

Sulfated Prism[6]arene as Water-Soluble Host for Molecular Recognition of Dye Pollutants in Aqueous Environments

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Supporting Information

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Copies of NMR and HR Mass Spectra of Sulfated-Prism[6]arene

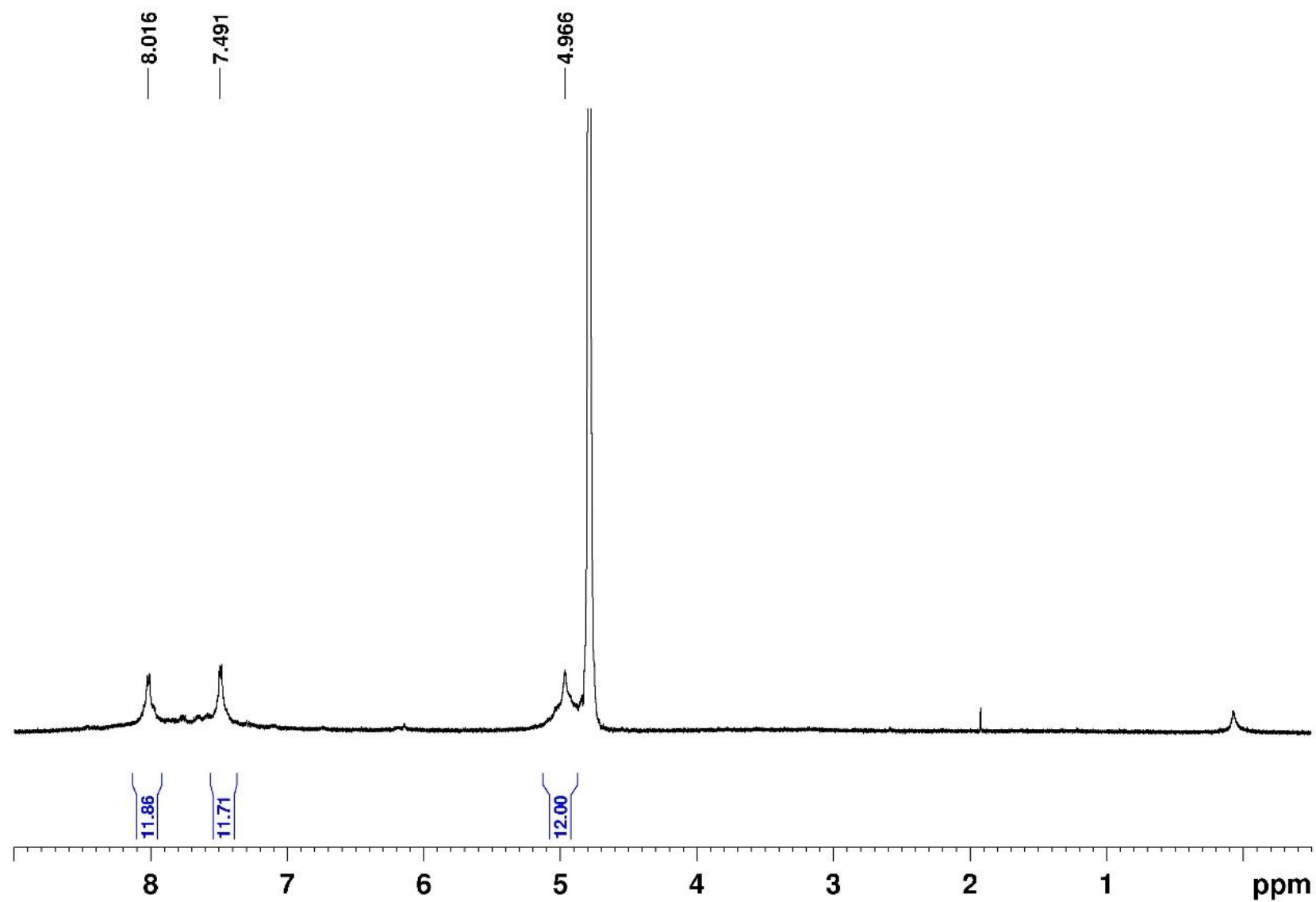


Figure S1. ^1H NMR spectrum of $\text{PrS[6]}^{\text{SO}_3\text{Na}}$ (D_2O , 600 MHz, 298 K).

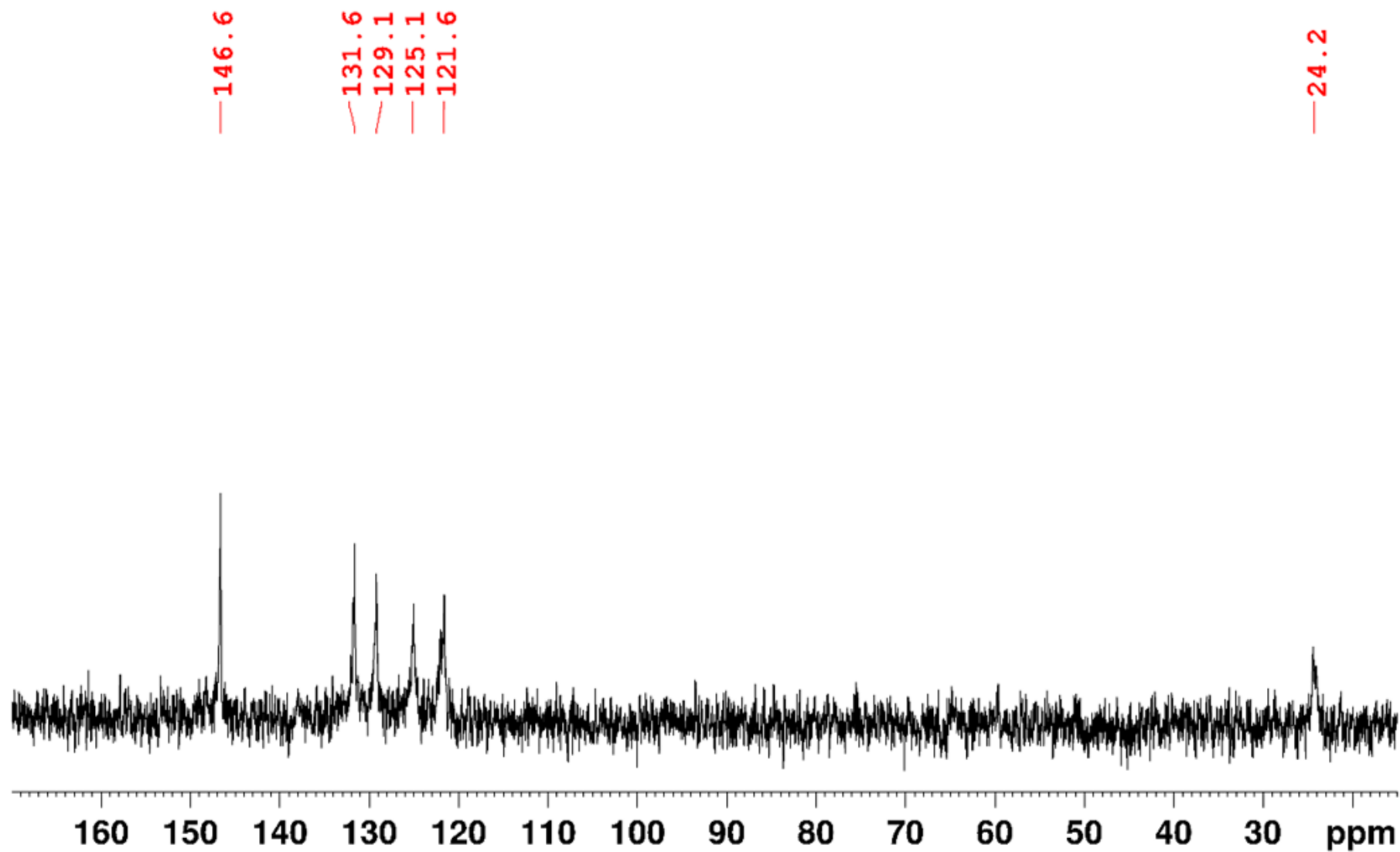


Figure S2. $\{^1\text{H}\} \text{ }^{13}\text{C}$ NMR spectrum of **PrS[6]^{SO₃Na}** (D_2O , 62.5 MHz, 298 K).

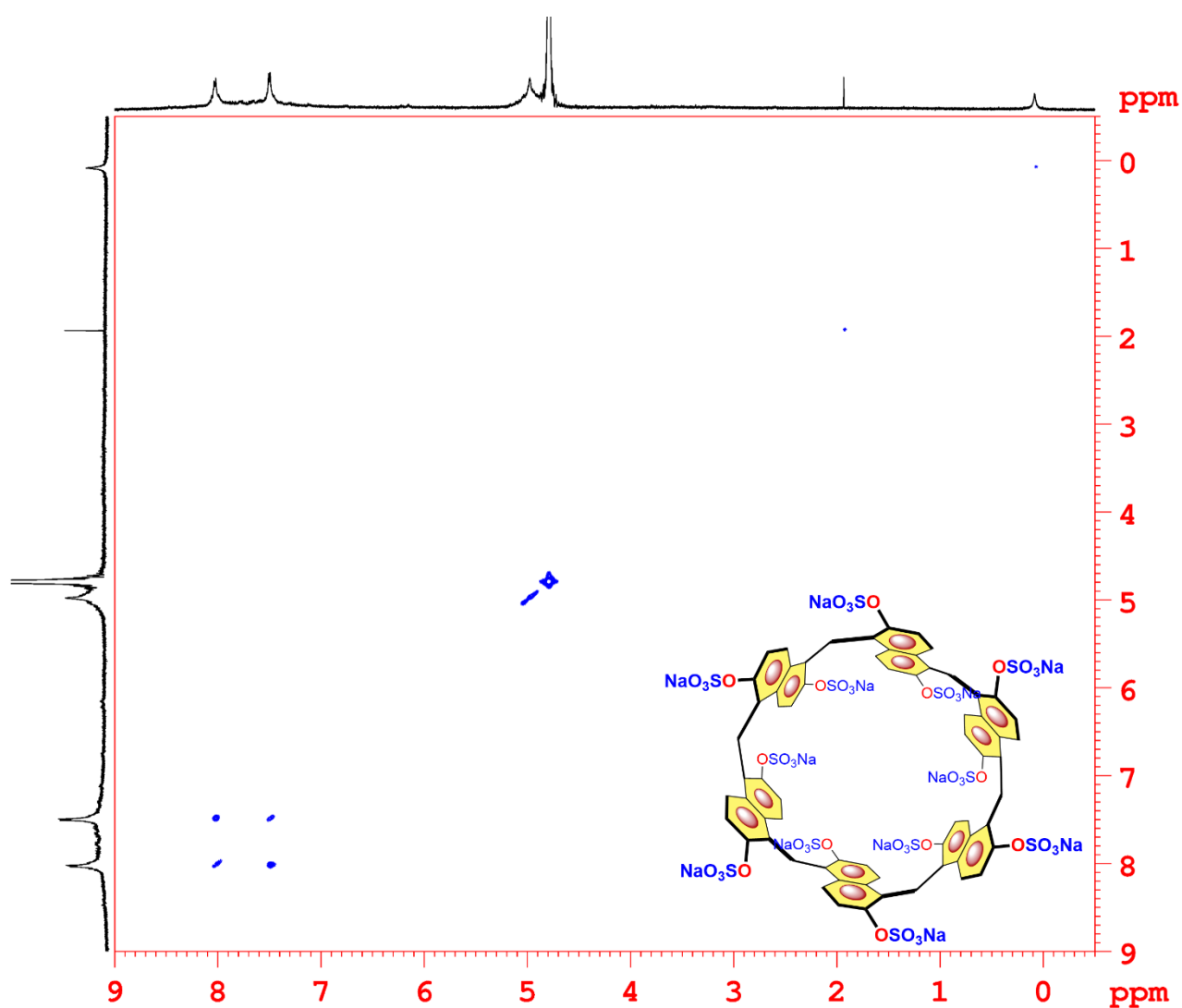


Figure S3. 2D-DQF COSY spectrum of $\text{PrS[6]SO}_3\text{Na}$ (D_2O , 600 MHz, 298 K).

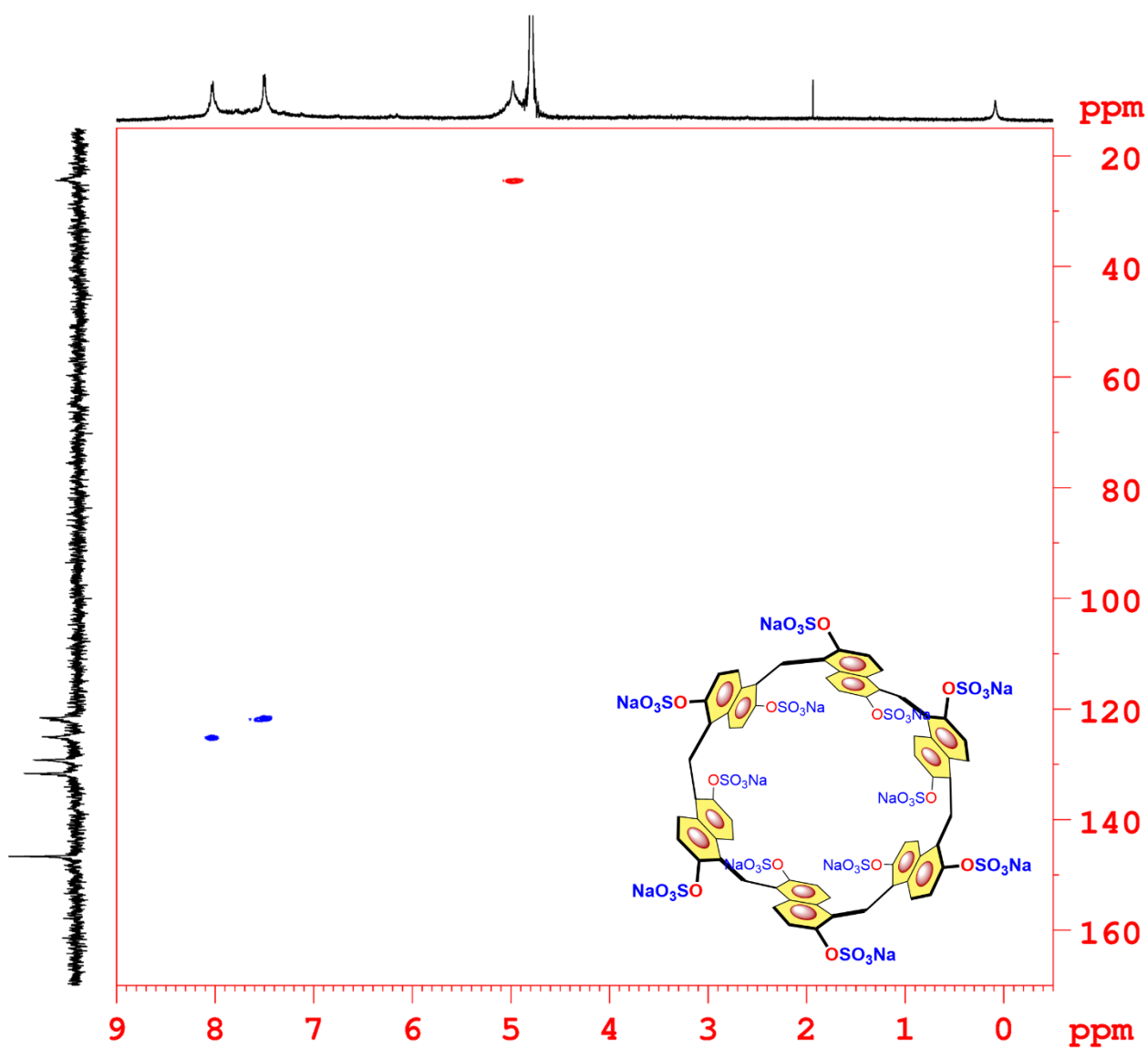


Figure S4. 2D-HSQC spectrum of $\text{PrS}[6]\text{SO}_3\text{Na}$ (D_2O , 600 MHz, 298 K).

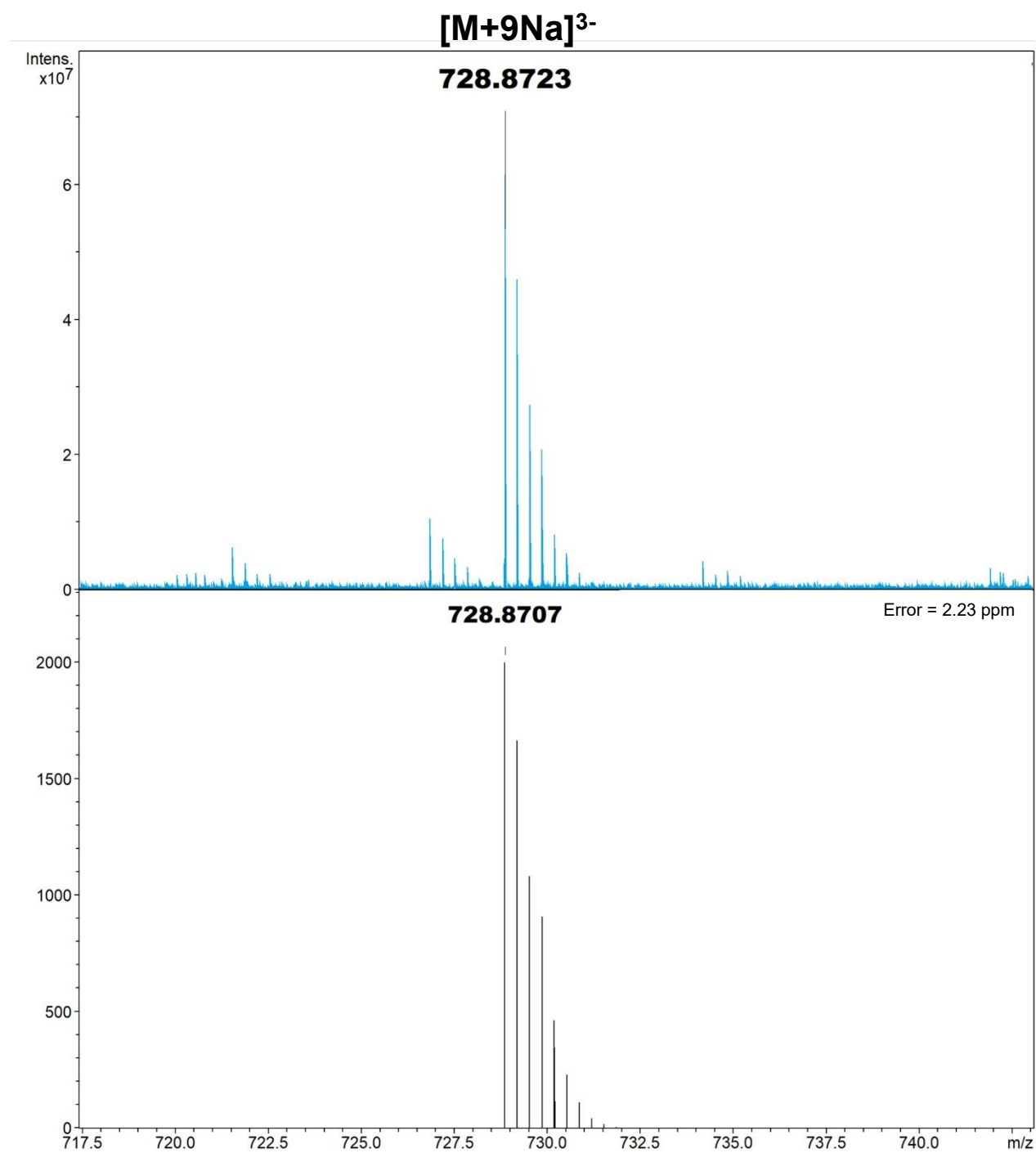


Figure S5. Comparison of experimental (top) and simulated (bottom) HR FT-ICR mass spectra of **PrS[6]^{SO3Na}**.

¹H NMR Studies on the Complexation of PrS[6]^{SO₃⁻} with RhodB and MethB

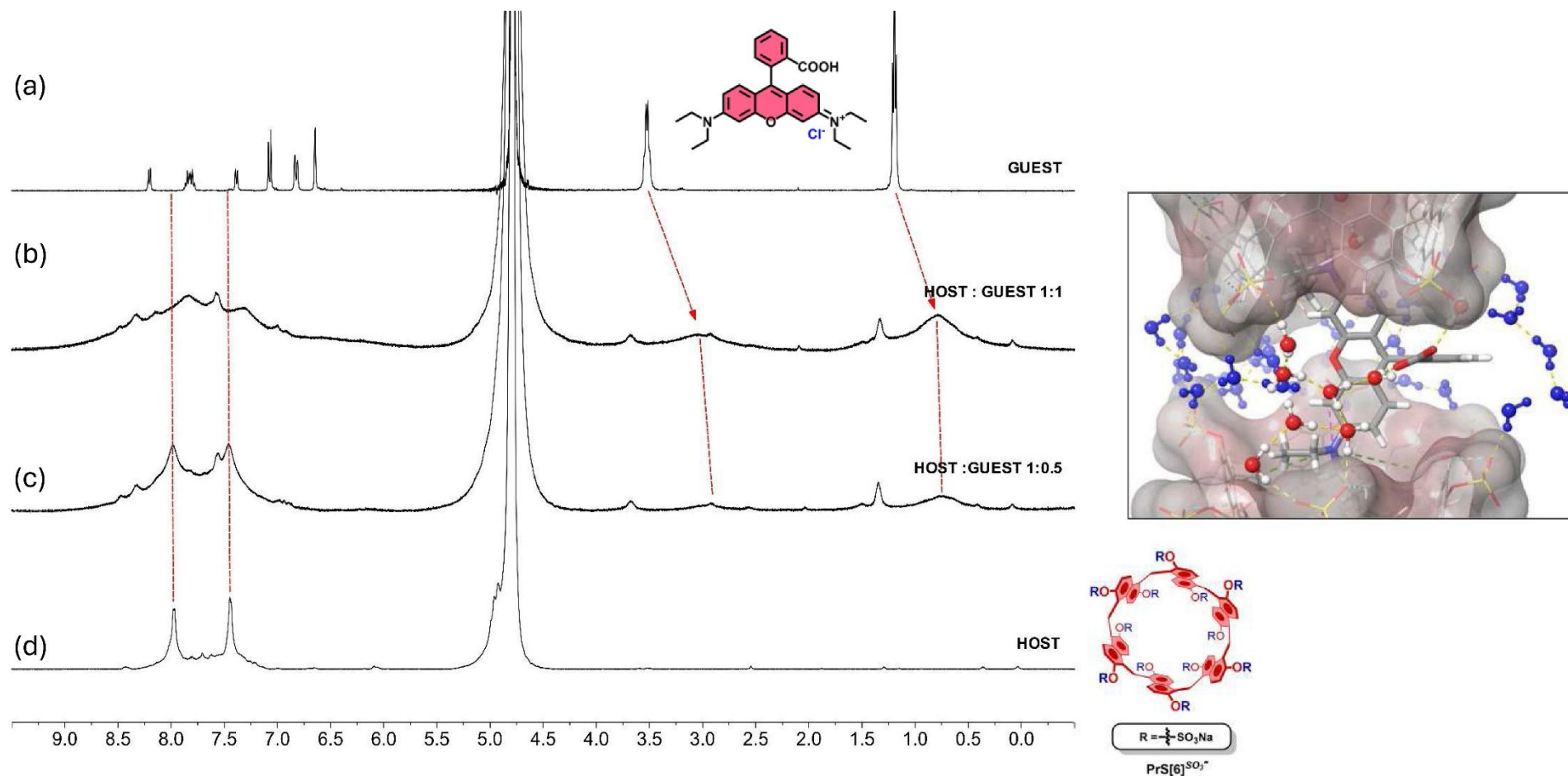


Figure S6. ¹H NMR spectra (D₂O, 400 MHz, 298 K) of: (a) **RhodB**, (b) an equimolar solution (3 mM) of **PrS[6]^{SO₃⁻}** and **RhodB**, (c) a 1:0.5 solution of **PrS[6]^{SO₃⁻}** (3 mM) and **RhodB** (1.5 mM). (d) **PrS[6]^{SO₃⁻}**. Inset: Detail of the dimeric capsule **RhodB**@(**PrS[6]^{SO₃⁻}**)₂ obtained by molecular dynamics simulations (see page S14), showing the inclusion of N(CH₂CH₃)₂ groups inside the aromatic cavity of **PrS[6]^{SO₃⁻}**, and consistent with the observed upfield shift of the ¹H NMR signals of N(CH₂CH₃)₂.

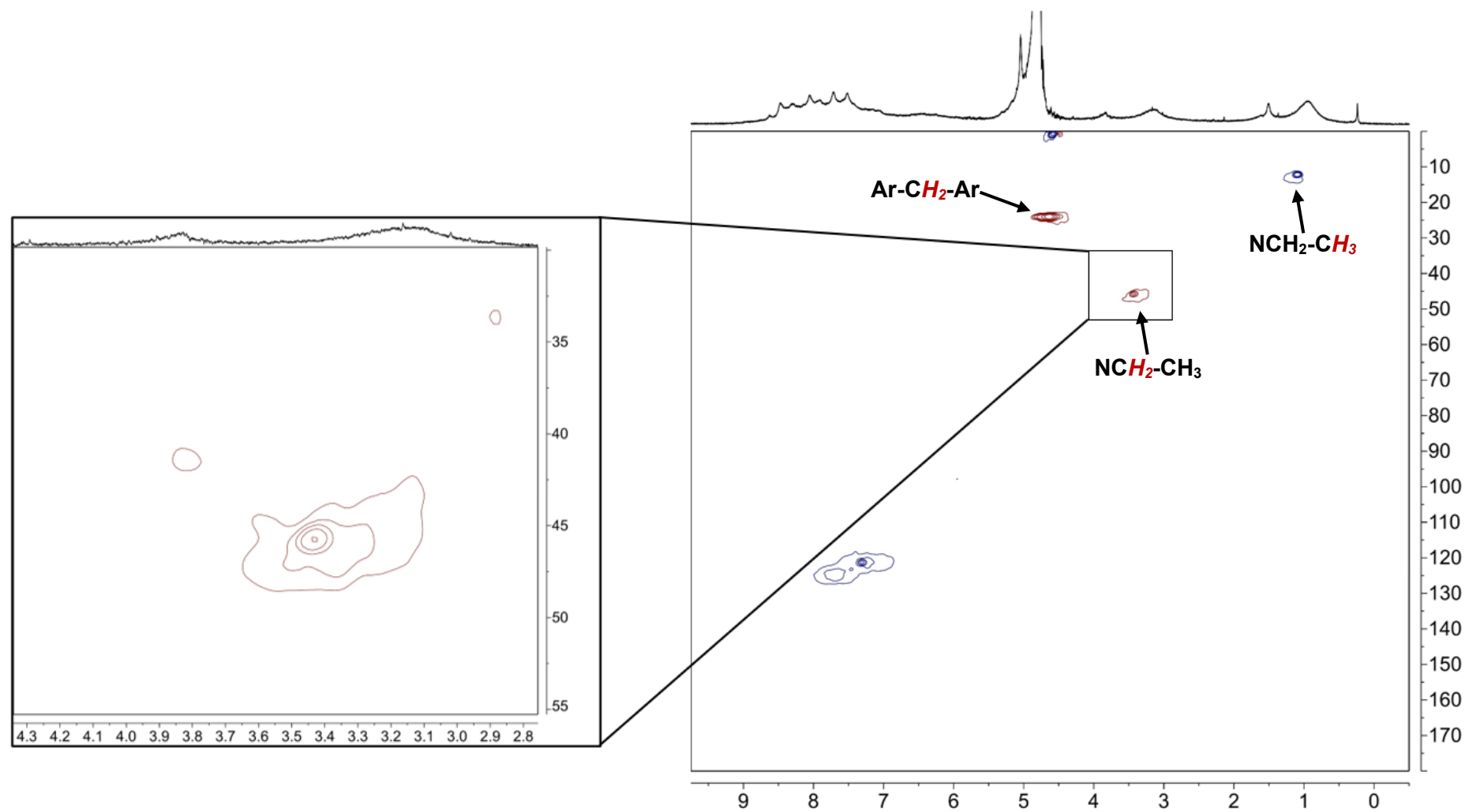


Figure S7. 2D-HSQC of RhodB @ PrS[6]^{SO3-} complex (D₂O, 600 MHz, 313 K).

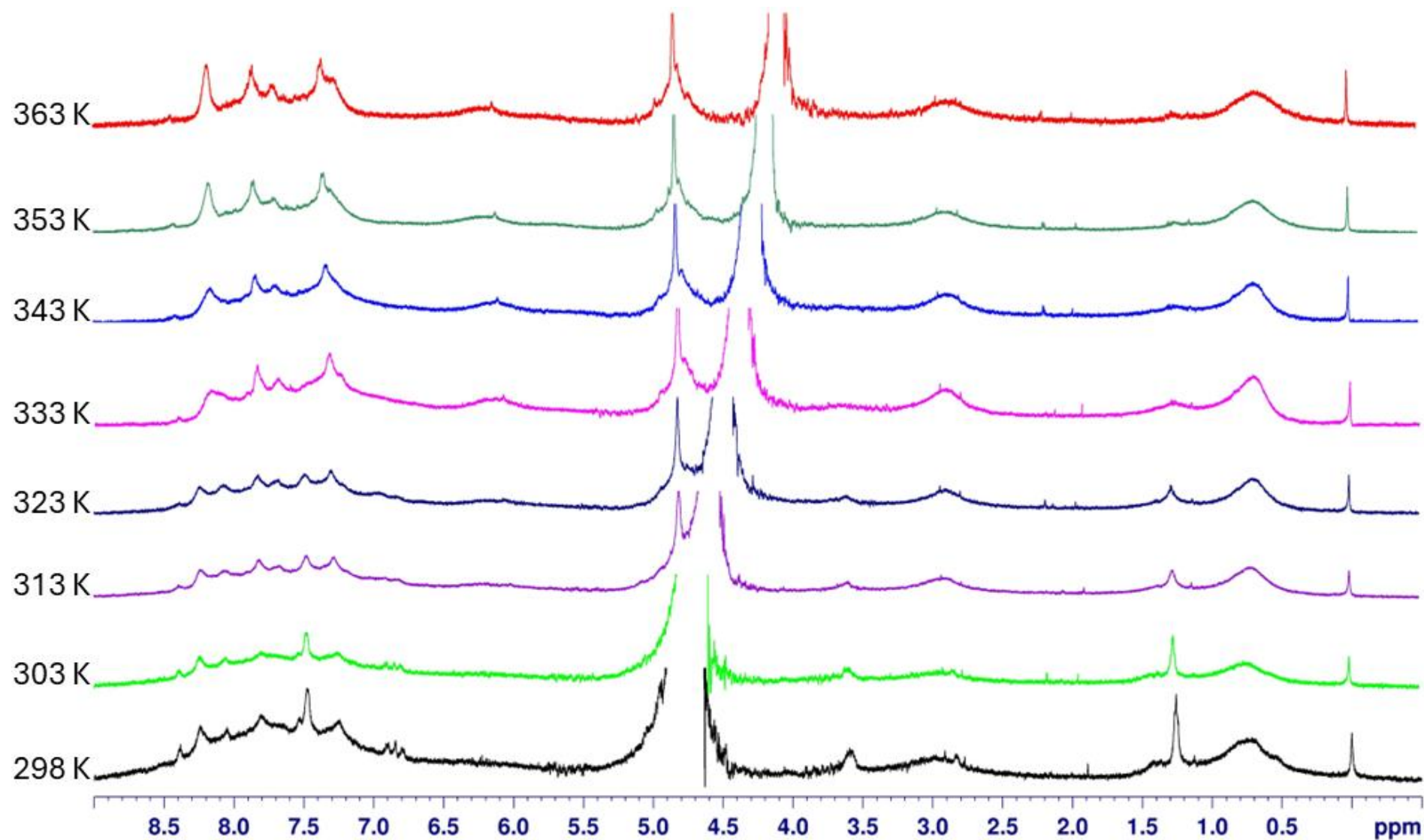


Figure S8. ¹H NMR spectra of an equimolar solution of **PrS[6]^{SO3-}** and **RhodB** (600 MHz, D₂O) at (from bottom to top): 298, 303, 313, 323, 333, 343, 353, 363 K.

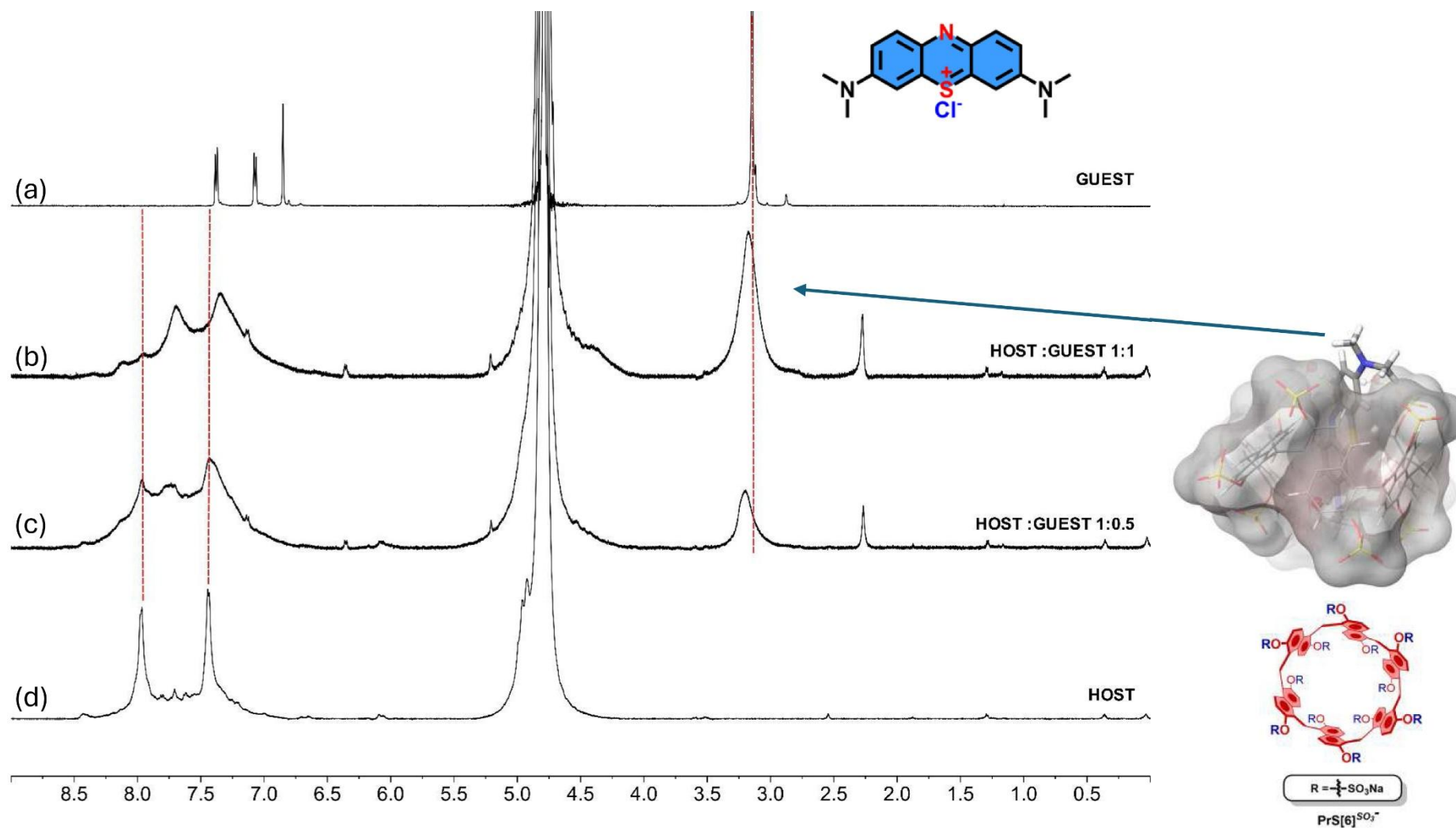


Figure S9. ¹H NMR spectra (D₂O, 600 MHz, 298 K) of: (a) **MethB**, (b) an equimolar (3 mM) solution of **PrS[6]^{SO₃-}** and **MethB**, (c) an 1:0.5 solution of **PrS[6]^{SO₃-}** (3 mM) and **MethB** (1.5 mM). (d) **PrS[6]^{SO₃-}**. Inset: Detail of the 1:1 **MethB@ PrS[6]^{SO₃-}** complex obtained from molecular dynamics simulations (see page S14), showing the N(CH₃)₂ groups protruding from the aromatic cavity of **PrS[6]^{SO₃-}**.

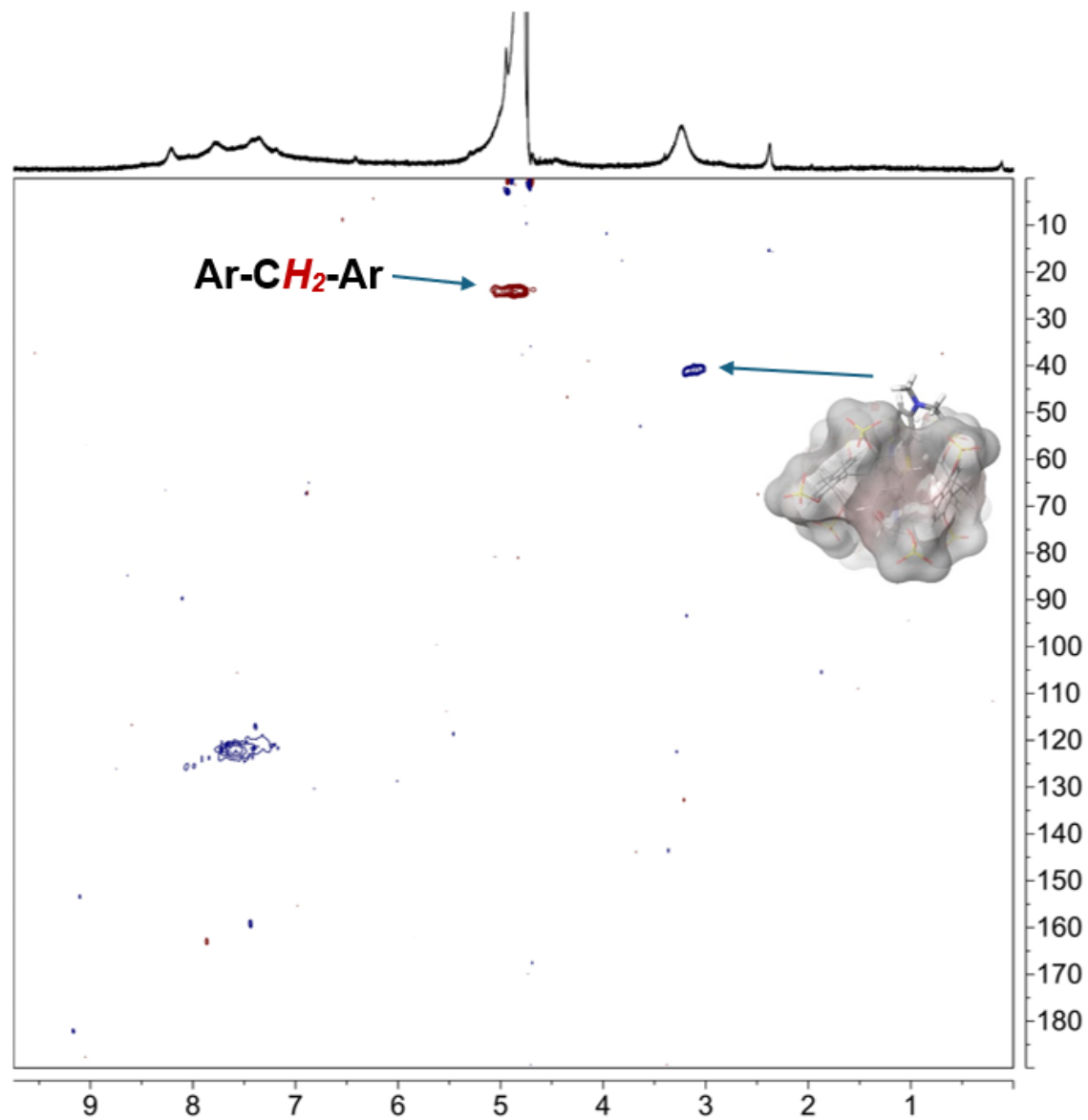


Figure S10. 2D-HSQC of **MethB@PrS[6]^{SO₃⁻}** complex (D₂O, 600 MHz, 298 K).

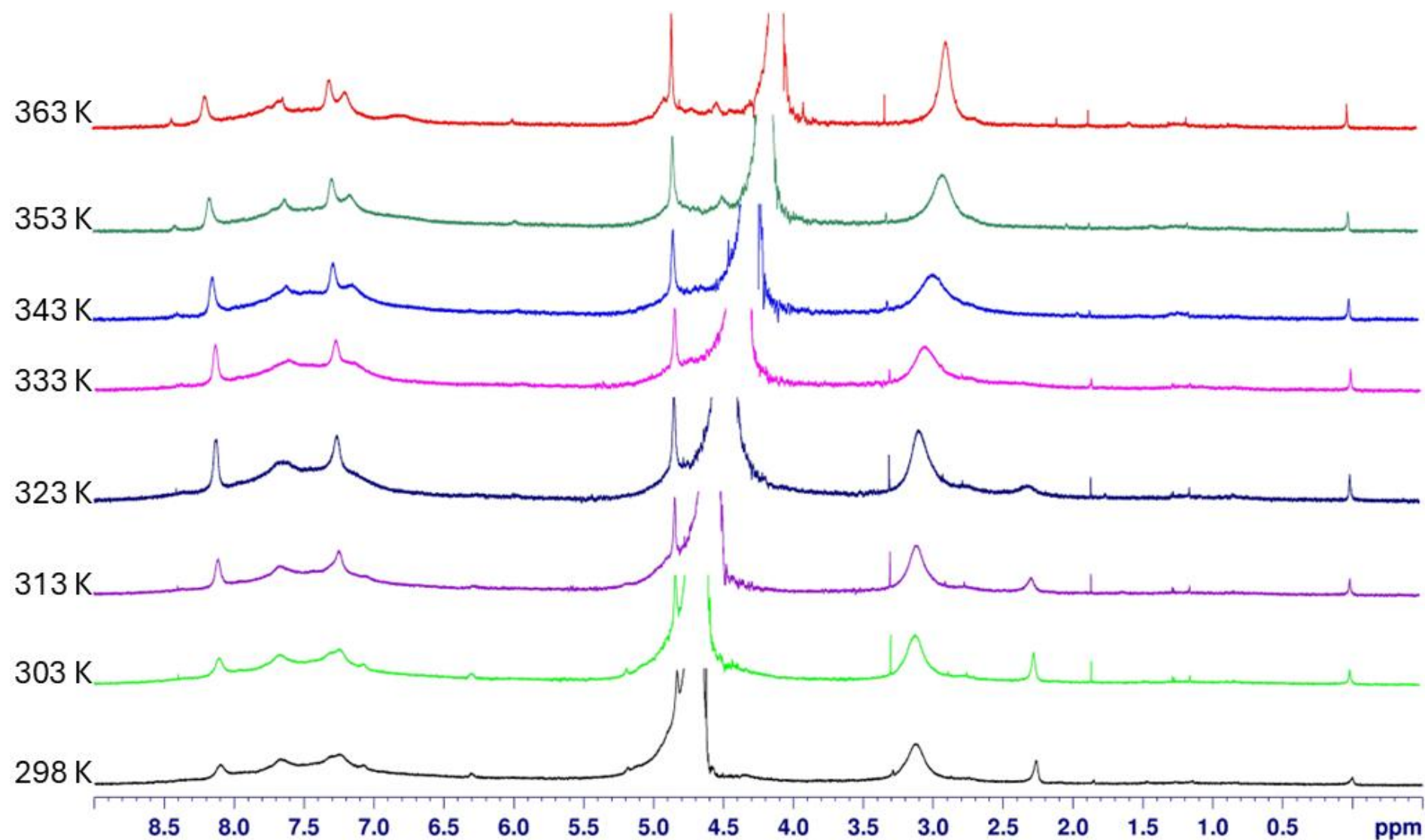


Figure S11. ¹H NMR spectra of an equimolar solution of **PrS[6]^{SO3-}** and **MethB** (600 MHz, D₂O) at (from bottom to top): 298, 303, 313, 323, 333, 343, 353, 363 K.

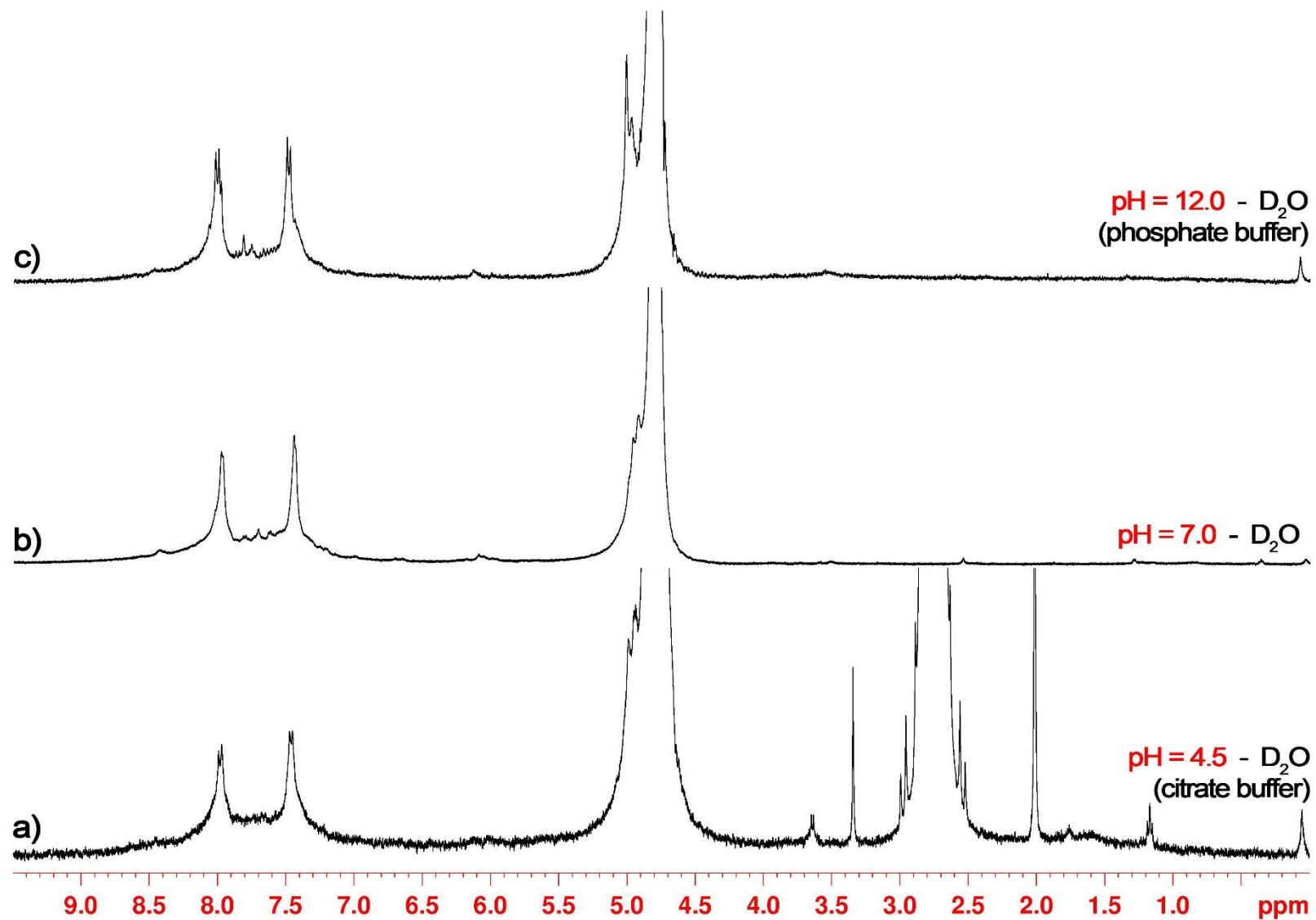


Figure S12. ^1H NMR spectra of $\text{PrS[6]}^{\text{SO}_3^-}$ recorded at: a) pH 4.5, b) pH 7, and c) pH 12 (400 MHz, D_2O , 298 K).

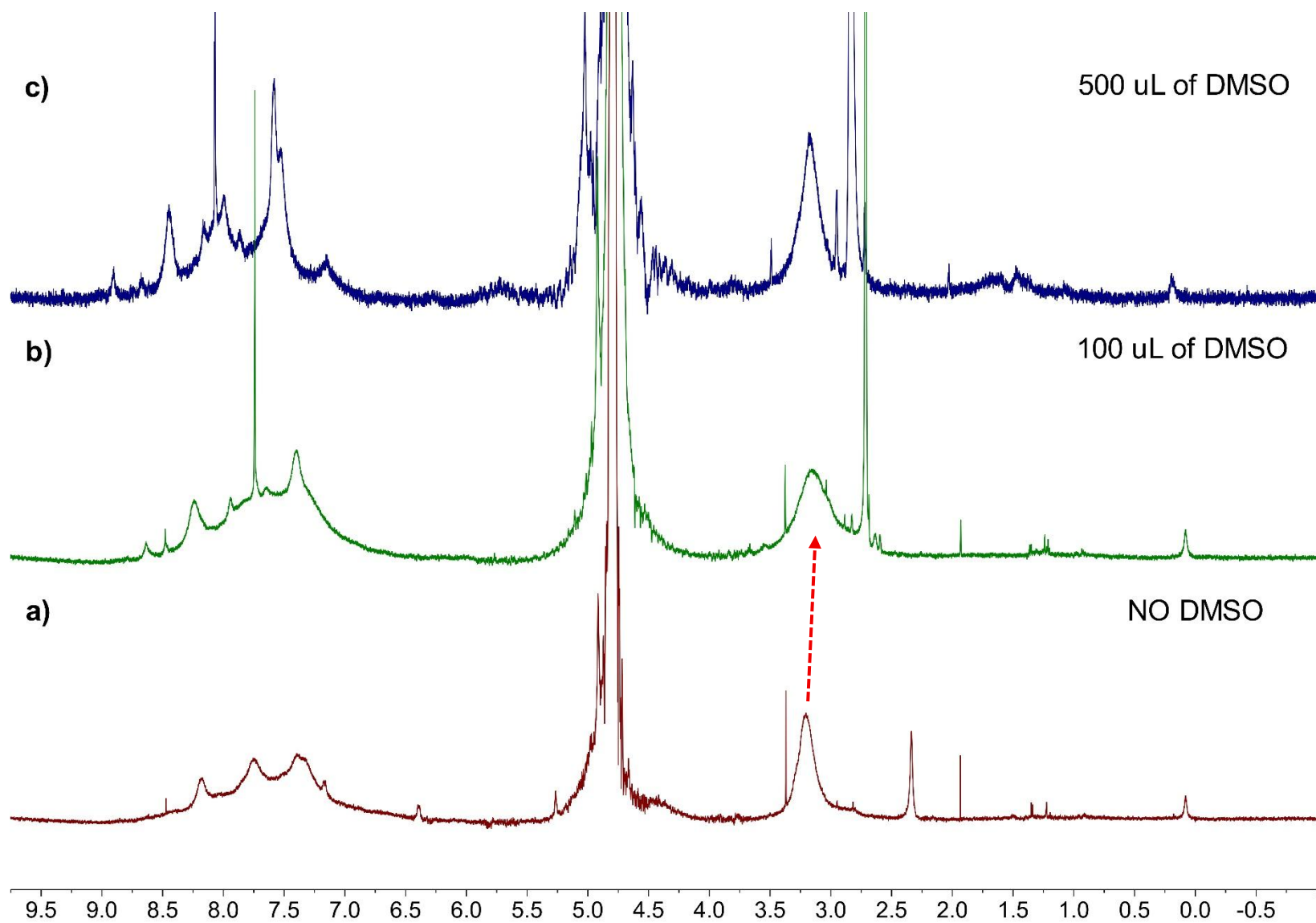


Figure S13. ^1H NMR spectra (600 MHz, D_2O , 298 K) of **MethB@PrS[6] $^{\text{SO}_3^-}$** in the presence of $\text{DMSO-}d_6$: a) 0 μL , b) 100 μL of $\text{DMSO-}d_6$, and c) 500 μL of $\text{DMSO-}d_6$.

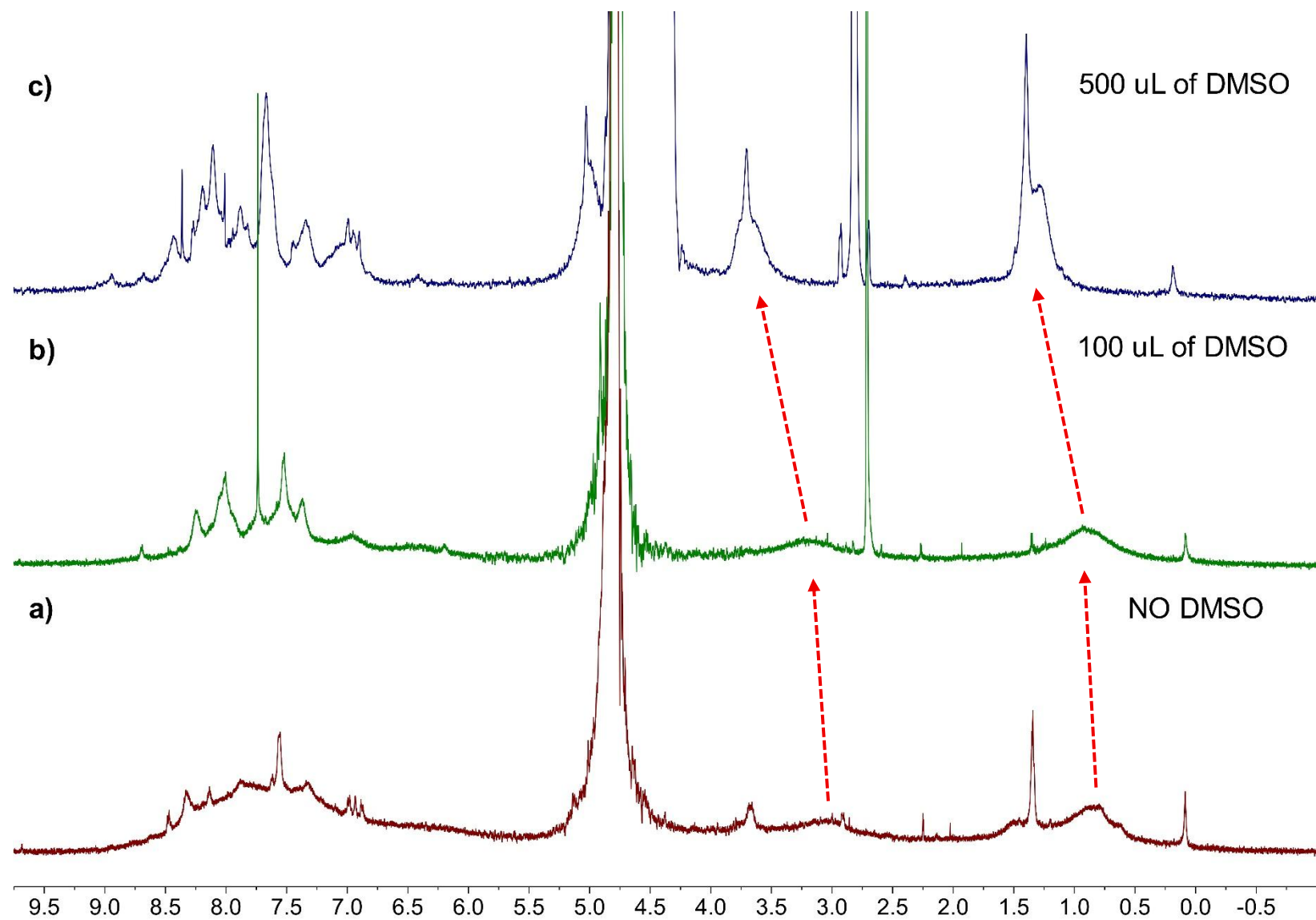


Figure S14. ^1H NMR spectra (600 MHz, D_2O , 298 K) of **RhodB@ PrS[6] $^{\text{SO}_3^-}$** in the presence of DMSO- d_6 : a) 0 μL , b) 100 μL of DMSO- d_6 , and c) 500 μL of DMSO- d_6 .

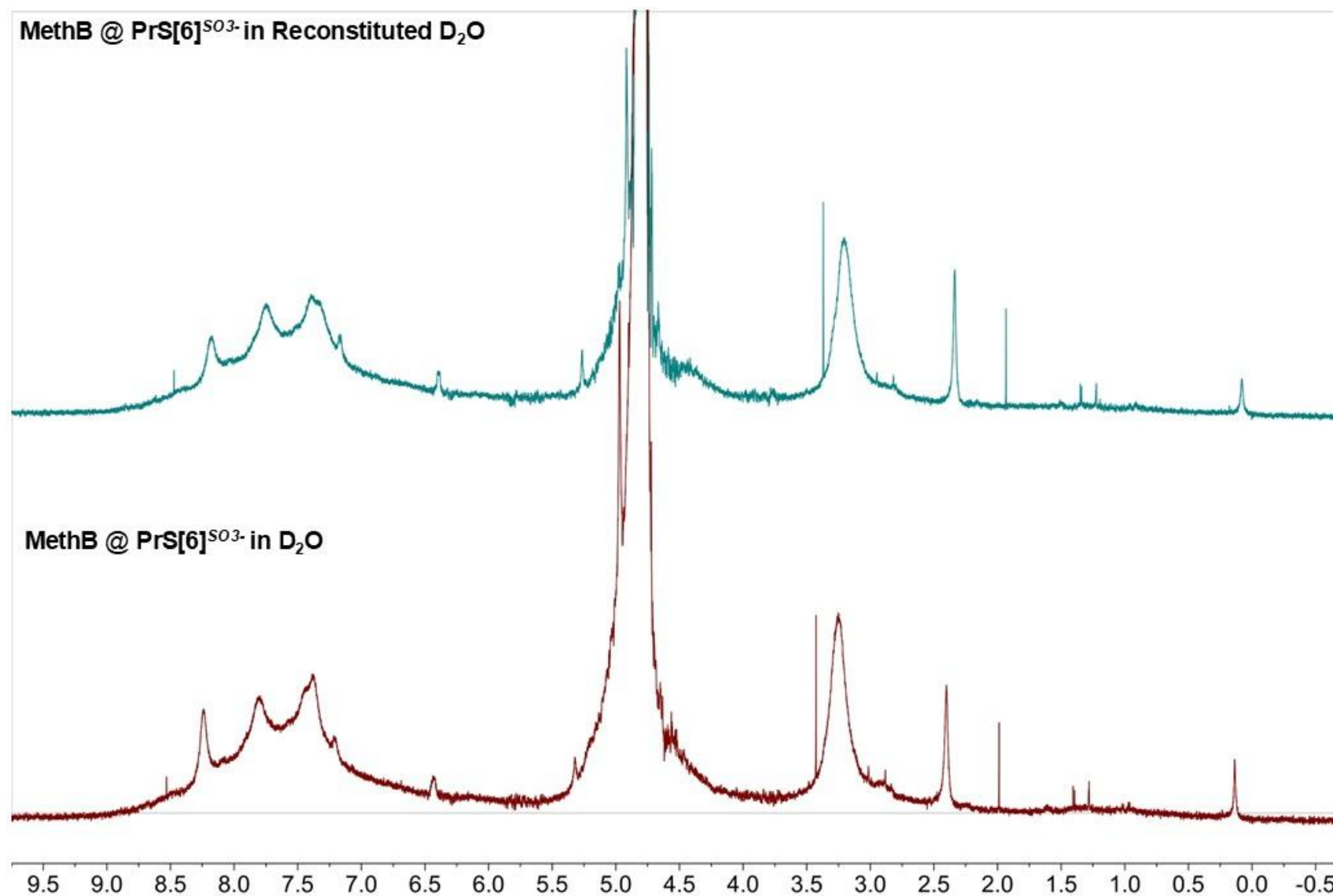


Figure S15. ^1H NMR spectra (600 MHz, 298 K) of **MethB@ PrS[6]^{SO3-}** recorded in: a) D₂O and b) in reconstituted water (Na^+ 710 g/L, K^+ 120 mg/L, Ca^{2+} 3.20 g/L, Mg^{2+} 480 mg/L, HCO_3^- 1.83 g/L, Cl^- 5.78 g/L, and SO_4^{2-} 1.92 g/L).

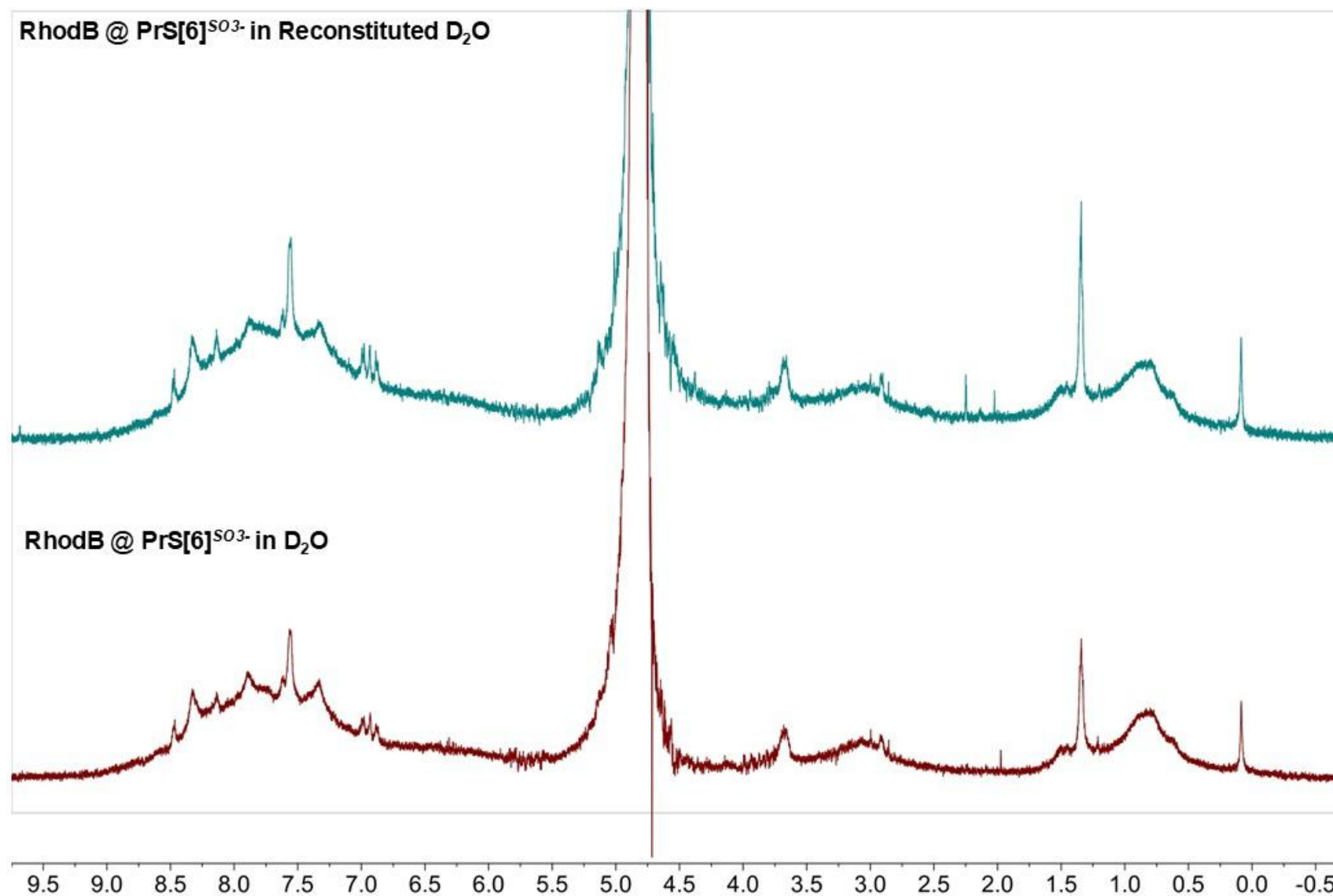


Figure S16. ¹H NMR spectra (600 MHz, 298 K) of **RhodB@ PrS[6]^{SO3-}** recorded in: a) D₂O and b) in reconstituted water (Na⁺ 710 g/L, K⁺ 120 mg/L, Ca²⁺ 3.20 g/L, Mg²⁺ 480 mg/L, HCO₃⁻ 1.83 g/L, Cl⁻ 5.78 g/L, and SO₄²⁻ 1.92 g/L).

Calorimetric investigation

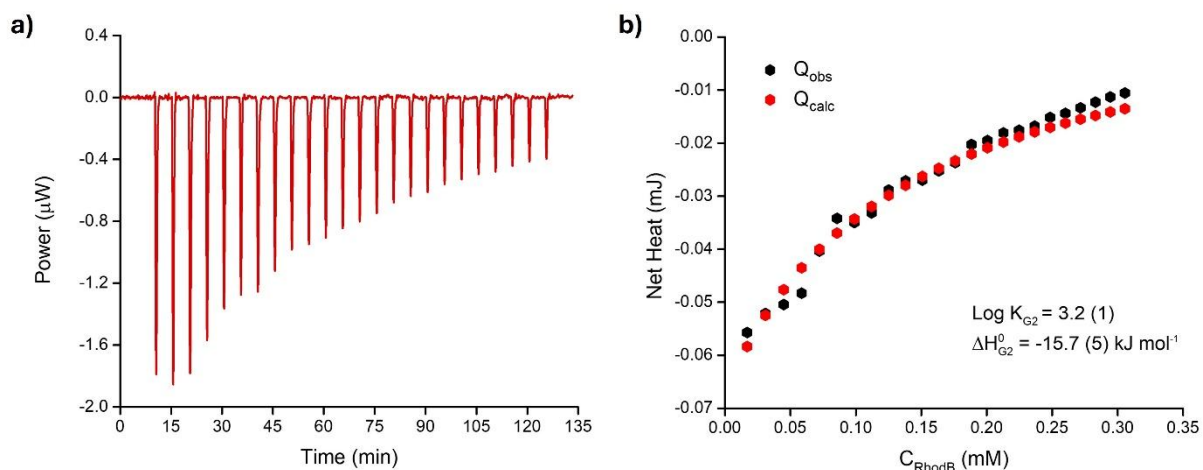


Figure S17. a) ITC titration of **RhodB** (1.40 mM) in 50 mM acetate buffer at 25 °C and pH 5.5; b) observed vs. calculated net heat data for dimer formation.

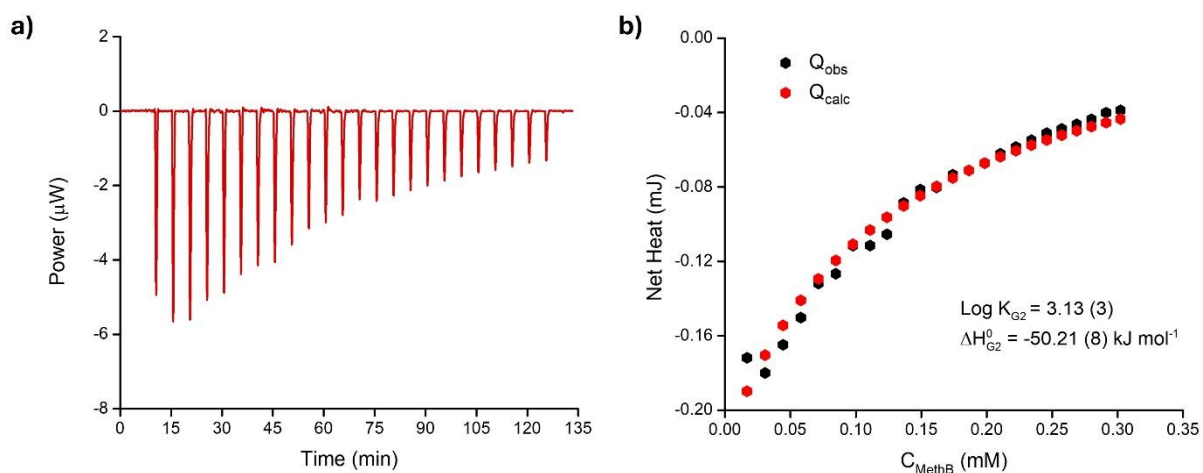


Figure S18. a) ITC titration of **MethB** (1.39 mM) in 50 mM acetate buffer at 25 °C and pH 5.5; b) observed vs. calculated net heat data for dimer formation.

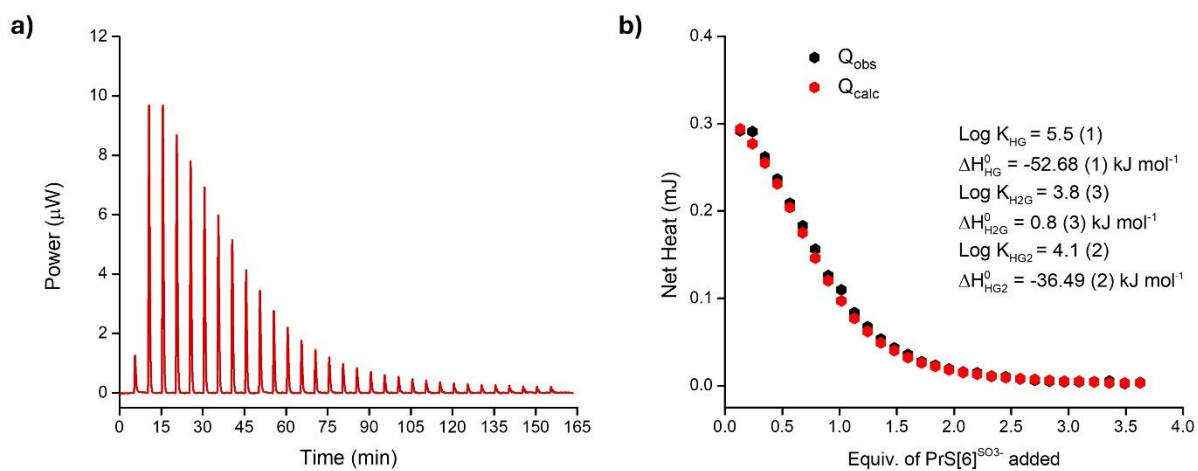


Figure S19. a) ITC titration of $\text{PrS[6]}^{\text{SO}_3^-}$ (0.65 mM) into **RhodB** (0.05 mM) at 25 °C and pH 5.5 (acetate buffer, 50 mM); b) observed vs. calculated net heat data.

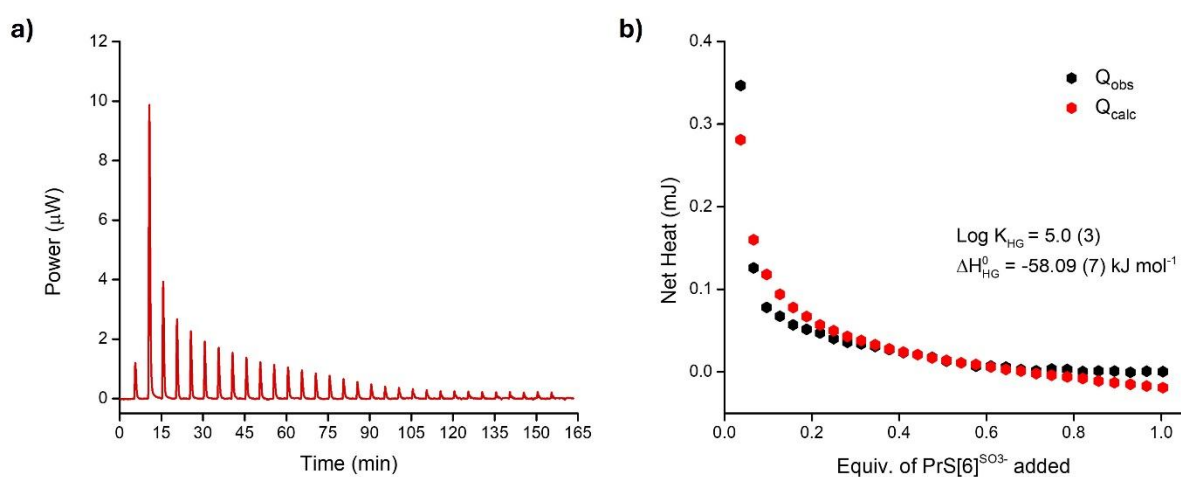


Figure S20. a) ITC titration of $\text{PrS[6]}^{\text{SO}_3^-}$ (0.18 mM) into **MethB** (0.05 mM) at 25 °C and pH 5.5 (acetate buffer, 50 mM); b) observed vs. calculated net heat data.

Dynamic Light Scattering (DLS)

Table S1. The tested solution $\text{PrS}[6]^{\text{SO}_3^-}$ at a concentration of 4.0×10^{-4} M was freshly prepared in CH_3COONa buffer (0.05 M) at pH 5.5 (410 mg CH_3COONa was dissolved in 100 ml MilliQ water, then glacial acetic acid was added dropwise to achieve pH 5.5).

System	d, nm	PDI
$\text{PrS}[6]$	1.61	0.288
$\text{PrS}[6]^{\text{SO}_3^-} + 0.1$ equiv. Rhod.B	1.51	0.302
$\text{PrS}[6]^{\text{SO}_3^-} + 0.3$ equiv. Rhod.B	4.32	0.378
$\text{PrS}[6]^{\text{SO}_3^-} + 0.5$ equiv. Rhod.B	2.48	0.543
$\text{PrS}[6]^{\text{SO}_3^-} + 0.7$ equiv. Rhod.B	1.85	0.367
$\text{PrS}[6]^{\text{SO}_3^-} + 1$ equiv. Rhod.B	1.68	0.336
$\text{PrS}[6]^{\text{SO}_3^-} + 3$ equiv. Rhod.B	1.48	0.625
$\text{PrS}[6]^{\text{SO}_3^-} + 5$ equiv. Rhod.B	0.23	0.653
$\text{PrS}[6]^{\text{SO}_3^-} + 0.3$ equiv. Meth.Blue	1.61	0.482
$\text{PrS}[6]^{\text{SO}_3^-} + 0.5$ equiv. Meth.Blue	1.19	0.249
$\text{PrS}[6]^{\text{SO}_3^-} + 1$ equiv. Meth.Blue	1.39	0.237
$\text{PrS}[6]^{\text{SO}_3^-} + 3$ equiv. Meth.Blue	1.27	0.602
$\text{PrS}[6]^{\text{SO}_3^-} + 5$ equiv. Meth.Blue	0.22	0.660

UV-Vis and fluorescence titration experiments

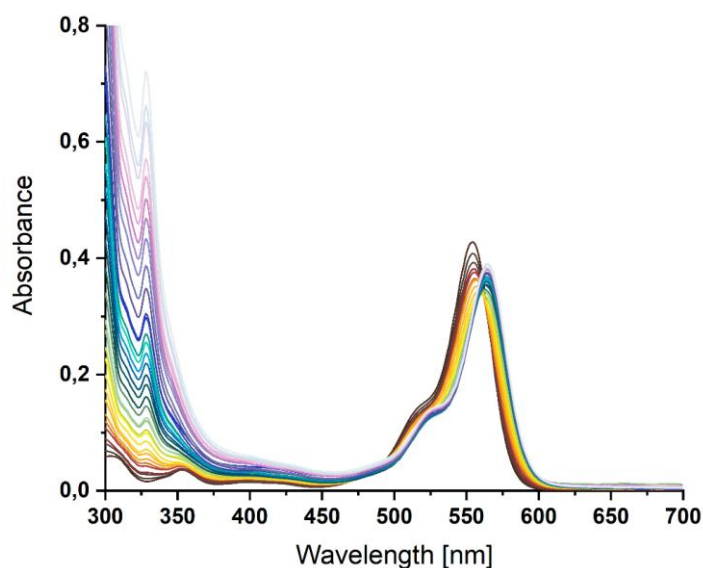


Figure S21. UV-Vis titration of Rhodamine B (4.7×10^{-6} M) with $\text{PrS}[6]^{\text{SO}_3^-}$ (2.2×10^{-4} M) in water buffered at pH 5.5.

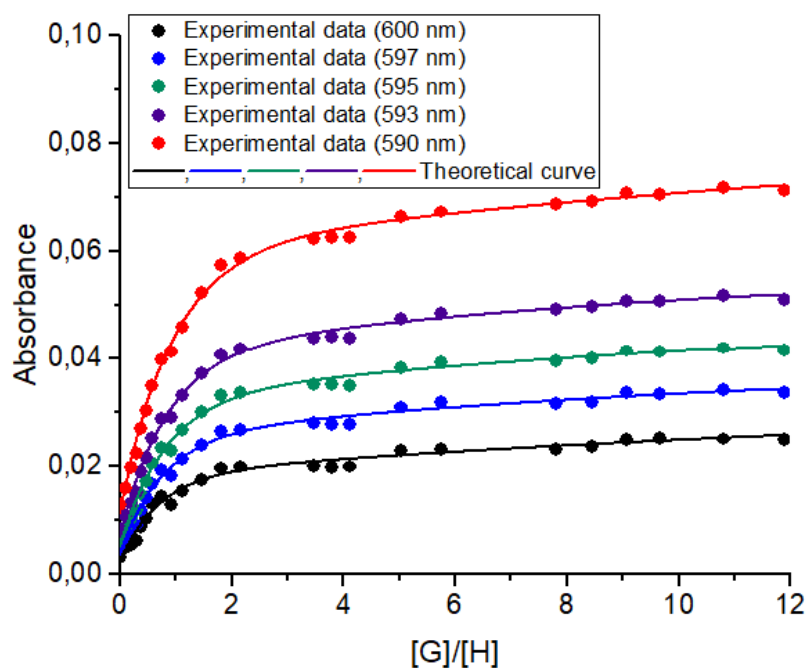


Figure S22. UV-Vis titration of Rhodamine B with **PrS[6]^{SO3-}** and selected binding isotherms.

The titration data were fitted using either a 1:1 (HG) or a 2:1 (H₂G) model. The results are reported in Table S2.

Table S2. Association constants determined by UV-Vis titrations of Rhodamine B and Methylene Blue with **PrS[6]^{SO3-}** in water buffered at pH 5.5 using a 1:1 or a 2:1 binding model.

Dye	Log K ₁₁	Log K ₂₁
Rhodamine B	5.67	4.39
Methylene Blue	5.22	----

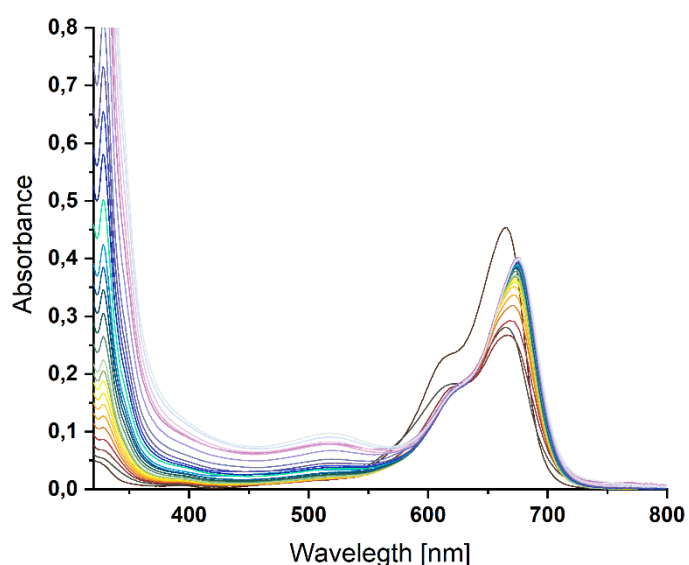


Figure S23. UV-Vis titration of Methylene Blue (5.0×10^{-6} M) with **PrS[6]^{SO3-}** (3.3×10^{-4} M) in water buffered at pH 5.5.

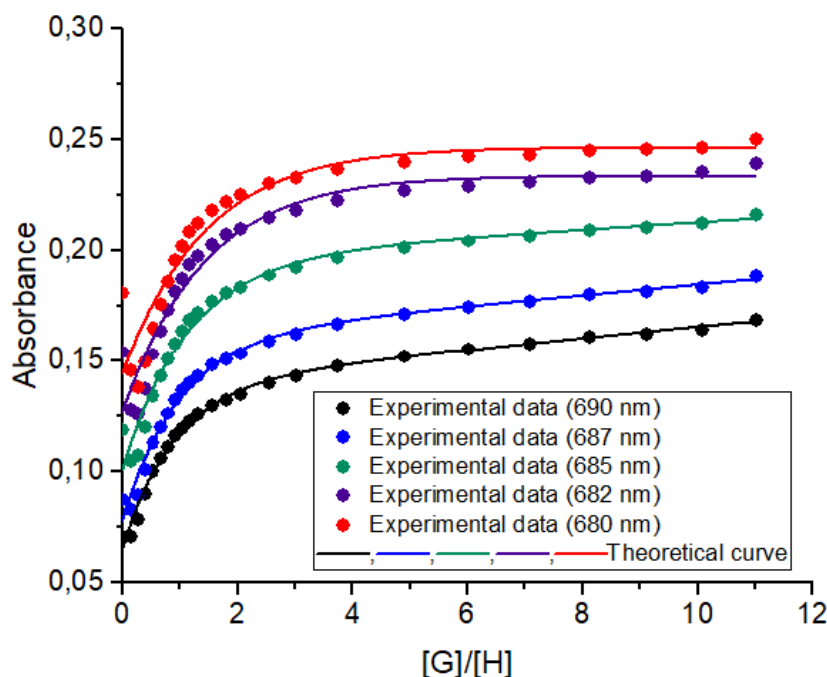


Figure S24. UV-Vis titration of Methylene Blue with $\text{PrS[6]}^{\text{SO}_3^-}$ and selected binding isotherms.

Preliminary fluorescence studies were performed by titrating a pH 5.5 buffered $\text{PrS[6]}^{\text{SO}_3^-}$ solution at a concentration of 1.0×10^{-6} M with a pH 5.5 buffered solution of RhodB at a concentration of 7.0×10^{-5} M. Figure S16 shows the fluorescence spectra obtained by exciting the sample at 300 nm. Neglectable changes were observed on the emission band of $\text{PrS[6]}^{\text{SO}_3^-}$ at 353 nm, while marked changes in the intensity of the RhodB emission at 584 nm were observed and attributed to the increased concentration of the dye in solution.

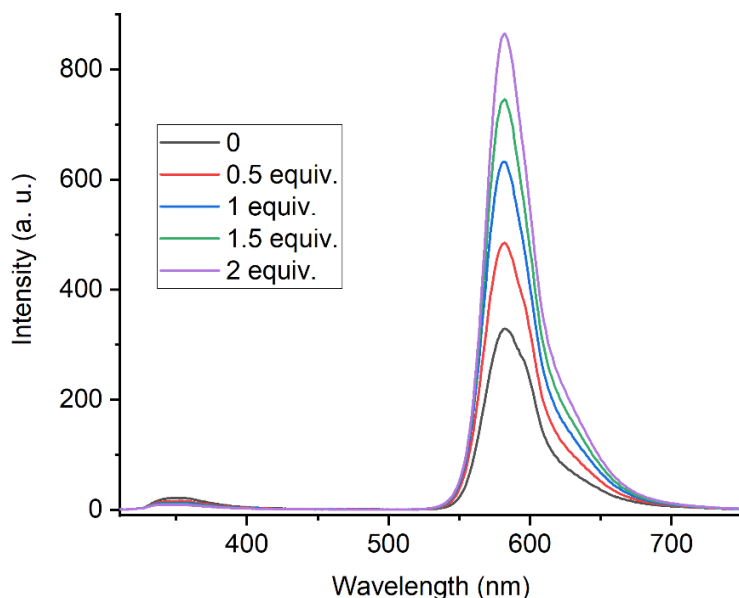


Figure S25. Emission spectra of $\text{PrS[6]}^{\text{SO}_3^-}$ (1.0×10^{-6} M) upon addition of increasing amount of **RhodB** (7.0×10^{-5} M) ($\lambda_{\text{exc}} = 300$ nm) in water buffered at pH 5.5. The band with a maximum at 353 nm corresponds to the $\text{PrS[6]}^{\text{SO}_3^-}$ emission, while the band at 589 nm corresponds to the **RhodB** emission.

Interestingly, when the experiment was repeated by monitoring the changes of the fluorescence emission of a pH 5.5 buffered solution of **RhodB** upon the addition of increasing amounts of a pH

5.5 buffered solution of **PrS[6]^{SO3-}** upon excitation at 500 nm, a partial quenching of the emission band accompanied by a bathochromic shift of 12 nm (from 584 nm to 596 nm) up to the addition of 2 equivalents of the prismarene guest was observed. This finding is in agreement with the formation of a 2:1 capsule complex (Figures S17 and S18 a and b). When an excess of **PrS[6]^{SO3-}** was added an enhancement of the emission intensity of **RhodB** was observed and attributed to the formation of dye aggregates (<https://doi.org/10.1016/j.apcatb.2015.02.040>, <https://doi.org/10.1016/j.molliq.2025.128481>).

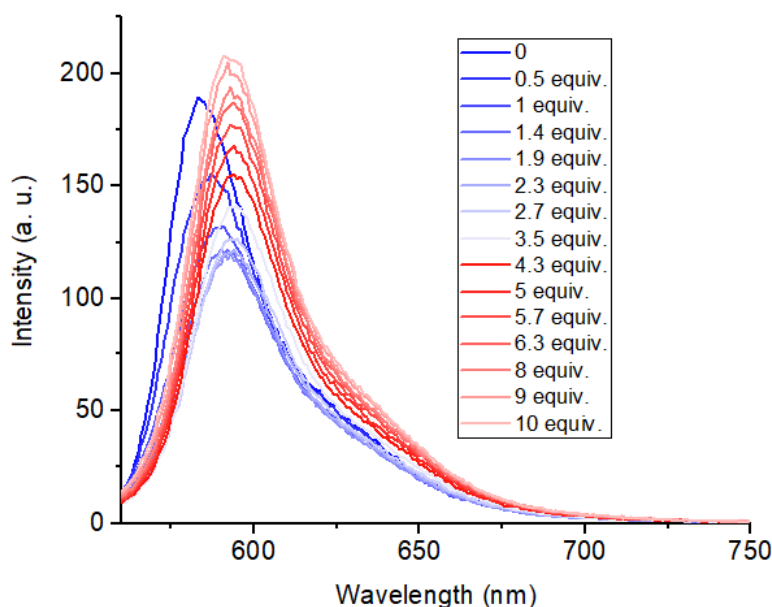


Figure S26. Changes in the emission spectra of **RhodB** (7.1×10^{-5} M) upon addition of increasing amounts **PrS[6]^{SO3-}** (2.1×10^{-4} M) ($\lambda_{\text{exc}} = 550$ nm) in water buffered at pH 5.5.

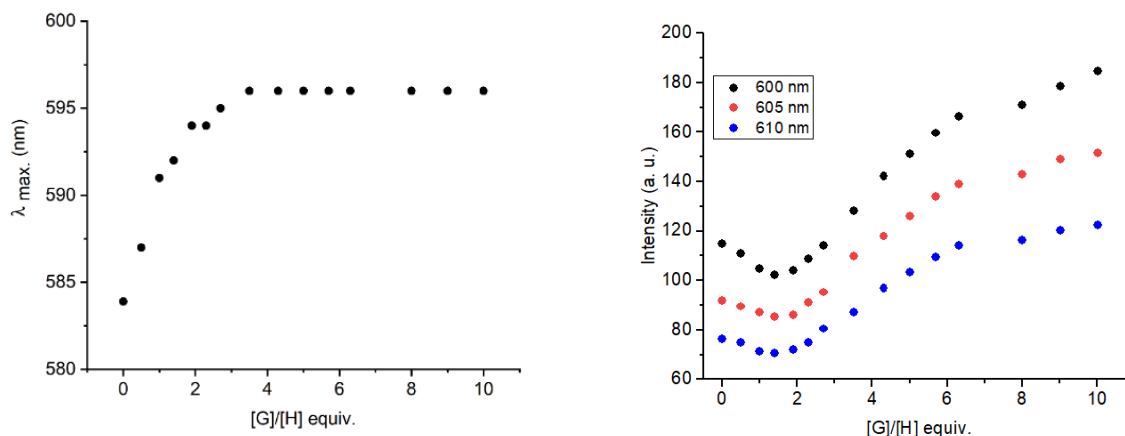


Figure S27. (a) Shifts in the maximum fluorescent intensity of **RhodB** due to the addition of **PrS[6]^{SO3-}** in buffered water at pH 5.5; (b) Shifts in the fluorescent intensity of **RhodB** upon the addition of **PrS[6]^{SO3-}** for selected wavelengths in buffered water at pH 5.5.

An analogous experiment was performed by monitoring the changes of the fluorescence emission of a pH 5.5 buffered solution of **MethB** upon the addition of increasing amounts of a pH 5.5 buffered solution of **PrS[6]^{SO3-}**. Upon excitation at 665 nm, an almost complete quenching of the emission band accompanied by a bathochromic shift (from 682 nm to 690 nm) upon the addition of one equivalent of the prismarene guest was observed in agreement with the formation of a 1:1 complex (Figure S19a and b).

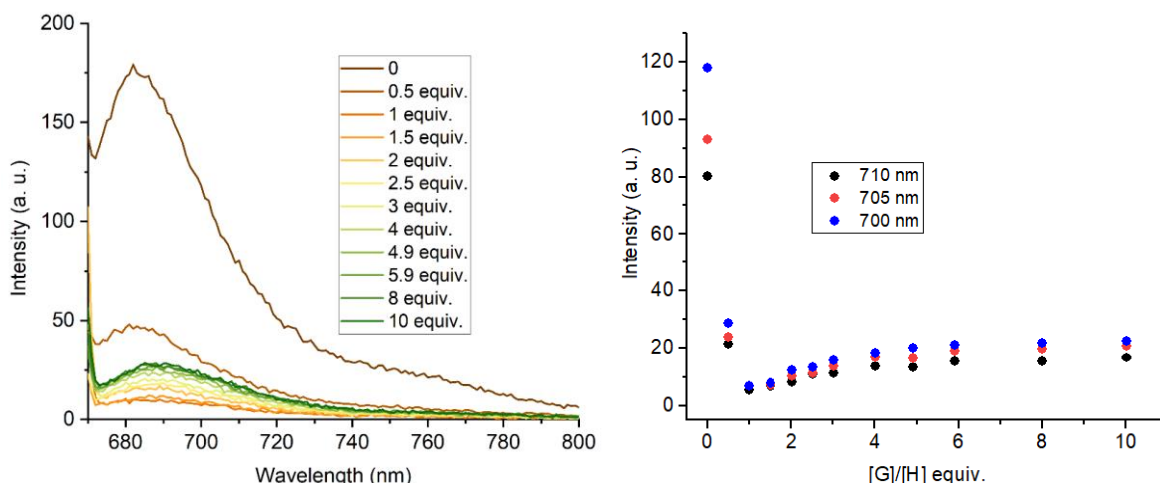


Figure S28. (a) Changes in the emission spectra of MethB (1.0×10^{-6} M) upon addition of increasing amounts $\text{PrS[6]}^{\text{SO}_3^-}$ (2.1×10^{-4} M) ($\lambda_{\text{exc}} = 665$ nm) in water buffered at pH 5.5; (b) Shifts in the fluorescent intensity of MethB upon the addition of $\text{PrS[6]}^{\text{SO}_3^-}$ for selected wavelengths in buffered water at pH 5.5.

***In Silico* Calculations**

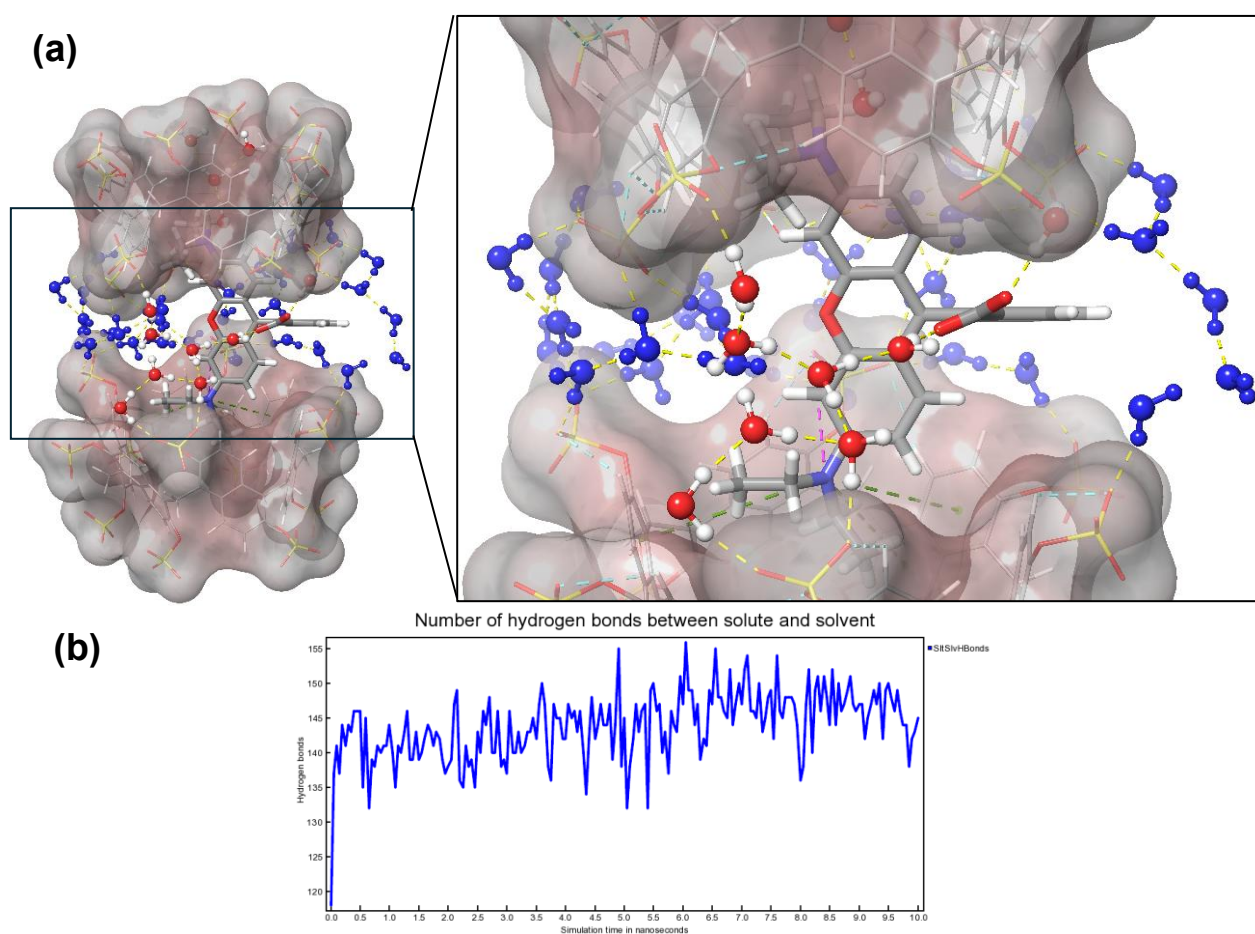


Figure S29. (a) Minimum-energy structure of the $\text{RhodB}@\text{(PrS[6]}^{\text{SO}_3^-})_2$ complex. Water molecules involved in the hydrogen-bonding network between the two macrocycles are highlighted in blue, while those bridging the guest and the macrocycle are shown in white and red. (b) Number of hydrogen bonds between $\text{RhodB}@\text{(PrS[6]}^{\text{SO}_3^-})_2$ complex and solvent as a function of simulation time.

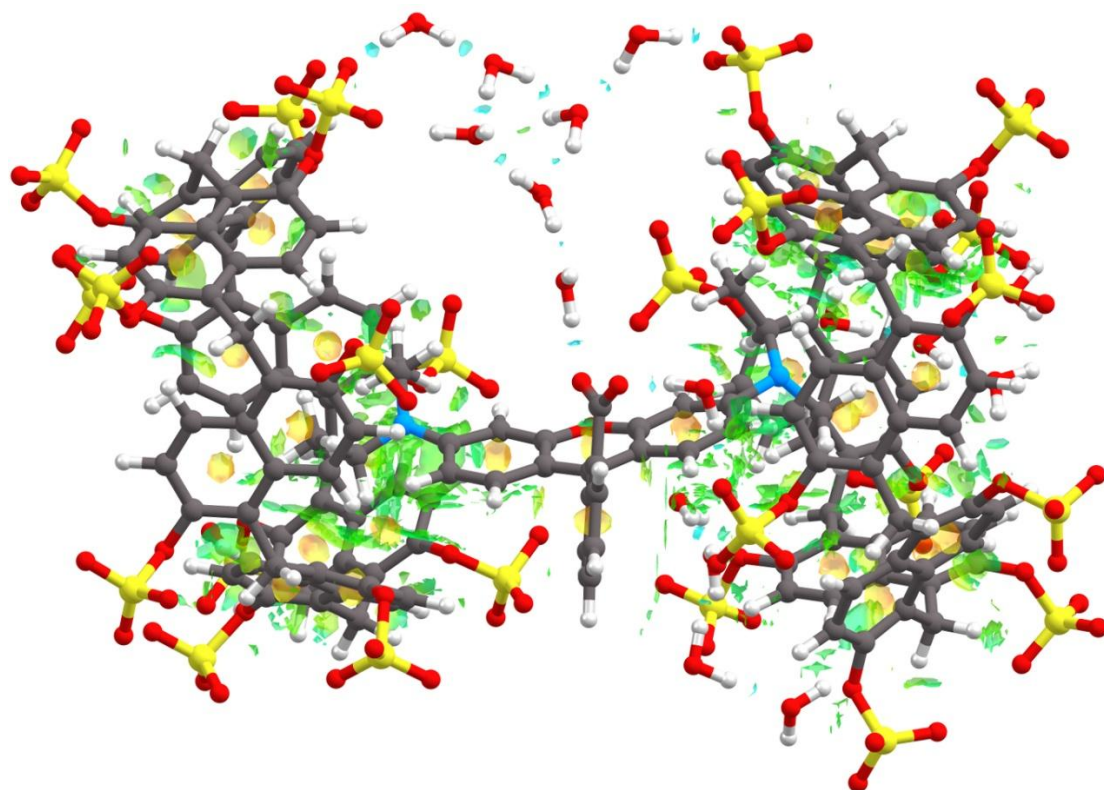


Figure S30. Gradient RDG isosurfaces (0.25) for the noncovalent interaction (NCI) regions of **RhodB @ (PrS[6]^{SO3-})₂** and the water molecules involved in stabilization through H-Bonds.

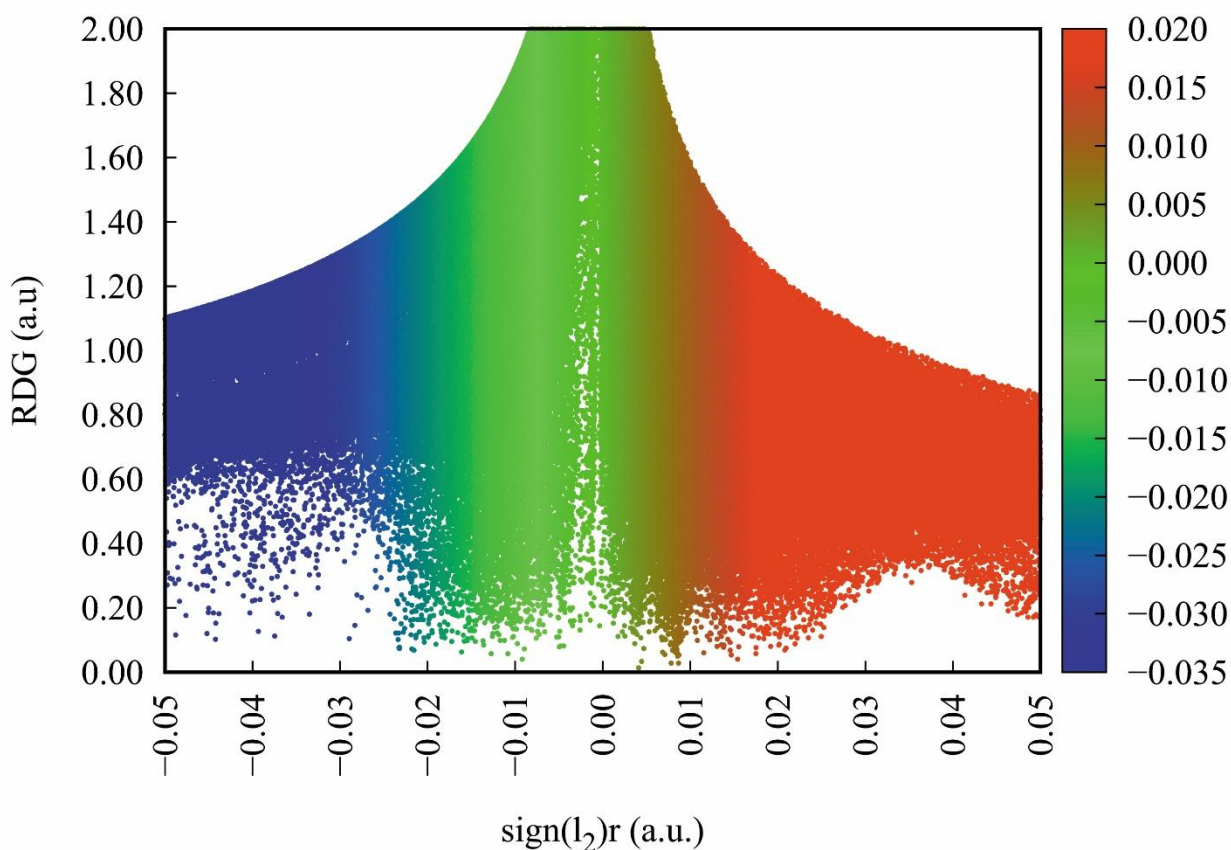


Figure S31. Plot of RDG versus $\text{sign}(I_2)r$ for **RhodB @ (PrS[6]^{SO3-})₂** complex (NCI-RDG isosurfaces with $S = 0.25$).

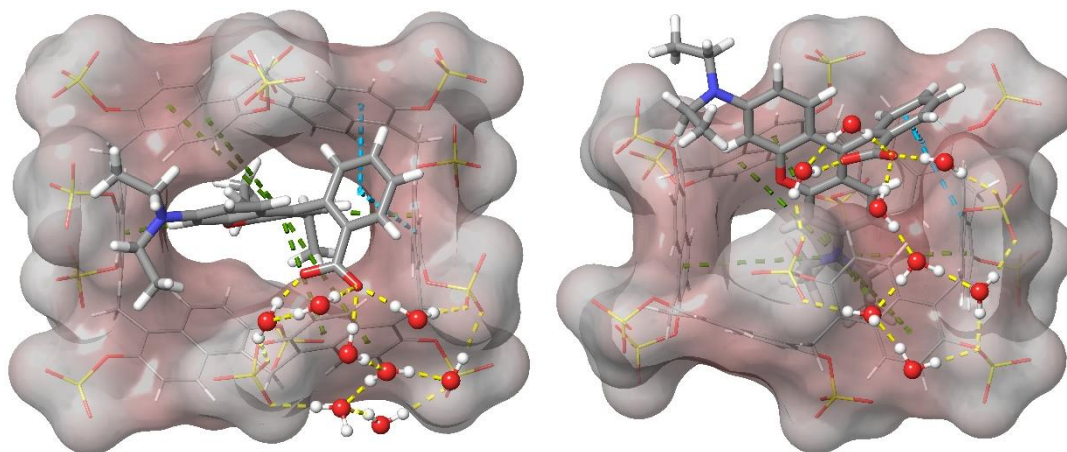


Figure S32. Minimum-energy structure of the **RhodB @ PrS[6]^{SO₃-}** complex. Water molecules that bridge the guest and the macrocycle are shown in white and red.

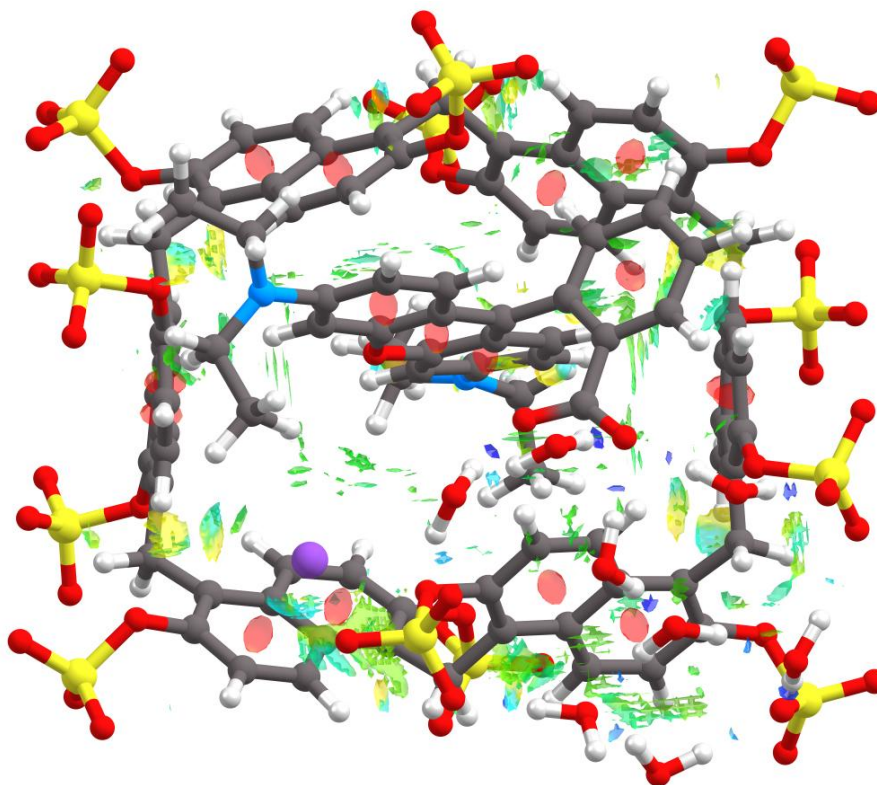


Figure S33. Gradient RDG isosurfaces (0.25) for the noncovalent interaction (NCI) regions of **RhodB @ PrS[6]^{SO₃-}** and the water molecules involved in stabilization through H-Bonds.

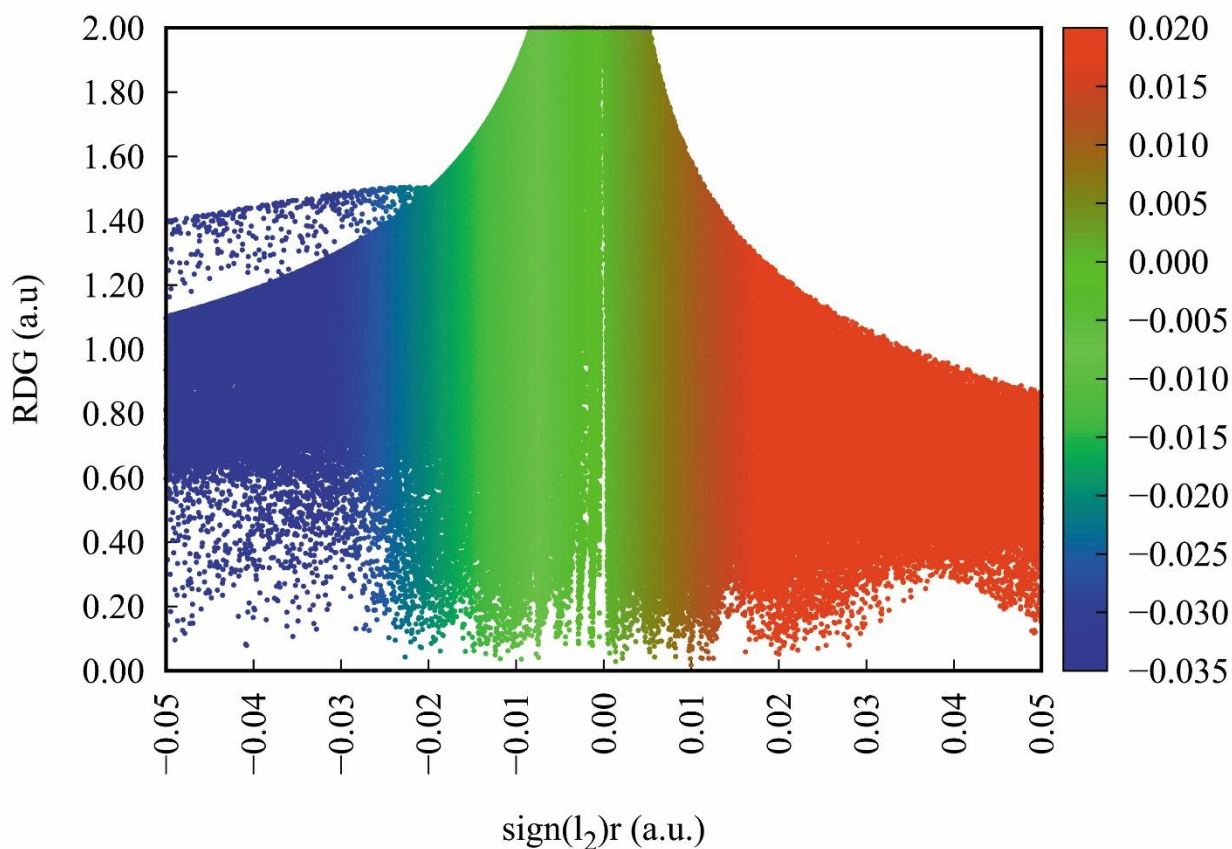


Figure S34. Plot of RDG versus $\text{sign}(I_2)r$ for **RhodB @ PrS[6]^{SO3-}** complex (NCI-RDG isosurfaces with $S = 0.25$).

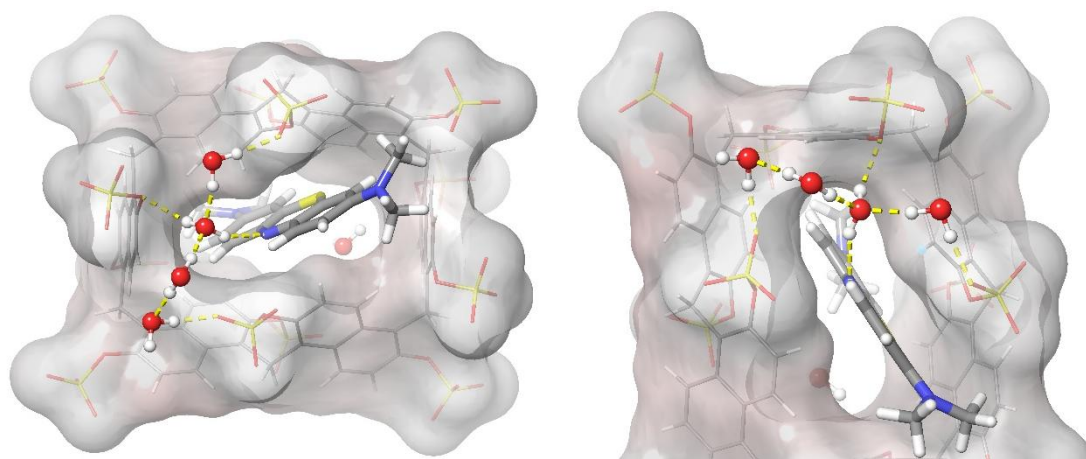


Figure S35. Minimum-energy structure of the **MethB @ PrS[6]^{SO3-}** complex. Water molecules that bridge the guest and the macrocycle are shown in white and red.

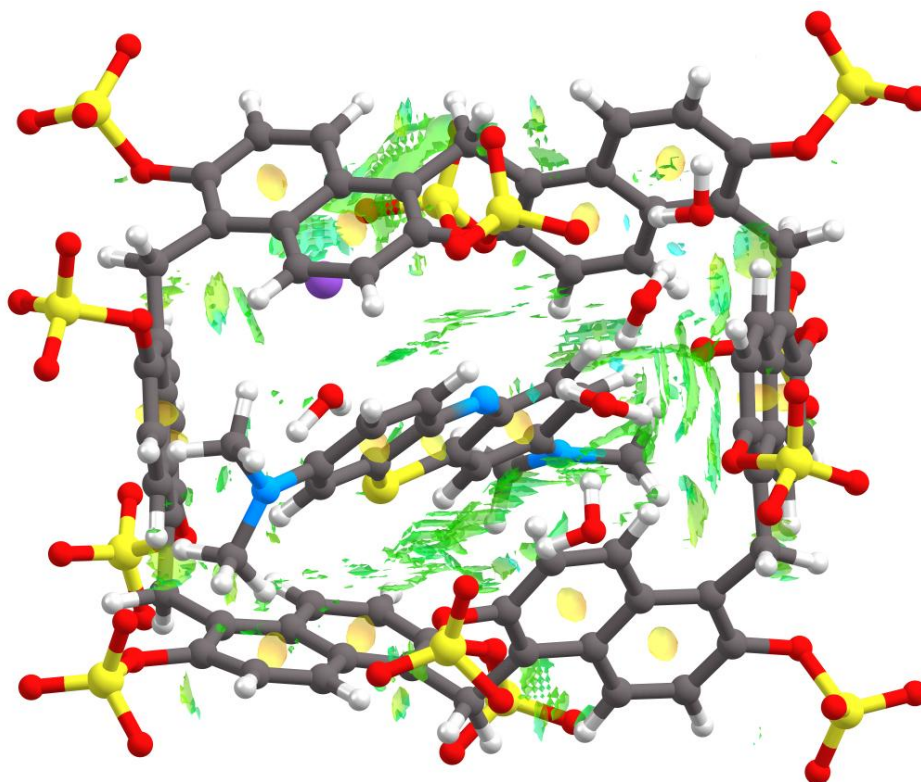


Figure S36. Gradient RDG isosurfaces (0.25) for the noncovalent interaction (NCI) regions of **MethB @ PrS[6]^{SO3-}** and the water molecules involved in stabilization through H-Bonds.

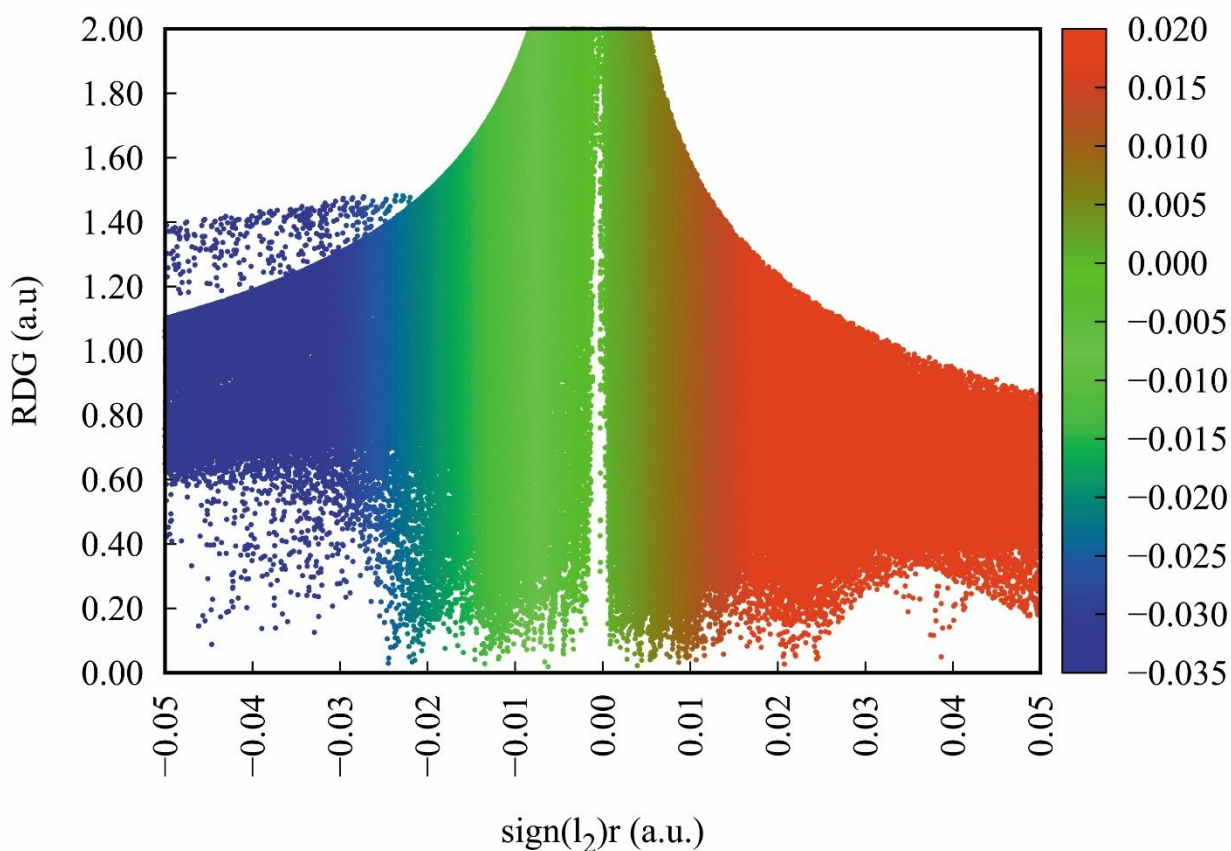


Figure S37. Plot of RDG versus $\text{sign}(I_2)r$ for **MethB @ PrS[6]^{SO3-}** complex (NCI-RDG isosurfaces with $S = 0.25$).

DOSY Studies

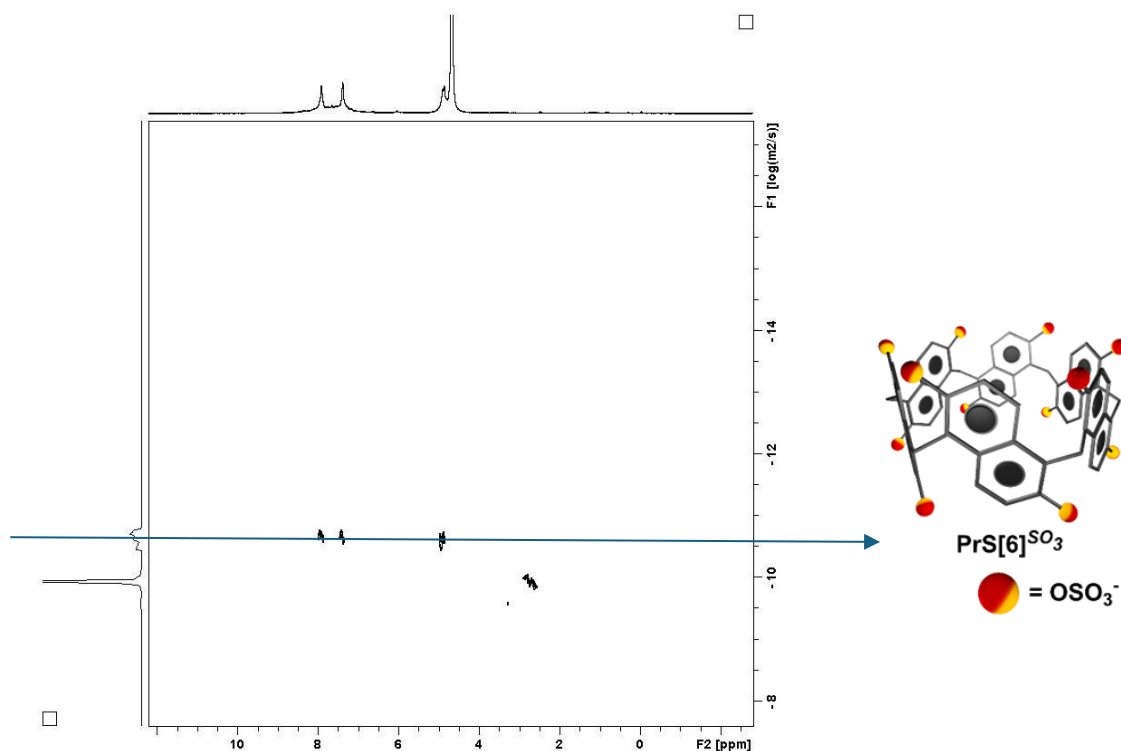


Figure S38. Pseudo 2D-DOSY representation of **PrS[6]^{SO₃⁻}** (4.17 mM).

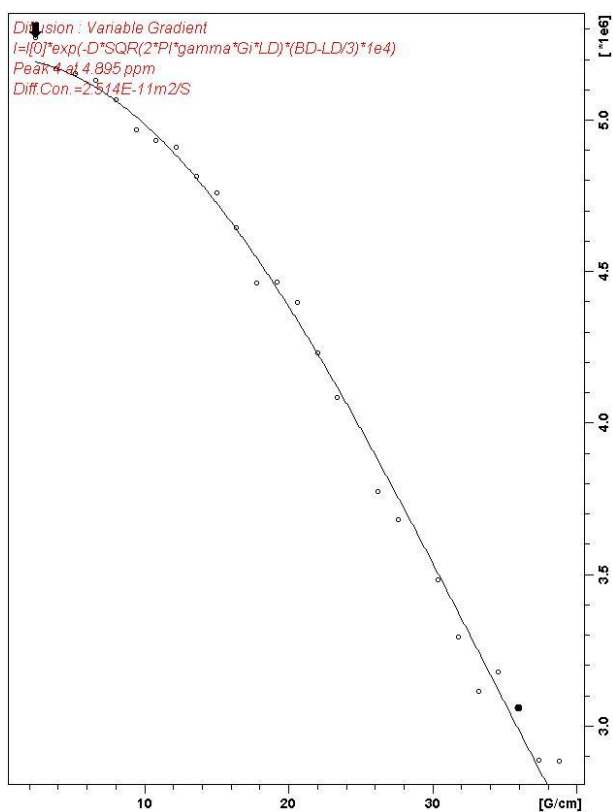


Figure S39. Mono-exponential Stejskal–Tanner fit of the DOSY signal attenuation for **PrS[6]^{SO₃⁻}** in the selected resonance region at **4.895 ppm**, giving a diffusion coefficient of **$D = 2.514 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$** .

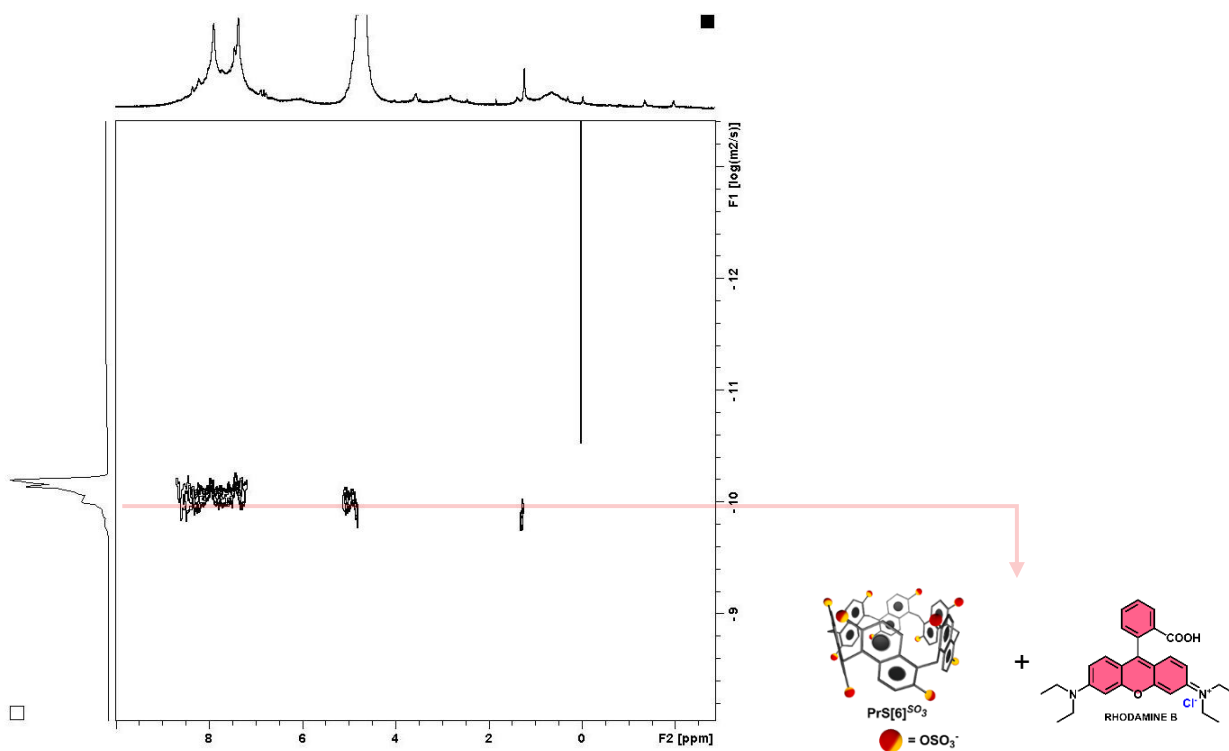


Figure S40. Pseudo-2D DOSY representation of an equimolar (4.17 mM) solution of **RhodB** and $\text{PrS[6]}^{\text{SO}_3^-}$.

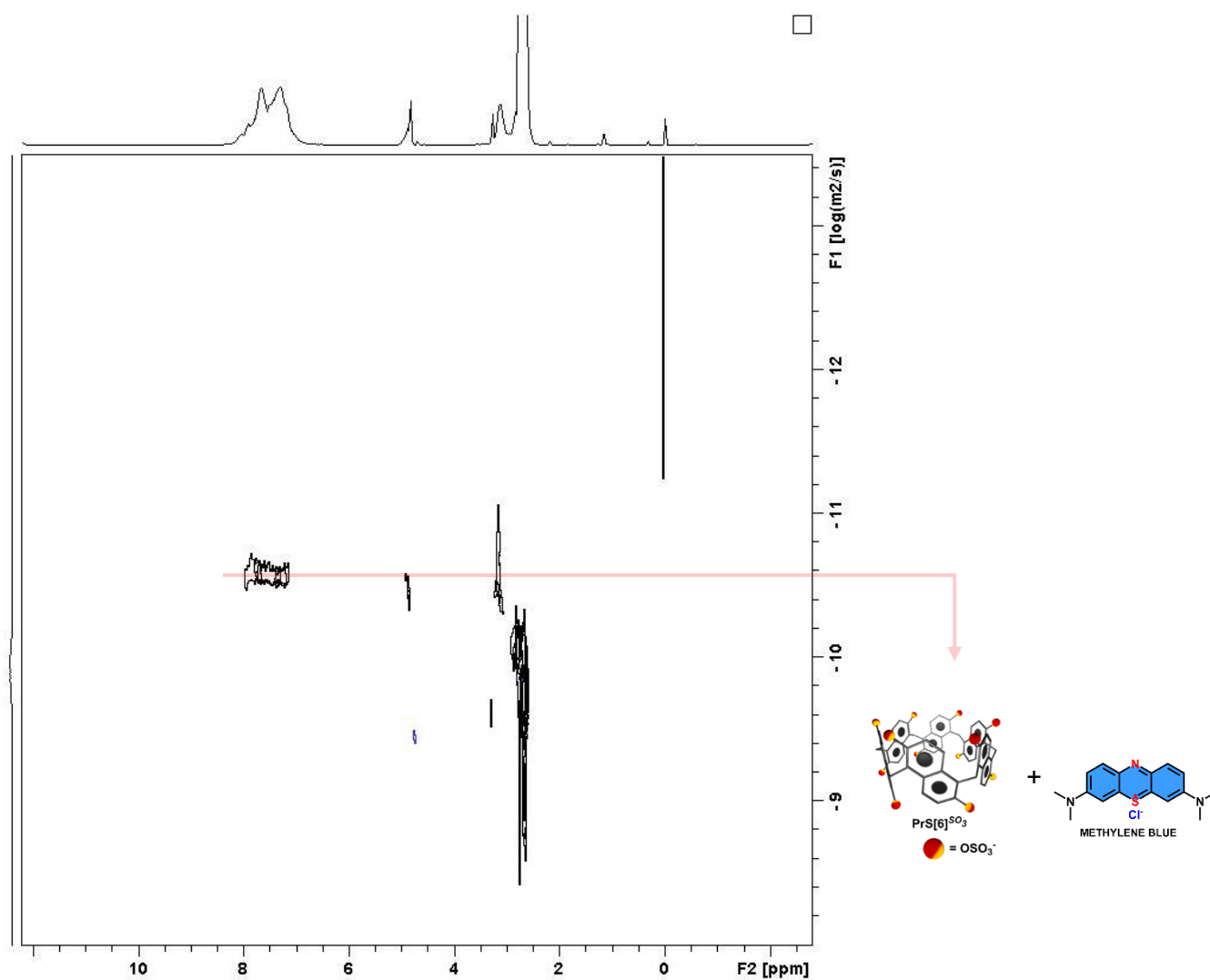


Figure S41. Pseudo-2D DOSY representation of an equimolar (4.17 mM) solution of **MethB** and $\text{PrS[6]}^{\text{SO}_3^-}$.

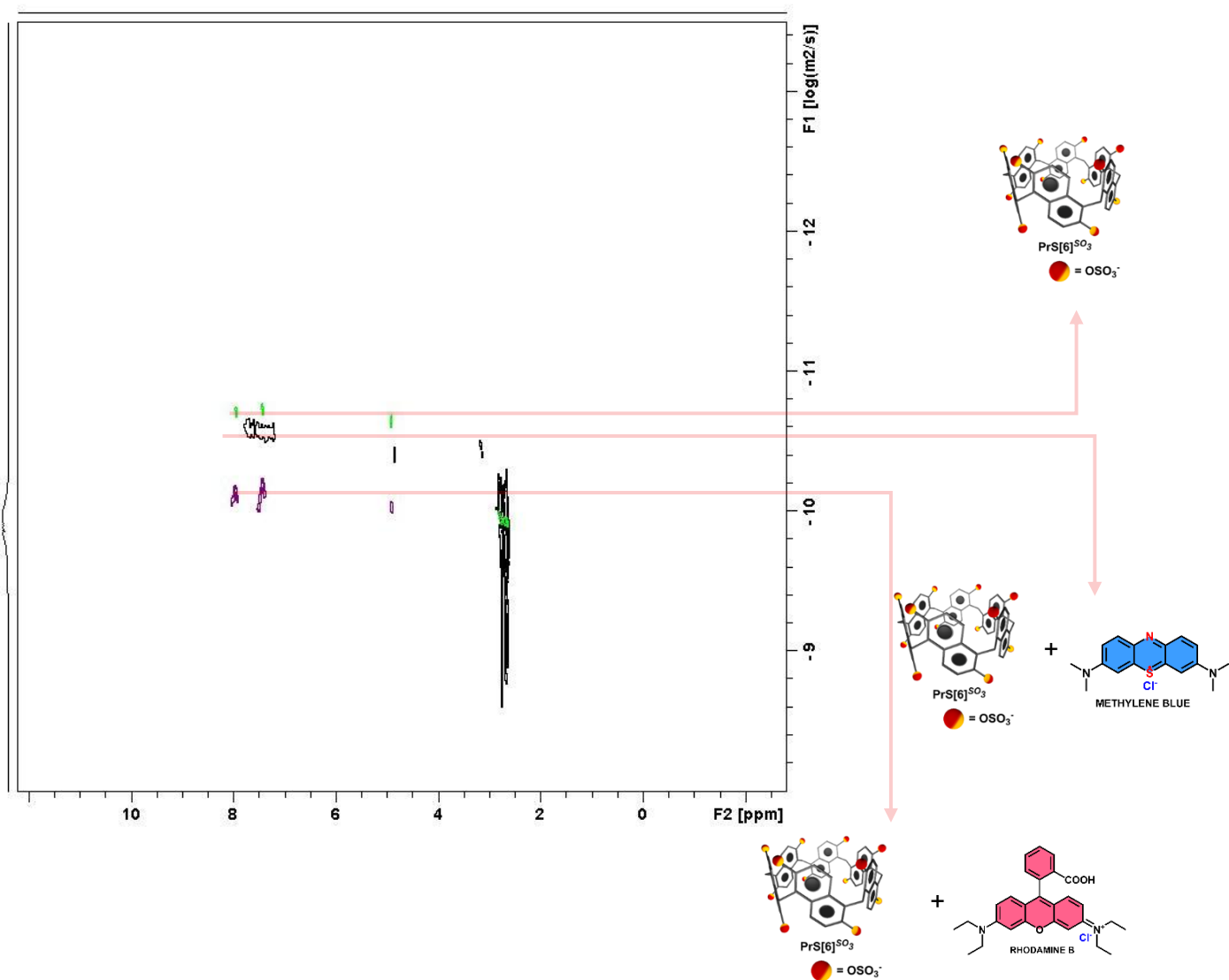


Figure S42. Superimposed pseudo-2D DOSY representations of $\text{PrS[6]}^{\text{SO}_3^-}$ alone (**green**), the **MethB**/ $\text{PrS[6]}^{\text{SO}_3^-}$ mixture (**black**), and the **RhodB**/ $\text{PrS[6]}^{\text{SO}_3^-}$ mixture (**purple**), shown for comparison of the diffusion behavior of the investigated systems.