Supporting Information

Unravelling the Interplay between Structure, Self-Assembly Patterns, AIEE and Chiroptical Properties of NDI-bis-Cholesteryl Systems

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Contents

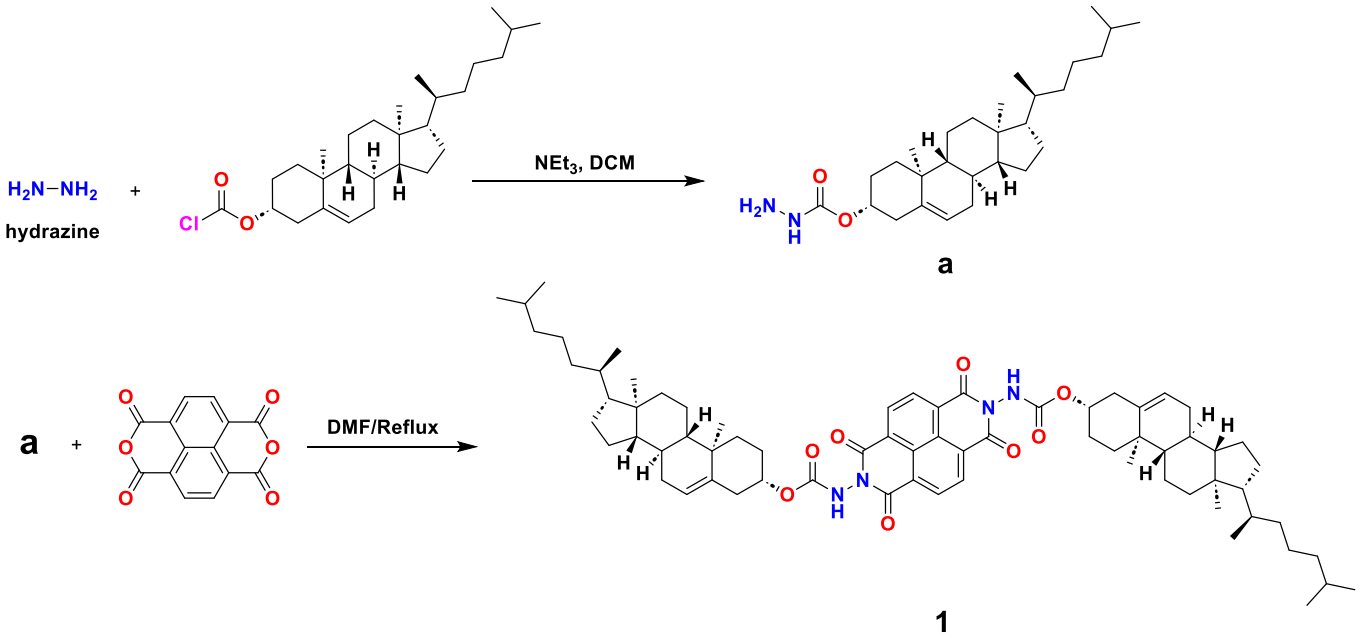
1. General Information

All the starting materials, reagents and requisite solvents were used as received. 1, 4, 5, 8-Naphthalenetetracarboxylic dianhydride (NTCDA), cholesterol chloroformate, Hydrazine hydrate, 4, 4'-methylenedianiline, 1, 4-diaminobutane and Boc-anhydride were purchased from TCI chemicals. Dichloromethane (DCM), Methanol (MeOH) Ethanol (EtOH), N, N-dimethylformamide (DMF), Chloroform ($CHCl_3$), n-hexane utilized in all the experiments were purchased from SRL Chemicals.

NMR was recorded on a Bruker 400 MHz spectrometer. MALDI-MS was done using Bruker Daltonics FLEX-PC (Autoflex- TOF) using α-cyano-4-hydroxycinnamic acid (CHCA) matrix. MALDI data was collected in negative ion reflector mode over a mass range of m/z 900-4500 Da. UV-Visible measurements were done using Agilent Cary 60 Spectrophotometer. Fluorescence measurements were conducted on Agilent Cary Eclipse Spectrophotometer. SSPL measurements were done using HORIBA Canada QM8450-22-C instrument. FE-SEM imaging was performed using a ZEISS EVO Series Scanning Electron Microscope EVO 50 and EVO 18. Powder X-ray diffraction measurements were performed using a Panalytical Empyrean Series 3 diffractometer, having a parallel beam geometry and a Cu Kα line focused radiation (λ = 1.540598 Å) at 45 kV, 40 mA power and detector with a 2.0 mm radiation entrance slit. CD experiment was performed on JASCO-CD POLARIMETER Time-resolved studies were performed in a HORIBA DeltaFlex Time-Correlated Single Photon Counting system using 320 nm Delta-diode as the excitation. The CPL measurements were done using JASCO CPL-300 CPL spectrometer. The rheological experiments were carried out in cone and plate geometry (diameter= 25 mm, 0.1 rad) on the rheometer plate using TA instruments Rheometer ARES G2. Dynamic Light Scattering experiments were done using Horiba SZ-100V2.

2. Synthetic procedure for compound 1, 2 and 3

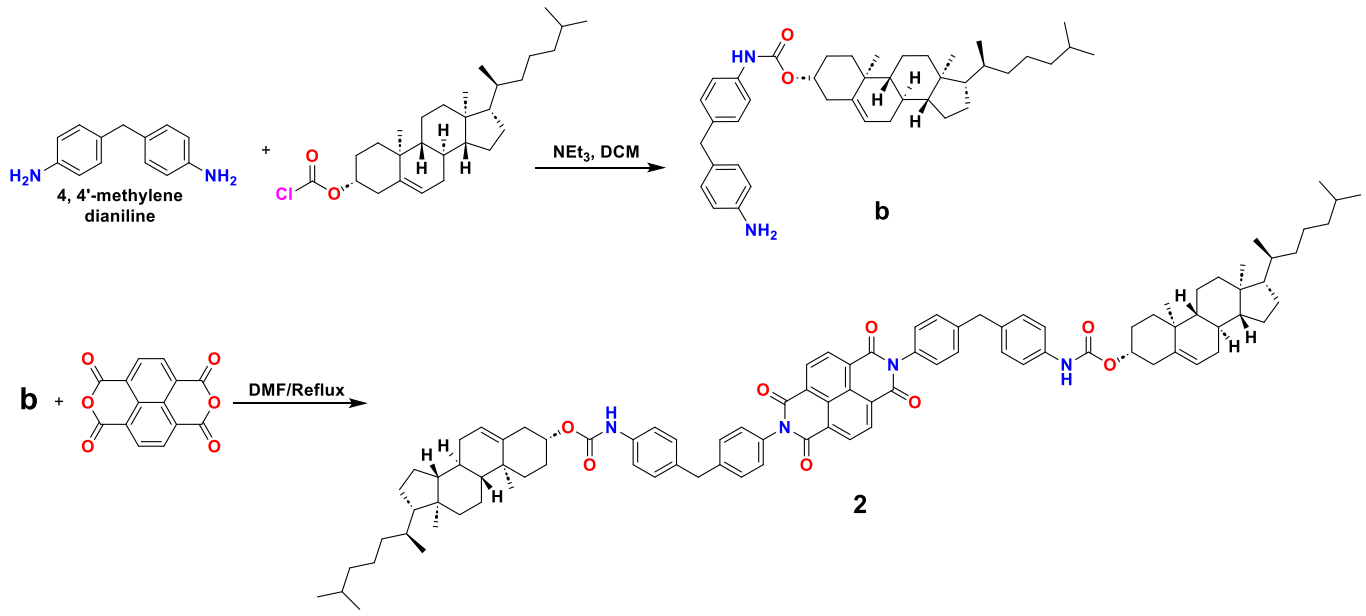
Synthesis of 1:



Synthetic Procedure: For the synthesis of **a**, Hydrazine hydrate was taken in a 100 mL round bottomed flask under nitrogen atmosphere. To it, freshly distilled 50 mL DCM was added. The stirred solution was cooled over an ice bath. To this solution 0.2 equivalent of cholesterol chloroformate was added dropwise for 2 hrs. The reaction mixture was stirred 5 hrs and was then extracted thrice using water. The organic phase so obtained was dried over anhydrous sodium sulphate. The mixture was then filtered and collected under vacuum to obtain **a** as the final product. **Yield:** 70 %. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 0.66-2.27 (cholesteryl protons), 3.79-4.30 (s, 2H), 4.25-4.42 (m, 1H), 5.21-5.43 (s, 1H), 8.08 (s, 1H).

For the synthesis of compound, **1** following procedure was followed: NTCDA was taken in 100 mL round bottomed flask and anhydrous DMF was added under nitrogen atmosphere. The reaction mixture was stirred for a few minutes. To this solution, compound **a** was added. The resultant mixture was refluxed under nitrogen for 10 hrs. The reaction mixture was then allowed to warm to the room temperature and extracted using DCM:water mixture. The organic phases so collected was dried over anhydrous Na_2SO_4. The mixture was then filtered off. Solvent was removed on rotary evaporator under reduced pressure to obtain the crude product which was further purified by column chromatography (silica, 60-120 mesh), using 2% $CHCl_3$/MeOH as the eluent to get a yellowish solid as a final product **1**. **Yield:** 60 %. **MP**: > 300 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 0.70 (s, 6H), 0.84-2.12 (cholesteryl protons), 4.60-4.76 (s, 2H), 5.32-5.47 (m, 2H), 6.92-7.12 (m, 2H), 8.85 (s, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ = 11.87, 18.72, 19.33, 22.57, 22.83, 23.83, 24.28, 28.02, 31.84, 35.80, 36.18, 39.52, 42.32, 49.97, 56.13, 56.33, 56.68, 58.19, 123.08, 126.88, 131.92, 161.05. **MALDI (TOF)-MS**: calculated for $C_{70}H_{95}N_4O_8(M-H)^-$, 1120.550; found, 1120.434.

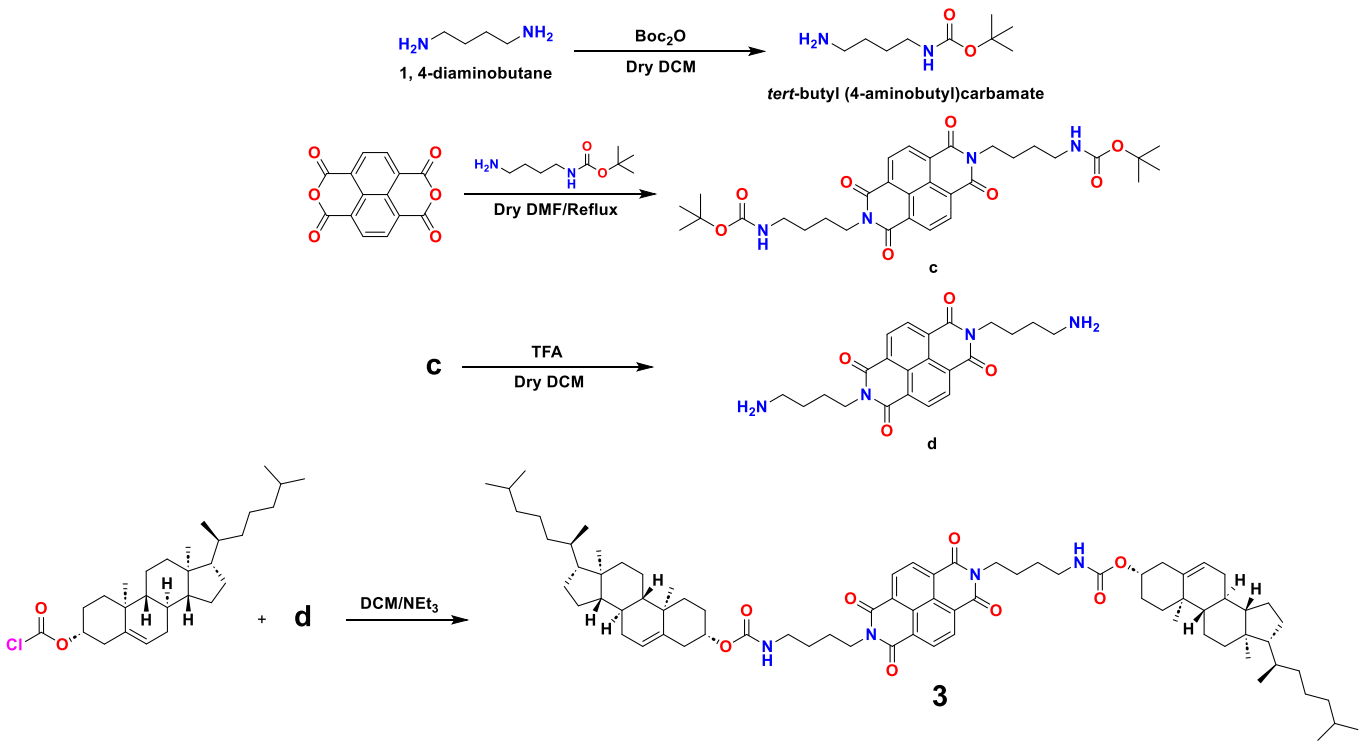
Synthesis of 2:



Synthetic Procedure: For the synthesis of **b**, 4, 4'-methylenedianiline was taken in a 100 mL round bottomed flask under nitrogen atmosphere. To it, freshly distilled 50 mL DCM was added. The stirred solution was cooled over an ice bath. To this solution 0.2 equivalent of cholesterol chloroformate was added dropwise for 2 hrs. The reaction mixture was stirred 5 hrs and was then extracted thrice using water. The organic phase so obtained was dried over anhydrous sodium sulphate. The mixture was then filtered and collected under vacuum to obtain **b** as the final product. **Yield:** 60 %. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 0.66-2.27 (cholesteryl protons), 3.57-3.73 (s, 2H), 4.36-4.48 (m, 1H), 4.74-4.92 (m, 2H), 5.29-5.43 (m, 1H), 6.35-6.60 (d, 2H), 6.63-6.87 (d, 2H), 6.91-7.16 (d, 2H), 7.19-7.46 (d, 2H), 9.48 (s, 1H).

For the synthesis of compound, **3** following procedure was followed: NTCDA was taken in 100 mL round bottomed flask and anhydrous DMF was added under nitrogen atmosphere. The reaction mixture was stirred for a few minutes. To this solution, compound **b** was added. The resultant mixture was refluxed under nitrogen for 10 hrs. The reaction mixture was then allowed to warm to the room temperature and extracted using DCM:water mixture. The organic phases so collected was dried over anhydrous Na_2SO_4. The mixture was then filtered off. Solvent was removed on rotary evaporator under reduced pressure to obtain the crude product which was further purified by column chromatography (silica, 60-120 mesh), using 2% $CHCl_3$/MeOH as the eluent to get a yellowish solid as a final product **2**. **Yield:** 60 %. **MP**: > 300 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 0.66-0.73 (s, 6H), 0.84-2.50 (cholesteryl protons), 3.98-4.14 (s, 4H), 4.53-4.71 (m, 2H), 5.37-5.46 (m, 2H), 6.56-6.64 (m, 2H), 7.14-7.47 (m, 16H), 8.79-8.91 (s, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ = 11.88, 18.73, 19.36, 22.57, 22.83, 28.03, 31.89, 35.81, 36.60, 38.48, 39.53, 42.33, 56.15, 56.70, 77.23, 128.35, 129.82, 129.97, 131.44, 163.02. **MALDI (TOF)-MS**: calculated for $C_{96}H_{115}N_4O_8$(M-H)$^-$, 1452.996; found, 1453.557.

Synthesis of 3:



Synthetic Procedure: For the synthesis of tert-butyl(4-aminobutyl)carbamate, 1, 4-diaminobutane was taken in a 100 mL round bottomed flask under nitrogen atmosphere. To it, freshly distilled 50 mL DCM was added. The stirred solution was cooled over an ice bath. To this solution 0.2 equivalent of Boc-anhydride was added dropwise. The reaction mixture was stirred overnight and was then extracted using water. The organic phase so obtained was dried over anhydrous sodium sulphate. The mixture was then filtered and collected under vacuum to obtain tert-butyl(4-aminobutyl)carbamate as the final product.

For the synthesis of compound **c** following procedure was followed: NTCDA was taken in 250 mL round bottomed flask and anhydrous DMF was added under nitrogen atmosphere. The reaction mixture was stirred for a few minutes. To this solution, tert-butyl(4-aminobutyl)carbamate was added. The resultant mixture was refluxed under nitrogen for 24 hrs. The reaction mixture was then allowed to warm to the room temperature and extracted using DCM:water mixture. The organic phases so collected was dried over anhydrous Na_2SO_4. The mixture was then filtered off. Solvent was removed on rotary evaporator under reduced pressure to obtain the crude product which was further purified by column chromatography (silica, 60-120 mesh), using 2% $CHCl_3$/MeOH as the eluent to get a yellowish solid as a final product **c**. **Yield:** 60 %. **MP**: 233 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 1.31-1.36 (s, 18H), 1.41-1.51 (m, 4H), 1.58-1.70 (m, 4H), 2.89-2.99 (m, 4H), 4.01-4.10 (m, 4H), 6.75-6.82 (m, 2H), 8.67 (s, 4H).

For the synthesis of compound **d** following procedure was followed: Compound **c**, was taken in a 100 ml round bottomed flask and anhydrous DCM was added under nitrogen atmosphere. The stirred solution was cooled over an ice bath. To this solution, TFA was added. The reaction mixture was then allowed to warm to the room temperature

under constant stirring and mixture was stirred at room temperature for 12 hrs. Reaction progress was monitored by TLC. The reaction was then washed with brine and organic layer was dried over Na_2SO_4. Solvent was removed on rotary evaporator under reduced pressure to obtain the product which was further washed by pet ether to obtain **d** as white colour product. **Yield:** 60 %.

For the synthesis of compound **3** following procedure was followed: Compound **d** was taken in a 250 ml two necked round bottomed flask and anhydrous $CHCl_3$ was added under nitrogen atmosphere. The stirred solution was cooled over an ice bath. To this solution triethylamine was added. The reaction mixture was then allowed to warm to the room temperature under constant stirring for 30 minutes. To the reaction mixture, cholesterol chloroformate was added under ice cold condition and the reaction mixture was stirred overnight under room temperature. Reaction progress was monitored by TLC. Solvent was removed on rotary evaporator under reduced pressure to obtain the crude product which was further purified by column chromatography (silica, 60-120 mesh), using 2% $CHCl_3$/MeOH as the eluent. **Yield:** 60 %. **MP**: > 300 °C. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ = 0.65-2.06 (cholesteryl protons), 2.19-2.40 (m, 4H), 3.17-3.38 (m, 4H), 4.17-4.31 (m, 4H), 4.40-4.55 (m, 2H), 4.66-4.79 (m, 2H), 5.26-5.41 (m, 2H), 8.67-8.84 (s, 4H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ = 11.87, 18.73, 19.34, 19.41, 21.05, 21.10, 22.57, 22.83, 23.84, 24.30, 28.02, 28.17, 28.24, 31.68, 31.92, 35.80, 36.20, 36.52, 36.56, 37.00, 37.27, 38.56, 39.53, 39.74, 39.80, 42.32, 50.01, 50.15, 56.17, 56.69, 56.78, 71.82, 76.71, 121.72, 122.46, 126.61, 126.72, 131.04, 140.78, 162.84. **MALDI (TOF)-MS**: calculated for $C_{78}H_{111}N_4O_8(M-H)^-$, 1232.766; found, 1232.553.

3. Absorption Studies

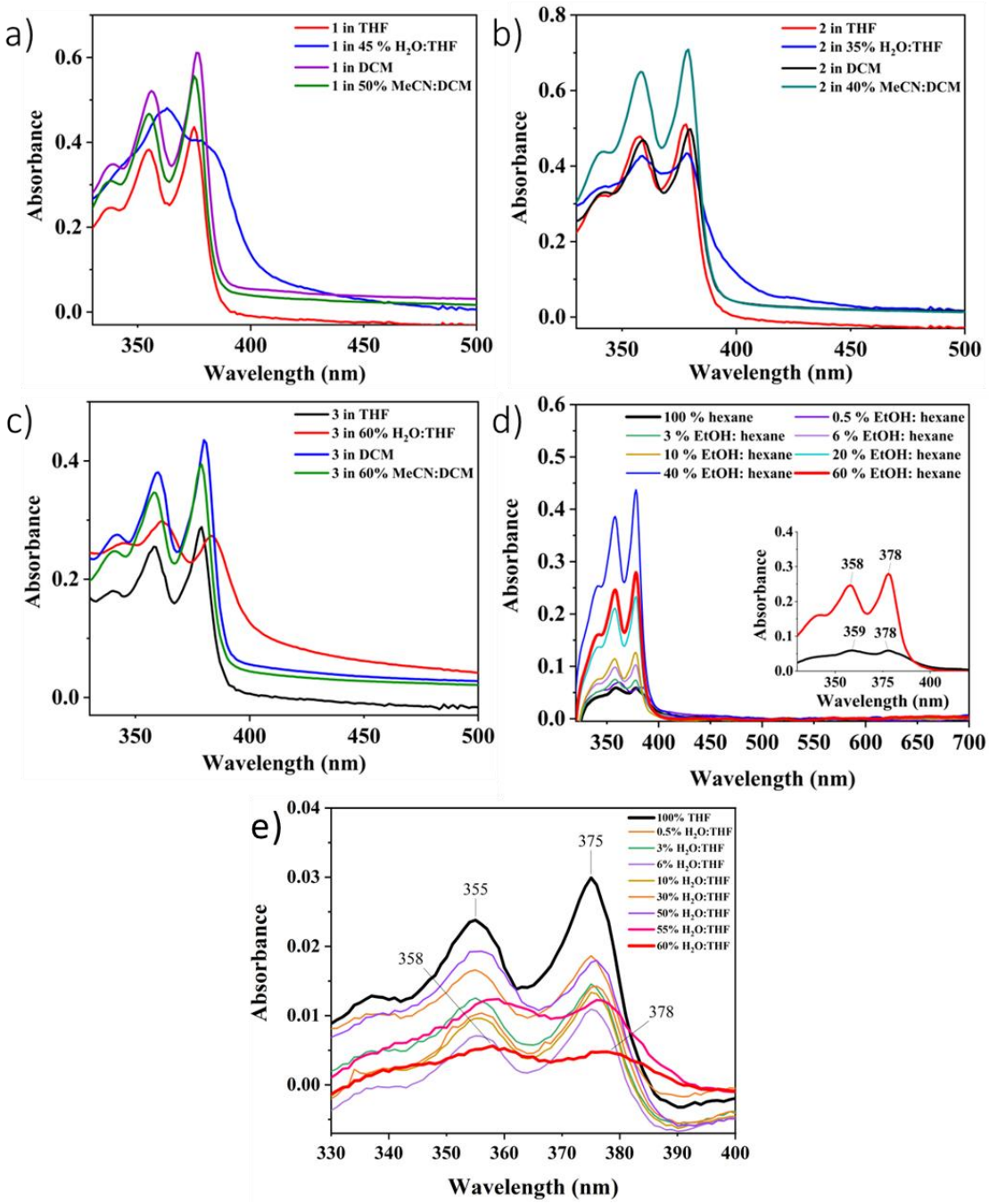


Fig. S1. Absorption spectra of compounds **1-3** [20 μM] in different solvent combinations (a-d), compound **1** [2 μM] in THF/H_2O (e).

Emission Studies

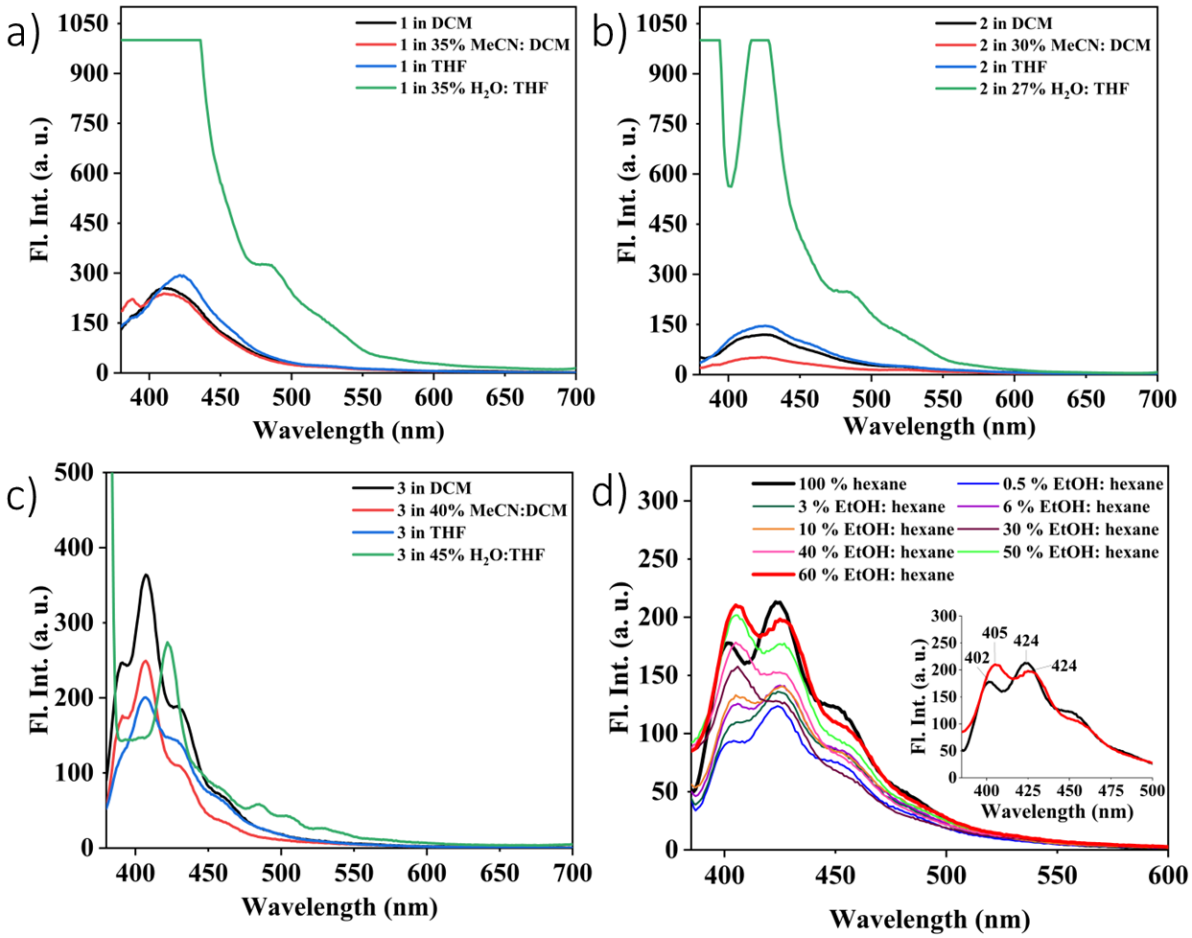


Fig. S2. Emission spectra of compound **1-3** [100 μM] in different solvent combinations (a-c), and compound **3** [100 μM] in hexane:EtOH (d).

4. Fluorescence spectra with integrated area under the curve

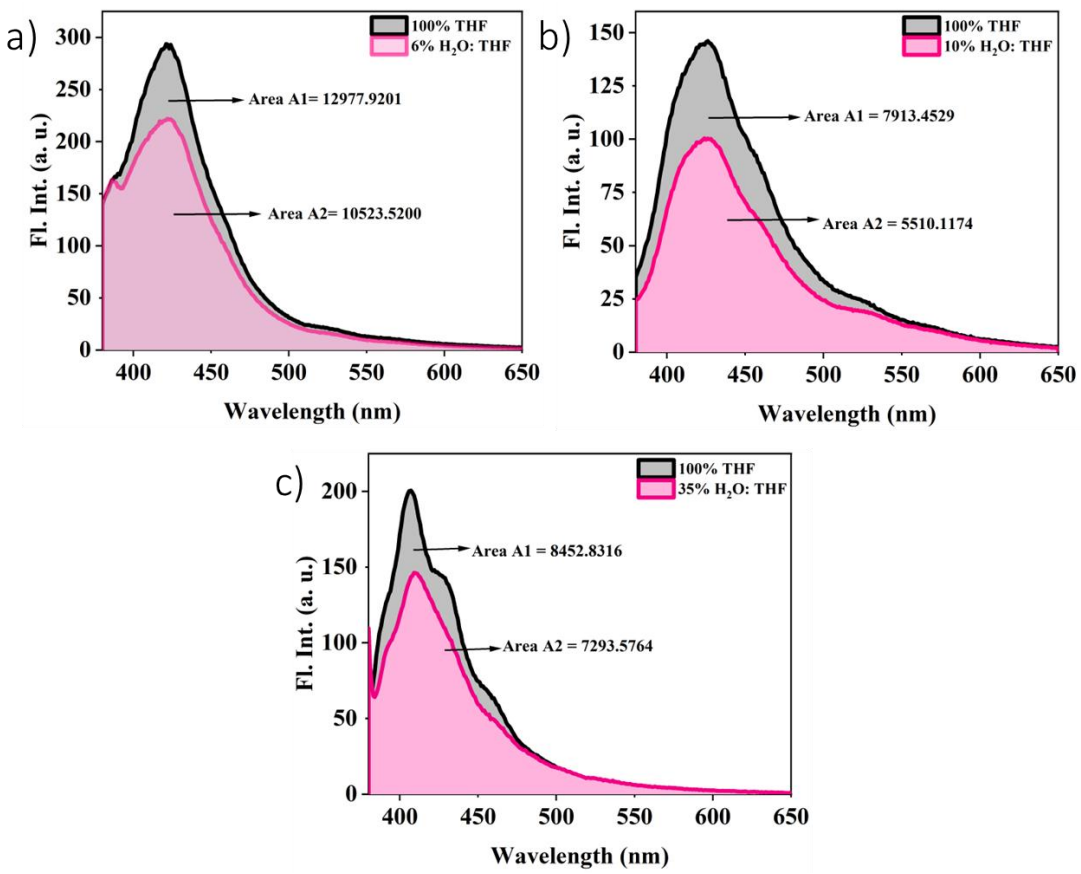


Fig. S3. Fluorescence spectra of the compound **1**, **2** and **3** recorded in THF/H_2O having water 6% (a), 10% (b), and 35% (c). The black curves represent spectra in THF, while the pink-filled spectra correspond to THF/H_2O mixtures.

5. FE-SEM images of molecules 1-3

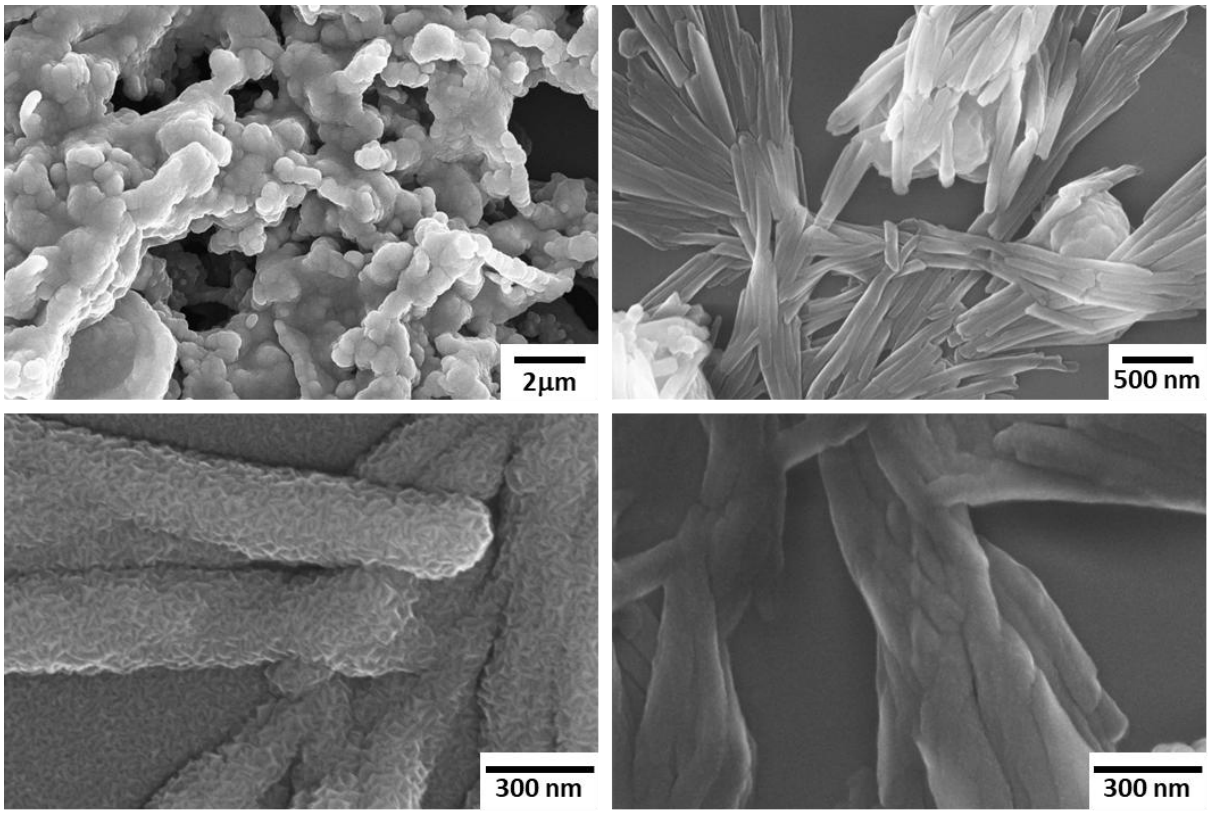


Fig. S4. FE-SEM images of compound **1** [100 μM] in THF:H_2O.

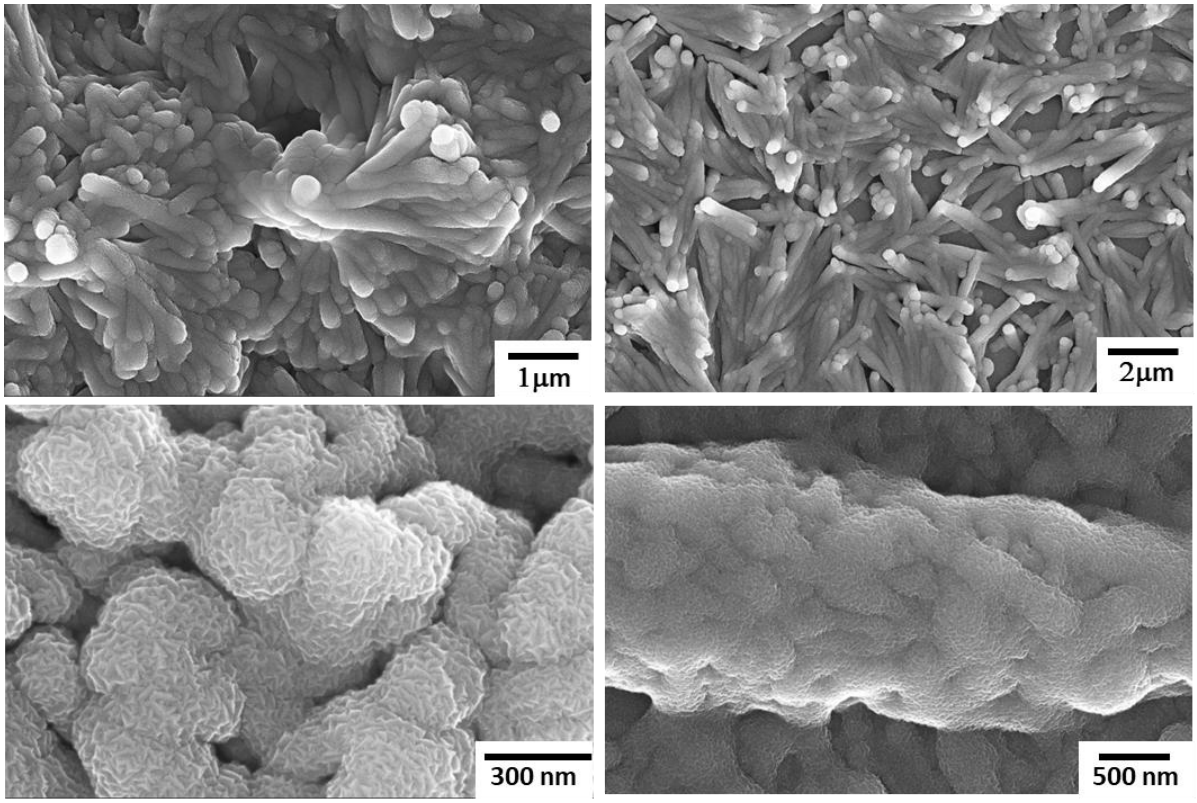


Fig. S5. FE-SEM images of compound **2** [100 μM] in THF:H_2O.

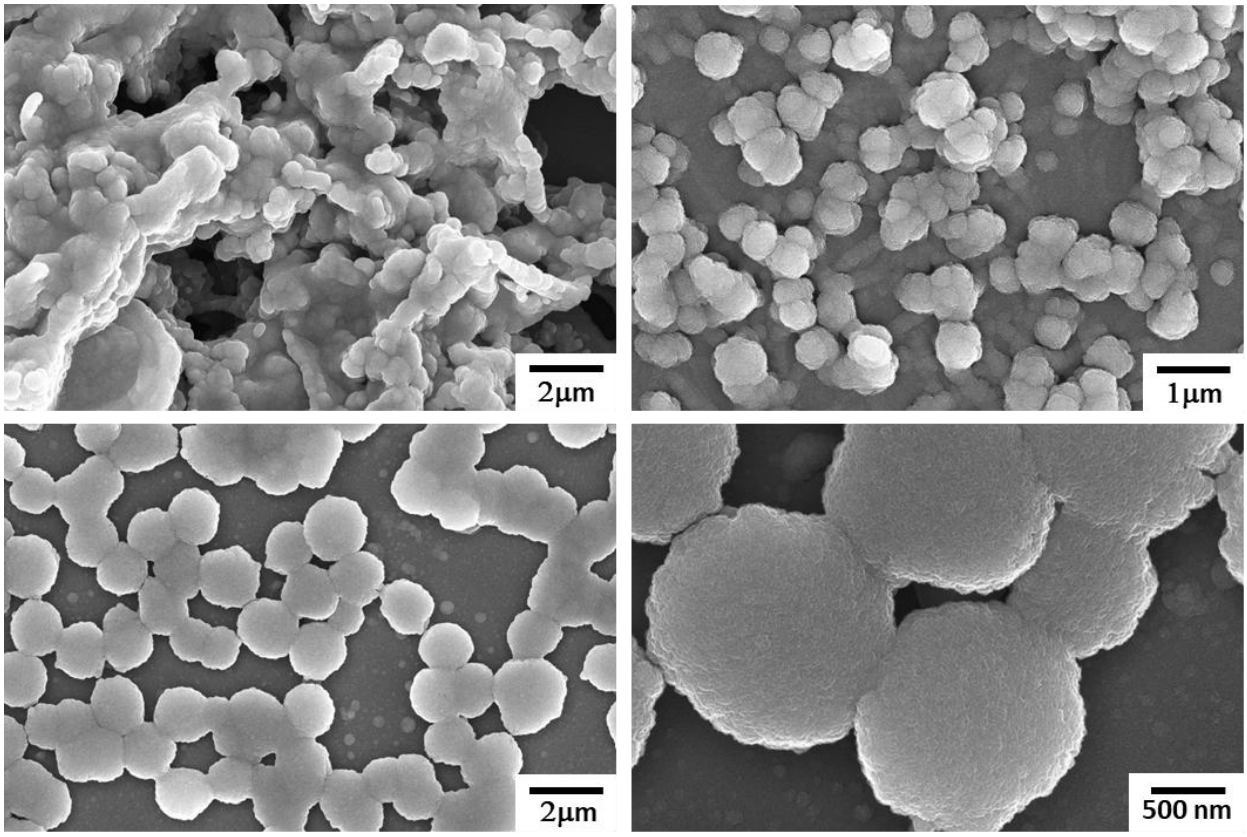


Fig. S6. FE-SEM images of compound **3** [100 μM] in THF:H_2O (9:1).

6. Circular Dichroism

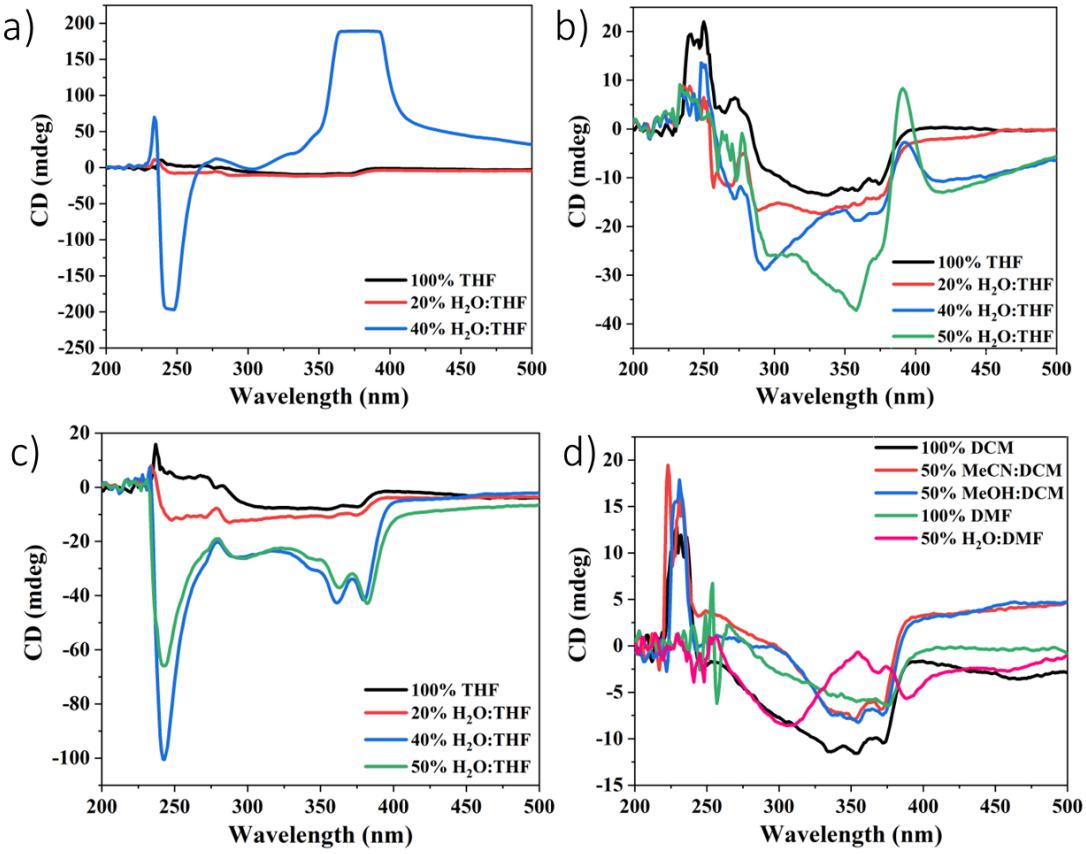


Fig. S7. Room temperature CD spectra of **1** [50 μM] (a), **2** [100 μM] (b), **3** [100 μM] (c) in 1:1 THF/H_2O. Plot (d) showing CD spectra of **1** [50 μM] in DCM, MeCN/DCM (1:1), MeOH/DCM (1:1), DMF, and DMF/H_2O (1:1).

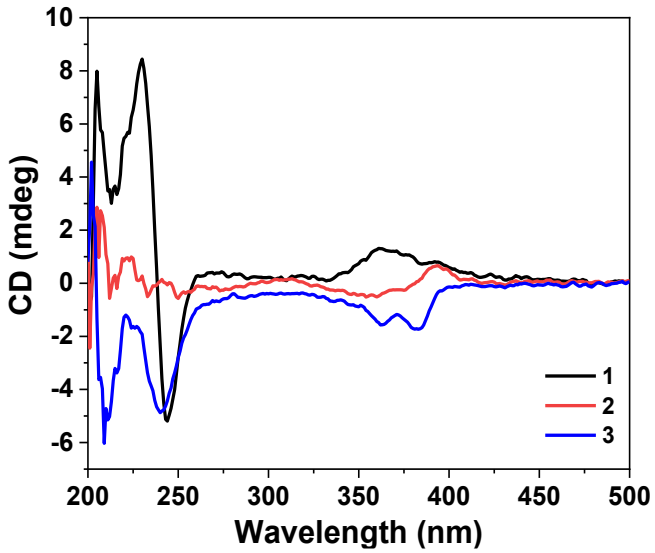


Fig. S8. Room temperature CD spectra of **1-3** [50 μM] in THF/H_2O (1:1) using cuvette of path length 1. mm.

7. Fluorescence Lifetime Measurements

Table T1. Summary of time resolved fluorescence lifetime measurement (λ_{ex} = 370 nm).

a) Compound **1** in THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.767569	0.857028	52.31	1.257450922	
4.19399	0.142972	47.69		0.839081
b) Compound **1** in 20 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
1.39697	0.805337	35.79	3.143196	
10.3674	0.194663	64.21		1.025183
c) Compound **1** in 45 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
4.97467	0.071202	31.50	1.124383	
0.829218	0.928798	68.50		0.662157

d) Compound **2** in THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
1.03986	0.790683	45.14	1.821297	
4.77314	0.209317	54.86		0.765099
e) Compound **2** in 20 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
1.02377	0.754657	34.01	2.271681	
6.11016	0.245343	65.99		0.716534
f) Compound **2** in 45 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
1.0496	0.925735	56.72	1.712987	
9.9823	0.074265	43.28		0.792926
g) Compound **3** in THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.856898	0.8085	44.71	1.549386	
4.47302	0.1915	55.29		0.990885

h) Compound **3** in 20 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.917371	0.81729	43.43	1.726397	
5.34531	0.18271	56.57		1.085147
i) Compound **3** in 45 % H_2O/THF	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.803325	0.916445	65.00	1.132673	
4.74501	0.083555	35.00		0.964962

j) Compound **3** in Hexane	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
1.04781	0.9506	80.89	1.231288	
4.76201	0.0494	19.11		1.070583
k) Compound **3** in 20 % EtOH/Hexane	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.995303	0.964652	84.33	1.13852	
5.04693	0.035348	15.67		0.928904
l) Compound **3** in 45 % EtOH/Hexane	Monitor Wavelength (λ_{em} = 421 nm)			
Fitted decay times (Γ^i)	**Pre-exponential coefficients (α^i)**	**Relative Amplitude %**	**Average Lifetime**	**χ^2**
0.967615	0.982809	91.11	1.043718	
5.3946	0.017191	8.89		0.830388

8. Gelation table for compound 1-3

Sr. No.	Solvent	Weight %	Observation
1.	Hexane	2.0	ppt (1), ppt (2), ppt (3)
2.	Hexane: EtOH (1:1)	1.0	viscous sol (1), insoluble (2), viscous sol (3)
3.	Hexane: EtOH (1:3)	2.5	viscous sol (1), viscous sol (2), viscous sol (3)
4.	Hexane: EtOH (1:6)	2.0	viscous sol (1), insoluble (2), opaque gel (3)
5.	$CHCl_3$: EtOH (1:1)	2.5	sol (1), sol (2), sol (3)
6.	DCM: MeCN (1:1)	1.25	viscous sol (1), viscous sol (2), viscous sol (3)
7.	THF: H_2O (1:1)	1.25	viscous sol (1), viscous sol (2), viscous sol (3)
8.	Toluene	2.5	sol (1), sol (2), sol (3)
9.	MeOH	1.0	insoluble (1), insoluble (2), insoluble (3)

Table T2. Gelation table for compounds **1-3.**

9. Dynamic Light Scattering (DLS)

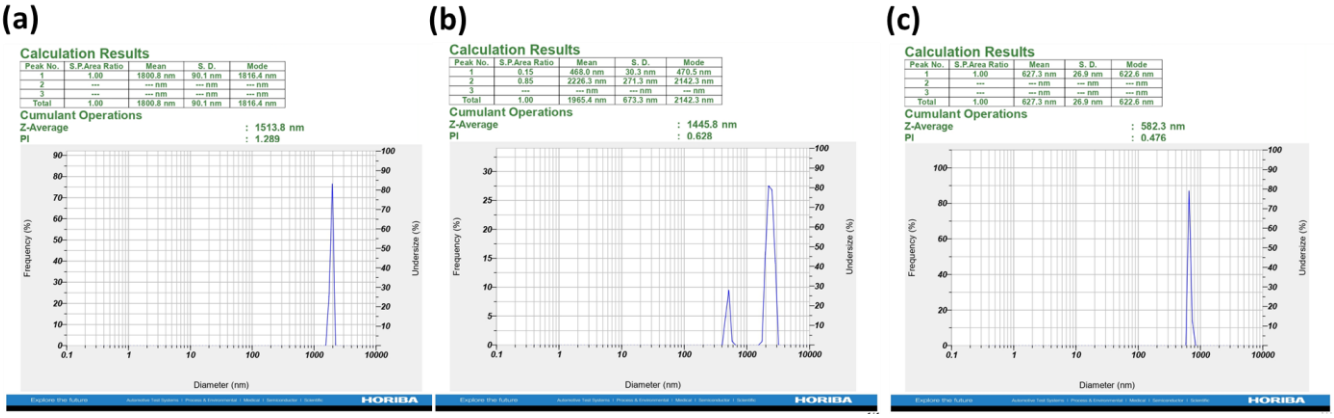


Fig. S9. Particle size determination of compounds **1-3** [50 μM] in THF/H_2O (1:1).

10. Rheological Measurements

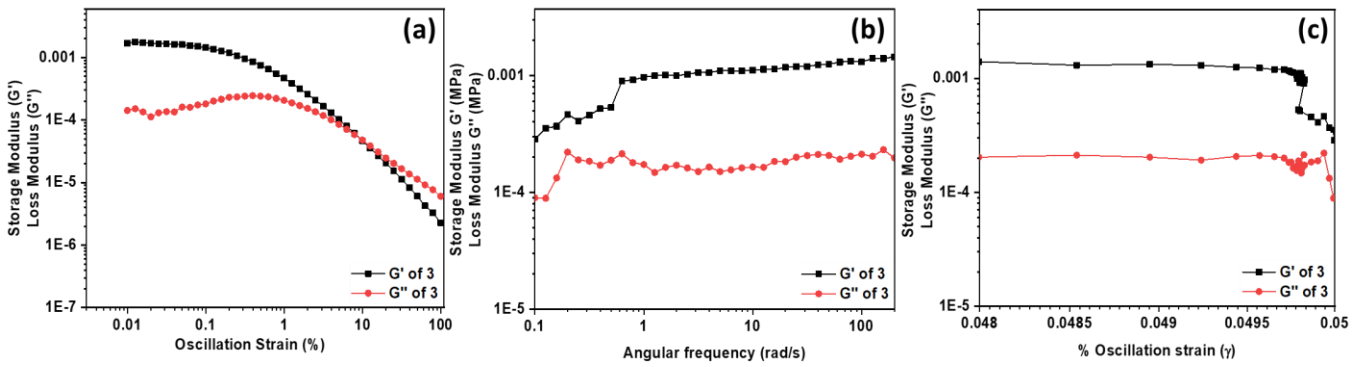


Fig. S10: Plots showing (a) change in *G′* and *G″* with respect to amplitude sweep (b) variation in G' and G" during frequency sweep from 0.1 to 100 rad s^{-1} (c) variation in G' and G" with respect to applied oscillatory strain for gelator **3**.

11. ^{1}H-NMR and ^{13}C-NMR Spectra of intermediates a-c and compounds 1-3:

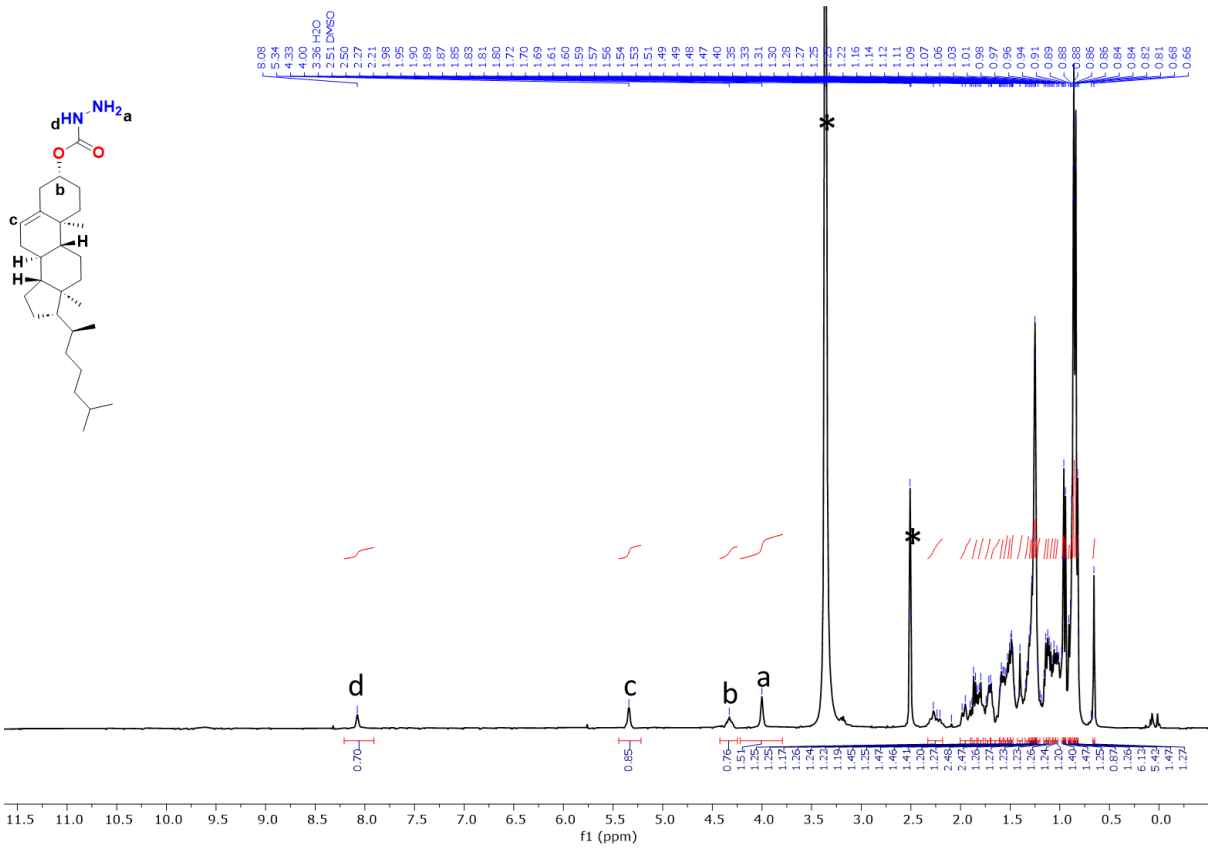


Fig. S11: ^{1}H NMR spectrum of compound **a** in DMSO-d_6. (* indicates peaks from residual solvent).

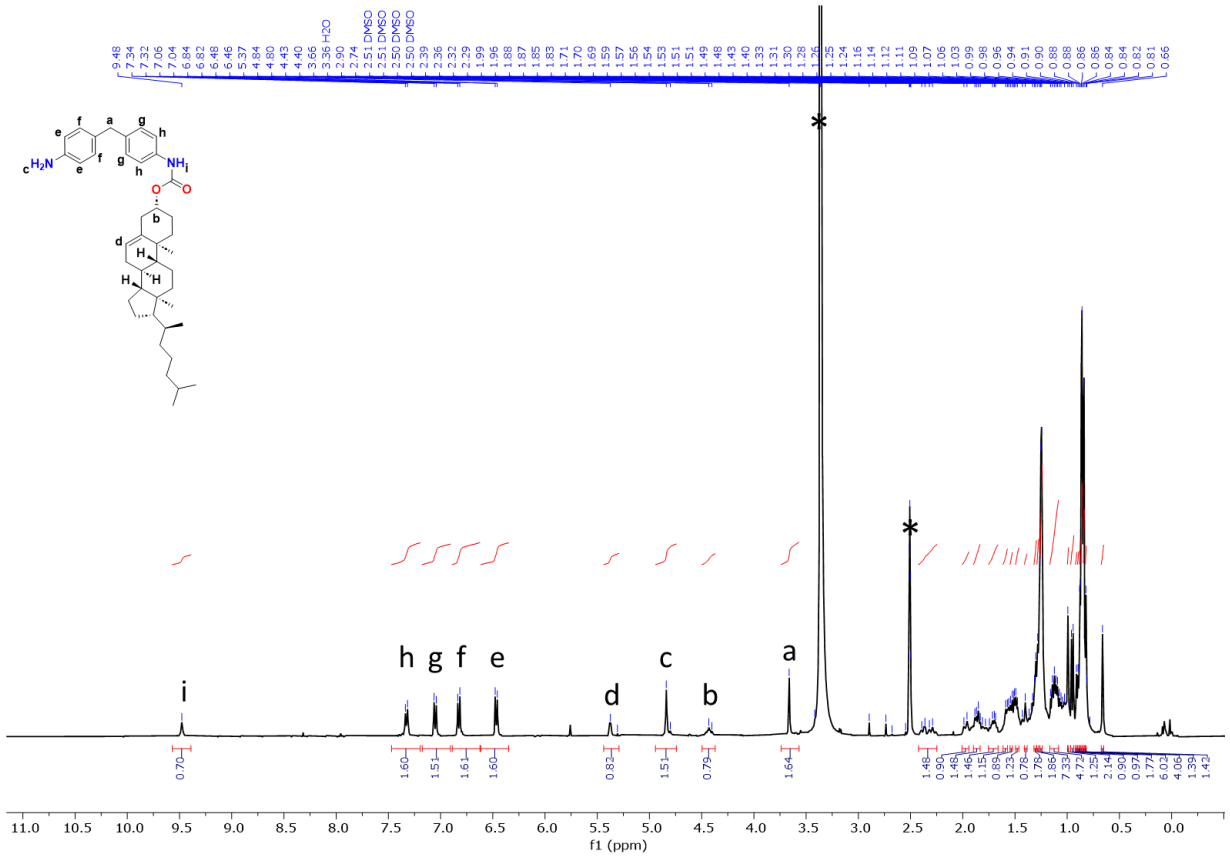


Fig. S12: ^{1}H NMR spectrum of compound **b** in DMSO-d_6. (* indicates peaks from residual solvent).

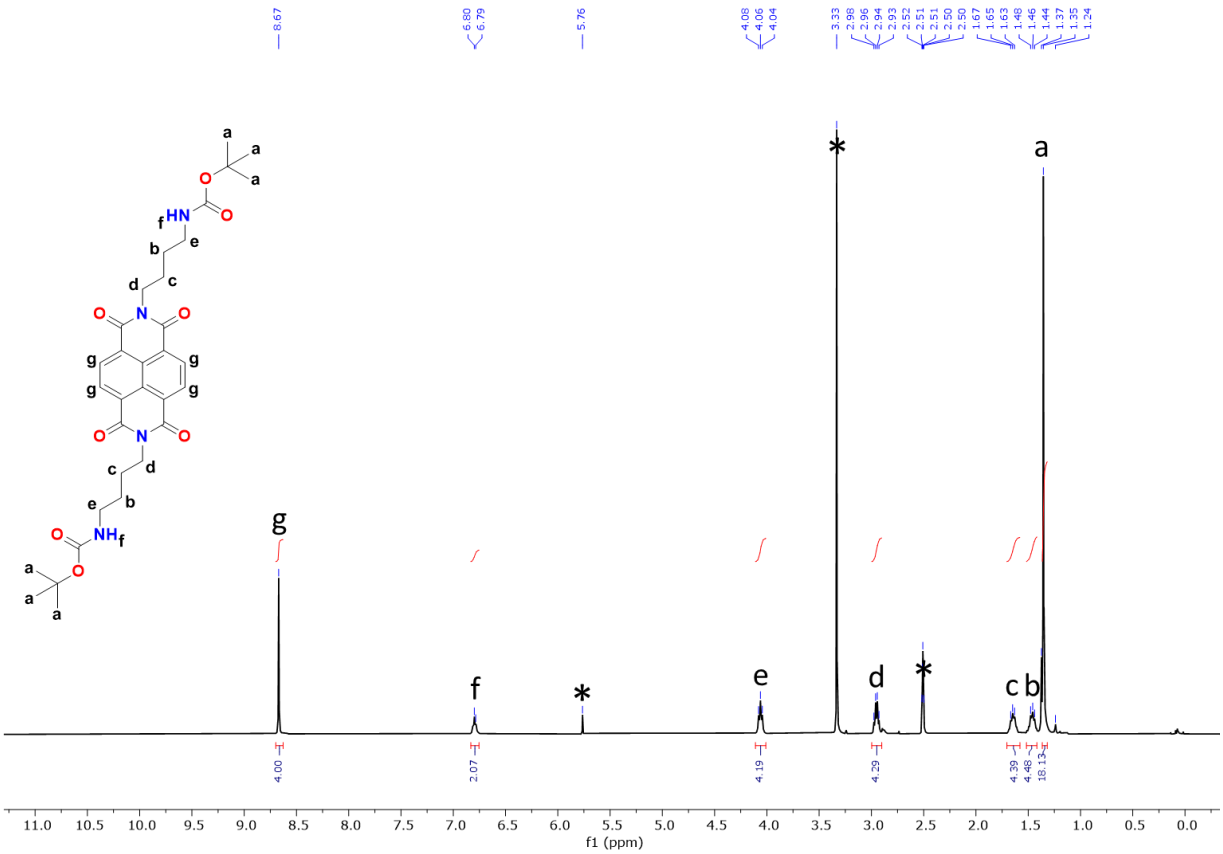


Fig. S13: ^{1}H NMR spectrum of compound **c** in DMSO-d_6. (* indicates peaks from residual solvent).

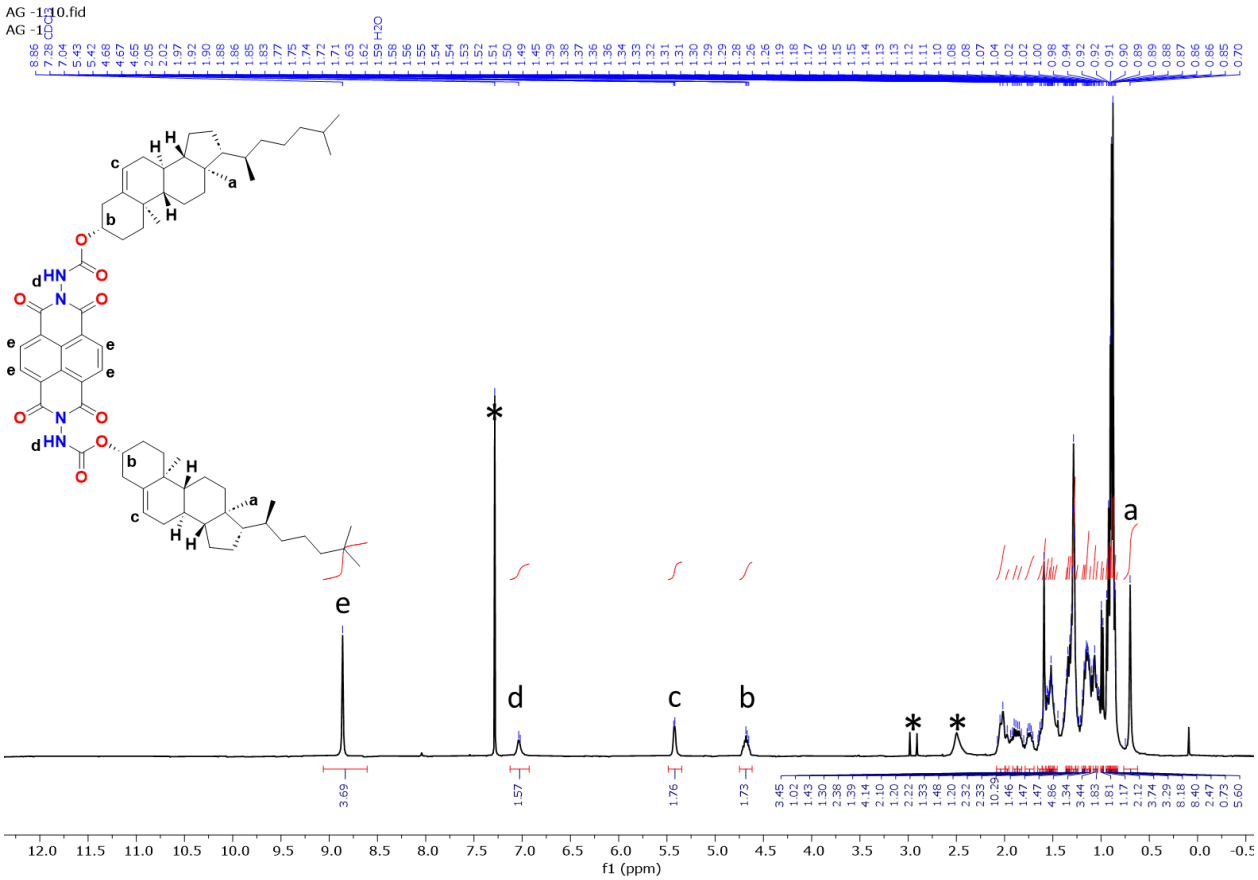


Fig. S14: ^{1}H NMR spectrum of compound **1** in $CDCl_3$. (* indicates peaks from residual solvent).

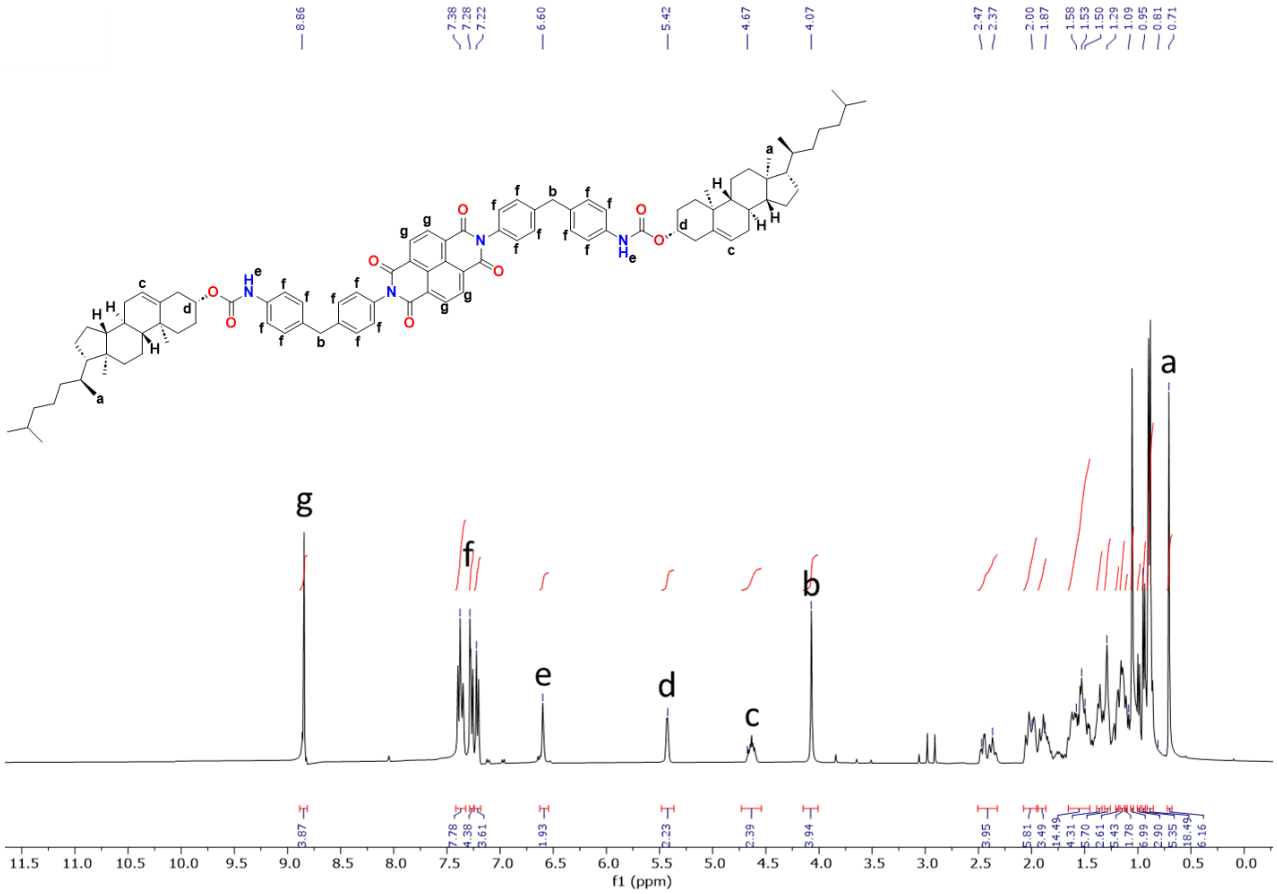


Fig. S15: ^{1}H NMR spectrum of compound **2** in $CDCl_3$. (* indicates peaks from residual solvent).

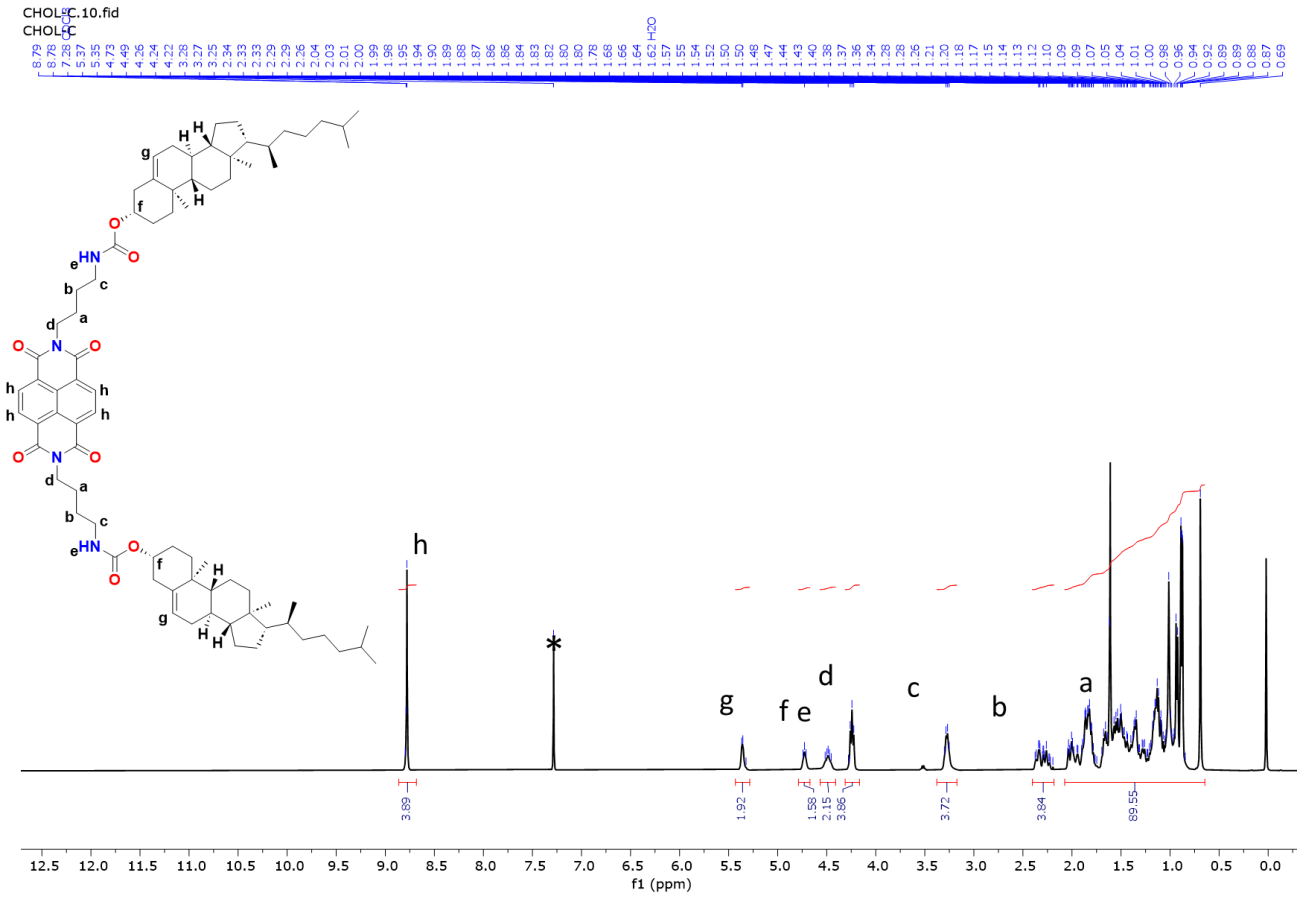


Fig. S16: ^{1}H NMR spectrum of compound **3** in $CDCl_3$. (* indicates peaks from residual solvent).

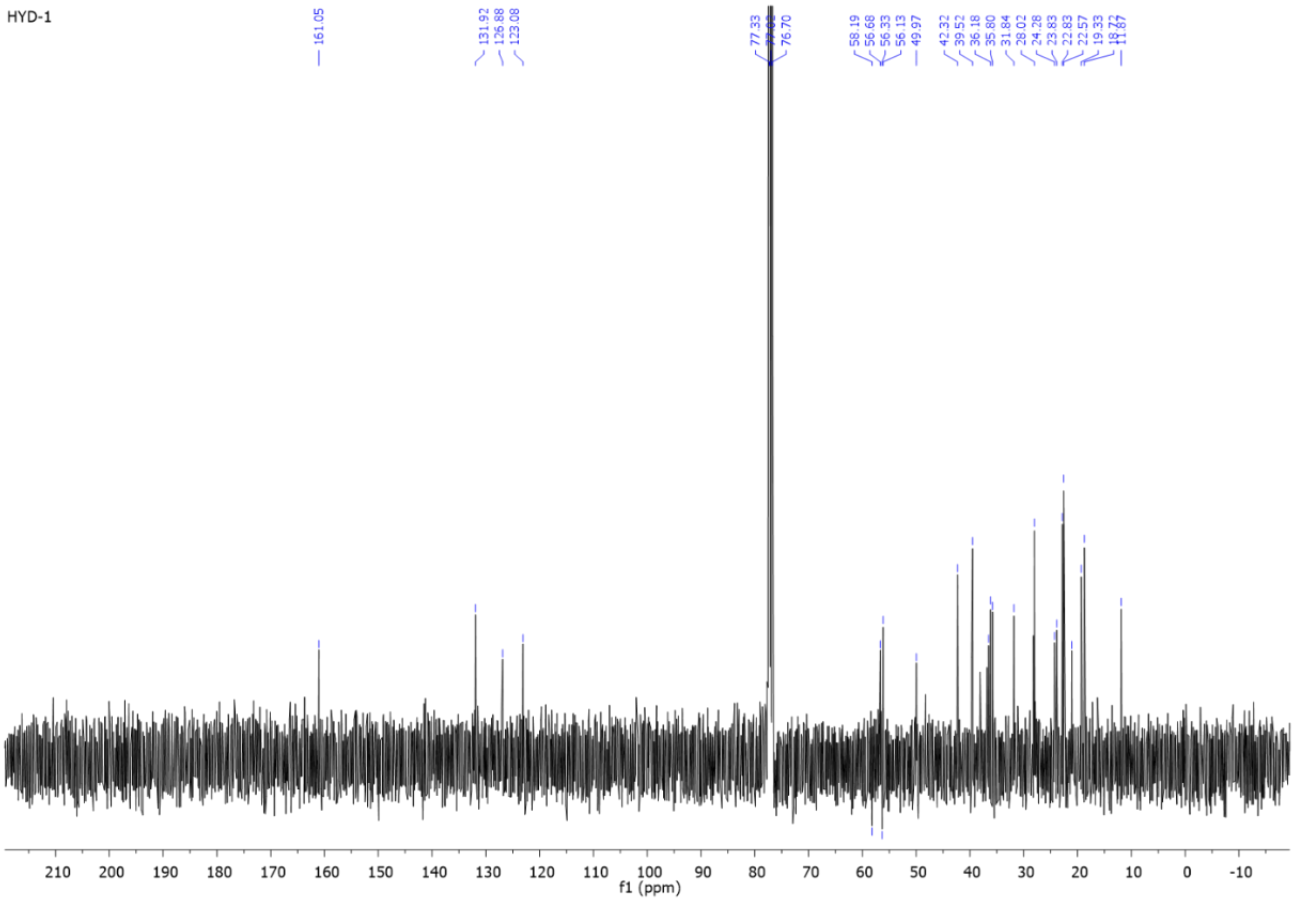


Fig. S17: ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

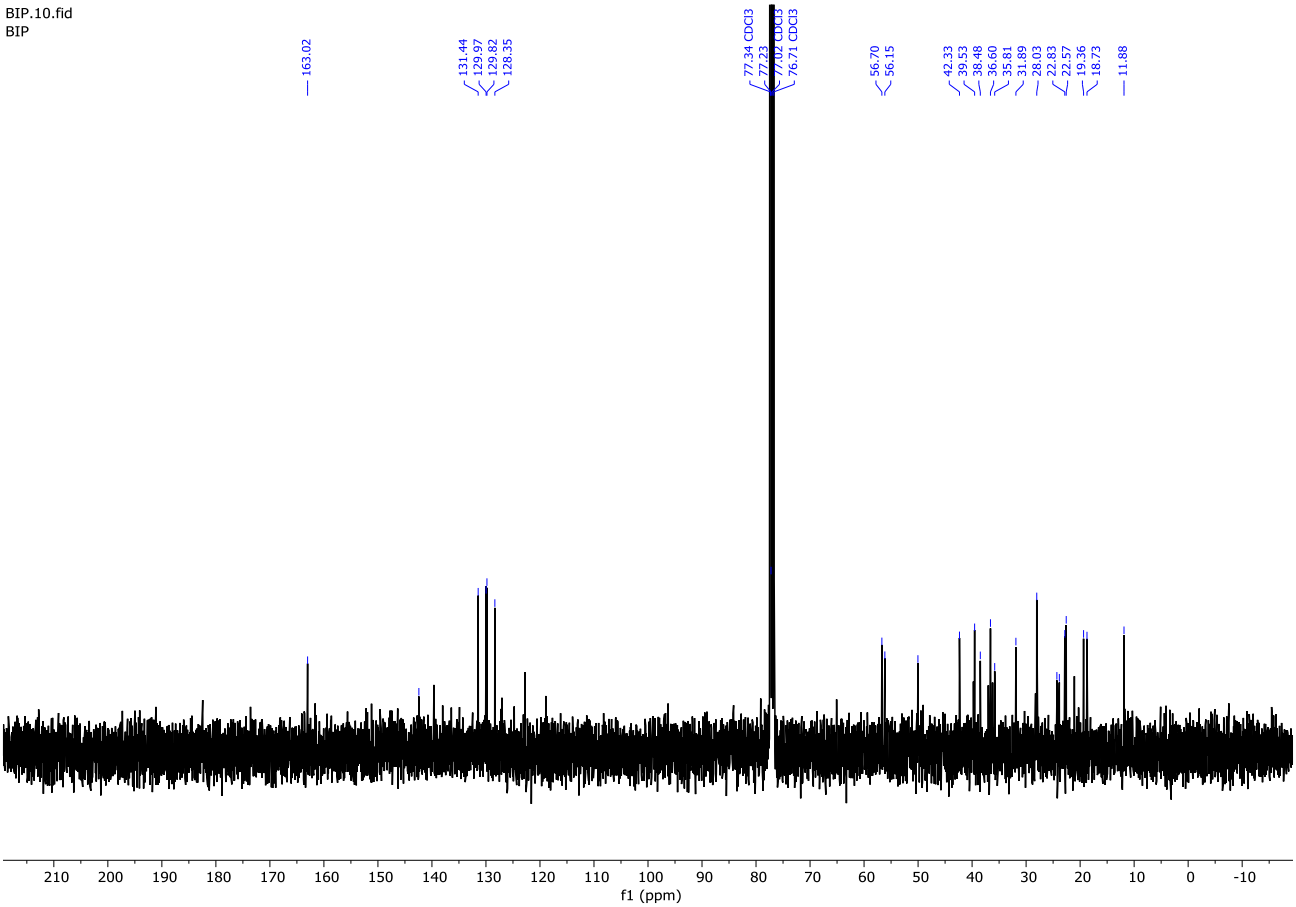


Fig. S18: ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

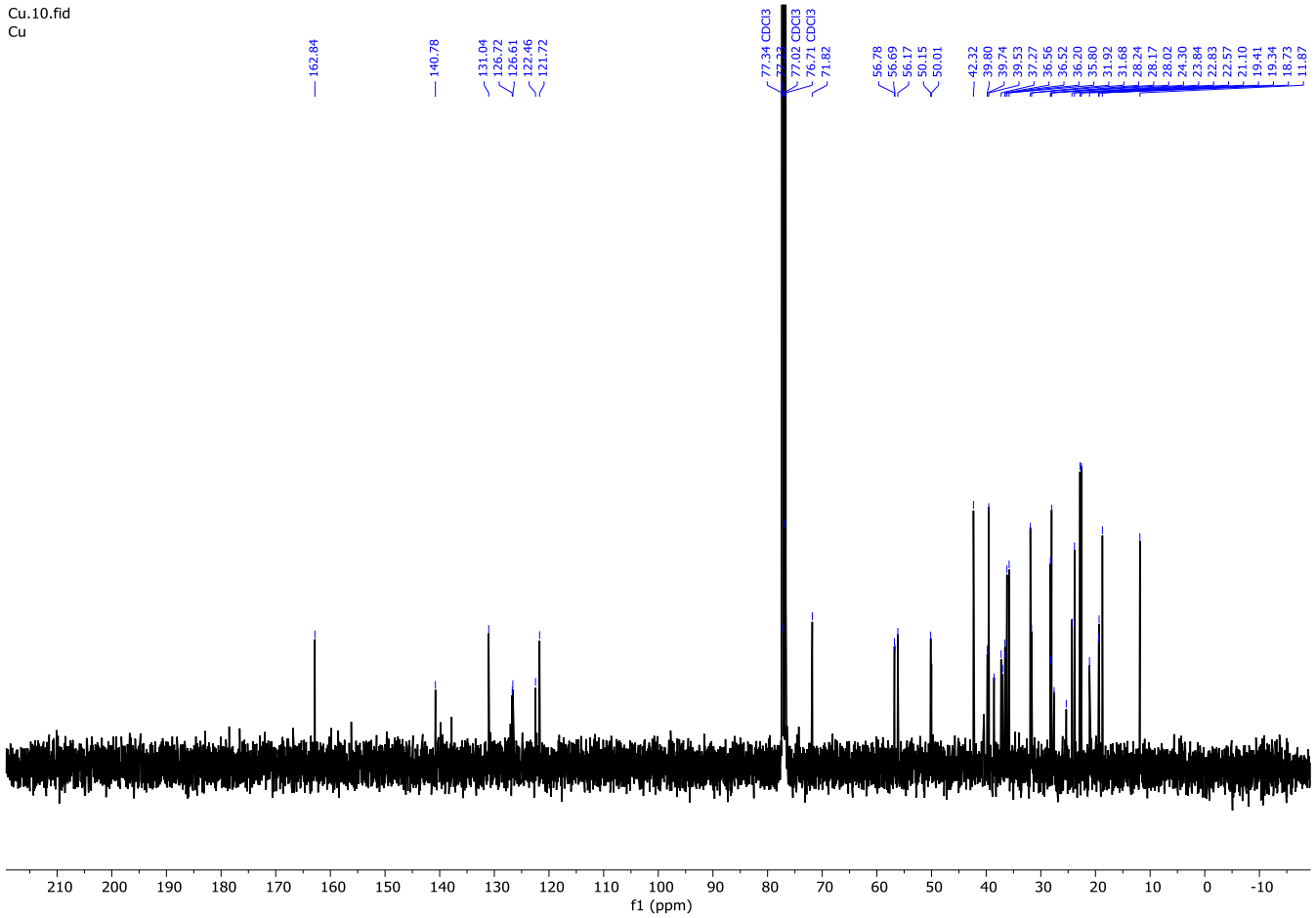


Fig. S19: ^{13}C NMR spectrum of compound **3** in $CDCl_3$.

12. MALDI-TOF Mass Spectra of Compounds 1-3:

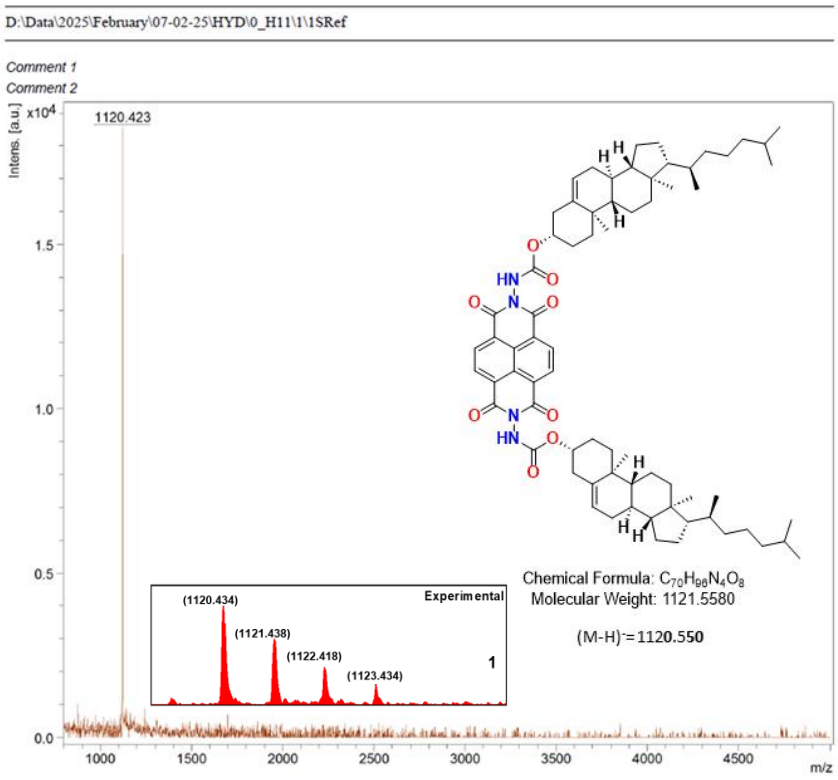


Fig. S20: MALDI-TOF mass spectrum with inset showing expanded peak for isotopic distribution of compound **1.**

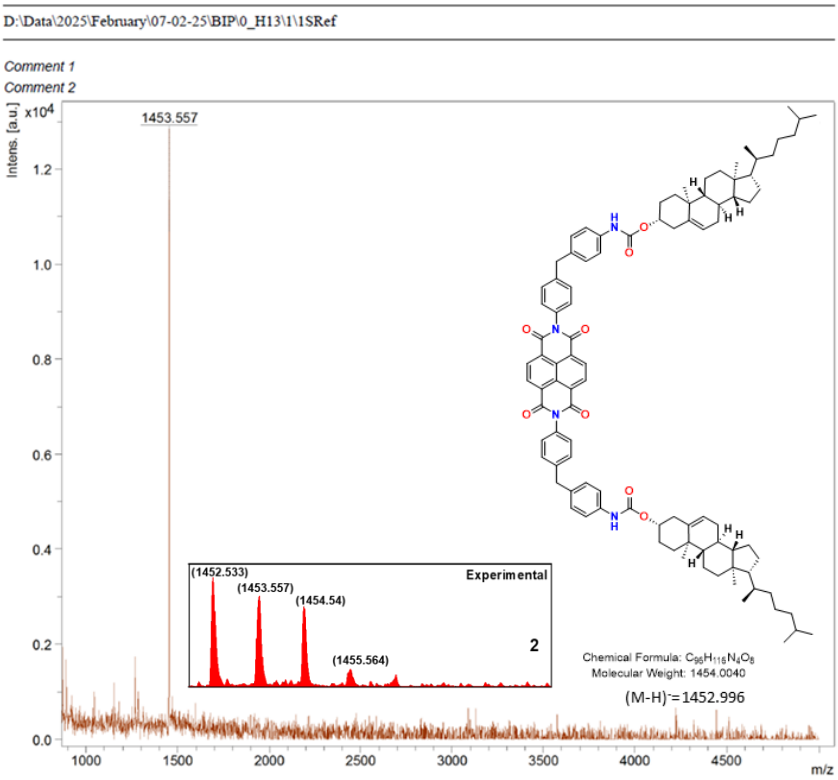


Fig. S21: MALDI-TOF mass spectrum with inset showing expanded peak for isotopic distribution of compound **2.**

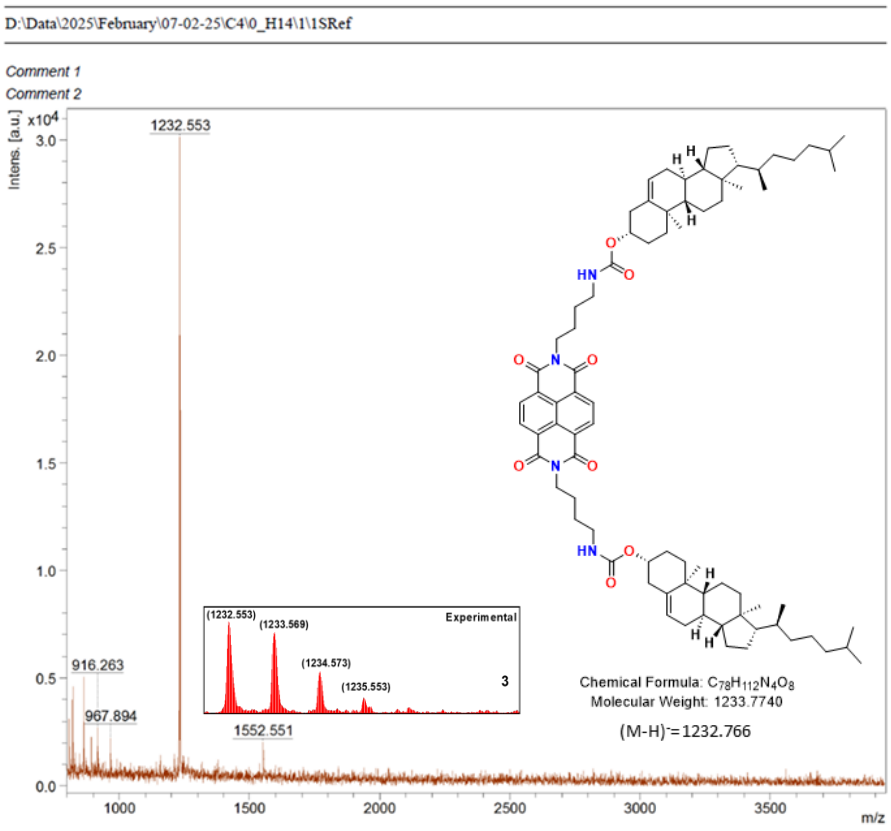


Fig. S22: MALDI-TOF mass spectrum with inset showing expanded peak for isotopic distribution of compound **3.**

13. IR Data

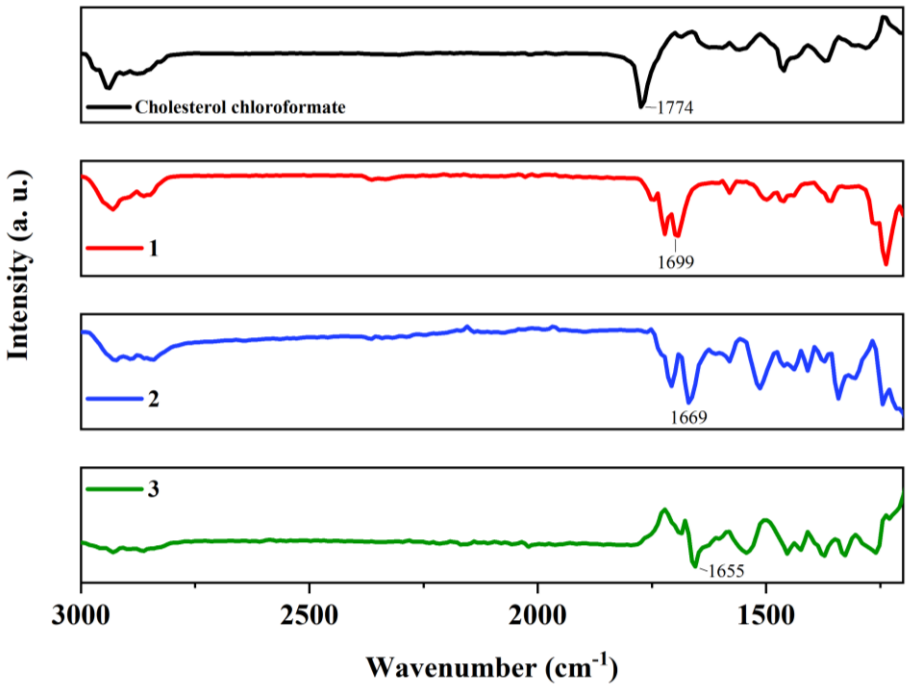


Fig. S23: FT-IR Spectra of compound **1-3** and starting material cholesteryl chloroformate.

14. Geometry optimised structures of compounds **1-3**

Geometry was optimized by performing Density Functional Theory calculations (DFT) using *Gaussian 09* software package.[1] The most stable ground state geometries of all the molecules were obtained using B3LYP hybrid exchange-correlation functional in conjugation with 6-311G basis set in gas phase.

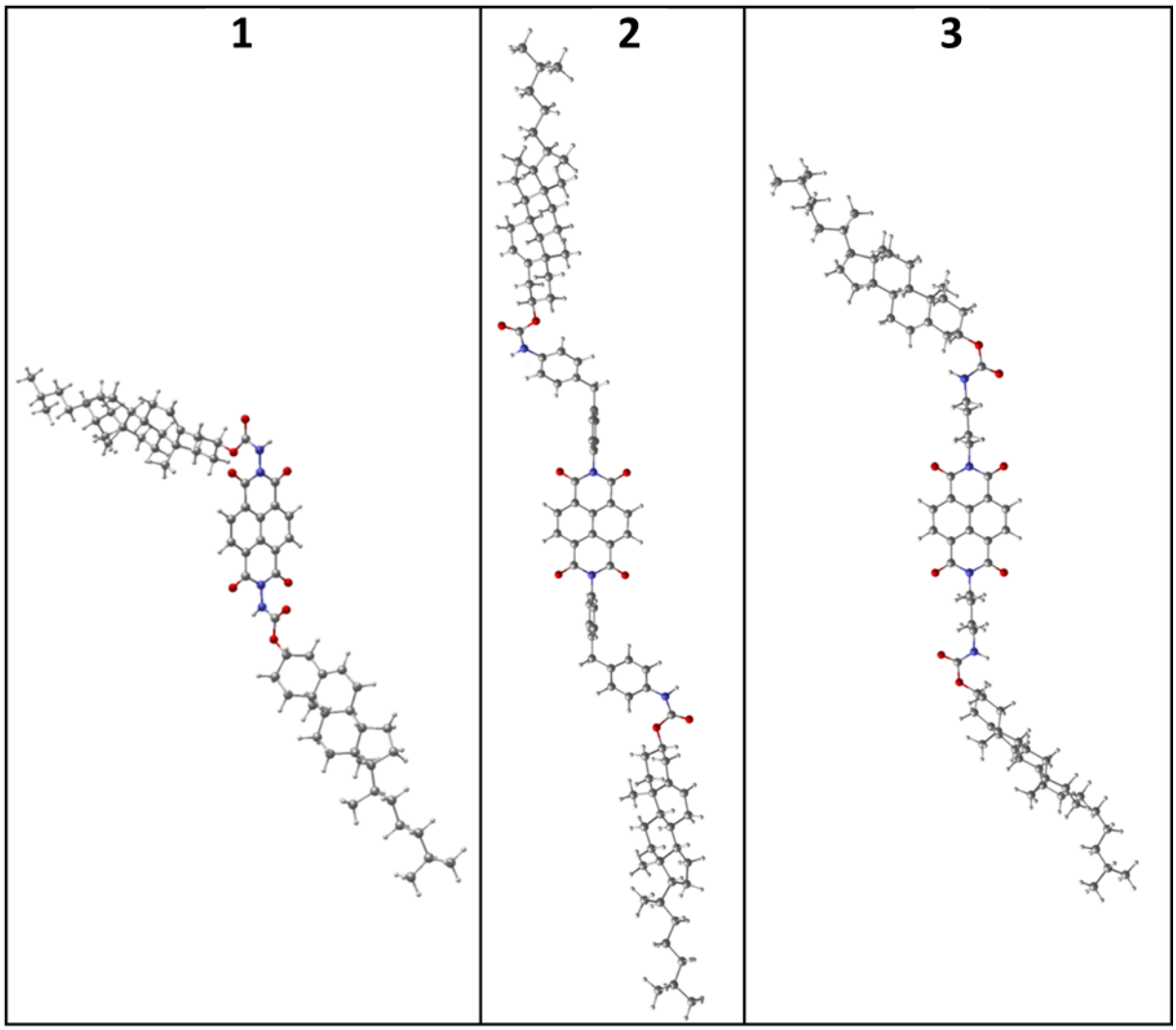


Fig. S24: Images show geometry optimized structures of compound **1**, **2**, and **3** respectively.

15. Reference

1. Gaussian 09, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.