Supplementary Information

for

A Chemical Strategy for Amphiphile Replacement in Membrane Protein Research

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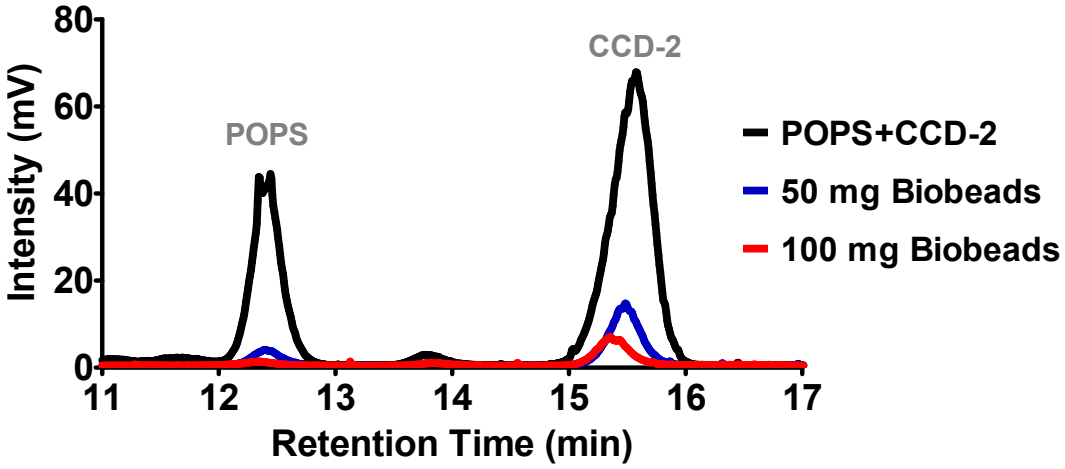


Figure S1. Physical adsorption of CCD-2 from POPS via BioBeads. The mixture containing 1 mM CCD-2, 0.5 mM POPS and 20 mM Hepes buffer (pH~7.5) was treated with BioBeads (SM-2, Bio-Rad, 50 mg or 100 mg/300 ul mixture) and then incubated at 4 °C with constant rotation for 2 hours. All samples were measured under an isocratic elution with 40% MeCN by ELSD-HPLC for three times.

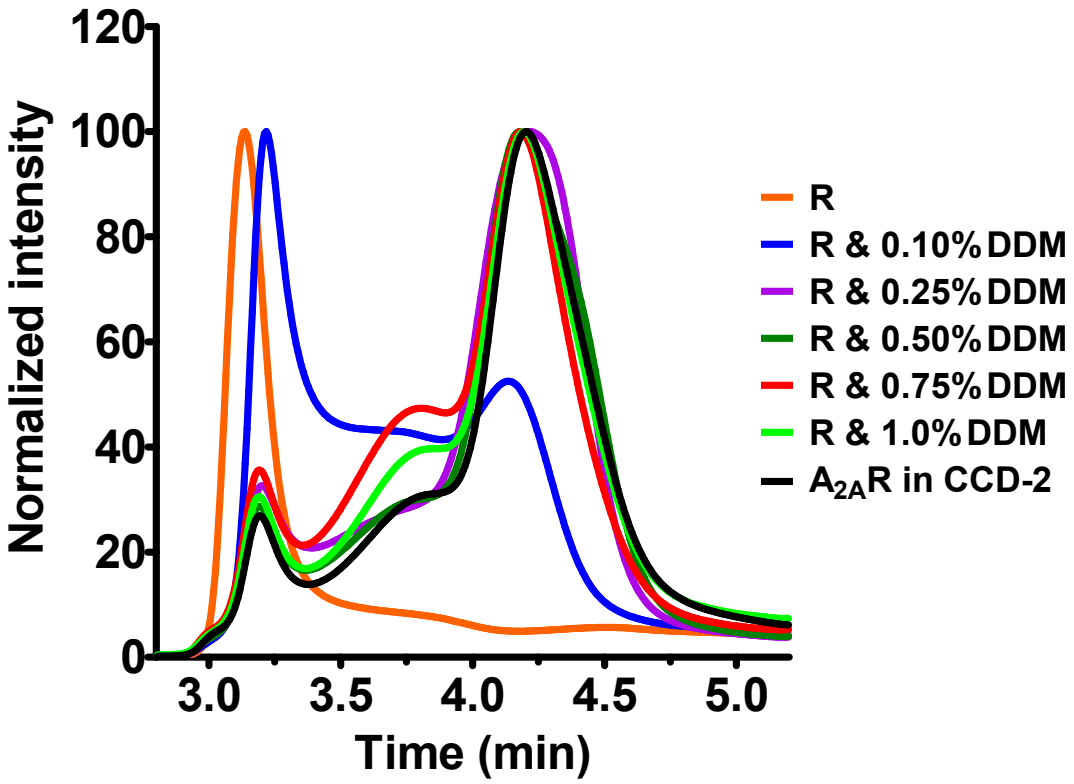
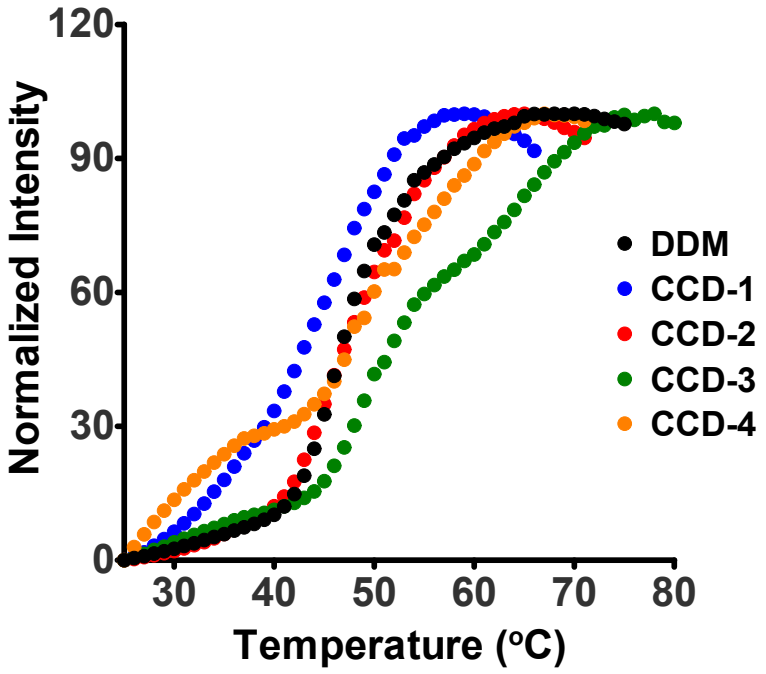


Figure S2. Higher concentration of DDM (test detergent) was required for CCD-2 (sacrificed detergent) mediated detergent exchange. 2.5 μM $A_{2A}R$ encapsulated in 0.025% CCD-2 was incubated with various concentration of DDM for 1 hour before treatment with 2.5 mM TCEP for 2 hours at 4 °C. $A_{2A}R$ maintained in monotopic state only at a concentration of DDM over 0.25%. $A_{2A}R$ aggregated when CCD-2 decomposed at low concentration of DDM (molar ratio: DDM/CCD-2 < 5), probably due to the delayed micelle re-organization during the fast cleavage. Therefore, we set a minimum concentration of the test detergent at roughly 10 times higher than the molar concentration of CCD-2 in the following performance of detergent screening for $A_{2A}R$.



Detergent	DDM	CCD-1	CCD-2	CCD-3	CCD-4
T_m/°C	47.4	43.3	47.7	53.6	49.3

Figure S3. Thermal stability of $A_{2A}R$ in CCDs and DDM assessed by thermal shift assay.

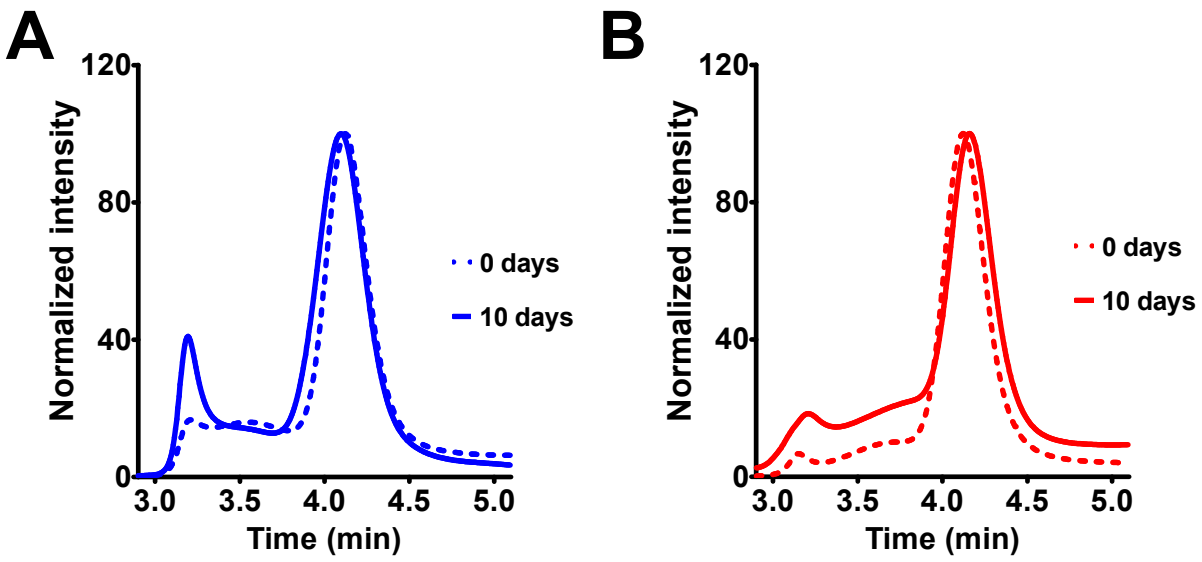


Figure S4. Long-term stability of $A_{2A}R$ encapsulated in DDM (A) or CCD-2 (B). $A_{2A}R$ was solubilized in 1% DDM or CCD-2 and then purified in 5 × CMC of corresponding detergents respectively. The monodispersity of $A_{2A}R$ was tested by aSEC from UV absorbance at 280 nm. These normalized chromatograms demonstrated $A_{2A}R$ encapsulated in CCD-2 exhibited superior stability to that in DDM over the course of 10 days at 4 °C.

Table S1. Reaction conditions for CCD-2 cleavage.

Reductant	Stoichiometry	Temperature	Time	Solvent	Decomposition% [b]
$NaBH_4$	10	r.t.	1 h	H_2O	2.5%
DTT[a]	10	r.t.	1 h	H_2O	72.3%
BME[a]	10	r.t.	1 h	H_2O	41.2%
TCEP[a]	10	r.t.	1 h	H_2O	> 99%
TCEP	1	r.t.	1 h	H_2O	28.1%
TCEP	1	r.t.	4 h	H_2O	85.0%
TCEP	2.5	r.t.	1 h	H_2O	92.5%
TCEP	2.5	r.t.	2 h	H_2O	> 99%
TCEP	5	r.t.	1 h	H_2O	> 99%
TCEP	5	4 °C	2 h	20 mM HEPES, pH 7.5	> 99%

[a]Abbreviation: dithiothreitol (DTT); 2-mercaptoethanol (BME), tris (2-carboxyethyl) phosphine (TCEP)
[b]The decomposition yield was measured under an isocratic elution with 40% MeCN by ELSD-HPLC at a flow rate of 1.0 mL/min.

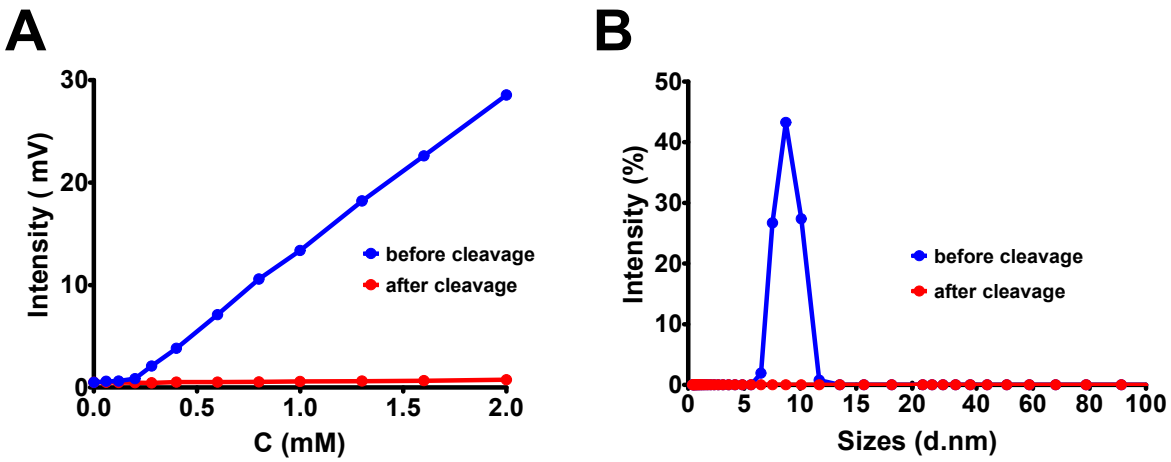


Figure S5. The CCD-2 degradation results in complete disappearance of micelles. (A) Fluorophore encapsulation assay. 2 mM CCD-2 was treated with 10 mM TCEP in 100 mM HEPES buffer (pH 7.5) containing 100 μM 8-Anilinonaphthalene-1-sulfonic acid (ANS) for 2 hours. The fluorescence intensity was measured at an excitation wavelength of 388 nm and an emission wavelength of 477 nm. (B) Dynamic light scattering. The hydrodynamic diameter was determined at 25 °C.

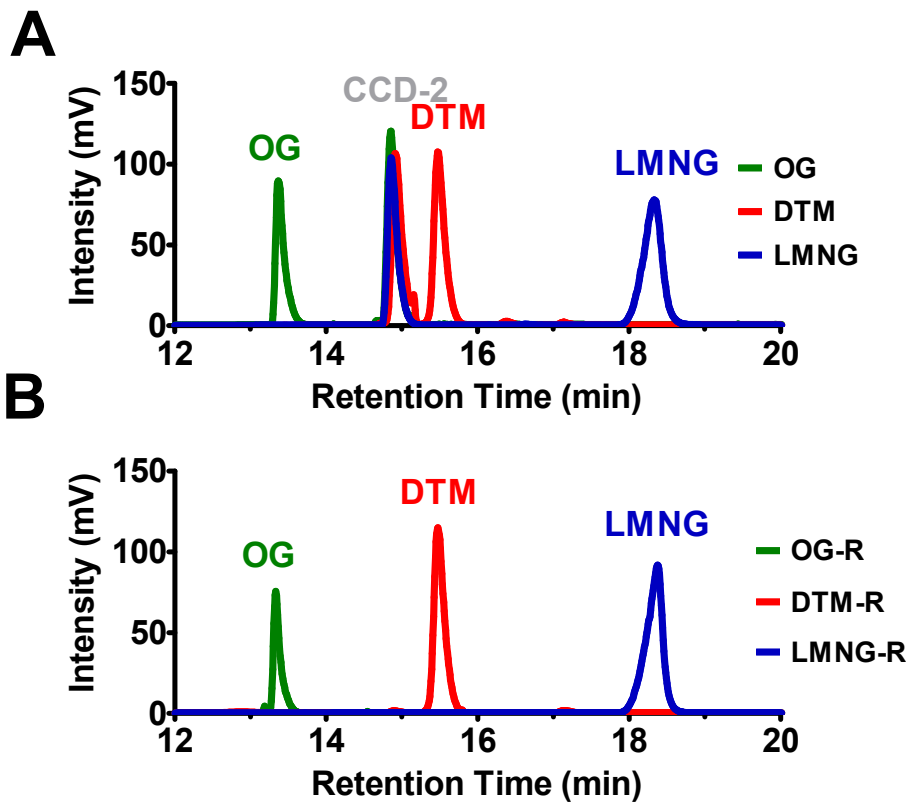


Figure S6. CCD-2 decomposition with commercial detergents detected by ELSD-HPLC. HPLC profile for the selected detergents in mixture with CCD-2 before (A) and after (B) the treatment with 2.5 mM TCEP in 100 mM HEPES buffer (pH ~7.5) for 2 hours. OG (green), DTM (red) and LMNG (blue), respectively. The complete decomposition of CCD-2 resulted in a new unitary system, demonstrating its feasibility for detergent replacement.

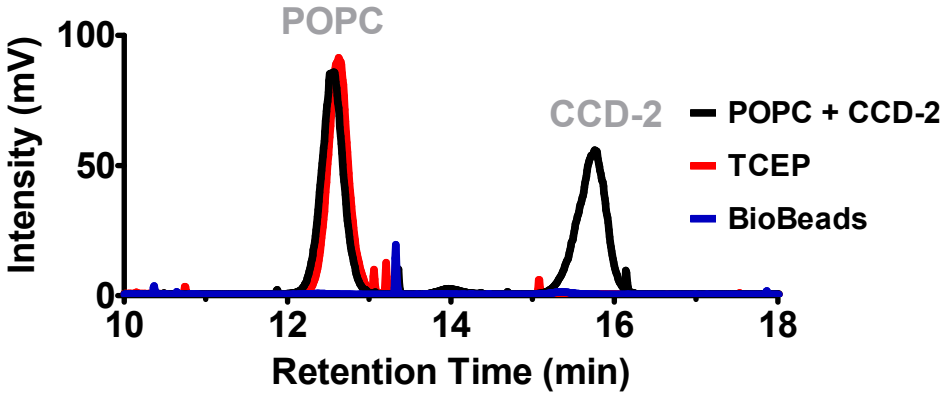


Figure S7. The replacement of CCD-2 with POPC via physical adsorption of BioBeads or chemical cleavage triggered by TCEP treatment. The mixture containing 1 mM CCD-2 , 0.5 mM POPC and 20 mM HEPES buffer (pH~7.5) was treated with BioBeads (SM-2, Bio-RAD, 50 mg/300 μL mixture) or TCEP (final concentration = 2.5 mM) and then incubated at 4 °C with constant rotation for 2 hours. All samples were measured under an isocratic elution with 40% MeCN by ELSD-HPLC for three times.

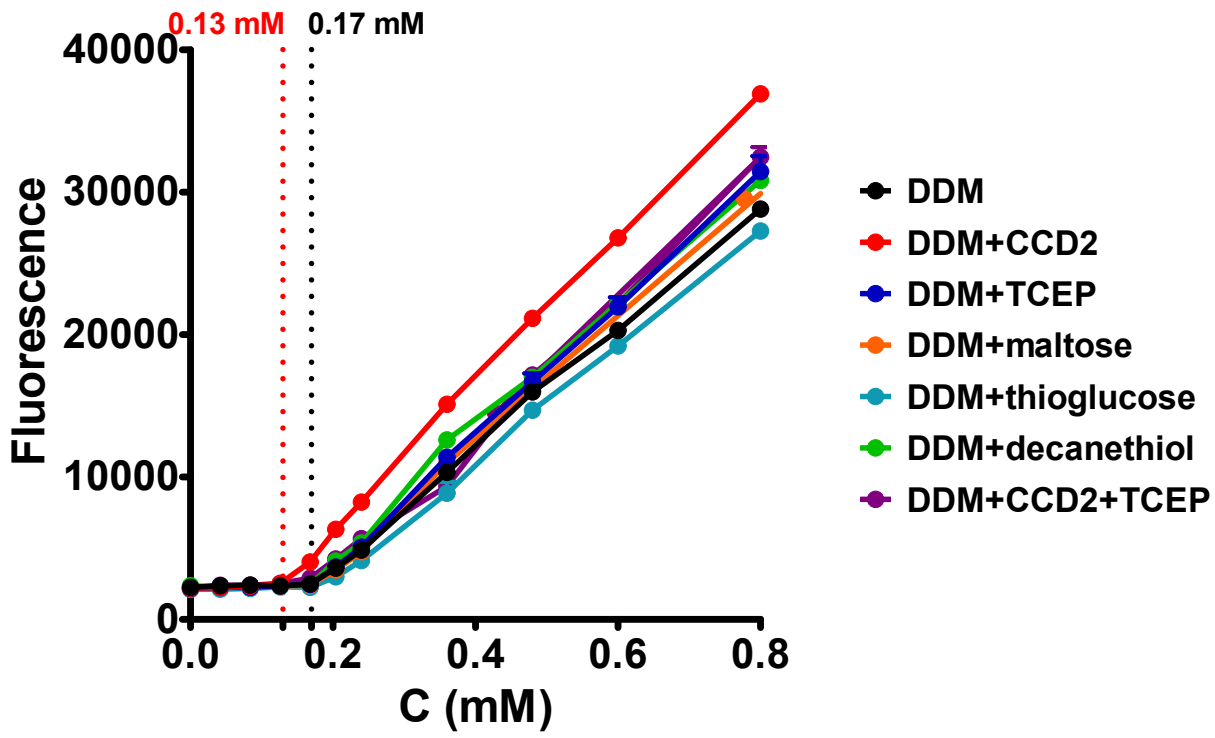


Figure S8. The influence of cleavage debris of CCD-2 on the CMC value of DDM. The CMC values are defined as the inflection point of fluorescence change of fluorescence intensity of 8-anilino-1-naphtalenesulfonic acid (ANS, 100 μM, λ_{ex} = 388 nm, λ_{em} = 477 nm). The molar ratios of CCD-2, saccharides, thiol compound and TCEP to DDM were 0.4, 0.4, 0.4 and 2.0, respectively.

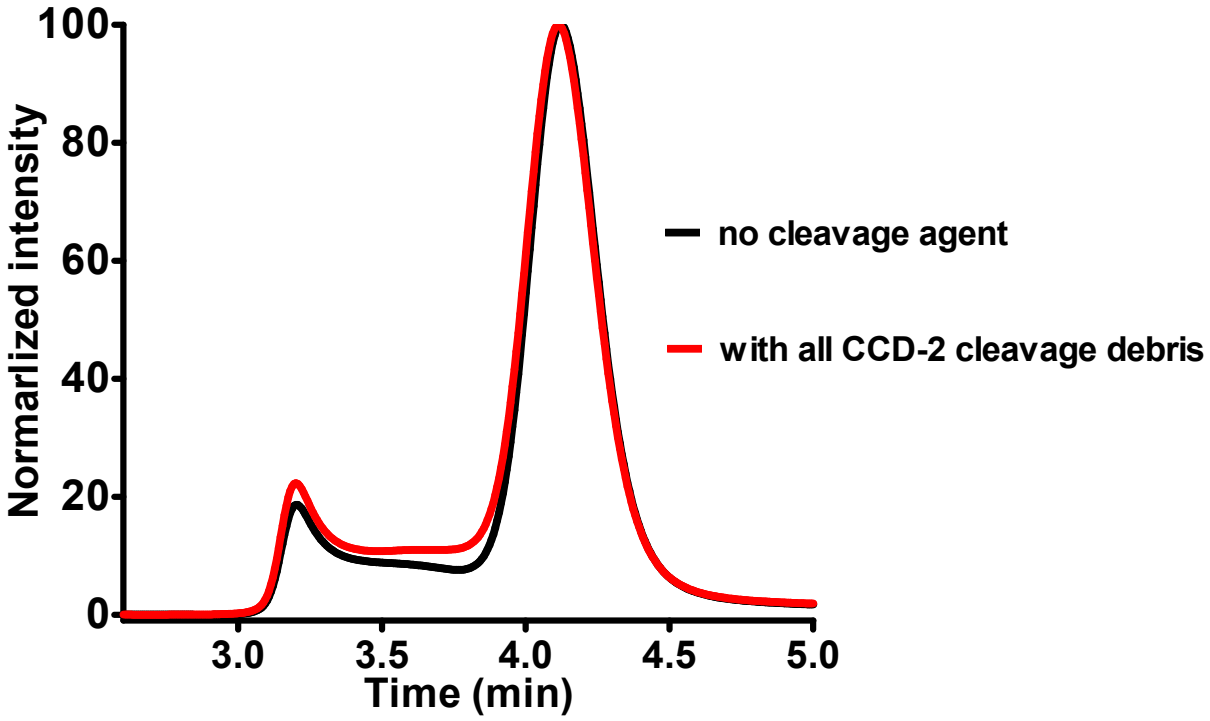


Figure S9. The influence of cleavage debris of CCD-2 on the homogeneity of $A_{2A}R$. 0.2 mg/mL $A_{2A}R$ encapsulated in DDM (0.025%, black line) was incubated with 0.025% CCD-2 at 4 °C. The resulting mixture was treated with 2.5 mM TCEP for 2 hours at 4 °C before being analyzed by aSEC.

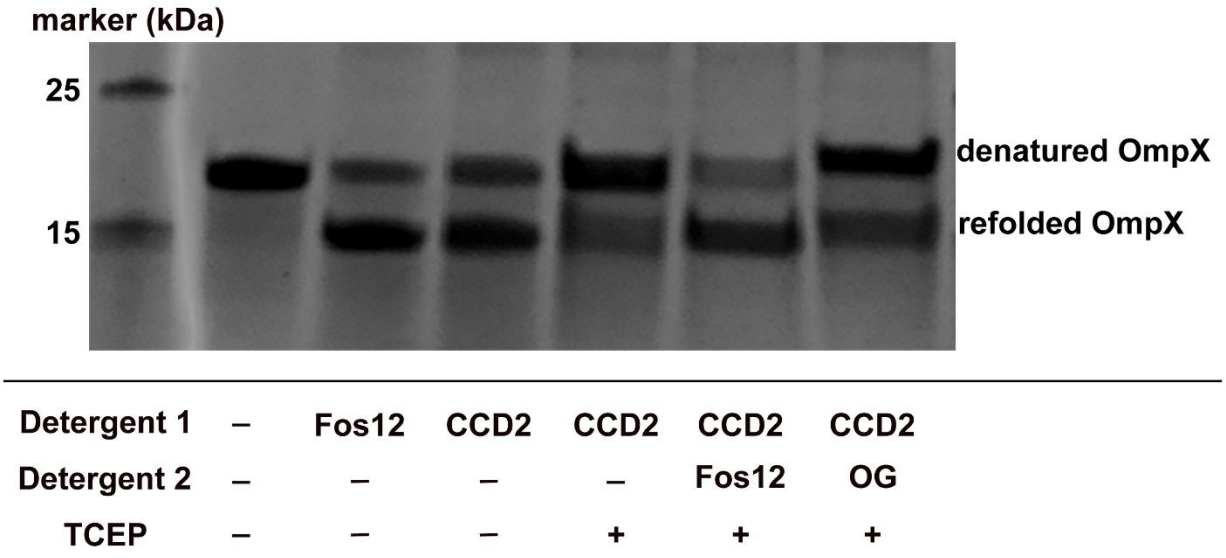


Figure S10. The influence of cleavage deris of CCD-2 on the refolding of OmpX. Refolding of OmpX performed well in Fos12 and CCD-2. When treated with TCEP, the refolded OmpX in CCD-2 denatured in the absence of new detergent or even in the presence of OG, but remained in the presence of Fos12. Final concentration of CCD-2 and other detergents was 1.0 % and the final concentration of TCEP was 100 mM (5 eq. of CCD-2).

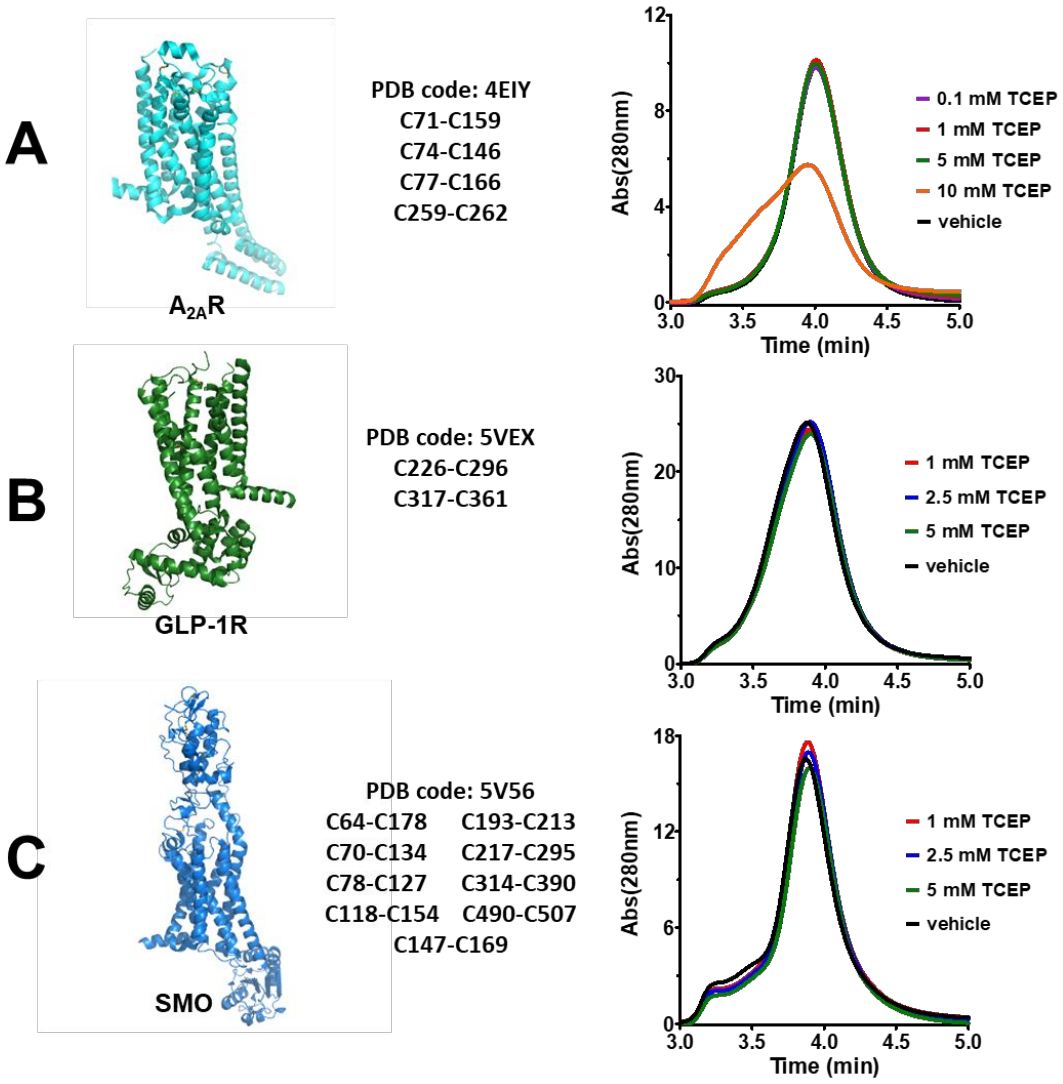


Figure S11. Three representative engineered GPCRs exhibit good tolerance to TCEP. (A) Class A GPCR: A_{2A}R-BRIL-ΔC with 4 pairs of disulfides. (B) Class B GPCR: GLP-1R-T4L with 2 pairs of disulfides. (C) Class F GPCR: SMO-FLA with 9 pairs of disulfides. The three representative GPCRs (0.1 ~ 0.3 mg/mL) were incubated in elute buffer (25 mM HEPES, pH 7.5, 500 mM NaCl, 10% glycerol, 250 mM imidazole, 0.025% DDM) with different concentration of TCEP for 6 hours at 4 °C before being analyzed by aSEC.

Table S2. Information of screened commercial detergents.

No.	Name	MW	CMC	
			mM	%
D310	n-Dodecyl-β-D-Maltopyranoside	510.6	0.17	0.0087
NG310	Lauryl Maltose Neopentyl Glycol	1005.21	0.01	0.0010
N324	n-Nonyl-β-D-Glucopyranoside	306.4	6.5	0.20
D360	n-Dodecyl-N,N-Dimethylamine-N-Oxide	229.4	2	0.050
C316	CHAPS	614.9	8	0.49
D322	n-Decyl-β-D-Maltopyranoside	482.6	1.8	0.087
TX-100	Triton X-100	646.85	0.9	0.058
APO129	Anapoe-C12E9	583	0.05	0.0029
D342	n-Dodecyl-β-D-thiomaltoside	526.7	0.05	0.0026
NG322	Decyl Maltose Neopentyl Glycol	949.08	0.036	0.0034
U300	n-Undecyl-β-D-Maltopyranoside	496.6	0.59	0.029
APO128	Anapoe-C12E8	538.77	0.11	0.0059
C327	CYMAL-7, Anagrade	522.64	0.19	0.0099
S350	Sucrose Monododecanoate, Anagrade	524.61	0.30	0.016
B310	Big Chap, Deoxy, Analytical Grade	862.1	1.4	0.12
N350	n-Nonyl-β-D-Thiomaltopyranoside, Anagrade	484.61	3.2	0.16
H110	HEGA-10, Anagrade	379.5	7.0	0.27
T350	Tetraethylene Glycol Monooctyl Ether (C8E4)	306.44	8.0	0.25
A340	Anameg-7	335.4	19.5	0.65
T730	Tripao	362.59	4.5	0.16
F308	Fos-Choline-12, Anagrade	351.47	1.5	0.053
F300F	Fos-Choline-8, Fluorinated, Anagrade	529.24	2.2	0.12
AZ312	Anzergent 3-12, Analytical Grade	335.55	4.0	0.13

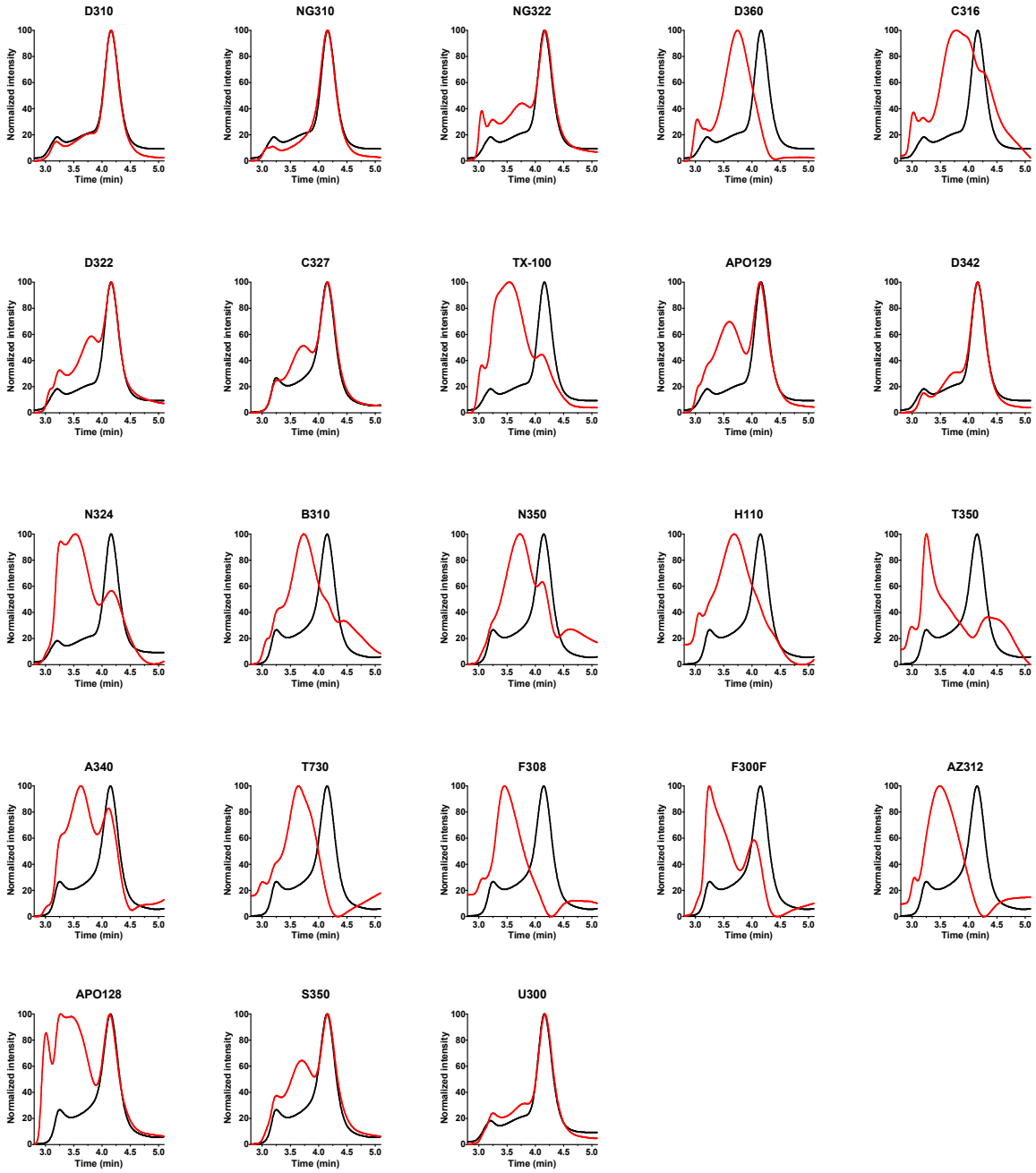


Figure S12. Preliminary results of detergent screening on stabilizing $A_{2A}R$ reconstituted in test detergents using sacrifice-replacement strategy. All samples were prepared according to the protocol presented in the method section (Red: test detergent; Black: DDM). The normalized HPLC profiles of $A_{2A}R$ encapsulated in various detergents were obtained from UV absorbance at 280 nm through size-exclusion chromatography with a flow at 0.5 mL/min.

Table S3. Homogeneity (monomer %) of $A_{2A}R$ in representative maltoside detergents.

No.	Detergent	MW	CMC (mM)	Homogeneity[a] (%)	
				4 °C	40 °C
D310	n-Dodecyl-β-D-Maltopyranoside	510.6	0.17	> 90 %	> 50 %
D342	n-Dodecyl-β-D-thiomaltoside	526.7	0.05		> 50 %
NG310	Lauryl Maltose Neopentyl Glycol	1005.21	0.01		~ 50 %
U300	n-Undecyl-β-D-Maltopyranoside	496.6	0.59		20 % ~ 30 %
C327	CYMAL-7	522.64	0.19	80 % ~ 90 %	
D322	n-Decyl-β-D-Maltopyranoside	482.6	1.8	70 % ~ 80 %	< 10 %
NG322	Decyl Maltose Neopentyl Glycol	949.08	0.036		

[a] Monomer %= (AUC (monomer peak, $t_R \approx 4.2$ min))/(AUC (t_R = 3~5 min)), where t_R represents the retention time, AUC represents the area under the curve of SEC-HPLC profiles.

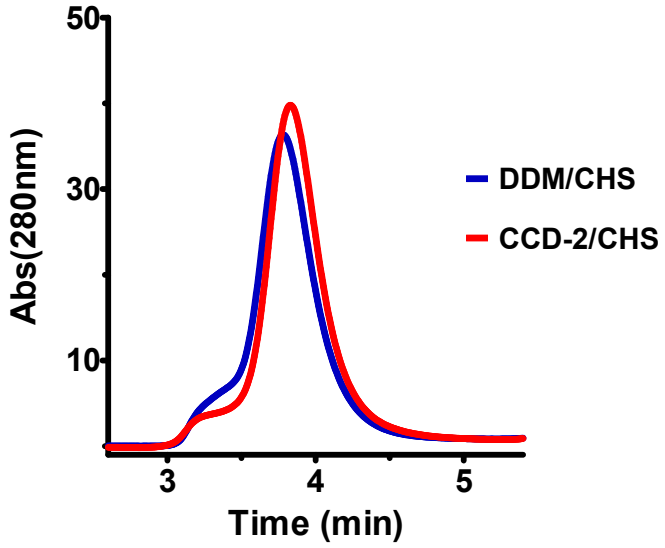


Figure S13. SEC-profiles of MBP-A_{2A}R-BRIL-ΔC purified by DDM/CHS or CCD-2/CHS. A_{2A}R was solubilized in 1% DDM or CCD-2 with the presence of 0.2% CHS. The solubilization mixture was then purified into 2.5 × CMC of corresponding detergents respectively. The chromatography was obtained with a flow at 0.5 mL/min from UV absorbance at 280 nm.

Table S4. The hydrodynamic diameter (D_h) (Mean ± S.D., n = 3) of nanodiscs by dynamic light scattering.

Groups		Physical method	Chemical method	No detergent
D_h (nm)	No protein	14.2 ± 1.7	14.5 ± 0.7	14.1 ± 1.0
	MBP-A2AR-BRIL-ΔC	36.5 ± 3.9	36.4 ± 1.4	

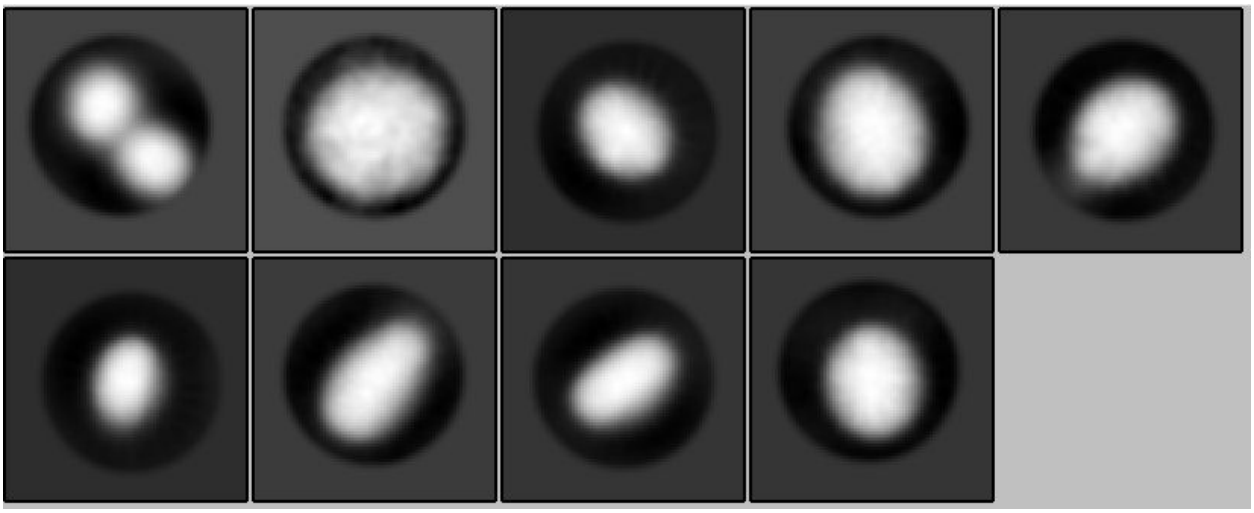
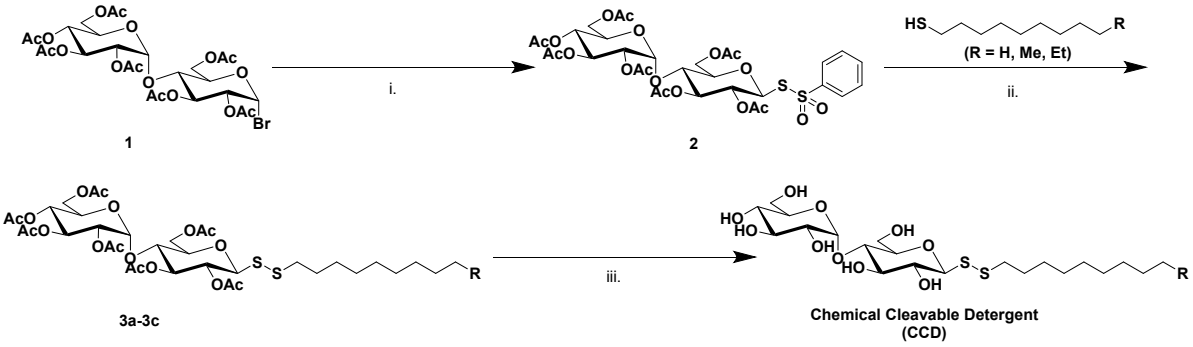


Figure S14. Representative classes of 2D classification of MBP-A_{2A}R-BRIL-ΔC in nanodiscs in different orientations.

General information for synthesis and characterization of compounds

All reagents and solvents were obtained from commercial sources (Adamas, Bide, Accela, J&K, Sigma-Aldrich etc.) and were used without further purification. Column chromatography was performed on silica gel 200-300 mesh. NMR spectra were recorded on a Bruker AVANCE III 500 or 800 spectrometer (FT, 500/800 MHz for ^{1}H NMR; 126/201 MHz for ^{13}C NMR) at room temperature with $CDCl_3$ or CD_3OD as the solvent. Chemical shifts were reported in units (ppm) by assigning $CDCl_3$ resonance in the ^{1}H spectrum as 7.26 ppm and CD_3OD resonance in the ^{1}H spectrum as 3.31 ppm, $CDCl_3$ resonance in the ^{13}C spectrum as 77.16 ppm and CD_3OD resonance in the ^{13}C spectrum as 49.00 ppm. All coupling constants (*J* values) were reported in Hertz (Hz). High-resolution mass spectra (HRMS) were recorded on an Agilent 6230 mass spectrometer using ESI (electrospray ionization).

Scheme S1. Synthesis of chemical cleavable detergents.



Reagents and conditions: i). sodium benzenethiosulphonate, tetrabutylammonium bromide, MeCN, 70 °C; ii). Et_3N, CH_2Cl_2, 0 °C to r.t.; iii). NaOMe, MeOH, r.t.

Synthesis of phenylthiosulfonate 2

Sodium benzenethiosulphonate (1.5 - 2 equiv.) and tetrabutylammonium bromide (0.1 equiv.) were added to the solution of peracetylated maltosylbromide **1** in anhydrous acetonitrile. The resulting mixture was stirred under nitrogen at 70 °C for 6 h. After TLC indicated the completion

of reaction, the solvent was removed by rotary evaporation. The residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/petrol) to afford desired product **2** as a white solid.

Compound **2** was prepared in 71 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.6, 1.4 Hz, 2H), 7.65 (tt, *J* = 7.5, 1.4 Hz, 1H), 7.56 (dd, *J* = 8.6, 7.2 Hz, 2H), 5.38 – 5.24 (m, 4H), 5.04 (t, *J* = 9.9 Hz, 1H), 4.86 (dd, *J* = 10.3, 9.0 Hz, 1H), 4.82 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.20 (ddd, *J* = 12.3, 4.8, 3.3 Hz, 2H), 4.06 (dd, *J* = 12.3, 3.8 Hz, 1H), 3.99 (dd, *J* = 12.5, 2.4 Hz, 1H), 3.97 – 3.86 (m, 2H), 3.70 (dt, *J* = 9.7, 3.4 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.70, 170.64, 170.32, 170.04, 169.97, 169.70, 169.53, 145.81, 134.19, 129.42, 127.13, 95.69, 86.41, 76.64, 75.85, 72.26, 70.10, 69.45, 69.25, 68.62, 68.02, 62.49, 61.51, 20.94, 20.92, 20.83, 20.82, 20.74, 20.72, 20.60. HRMS (ESI): calcd. for $C_{32}H_{40}O_{19}S_2$ $[M+Na]^+$ 815.1503, found 815.1498.

General experimental method for disulfide formation reaction

A solution of thiol (2 equiv.) in CH_2Cl_2 was added dropwise over 30 min to a solution of phenylthiosulfonate **2** and triethylamine (2 equiv.) in CH_2Cl_2 with stirring at 0 °C, after which the reaction mixture was allowed to warm to room temperature. After a further 2 h, TLC indicated the consumption of starting material. The solvent was removed in vacuo and the residue purified by flash chromatography (EtOAc/petrol) to give desired product **3** as a clear oil.

Compound **3a** was prepared in 84 % yield according to the general procedure for disulfide formation reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 (d, *J* = 4.0 Hz, 1H), 5.30 (dt, *J* = 17.8, 9.5 Hz, 2H), 5.09 (t, *J* = 9.6 Hz, 1H), 5.03 (t, *J* = 9.9 Hz, 1H), 4.83 (dd, *J* = 10.6, 4.0 Hz, 1H), 4.53 – 4.47 (m, 2H), 4.22 (dd, *J* = 12.5, 3.6 Hz, 1H), 4.17 (dd, *J* = 12.2, 4.2 Hz, 1H), 4.03 – 3.96 (m, 2H), 3.90 (dt, *J* = 10.3, 3.0 Hz, 1H), 3.68 (ddd, *J* = 9.8, 4.3, 2.6 Hz, 1H), 2.70 (qt, *J* = 12.7, 7.4 Hz, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (d, *J* = 4.3 Hz, 9H), 1.60 (ddt, *J* = 17.5, 12.2, 8.5 Hz, 2H), 1.32 – 1.20 (m, 12H), 0.84 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 170.63, 170.60, 170.34, 170.31, 170.01, 169.54, 169.49, 95.67, 87.41, 76.47, 76.36, 72.35, 70.03,

69.83, 69.34, 68.55, 67.98, 62.77, 61.46, 40.09, 31.92, 29.54, 29.31, 29.29, 28.96, 28.47, 22.73, 21.02, 20.87, 20.77, 20.74, 20.69, 20.67, 14.20. HRMS (ESI): calcd. for $C_{35}H_{54}O_{17}S_2$ $[M+Na]^+$ 833.2700, found 833.2694.

Compound **3b** was prepared in 73 % yield according to the general procedure for disulfide formation reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 (d, *J* = 4.0 Hz, 1H), 5.33 (dt, *J* = 18.5, 9.6 Hz, 2H), 5.11 (t, *J* = 9.6 Hz, 1H), 5.06 (t, *J* = 9.9 Hz, 1H), 4.85 (dd, *J* = 10.6, 4.0 Hz, 1H), 4.55 – 4.50 (m, 2H), 4.24 (dd, *J* = 12.5, 3.6 Hz, 1H), 4.20 (dd, *J* = 12.2, 4.2 Hz, 1H), 4.06 – 3.98 (m, 2H), 3.93 (dt, *J* = 10.2, 3.0 Hz, 1H), 3.75 – 3.67 (m, 1H), 2.73 (qt, *J* = 12.7, 7.4 Hz, 2H), 1.70 – 1.59 (m, 4H), 1.25 (s, 14H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 170.72, 170.70, 170.43, 170.39, 170.10, 169.63, 169.58, 95.75, 87.52, 76.55, 76.44, 72.43, 70.10, 69.92, 69.42, 68.62, 68.05, 62.84, 61.53, 40.18, 32.01, 29.68, 29.65, 29.44, 29.36, 29.03, 28.55, 22.81, 21.09, 20.94, 20.84, 20.80, 20.76, 20.74, 14.26. 14.20. HRMS (ESI): calcd. for $C_{36}H_{56}O_{17}S_2$ $[M+Na]^+$ 847.2857, found 847.2855.

Compound **3c** was prepared in 76 % yield according to the general procedure for disulfide formation reaction. ^{1}H NMR (500 MHz, $CDCl_3$) 1H NMR (500 MHz, Chloroform-d) δ 5.42 (d, *J* = 4.0 Hz, 1H), 5.37 – 5.27 (m, 2H), 5.11 (t, *J* = 9.5 Hz, 1H), 5.05 (t, *J* = 9.9 Hz, 1H), 4.85 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.55 – 4.49 (m, 2H), 4.24 (dd, *J* = 12.5, 3.7 Hz, 1H), 4.19 (dd, *J* = 12.2, 4.3 Hz, 1H), 4.06 – 3.98 (m, 2H), 3.92 (ddd, *J* = 10.3, 3.7, 2.4 Hz, 1H), 3.70 (ddd, *J* = 9.7, 4.2, 2.5 Hz, 1H), 2.72 (qt, *J* = 12.7, 7.3 Hz, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 6H), 1.99 (s, 3H), 1.63 (dtd, *J* = 15.0, 7.7, 2.2 Hz, 2H), 1.32 (q, *J* = 7.6 Hz, 2H), 1.24 (s, 14H), 0.86 (t, *J* = 6.9 Hz, 3H). 13C NMR (126 MHz, $CDCl_3$) δ 170.69, 170.66, 170.40, 170.37, 170.07, 169.60, 169.55, 95.74, 87.51, 76.53, 76.43, 72.44, 70.09, 69.91, 69.41, 68.62, 68.05, 62.83, 61.52, 40.17, 32.02, 29.72, 29.71, 29.64, 29.44, 29.35, 29.02, 28.53, 22.80, 21.07, 20.92, 20.82, 20.78, 20.74, 20.72, 14.25. HRMS (ESI): calcd. for $C_{37}H_{58}O_{17}S_2$ $[M+Na]^+$ 861.3013, found 861.3015.

Compound **3d** was prepared in 89 % yield according to the general procedure for disulfide formation reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41 (d, *J* = 4.0 Hz, 1H), 5.32 (dt, *J* = 18.8, 9.5 Hz, 2H), 5.10 (t, *J* = 9.6 Hz, 1H), 5.05 (t, *J* = 9.9 Hz, 1H), 4.85 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.56 – 4.48 (m, 2H), 4.21 (ddd, *J* = 22.1, 12.3, 3.9 Hz, 2H), 4.07 – 3.96 (m, 2H), 3.92 (dt, *J* = 10.3, 3.1

Hz, 1H), 3.70 (dt, *J* = 9.9, 3.4 Hz, 1H), 2.79 – 2.66 (m, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.00 (t, *J* = 5.5 Hz, 12H), 1.63 (p, *J* = 8.1 Hz, 2H), 1.24 (s, 18H), 0.86 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.62, 170.58, 170.32, 170.30, 169.99, 169.52, 169.48, 95.66, 87.38, 76.46, 76.35, 72.34, 70.02, 69.82, 69.33, 68.54, 67.97, 62.75, 61.45, 40.08, 31.96, 29.71, 29.68, 29.64, 29.58, 29.40, 29.29, 28.95, 28.47, 22.75, 21.01, 20.86, 20.76, 20.73, 20.69, 20.66, 14.21. HRMS (ESI): calcd. for $C_{38}H_{60}O_{17}S_2$ $[M+Na]^+$ 875.3170, found 875.3166.

General Procedure for the deacetylation reaction

The O-acetylated compounds **3** were dissolved in MeOH and then treated with NaOMe (30% in MeOH, 0.5 equiv.) The reaction mixture was left stirring for 6 h at room temperature, and then neutralized with Amberlite IR-120 (H^+ form) resin. The resin was removed by filtration and washed with MeOH and solvent was removed in vacuo. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$). Further purification carried out by Sephadex LH-20 chromatography (MeOH) afforded CCD as a white solid.

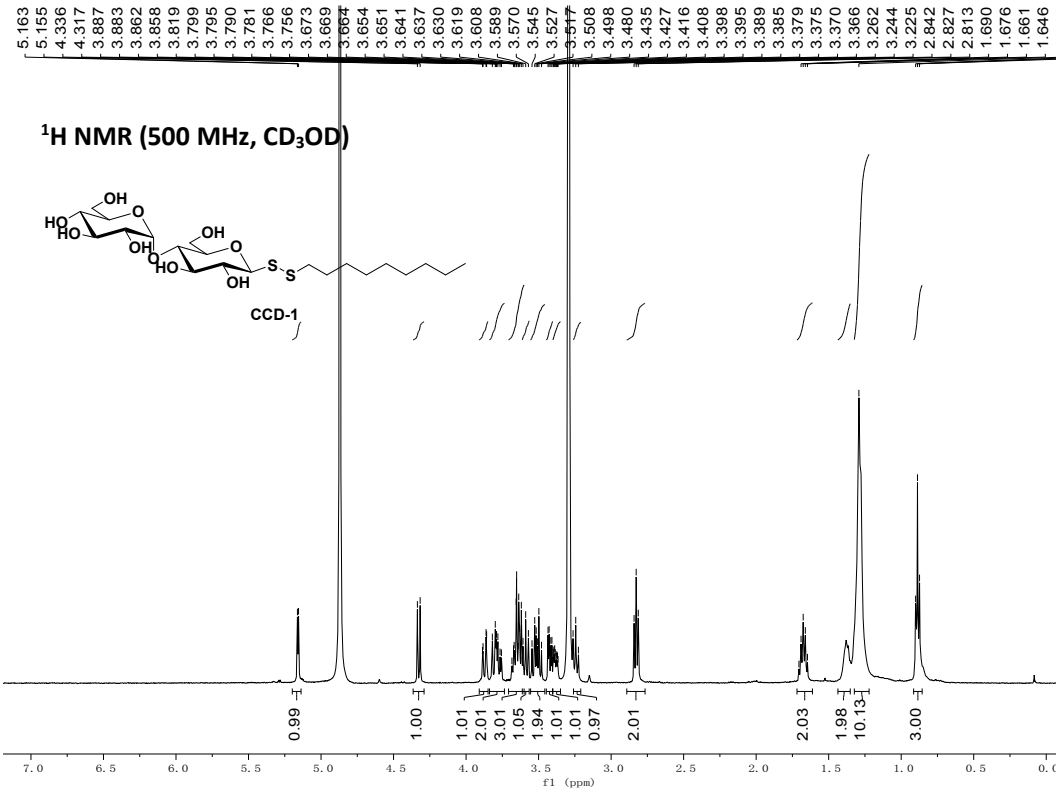
CCD-1 was prepared in 99 % yield according to the general procedure for deacetylation. ^{1}H NMR (500 MHz, CD_3OD) δ 5.16 (d, *J* = 3.8 Hz, 1H), 4.33 (d, *J* = 9.5 Hz, 1H), 3.87 (dd, *J* = 12.3, 2.0 Hz, 1H), 3.83 – 3.75 (m, 2H), 3.69 – 3.56 (m, 4H), 3.51 (dt, *J* = 14.2, 9.3 Hz, 2H), 3.42 (dd, *J* = 9.7, 3.7 Hz, 1H), 3.38 (ddd, *J* = 9.7, 4.7, 1.9 Hz, 1H), 3.24 (t, *J* = 9.2 Hz, 1H), 2.83 (t, *J* = 7.3 Hz, 2H), 1.68 (p, *J* = 7.3 Hz, 2H), 1.42 – 1.22 (m, 12H), 0.89 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 102.88, 92.39, 81.10, 80.90, 79.33, 75.08, 74.80, 74.20, 72.46, 71.48, 62.73, 62.43, 41.04, 33.08, 30.69, 30.43, 30.40, 30.21, 29.50, 23.76, 14.46. HRMS (ESI): calcd. for $C_{21}H_{40}O_{10}S_2$ $[M+Na]^+$ 539.1961, found 539.1958.

CCD-2 was prepared in 99 % yield according to the general procedure for deacetylation. ^{1}H NMR (500 MHz, CD_3OD) δ 5.16 (d, *J* = 3.8 Hz, 1H), 4.33 (d, *J* = 9.5 Hz, 1H), 3.87 (dd, *J* = 12.3, 2.0 Hz, 1H), 3.82 – 3.75 (m, 2H), 3.69 – 3.61 (m, 3H), 3.59 (t, *J* = 9.3 Hz, 1H), 3.51 (dt, *J* = 13.9, 9.3 Hz, 2H), 3.42 (dd, *J* = 9.7, 3.8 Hz, 1H), 3.38 (ddd, *J* = 9.7, 4.6, 1.9 Hz, 1H), 3.24 (t, *J* = 9.2 Hz, 1H), 2.83 (t, *J* = 7.3 Hz, 2H), 1.68 (p, *J* = 7.3 Hz, 2H), 1.41 – 1.24 (m, 14H), 0.89 (t, *J* = 6.8 Hz,

3H). ^{13}C NMR (126 MHz, CD_3OD) δ 102.86, 92.36, 81.08, 80.88, 79.31, 75.07, 74.78, 74.18, 72.44, 71.46, 62.72, 62.42, 41.03, 33.09, 30.72, 30.71, 30.48, 30.39, 30.20, 29.49, 23.75, 14.47. HRMS (ESI): calcd. for $C_{22}H_{42}O_{10}S_2$ $[M+Na]^+$ 553.2117, found 553.2113.

CCD-3 was prepared in 99 % yield according to the general procedure for deacetylation. ^{1}H NMR (500 MHz, CD_3OD) δ 5.14 (d, *J* = 3.8 Hz, 1H), 4.31 (d, *J* = 9.5 Hz, 1H), 3.86 (dd, *J* = 12.3, 2.0 Hz, 1H), 3.81 – 3.73 (m, 2H), 3.67 – 3.60 (m, 3H), 3.57 (t, *J* = 9.4 Hz, 1H), 3.50 (dt, *J* = 13.8, 9.4 Hz, 2H), 3.41 (dd, *J* = 9.7, 3.7 Hz, 1H), 3.37 (ddd, *J* = 9.7, 4.7, 1.9 Hz, 1H), 3.23 (t, *J* = 9.2 Hz, 1H), 2.81 (t, *J* = 7.3 Hz, 2H), 1.66 (p, *J* = 7.3 Hz, 2H), 1.37 (q, *J* = 7.2 Hz, 2H), 1.27 (d, *J* = 4.4 Hz, 14H), 0.87 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (126 MHz, CD_3OD) δ 102.81, 92.30, 81.02, 80.82, 79.27, 75.03, 74.73, 74.14, 72.41, 71.43, 62.69, 62.40, 41.01, 33.06, 30.75, 30.73, 30.70, 30.47, 30.37, 30.18, 29.48, 23.73, 14.47. HRMS (ESI): calcd. for $C_{23}H_{44}O_{10}S_2$ $[M+Na]^+$ 567.2274, found 567.2271.

CCD-4 was prepared in 99 % yield according to the general procedure for deacetylation. ^{1}H NMR (800 MHz, CD_3OD) δ 5.20 (d, *J* = 3.8 Hz, 1H), 4.36 (d, *J* = 9.5 Hz, 1H), 3.91 (dd, *J* = 12.3, 2.0 Hz, 1H), 3.84 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.81 (dd, *J* = 12.3, 4.7 Hz, 1H), 3.72 – 3.66 (m, 3H), 3.63 (t, *J* = 9.3 Hz, 1H), 3.56 (t, *J* = 9.3 Hz, 1H), 3.54 (t, *J* = 9.3 Hz, 1H), 3.46 (dd, *J* = 9.7, 3.8 Hz, 1H), 3.42 (ddd, *J* = 9.7, 4.7, 1.9 Hz, 1H), 3.28 (t, *J* = 9.4 Hz, 1H), 2.86 (t, *J* = 7.3 Hz, 2H), 1.71 (p, *J* = 7.4 Hz, 2H), 1.41 (p, *J* = 7.2 Hz, 2H), 1.37 – 1.26 (m, 16H), 0.92 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (201 MHz, CD_3OD) δ 102.86, 92.37, 81.10, 80.90, 79.33, 75.09, 74.79, 74.20, 72.48, 71.51, 62.74, 62.45, 41.06, 33.07, 30.79, 30.76, 30.72, 30.69, 30.47, 30.37, 30.20, 29.49, 23.73, 14.44. HRMS (ESI): calcd. for $C_{24}H_{46}O_{10}S_2$ $[M+Na]^+$ 581.2430, found 581.2426。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3.517
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3.379
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3.370
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3.262
3.244
3.225
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1.676
1.661
1.646
1H NMR (500 MHz, CD3OD)
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1.01
2.01
3.01
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0.97
2.01
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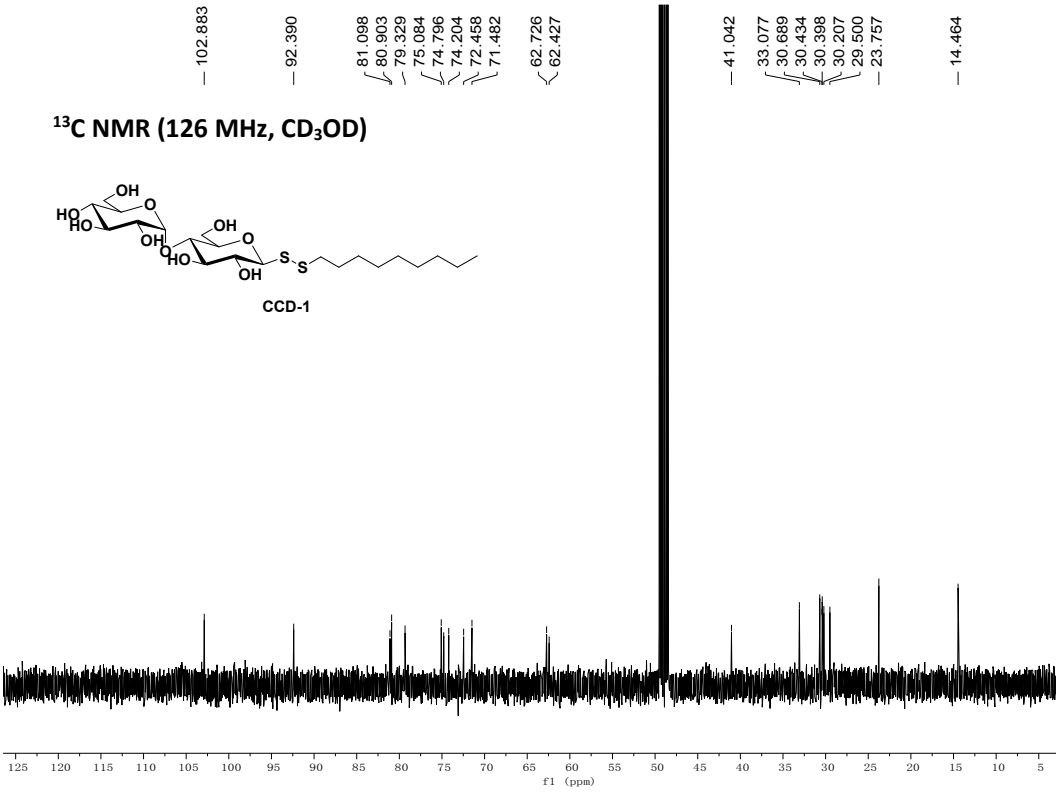
10.13
3.00
f1 (ppm)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33.077
30.689
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30.207
29.500
23.757
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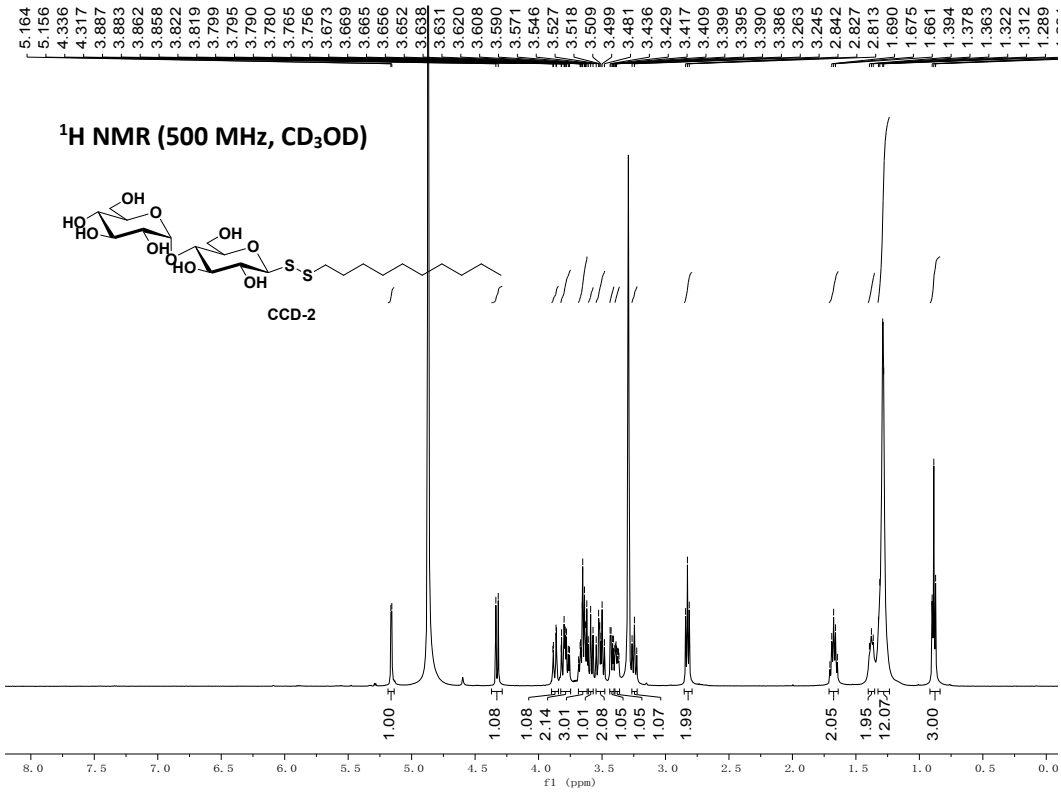
13C NMR (126 MHz, CD3OD)
CCD-1
f1 (ppm)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3.765
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3.656
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3.638
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3.509
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3.436
3.429
3.417
3.409
3.399
3.395
3.390
3.386
3.263
3.245
2.842
2.827
2.813
1.690
1.675
1.661
1.394
1.378
1.363
1.322
1.312
1.289
1H NMR (500 MHz, CD3OD)
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1.08
2.14
3.01
1.01
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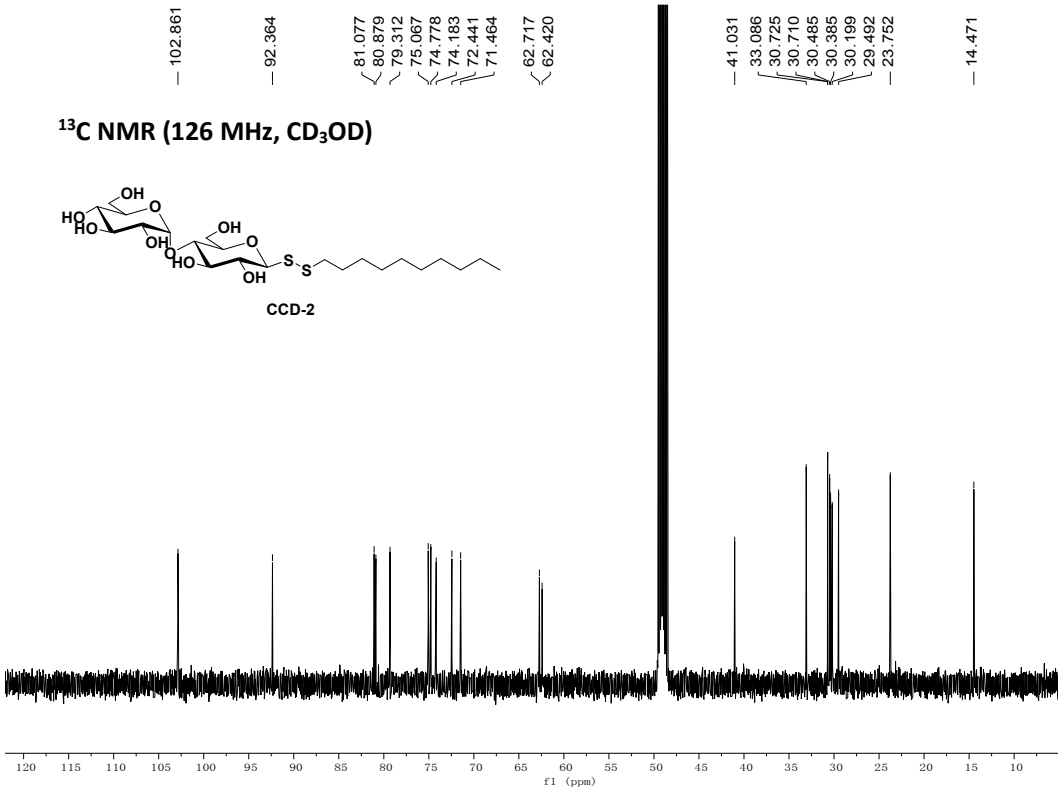
1.05
1.05
1.07
1.99
2.05
1.95
12.07
3.00
f1 (ppm)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33.086
30.725
30.710
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23.752
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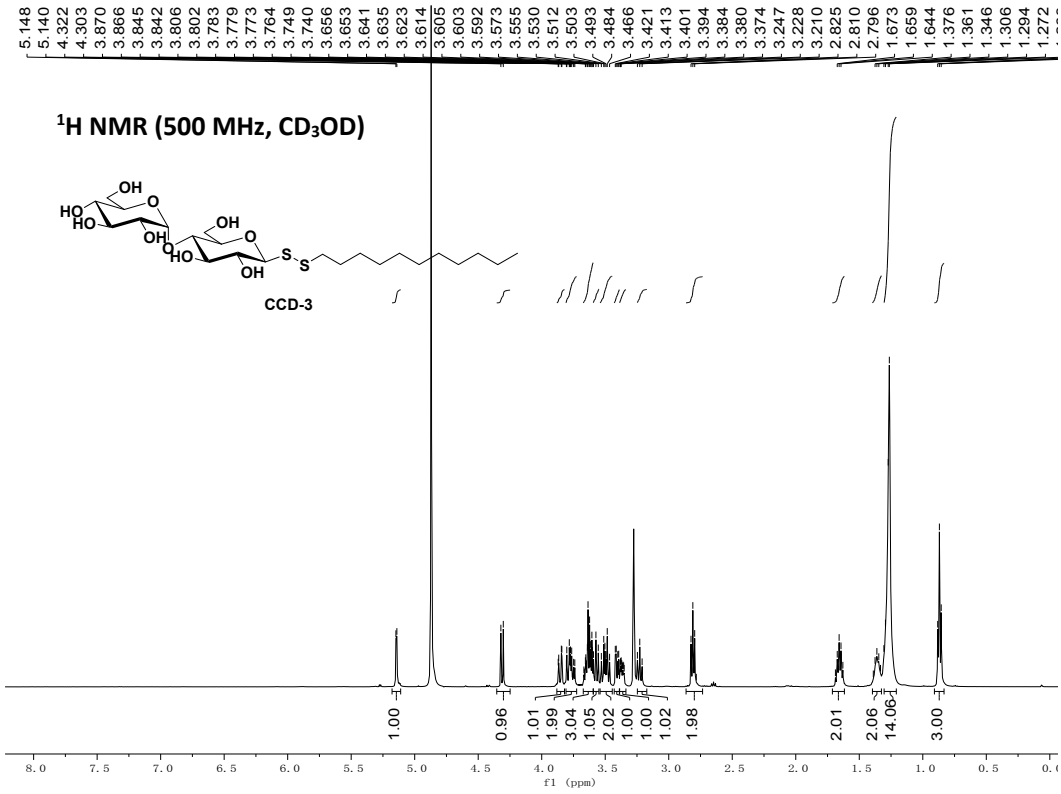
13C NMR (126 MHz, CD3OD)
CCD-2
f1 (ppm)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3.653
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3.635
3.623
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1.644
1.376
1.361
1.346
1.306
1.294
1.272
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1.01
1.99
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1.00
1.00
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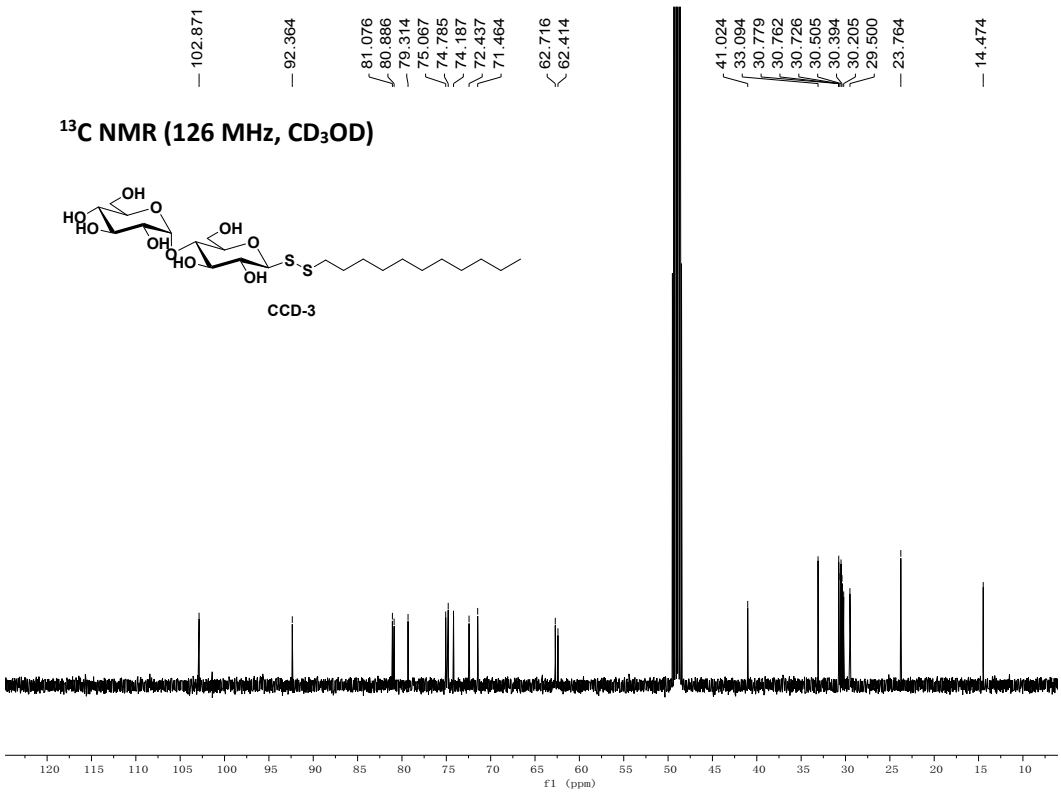
14.06
3.00
f1 (ppm)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30.779
30.762
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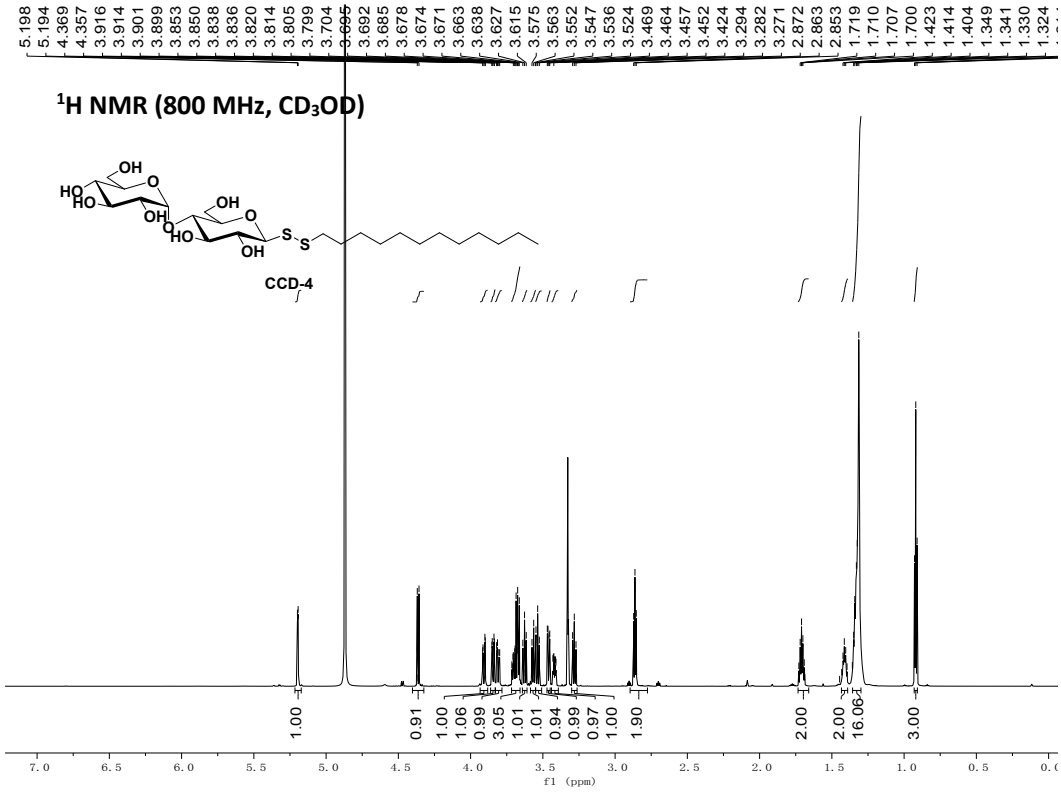
13C NMR (126 MHz, CD3OD)
CCD-3
f1 (ppm)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3.674
3.671
3.663
3.638
3.627
3.615
3.575
3.563
3.552
3.547
3.536
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3.464
3.457
3.452
3.424
3.294
3.282
3.271
2.872
2.863
2.853
1.719
1.710
1.707
1.700
1.423
1.414
1.404
1.349
1.341
1.330
1.324
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CCD-4
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1.00
1.06
0.99
3.05
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1.01
0.94
0.99
0.97
1.00
1.90
2.00
2.00
16.06
3.00
7.0
6.5
6.0
5.5
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4.0
3.5
3.0
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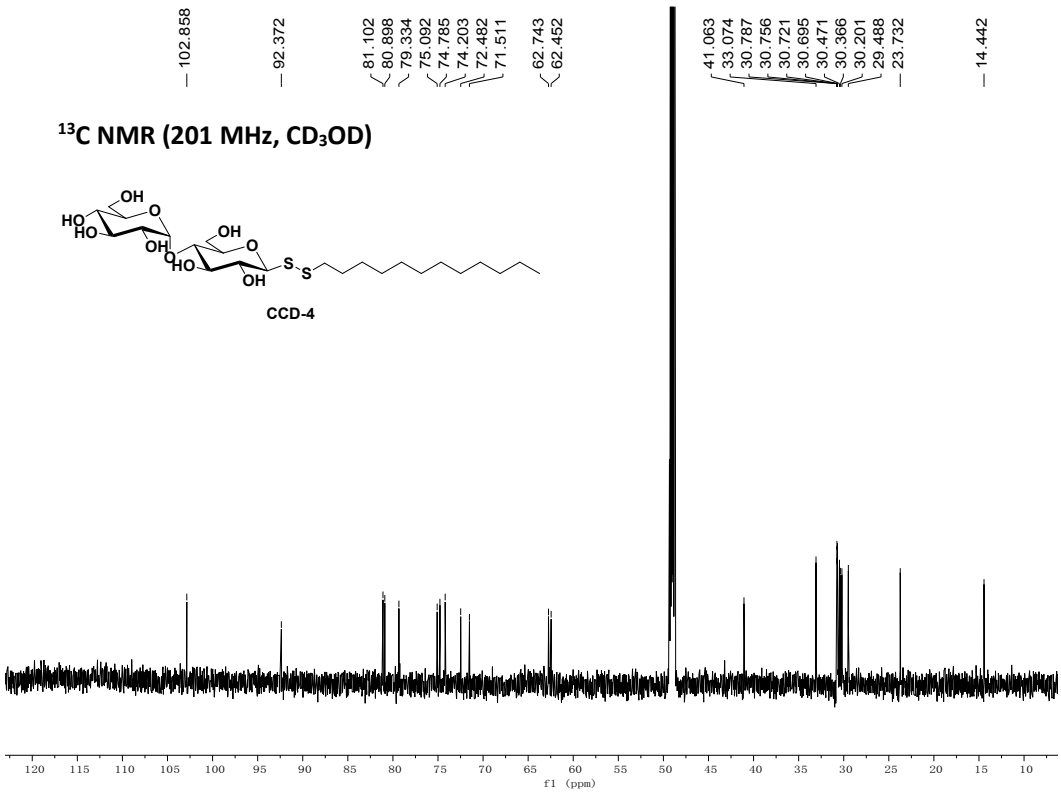
1.5
1.0
0.5
f1 (ppm)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33.074
30.787
30.756
30.721
30.695
30.471
30.366
30.201
29.488
23.732
14.442
13C NMR (201 MHz, CD3OD)
CCD-4
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
f1 (ppm)