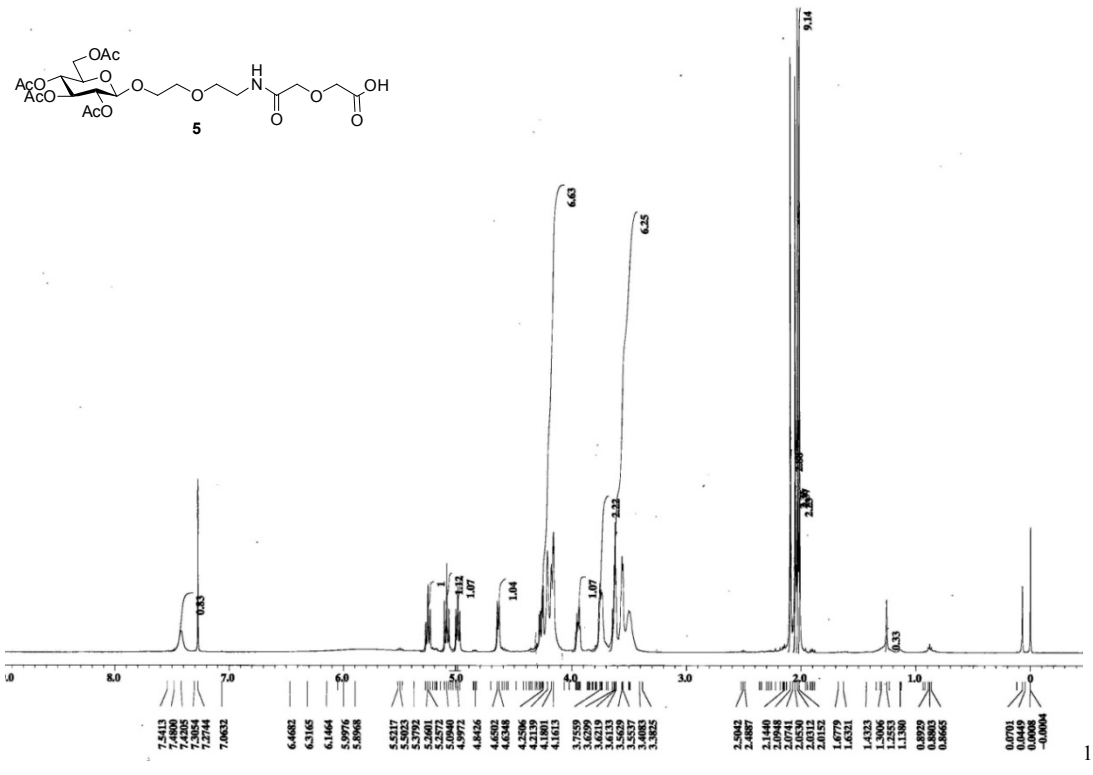
3 H. LeVine, III, *Protein Sci.,* 1993, **2**, 404.

4 V. Rangachari, B. D. Moore, D. K. Reed, L. K. Sonoda, A. W. Bridges, E. Conboy, D. Hartigan and T. Rosenberry, *Biochemistry,* 2007, **46**, 12451.

5 F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy and G. M. Cole, *J. Biol. Chem.,* 2005, **280**, 5892.

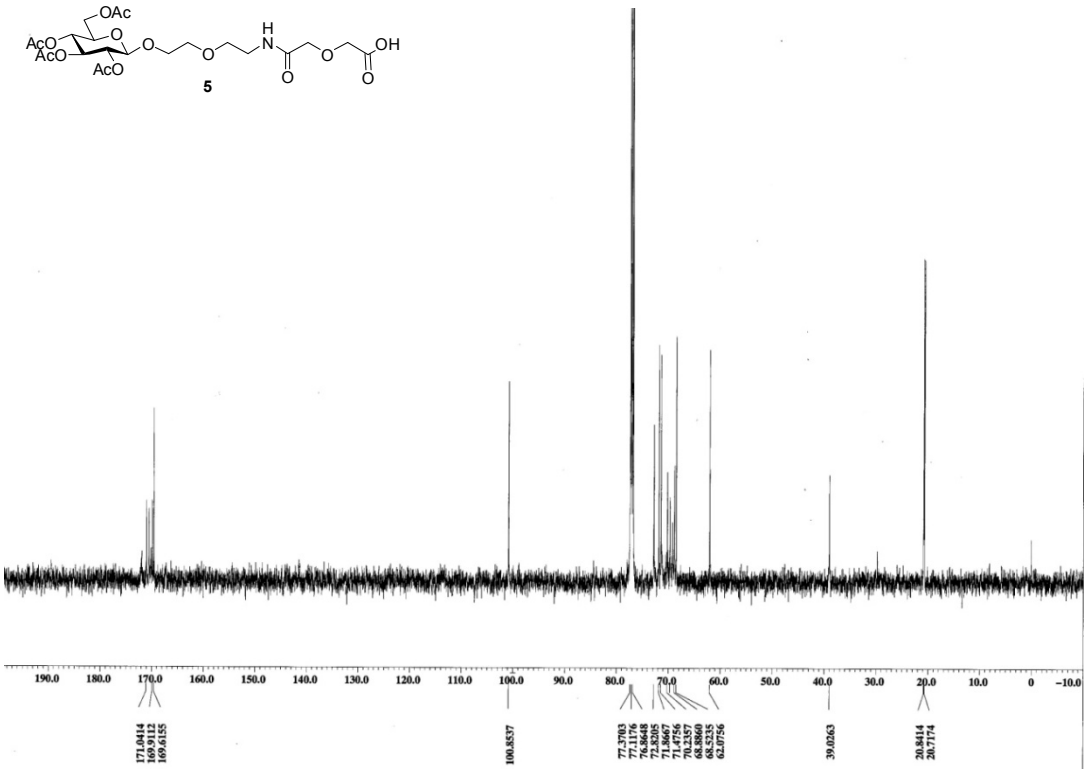
6 Y. Yamakoshi, N. Umezawa, A. Ryu, K. Arakane, N. Miyata, Y. Goda, T. Masumizu and T. Nagano, *J. Am. Chem. Soc.,* 2003, **125**, 12803.

^{1}H- and ^{13}C-NMR spectrum charts

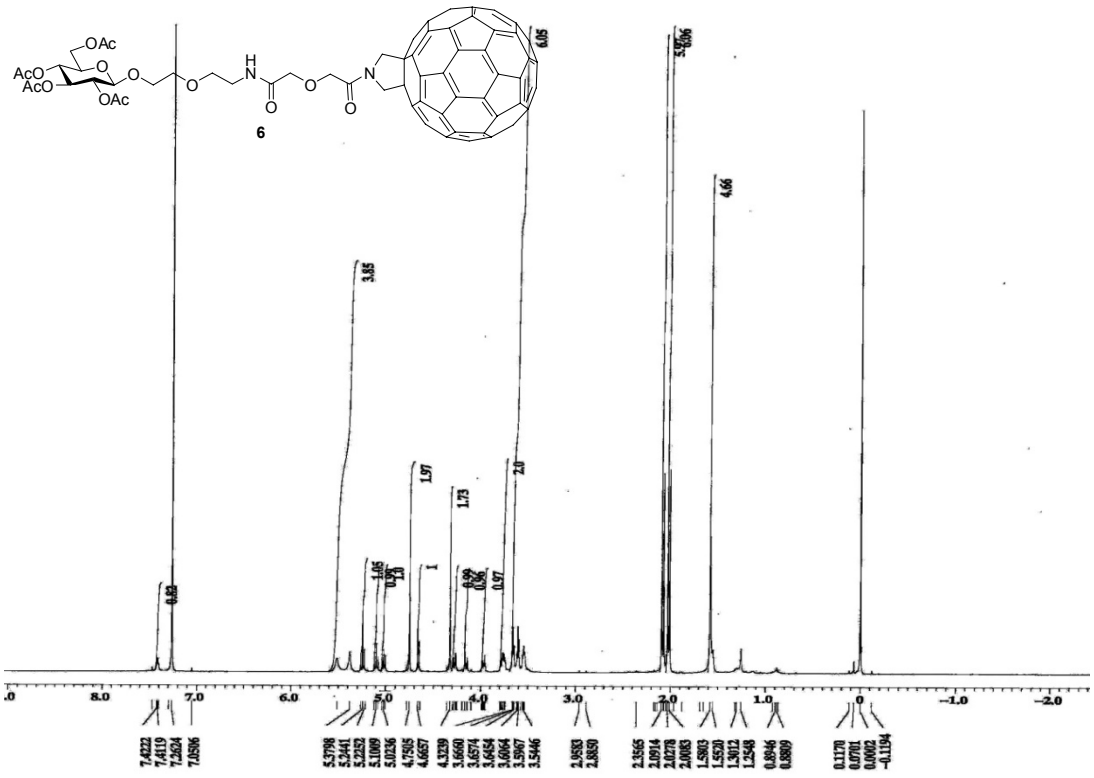


^{1}H

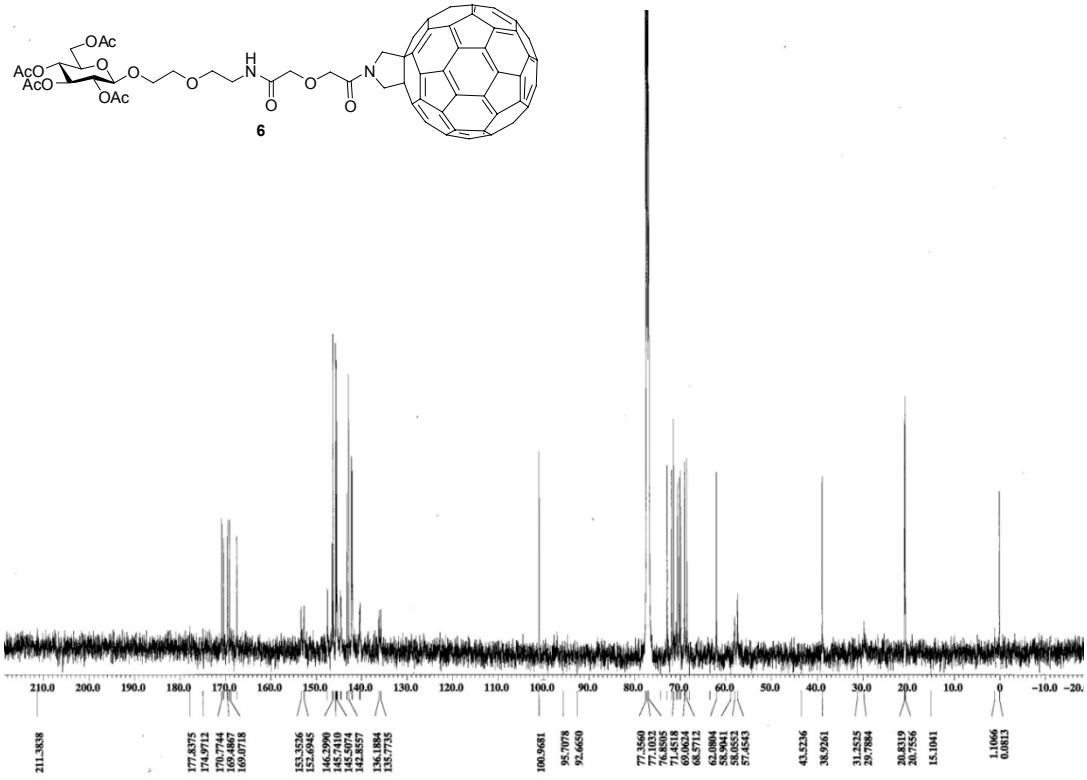
NMR spectrum of **5**



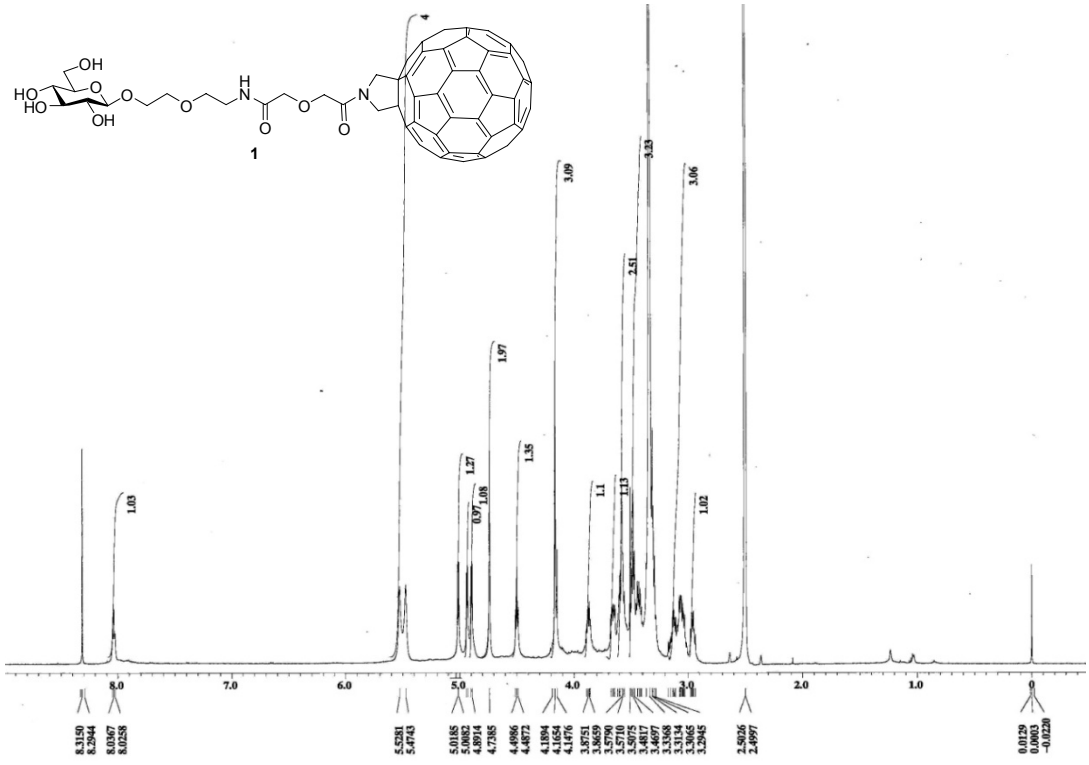
^{13}C NMR spectrum of **5**



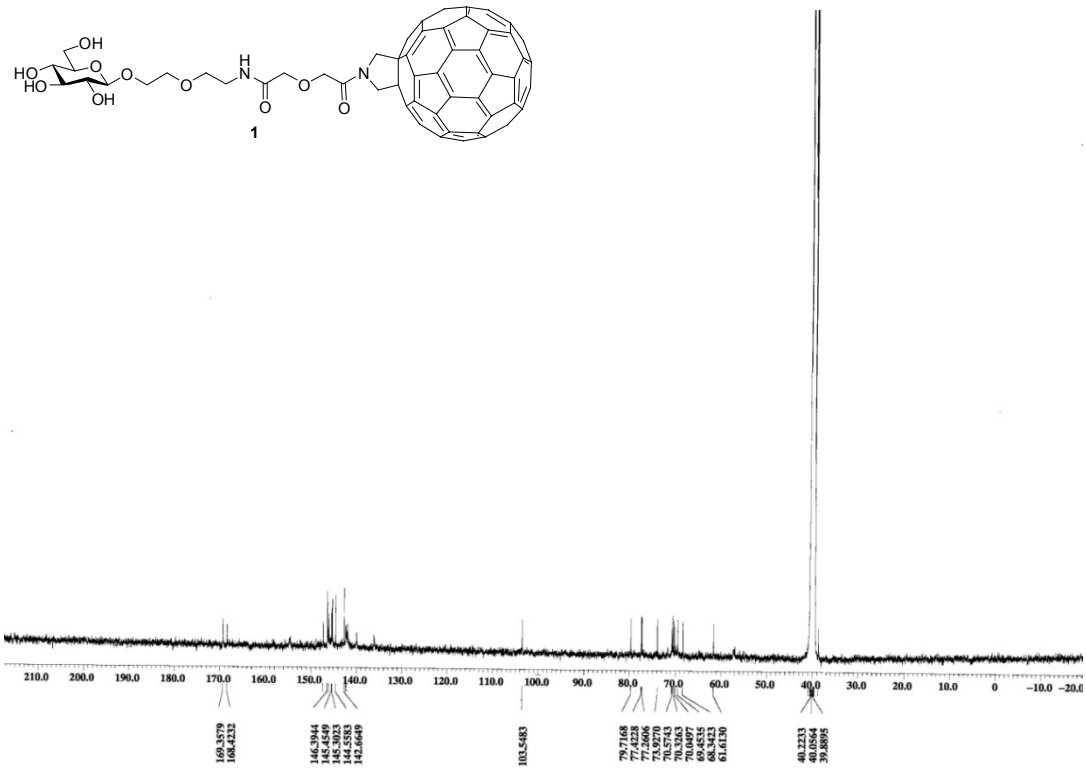
^{1}H NMR spectrum of **6**



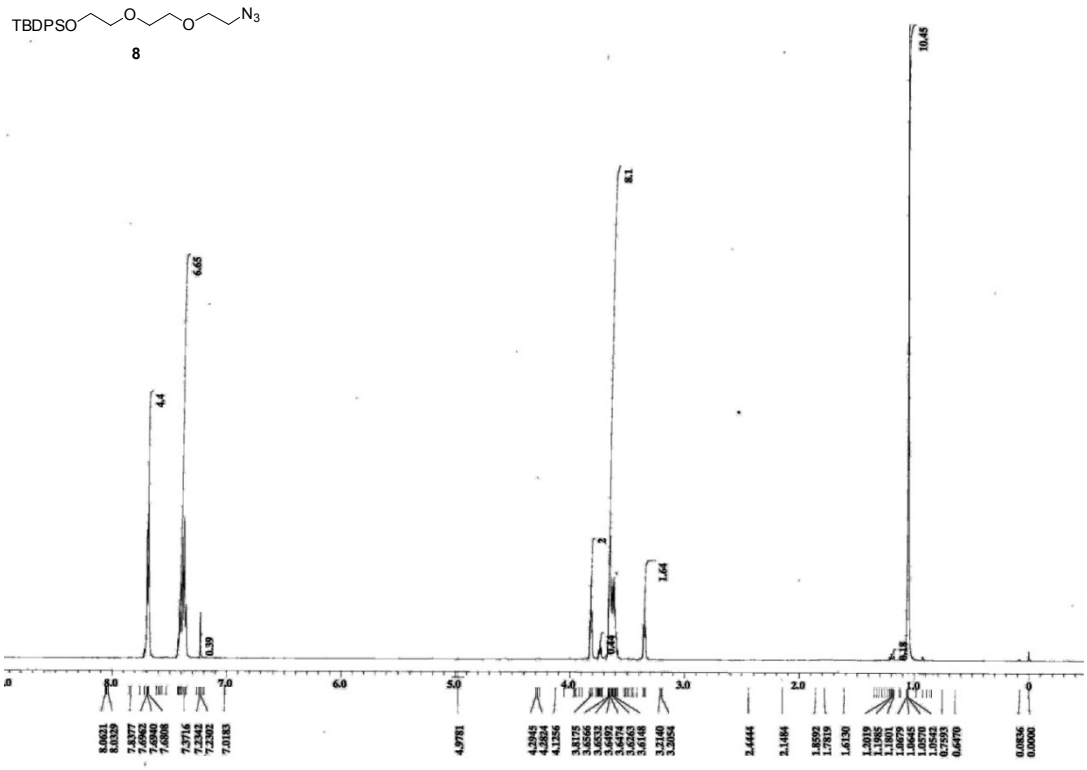
^{13}C NMR spectrum of **6**



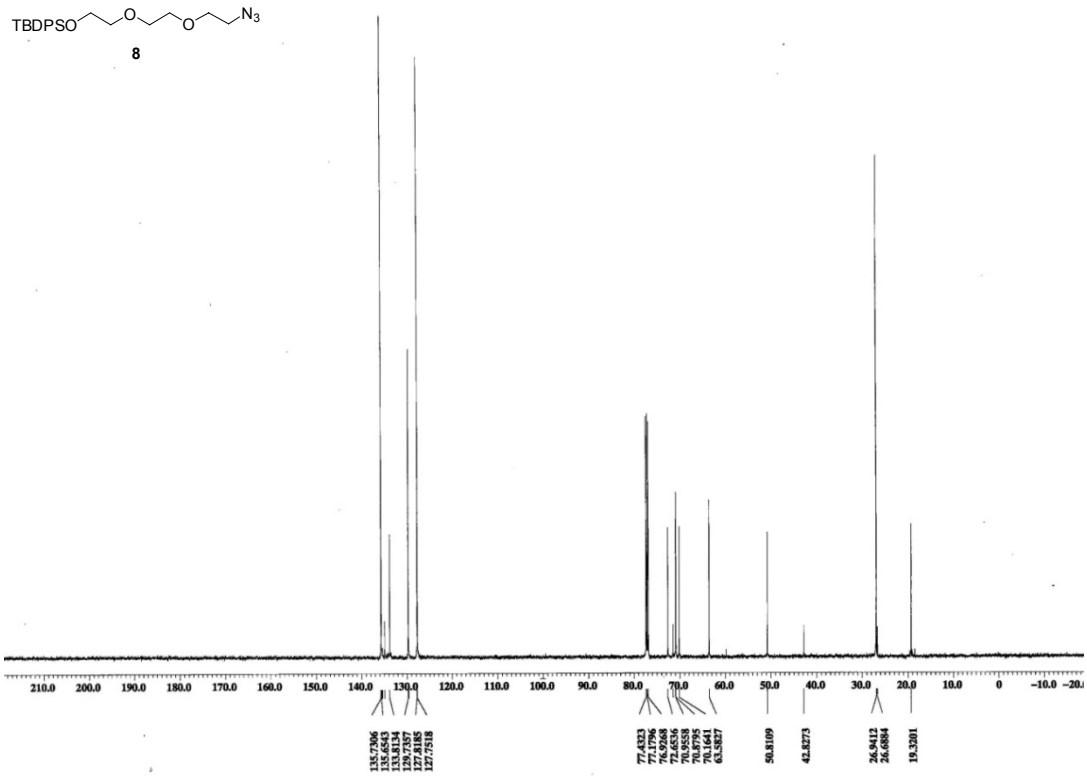
^{1}H NMR spectrum of **1**



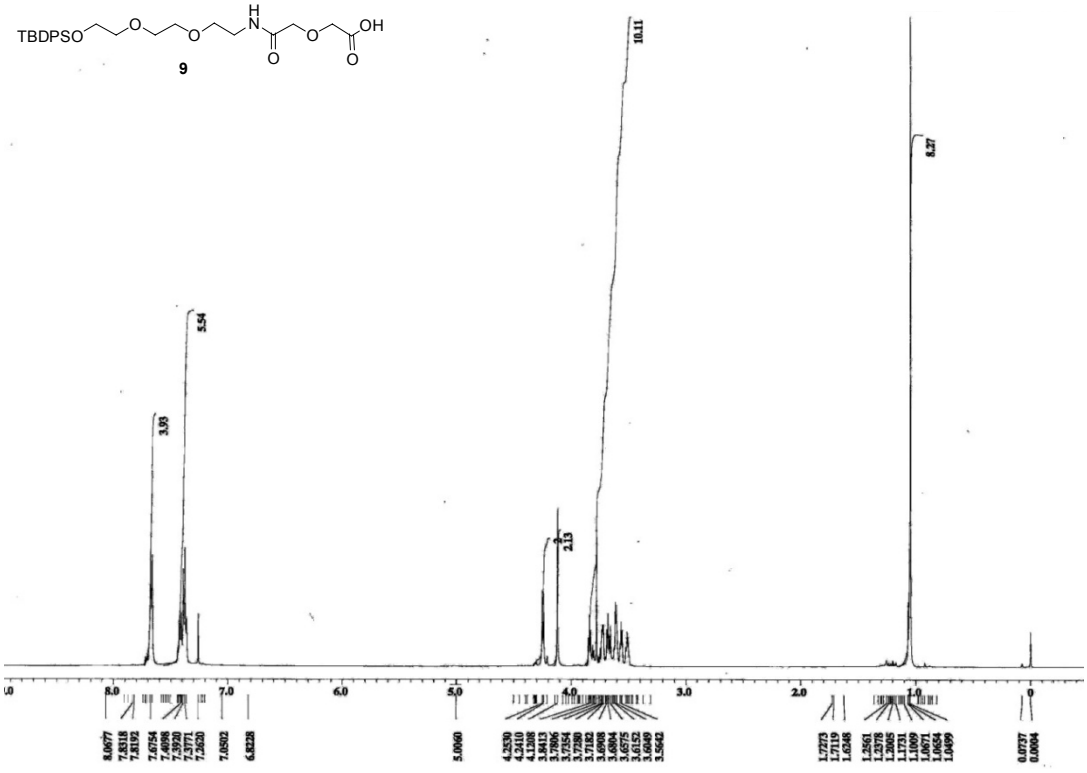
^{13}C NMR spectrum of **1**



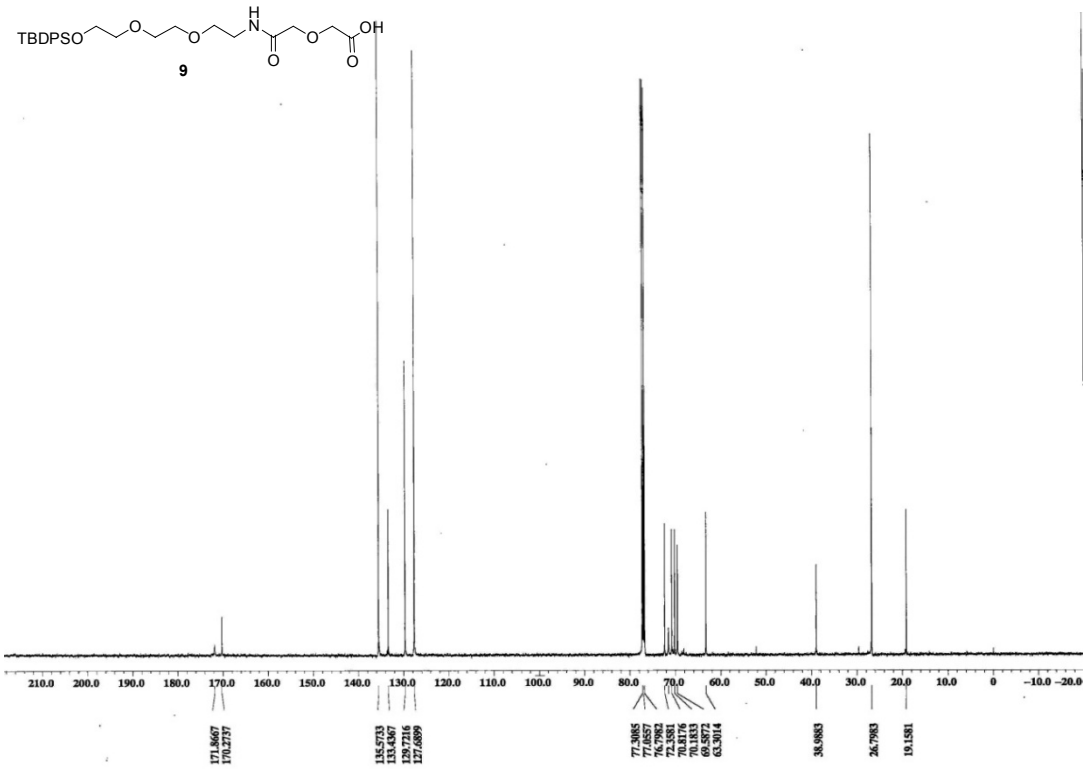
^{1}H NMR spectrum of **8**



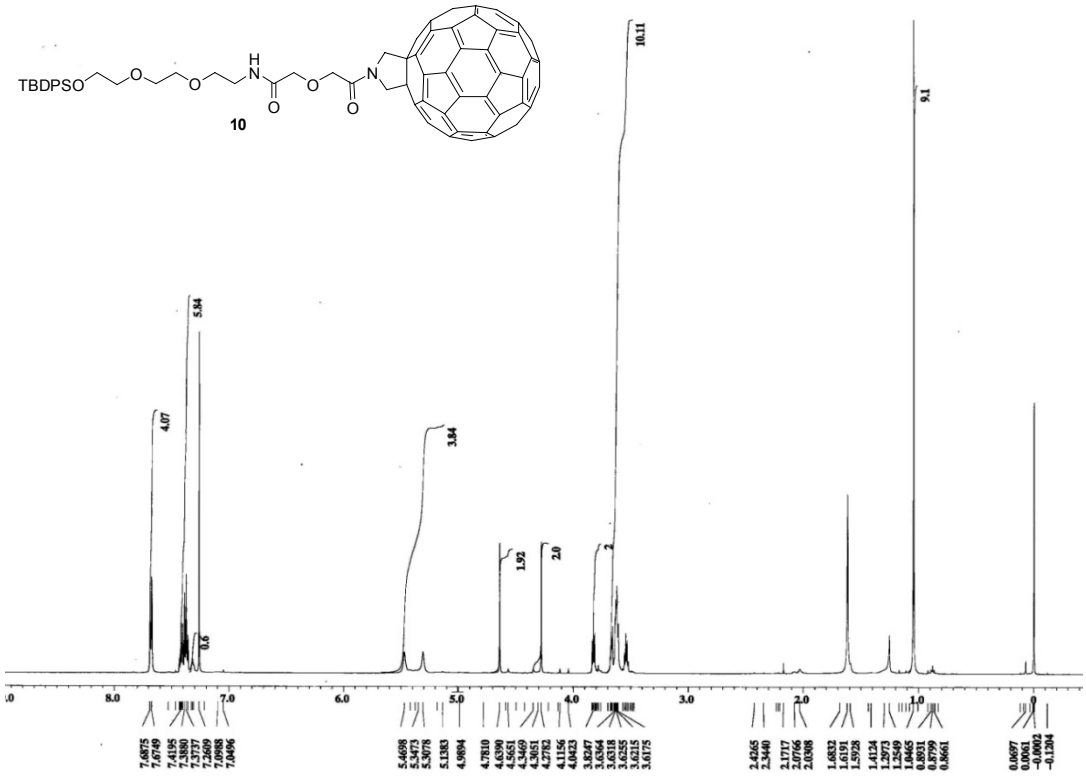
^{13}C NMR spectrum of **8**



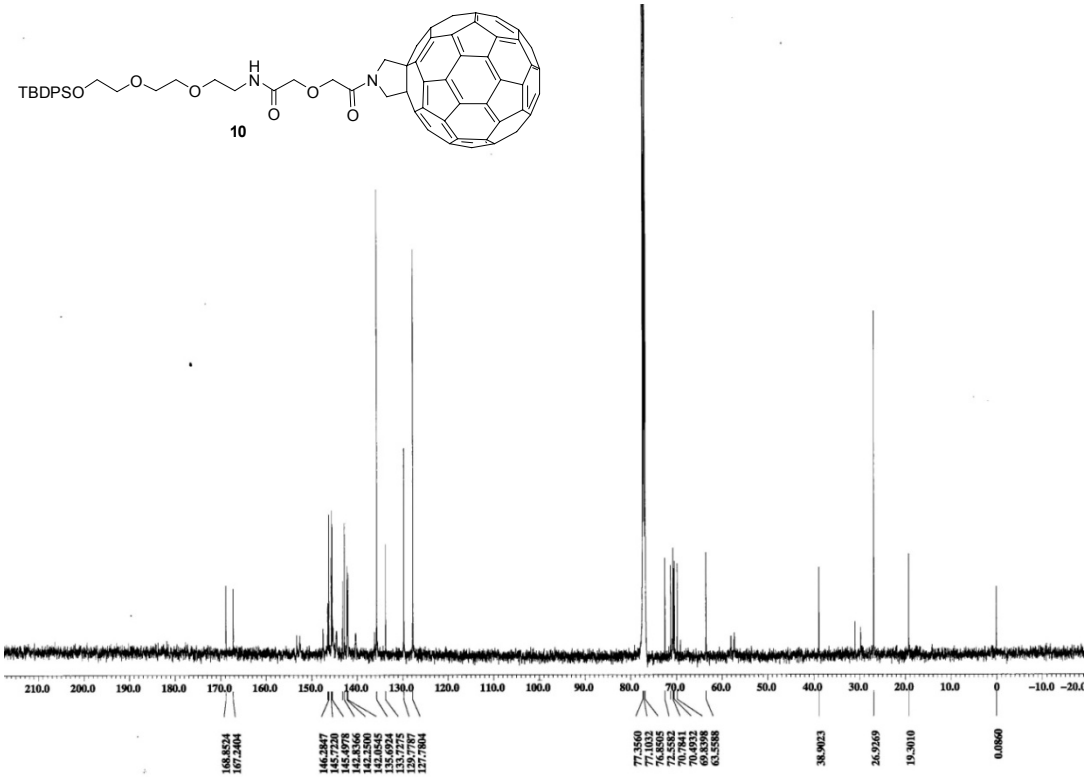
^{1}H NMR spectrum of **9**



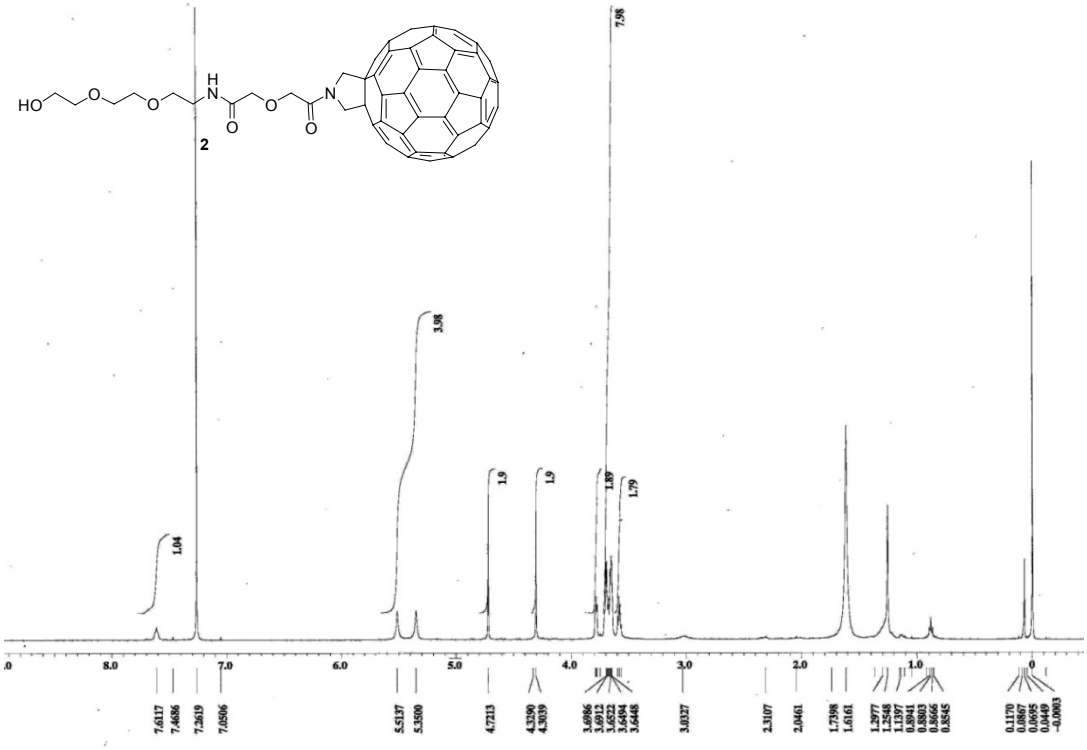
^{13}C NMR spectrum of **9**



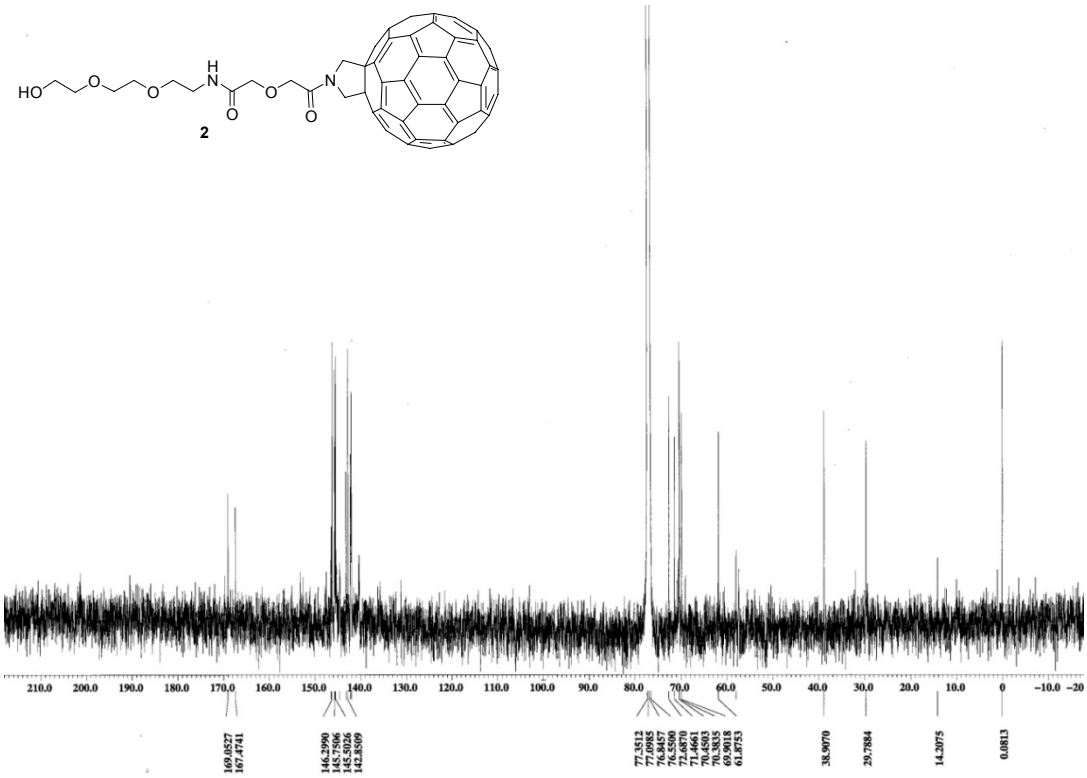
^{1}H NMR spectrum of **10**



^{13}C NMR spectrum of **10**



^{1}H NMR spectrum of **2**



^{13}C NMR spectrum of **2**