Supplementary Information (SI) for ChemComm.

Light-responsive aggregation of vesicles using host-guest interaction of β-cyclodextrin and diazocine

Ashutosh Kanojiya[1], Anurag Mukherjee[1], Dominik Mählmann[3], Bart Jan Ravoo[1,3]
[1]Organic Chemistry Institute, University of Münster, Germany
[3]Center for Soft Nanoscience, University of Münster, Germany

Materials and Methods

General

Amphiphilic β-cyclodextrin[1, 2] was prepared according to the previously reported procedure. 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 60 mM NaCl (Sigma-Aldrich), and Milli-Q water (Pure lab UHQ water purification system) were used to prepare a buffer with pH = 7. DPBS (Dulbecco's Phosphate Buffered Saline) was purchased from Sigma. NMR spectra were recorded using Bruker AV300 (300 MHz), AV400 (400 MHz), or Agilent DD2 (600 MHz) spectrometers. Chemical shifts were referenced to internal standards.

Preparation of Cyclodextrin Vesicles (CDV)

Vesicles were prepared as described previously.[1, 2] 2 mM 100 µL stock of amphiphilic β-cyclodextrin in chloroform was taken in a round-bottom flask and evaporated under the stream of argon to form a thin layer on the walls of the flask. 2 mL HEPES buffer (or water or DPBS) was added to obtain empty vesicles (100 µM). The vesicles were then sonicated for 2 min and kept stirring overnight. The solution was then repeatedly filtered through a polycarbonate membrane with 100 nm pores (AVESTIN) in a Liposofast manual extruder (AVESTIN) to obtain CDV. CDV was prepared in DPBS for cell experiments.

Dynamic Light Scattering (DLS)

DLS measurements were performed on a Nano ZS Zetasizer (Malvern Instruments) at 25 °C. The samples were prepared in disposable 1 mL semi-micro PMMA cuvettes (BRAND) or in disposable DTS 1070 capillary cells (Malvern Instruments). Data analysis was performed with Zetasizer Software Version 7.12 (Malvern Instruments) and OriginPro 2023 10.0.0.154 (Academic). Solutions were prepared in water. The detector angle was 173 degrees. Equilibrium time was 10 s. Temperature was 20 °C. Number-weighted distribution was used for analysis.

Atomic Force Microscopy (AFM)

The device that was used for acquiring images was Nanowizard3 (JPK Instruments AG, Berlin, Germany; software: JPK SPM desktop (version: 4.3.48)). The sample was taken and dried on a cleaned quartz surface, which was analysed in tapping mode. For image analysis, the software Gwyddion (version 2.55) was used.

Transmission Electron Microscopy (TEM)

The device used to acquire images was a Thermo Fisher Scientific FEI TITAN Themis G3 60-300 transmission electron microscope (Thermo Fisher, Waltham, Massachusetts, USA). Samples were prepared by drop-casting aqueous solutions (5 µL) onto carbon-coated copper grids

(Plano EM, S162). Images were analyzed using TIA version 5.2 (FEI) and ImageJ version 1.54g (National Institutes of Health, USA, Java 1.8.0_345). The microscope was operated at an acceleration voltage of 300 kV.

UV-Vis Spectroscopy

UV-vis measurements were carried out on a double-beam JASCO V-650 spectrophotometer of Jasco Deutschland GmbH (Pfungstadt, Germany) using disposable PMMA semi-micro cuvettes from Brand GmbH and Co&KG (Weinheim, Germany). Milli-Q grade water was used as the solvent for the measurements.

Irradiation Experiments

The samples were irradiated from a distance of 5 cm at room temperature. LED 515 nm (SMB1N-515N-02) and 390 nm (H2A1-H395) were purchased from Roithner Laser Technic GmbH.

Isothermal Titration Calorimetry (ITC)

ITC measurements were performed using a TA Instruments Nano ITC low Volume (Waters Corp., Milford, Massachusetts, USA) with a cell volume of 170 µL and a syringe volume of 50 µL. All titrations were performed in 20 injections of 2.5 µL each at 25 °C with a stirring speed of 300 rpm. In all titrations, 10 mM β-CD was used in the syringe. 0.3mM Dz3 in HEPES buffer (pH 7) was in the syringe. The obtained data were analysed using the software NanoAnalyse Version 4.1.0 (TA Instruments, Waters Corp., Milford, Massachusetts, USA) and OriginPro 2023 10.0.0.154 (Academic). Prior to data fitting, a blank measurement of the CD solution titrated into PBS buffer was subtracted from the data to account for dilution enthalpy.

Critical Aggregation Concentration (CAC)

The CAC of Dz3 was measured by monitoring the change in absorbance as the Dz3 concentration increased from 0 to 50 µM. The change in absorbance at 500 nm indicates the point of deflection (CAC) caused by assembly formation.[3]

Determination of Photostationary State (PSS)

^{1}H-NMR spectroscopy was used to determine the PSS for the *Z-E* and *E-Z* isomerization process. Dz3 solution was prepared in CD_2Cl_2. After the initial sample was measured, the sample was irradiated with UV light (390 nm, 30 min), and the NMR spectrum was measured again. Lastly the sample was irradiated with green light (515 nm, 30 min), and the NMR spectrum was measured again. By integration of the proton signals in the aromatic region, the ratio between both isomers could be determined at the PSS. The survey spectra and the zoom-in for the aromatic region, which was used for the determination of the PSS, are shown in Figures S14 and S15.[7]

Additional Experimental Data

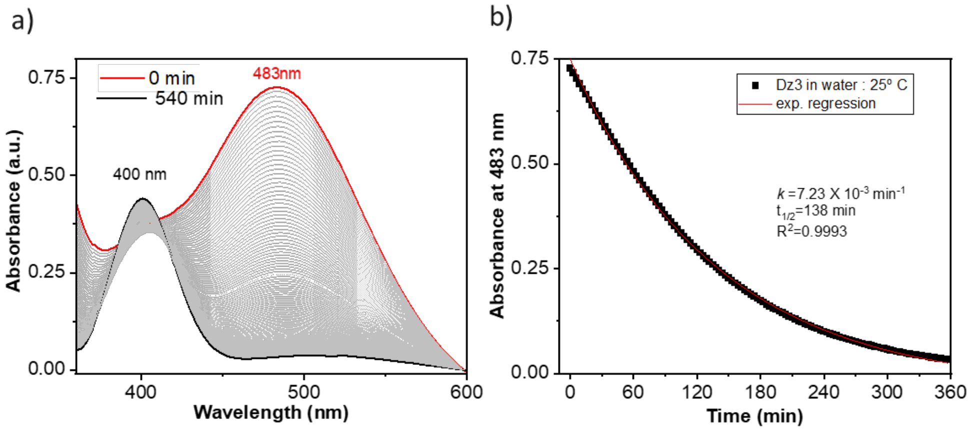


Figure S1. Thermal *Z* to *E* isomerization of Dz3. a) UV-*vis* spectra with 10% dioxane in water (0.15 mM) at room temperature, recorded at 10 min intervals after irradiation with UV light (390 nm). b) Plot of absorbance at 483 nm versus time and exponential fit to provide half-life time.

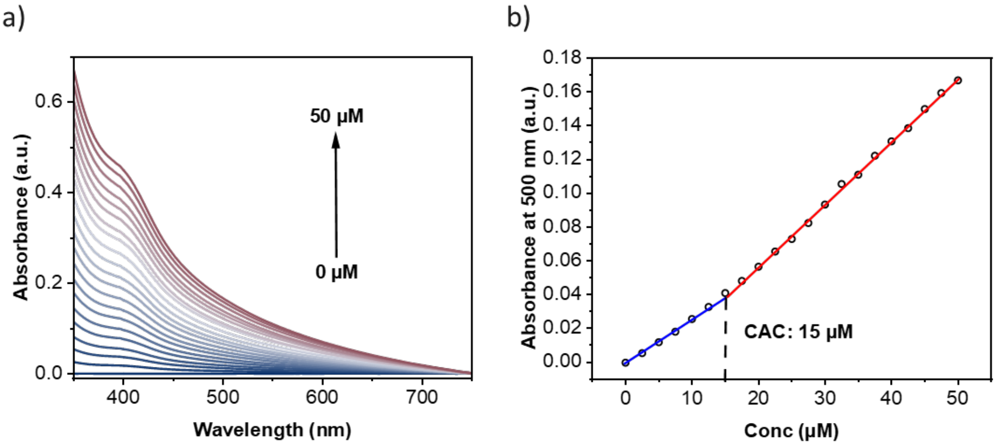


Figure S2. Critical aggregation concentration (CAC) measurement by measuring change of absorbance value obtained by varying concentration of Dz3 from 0 to 50 µM in water.

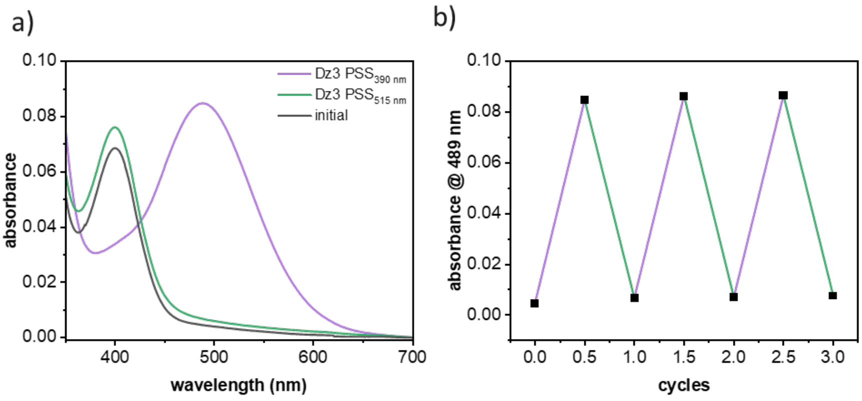


Figure S3 Photoisomerization of Dz3. a) Absorbance spectrum of Dz3 in DCM after irradiation with UV light (390 nm, 3 W) and green light (515 nm, 3 W), each for 30 min. b) Absorbance maxima at 489 nm after irradiation with UV light and green light. Concentration of Dz3: 50 µM in DCM.

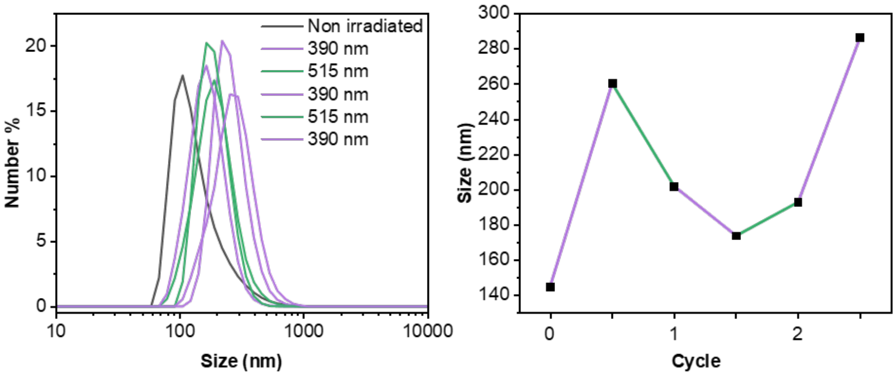


Figure S4. a), b) Size analysis by DLS measurements of 50 µM solution of Dz3 in Milli-Q with alternating irradiation UV light (390 nm) and green light (515 nm) each for 30 min.

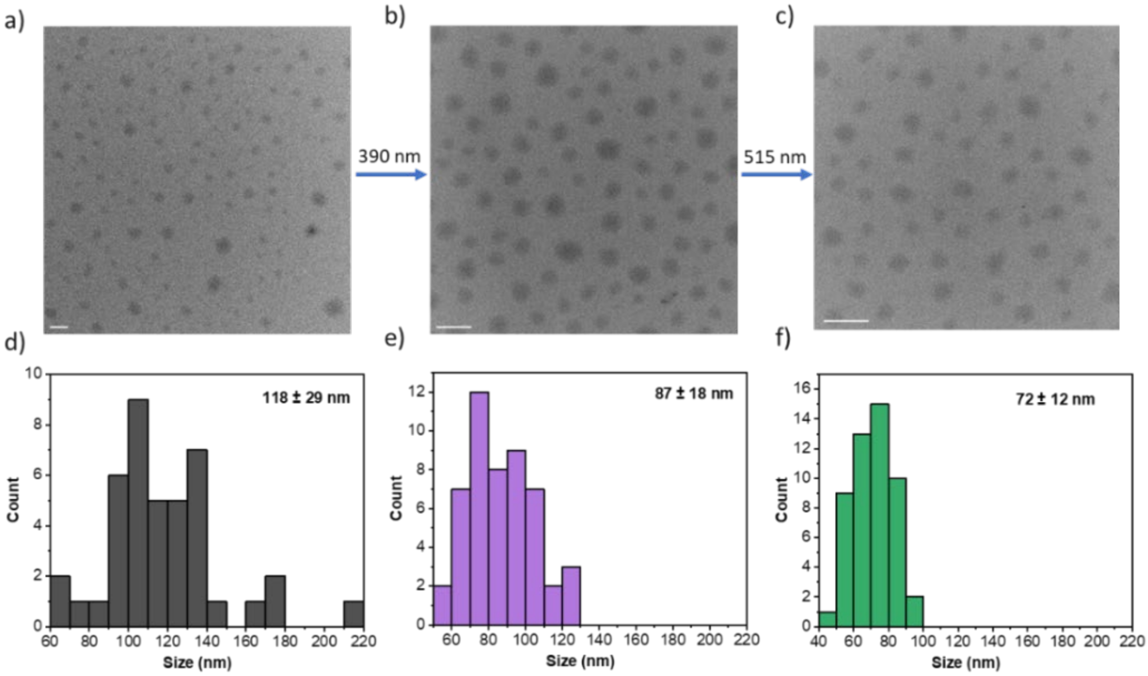


Figure S5. Size and morphology analysis of 50 µM Dz3 in Milli-Q. a) without irradiation b) 390 nm light irradiation c) and 515 nm light irradiation. d), e), f) are size distribution data of a), b), and c), respectively. Scale bar: 200 nm. Irradiation time: 30 min.

Although the size of the supramolecular nanoparticles fluctuated during irradiation, no pattern in the size change was observed with respect to the corresponding wavelength of light (see Figures S4 and S5)

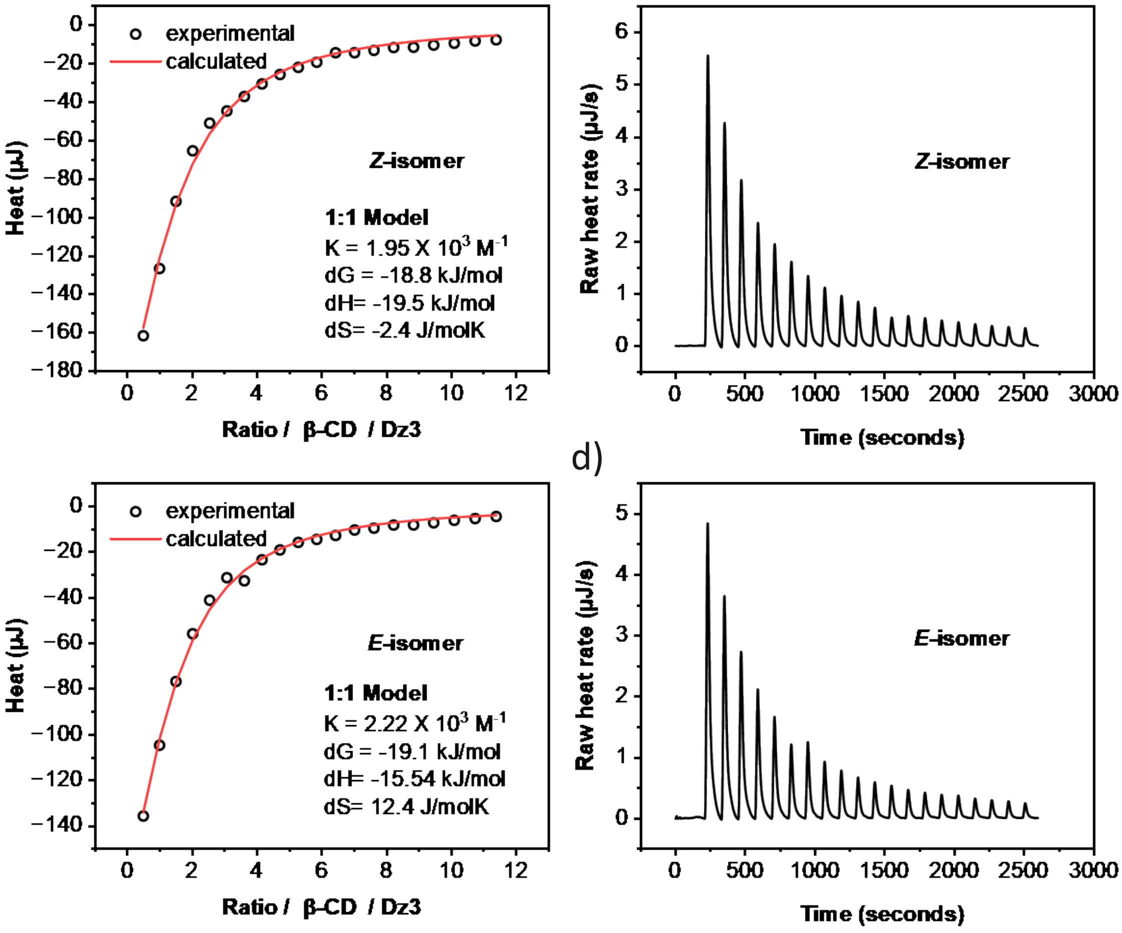


Figure S6 Isothermal titration calorimetry for host guest interaction of β-CD and Dz3. a), b) ITC of the *Z*-isomer of Dz3 with β-CD (irradiated with 515 nm, 3 W light for 30 min. c), d) ITC of the *E*-isomer of Dz3 with β-CD (irradiated with 390 nm ,3 W light for 30 min). ITC performed in HEPES buffer (pH = 7). Concentration of host and guest: cell: Dz3: 0.3 mM, syringe: β-CD: 10 mM.

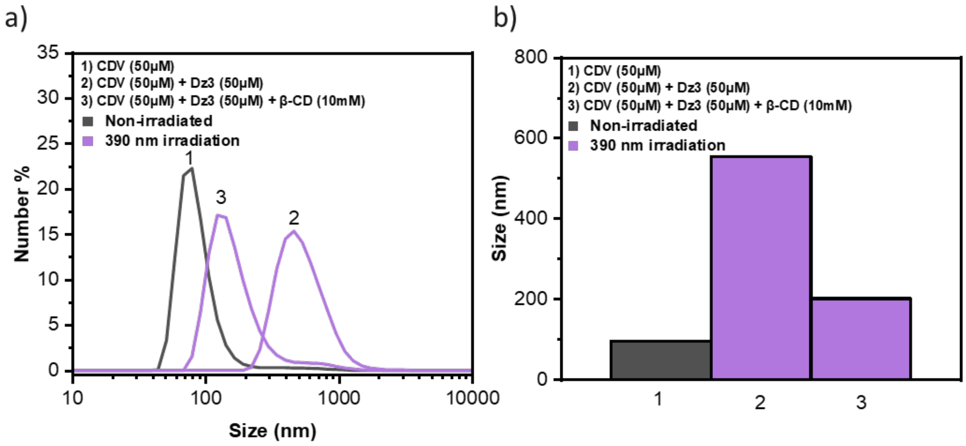


Figure S7. Competitive binding studies with β-CD as competitive inhibitor. Addition of 10 mM β-CD to the mixture of CDV and Dz3 (50 µM each) under 390 nm irradiation for 30 min. a) Size distribution by DLS, b) Average size by DLS of sample 1,2,3.

Synthesis

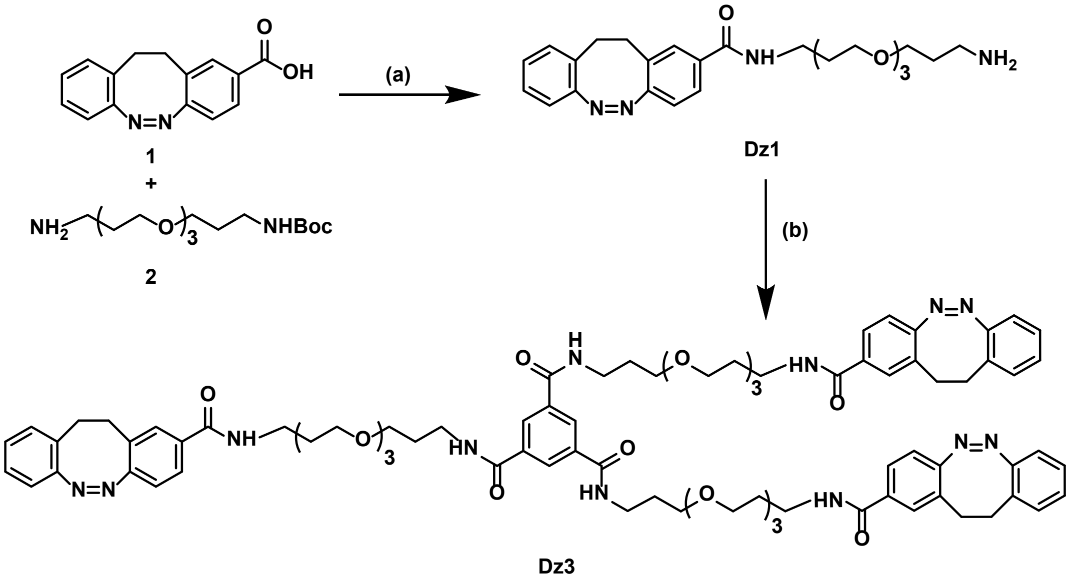
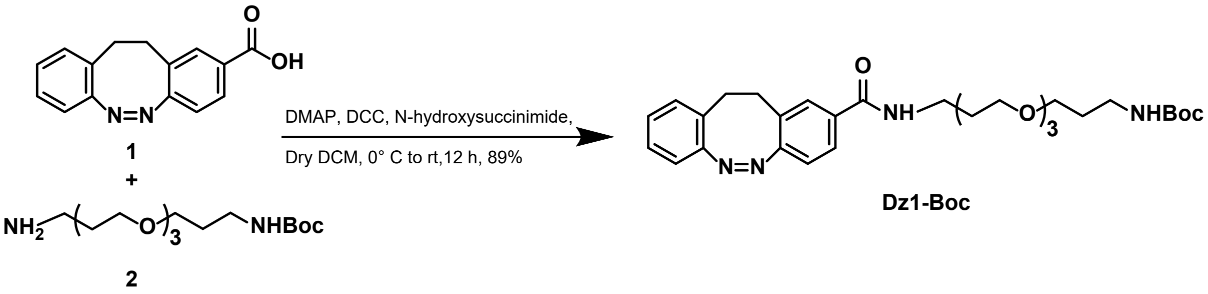


Figure S8 Synthesis of Dz3. Reagents and conditions: (a)(i) DMAP, DCC, N-hydroxy succinimide, Dry DCM, 0 °C to rt,12 h, 89%, (ii) TFA, dry DCM, 0 °C to rt, 6 h, 93% ;(b) ET3, benzene-1,3,5-tricarbonyl trichloride, dry DCM, 0° C to rt, 6 h, 75%.

Synthesis of Dz1-Boc

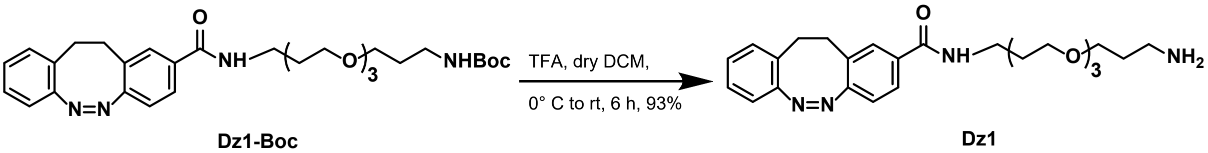


Compound 1 (150 mg, 0.59 mmol) (synthesized via reported procedure)[4, 5] and compound 2 (290 mg, 0.89 mmol) were dissolved together in 2 mL dry DCM and stirred, followed by DMAP (73 mg, 0.59 mmol) addition for 15 min. The reaction mixture was then cooled to 0 °C, followed by the addition of DDC (135 mg, 0.65mmol) and N-hydroxy succinimide (75 mg, 0.65 mmol). The reaction was purged with argon and left for stirring overnight at room temperature. Then, reaction mixture was diluted by 50 mL DCM. There was a white precipitate formation (DCU), that is removed via filtration. Further, the organic part was washed over brine and dried over $MgSO_4$ and concentrated over vacuum. Further, the purification was performed via column chromatography (2% MeOH/DCM) that yielded light yellow sticky solid as product(yield = 89%).[6]

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.58 – 7.46 (m, 2H), 7.13 (td, *J* = 7.4, 1.6 Hz, 1H), 7.04 – 6.95 (m, 2H), 6.84 (td, *J* = 7.2, 1.6 Hz, 2H), 4.90 (s, 1H), 3.68 – 3.59 (m, 6H), 3.56 – 3.49 (m, 4H), 3.49 – 3.39 (m, 4H), 3.17 (d, *J* = 6.2 Hz, 2H), 3.08 – 2.78 (m, 4H), 1.86 (q, *J* = 5.8 Hz, 2H), 1.69 (p, *J* = 6.3 Hz, 2H), 1.43 (d, *J* = 4.8 Hz, 9H).

MS (ESI+, MeOH): *m/z* calculated for $[M+Na]^+$: 577.29966; found: 577.29914.

Synthesis of Dz1



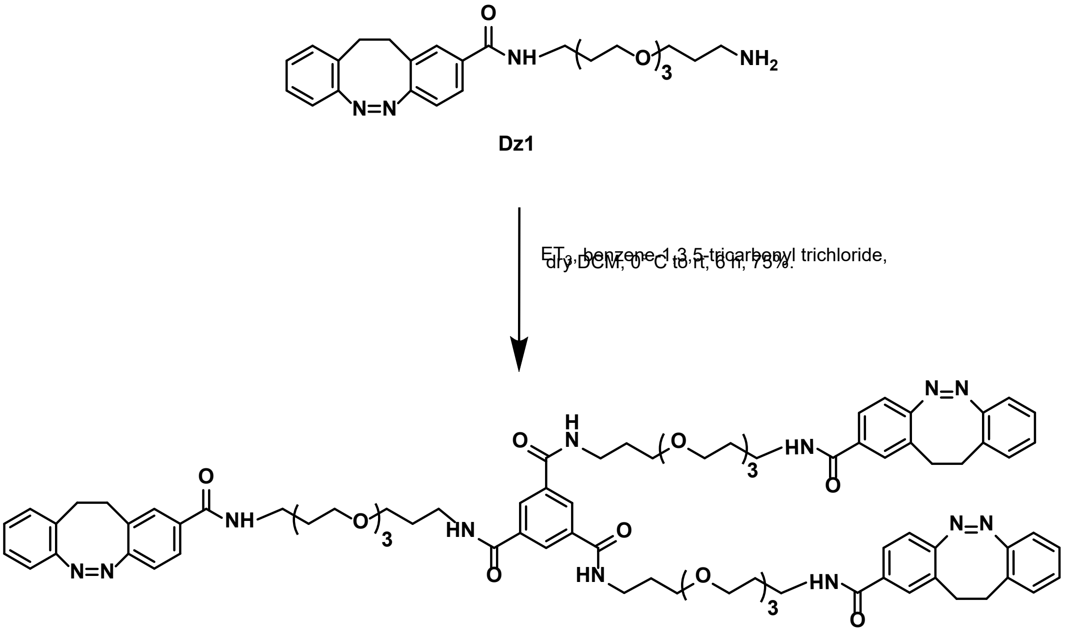
Dz1-Boc (240 mg, 0.53mmol) was dissolved in 3 mL dry DCM and cooled to 0 °C. Further, TFA (20%, 1 mL, 2.61 mmol) was dissolved in 2 mL dry DCM and was added dropwise to the reaction mixture, then reaction was left on stirring at room temperature for 6 hours. Then, reaction mixture was concentrated using rotary evaporator to remove the residual TFA. Further, the purification was performed via column chromatography (2% MeOH/DCM) that yielded light brownish yellow solid as product(yield = 93%).[4]

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.64 – 7.52 (m, 2H), 7.11 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.05 – 6.93 (m, 2H), 6.90 – 6.78 (m, 2H), 3.60 (q, *J* = 5.1 Hz, 6H), 3.56 – 3.42 (m, 8H), 3.03 – 2.85 (m, 4H), 2.79 (dd, *J* = 17.9, 11.0 Hz, 2H), 1.89 – 1.80 (m, 2H), 1.74 (p, *J* = 6.1 Hz, 2H).

^{13}C NMR (101 MHz, CDCl$_3$): δ 166.67, 157.75, 155.51, 133.38, 130.02, 129.36, 128.74, 127.80, 127.48, 126.95, 118.87, 118.77, 70.47, 70.28, 70.23, 70.10, 70.04, 39.94, 38.54, 34.09, 31.78, 31.63, 31.32, 29.02, 25.76, 25.08.

MS (ESI+, MeOH): *m/z* calculated for $[M+H]^+$: 455.26528; found: 455.26461.

Synthesis of Dz3



Dz1

Dz3

Dz1(140 mg, 0.3 mmol) was dissolved in 1.5 mL dry DCM followed by addition of triethly amine(ET$_3$, 31 mg, 0.3 mmol) while stirring. Further, 1,3,5-tricarbonyl trichloride (27 mg, 0.1 mmol) was dissloved in 200 µL dry DCM and then added dropwise at 0^{o}C. Then reaction mixture was kept on stirring for 6 h at room temperature. Further, the purification was performed via column chromatography (1% MeOH/DCM) that yielded light brownish yellow solid as product (yield = 75%).[6]

^{1}H NMR (599 MHz, CDCl$_3$): δ 7.57 – 7.47 (m, 2H), 7.12 (ddt, *J* = 9.0, 7.5, 4.4 Hz, 1H), 7.05 – 6.91 (m, 2H), 6.83 (ddd, *J* = 14.5, 10.5, 7.4 Hz, 2H), 3.75 – 3.37 (m, 14H), 3.06 – 2.90 (m, 2H), 2.79 (td, *J* = 22.6, 11.8 Hz, 2H), 1.89 – 1.63 (m, 6H).

^{13}C NMR (151 MHz, CDCl$_3$): δ 166.60, 166.09, 155.49, 133.33, 130.02, 129.38, 129.29, 128.81, 127.76, 127.52, 126.98, 118.89, 118.79, 70.57, 70.46, 70.32, 70.15, 70.07, 38.95, 33.68, 31.84, 31.80, 31.62, 29.85, 29.19, 28.95.

MS (ESI+, MeOH): *m/z* calculated for [M+Na]$^+$: 1541.74798; found: 1541.74901.

NMR Spectra

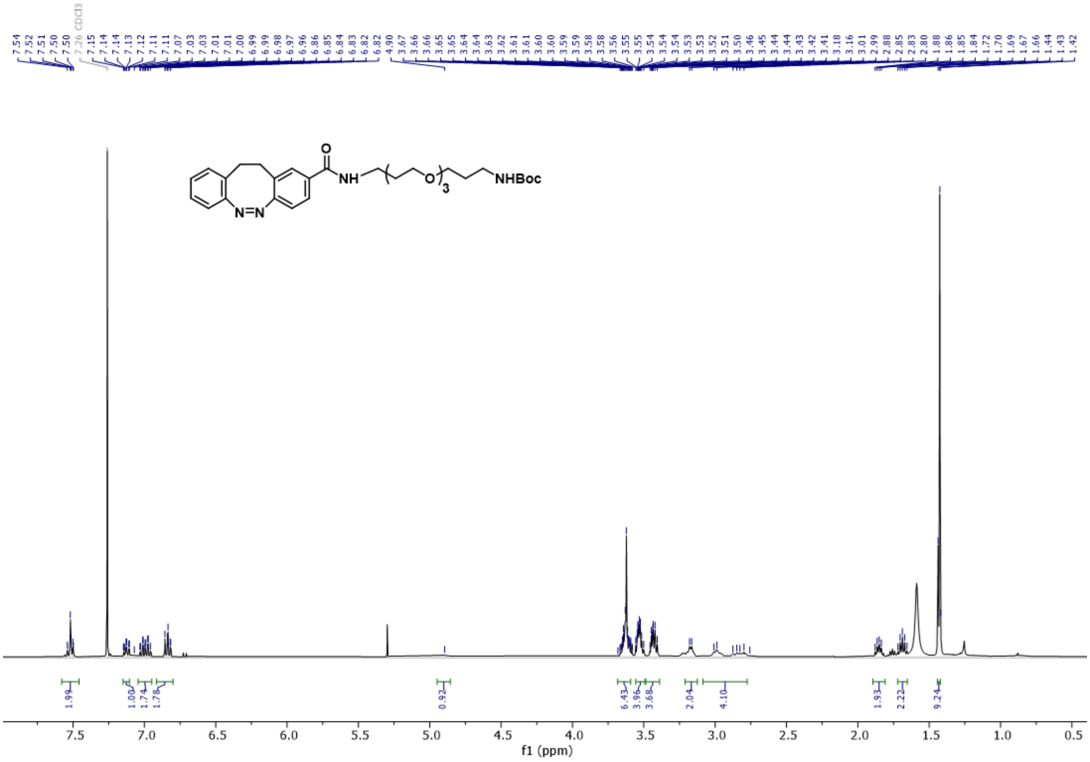


Figure S9. ^{1}H NMR of Dz1-Boc ($CDCl_3$, 400 MHz, 298 K).

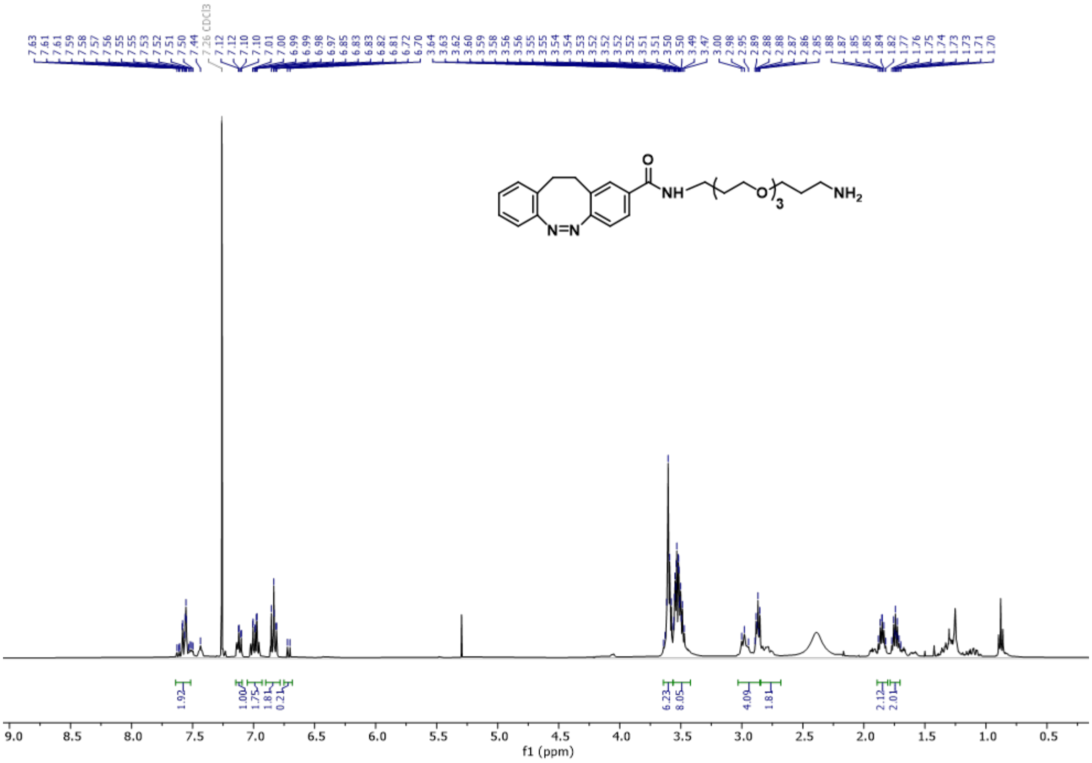


Figure S10. ^{1}H NMR of Dz1 ($CDCl_3$, 400 MHz, 298 K).

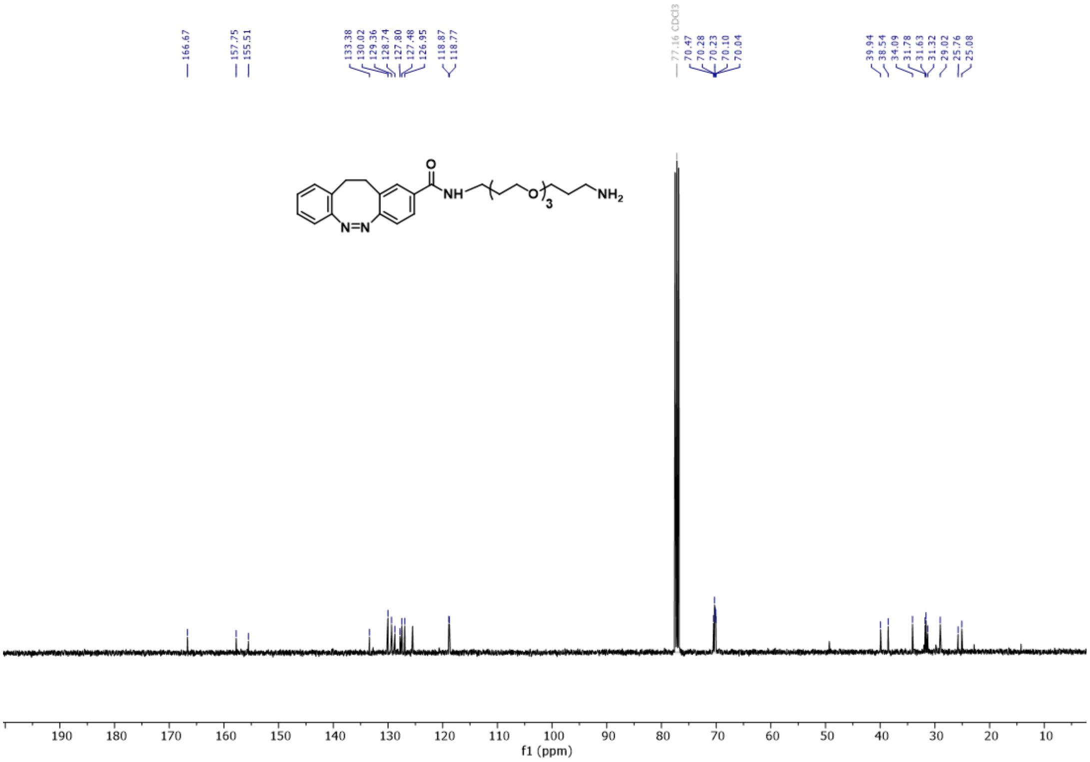


Figure S11. ^{13}C NMR of Dz3 ($CDCl_3$, 400 MHz, 298 K).

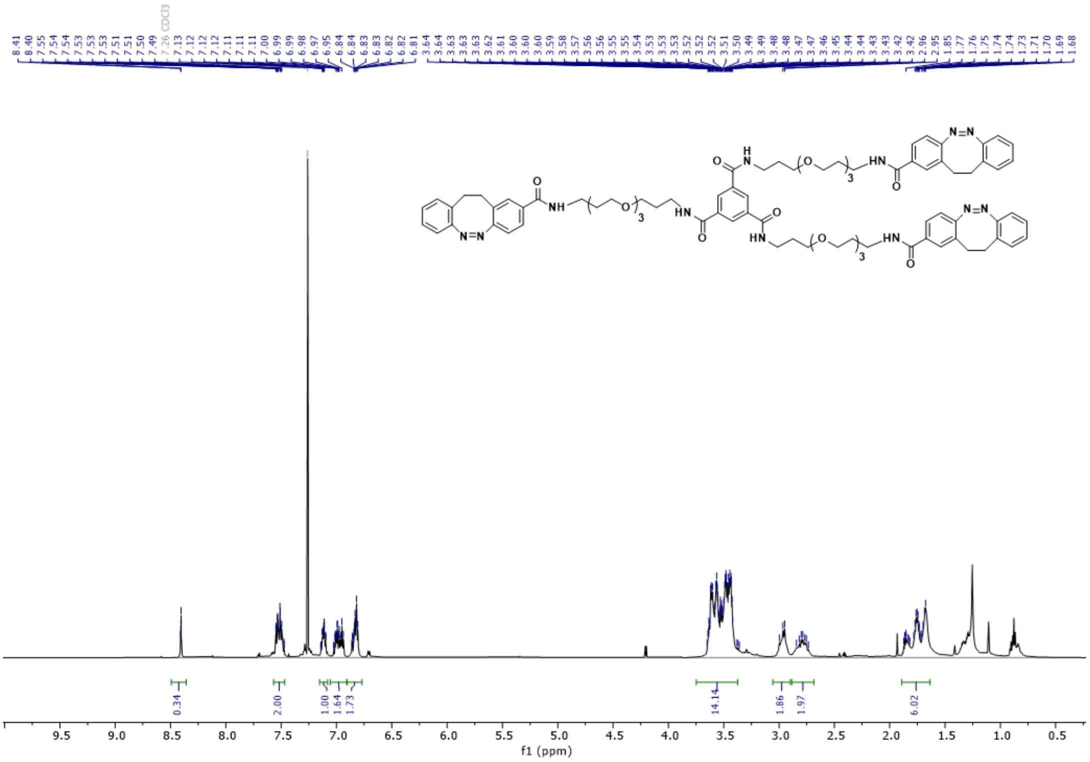


Figure S12. ^{1}H NMR of Dz3 ($CDCl_3$, 600 MHz, 298 K).

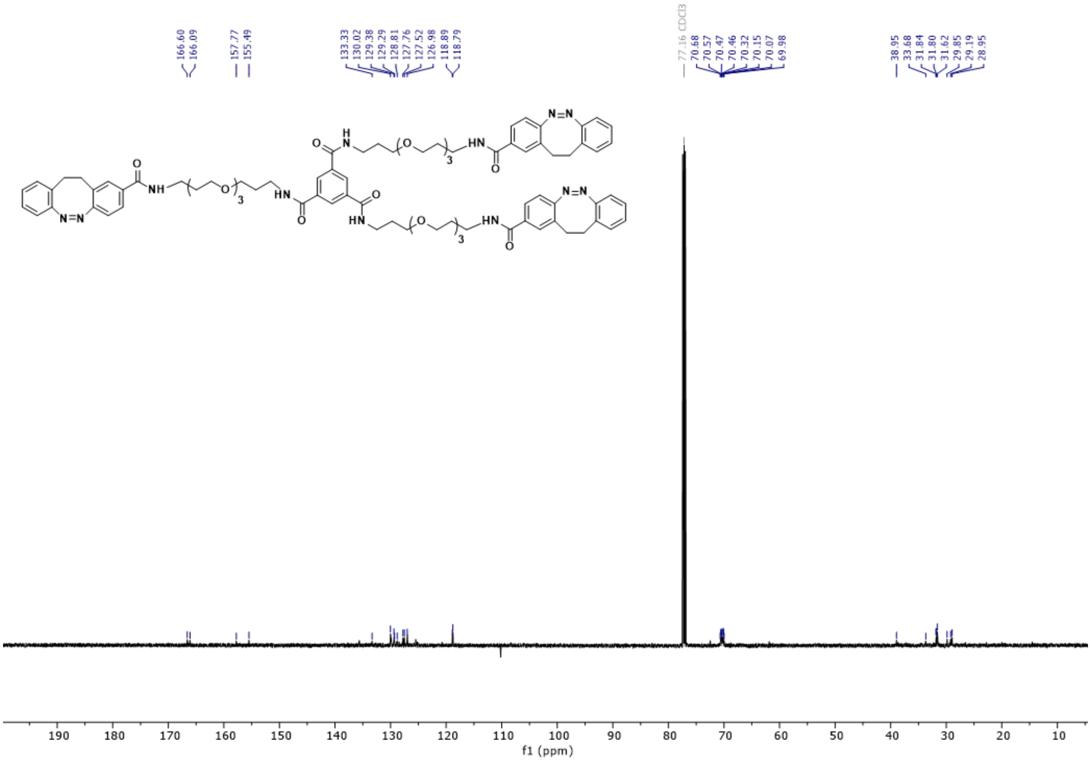


Figure S13. ^{13}C NMR of Dz3 ($CDCl_3$, 600 MHz, 298 K).

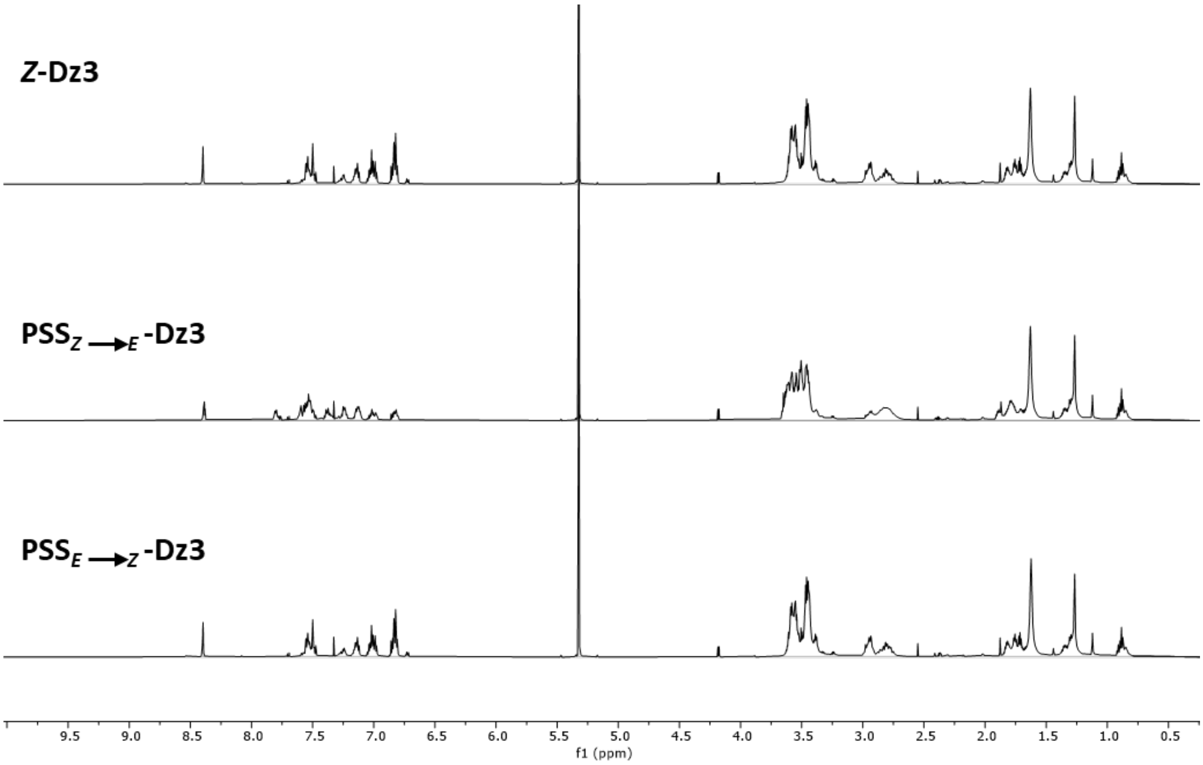


Figure S14. ^{1}H NMR spectra of the *Z*-isomer, PSS$_{Z\text{ to }E}$, and PSS$_{E\text{ to }Z}$ of Dz3 recorded in CD_2Cl_2 (irradiation time:30 min)

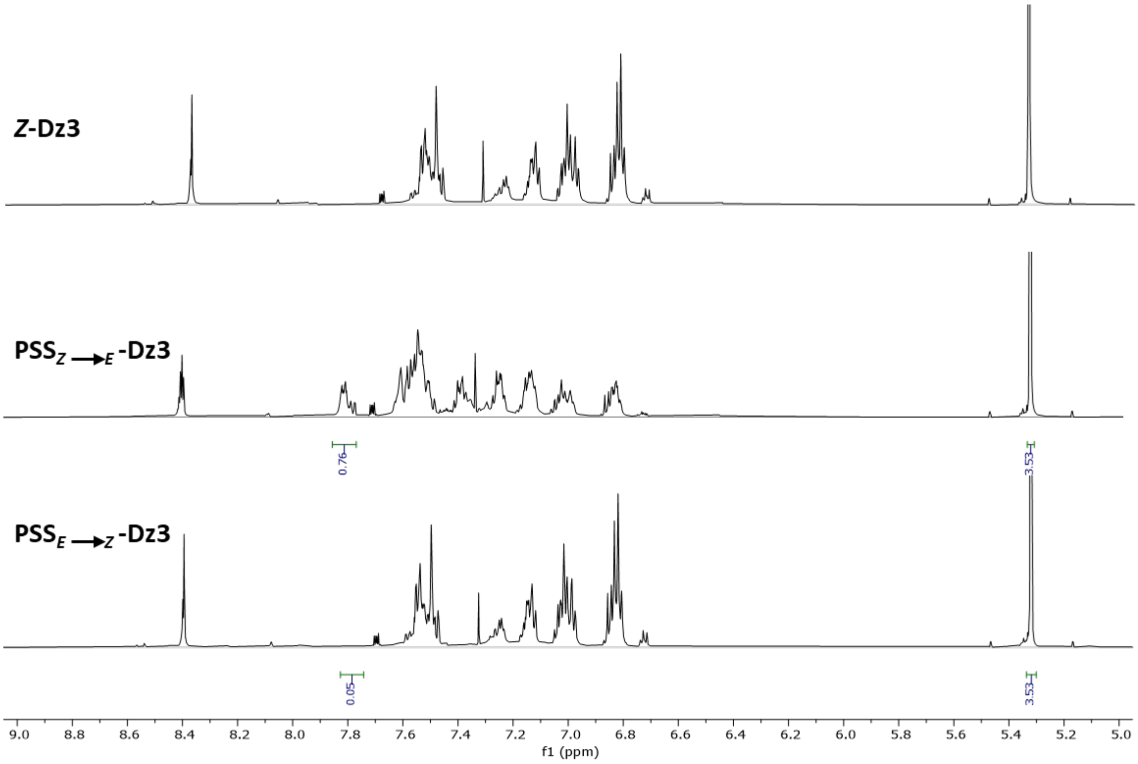


Figure S15. Aromatic region of the ^{1}H NMR spectra of the *Z*-isomer, PSS$_{Z\text{ to }E}$, and PSS$_{E\text{ to }Z}$ of Dz3 recorded in CD_2Cl_2 (irradiation time:30 min)

References

1. B. J. Ravoo and R. Darcy, *Angew. Chem. Int. Ed*, 2000, **39**, 4324-4326.
2. A. Kanojiya, J. Terglane, V. Gerke and B. J. Ravoo, *Soft Matter,* 2025, **21**, 1639-1645.
3. X.-F. Hou, Y. Chen and Y. Liu, *Soft Matter*, 2015, **11**, 2488-2493.
4. D. K. Joshi, M. J. Mitchell, D. Bruce, A. J. Lough and H. Yan, *Tetrahedron*, 2012, **68**, 8670-8676.
5. M. S. Maier, K. Hüll, M. Reynders, B. S. Matsuura, P. Leippe, T. Ko, L. Schäffer and D. Trauner, *J. Am. Chem. Soc.*, 2019, **141**, 17295-17304.
6. L. Zhang, Y. Wu and L. Brunsveld, *Angew. Chem. Int. Ed*, 2007, **46**, 1798-1802.
7. L. Stricker, M. Böckmann, T. M. Kirse, N. L. Doltsinis, B. J. Ravoo, *Chem. Eur. J.,*2018, **24**, 8639 - 8647.