Electronic Supplementary Information

Self-Assembly of a Fluorescent Hydrogen-Bonded Capsule Based on an Amino-Acid Functionalised Tetraphenylethylene

Anna Brzechwa-Chodzyńska, ‡[a,b] Grzegorz Markiewicz, ‡[a,b] Piotr Cecot,[a,b] Jack Harrowfield,[c] and Artur R. Stefankiewicz[a,b]*

[a] Faculty of Chemistry, Adam Mickiewicz University, Uniwersytetu Poznańskiego 8, 61-614 Poznań, Poland E-mail: ars@amu.edu.pl

[b] Center for Advanced Technologies, Uniwersytetu Poznańskiego 10, 61-614 Poznań, Poland

[c] Université de Strasbourg, ISIS, 8 allée Gaspard Monge, 67083 Strasbourg, France

‡ These authors contributed equally in this work.

Table of contents

1. General information

All commercially available reagents including TPE, *S*-Trityl-L-cysteine, and solvents were obtained from commercial sources (mainly Sigma-Aldrich/Merck) and used without further purification. Quinine bisulfate was purchased at fluorescence-grade from Sigma-Aldrich. Water was obtained from a Merck MiliiQ purification system. NMR solvents were purchased from Deutero GmbH and used as received. The tetrabromide[1] **(TPE-Br)** and tetracarboxylic acid[2] **(TPE-COOH)** derivatives of tetraphenylethylene **(TPE)**, were prepared following the published procedures with some modification.

^{1}H, ^{13}C, HSQC, COSY NMR spectra were recorded on Bruker Fourier 300 MHz and Bruker Avance IIIHD 600 MHz NMR spectrometers equipped with a 5 mm probes and referenced on the solvent residual peaks. All spectra were acquired at 298 K unless otherwise stated and chemical shifts are expressed in ppm. DOSY NMR spectra were recorded using 2D LEDbp-pulse sequence (ledbpgp2s). Δ and δ were optimised with 1D sequence (ledbpgp2s1d) to achieve sufficient signal attenuation at 95% gradient strength. Diffusion coefficients were obtained from T1/T2 analysis and cross-checked with Bayesian transformation.

Elemental analysis was recorded on ThermoFisher Flash 2000 analyser in CHNS mode. Samples were dried for 24 h under high vacuo prior to the analysis.

MALDI MS spectra were recorded on Bruker ultrafleXtreme operating in linear mode using DCTB as a matrix.

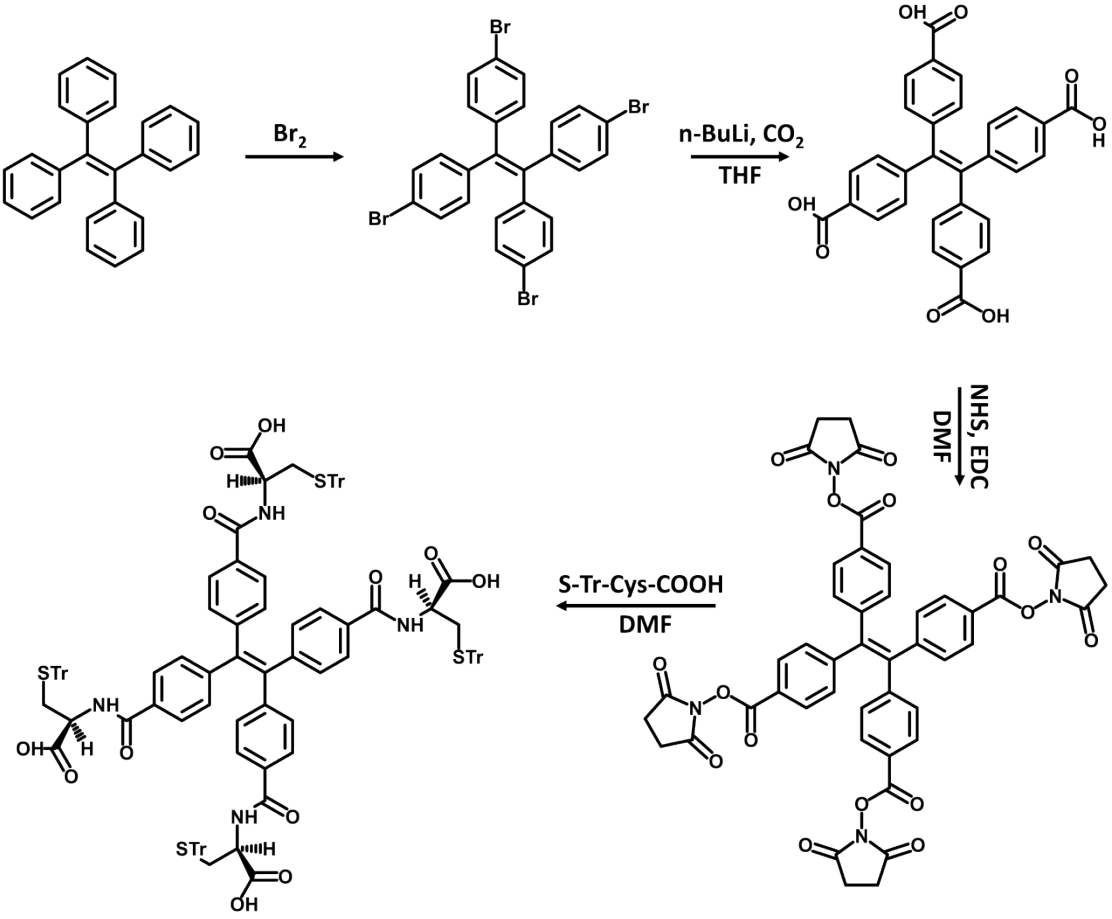
FT-IR spectra were recorded on Jasco FT/IR-4700 spectrometer in the airtight CaF_2 cuvette of 0.2 mm pathlength. Spectra of the pure solvents were used for subtraction.

Electronic absorption spectra were recorded on Jasco V-750 spectrophotometer equipped with Peltier-type temperature control unit in Helma quartz cuvettes of 5×5 mm pathlength. Photoluminescence spectra were recorded on Jasco FP-8300 spectrofluorometer equipped with Peltier-type temperature control unit in Helma quartz cuvettes of 5×5 mm pathlength. For PL quantum yield determination, both spectrometers were set up at constant ABS, E_x and E_m slits of 5 nm. An excitation wavelength of λ_{ex} = 390 nm and PL detector sensitivity of PMT = 275 V were constant for all measurements.

ECD and FD-CD spectra were recorded on Jasco J-1500 spectropolarimeter equipped with Peltier-type temperature control unit in Helma quartz cuvettes of 2x10 mm and 10×10 mm pathlengths respectively. FD-CD spectra were recorded in manual mode at constant HT voltage on the emission side of 700 V, and low-pass filter of 420 nm. For noise reduction, both ECD and FDCD the spectra were accumulated, with 3 acc./spectrum for ECD and 10 acc./spectrum for FDCD.

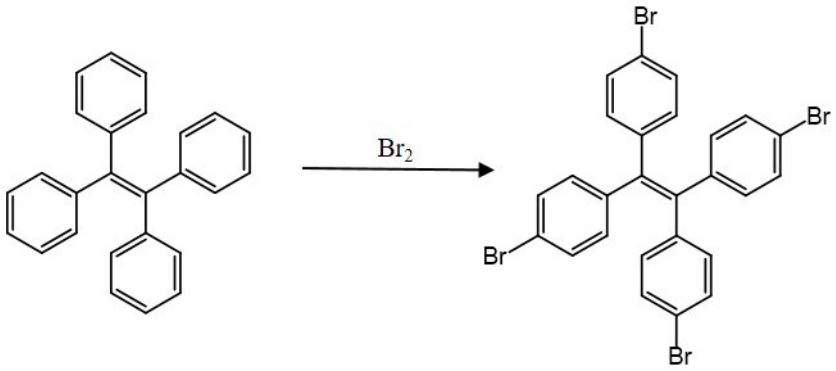
DLS analysis was recorded on Malvern Zetasizer Nano analyser in Helma quartz cuvettes of 10x10 mm pathlength at 298 K and at a scattering angle of 173°. In order to remove any dust particles, THF and H_2O were filtered 2 times via syringe filters (0.2 µm) and the cuvettes were cleaned with hot aqua regia and the above filtered H_2O.

2. Synthetic Procedures



Scheme S1. The four-step synthesis of **TPE-S-Trityl-L-cysteine** (**1**).

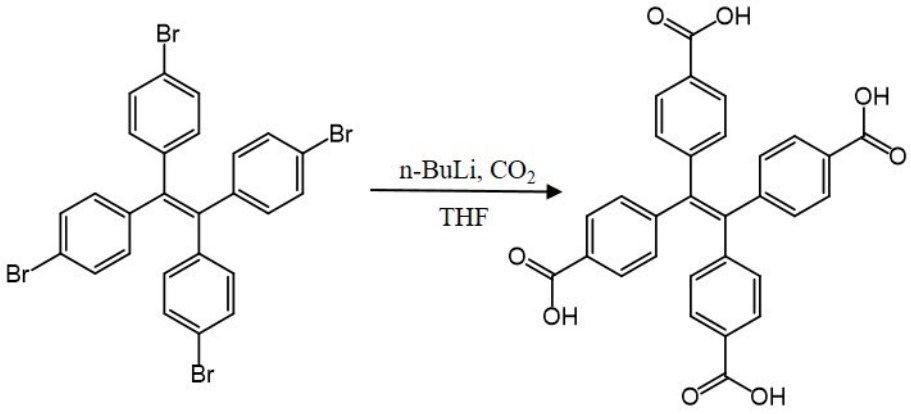
*Preparation of tetra-(4-bromo-phenyl)ethylene - **TPE-Br***



Scheme S2. Synthesis of **TPE-Br**.

TPE-Br was prepared by a known procedure[1] with some modification. Tetraphenylethylene (2.5 g, 7.5 mmol) was thoroughly powdered in a mortar and a thin layer of powder was placed on a Petri dish in a desiccator over liquid Br_2 (3 ml) for 3 days. The desiccator was slightly glazed to allow the HBr to escape. The crude green product was kept under a high vacuum for 10 hours. TPE-Br was obtained as a light green powder (3.7 g, 95 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.34 Hz, 8H), 6.84 (d, *J* = 8.34 Hz, 8H).

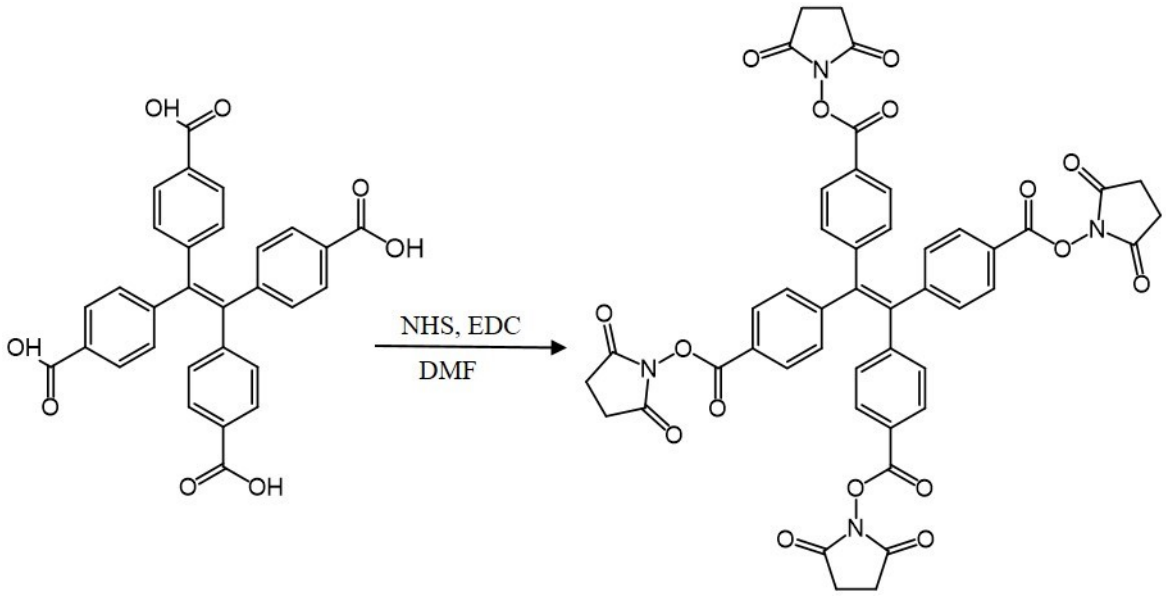
*Preparation of tetrakis(4-benzoic acid)ethylene - **TPE-COOH***



Scheme S3. Synthesis of **TPE-COOH**.

TPE-COOH was prepared by a known procedure[2] with some modification. To **TPE-Br** (2.7 g, 4.2 mmol) placed in a flask and degassed under Ar for 30 minutes, freshly distilled dry THF (50 ml) was added and the substance allowed to dissolve to form a clear solution. This was cooled to -80 ^{o}C (liquid nitrogen/technical acetone bath) and *n*-butyllithium (1.6 M in hexane, 13.2 ml, 21 mmol) was added dropwise under Ar. After addition, the mixture was stirred for 1 hour under the same conditions before solid carbon dioxide (25 g) was added in portions. The mixture was allowed to warm to room temperature and stirred overnight. Distilled water (20 ml) was added, THF evaporated out and the remaining aqueous solution was acidified with 1 M HCl (12 ml). The addition of chloroform provided a creamy precipitate, which was collected by filtration and washed with chloroform and water. The material collected was then sonicated in chloroform and the solid produced was dried under a high vacuum to give TPE-COOH as a light yellow powder (1.2 g, 57 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.93 (s, 4H), 7.74 (d, J = 8.27 Hz, 8H), 7.11 (d, J = 8.26 Hz, 8H).

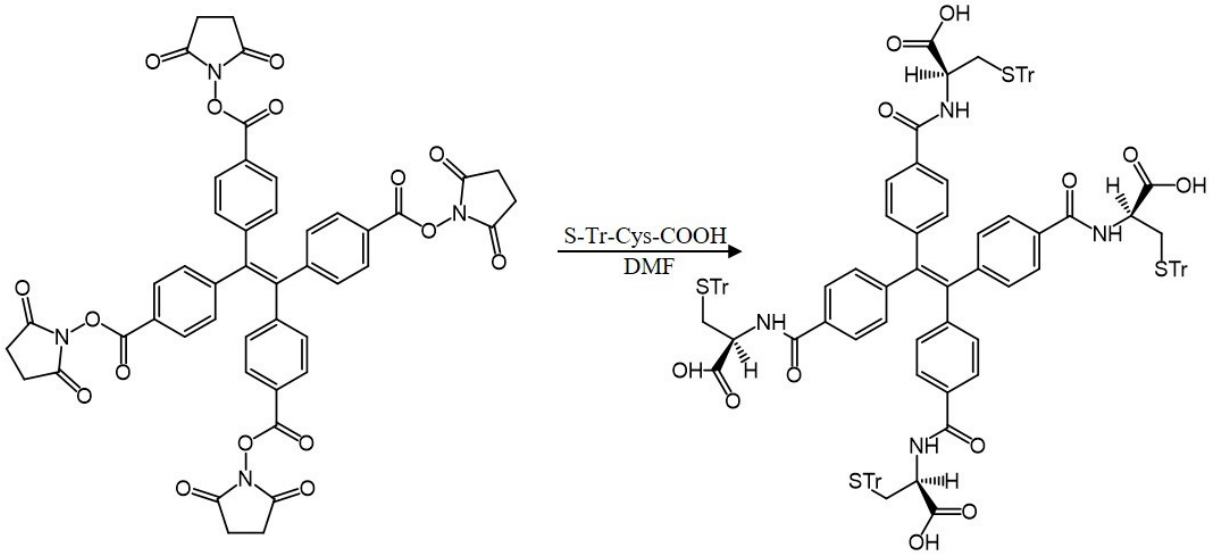
*Preparation of tetrakis (4-carboxylic acid NHS ester)ethylene - **TPE-NHS ester***



Scheme S4. Synthesis of **TPE-NHS ester**.

A solution of **TPE-COOH** (300 mg, 0.6 mmol), *N*-hydroxy succinimide (414 mg, 3.6 mmol), EDC·HCL (688 mg, 3.6 mmol) in DMF (50 mL) was stirred at room temperature for 24 hours. The solvent was removed and the residue redissolved in a small amount of acetone. This solution was slowly added dropwise to 1 M HCl (100 ml) to give a light brown precipitate that was collected by filtration, washed with water, and dried under a high vacuum to give TPE-NHS ester as an intensely yellow powder (380 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.98 (d, J = 8.44 Hz, 8H), 7.36 (d, J = 8.37 Hz, 8H), 2.88 (s, 16H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.29, 161.32, 148.34, 141.41, 132.01, 130.12, 123.44, 25.55.

*Preparation of tetrakis (4-S-Tr-Cys-amid)ethylene - **TPE-S-Trityl-L-cysteine***



Scheme S5. Synthesis of **TPE-S-Trityl-L-cysteine**.

To a solution of **TPE-NHS ester** (200 mg, 0.2 mmol) in DMF (35 ml), *S*-Trityl-L-cysteine (437 mg, 1.2 mmol) and triethylamine (0.5 ml) were added. The mixture was stirred for 24 hours at room temperature. The solvent was evaporated and the residue was dissolved in a small amount of acetone. This solution was added dropwise to 1 M HCl to precipitate a light brown solid, which was then filtered off and washed with water. To remove the excess *S*-Trityl-L-cysteine, the solid was recrystallized from a mixture of DCM and *n*-hexane. The precipitate was filtered off, washed with water (50 ml), and dried in a desiccator. Because of solvent encapsulation, this material was heated in acetonitrile, sonicated, filtered off, and heated again in water before being dried under high vacuum to give TPE-S-Tr-Cys-amide as a light cream powder (266 mg, 65%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.70 (s, 4H), 8.75 (d, J = 7.50 Hz, 4H), 7.70 (d, J = 8.08 Hz, 8H), 7.30 (m, 60H), 7.14 (d, J = 8.08 Hz, 8H), 4.23 (m, 4H), 2.74 (m, 4H), 2.47 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.70, 165.56, 145.66, 144.25, 132.06, 130.77, 129.09, 128.07, 127.36, 126.82, 66.30, 52.21, 39.52, 32.73. ESI-MS Calcd for $C_{118}H_{95}N_4O_{12}S_4$=[M-H]$^-$ =1888.587, Found: [M-H]$^-$ 1888.571 m/z. EA calcd for $C_{118}H_{96}N_4O_{12}S_4$: C 74.98, H 5.12, N 2.96, S 6.78%; Found: C 74.65, H 5.24, N 3.04, S 6.61%.

3. Experimental data for TPE-NHS ester

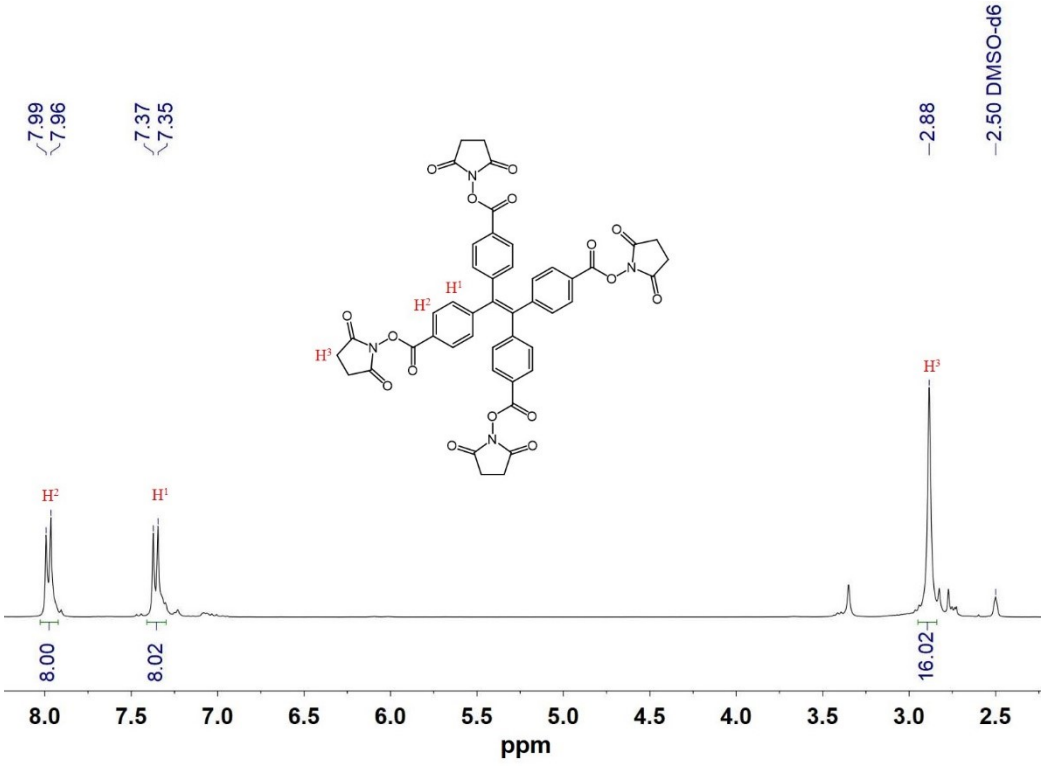


Figure S1. ^{1}H NMR spectrum of **TPE-NHS ester** in DMSO-d_6.

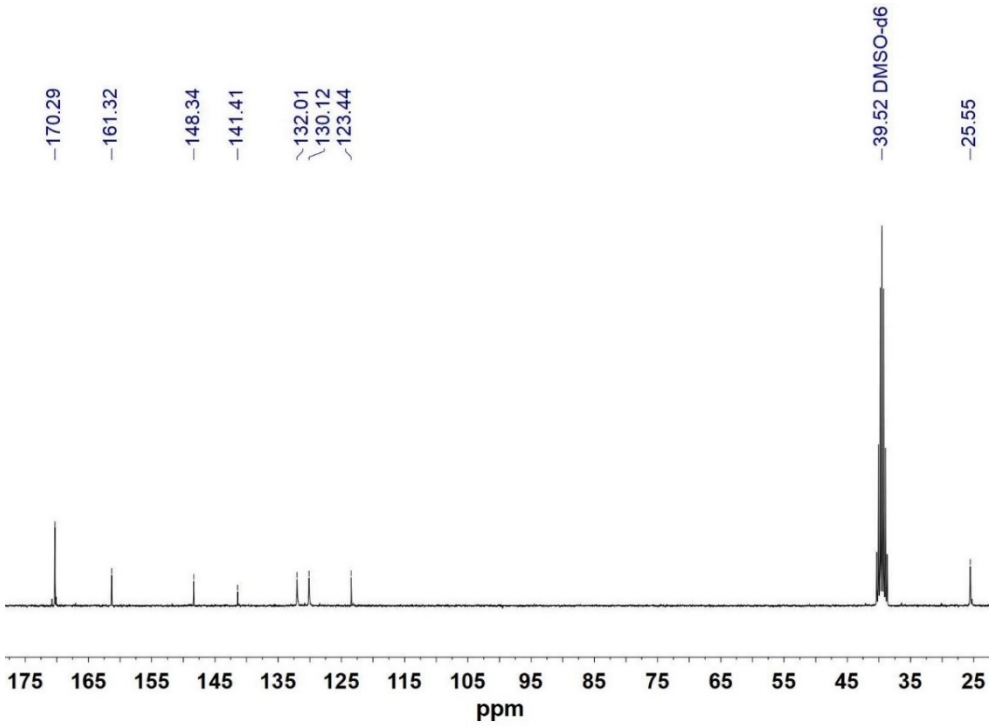


Figure S2. ^{13}C NMR spectrum of **TPE-NHS ester** in DMSO-d_6.

4. Experimental data for TPE-S-Tr-cysteine

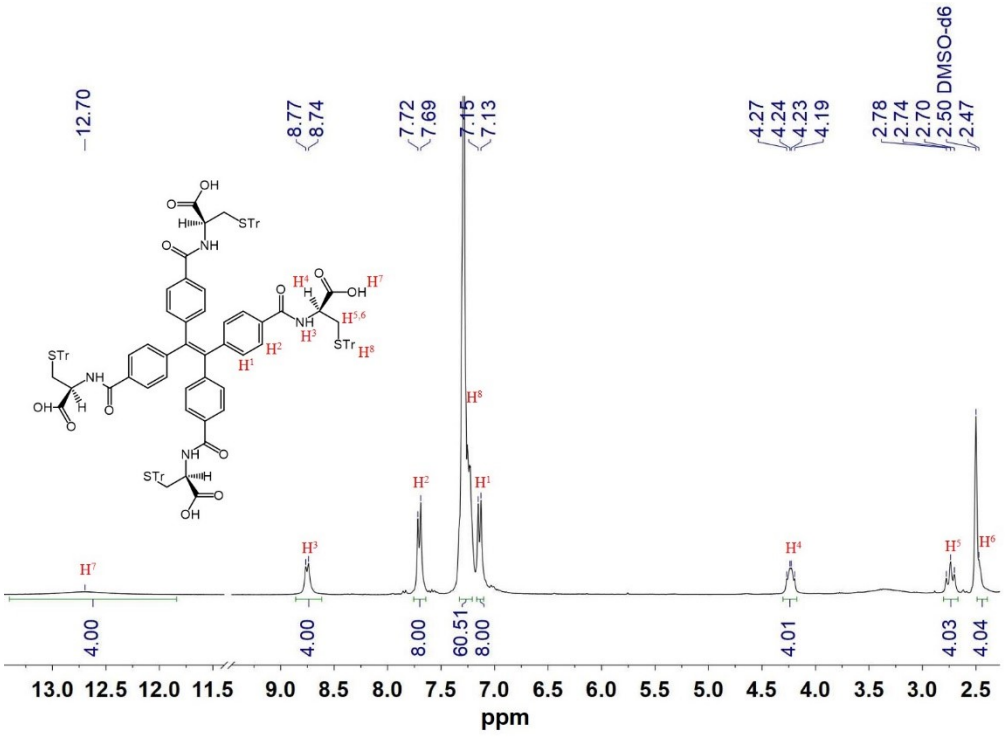


Figure S3. ^{1}H NMR spectrum of **TPE-S-Tr- L-cysteine** in DMSO-d_6.

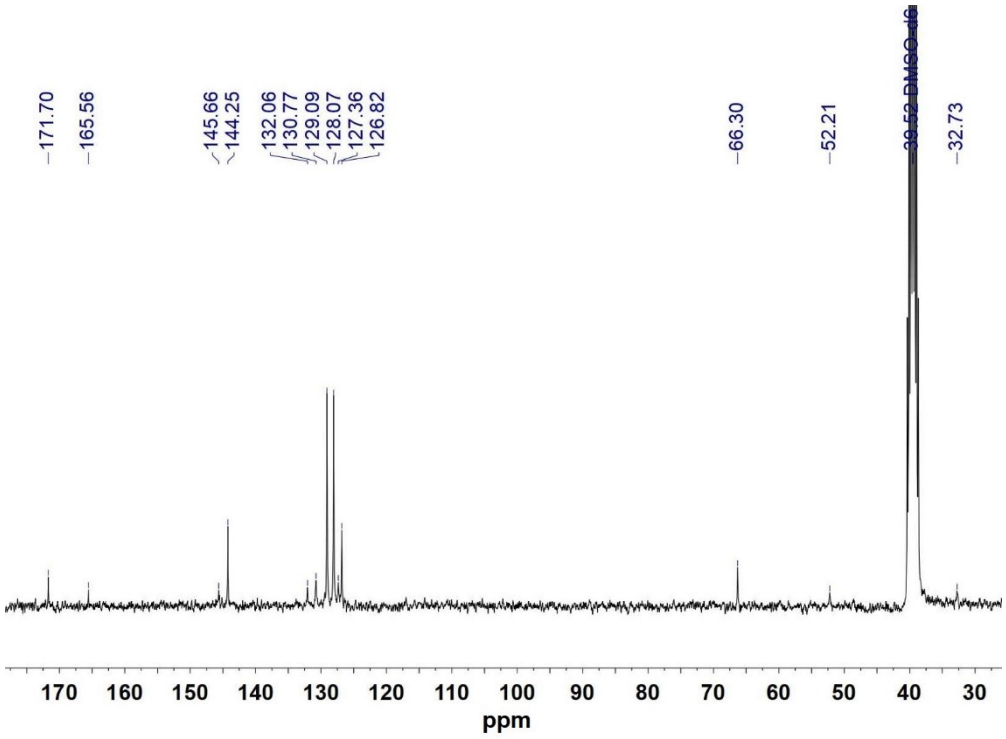


Figure S4. ^{13}C NMR spectrum of **TPE-S-Tr- L-cysteine** in DMSO-d_6.

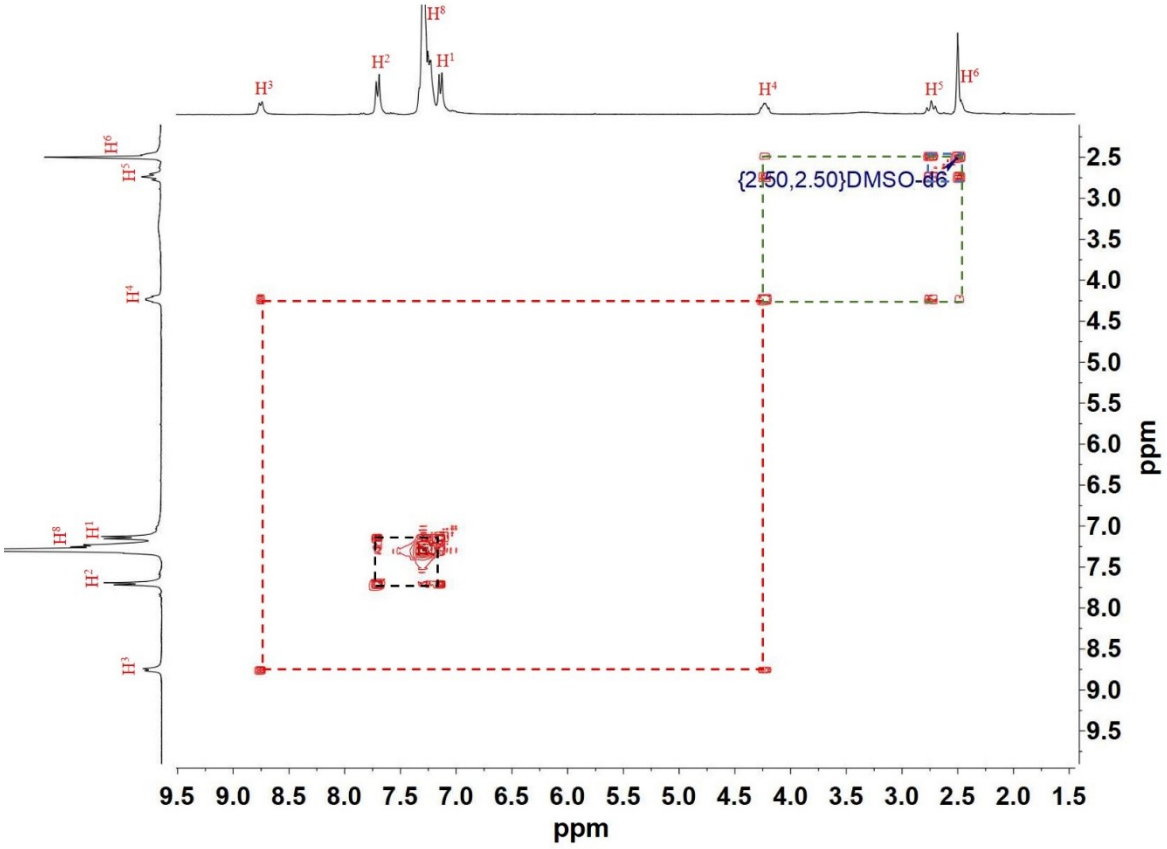


Figure S5. ^{1}H-^{1}H COSY NMR spectrum of **TPE-S-Tr- L-cysteine** in DMSO-d_6.

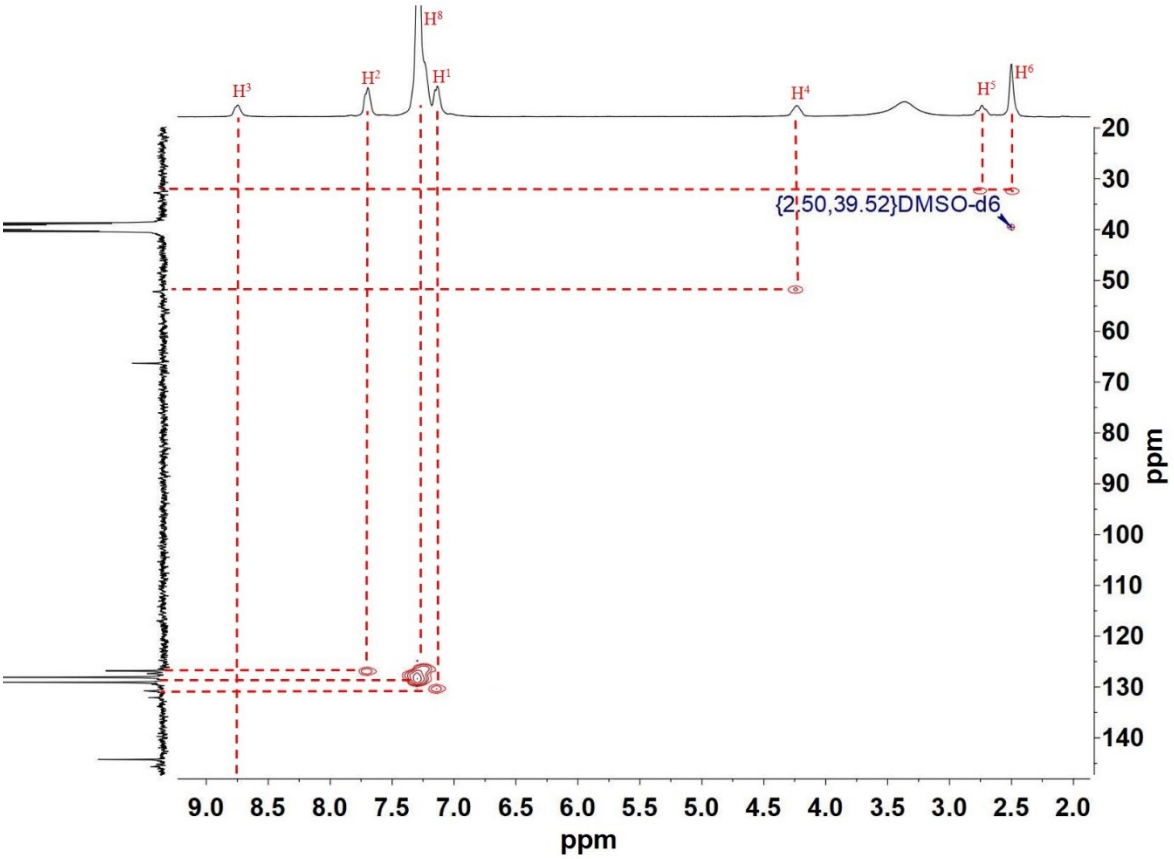


Figure S6. ^{1}H-^{13}C HSQC NMR spectrum of **TPE-S-Tr-L-cysteine** in DMSO-d_6.

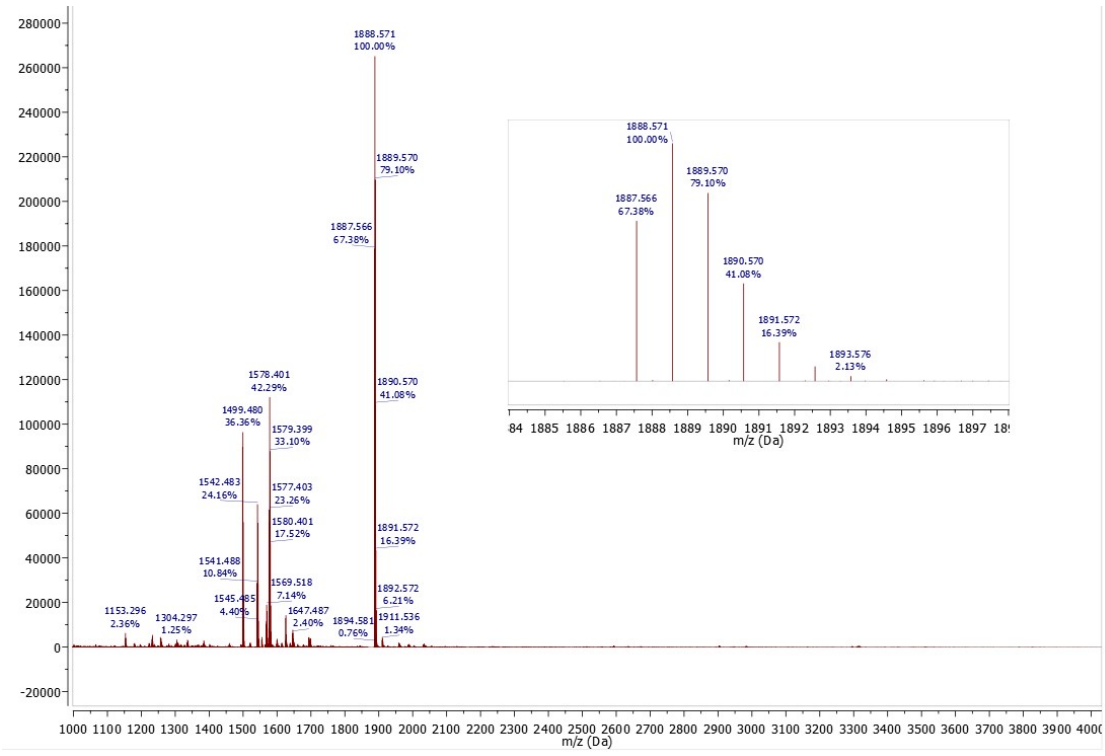


Figure S7. ESI-TOF-MS spectrum of **TPE-S-Tr-L-cysteine** (negative ion mode).

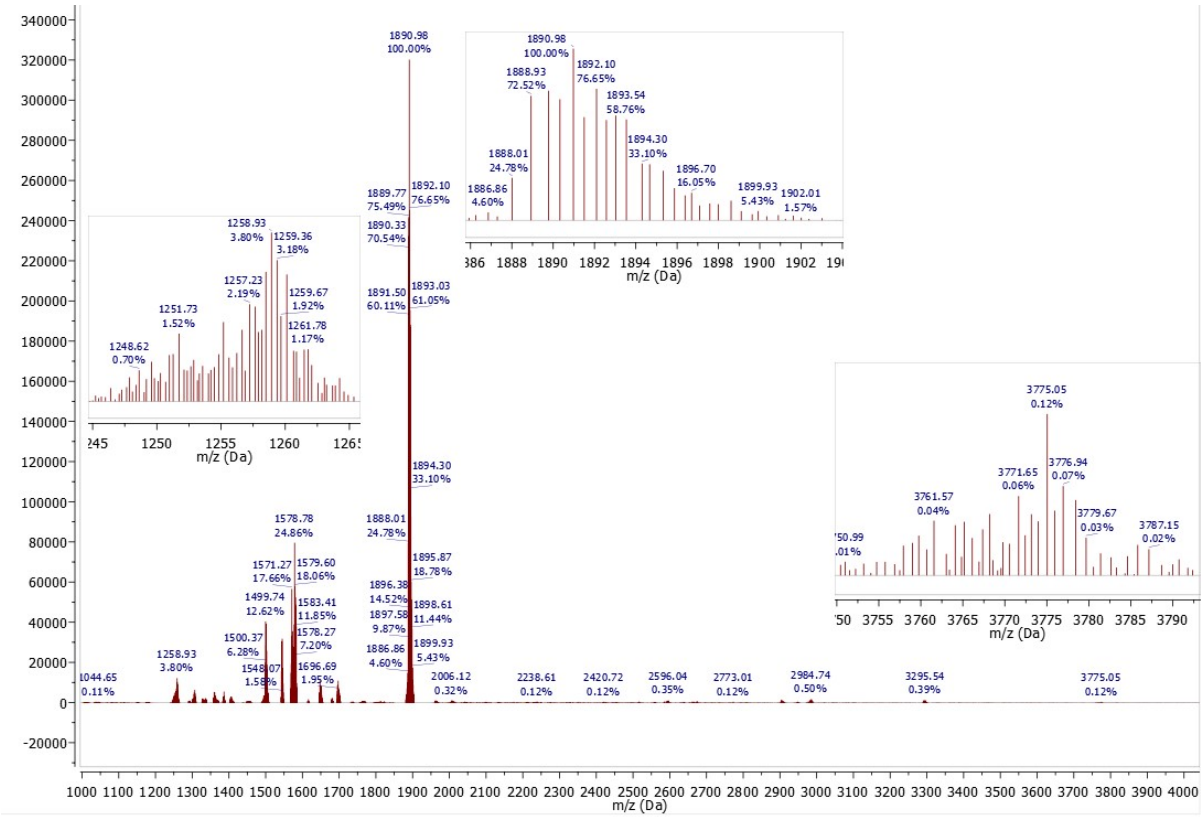


Figure S8. MALDI MS spectrum of **TPE-S-Tr-L-cysteine** recorded in linear mode on DCTB matrix.

5. Molecular modelling and determination of the cavity volume

The molecular model for the self-assembled dimeric capsule was prepared based on methodology proposed by Mastalerz et. al.[16, main text] in their work. The calculations were performed using Gaussian 16[3] and Wavefunction Spartan'14[4] programs. Potential hydrogen bonding structural motifs were identified inside the carbonyl, amine and carboxylic functions. The starting geometry of a monomeric structure was pre-optimised with the MMFF method and two monomers were stacked over each other, with potential H-bond acceptors/donors were placed over each other close enough so that hydrogen bonds were detected by the software. The dimeric structure was then optimised fully using the RM1 semiempirical method[5]. The final structure of dimer involved the formation of 8 hydrogen bonds between pairs of carboxylic groups, while the structural constraints prevented formation of hydrogen bonds between other donor-acceptor groups in the assembly. The structure has the shape of an oblate spheroid with an equatorial radius of ~14 Å and axial radius of ~4 Å

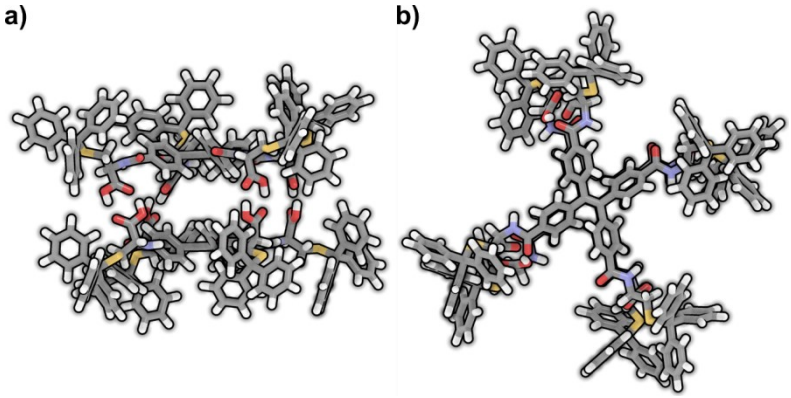


Figure S9. Optimised structure of a dimeric capsule: a) side view, b) top view.

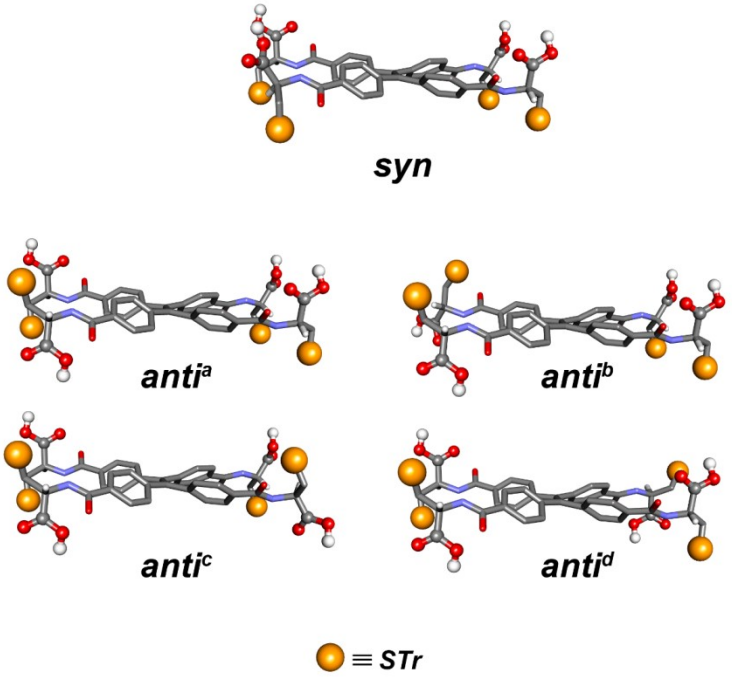


Figure S10. Five possible conformations of COOH binding groups of **TPE-S-Tr- L-cysteine**.

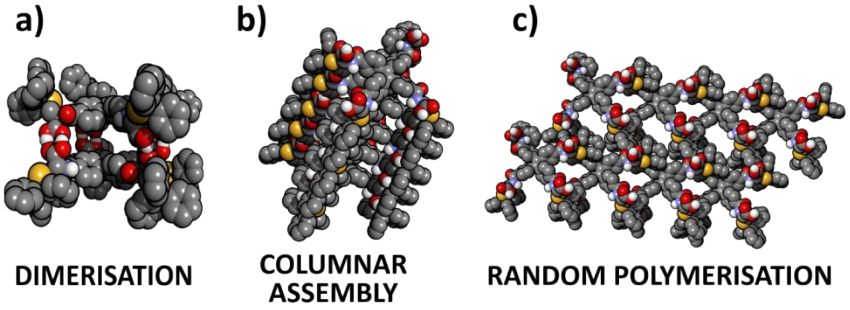


Figure S11. Three possible aggregated products of **TPE-S-Tr- L-cysteine**. The monomer of **TPE-S-Tr-cysteine** might be expected to aggregate in solution via COOH-HOOC hydrogen bonds, although amide NH···O interactions are another possible source of aggregation. The TPE core is not planar and favours twisted-propeller type conformations, so extended π-stacking interactions are not possible.

6. Estimation of hydrodynamic radii and spherical volumes by DOSY NMR and DLS experiments.

DOSY NMR experiments were recorded at 298 K. The hydrodynamic radii were estimated using the Stokes-Einstein equation:

$$D = \frac{k_B \times T}{6\pi\eta r}$$

D - the measured diffusion coefficient ($m^2 \cdot s^{-1}$)

k_B - Boltzmann constant ($1.3806485 \times 10^{-23} m^2 \cdot kg \cdot s^{-2} \cdot K^{-1}$)

T - the temperature (K)

r - the hydrodynamic radius of the analyte (m)

η – the viscosity of the solvent at temperature T ($kg \cdot m^{-1} \cdot s^{-1}$)

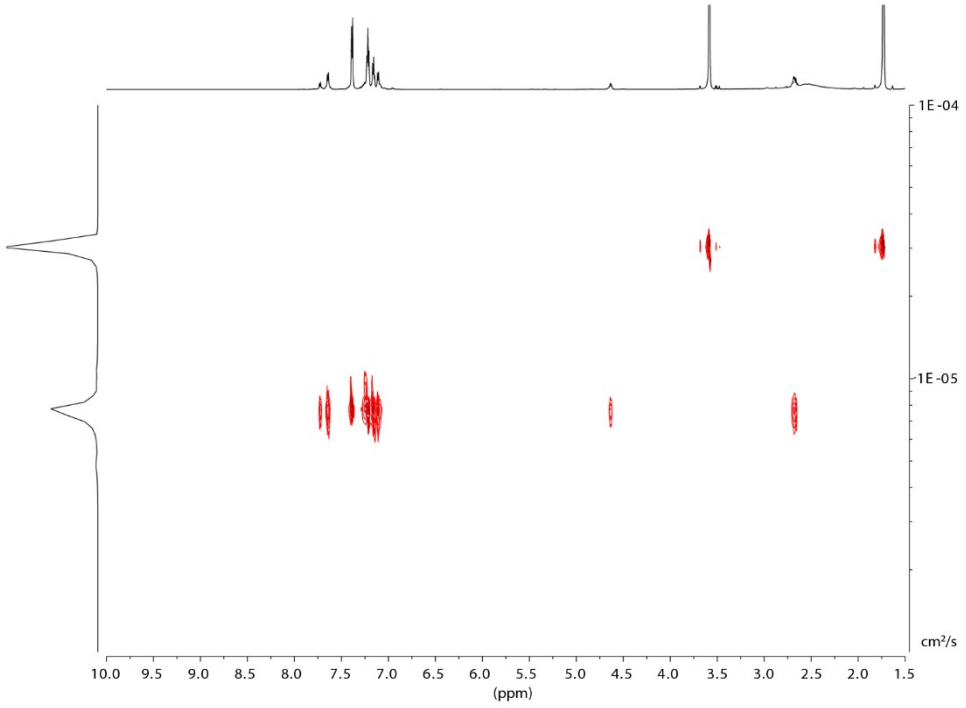


Figure S12. DOSY NMR spectrum (THF-d_8, 600 MHz) of **TPE-S-Tr-L-cysteine**.

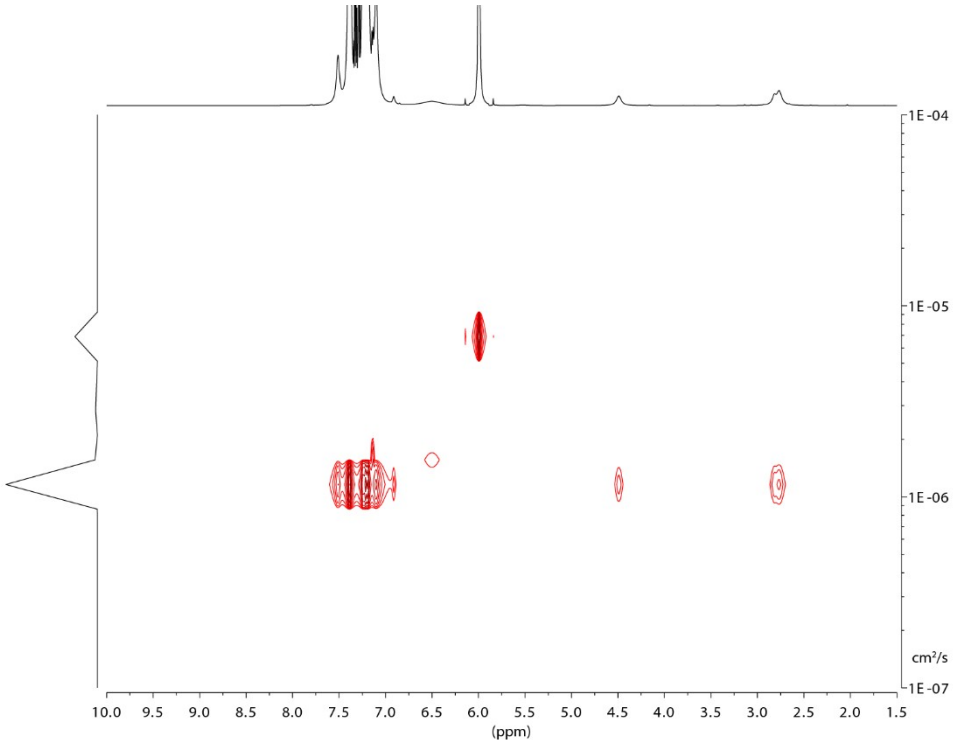


Figure S13. DOSY NMR spectrum (TCE-d_2, 600 MHz) of **TPE-S-Tr-L-cysteine**.

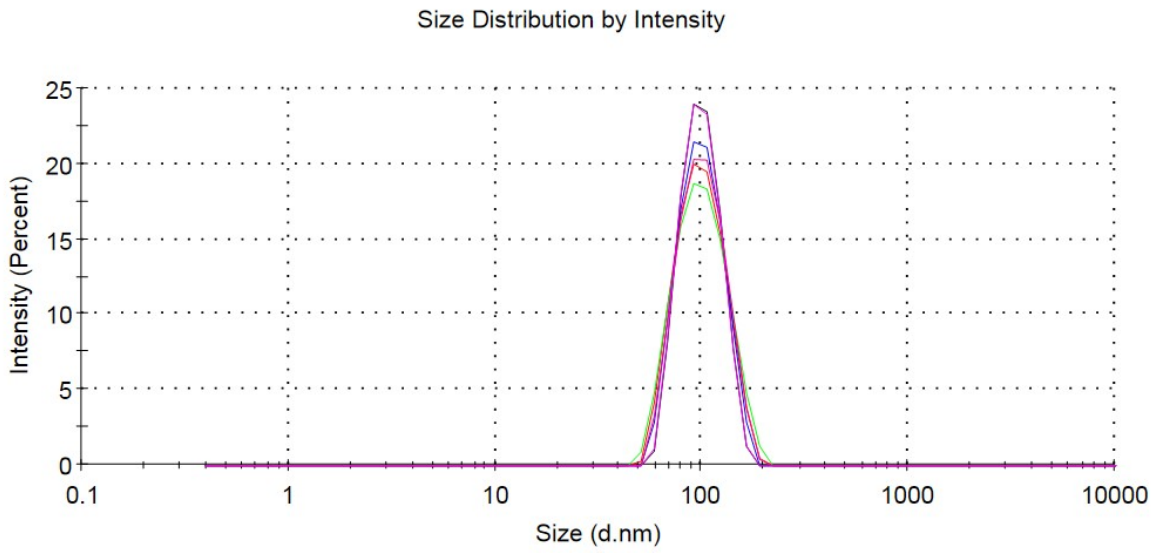


Figure S14: DLS analysis of **TPE-S-Tr-L-cysteine** aggregates in THF:water mixture (9:1 v/v) at 1.0×10^{-5} M and 298 K. Equilibration time t_{eq} = 30 min. 6 consecutive measurements are shown.

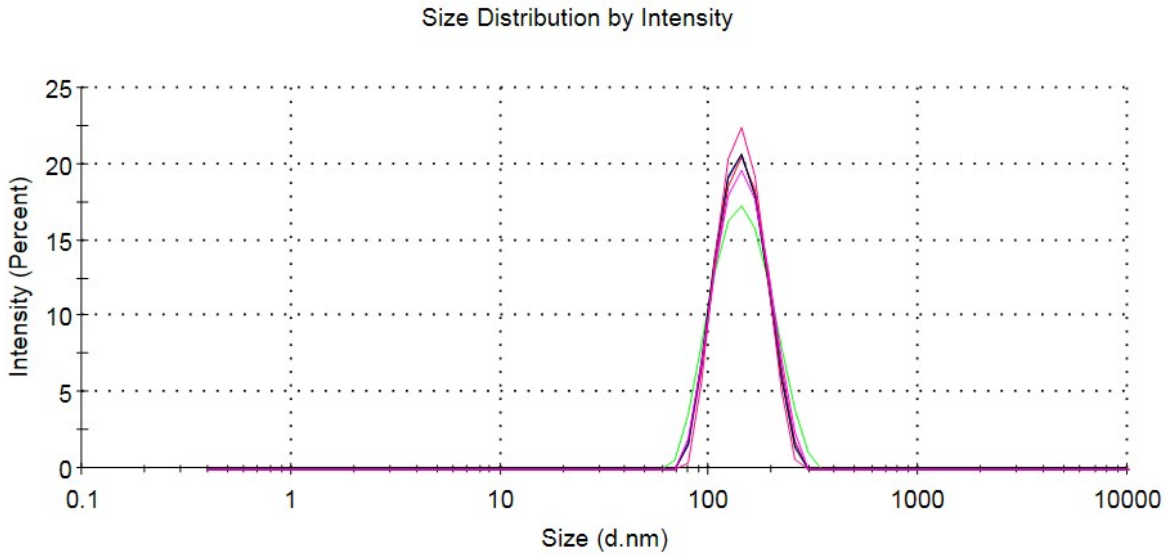


Figure S15: DLS analysis of **TPE-S-Tr-L-cysteine** aggregates in THF:water mixture (9:1 v/v) at 1.0×10^{-4} M and 298 K. Equilibration time t_{eq} = 30 min. 6 consecutive measurements are shown.

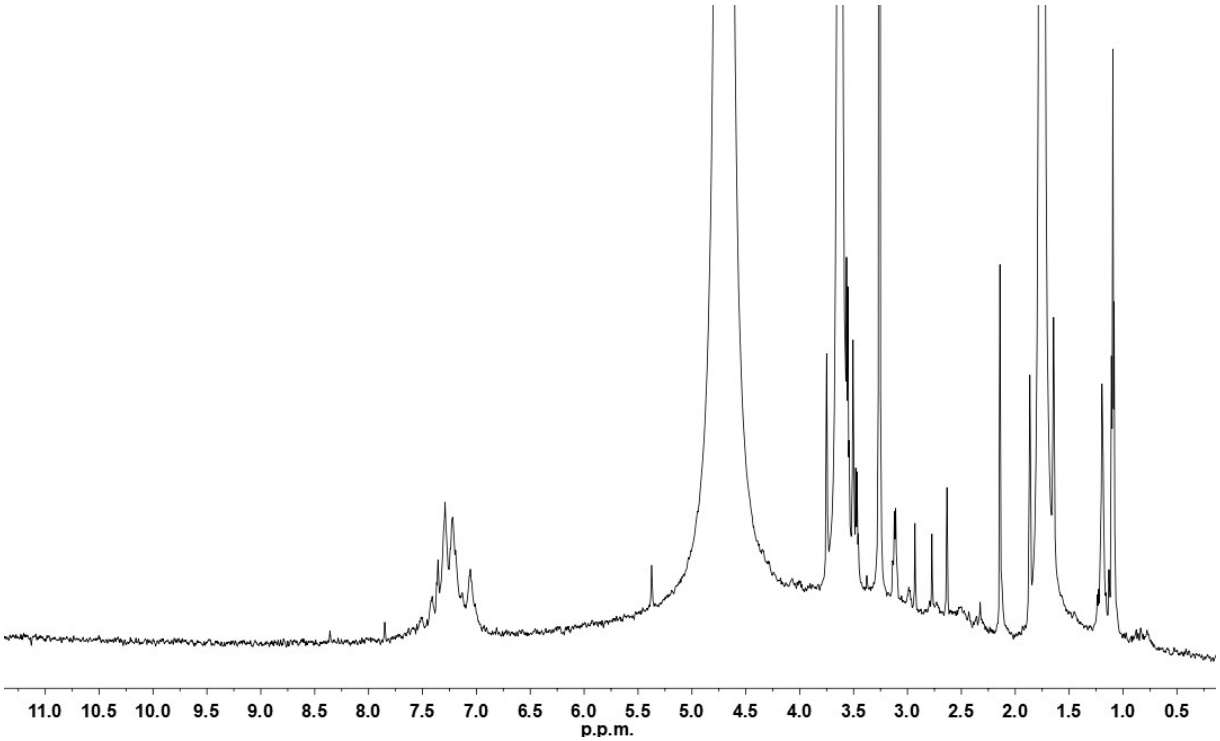


Figure S16. ^{1}H NMR spectrum of **TPE-S-Tr- L-cysteine** aggregate in THF-d_8:D_2O mixture (9:1 v/v) 1.0×10^{-4} M and 298 K. Experimental time≈2 h, apodization lb=3.0 Hz.

7. Variable temperature ^{1}H NMR spectra

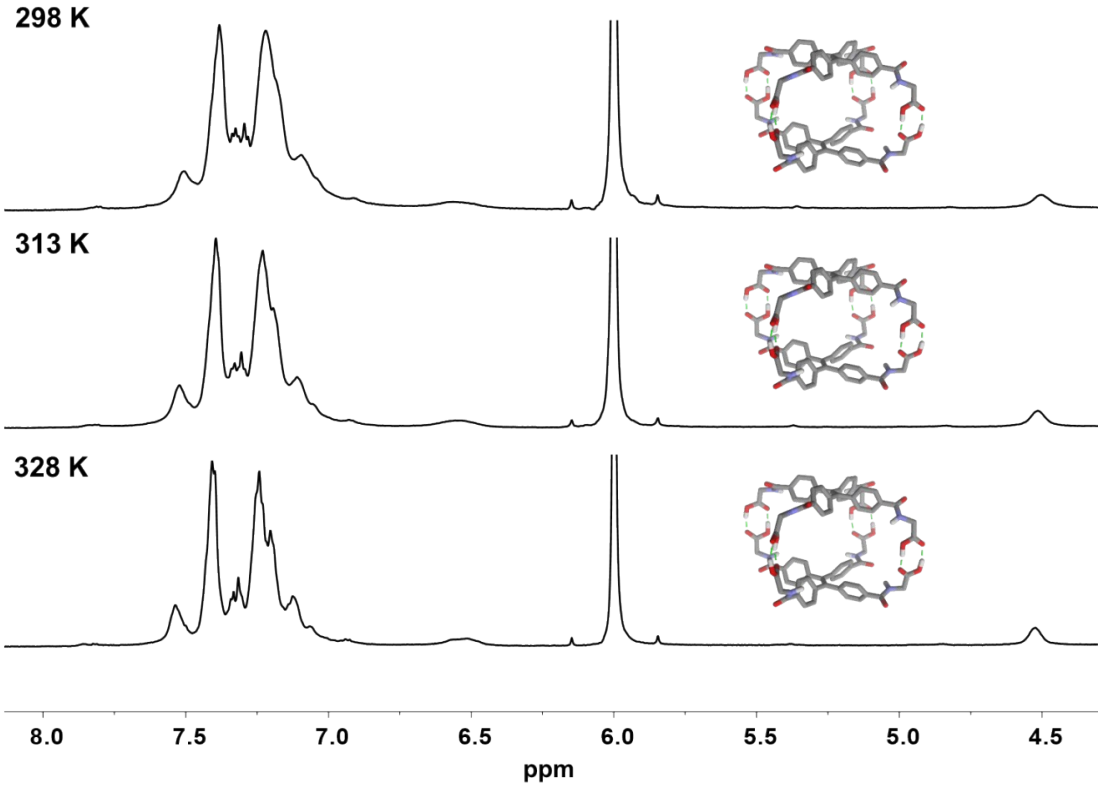


Figure S17. ^{1}H NMR spectrum (TCE-d_2, 600 MHz) of **TPE-S-Tr-L-cysteine** recorded between 298-328 K.

8. FT-IR spectra

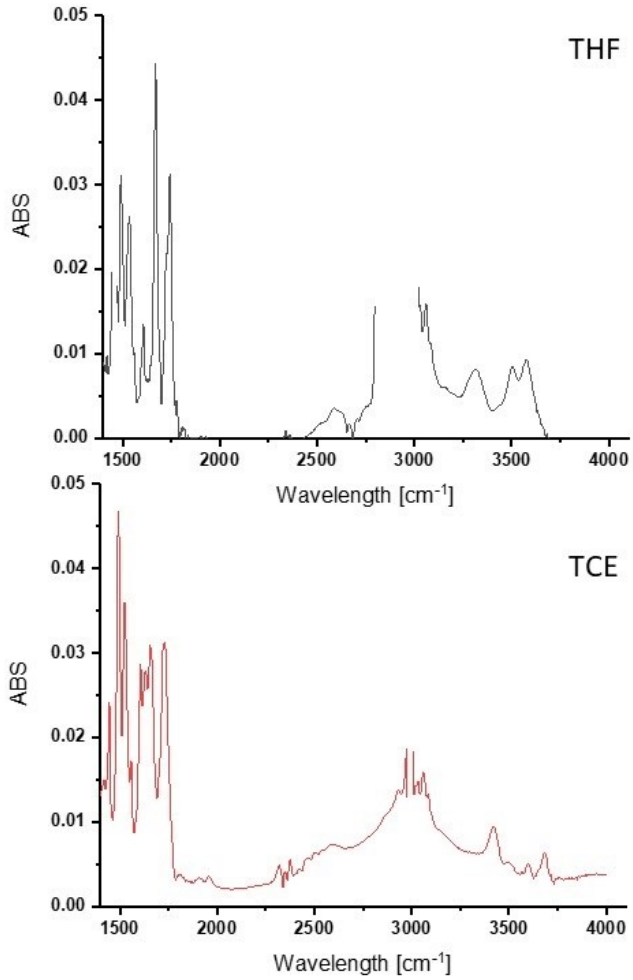


Figure S18. FT-IR spectra of **TPE-S-Tr- L-cysteine** in THF (top) and TCE (bottom) recorded at 1.0×10^{-3} M and 298 K.

9. Thermodynamic study

The dimerization equilibrium constant K_D was defined as follows:

$$K_D = \frac{[\mathbf{1}_2]}{[\mathbf{1}]^2}$$

Where [$\mathbf{1}_2$] and [**1**] denote the concentrations of the individual species at equilibrium, and the total concentration of the sample C_T is defined as:

$$C_T = 2[\mathbf{1}_2] + [\mathbf{1}]$$

ΔCD at λ = 300 nm was plotted as a function of temperature *T* and fitted using the *T*-dependent equal-K (isodesmic) assembly model.[S6] The thus obtained plot was then normalized within aggregation degree range α = 0.0 and α = 1.0, where:

$$\alpha = 0.0 \quad \equiv \quad [\mathbf{1}] = C_T \qquad \alpha = 1.0 \quad \equiv \quad [\mathbf{1_2}] = \frac{C_T}{2}$$

With the assumption, that the CD at α = 0.0 represents the molar ellipticity of a monomer, and the CD at α =1.0 represents the molar ellipticity of a dimer.

The individual concentrations of [$\mathbf{1}_2$] and [**1**] and subsequently K_D at particular temperature *T,* were calculated from the observed α and material distribution. K_D was determined for six temperatures *T* in 270-360 K range, and subsequently $\ln K_D$ was plotted as a function of 1/*T* according to the linear Van't Hoff relation.

$$lnK_D = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

ΔH and *ΔS* were determined from the slope and intercept respectively.

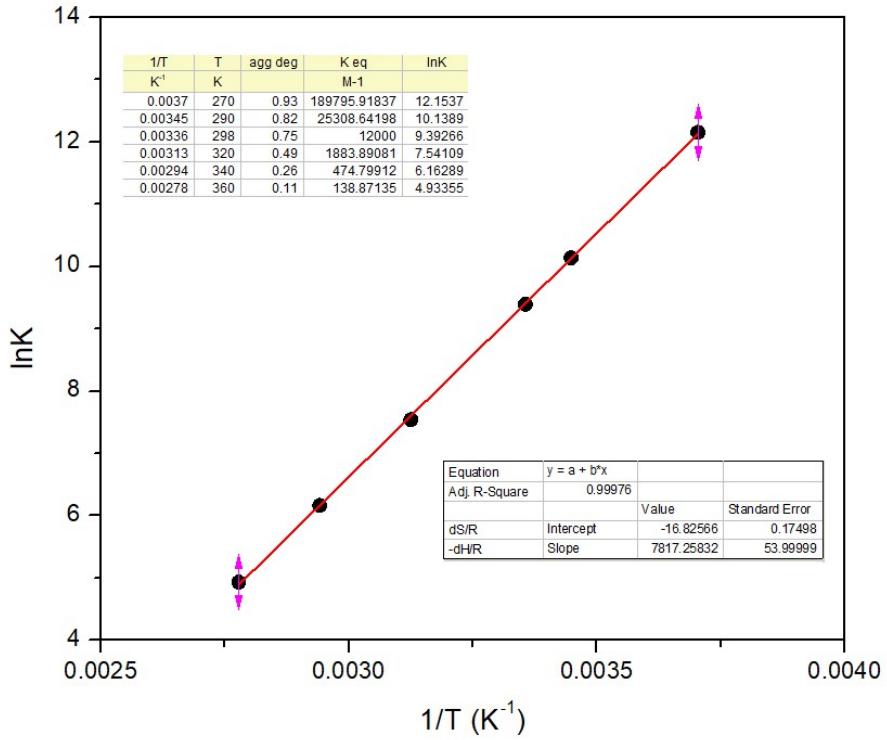


Figure S19. Van't Hoff plot for the dimerization of **TPE-S-Tr- L-cysteine** in TCE (C=5.0 ×10^{-4} M) calculated from the fitting of VT CD measurements.

10. References

[1] B. J. Deibert, E. Velasco, W. Liu, S. J. Teat, W. P. Lustig, J. Li, *Crystal Growth & Design* **2016**, *16*, 4178-4182.

[2] H.-L. Sun, R. Jiang, Z. Li, Y. Q. Dong, M. Du, *CrystEngComm* **2013**, *15*, 1669-1672.

[3] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, Wallingford, CT, **2016**.

[4] I. Wavefunction, *Irvine, CA* **2014**.

[5] G. B. Rocha, R. O. Freire, A. M. Simas, J. J. Stewart, *Journal of computational chemistry* **2006**, *27*, 1101-1111.

[6] M. M. J. Smulders, M. M. L. Nieuwenhuizen, T. F. A. de Greef, P. van der Schoot, A. P. H. J. Schenning, E. W. Meijer, Chem. Eur. J. 2010, 16, 362.