Electronic Supplementary Information

Molecular Recognition and Photoprotection of Riboflavin in Water by a Biomimetic Host

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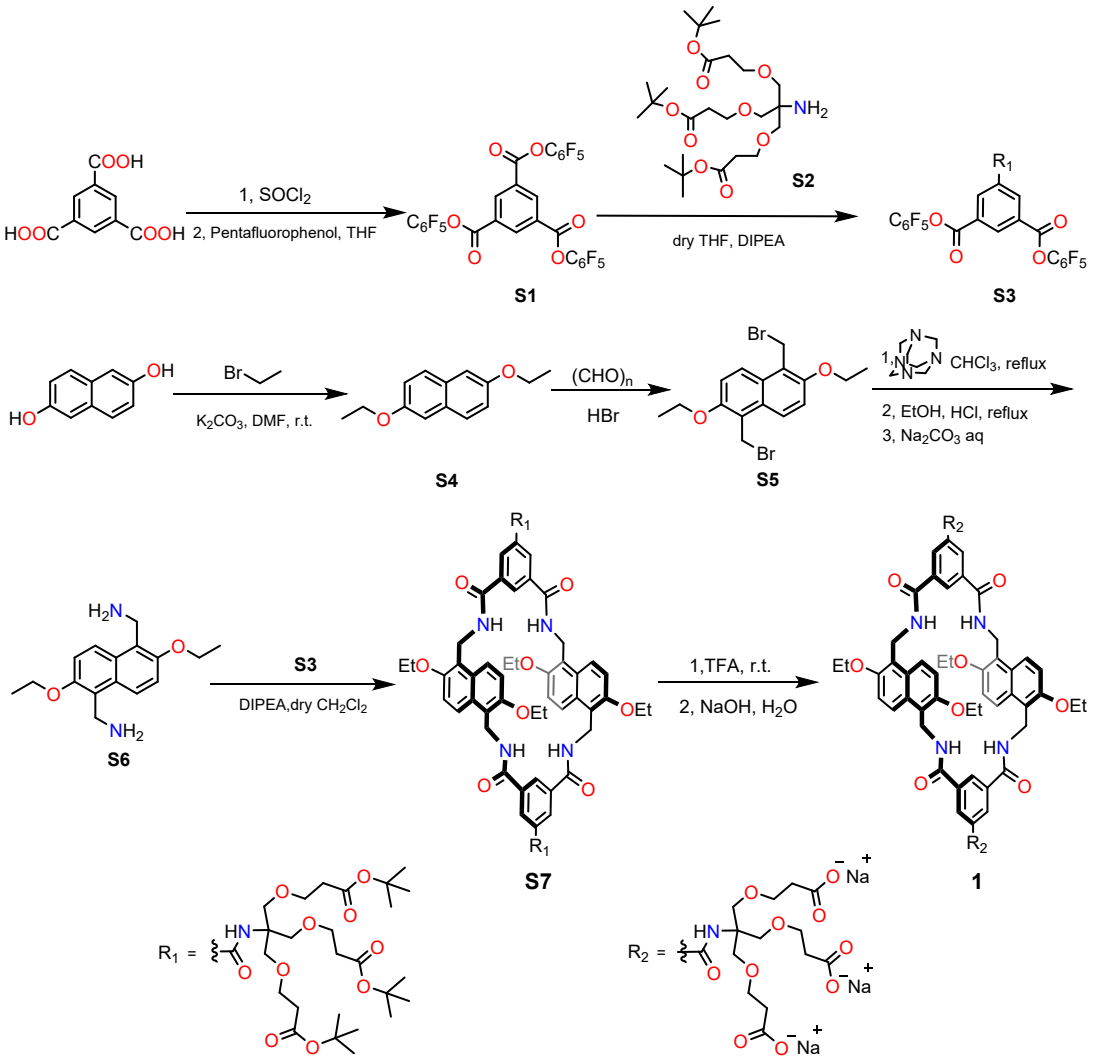
1. Experimental Section

1.1 General. All the reagents involved in this research were commercially available and used without further purification unless otherwise noted. Solvents were either employed as purchased or dried prior to use by standard laboratory procedures. Thin-layer chromatography (TLC) was carried out on 0.25 mm Yantai silica gel plates (60F-254). Column chromatography was performed on silica gel 60 (Tsingdao 40 – 63 nm, 200 – 300 mesh). ^{1}H, ^{13}C NMR spectra were recorded on Bruker Avance-400 or 500 spectrometers. All chemical shifts are reported in *ppm* with residual solvents as the internal standards. The following abbreviations were used for signal multiplicities: s, singlet; d, doublet; t triplet; m, multiplet. Electrospray-ionization time-of-flight high-resolution mass spectrometry (ESI-HRMS) experiments were conducted on an applied Q EXACTIVE mass spectrometry system. Circular dichroism (CD) spectra were recorded on an Applied PhotoPhysics Chirascan CD spectropolarimeter, using a 1 cm quartz cuvette. UV−vis absorption spectra were obtained on a Shimadzu UV-2600 spectrophotometer. Fluorescence spectra (FL) was obtained on a Shimadzu RF-5301pc spectrometer. Isothermal Titration Calorimetry (ITC) experiments were performed in deionized H_2O at 25 °C on a Malvern MicroCal PEAQ-ITC Automated instrument.

Quantum chemistry calculations were performed using Gaussian 09 package.[1] The host-guest complexes have been optimized employing density functional theory (DFT) with dispersion corrected method (ωB97XD)[2]. All other atoms were modeled at the 6-31G(d) level of theory. Geometry optimizations were performed by considering the solvent effects (SMD, water) without applying any geometry Constraints (C1 symmetry). Minima were characterized by the absence of imaginary frequencies.

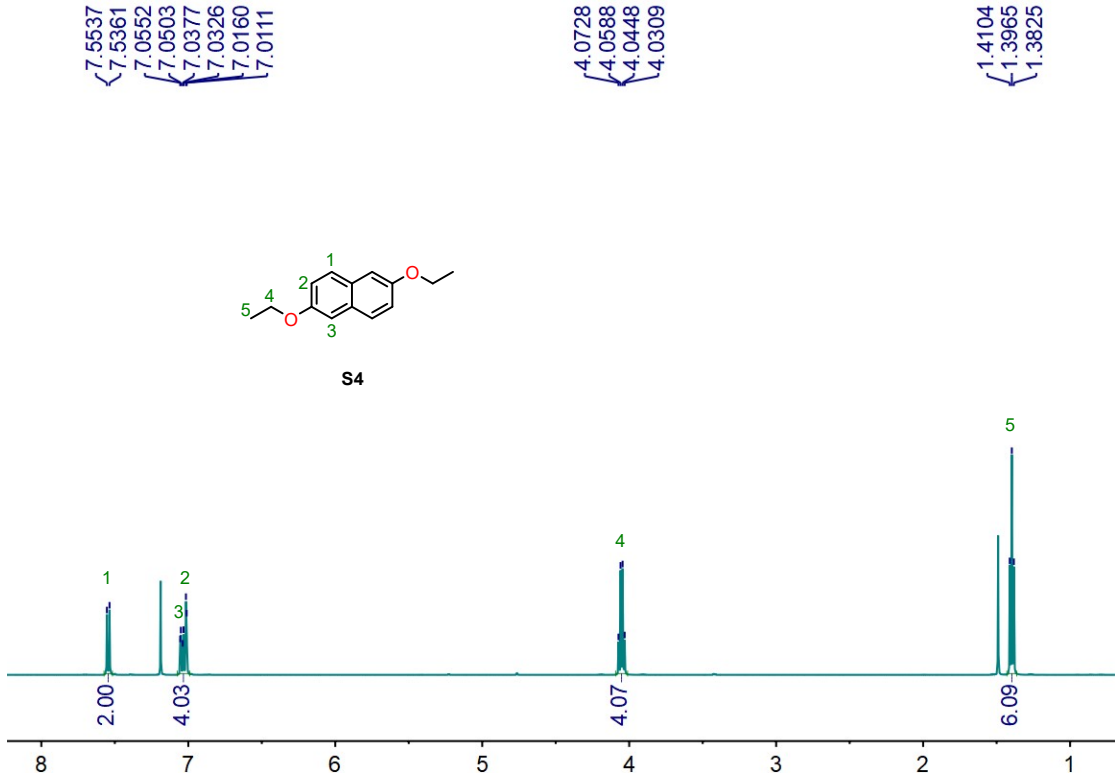
2. Synthetic procedure for Host 1



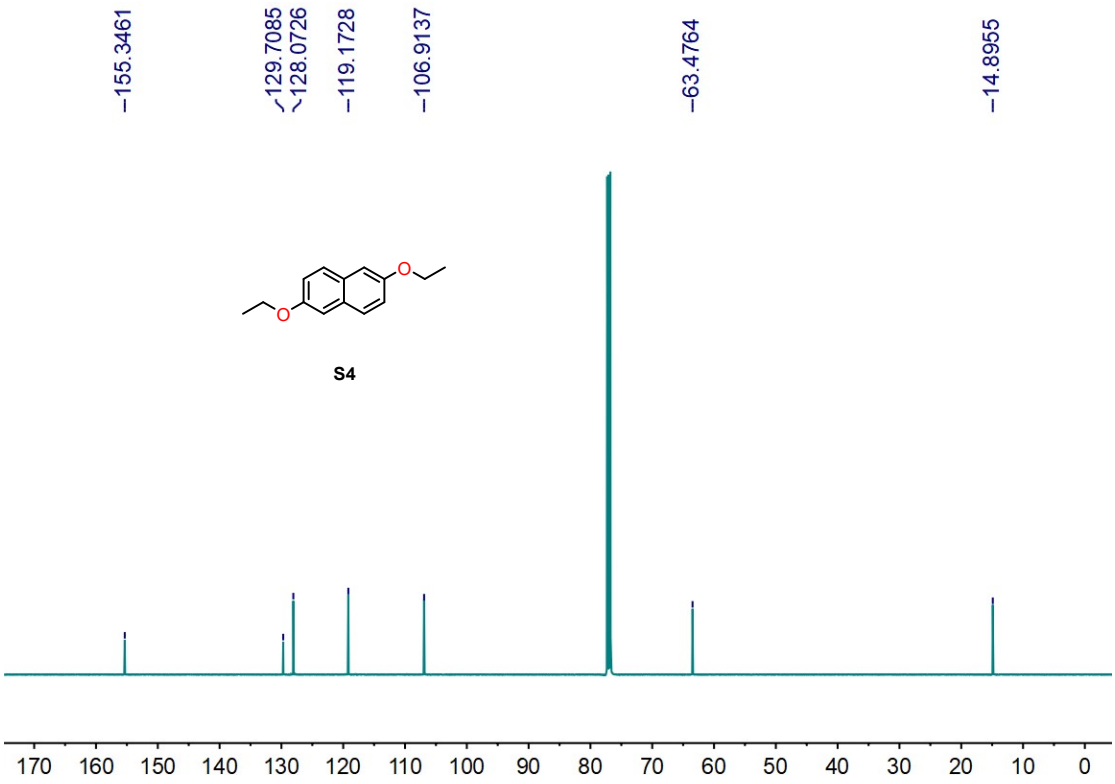
Compound S1, **S2** and **S3** were prepared followed a reported procedure.[3]

Compound S4: 2,6-Dihydroxy naphthalene (16.0 g, 100 mmol), ethyl bromide (43.2 g, 400 mmol), and K_2CO_3 (69.0 g, 500 mmol) were mixed in dry CH_3CN (600 mL). After stirred for 10 h at 80 °C under Argon protection, the solvent was evaporated under reduced pressure, and the residue was poured into H_2O (400 mL), then extracted with dichloromethane (100 mL × 3). The organic phase was collected and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give compound **S4** as off-white solid (16.0 g, 74%).^{1}H NMR (500 MHz, $CDCl_3$,

298 K) δ [ppm] = 7.54 (d, *J* = 8.8 Hz, 2H), 7.08 – 6.99 (m, 4H), 4.05 (q, *J* = 7.0 Hz, 4H), 1.40 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, 298 K) δ [ppm] = 155.35, 129.71, 128.07, 119.17, 106.91, 63.48, 14.90.

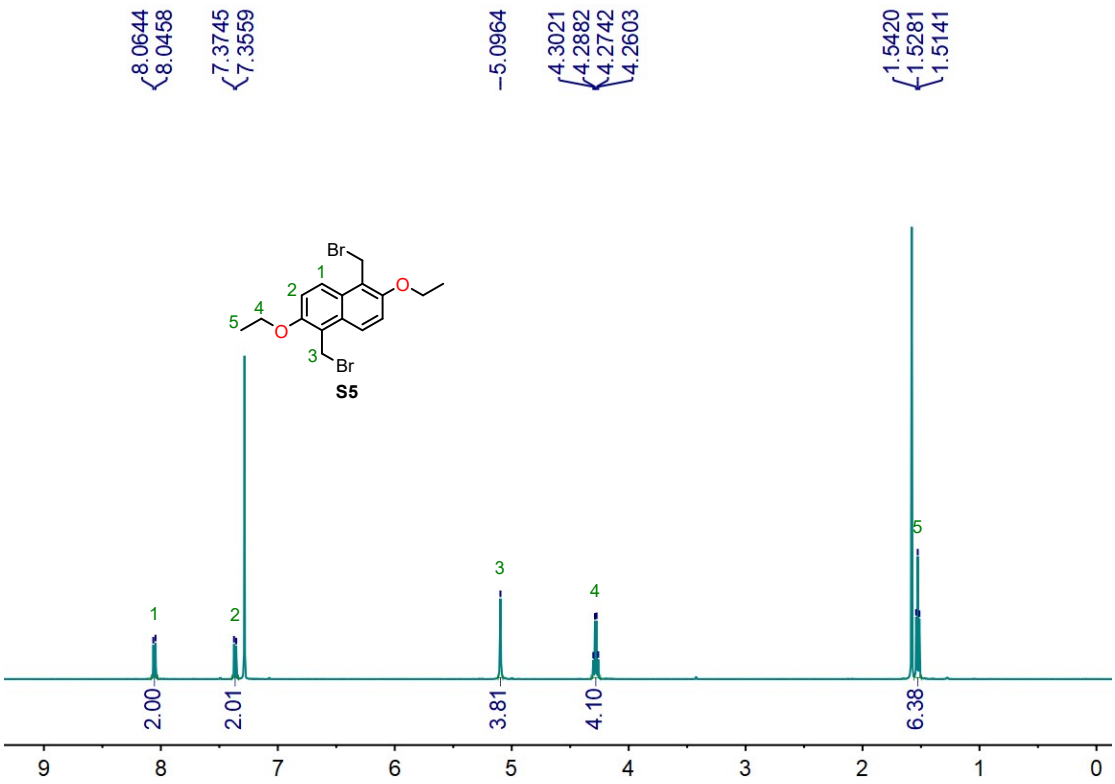


^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **S4**

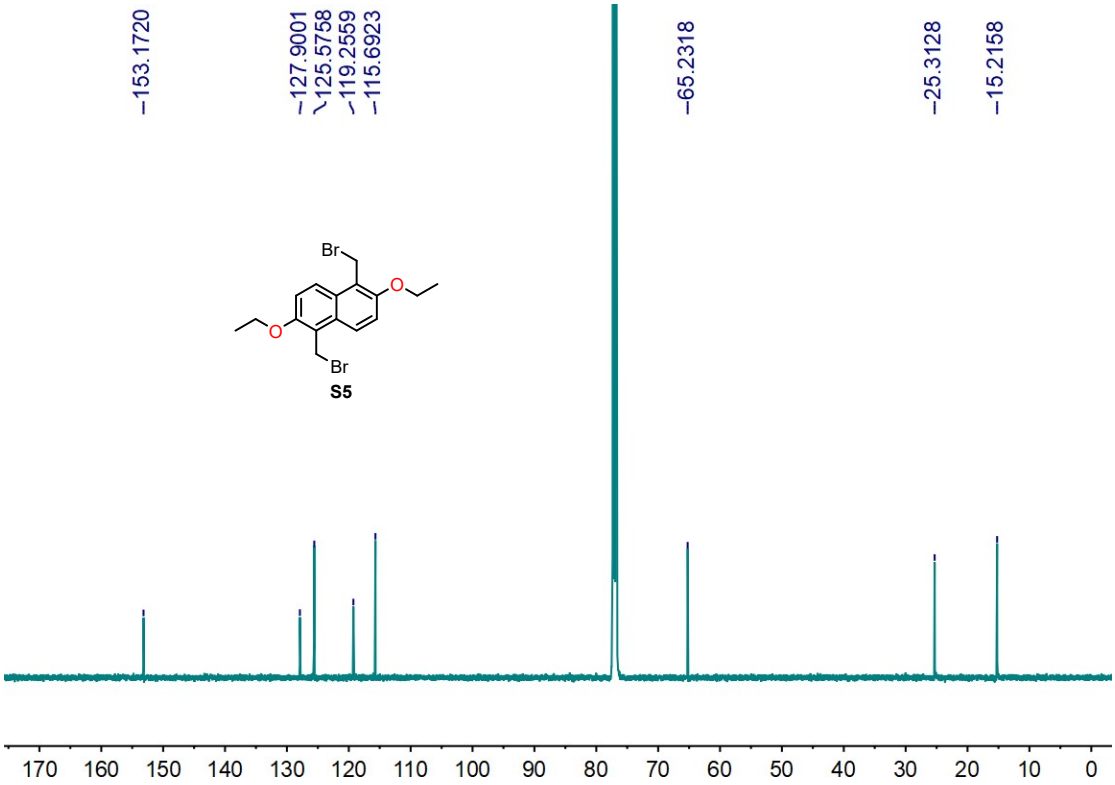


^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **S4**

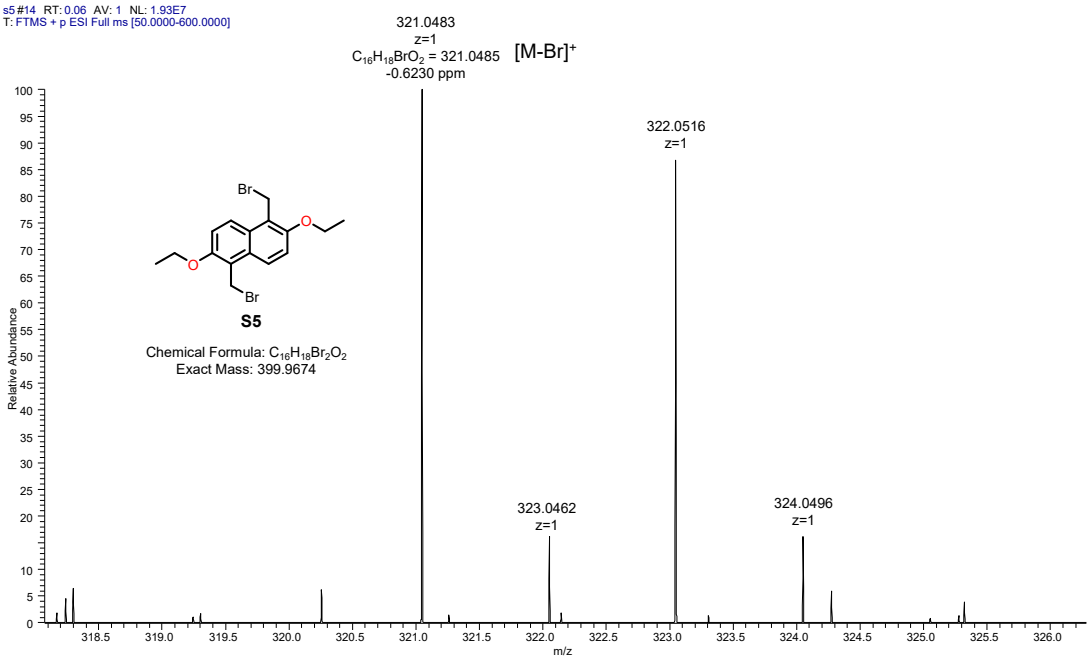
Compound S5: To the solution of mixture of **S4** (6.9 g, 32 mmol) and paraformaldehyde (5.7 g, 170 mmol) in HBr/AcOH (33% w/w, 100 mL). The resulting mixture was heated to 50 °C and stirred for 5 h. The solution was cooled to room temperature. The light purple precipitate was filtered off and washed with copious methanol. The crude product was dissolved in minimal amount of dichloromethane and then added dropwise into methanol (50 mL) during stirring. The precipitate was collected through filtration and dried to afford dibromide **S5** (7.0 g, 55%) as off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ [ppm] = 8.06 (d, J = 9.3 Hz, 2H), 7.37 (d, J = 9.3 Hz, 2H), 5.10 (s, 4H), 4.28 (q, J = 7.0 Hz, 4H), 1.53 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, 298 K) δ [ppm] = 153.17, 127.90, 125.58, 119.26, 115.69, 65.23, 25.31, 15.22. ESI-TOF-HRMS: m/z calcd for $[M\text{-}Br]^+$ $C_{16}H_{18}BrO_2^+$, 321.0485; found 321.0483 (error = -0.6 ppm).



^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **S5**

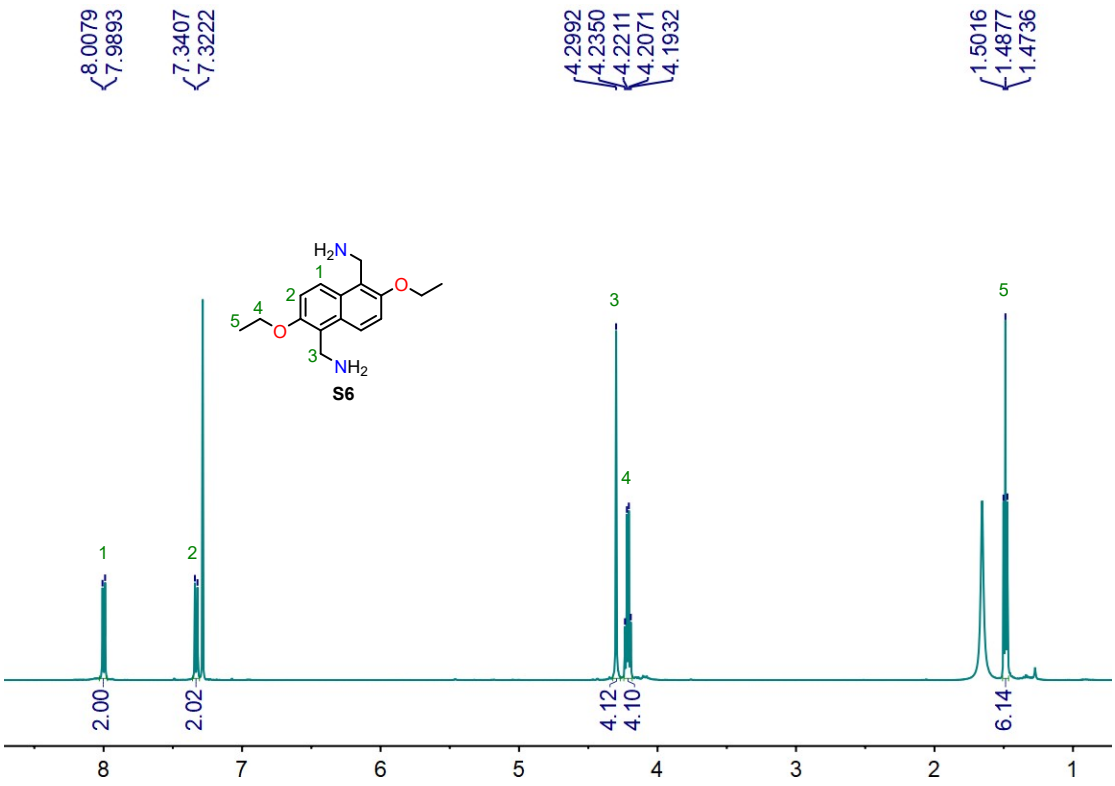


^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **S5**

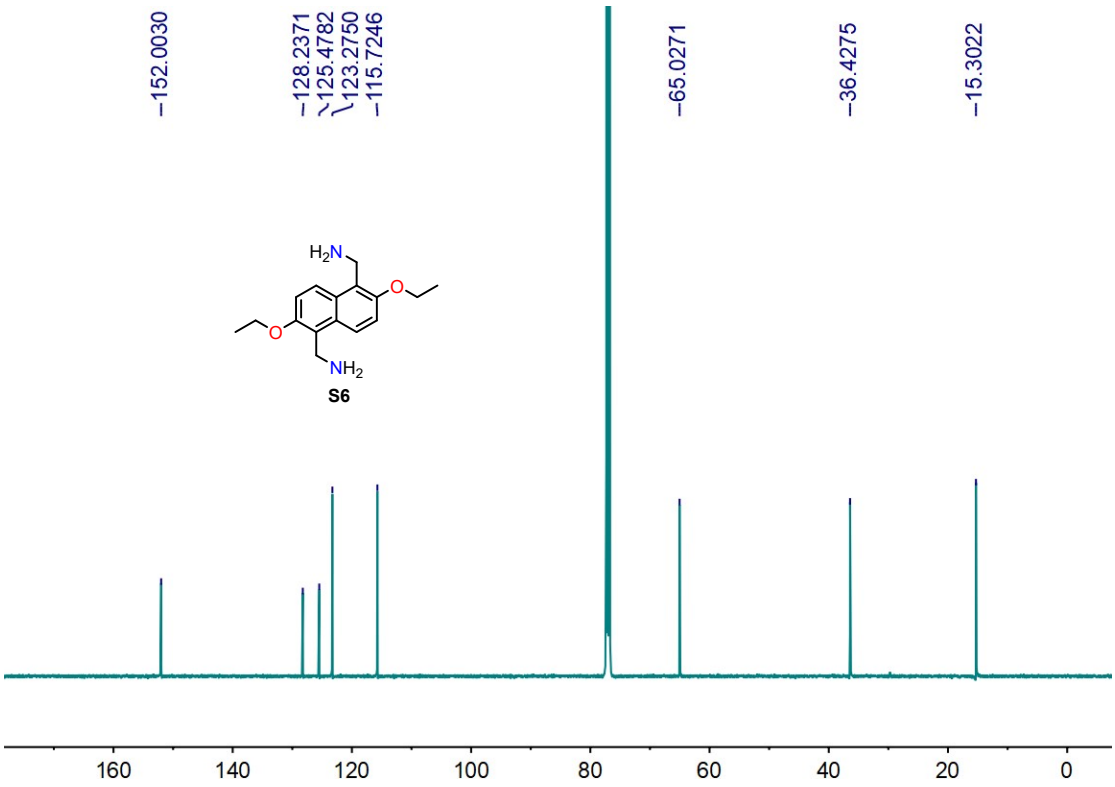


ESI mass spectrum of compound **S5**

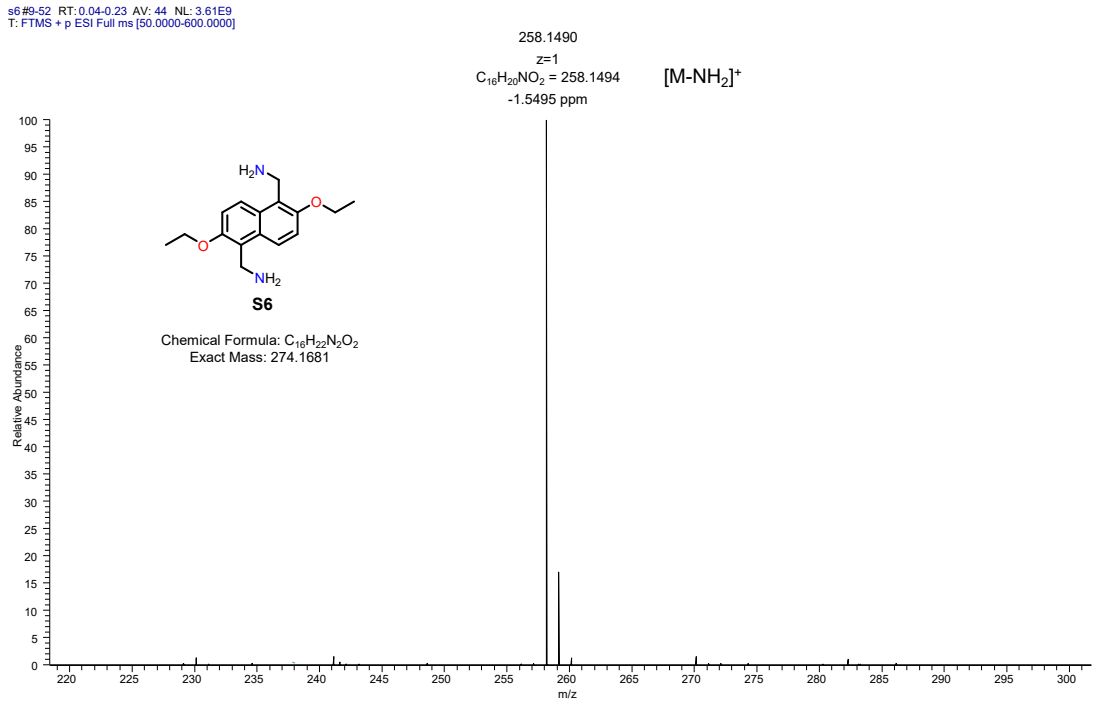
Compound S6: Compound **S5** (4.1 g, 2.7 mmol) and hexamethylenetetramine (4.0 g, 28 mmol) were suspended in chloroform (150 mL) and reaction was refluxed for 48 h. The mixture was allowed to cool and the precipitate was collected by filtration. The solid was air-dried then suspended in ethanol (500 mL). Concentrated HCl aq. (100 mL) was added and the mixture was refluxed for 48 h. The mixture was cooled to 0 ^{o}C, and the resulting precipitate was collected by filtration and washed with cold ethanol. The solid was suspended in Na_2CO_3 aq. (2 M, 250 mL) with stirring. Chloroform (600 mL) was added to extract the resulting amine. The aqueous phrase was extracted with additional chloroform (200 mL). The organic phases were combined and dried over Na_2SO_4. The solvent was removed to give **S6** (2.2g, 77%) as off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ [ppm] = 8.00 (d, J = 9.3 Hz, 2H), 7.33 (d, J = 9.3 Hz, 2H), 4.30 (s, 4H), 4.21 (q, J = 7.0 Hz, 4H), 1.49 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, 298 K) δ [ppm] = 152.00, 128.24, 125.48, 123.27, 115.72, 65.03, 36.43, 15.30. ESI-TOF-HRMS: m/z calcd for $[M\text{-}NH_2]^+$ $C_{16}H_{20}NO_2^+$, 258.1494; found 258.1490 (error = -1.5 ppm).



^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **S6**



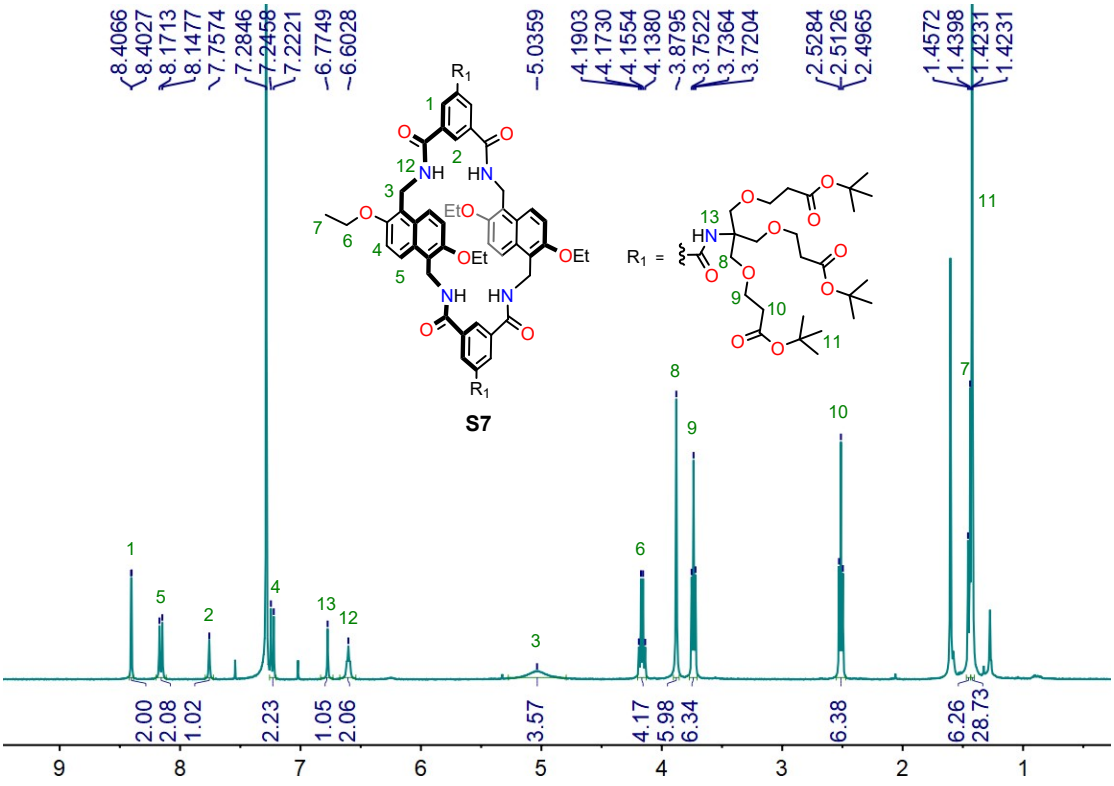
^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **S6**



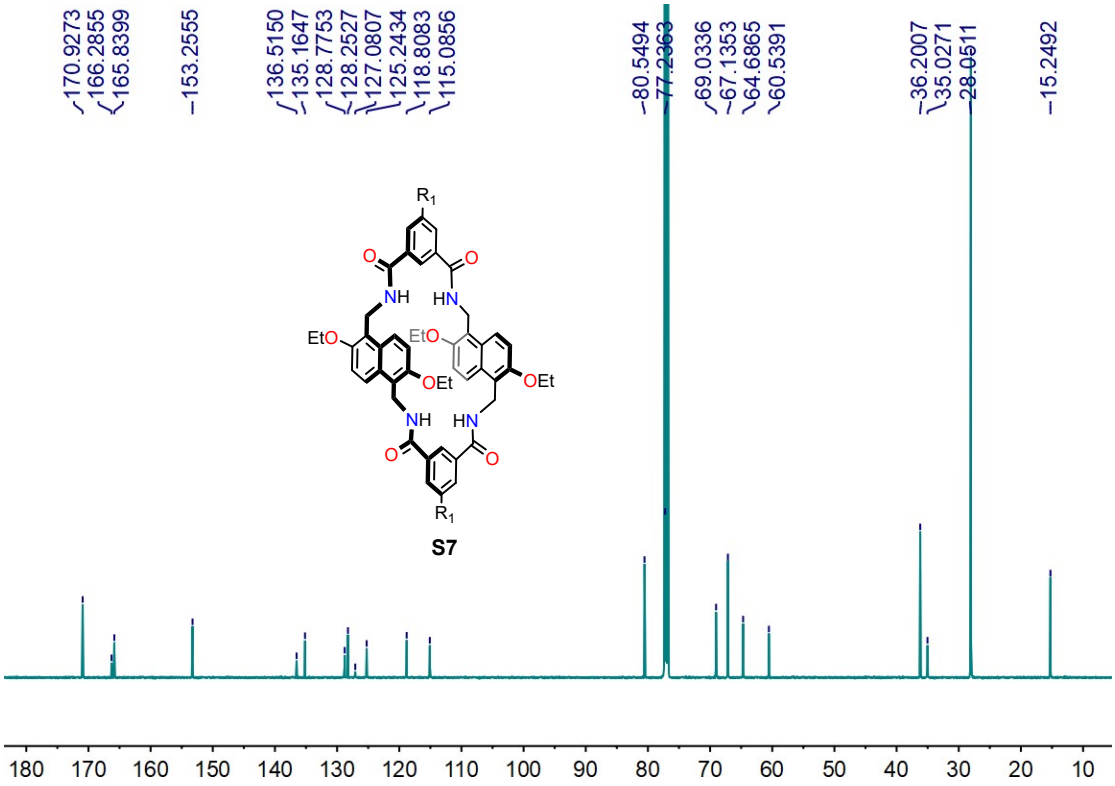
ESI mass spectrum of compound **S6**

Macrocycle S7: A solution of bis-pentafluorophenyl ester **S3** (1.03 g, 1.0 mmol) in anhydrous dichloromethane (50 mL) was added dropwise over 30 h (syringe pump) to a solution of **S6** (274 mg, 1.50 mmol) and DIPEA (5 mL) in anhydrous dichloromethane (500 mL) under nitrogen. After stirring for a further 24 h, the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (100 mL) and washed with water (100 mL) and brine (100 mL). The organic solution was dried over Na_2SO_4 and evaporated in vacuo, then the solid was purified by column chromatography (CH_2Cl_2:MeOH = 100:1) to afford compound **S7** as a white solid (310 mg, yield: 17%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ [ppm] = 8.40 (d, *J* = 1.6 Hz, 2H), 8.16 (d, *J* = 9.4 Hz, 2H), 7.76 (s, 1H), 7.23 (d, *J* = 9.5 Hz, 2H), 6.77 (s, 1H), 6.60 (s, 2H), 5.04 (s, 4H), 4.16 (q, *J* = 6.9 Hz, 4H), 3.88 (s, 6H), 3.74 (t, *J* = 6.4 Hz, 6H), 2.51 (t, *J* = 6.4 Hz, 6H), 1.45 (d, *J* = 7.0 Hz, 6H), 1.42 (s, 29H). ^{13}C NMR (126 MHz, $CDCl_3$, 298 K) δ [ppm] = 170.93, 166.29, 165.84, 153.26, 136.52, 135.16, 128.78, 128.25, 127.08, 125.24, 118.81, 115.09, 80.55, 77.24, 69.03, 67.14, 64.69,

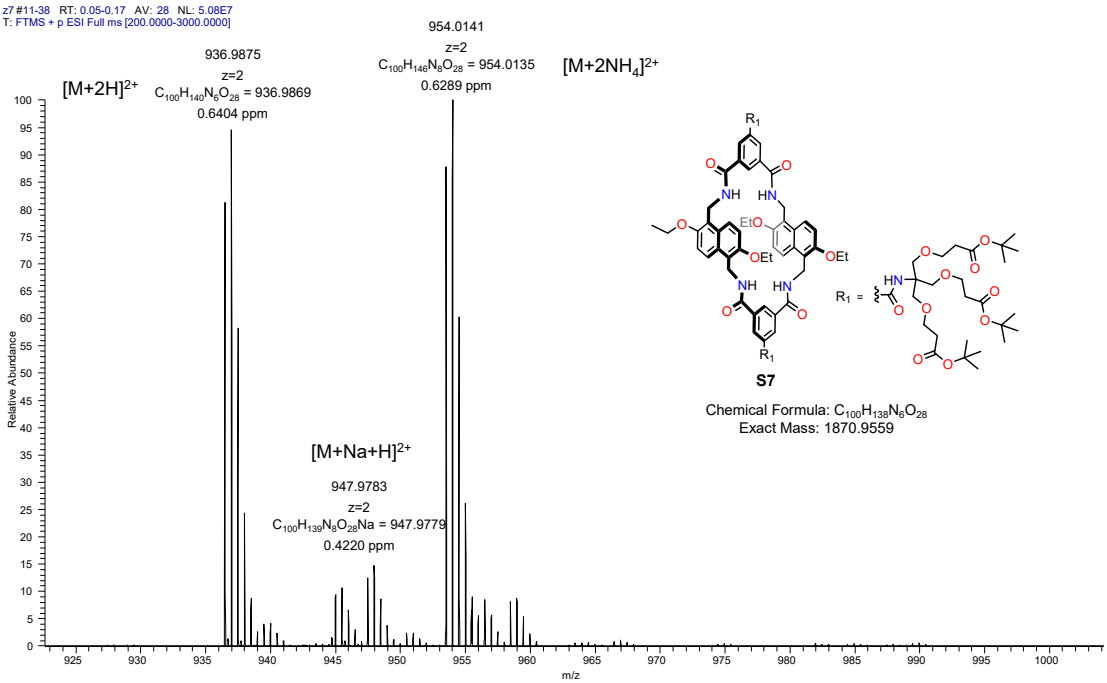
60.54, 36.20, 35.03, 28.05, 15.25. ESI-TOF-HRMS: m/z calcd for $[M+2H]^{2+}$ $C_{100}H_{140}N_6O_{28}^{2+}$, 936.9869; found 936.9875 (error = 0.6 ppm).



^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **S7**

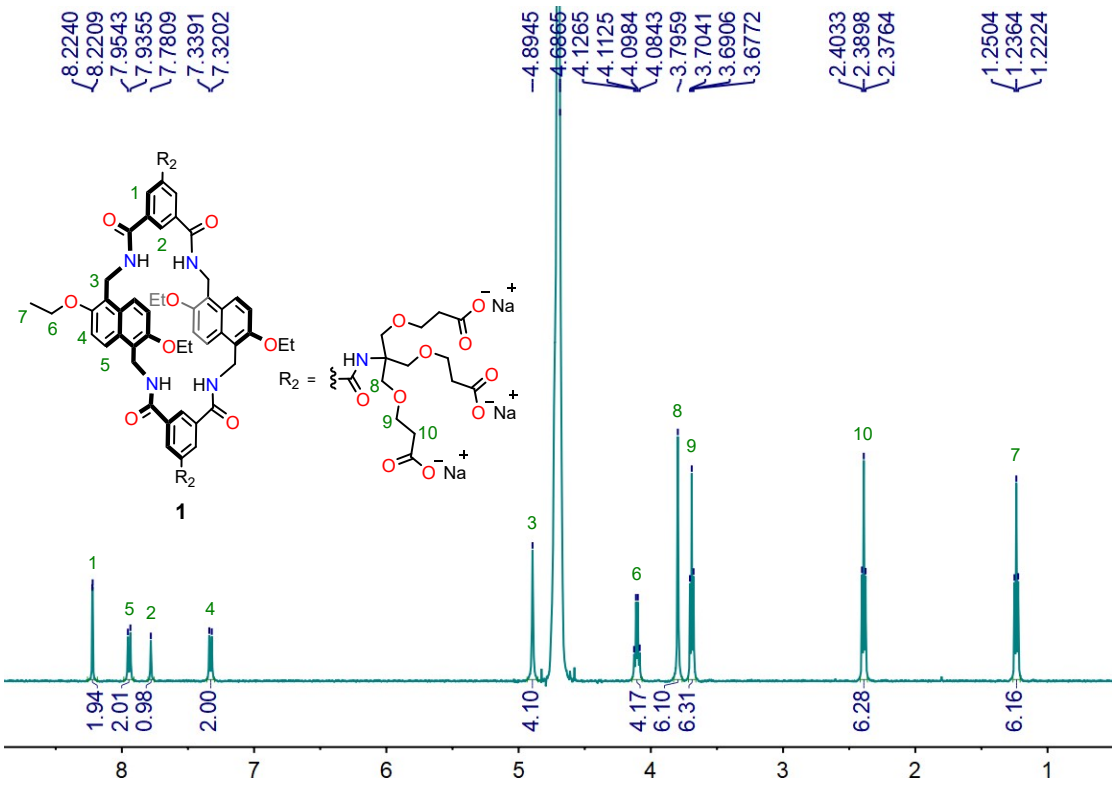


^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **S7**

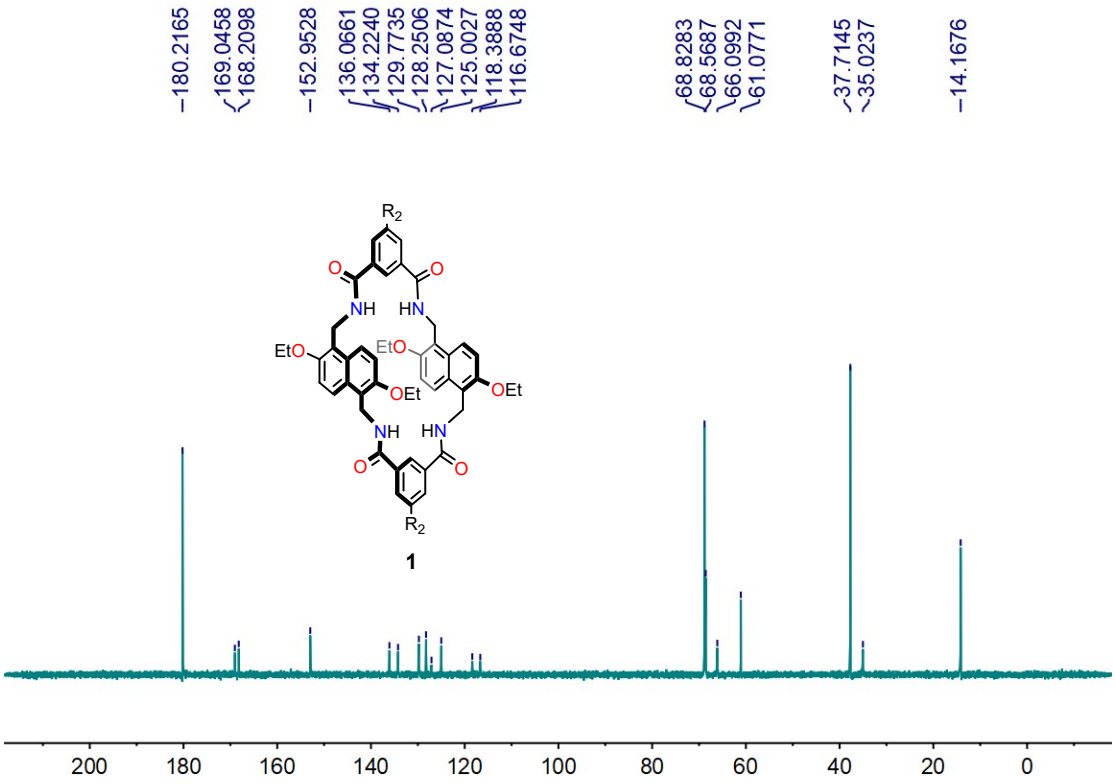


ESI mass spectrum of compound **S7**

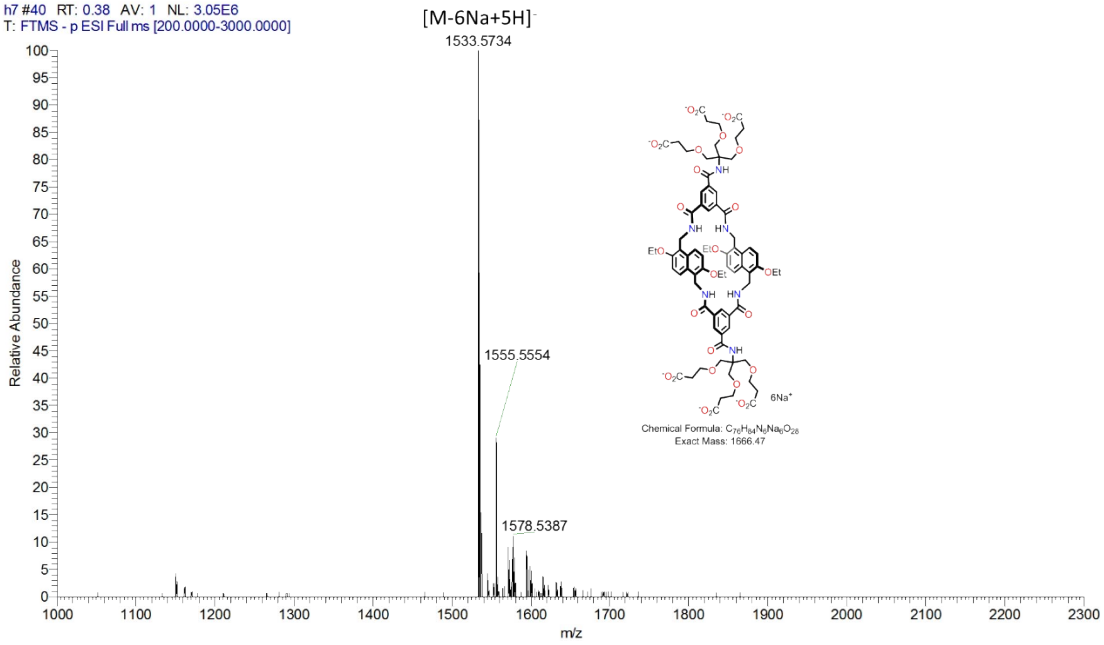
Macrocycle 1: Macrocycle **S7** (100 mg, 0.05 mmol) was dissolved in dichloromethane (20 mL) and cooled in ice water. Trifluoroacetic acid (5 mL) was added dropwise to the solution. The reaction was allowed to warm to room temperature and stirred for 3 h. The dichloromethane was removed in vacuo, and the precipitate was obtained by filtration. The solid was washed with water and then dissolved in NaOH solution. The solution was freeze-dried to yield macrocycle **1** (63 mg, yield: 71%). ^{1}H NMR (500 MHz, D_2O, 298 K) δ [ppm] = 8.22 (d, J = 1.6 Hz, 2H), 7.94 (d, J = 9.4 Hz, 2H), 7.78 (s, 1H), 7.33 (d, J = 9.4 Hz, 2H), 4.89 (s, 4H), 4.11 (q, J = 7.0 Hz, 4H), 3.80 (s, 6H), 3.69 (t, J = 6.7 Hz, 6H), 2.39 (t, J = 6.7 Hz, 6H), 1.24 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, D_2O, 298 K) δ [ppm] = 180.22, 169.05, 168.21, 152.95, 136.07, 134.22, 129.77, 128.25, 127.09, 125.00, 118.39, 116.67, 68.83, 68.57, 66.10, 61.08, 37.71, 35.02, 14.17. ESI-TOF-HRMS: m/z calcd for [M-6Na+5H]$^-$ $C_{76}H_{89}N_6O_{28}^-$, 1533.5730; found 1533.5734 (error = 0.3 ppm).



^{1}H NMR spectrum (500 MHz, D_2O, 298 K) of **1**



^{13}C NMR spectrum (126 MHz, D_2O, 298 K) of **1**



ESI mass spectrum of compound **1**

3. Energy-Minimized Structures

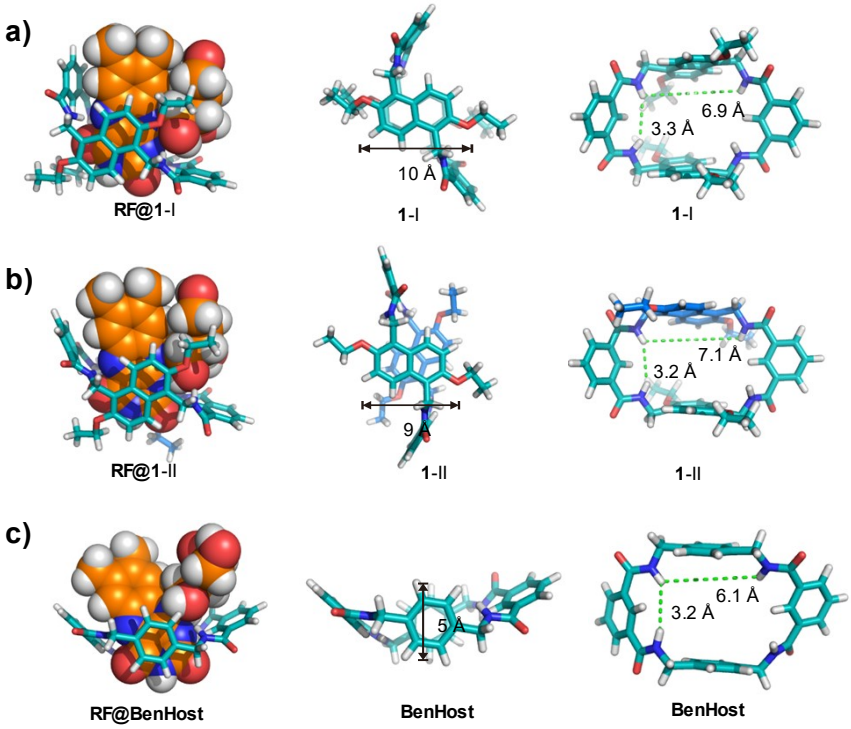


Fig. S1. Energy-minimized structures of **1-I@RF** (a) and **1-II@RF** (b), **RF** was omitted for showing the cavity in the most expanded forms for **1-I** and **1-II** in middle and right images. c) Energy-minimized structure of host-guest complex of a similar tetralactam macrocycle with phenyl spacers (**BenHost**) with **RF**, **RF** was omitted for showing the cavity in the most expanded form in middle and right images. As shown in ***Fig. S1a-1c***, the tetralactam macrocycle **BenHost** has obviously narrower and shallower cavity than macrocycle with naphthalene spacers. **RF** is only partly encapsulated in the cavity, which is in contrast to the good encapsulation in the cavity of naphthalene macrocycle **1**. This suggests that the larger cavity of naphthalene macrocycle **1** is better fit to riboflavin.

4. Mass Spectra of the Complex

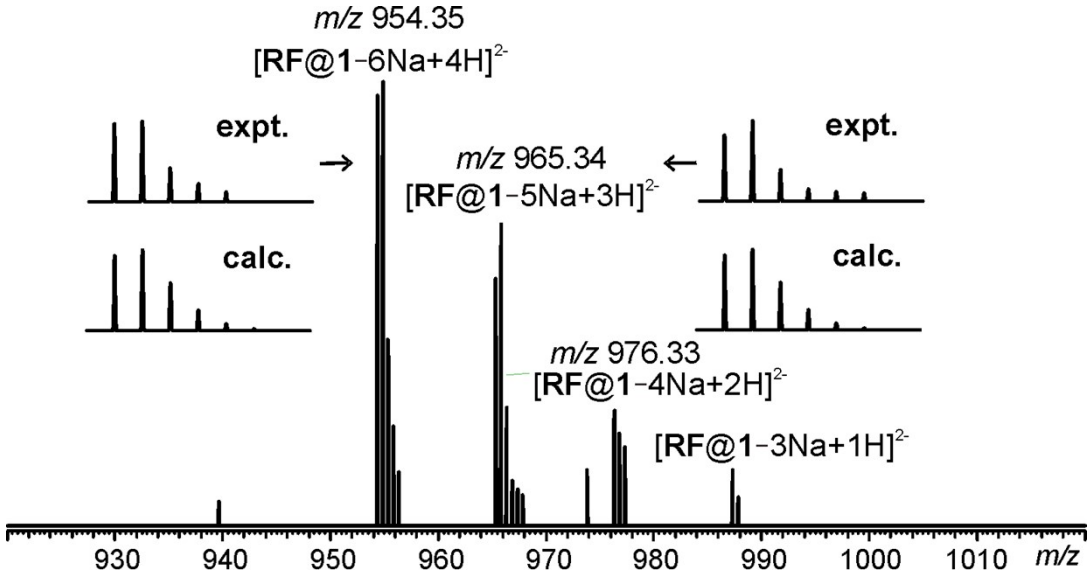


Fig. S2. ESI mass spectrum of **RF@1**. The result indicates **RF** and **1** form a 1:1 complex.

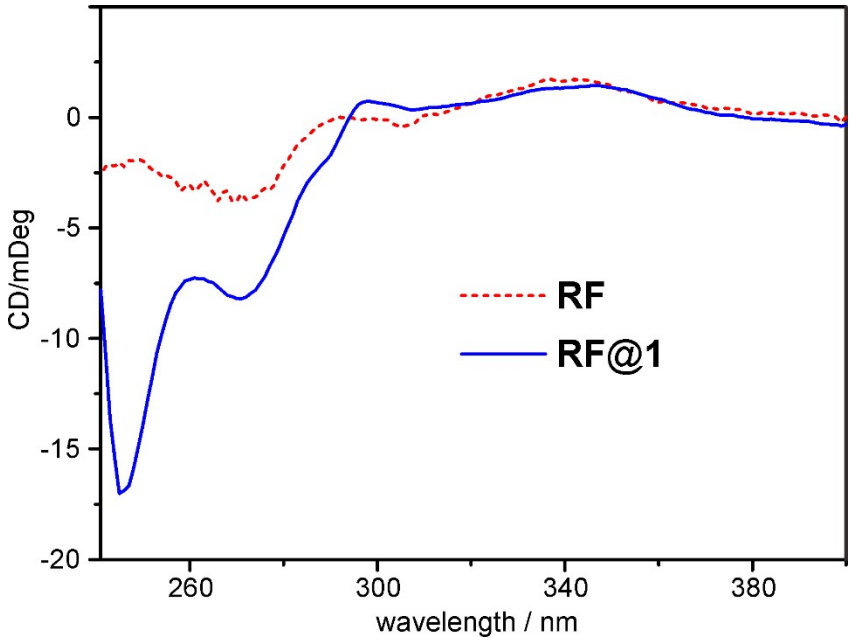


Fig. S3. CD spectra of riboflavin (26 μM, H_2O) and 1:1 mixture of host **1** and riboflavin.

5. Photoprotection Control Experiments

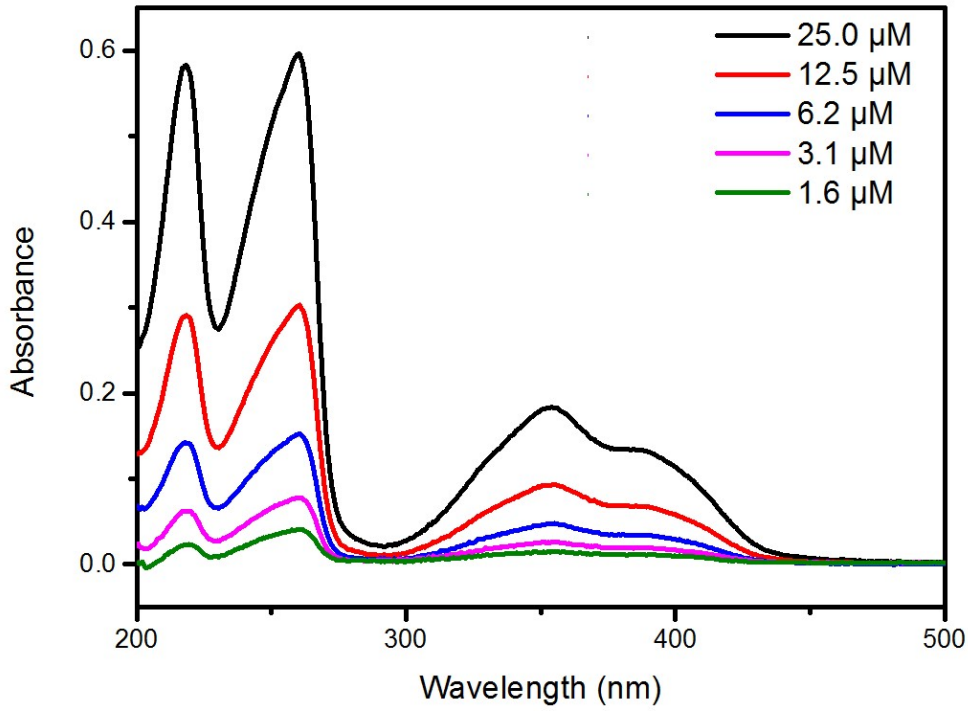


Fig. S4. UV-Vis spectra of Lumichrome with different concentrations in PB buffer (pH 7.4) at 25 °C.

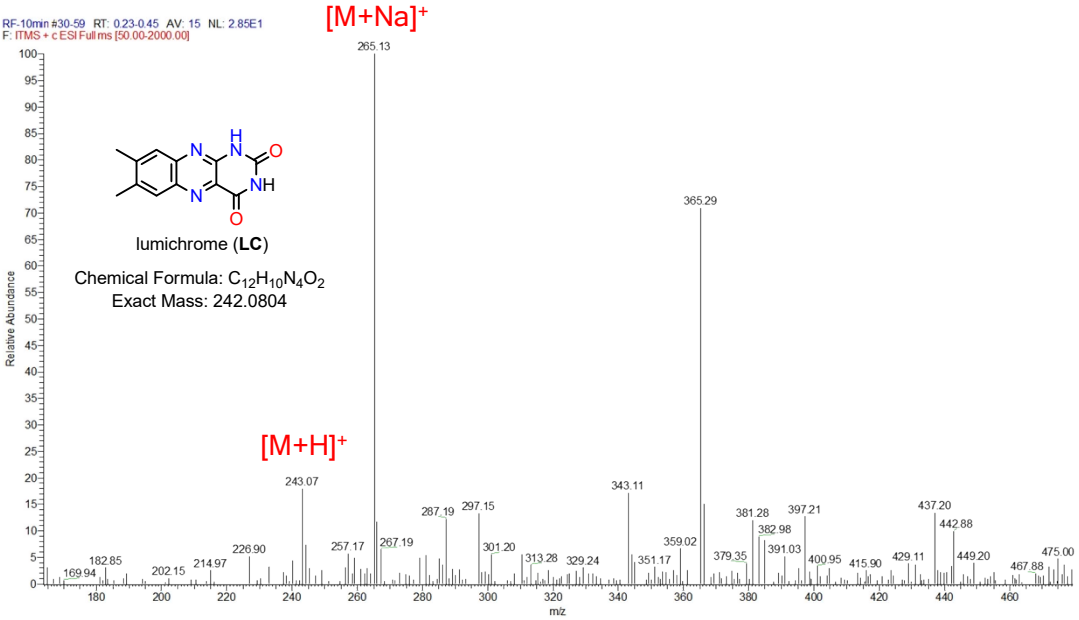


Fig. S5. ESI mass spectrum of riboflavin after exposing to UV light in PB buffer (pH 7.4).

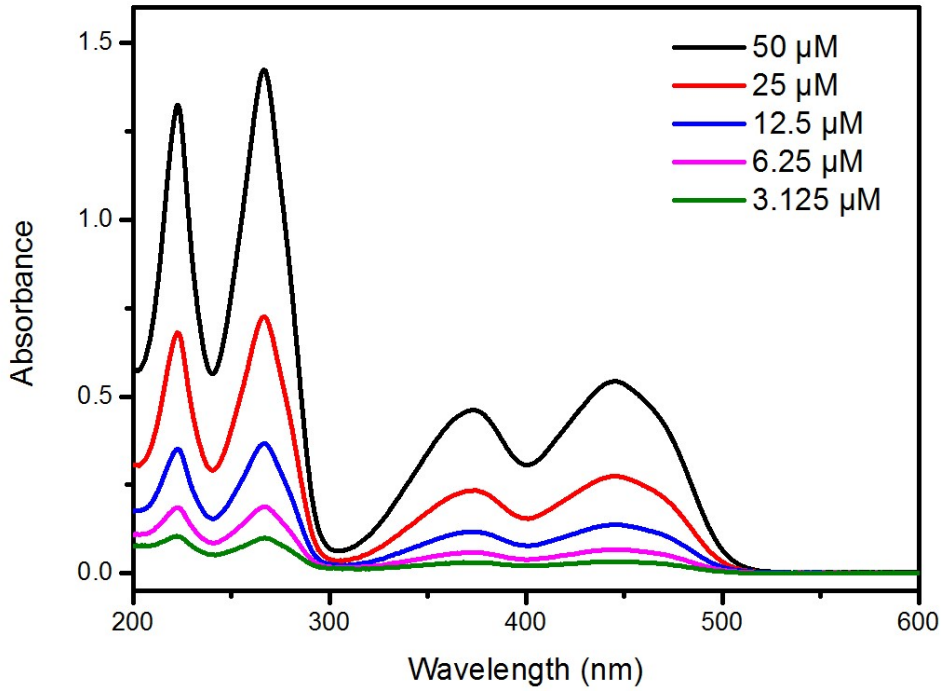


Fig. S6. UV-Vis spectra of riboflavin with different concentrations in PB buffer (pH 7.4) at 25 °C.

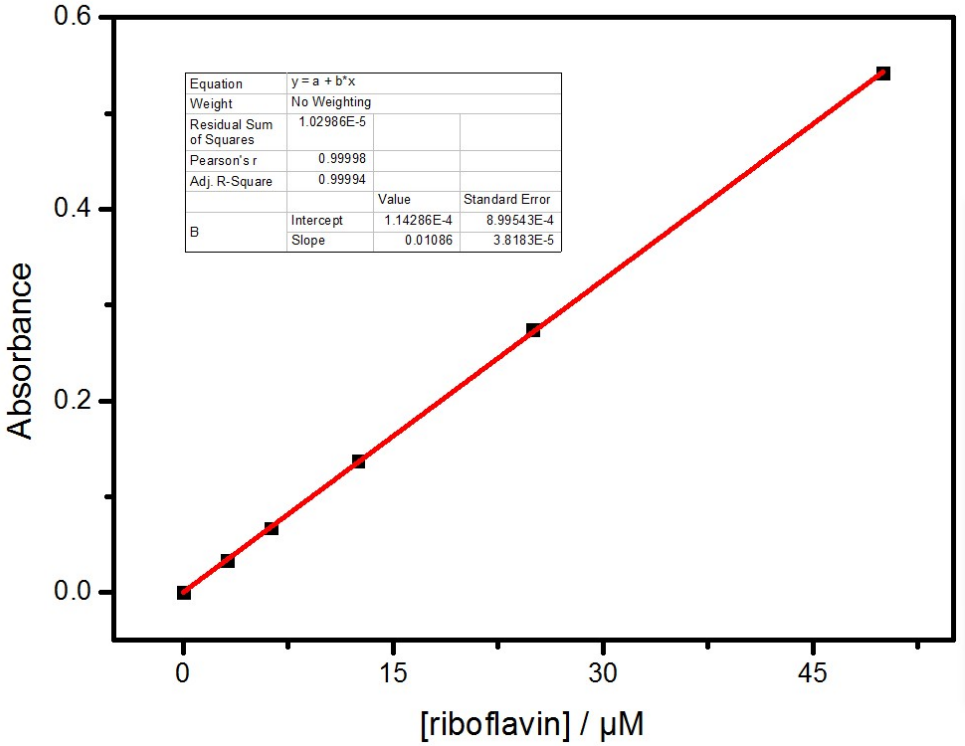


Fig. S7. Calibration curve of riboflavin plotted based on the absorbance at 444 nm in ***Fig. S4.***

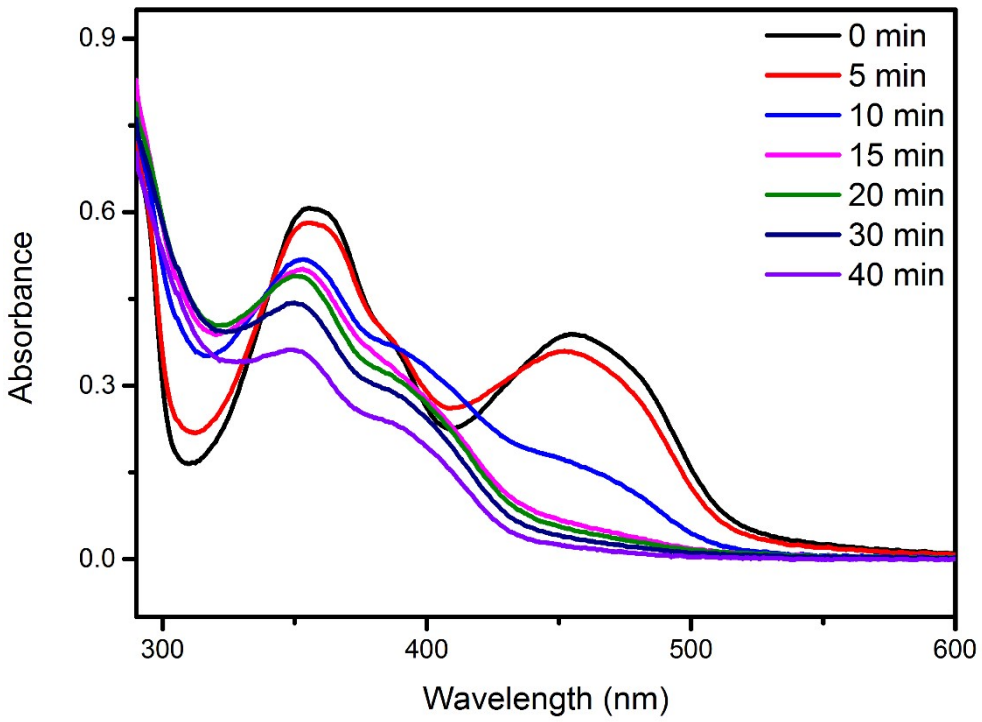


Fig. S8. UV−vis spectra of riboflavin (50 μM) in the presence of host **1** (1.0 equiv) in PB buffer (pH 7.4) under constant light irradiation at 365 nm.

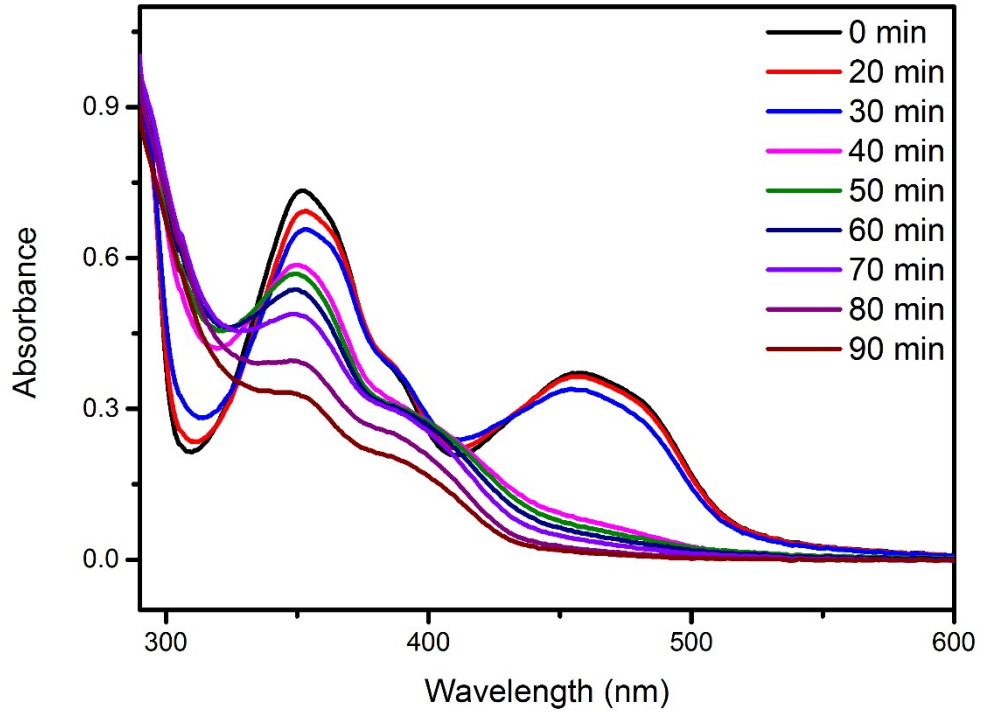


Fig. S9. UV−vis spectra of riboflavin (50 μM) in the presence of host **1** (1.5 equiv) in PB buffer (pH 7.4) under constant light irradiation at 365 nm.

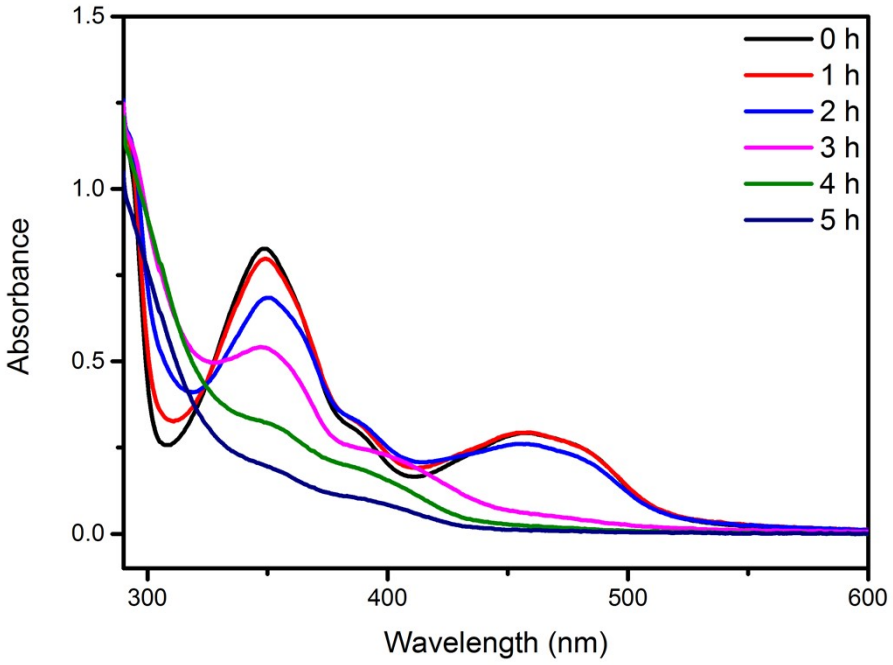


Fig. S10. a) UV−vis spectra of riboflavin (50 μM) in the presence of host **1** (2.0 equiv) in PB buffer (pH 7.4) under constant light irradiation at 365 nm.

6. References

1. *Gaussian 09; Revision D.01;* Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Li, M.; Caricato, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.;. Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Gaussian, Inc.: Wallingford, CT, USA, 2013.

2. (a) J.-D. Chai, M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615; (b) A. V. Marenich, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B.*, 2009, **113**, 6378.

3. E. Klein, M. P. Crump A. P. Davis, *Angew. Chem. Int. Ed.*, 2005, **44**, 298.