Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Modified Cyclodextrins Solubilize Elemental Sulfur in Water and Enable Biological Sulfane Sulfur Delivery

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Experimental Section

Materials and Methods

Reagents were purchased from Fisher, Oakwood, and Tokyo Chemical Industry (TCI) and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories. ^{31}P NMR spectra were recorded on a Bruker 500 MHz instrument. UV-vis spectra were recorded on an Agilent Cary 100 UV-vis spectrophotometer under standard conditions. Air-free techniques were performed under an inert N_2 atmosphere using Schlenk technique or in an Innovative Atmospheres glove box.

Procedures

CD/S_8 Complex Formation. Different weight percentages (0-45%) of 2-hydroxypropyl β cyclodextrin (2HPβ) were added to pH 7.4 PBS buffer prepared from Millipore tablets in nanopure water in a stirred scintillation vial. A 10x molar equivalent of solid elemental sulfur (S_8) (Fisher) was added to this solution and shaken by hand until mixed. The vial was then stirred for several days at room temperature. After stirring, the solutions were taken up in plastic syringes and filtered through syringe filters (0.45 or 0.2 μm) to yield a clear solution that is colorless at low sulfur/cyclodextrin (CD) concentrations and yellow at higher concentrations. The S_8 concentration of each solution was quantified by UV-vis spectrophotometry in quartz cuvettes using the extinction coefficient of S_8 in methanol (λ_{max} = 263 nm, ε = 6730 $M^{-1}cm^{-1}$).

S_8 Absorbance in MeOH versus buffer. To measure the absorbance of S_8 in methanol, 1.0 mg of S_8 was added to 7.0 mL of MeOH and shaken vigorously. The solution was then filtered into a quartz cuvette and the S_8 concentration was measured using the UV-vis absorbance at 263 nm. A stock solution of 2HPβ/S_8 in water was diluted to the same S_8 concentration and also measured by UV-vis. Both spectra were then overlaid to determine the percent difference of the S_8 concentration value.

Comparison of S_8 Solvation Between 2HPβ and Other Saccharides. Scintillations vial were charged with 640 mg S_8 followed by 10 mL of pH 7.4 PBS buffer or 10 mL of pH 7.4 PBS buffer containing 365 mg of either 2HPβ, hydroxypropyl cellulose (HPC), or glucose. The vials were sealed and stirred for three days, after which they were filtered and analyzed by UV-vis. Similar experiments were also performed with a 1.56% w/w solution of the parent non-functionalized β-CD in 8 mL pH 7.4 PBS buffer and 100 mg S_8. The resultant heterogenous solution was stirred for one month, filtered, and measured as described above. To evaluate S_8 solubilization in 2-hydroxypropyl γ-CD (2HPγ), a 25% w/w solution of 2HPγ or 2HPβ was prepared in 7.5 mL water or pH 7.4 PBS buffer. To each solution, 200 mg S_8 was added and solutions were stirred for two weeks, after which they were filtered and analyzed as described above.

Measurement of Binding Affinity of Elemental Sulfur to 2HPβ. Solutions of 2HPβ or other CDs of increasing weight percentage from 0 to 45% in pH 7.4 PBS were prepared from a 50% w/w stock solution of 2HPβ. To each of these vials were added 10-fold molar equivalents of S_8, and the resultant solutions were stirred for several days. After stirring, the solutions were filtered and the S_8 content quantified as described above. The measured S_8 concentrations from each sample were plotted against the 2HPβ concentration in a log-log plot described by equation (1). The apparent

K_a value was obtained by linear regression analysis using Microsoft Excel's LINEST function. These experiments were performed in triplicate.

^{31}P NMR Spectroscopy of Trapped Sulfur. NMR tubes were charged with 25% w/w 2HPβ/S_8 solutions in pH 7.4 PBS buffer and ^{31}P NMR spectra were recorded, after which a 30-fold molar excess of TCEP was added. The NMR tubes were incubated overnight at room temperature, after which additional ^{31}P NMR spectra were recorded. The presence of reductant-labile sulfur was characterized by the formation of TCEP oxidation products, including TCEP sulfide or TCEP oxide formed by hydrolysis. The parent TCEP peak is at $\delta = 15.2\text{-}15.8$ ppm, and the oxidized oxygen-containing and sulfur-containing product peaks are at $\delta = 53.0$ (P=O) and 51.5 (P=S) ppm, respectively. To ensure that 2HPβ was not causing TCEP oxidation directly, the above experiments were performed with a solution containing only 25% w/w 2HPβ in pH 7.4 PBS and no oxidized TCEP products were observed.

Evaluating Sulfur Content of Liquid and Precipitated 2HPβ/S_8 Complex. Two scintillation vials were charged with 25% w/w 2HPβ and a 10-fold excess of S_8 in 10 mL pH 7.4 PBS buffer and stirred overnight. One solution was filtered and evaluated as described above. The other solution was filtered into a recrystallization dish filled with an excess of acetone, which results in formation of a white precipitate. The solvent was evaporated, and the resultant solid was collected and used to make a new 25% w/w solution, which was analyzed by UV-vis spectroscopy as described above.

Measurement of H_2S Release from 2HPβ/S_8 Complex in the Presence of Biological Thiols. A round bottom flask was charged with 40 mL of degassed pH 7.4 PBS buffer, a stir bar, and either 1 mM L-cysteine or reduced GSH in an N_2-filled glove box. The solutions were removed from the glovebox and 500 μL of 2.0 mM S_8 in 50% w/w 2HPβ (for a final S_8 concentration of 25 μM) was added by syringe. For each time point, a 500 μL aliquot was removed by syringe and added to 500 μL of the methylene blue cocktail in a 1.5 mL plastic cuvette. (Methylene blue cocktail contains: 200 μL 30 mM $FeCl_3$ in 1.2 M HCl, 200 μL of 20 mM *N,N*-dimethyl-*p*-phenylene diamine in 7.2 M HCl, and 100 μL of 1% (w/v) $Zn(OAc)_2$). The methylene blue reactions were incubated at room temperature for 1 hour, after which the absorbance at 670 nm was measured. For control experiments, the above procedure was repeated but with either 0.5 mg S_8 (50 μM if fully soluble) or 0.9% w/w 2HPβ. All time points were collected in triplicate.

Calibration Curve for Sulfide Measurement. Solutions containing 500 μL of the methylene blue cocktail and 0.5 mL PBS containing 0.9% w/w 2HPβ were prepared in plastic cuvettes. A 100 mM stock solution of NaSH was prepared in 1.0 mL PBS under inert atmosphere. Solution was then diluted to 1 mM and added to the 1.0 mL solutions for final concentrations of 10, 20, 30, 40, 50, and 70 μM. The cuvettes were incubated at room temperature for 1 hour and absorbance at 670 nm was measured. All data points were measured in triplicate

Fluorescent S^0 Imaging. HeLa human cervical cancer cells (ATCC CCL-2) were cultured in DMEM containing phenol red, 10% premium grade fetal bovine serum (FBS), and 1% penicillin-streptomycin (PS) (10,000 units/mL penicillin and 10,000 μg/mL streptomycin). Cells were maintained at 37 ˚C under 5% CO_2. Cells were seeded in glass-bottomed 2 mL imaging dishes (MatTek) in DMEM containing 10% FBS, 1% PS and incubated overnight. The next day dishes were rinsed twice with FBS-free DMEM and incubated with test substances (solvated in PBS) in FBS-free DMEM for 24 hours. 2HPβ and 2HPβ/S_8 stock solutions both contained 50% w/w 2HPβ, with equal volumes added to control and test dishes respectively. K_2S_5 was solvated in a ~1 mg/mL stock solution in water, and 10 μL were added to positive control dish. After treatment, dishes were again rinsed twice with FBS-free DMEM and incubated with NucRed and 10 μM SSP4 (Dojindo) separately in FBS-free DMEM according to manufacturer instructions for 15 minutes each. The NucRed (Invitrogen) concentration was two drops per 10 mL FBS-free DMEM. Dishes were rinsed twice with FluoroBrite DMEM (Thermo Fisher), and then imaged in this media. Image workup done with FIJI. 2HPβ/S_8 SSP4 image normalized to 2HPβ only image, where this image's intensity was turned down to background, and these identical settings applied to the 2HPβ/S_8 image.

Griess Assay. RAW 264.7 murine macrophage cells (ATCC TIB-71) were cultured in DMEM containing phenol red, 10% premium grade fetal bovine serum (FBS), and 1% penicillin-streptomycin (PS) (10,000 units/mL penicillin and 10,000 μg/mL streptomycin). Cells were maintained at 37 ˚C under 5% CO_2. For the Griess assay, RAW 264.7 cells were seeded in sterile 12 well culture plates in DMEM containing 10% FBS, 1% PS and incubated overnight. The next day, the media was removed, and the cells were washed with PBS. The media was replaced with media containing either the 2HPβ/S_8 complex or an equivalent w/w percentage of 2HPβ in FBS-free, phenol red-free DMEM. After a 24-hour incubation, cells are again rinsed and media is replaced with FBS-free, phenol red-free DMEM containing 1 μg/mL lipopolysaccharide (LPS). After another 24-hour incubation, 150 μL of media from each well was pipetted into a 96 well plate containing 20 μL mixed Griess reagent (Invitrogen) and then mixed with 150 μL DI water. After incubation at room temperature for 30 minutes, plates were analyzed on a plate reader (BioTek Synergy 2)

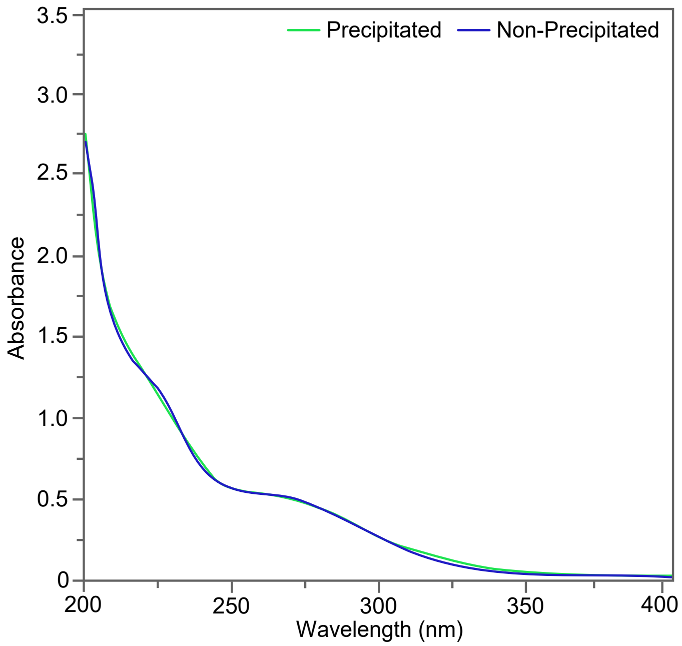

Figure S1. UV-vis spectra of 2HPβ/S_8 solutions in pH 7.4 PBS that have been precipitated (cyan) or not precipitated (blue) before scanning. When precipitated solutions are reconstituted to the same w/w percentage, the [S_8] does not change.

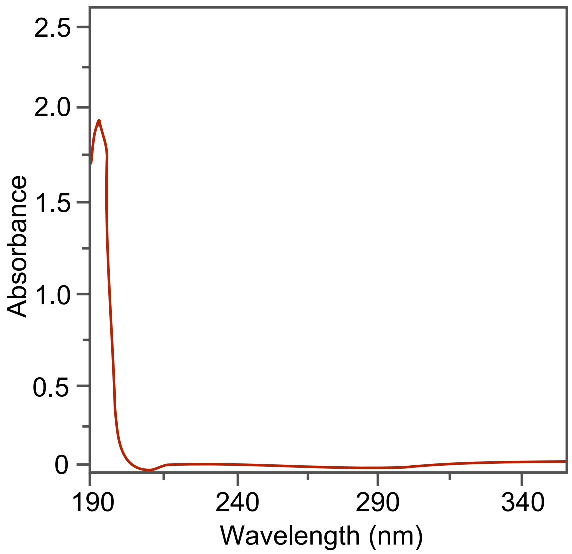

Figure S2. UV-vis spectrum of βCD treated with S_8 in pH 7.4 PBS. No S_8 was observed in solution based on the lack of an absorbance peak at 263 nm.

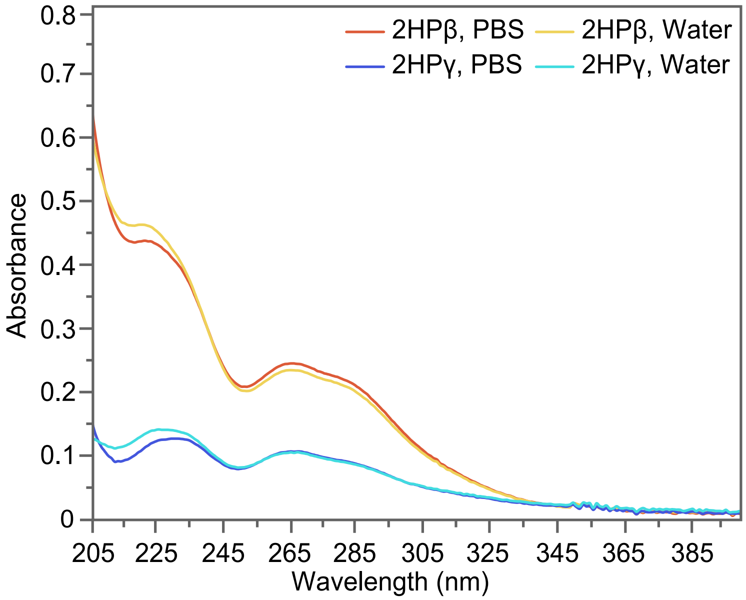

Figure S3. UV-vis spectra of S_8 with 2HPβ (yellow, orange) and 2HPγ (cyan, blue) in water and PBS. 2HPβ demonstrates superior S_8 solvation ability over 2HPγ.

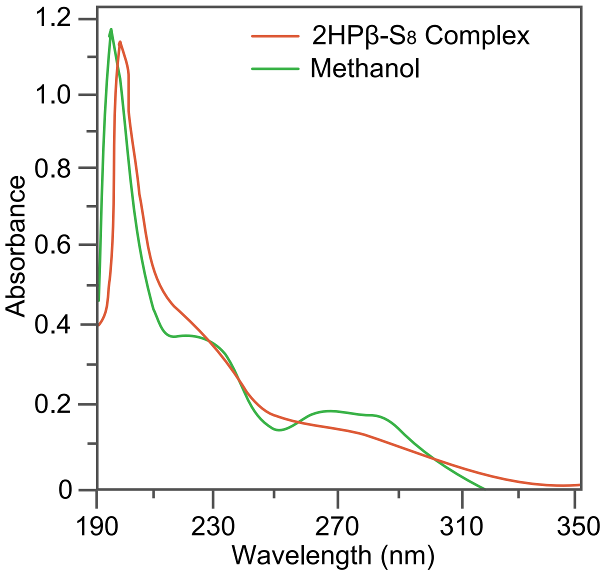

Figure S4. UV-vis spectra of equimolar concentrations of S_8 solvated in methanol (green) and 2HPβ in pH 7.4 PBS (orange). There is a 12% difference in the absorbance at 263 nm, suggesting that at most the extinction coefficient differs in these two solvents by 12%, and that the MeOH extinction coefficient for S_8 in MeOH can be used to approximate the amount of S_8 in the 2HPβ systems.

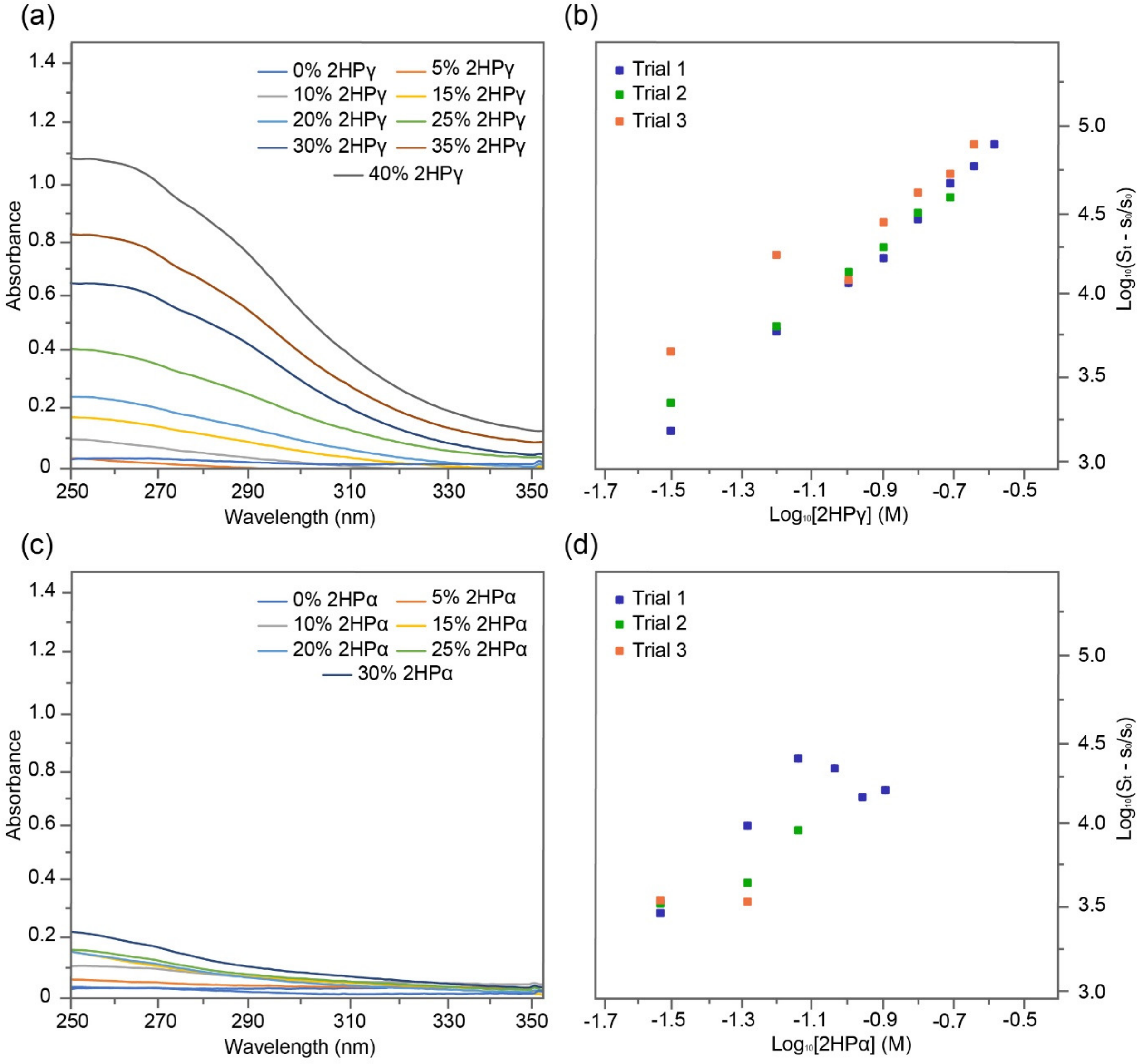

Figure S5. UV-vis spectra of S_8 binding to (a) 2HPγ and (c) 2HPα, log-log plots (b) and (d) demonstrate the binding relationship of 2HPγ and 2HPα respectively. By comparison to 2HPβ, less S_8 is solubilized in 2HPγ and 2HPα. Similarly, the plots in (b) and (d) are significantly less linear than the corresponding plot for 2HPβ (Figure 4b), suggesting that a more complex binding relationship is present.

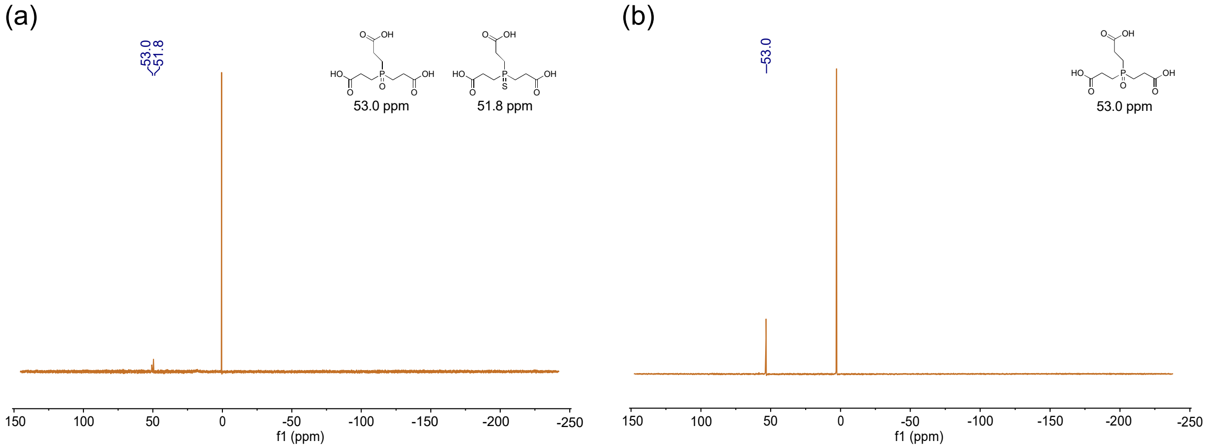

Figure S6. $^{31}P\{^{1}H\}$ NMR spectra of (a) S_8 in MeOD and (b) K_2S_5 in D_2O demonstrating the formation of oxidized TCEP product peaks.

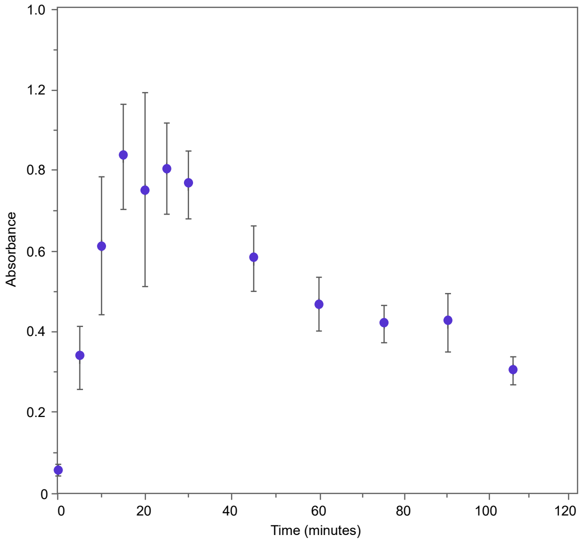

Figure S7. Methylene blue curve of H_2S release from cysteine and solid 2HPβ and S_8 added separately rather than pre-solvated in a 2HPβ- S_8 complex. These data demonstrate that the 2HPβ-S_8 complex does not need to be pre-formed for efficient reduction of S_8 to H_2S by cysteine.

% CD	Absorbance (263 nm)	$[S_8]$ (mM)
0	0.012	0.01
5	0.106	0.16
10	0.212	0.32
15	0.282	0.42
20	0.422	0.62
25	0.605	0.90
30	0.717	1.06
35	0.991	1.47
40	1.140	1.69
45	1.286	1.91

Table S1. Tabulated UV-vis spectral data for solubilized S_8 with increasing concentrations of 2HPβ with a 10-fold excess of S_8. UV-vis spectra are shown in Figure 4a.