Supporting Information

# Thermoresponsive cyclodextrins with benzenesulfonamide showing tunable inhibition for carbonic anhydrase

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**Content**

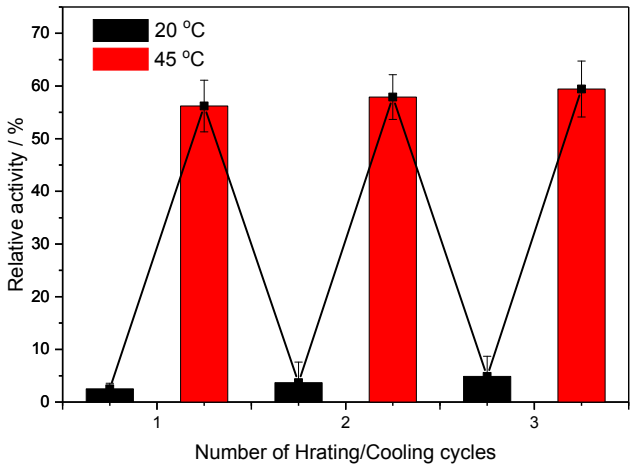


Fig. S1 CAB activity switched by **CD-I1** under thermal cycling between 20 and 45°C.

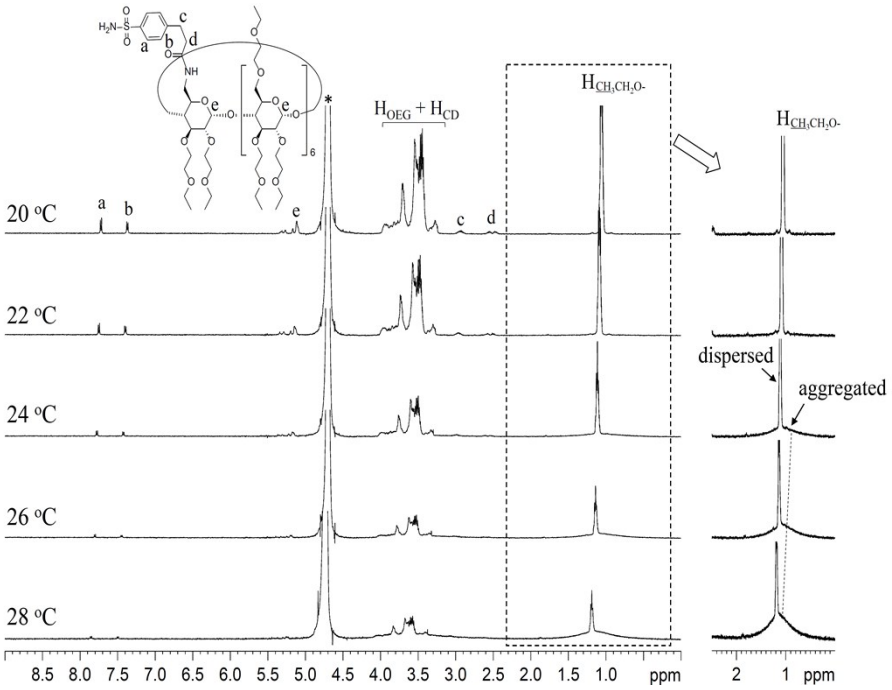


Fig. S2 Temperature varied $^{1}$H NMR spectra for 500 μM **CD-I1** in pH 7.4 buffer.

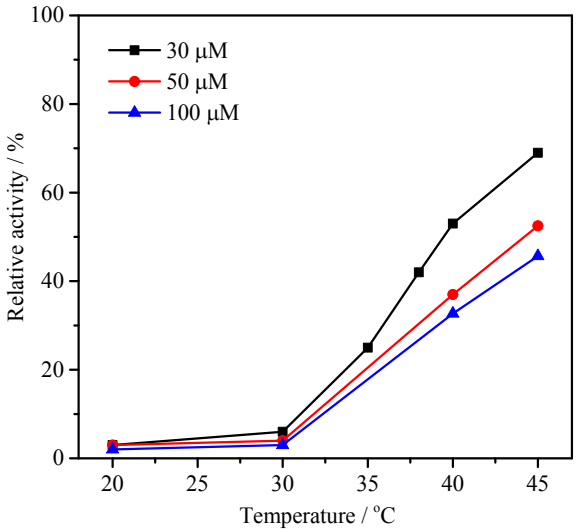


Fig. S3 Recovery of CAB activity inhibited by **CD-I1** above $T_c$ at different concentrations.

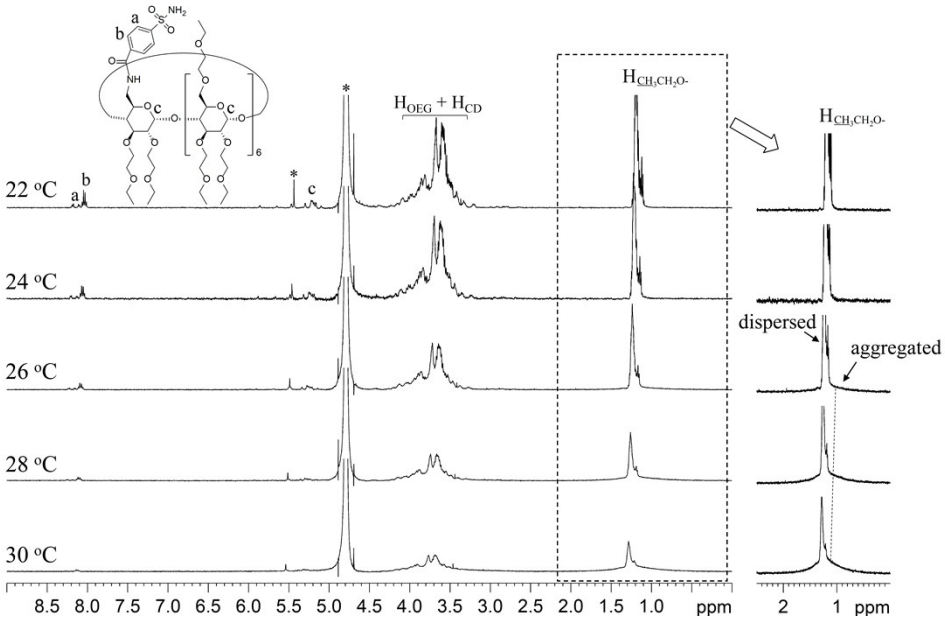


Fig. S4 Temperature varied $^1$H NMR spectra for 500 μM **CD-I2** in pH 7.4 buffer.

## Experimental Section

**Materials.** Dry dichloromethane (DCM) was prepared by distillation from $CaH_2$. Dimethylformamide (DMF) was dried over $CaH_2$. Triethylamine was dried over NaOH pieces. Other reagents and solvents were purchased at reagent grade and used without further purification. All reactions were run under a nitrogen atmosphere. Macherey-Nagel precoated TLC plates (silica gel 60 G/UV254, 0.25 mm) were used for thin-layer chromatography (TLC) analysis. Silica gel 60 M (Macherey-Nagel, 0.04–0.063 mm, 200–300 mesh) was used as the stationary phase for column chromatography.

**Instrumentation.** $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV 500 ($^{1}H$:500 MHz, $^{13}C$: 125 MHz) spectrometer and chemical shifts are reported as δ values (ppm) relative to internal $Me_4Si$. UV/vis turbidity measurements were carried out on a JASCO UV/vis spectrophotometer V-750 equipped with a thermos-regulated bath. Sample solutions were placed in the spectrophotometer (path length 1 cm), and heated or cooled at a rate of 0.2 K $min^{-1}$. The absorptions of the solution at $\lambda$ = 500 nm were recorded every five seconds. The cloud point temperature ($T_c$) was determined as the one at which the transmittance had reached 50% of the values between the initial and final stages.

**Synthesis.**

Compound **2**. Sodium hydride (2.5 g, 100 mmol) was added into a mixture of mono-6-deoxy-6-azido-β-CD (2.0 g,1.7 mmol) and potassium iodide (2.9 g， 17 mmol) in DMF (60 mL) at 0 °C. After stirring for 30 minutes, tosylated ethylene glycol monoethyl ether (21.1 g, 0.86 mmol) in DMF was added slowly. The mixture was stirred over 24 h at 25 °C. Methanol was added to terminate the reaction. The solvents were evaporated in vacuum and the residue was dissolved in DCM. The organic phase was washed with brine for three times, then dried over $MgSO_4$. Purification by column chromatography with DCM–MeOH (v/v, 20:1) afforded the title compound as a colorless liquid (1.3 g,

30%). $^1$H NMR ($CDCl_3$): δ = 1.18-1.21 (m, 60H, $CH_3$), 3.32-3.35 (m, 7H, CH), 3.50-3.90 (m, 141H, $CH_2$ + CH), 4.03-4.16 (m, 14H, CH), 5.18 -5.37 (m, 7H, CH).

Compound **3**. A mixture of compound **2** (1 g, 0.38 mmol) and triphenylphosphine (151 mg, 0.57 mmol) in DMF (10 mL) and ammonia water (0.5 mL) was stirred at 50 °C overnight. The solvent was evaporated off. Purification by column chromatography with DCM–MeOH (v/v: 30:1 to 10:1) afforded the product as a colorless liquid (0.77 g, 77%). $^1$H NMR ($CDCl_3$): δ = 1.17-1.21 (m, 60H, $CH_3$), 3.30-3.34 (m, 7H, CH), 3.49-3.86 (m, 141H, $CH_2$ + CH), 4.05-4.16 (m, 14H, CH), 5.14 -5.44 (m, 7H, CH).

**CD-I1**. A mixture of 3-(4-sulfamoylphenyl)propionic acid (48 mg, 0.21 mmol), $Et_3N$ (98 mg, 0.97 mmol) and HOBt (52 mg, 0.39 mmol) were dissolved in the mixed solvents of DCM (10 mL) and DMF (2 mL). After cooling the solution to -15 °C, EDC (74 mg, 0.39 mmol) was added in one portion. Compound **3** (500 mg, 0.19 mmol) in DCM (10 mL) was then added dropwise under $N_2$. The reaction solution was gradually warmed to room temperature, and further stirred overnight. After washing with saturated $NaHCO_3$ and brine, the organic phase was dried with $MgSO_4$ and concentrated. Purification with column chromatograph afforded **CD-I1** as a colorless oil (250 mg, 46%). $^1$H NMR ($d_6$-DMSO): δ = 1.06-1.12 (m, 60H, $CH_3$), 3.14-3.28 (s, 7H, $CH_3$), 3.55-3.58 (m, 162H, $CH_2$ + CH), 3.94-4.07 (m, 14H, CH), 5.12-5.37 (m, 7H, CH), 7.5 (d, 2H, $NH_2$), 7.88 (d, 2H, Ar-H), 8.01 (d, 2H, Ar-H), 8.31(s, 1H, NH). $^{13}$C NMR ($d_6$-DMSO): δ = 15.1, 30.9, 36.4, 54.9, 65.4, 65.6, 69.0, 69.1, 69.4, 69.5, 69.7 70.0, 70.1, 70.5, 70.7, 70.8, 71.8, 77.1, 77.4, 78.0, 78.3, 79.3, 79.7, 80.2, 80.4, 80.6, 81.0, 96.5, 96.9, 97.2, 125.7, 128.5, 141.9, 145.7, 171.1. HR-MS: m/z calcd for $C_{131}H_{241}N_2O_{57}S$ $[M + H]^+$ 2786.57; found 2786.5736.

**CD-I2**. A mixture of 4-sulfamoylbenzoic acid (42 mg，0.20 mmol), $Et_3N$ (98 mg, 0.97 mmol) and HOBt (52 mg, 0.39 mmol) were dissolved in the mixed solvents of DCM (10 mL) and DMF (2 mL). After cooling the solution to -15 °C, EDC (74 mg, 0.39

mmol) was added in one portion. Compound **3** (500 mg, 0.19 mmol) in DCM (10 mL) was then added dropwise under $N_2$. The reaction solution was gradually warmed to room temperature, and further stirred overnight. After washing with saturated $NaHCO_3$ and brine, the organic phase was dried with $MgSO_4$ and concentrated. Purification with column chromatograph afforded **CD-I2** as a colorless oil (283 mg, 53%). $^1$H NMR ($d_6$-DMSO): δ = 1.06-1.12 (m, 60H, $CH_3$), 3.14-3.28 (s, 7H, $CH_3$), 3.55-3.58 (m, 162H, $CH_2$+CH), 3.94-4.07 (m, 14H, CH), 5.12-5.37 (m, 7H, CH), 7.5 (d, 2H, $NH_2$), 7.88 (d, 2H, Ar-H), 8.01 (d, 2H, Ar-H), 8.31(s, 1H, NH). $^{13}$C NMR ($d_6$-DMSO): δ = 15.1, 65.4, 65.6, 68.8, 68.9, 69.1, 69.5, 69.7, 69. 9, 70.1, 70.2, 70.6, 70.7, 71.0, 71.8, 76.4, 76. 9, 77.4, 78.5, 79.5, 79.6, 79.7, 80.0, 80.2, 80.4, 80.5, 80.6, 80.7, 96.7, 97.2, 125.4, 127.9, 137.3, 146.3, 165.4. HR-MS: m/z calcd for $C_{129}H_{237}N_2O_{57}S$ $[M + H]^+$ 2758.54; found 2758.5423.

**Assay for enzymatic activity of CAB.** The CAB activity was determined using a p-nitrophenyl acetate (p-NPA) esterase assay, in which 10 μL of 55 mM p-NPA in acetonitrile was added into 2 mL of 3 μM CAB solution in pH = 7.4 phosphate buffer containing varied concentrations of inhibitors. p-NPA hydrolysis was monitored by measuring the absorbance of solution at 400 nm (corresponding to p-nitrophenolate) every 0.5 s for 5 min. After deducting the background absorbance at 400 nm from non-enzymatic hydrolysis without CAB, the net absorbance at 400 nm was plotted as a function of time. The initial curve from previous 100 seconds is linear, and its slope was taken as the enzyme activity. For temperature-varied measurements, the samples was kept to equilibrium at a predetermined temperature for around 25 min before data collection. The relative activity of CAB was then calculated by dividing the enzyme activity in the presence of CD inhibitors by that of the free enzyme (100% actvity) under the same condition.

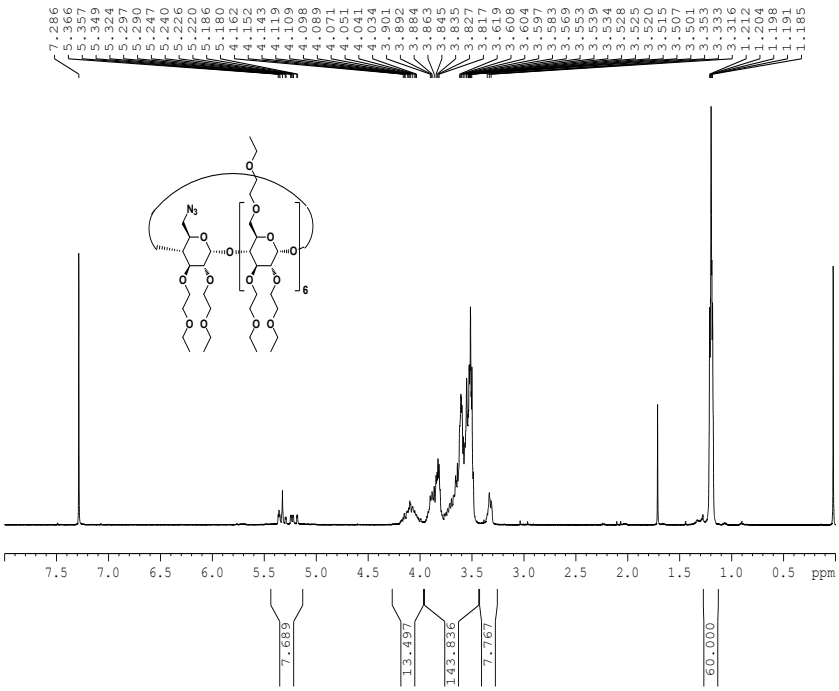


Fig. S5. $^{1}H$ NMR spectrum of compound **2** in $CDCl_3$.

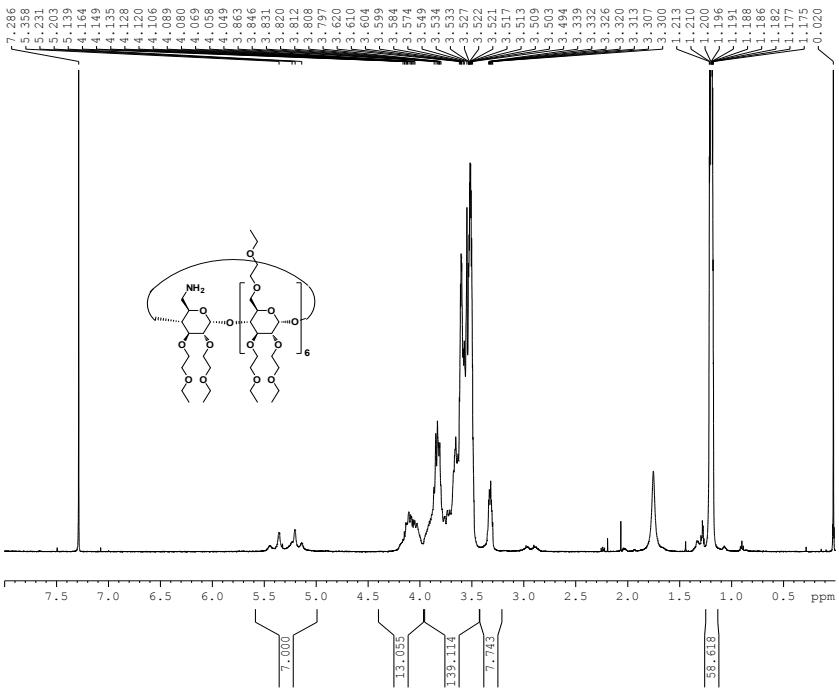


Fig. S6 $^{1}H$ NMR spectrum of compound **3** in $CDCl_3$.

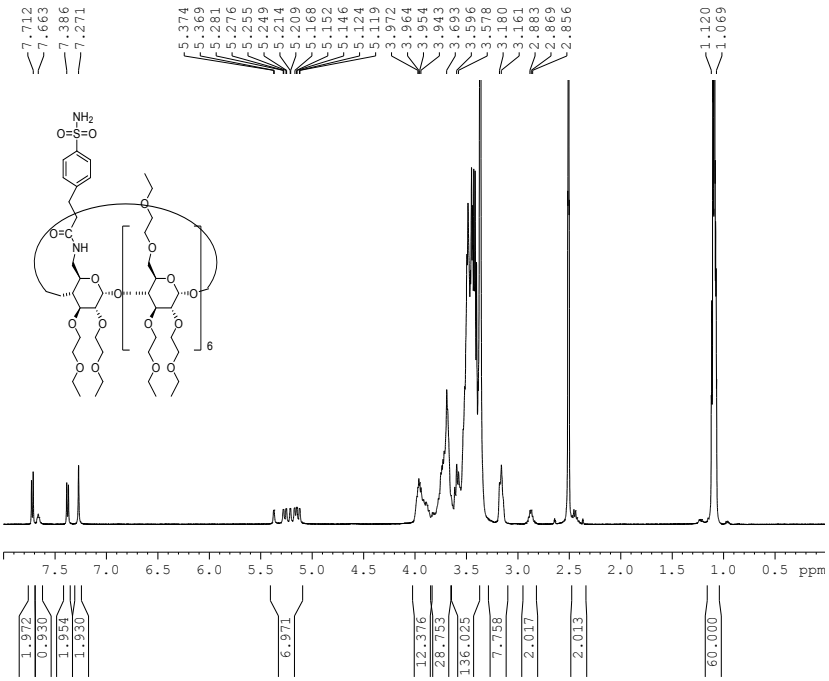

Fig S7. $^{1}$H NMR spectrum of **CD-I1** in $d_6$-DMSO.

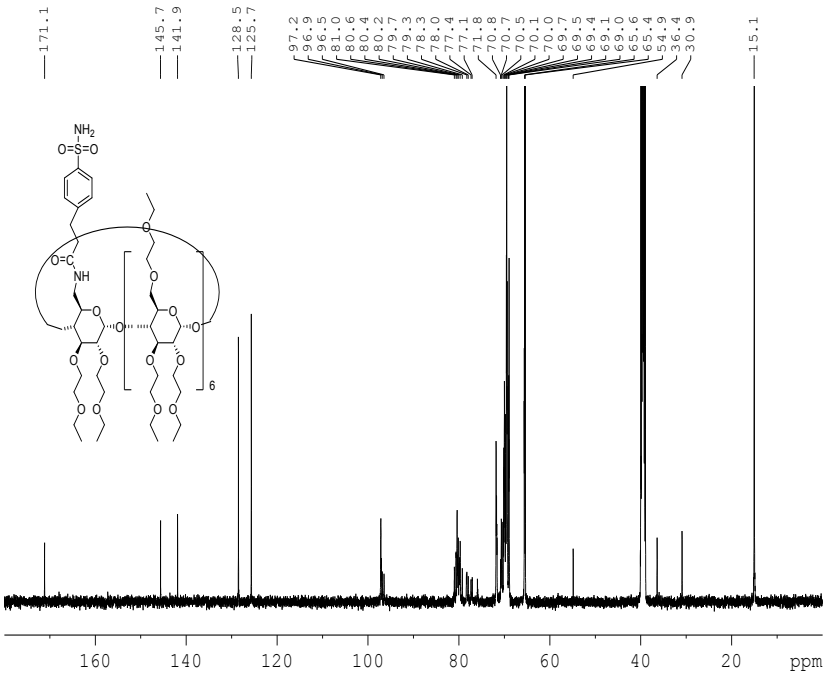

Fig. S8 $^{13}$C NMR spectrum of **CD-I1** in $d_6$-DMSO

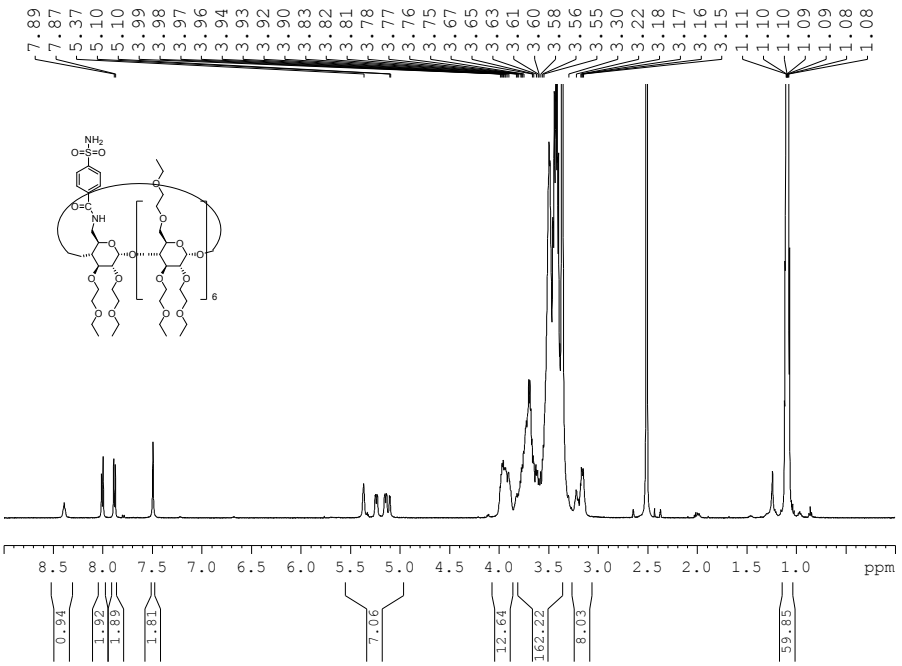


Fig. S9 $^{1}$H NMR spectrum of **CD-I2** in $d_6$-DMSO.

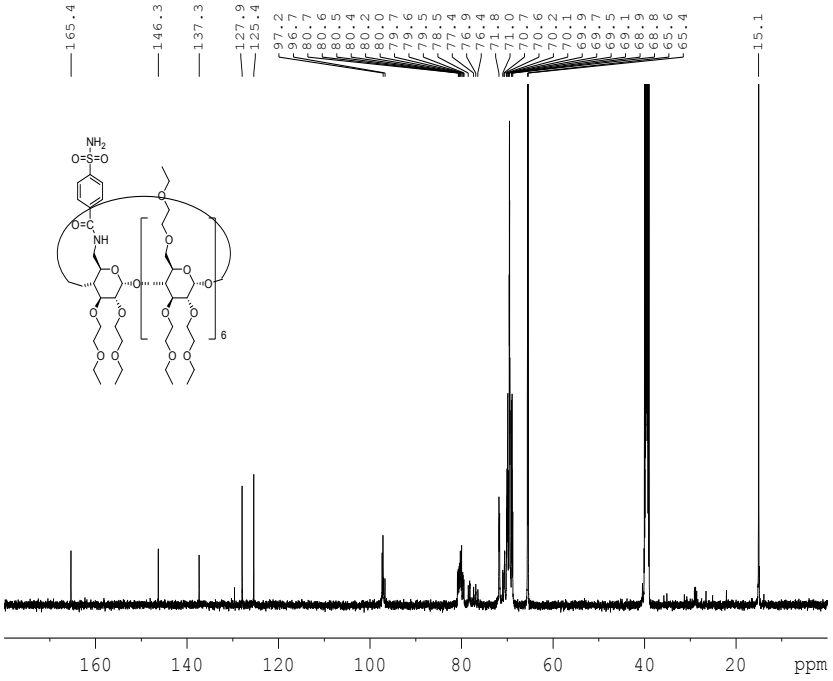


Fig. S10 $^{13}$C NMR spectrum of **CD-I2** in $d_6$-DMSO.