# Electronic Supplementary Information

## A pillar[5]arene-based three-component supramolecular copolymer for the fluorescence detection of spermine

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## Experimental section



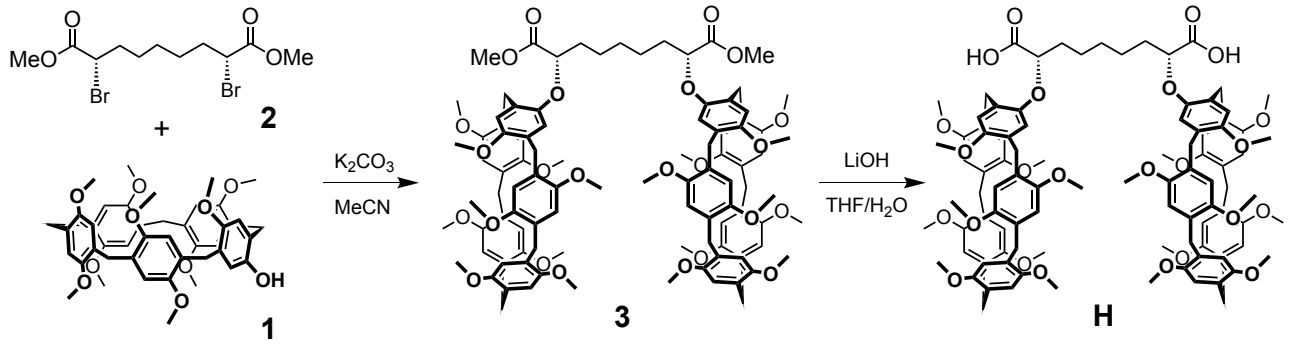
## Additional figures

**General**

$^{1}$H NMR spectra (500 MHz) were recorded at 298 K in either in $CDCl_3$ or $CDCl_3$/TFE, 97:3, v/v. Chemical shifts are reported in ppm and are referenced to the residual solvent (7.26 ppm). $^{13}$C NMR spectra were recorded at 25 °C in $CDCl_3$, at 125 MHz. $^{1}$H NMR spectra in $CDCl_3$/TFE, 97:3, v/v were recorded using solvent suppression pulse sequences (PRESAT) irradiating at $\delta$ = 3.92 ppm ($CF_3C\underline{H_2}OH$). Chemical shifts are reported in ppm and are referenced to the residual solvent (77.0 ppm). Where present, $^{1}$H NMR peak assignments follow from COSY experiments. Mass spectra were measured on an ion trap electrospray instrument. ESI(+)-MS measurements were performed on a triple-quadrupole mass spectrometer equipped with an electrospray ion source, using $CHCl_3$ as the solvent. Anhydrous solvents were either obtained commercially or dried by standard methods prior to use, while other chemicals were reagent grade, routinely used without any further purification. Column chromatography was performed on silica gel (Merck, 230–400 mesh).

All MS experiments were performed using the LTQ XL mass spectrometer, equipped with H-ESI II source, (ThermoFisher, San Jose, CA, USA) in full scan mode from m/z 150–2000 range. All measurements were performed in positive mode with a spray voltage of 2–3 kV. The capillary temperature was set to 250 °C, the capillary voltage to 20 V and the tube lens to 120 V. Ion trap CID (collision induced dissociation) measurements was performed with a precursor ion selection window of 1–2 m/z with an activation time of 30 ms and helium as collision gas. External calibration was performed using the Pierce LTQ ESI Positive Ion Calibration Solution. Data proceeding was performed using the FreeStyle Software ver. 1.6 SP1 (Thermo Fisher Scientific).

Photophysical studies were conducted in air-equilibrated solutions at room temperature using quartz cuvettes with a 1.0 cm path length. The UV/Vis absorption spectra were recorded on a Jasco V-560 spectrophotometer. Luminescence measurements were performed using a Jobin Yvon-Spex Fluoromax P spectrofluorimeter, equipped with a Hamamatsu R3896 photomultiplier. To measure the luminescence lifetimes, an Edinburgh OB 900 time-correlated single-photon-counting spectrometer was used, equipped with a Hamamatsu PLP 2 laser diode (59 ps pulse width at 408 nm) as the excitation source. The uncertainties in the lifetimes are approximately 10%, and the absorption spectra have an uncertainty of 2 nm. Time-resolved transient absorption experiments were conducted using a pump-probe setup with a Spectra-Physics MAI-TAI Ti laser system as the light source and an Ultrafast Systems Helios spectrometer as the detector. The laser beam output was divided into pump and probe beam pulses using a beam splitter (85% for the pump and 15% for the probe). The pump pulse (400 nm, 1–4 μJ) was generated with a Spectra-Physics 800 FP OPA and focused onto the sample cuvette. The probe beam was delayed using a computer-controlled motion controller and then focused into a 2-mm sapphire plate to produce a white light continuum (spectral range 450–800 nm). This white light was subsequently overlapped with the pump beam in a 2-mm quartz cuvette containing the sample. The effective time resolution was approximately 200 fs, with a temporal chirp of about 150 fs over the 450–750 nm range; the optical delay stage had a temporal window of 0–3200 ps. All transient spectra presented in this paper are chirp-corrected, using the pump-induced absorption signals under identical conditions (same cuvette, solvent, temperature, stirring frequency, etc.) for each experiment. The time-resolved data were analysed using the Ultrafast Systems Surface Explorer Pro software.




**Scheme S1**. Synthesis of bis-pillar[5]arene dicarboxylic acid **H**.

***meso*-Bis-pillar[5]arene dimethyl diester (3)**

Nonamethyl-pillar[*5*]arene **1**[S1] (200 mg, 0.27 mmol), *meso*-2,8-nonanedioic dimethyl ester **2**[S2] (111 mg, 0.27 mmol) and $K_2CO_3$ (47 mg, 0.33 mmol) were suspended in MeCN (30 mL). The reaction mixture was heated to reflux under a $N_2$ atmosphere. After a week the reaction was completed. After cooling, the solvent was evaporated under reduced pressure, and the resulting crude mixture, dissolved in $CH_2Cl_2$ (20 mL). was washed with 1 M HCl (3 x 15 mL). The resulting organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude was then purified by crystallization ($CHCl_3/CH_3OH$) giving **3** (100 mg, 46%). M.p. 160 °C (dec). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.83, 6.82, 6.802, 6.797, 6.73, 6.70, 6.68, 6.62, 6.41 (9×s, ratio 2:1:1:1:1:1:1:1:1, ArH, 20 H), 4.39 (t, *J* = 6.4 Hz, OCH, 2 H), 3.90–3.58 (m, $ArCH_2Ar$, 20 H), 3.75, 3.74, 3.71, 3.68, 3.64, 3.63, 3.582, 3.580, 3.56, 3.55 (10×s, ratio 1:1:1:1:1:3:1:1, $OCH_3$, 60 H), 1.84–1.79 (m, $CHCH_2$, 4 H), 1.66–1.40 (m, $CH_2$, 6 H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 172.4, 151.1, 150.7, 150.6 (×3), 150.5 (×3), 150.3, 149.3, 128.9, 128.8, 128.5, 128.4, 128.3, 128.1 (×2), 128.0, 127.9, 127.7, 115.1, 114.8, 114.2, 114.1, 113.8 (×3), 113.5(×2), 113.0, 56.3, 55.9, 55.8, 55.7, 55.6, 55.5, 50.5, 32.9 (×2), 31.0, 29.6(×2), 29.5, 29.0, 28.2, 25.7 (×2) ppm. ESI/MS calculated for $C_{99}H_{113}O_{24}^+$ is 1686.7$[M+H]_+$ found 1703.9 $[M+H_2O+H]^+$. Anal. Calcd for $C_{99}H_{112}O_{24}$: C, 70.53; H, 6.70. Found: C, 70.49; H, 6.73.

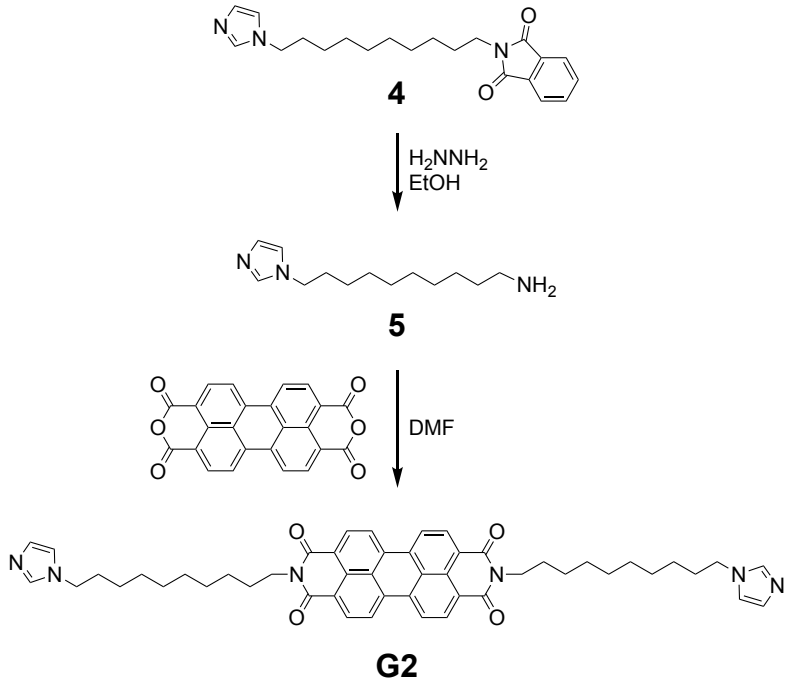
***meso*-Bis-pillar[*5*]arene dicarboxylic acid (H)**

Bis-pillar[5]arene dimethyl diester **3** (100 mg, 0.06 mmol) was dissolved in 30 mL of THF, then an aqueous solution of LiOH was added (2.5 mL, 2 M). The reaction was heated in a thermostated oil bath at 50 °C for 24 hours. 1 M of HCl (3 mL) was added and the resulting solution was concentrated under reduced pressure. The crude was poured in $CHCl_3$ and washed whit $H_2O$ (3 x 15 mL). The resulting organic layer was dried whit $MgSO_4$, filtered and evaporated under reduced pressure to give a precipitate that was crystallized from $CH_3OH/CH_2Cl_2$ to yield the desired compound; yield: 30 mg, 30%. M.p. 124–126 °C (from $CH_3OH/CH_2Cl_2$) $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.77, 6.76, 6.75, 6.74, 6.726, 6.722, 6.70, 6.69, 6.68, 6.67, 6.66, 6.65, 6.64, 6.63, 6.61, 6.58, 6.57 (18xs, ratio 1:1:1:1:1:1:2:1:1:1:1:1:2:1:1:1:1:1, ArH, 20H), 4.50(dd, $J_1$ = 11.3,5.6 Hz, OCH, 2 H), 3.94-3.49 (m, Ar-$CH_2$-Ar, 20H), 3.78, 3.77, 3.76, 3.739, 3.737, 3.707, 3.702, 3.678, 3.672, 3.66, 3.65, 3.63, 3.61, 3.60, 3.59, 3.58, 3.54, 3.53, (18xs, $OCH_3$, 54H), 1.87-1.82 (m, $CHCH_2$, 4H), 1.49–1.20 (m, $CH_2$, 6 H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 173.4, 151.3, 151.1, 150.9 (×2), 150.8, 150.8 (×2), 150.6 (×2), 148.3, 128.8 (×2), 128.5 (×2),

S1 T. Ogoshi, K. Demachi, K. Kitajima and T. Yamagishi. Monofunctionalized pillar[5]arenes: synthesis and supramolecular structure. *Chem. Commun.*, 2011, **47**, 7164–7166.

S2 C. T. Nieto, M. M. Salgado, S. H. Domínguez, D. Díez and N. M. Garrido. Rapid access with diversity to enantiopure flexible PNA monomers following asymmetric orthogonal strategies. *Tetrahedron: Asymm.*, 2014, **25**, 1046–1060.

128.4 (×3), 128.2 (×2), 127.9, 114.8, 114.7, 114.6, 114.4 (×2), 114.3, 114.2, 114.1 (×2), 114.9, 56.3, 56.2, 56.2, 56.0 (×4), 55.8, 55.7, 32.6, 30.1, 29.8 (×3), 29.4, 29.1, 28.9, 25.1, 25.0 ppm. ESI/MS calculated for $C_{97}H_{109}O_{24}^{+}$ is 1658.7$[M+H]^{+}$ found 1676.8 $[M+H_2O+H]^{+}$. Anal. Calcd for $C_{97}H_{108}O_{24}$: C, 70.27; H, 6.57. Found: C, 70.22; H, 6.62.




**Scheme S2**. Synthesis of bis-*N*,*N*'-(6-(1*H*-imidazole)decyl)-perilene-bisimide **G2**.

**bis-*N*,*N*'-(6-(1*H*-imidazole)decyl)-perilene-bisimide (G2):** perylene-3,4,9,10-tetracarboxylic dianhydride (281 mg, 0.71 mmol) and 10-amino-decan-imidazolium (320 mg, 1.41 mmol) were poured in 20 mL of DMF. The mixture was heated to 160 °C for 2 days under a $N_2$ atmosphere. After cooling, the mixture was filtered and washed with plentiful acetone. The crude was then further purified by recrystallization with MeOH/THF to afford the desired compound; yield: 460 mg, 80%. M.p. >300 °C (from MeOH/THF). $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.70 (d, *J* = 7.9 Hz, ArH, 4H), 8.64 (d, *J* = 8.1 Hz, ArH, 4H), 7.45 (s, NCHN, 2H), 7.04 (s, NCHC, 2H), 6,89 (s, NCHC, 2H), 4.20 (t, *J* = 7.1, $CH_2N$, 2H), 3.92 (t, *J* = 7.73, $CH_2N$, 2H), 1.79-1.72 (m, $CHCH_2$, 10H), 1.47-1.25 (m, $CHCH_2$, 30H). $^{13}$C NMR (125 MHz, $CDCl_3$/TFE, 97:3, v/v) $\delta$ 163.6, 136.5, 134.7, 131.5, 129.3, 128.0, 12.4, 123.2, 119.1, 47.4, 40.8, 30.9, 29.31, 29.26, 29.20, 28.9, 28.0, 27.0, 26.4. ESI/MS calculated for $C_{50}H_{55}N_6O_4^{+}$ $[M+H]^{+}$ found 803.4 $[M+H]^{+}$ and 403.2 $[M+2H]^{2+}$. Anal. Calcd for $C_{50}H_{54}N_6O_4$: C, 74.79; H, 6.78; N, 10.47. Found: C, 74.70; H, 10.62, N, 10.44.

**Preparation of H/G1/G2 copolymer**

A 10 mM solution of copolymer was prepared by dissolving **H** (16.36 mg, 0.01 mmol) and **G1** (2.72 mg,0.009 mmol) of in $CDCl_3$/TFE (97:3 v/v, 1 mL). Then, solid **G2** (1 mg, 1.2 μmol) was added to the solution and the suspension was stirred for 2 h at rt. Successively, the suspension was filtered on a PTFE filter (0.4 μm) to remove the excess of **G2**, in order to obtain a stoichiometric ratio of 1:0.9:0.1 for **H**/**G1**/**G2**.

**UV-vis/emission titrations**

Titration experiments of [**G2**] = $7.83 \times 10^{-6}$ M in $CHCl_3$/TFE (97:3 v/v) were carried out with a [**H**] = $2.46 \times 10^{-4}$ M solution in $CHCl_3$/TFE (97:3 v/v, titrant concentration from $7.78 \times 10^{-7}$ to $1.54 \times 10^{-5}$ M). Titration of a solution containing [**G2**] = $7.34 \times 10^{-4}$ M and [**H**] = 2.19 x $10^{-3}$ M in $CHCl_3$/TFE (97:3 v/v) was carried out with a [**S**] = $2.3 \times 10^{-4}$ M solution in $CHCl_3$/TFE (97:3 v/v, titrant concentration from $7.73 \times 10^{-7}$ to $1.57 \times 10^{-5}$ M). The excitation wavelength was set at $\lambda_{ex}$ = 456 nm.

Titration of a solution containing [**G1**] = $1.01 \times 10^{-5}$ M, [**G2**] = $1.13 \times 10^{-6}$ M and [**H**] = $1.13 \times 10^{-5}$ M in $CHCl_3$/TFE (97:3 v/v) was carried out with a [**S**] = $1.54 \times 10^{-3}$ M solution in $CHCl_3$/TFE (97:3 v/v, titrant concentration from $6 \times 10^{-6}$ to $1 \times 10^{-4}$ M). All titrations were performed by adding small aliquots of a titrant solution (maximum volume added 100 μL, in order to avoid dilution effects) to a quartz cuvette (1 cm path) containing 2.5 mL of the titrated solution. In all emission experiments, the excitation wavelength was set at $\lambda_{ex}$ = 456 nm.

**$^1$H NMR titrations**

$^1$H NMR titration studies on **H**/**G1** were carried out at a fixed concentration of [**H**] = 1 mM in $CDCl_3$/TFE (97:3 v/v). Such solution was titrated with a [**G1**] = 10 mM solution dissolved in the same [**H**] = 1 mM solution in $CDCl_3$/TFE 97:3 v/v, so that, during the titration, the concentration of **H** did not vary upon addition of increasing aliquots of the **G1**. Studies on **H**/**S** were carried out at a fixed concentration of [**S**] = 2 mM in $CDCl_3$/TFE (97:3 v/v). Such solution was titrated with a [**H**] = 10 mM solution dissolved in a [**S**] = 1 mM solution in $CDCl_3$/TFE 97:3 v/v.

**DOSY NMR**

DOSY experiments were carried out on a 500 MHz NMR spectrometer equipped with a z-gradient system capable of producing pulse gradients up to 50 gauss × $cm^{-1}$. Spectra were recorded either in $CDCl_3$ or in $CDCl_3$/TFE (97:3 v/v) at 25 ± 0.1 °C, using a gradient stimulated echo with spin-lock and a convection compensation pulse sequence.

**Atomic Force Microscopy**

Spin-coated **H**, **G1**, **G2** and **H**/**G1**/**G2** thin films for AFM analysis were prepared at room temperature by casting a few drops of [**H**] = 1 mM, [**G1**] = 1 mM, [**G2**] = 0.1 mM and [**H**] = 1 mM, [**G1**] = 0.9 mM, [**G2**] = 0.1 mM copolymer solutions ($CHCl_3$/TFE 97:3 v/v) onto a silica surface while spinning at about 2000 RPM. The images were acquired using an AFM SMENA (NT-MDT) apparatus equipped with a silicon probe working in semi contact mode (NSG30, 6 nm typical curvature radius). All the images were post processed by a plane removal numerical procedure.

**Fluorescence and Raman spectroscopy**

The photoluminescence data were acquired by exciting the sample via a 472nm DPSS laser source focused on the sample through a 100X SLWD Mitutoyo objective. The same objective was used, in reflection configuration, to collect the photoluminescence emitted by the sample. To filter out the intense scattered laser light, a Semrock Razor Edge filter (BLP01-473R-25) was inserted into the optical path. The light was dispersed by a Sol MS350I spectrometer and detected using an ADOS IDUS 401 camera.

The Raman spectroscopy was performed using the same optical setup as for photoluminescence but the sample with a DPSS laser at 532nm for excitation. The laser power was kept as low as possible (below 1 mW/$cm^2$) to avoid any thermal damage to the organic material. Additionally, two Semrock ultrasteep long pass filters (LP03-532RE-25) were inserted into the optical path.

**Molecular modelling**

Computational investigation of the **H**/**G1** polymer was carried out on a simplified model composed of a **H**/**G1** complex whose free cavity and imidazole ends were complexed with an hexyl-imidazole and a carboxyl pillar[5]arene, respectively, to simulate the occupancy of both ends of the **H**/**G1** repeating unit (in other words, an ABBAAB fragment). The conformational analysis was optimized with the classical molecular mechanics force field (MMFF) and the conformer obtained was further refined by semiempirical methods at the PM6 level. All calculations were performed using Spartan'10 (Wavefunction, Inc., Irvine, CA, USA).

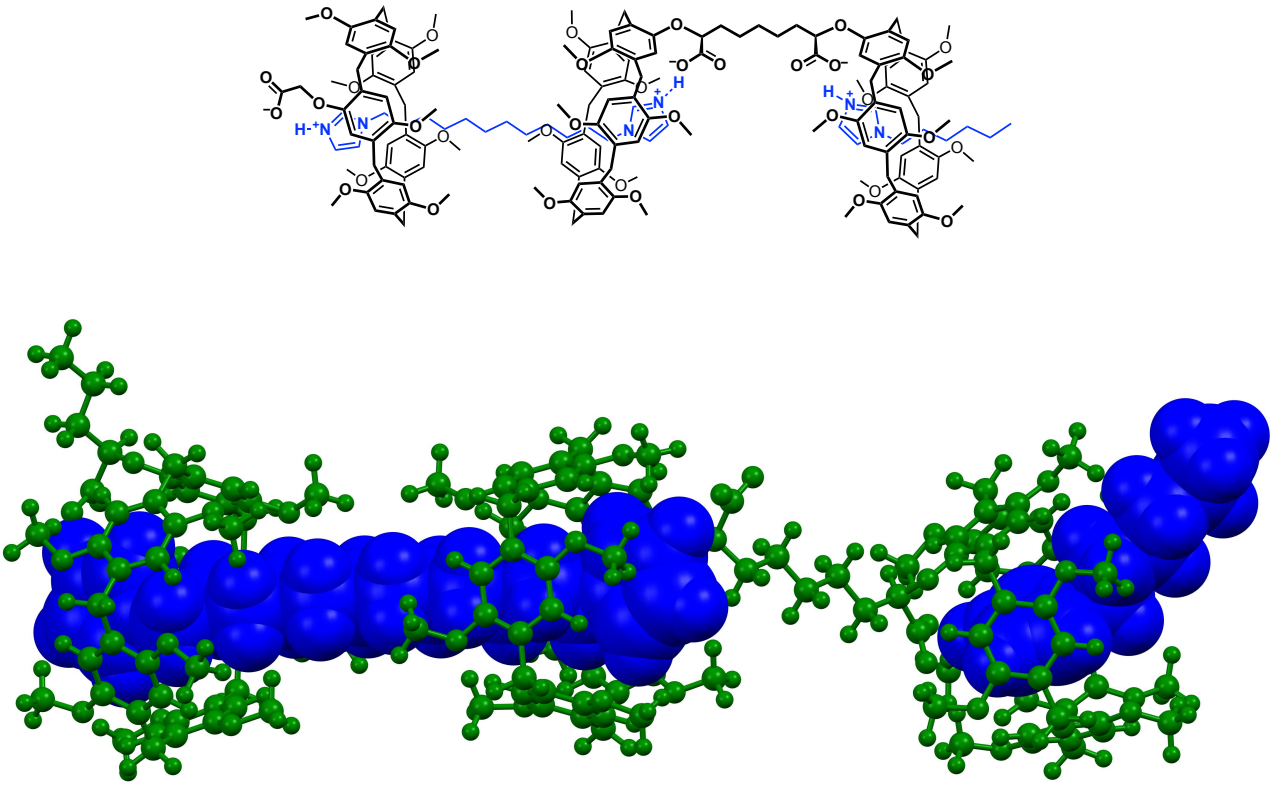


**Fig. S1** Optimized geometry of the repeating unit of the **H**/**G1** aggregate (semiempirical level, PM6).

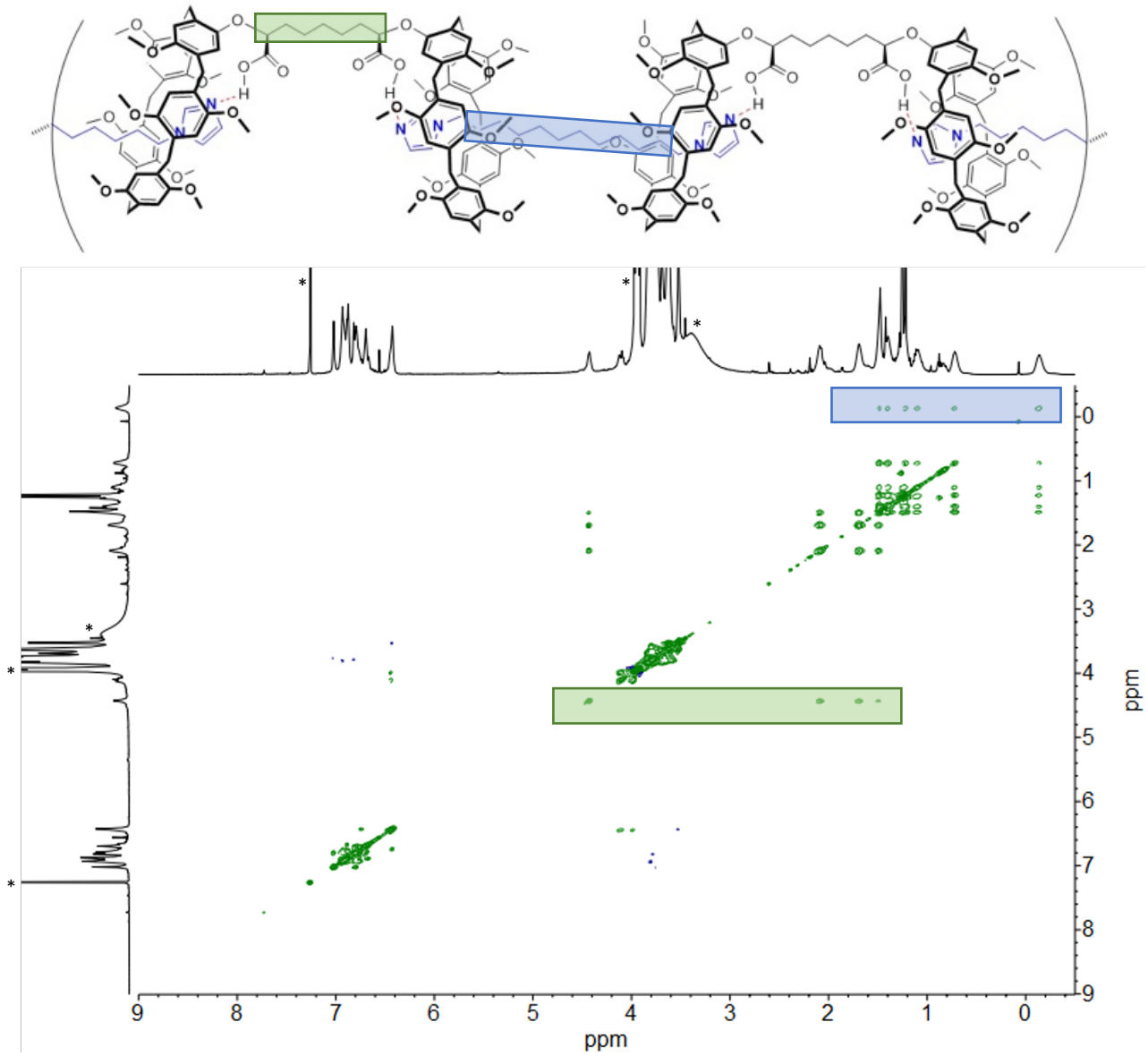



**Fig. S2**. 2D TOCSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = [**G1**] = 10 mM. *Asterisks indicate residual solvent peaks.

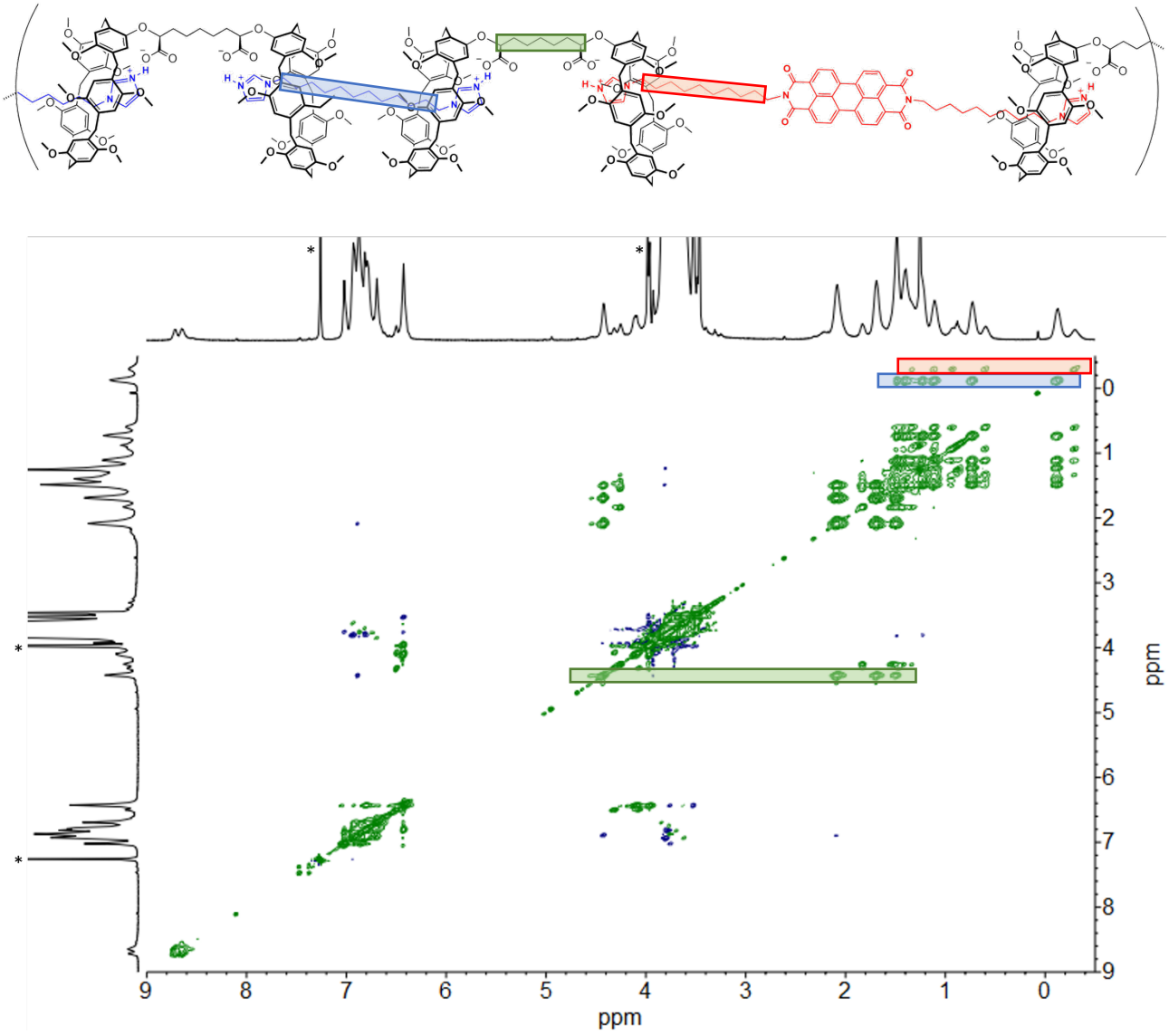



**Fig. S3**. 2D TOCSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = 10 mM, [**G1**] = 9 mM, [**G2**] = 1 mM. *Asterisks indicate residual solvent peaks.

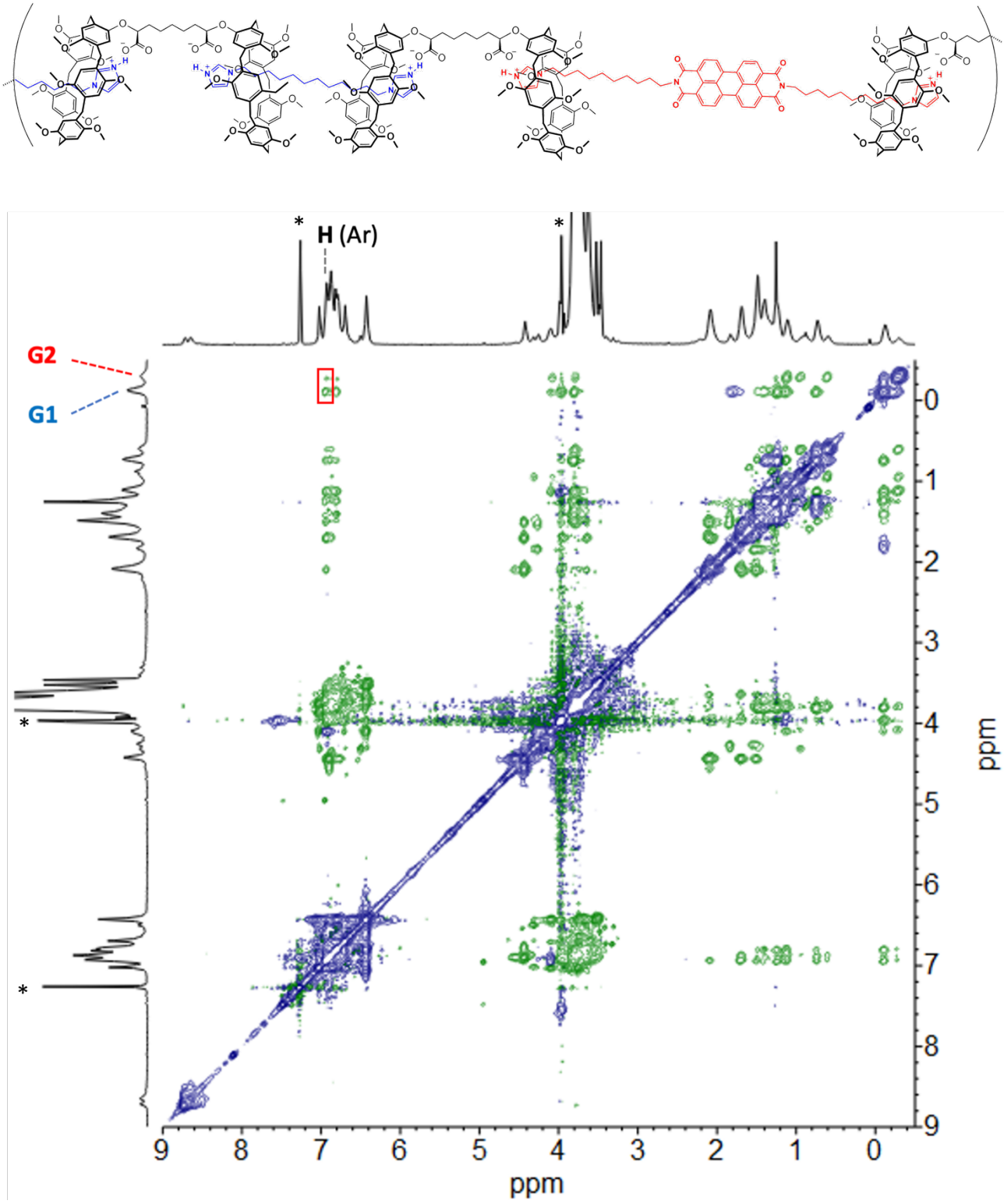



**Fig. S4**. 2D ROESY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = 10 mM, [**G1**] = 9 mM, [**G2**] = 1 mM. *Asterisks indicate residual solvent peaks.

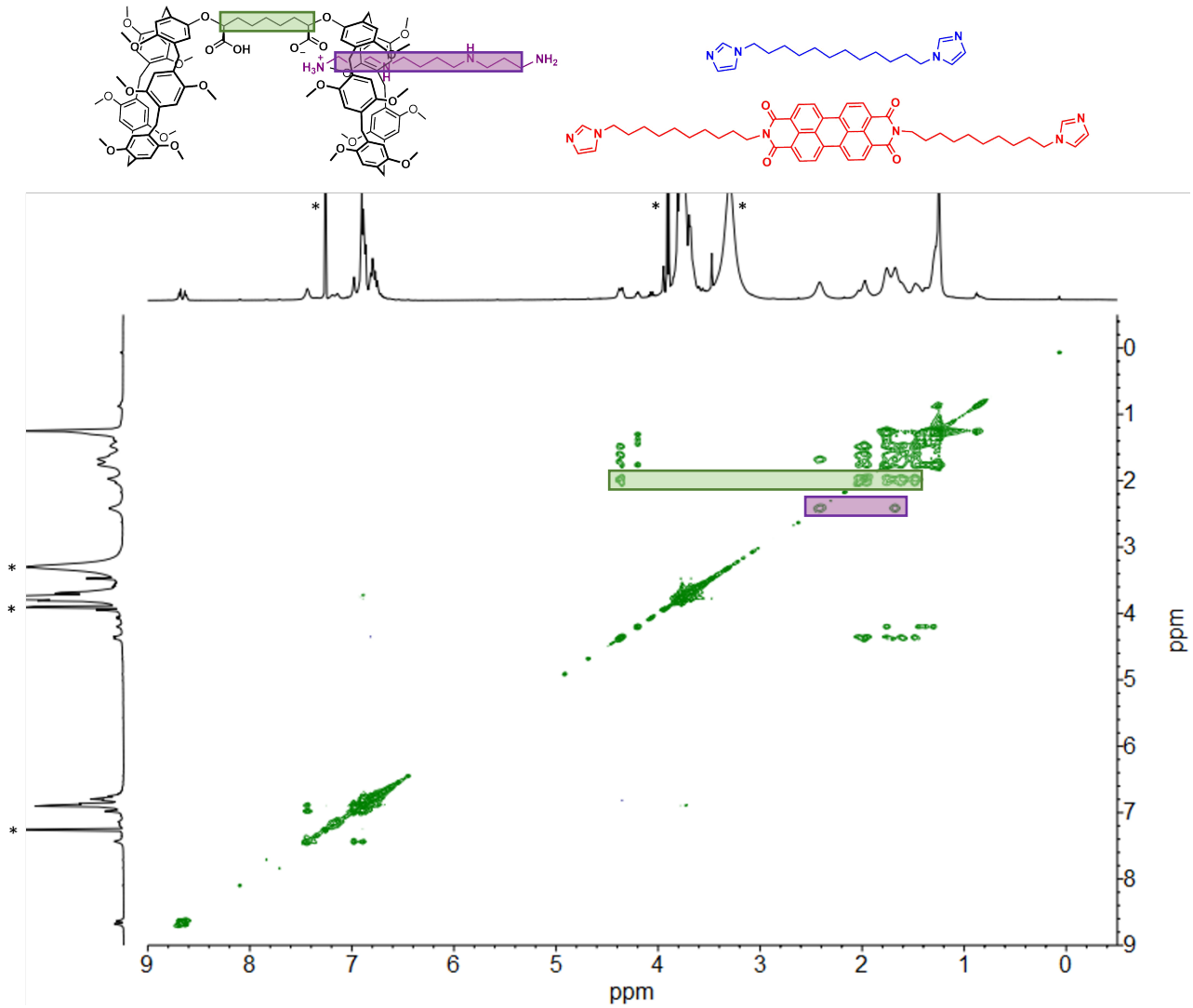



**Fig. S5**. 2D TOCSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = 5 mM, [**G1**] = 4.5 mM, [**G2**] = 0.5 mM, [**S**] = 10 mM. *Asterisks indicate residual solvent peaks.

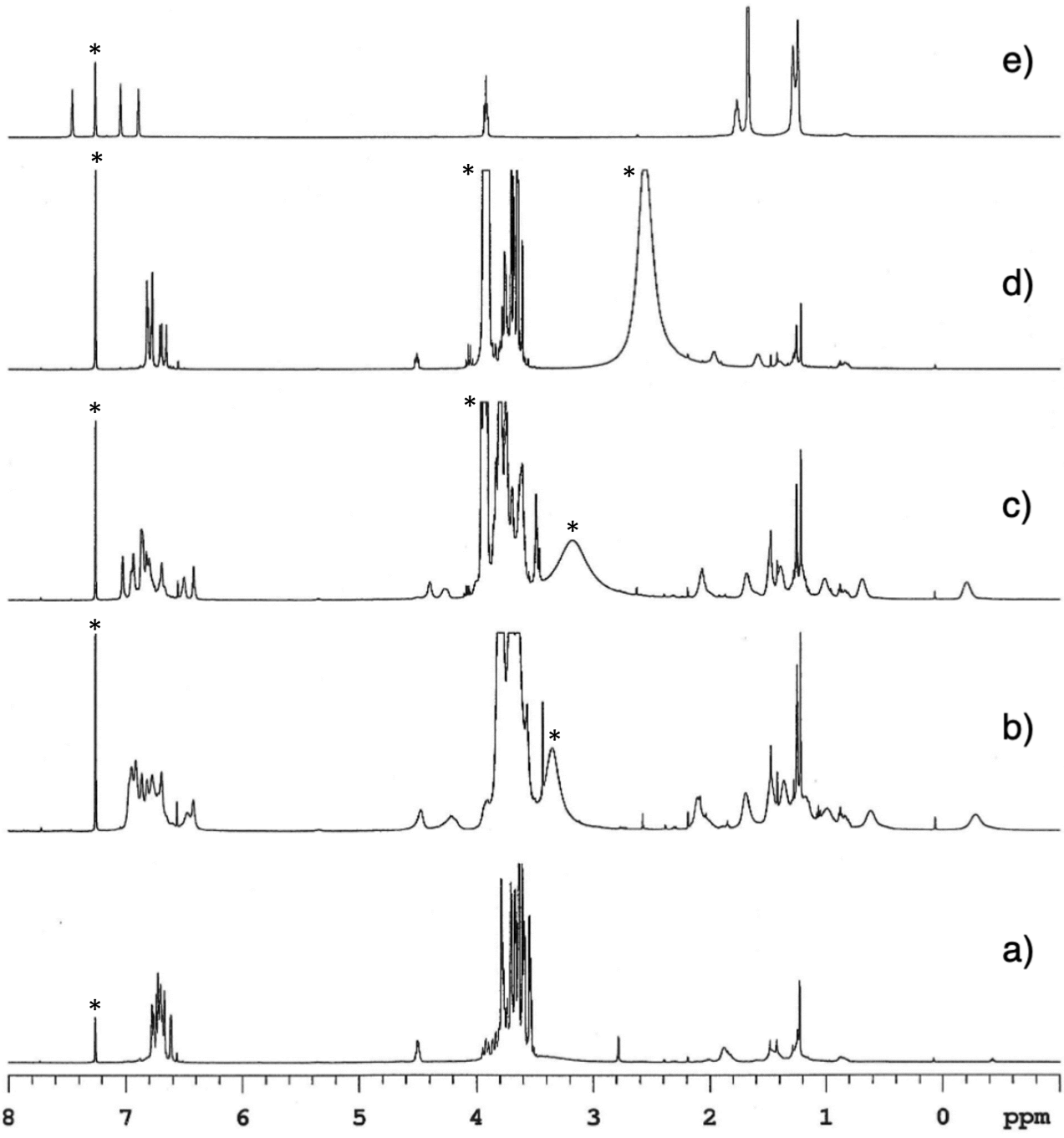



**Fig. S6.** $^{1}$H NMR spectra (500 MHz, 25 °C) of: a) [**H**] = 10 mM in $CDCl_3$; b) [**H**] = [**G1**] = 10 mM in $CDCl_3$; c) [**H**] = [**G1**] = 10 mM in $CDCl_3$/TFE 97:3 v/v; d) [**H**] = 10 mM in $CDCl_3$/TFE 97:3 v/v; e) [**G1**] = 10 mM in $CDCl_3$. *Asterisks indicate residual solvent peaks.

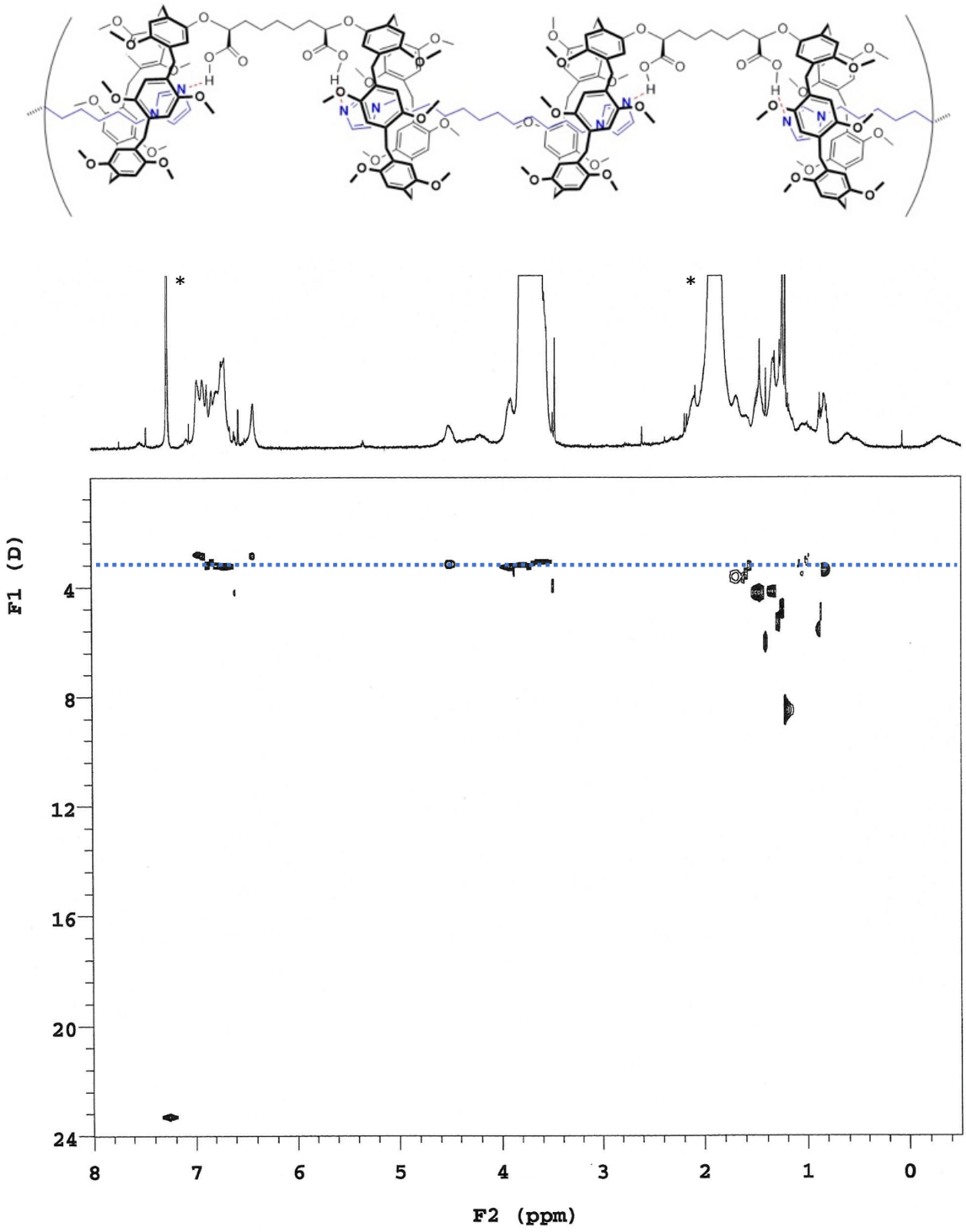



**Fig. S7**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$) of [**H**] = [**G1**] = 1 mM. *Asterisks indicate residual solvent peak and $H_2O$.

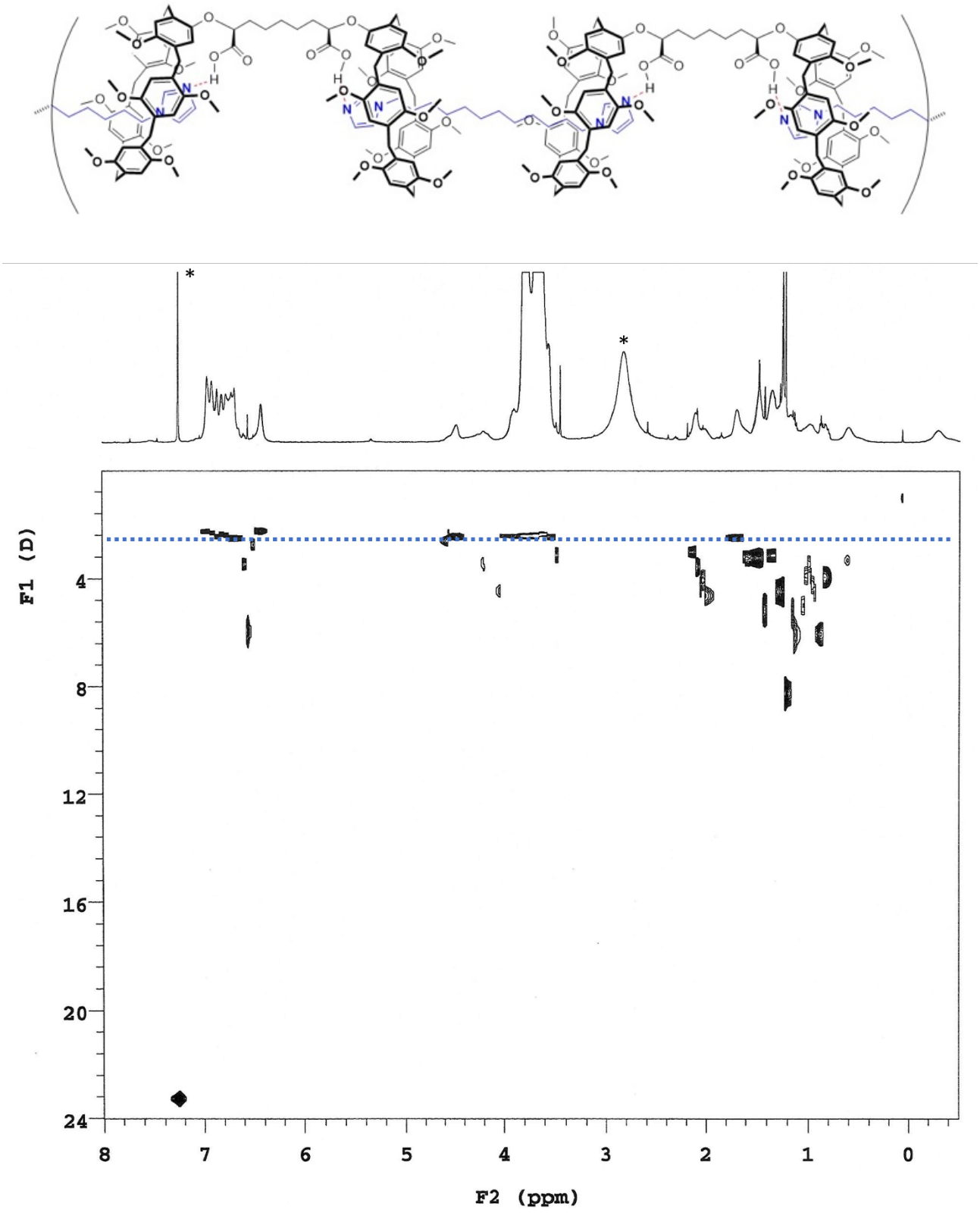



**Fig. S8**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$) of [**H**] = [**G1**] = 5 mM. *Asterisks indicate residual solvent peak and $H_2O$.

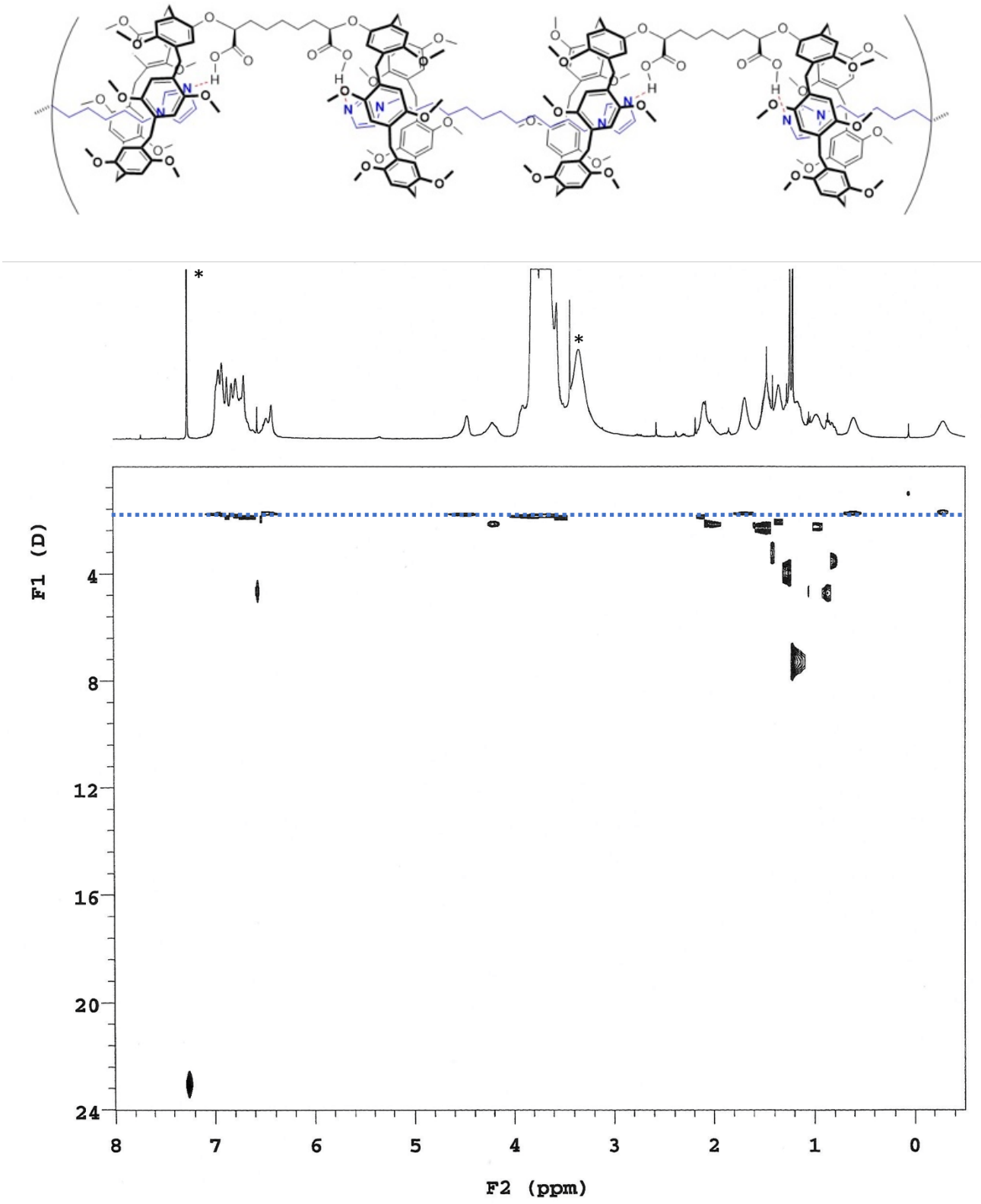



**Fig. S9**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$) of [**H**] = [**G1**] = 10 mM. *Asterisks indicate residual solvent peak and $H_2O$.

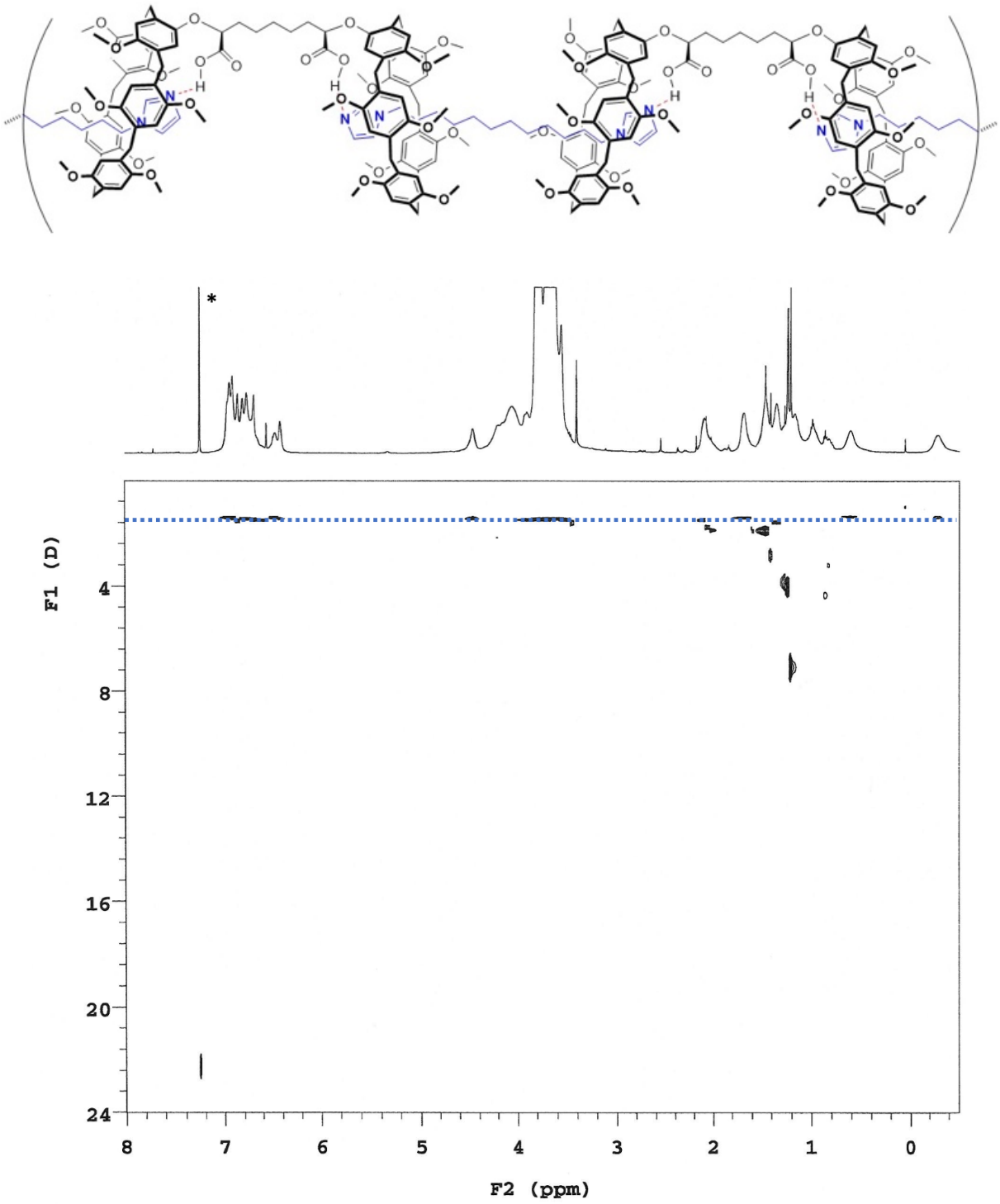



**Fig. S10**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$) of [**H**] = [**G1**] = 16.6 mM. *The asterisks indicates the residual solvent peak.

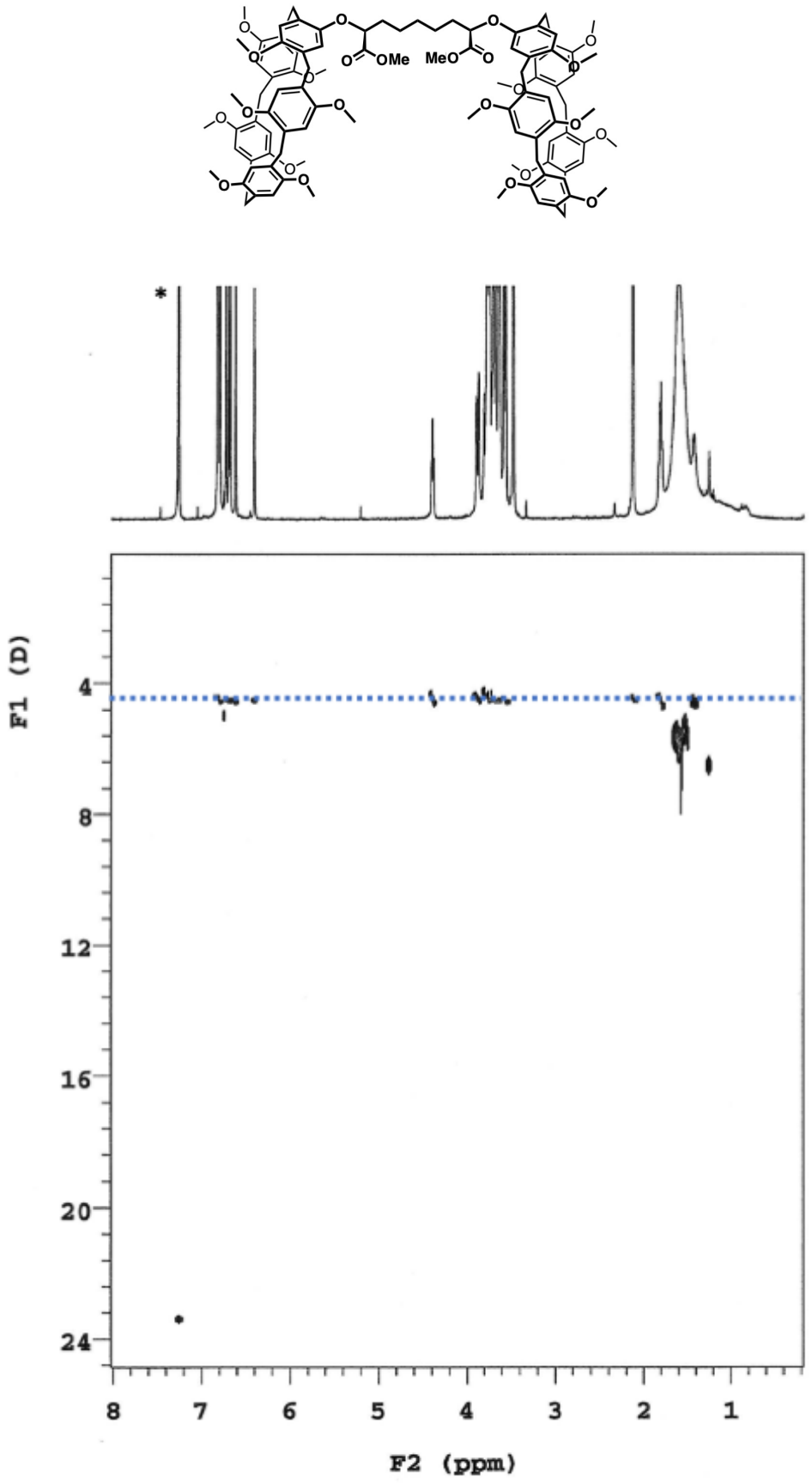



**Fig. S11**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$) of [**3**] = 16.6 mM. *The asterisk indicates the residual solvent peak.

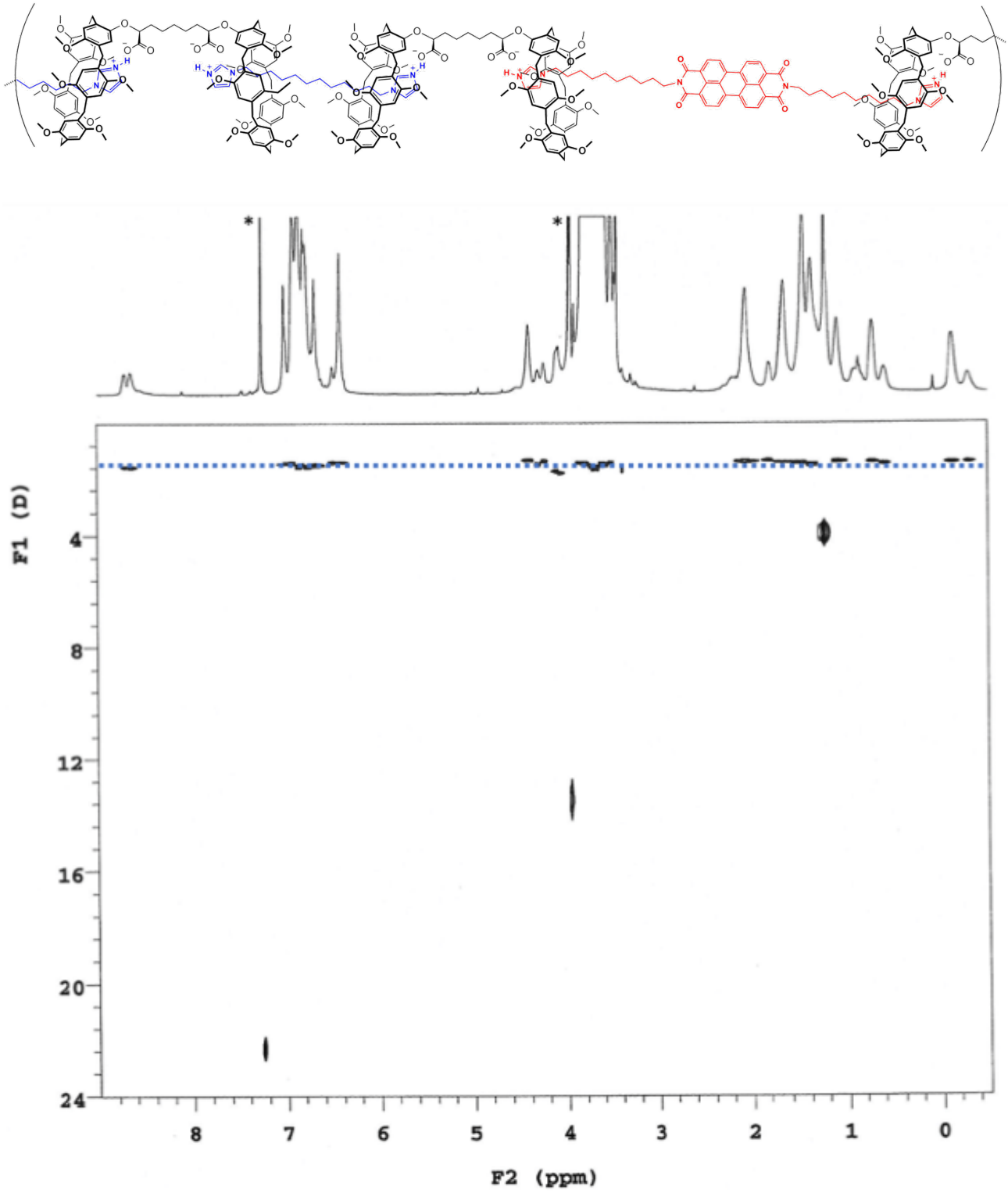



**Fig. S12**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = 10 mM, [**G1**] = 9 mM, [**G2**] = 1 mM. *Asterisks indicate residual solvent peaks.

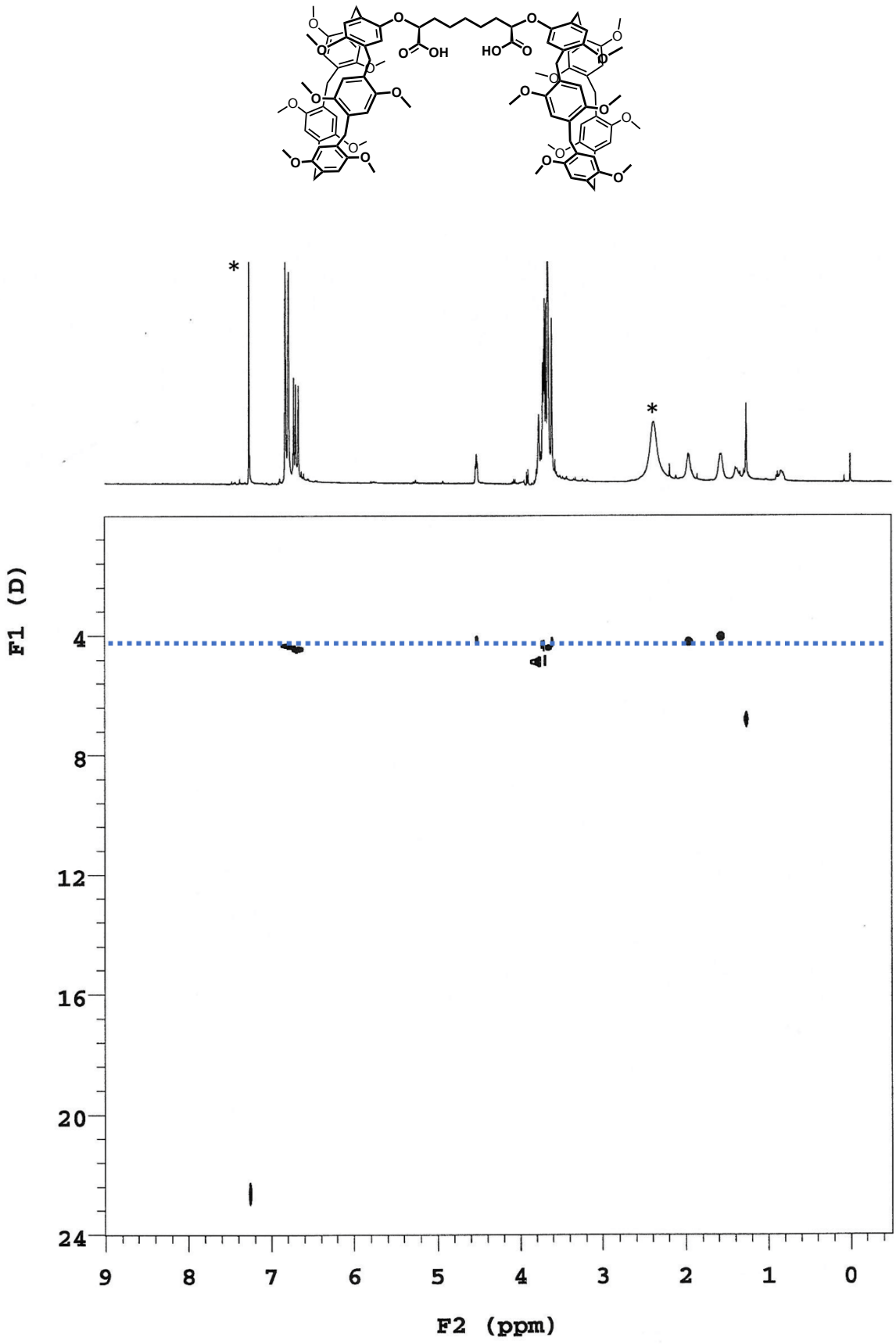



**Fig. S13**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**H**] = 10 mM. *Asterisks indicate residual solvent peaks.

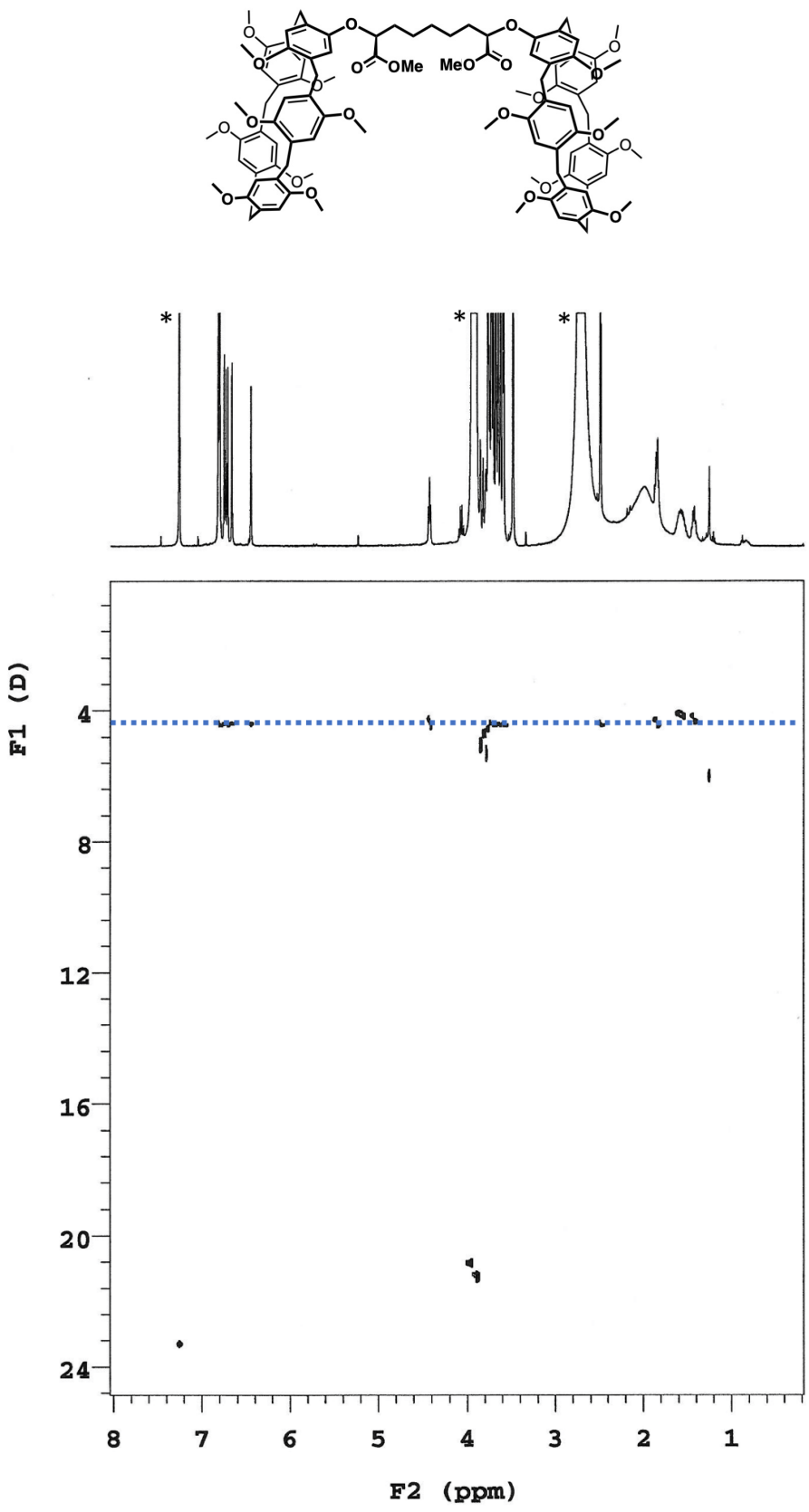



**Fig. S14**. DOSY spectrum (500 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of [**3**] = 10 mM. *Asterisks indicate residual solvent peaks.

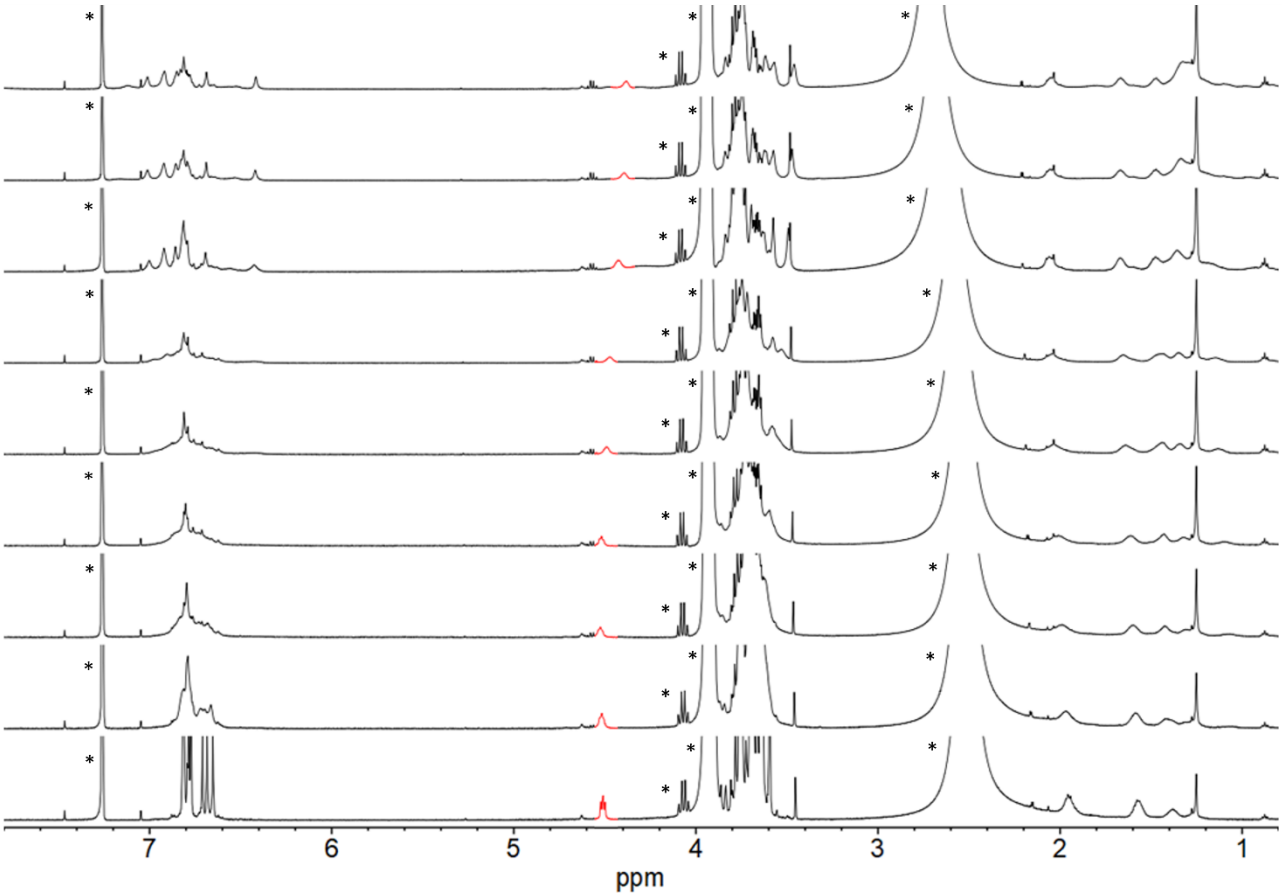



**Fig. S15**. $^{1}$H NMR spectra (500 MHz, 25°C, $CDCl_3$/TFE 97:3 v/v) of the titration of [**H**] = 1 mM with [**G1**] = 10 mM. *Asterisks indicate residual solvent peaks.

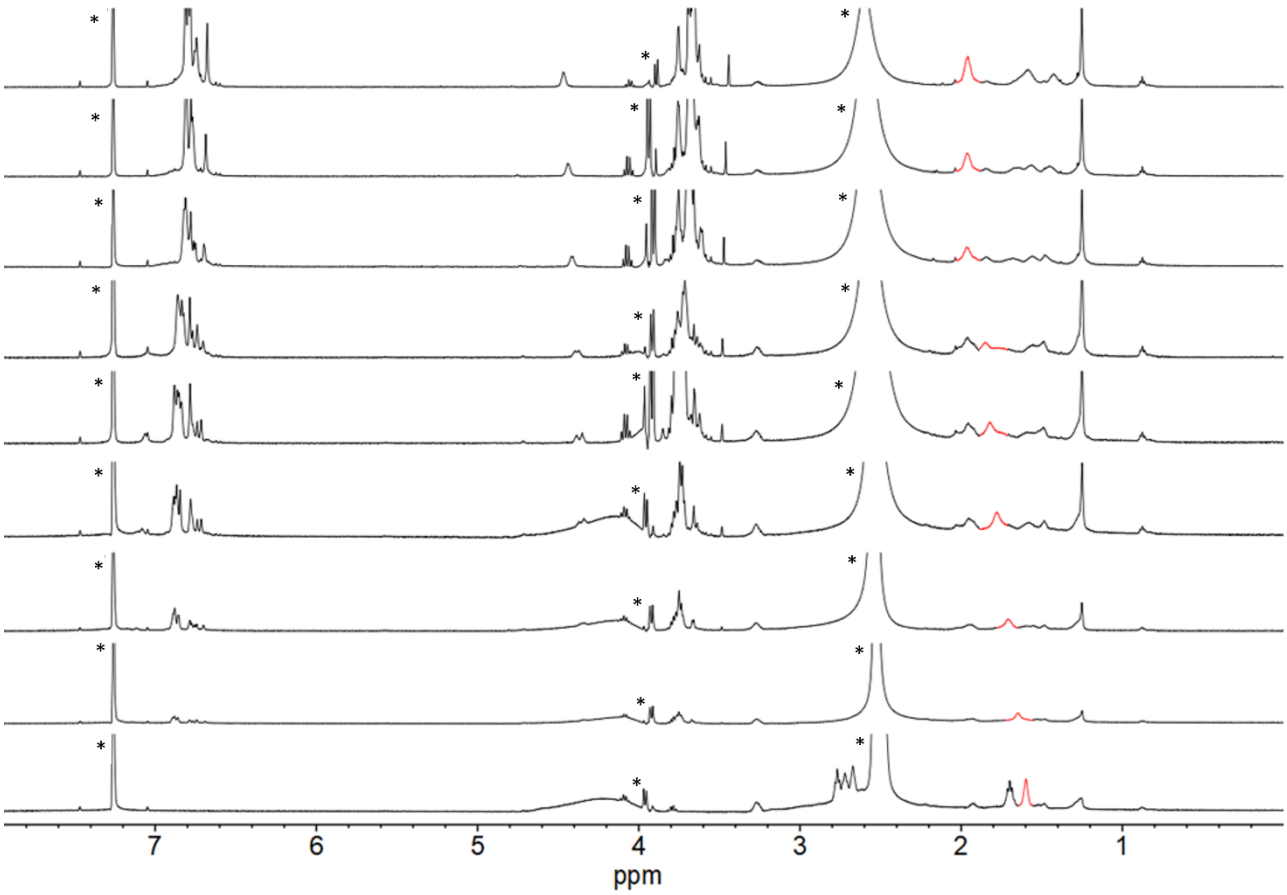



**Fig. S16**. $^{1}$H NMR spectra (500 MHz, 25°C, $CDCl_3$/TFE 97:3 v/v) of the titration of [**S**] = 2 mM with [**H**] = 10 mM. *Asterisks indicate residual solvent peaks.

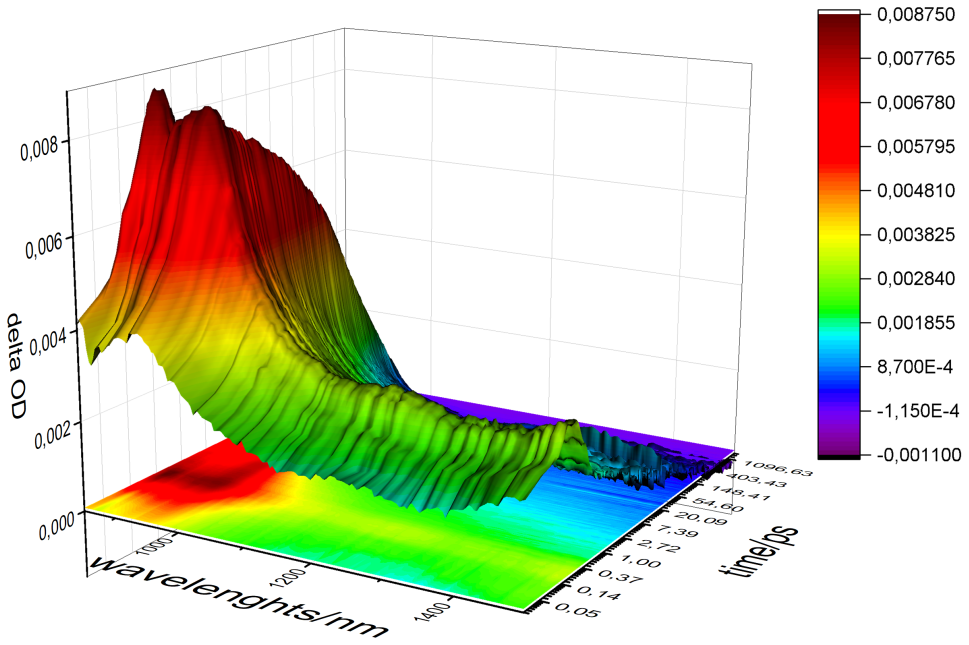



**Fig. S17.** TAS matrix in the near IR region of [**H**/**G2**] = 3.83 × $10^{-5}$ M in $C_2H_2Cl_4$/$CF_3CH_2OH$ 97:3 v/v solution ($\lambda_{exc}$ 400 nm, pulse 100 fs, 100 mJ).

**Binding data treatment**

The HypNMR[S3] computer program was employed to treat the $^1$H NMR data for the determination of the **H/S** and **H/G1** association constant values. Equilibrium was considered to be rapidly reached on the NMR time scale. The experimental (observed) chemical shifts for each nucleus are the averages of its signals across the various species present in the investigated system. HypSpec[S4] was used for the refinement of **H/G2** formation constants from emission data. The distribution diagrams were drawn using the HySS program.[S5]

**Table S1.** Overall ($\beta$) and stepwise ($K$) formation constants and calculated chemical shifts determined using HypNMR[S3] for the refined **H/S** and **H/G1** species.

| Complex | log$\beta$ | $\beta$ | δ (ppm) | log$K$ | $K$ |
|---|---|---|---|---|---|
| **S** | - | - | 1.55 ± 0.01[a)] | - | - |
| **H/S** | 6.39 ± 0.01[a)] | 2.45 ± 0.06[a)] × $10^6$ | 1.84 ± 0.01 | 6.39 ± 0.01[a)] | 2.45±0.06[a)] × $10^6$ |
| **S/H/S** | 10.08 ± 0.01 | 1.20 ± 0.03 × $10^{10}$ | 1.77 ± 0.01 | 3.69 ± 0.02 | 4.90±0.20 × $10^3$ |
| | | | | | |
| **H** | - | - | 4.50 ± 0.01[a)] | - | - |
| **H/G1** | 6.03 ± 0.01[a)] | 1.07±0.05[a)] × $10^6$ | 4.41 ± 0.01 | 6.03 ± 0.01[a)] | 1.07±0.05[a)] × $10^6$ |
| **H/G1/H** | 8.51 ± 0.07 | 3.24±0.50 × $10^8$ | 4.80 ± 0.01 | 2.48 ± 0.08 | 3.02±0.56 × $10^2$ |
| **G1/H/G1** | 11.10 ± 0.02 | 1.26±0.06 × $10^{11}$ | 4.38 ± 0.01 | 5.07 ± 0.03 | 1.17±0.08 × $10^5$ |

[a)] ±Std. Deviation.

**Table S2**. Overall ($\beta$) and stepwise ($K$) formation constants determined using HypSpec[S4] for the refined **H/G2** species.

| Complex | log$\beta$ | $\beta$ | log$K$ | $K$ |
|---|---|---|---|---|
| **H/G2** | 6.42 ± 0.01[a)] | 2.69 ± 0.06[a)] × $10^6$ | 6.43 ± 0.01[a)] | 2.69 ± 0.06[a)] × $10^6$ |
| **H/G2/H** | 12.66 ± 0.02 | 4.57 ± 0.21 × $10^{13}$ | 6.24 ± 0.03 | 1.74 ± 0.12 × $10^6$ |
| **G2/H/G2** | 10.80 ± 0.02 | 6.61 ± 0.30 × $10^{10}$ | 4.40 ± 0.03 | 2.51 ± 0.17 × $10^4$ |

[a)] ±Std. Deviation.


S3 C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M.S. Moruzzi and A. Vacca, Nuclear Magnetic Resonance as a Tool for Determining Protonation Constants of Natural Polyprotic Bases in Solution. *Anal. Biochem.*, 1995, **231**, 374–382

S4 P. Gans, P.; A. Sabatini and A. Vacca, Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite programs. *Talanta*, 1996, **43**, 1739–1753.

S5 L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca. Hyperquad simulation and speciation (HySS): A utility program for the investigation of equilibria involving soluble and partially soluble species. *Coord. Chem. Rev.*, 1999, **184**, 311–318.

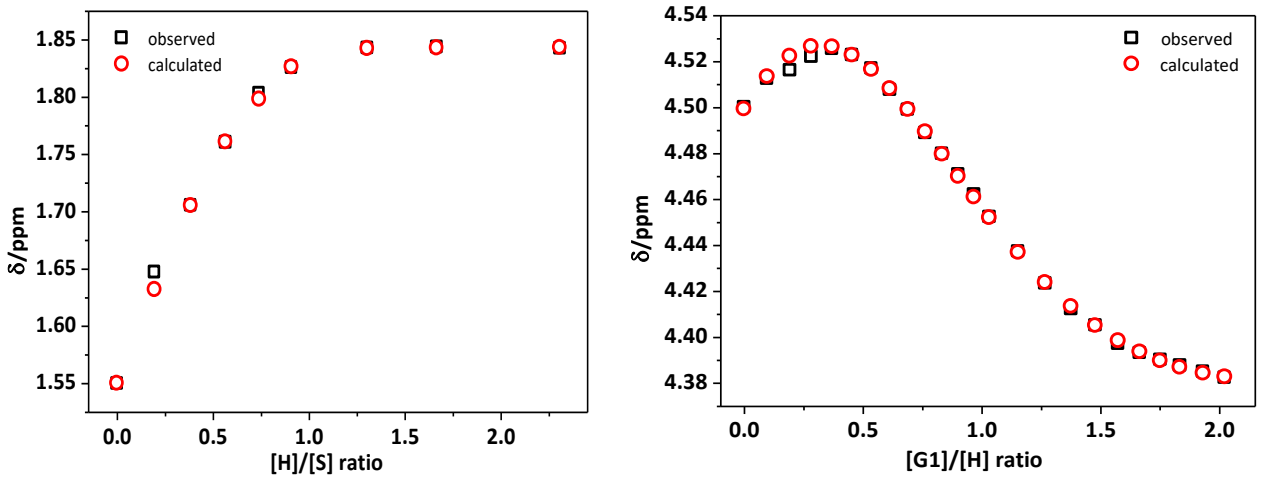



**Figure S18.** Observed and calculated chemical shifts for the **S** (left) and **H** (right) nuclei at varying the components concentration ratios. Experimental conditions: a) **H**/**S** system, [**H**] = 0.00-3.75 mM, [**S**] = 1.63-2.00 mM; b) **H**/**G1** system, [**H**] = 2.00 mM, [**G1**] = 0.00-4.04 mM.

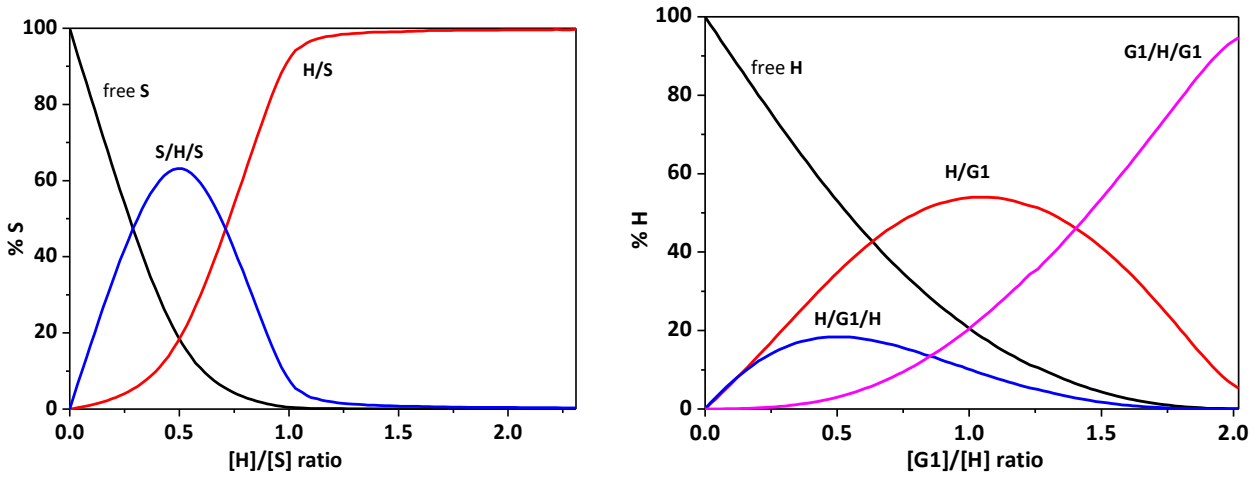



**Figure S19.** Distribution diagrams of **H**/**S** and **H**/**G1** species at varying the components concentration ratios. Experimental conditions: left) [**H**] = 0.00-3.75 mM, [**S**] = 1.63-2.00 mM; right) [**H**] = 2.00 mM, [**G1**] = 0.00-4.04 mM.

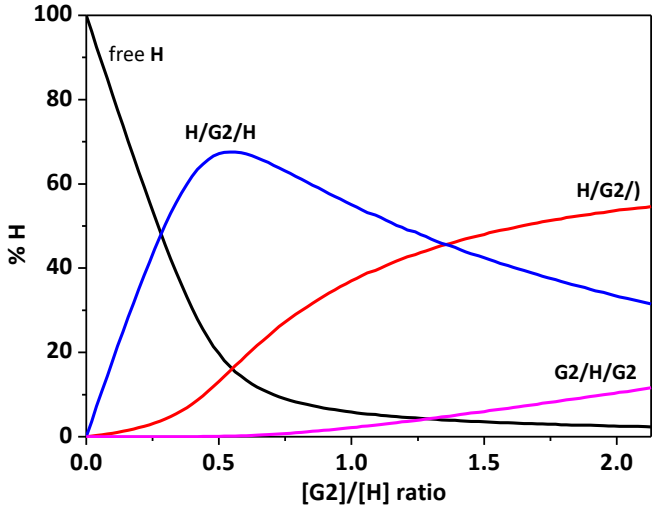



**Figure S20.** Distribution diagram of **H**/**G2** species at varying the components concentration ratio. Experimental conditions: [**H**] = (7.22–7.73) × $10^{-6}$ M, [**G2**] = (0.00-1.57) × $10^{-5}$ M.

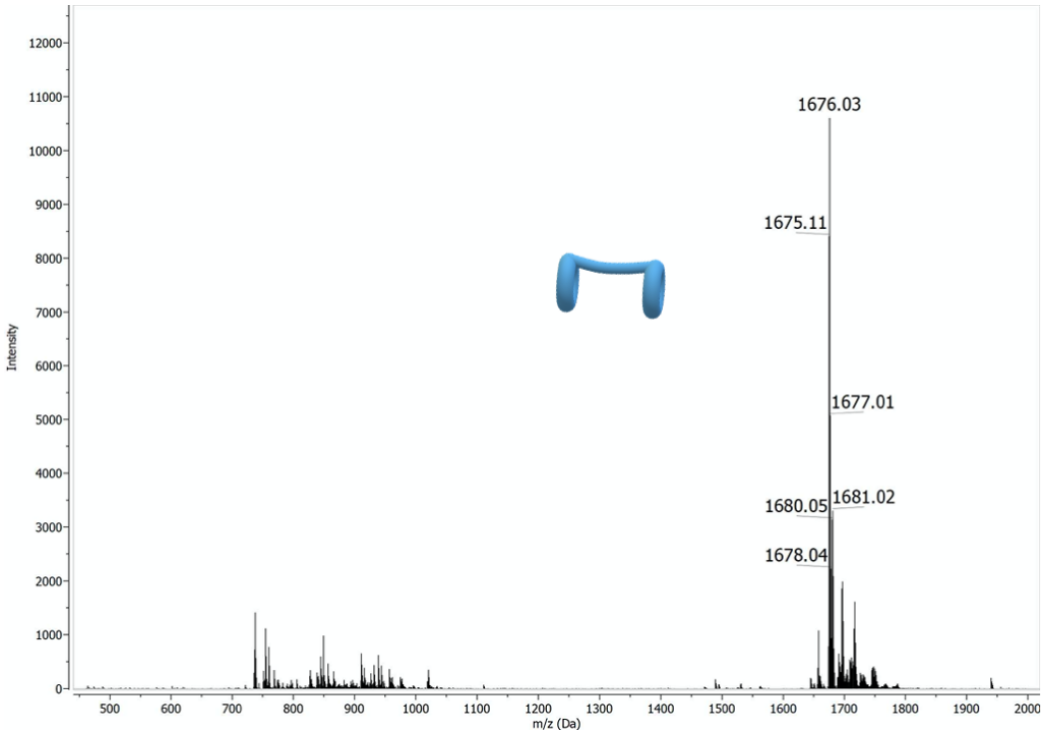



**Fig. S21**. ESI/MS(+) spectrum of [**H**] = 10 µM in MeOH.

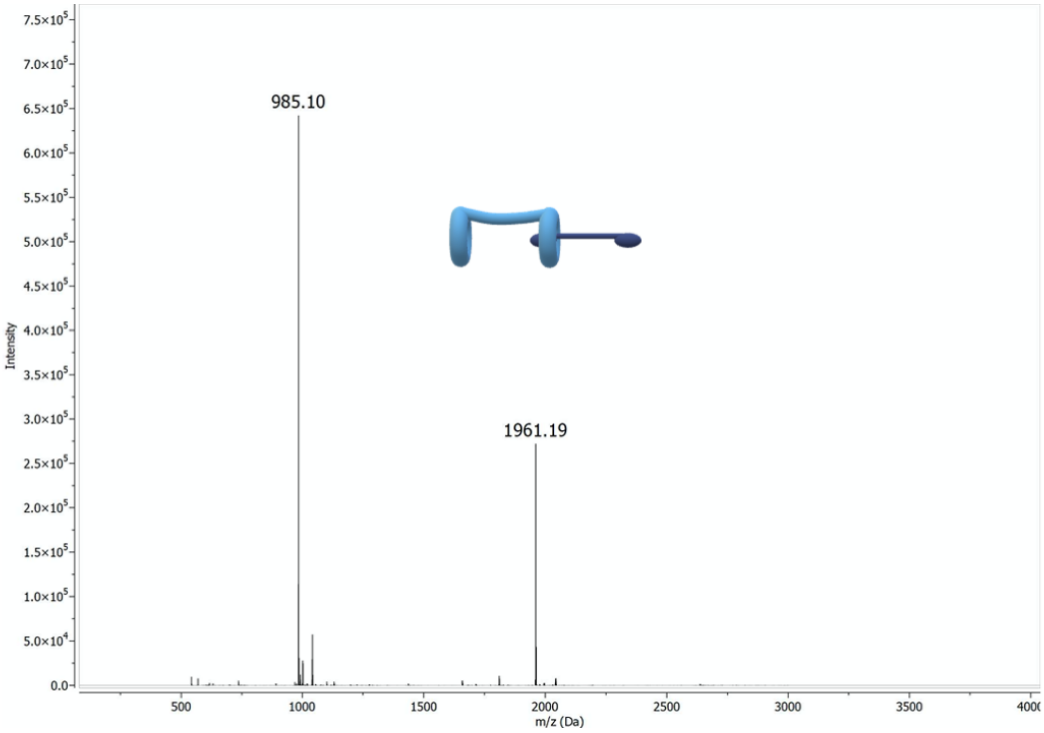



**Fig. S22**. ESI/MS(+) spectrum of [**H**] = 10 µM and [**G1**] = 10 µM in MeOH.

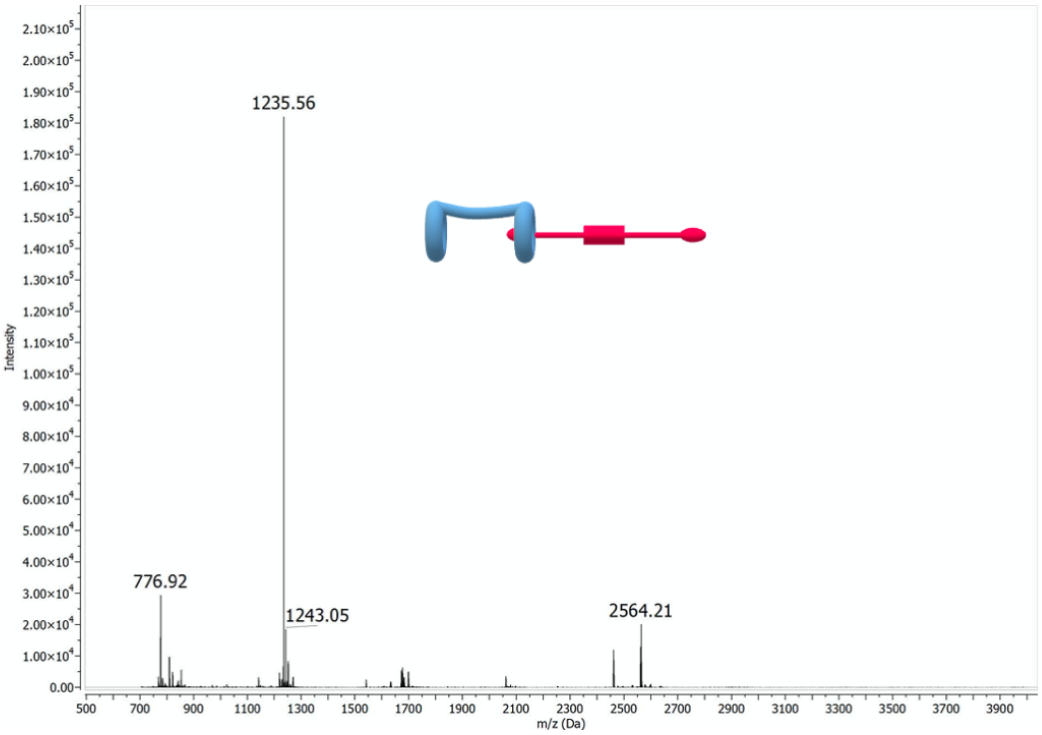



**Fig. S23**. ESI/MS(+) spectrum of [**H**] = 10 µM and [**G2**] = 10 µM in MeOH.

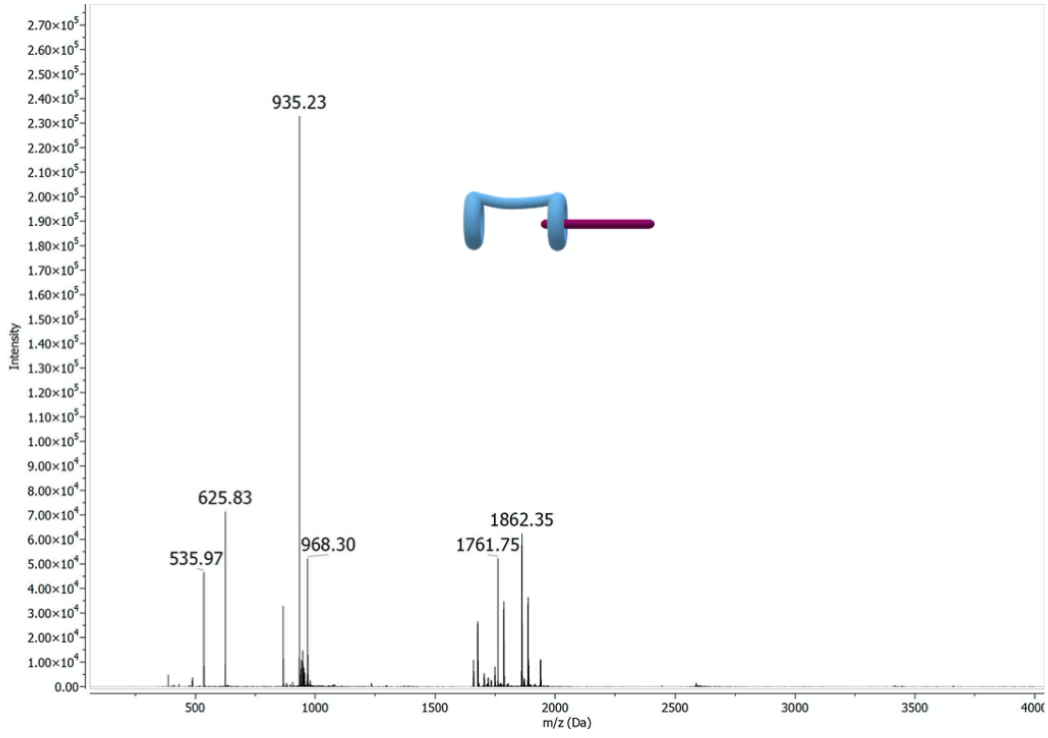



**Fig. S24**. ESI/MS(+) spectrum of [**H**] = 10 µM and [**S**] = 10 µM in MeOH.

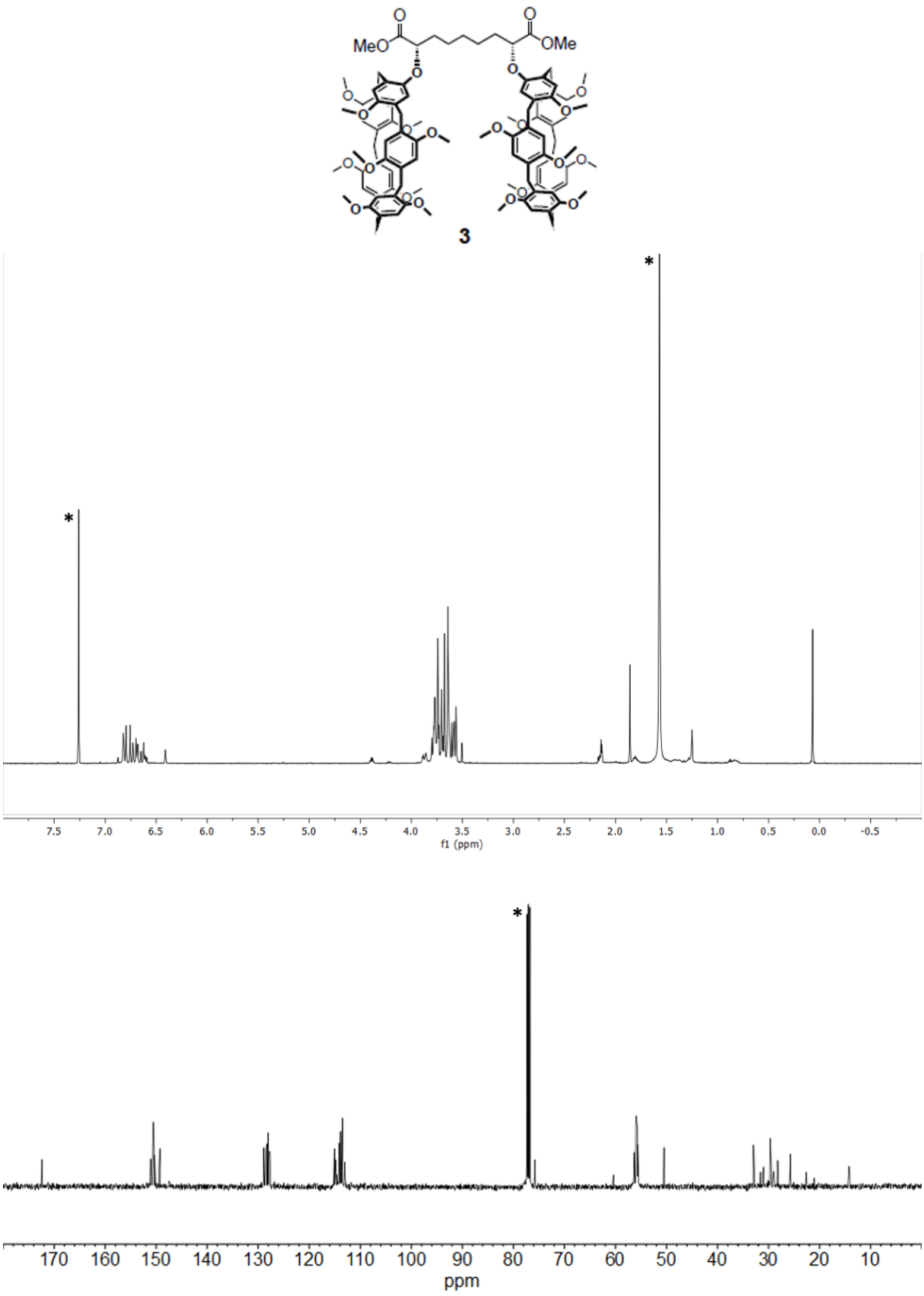



**Fig. S25**. Top: $^{1}$H NMR spectrum (500 MHz, 25°C, $CDCl_3$); and bottom: $^{13}$C NMR spectrum (125 MHz, 25°C, $CDCl_3$) of **3**. *Asterisks indicate residual solvent peaks.

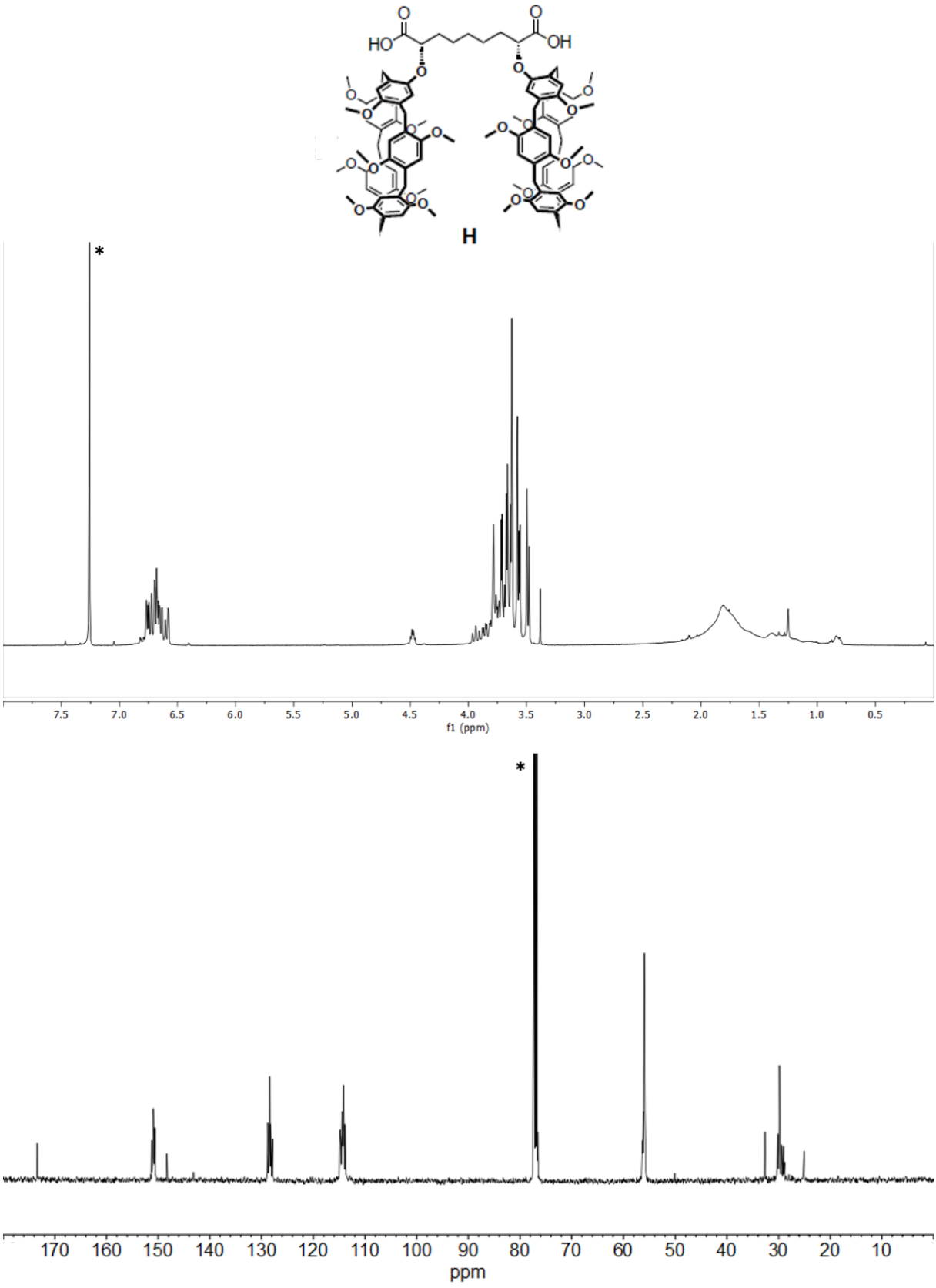



**Fig. S26**. Top: $^{1}$H NMR spectrum (500 MHz, 25°C, $CDCl_3$); and bottom: $^{13}$C NMR spectrum (125 MHz, 25°C, $CDCl_3$) of **H**. *Asterisks indicate residual solvent peaks.

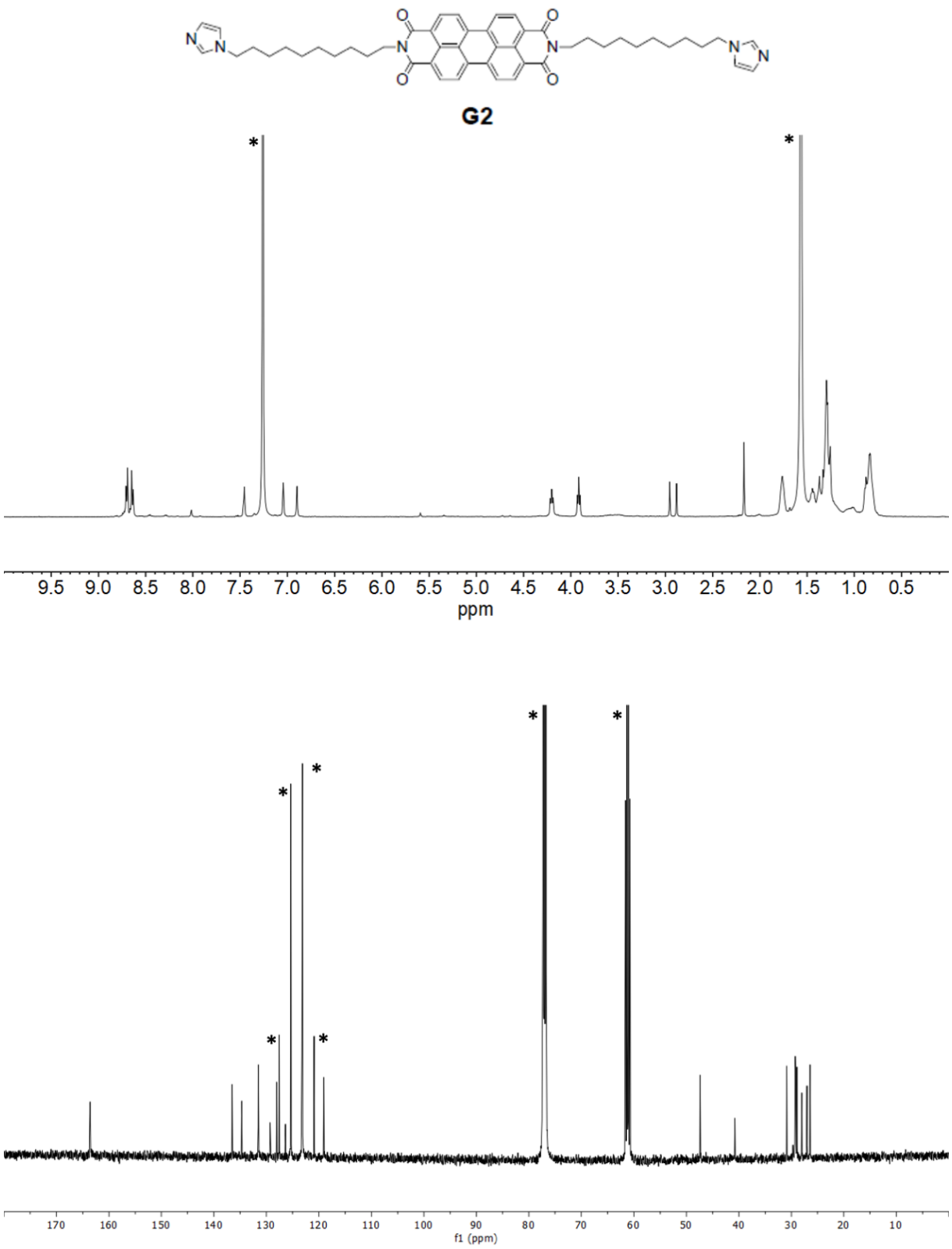



**Fig. S27**. Top: $^{1}$H NMR spectrum (500 MHz, 25°C, $CDCl_3$); and bottom: $^{13}$C NMR spectrum (125 MHz, 25°C, $CDCl_3$/TFE, 97:3, v/v) of **G2**. *Asterisks indicate residual solvent peaks and $H_2O$.