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Published in:
Angewandte Chemie-International Edition

DOI:
10.1002/anie.201103707

Publication date:
2011

Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Light-Responsive Capture and Release of DNA in a Ternary Supramolecular Complex**

*Siva Krishna Mohan Nalluri, Jens Voskuhl, Jelle B. Bultema, Egbert J. Boekema, and Bart Jan Ravoo**

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Materials and Methods

Materials and analytical methods: Chemicals were purchased from Acros Organics (Schwerte, Germany) or Sigma-Aldrich (Taufkirchen, Germany) and used without further purification, unless otherwise noted. α-CD and β-CD were kindly donated by WackerChemie (Burghausen, Germany). Double stranded DNA from salmon testes Type III (Molecular weight of 1.3×10^6 g mol^{-1} with approximately 2000 bp) was purchased from Sigma-Aldrich (Taufkirchen, Germany). Single stranded DNA (5'-AATCGTTGGATCGTAGATCGCTTGCTGATAGATGCTCGATGCTGCTTTTA-3') (MW 15427 g mol^{-1}) was purchased from Eurofins MWG Operon (Ebersberg, Germany). All solvents were dried according to the conventional methods before use. All aqueous solutions were prepared in Milli-Q water. All reactions were carried out in oven-dried glassware and stirred magnetically under an inert gas atmosphere. Analytical TLC was performed on Merck silica gel 60 F_{254} plates. All compounds were visualized either by UV light or by dipping in basic permanganate solution. Column chromatography was carried out by using silica gel 60 (230–400 mesh). ^{1}H-NMR and ^{13}C-NMR spectroscopic measurements were carried out by using Bruker ARX 300MHz or 400MHz. Chemical shifts were referenced to internal standards $CDCl_3$ (δ = 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) or TMS (δ = 0.00 ppm for ^{1}H and ^{13}C). High-resolution mass spectrometry (HR-MS) was performed by using a MicroTof spectrometer (Bruker).

Preparation of CD vesicles: Unilamellar CD bilayer vesicles of α-CD **1a** and β-CD **1b** were prepared by extrusion. In short, several milligrams of **1a** (or **1b**) in 1 ml of chloroform was dried by slow rotary evaporation to yield a thin film in a glass vial. Residual solvent was removed under high vacuum. 10 ml of aqueous buffer (2 mM HEPES and 10 μM EDTA, pH

7.2) was added and stirred overnight. The resulting suspension was repeatedly passed through a polycarbonate membrane with 100 nm pore size in a Liposofast manual extruder.

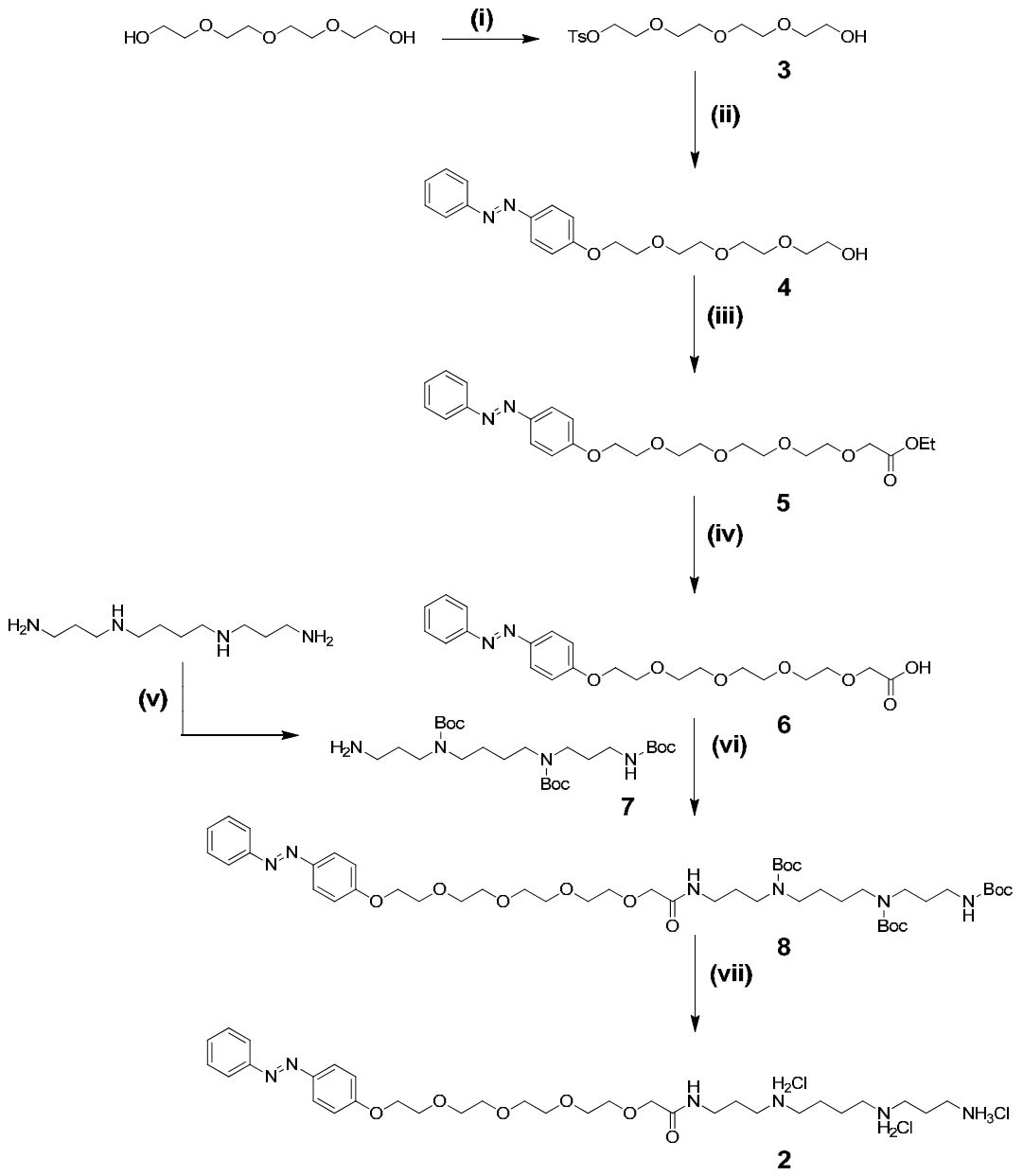
Irradiation experiments: Two different light sources were utilized for light irradiation experiments. One source was a Rayonet photochemical reactor (The Southern New England Ultraviolet Company) equipped with 16 RPR- 3500 lamps used to generate UV light (350 nm) to isomerize azobenzene moieties from *trans* to *cis* state. The other source was a Philips Lumileds royal blue LUXEON K2 emitter (LXK2-PR14-Q00) used to generate visible light (455 nm) to isomerize azobenzene moieties from *cis* to *trans*- state.

UV – Vis spectroscopy: Optical density measurements were carried out at in 1.5 ml disposable cuvettes with dimensions 12.5 × 12.5 × 45 mm and 10 mm path length using a Uvikon 923 double-beam spectrophotometer. The optical density was measured at λ = 600 nm (OD600), which is far from absorption of the azobenzene chromophore. Measurements were performed for 30 min to 60 min, unless otherwise noted, with data points collected every 12 s. The freshly prepared vesicles and DNA solutions were used for each measurement and the measurement procedure was as follows. For example, 1 ml solution of vesicles of **1a** or **1b** (30 μM) in aqueous buffer (2 mM HEPES and 10 μM EDTA, pH 7.2) were taken in a cuvette and OD600 was measured for 1 min. After 1 min, 15 μl of conjugate *trans*-**2** (2 mM, prepared in Millipore water) was added to the solution in the cuvette to make the resultant conjugate concentration 30 μM (this addition was done with slight mixing within one interval of 12 sec) and OD600 was measured for 3 min. After 3 min, 10 μl of 50-mer **ssDNA** or **dsDNA** (1 mg ml^{-1} in Millipore water) was added to the above solution and OD600 was measured for at least 30 min. The same measurements were performed with the other samples by following the above procedure. Typical concentrations: **[1a]** = **[1b]** = 30 μM and **[***trans*-**2]** = from 10 μM to 100 μM range.

Dynamic light scattering and ζ–potential measurements: DLS measurements were performed by using a Malvern Nano-ZS instrument (Malvern Instruments) with low-volume disposable cuvettes kept at 25^{o} C. The average size of the free vesicles of **1a** or **1b,** binary mixture of vesicles of **1a** or **1b** and bifunctional conjugate *trans*-**2** and the ternary complex of vesicles of **1a** or **1b**, bifunctional conjugate *trans*-**2** and dsDNA or 50-mer ssDNA was measured after 60 min after mixing the corresponding components. Immediately after alternate UV and visible light irradiations, the corresponding average size of the ternary complex was measured. Typical concentrations: **[1a]** = **[1b]** = 30 μM and **[***trans*-**2]** = from 10 μM to 100 μM range, in aqueous buffer (2 mM HEPES and 10 μM EDTA, pH 7.2). ζ-Potential measurements were also performed by using a Malvern Nano-ZS instrument (Malvern Instruments) at 25^{o} C.

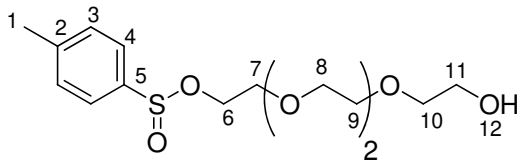
Cryogenic-Transmission Electron Microscopy: Samples for cryo-TEM were prepared by deposition of a few μL of free vesicles or a ternary complex (after 15 min after the addition of the corresponding components and OD600 of the ternary complex is ca. 1.0) on glow-discharged holey carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid at 100% humidity and 22 ^{0}C, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands). The vitrified specimens were mounted in a liquid nitrogen cooled Gatan 626 cryo-holder (Gatan Inc., Pleasanton, U.S.A.) and inserted in the electron microscope. Low-dose images were recorded with a Gatan 4K slow-scan CCD camera (Pleasanton, CA) on a Philips CM 120 electron microscope (FEI, Eindhoven, The Netherlands) equipped with a LaB6 tip operated at 120 kV. Typical concentrations: **[1a]** = **[1b]** = 1 mM, **[***trans*-**2]** = 0.5 mM and **[dsDNA]** = 0.5 μM in 10 mM phosphate buffer (pH = 7.2).

Synthesis



Scheme S1. Reagents and conditions: (i) TsCl, Et_3N, DMAP, DCM, 5^0 C, 3 h, rt, 18 h, 70%; (ii) 4-phenylazophenol, K_2CO_3, LiBr, MeCN, reflux, 2 days, 91%; (iii) Ethyl diazoacetate, BF_3•Et_2O, DCM, 0^o C to rt, 1.5 h, 40%; (iv) NaOH, MeOH/H_2O, 0^0 C to rt, 2 h, 87%; (v) Ethyl trifluoroacetate, MeOH, -78^o C, 1 h, 0^o C, 1 h, BOC_2O, rt, 1 h, NH_4OH, rt, 15 h, 38 %; (vi) tri-BOC-spermine **7**, DCC, HOBt, Et_3N, EtOAc, 0^0 C, 1 h, rt, 2 days, 24%; (vii) Acetyl chloride, MeOH, rt, overnight, 83%.

2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3)



The synthesis was carried out as described in the literature.[S1] To a stirred solution of tetraethyleneglycol (30.0 g, 154 mmol) and DMAP (100 mg cat.) and Et_3N (5.6 mL, 40 mmol) in 300 ml of DCM was added *p*-toluenesulfonylchlorde (7.24 g, 39 mmol) portionwise at 5^o C and stirred for about 3 h at this temperature. After that the resulting solution was continued to stir at room temperature for 18 h and finally extracted 1 M HCl (2 × 150 ml), water (2 × 150 ml) and brine (2 × 150 ml). The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc) to afford of the title compound.

Molecular formula $C_{15}H_{24}O_7S$ (colorless oil)

Yield: 9.51 g (27.3 mmol, 70 %).

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 2.37 (s, 3H, 1-H), 3.44-3.51 (m, 10H, 8-11-H), 3.61 (t, 2H, 7-H), 4.09 (*t*, 2H, 6-H), 7.27 (d, *J* = 6.0 Hz, 2H, 3-H), 7.73 (d, *J* = 6.0 Hz, 2H, 4-H).

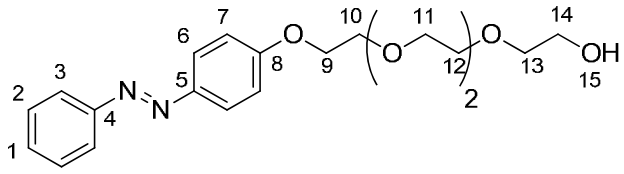
^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ = 21.59 (CH_3, 1-C), 68.66 (CH_2, 6-C), 69.24-70.72 (CH2, 6-9-C), 71.90 (CH_2, 11-C), 127.95 (C_q, 4-C), 129.81 (C_q, 3-C), 133.08 (C_q, 2-C), 144.77 (C_q, 5-C).

IR (neat): ν = 553 (s), 582 (w), 662 (m), 774 (m), 816 (m), 918 (s), 1011 (m), 1069 (w), 1096 (m), 1121 (m), 1175 (s), 1248 (w), 1352 (m), 1453 (w), 2872 (br), 3441 (br).

ESI-HRMS (m/z): Calculated for $[C_{13}H_{20}O_6SNa]^+$: 329.1814, found: 329.1811.

[S1] M. D. Lankshear, I. M. Dudley, K. M. Chan, A. R. Cowley, S. M. Santos, V. Felix, P. D. Beer, *Chem. Eur. J.* **2008,** *14,* 2248–2263.

(*E*)-2-(2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethoxy)ethanol (4)



To a stirred solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **(3)** (5.23 g, 15 mmol) in 150 ml of dry acetonitrile, containing of K_2CO_3 (10.37 g, 75 mmol) and catalytic amounts of LiBr, (3.17 g 16 mmol) of 4-phenylazophenol dissolved in 50 ml of acetonitrile was added and the reaction mixture was refluxed for 2 days under argon. It was then allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in 100 ml of DCM, washed once with 100 ml of water and thrice with 100 ml of brine. The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (DCM / methanol 95:5) to afford of the title compound.

Molecular formula: $C_{20}H_{26}N_2O_5$ (orange oil).

Yield: 5.11 g (14.3 mmol, 91 %).

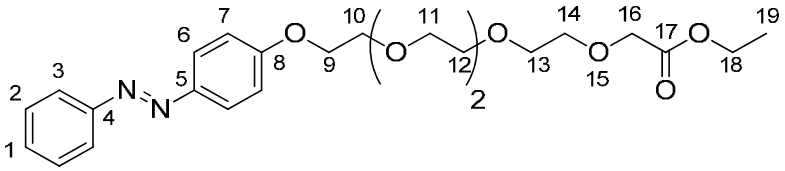
^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 2.57 (s, 1H, 15-H), 3.58 – 3.50 (m, 2H, 14-H), 3.72 – 3.61 (m, 10H, 11,12,13-H), 3.83 (m, 2H, 10-H), 4.16 (m, 2H, 9-H), 7.03 – 6.95 (m, 2H, 10,11-H), 7.50 – 7.35 (m, 3H, 1,2,-H), 7.92 – 7.80 (m, 4H, 3,6-H).

^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 61.56 (CH_2, 14-C), 69.52 (CH_2, 9-C), 70.45, 70.55, 70.17, 70.16 (4 CH_2, 11,12-C), 70.73 (CH_2, 10-C), 72.60 (CH_2, 13-C), 114.80 (CH, 7-C), 122.53 (CH, 3-C), 124.69 (CH, 6-C), 129.01 (CH, 2-C), 130.39 (CH, 1-C), 147.01 (C_q, 5-C), 152.65 (C_q, 4-C), 161.17 (C_q, 8-C).

IR (neat): ν = 549 (m), 689 (m), 723 (w), 768 (m), 839 (m), 925 (w), 1059 (s), 1105 (s), 1138 (s), 1250 (s), 1300 (w), 1357 (w), 1500 (m), 1582 (w), 1599 (m), 1701 (w), 2874 (br), 3458 (br).

ESI-HRMS (m/z): Calculated for $[C_{20}H_{26}N_2O_5Na]^+$: 397.1734, Found: 397.1736.

(*E*)-ethyl 14-(4-(phenyldiazenyl)phenoxy)-3,6,9,12-tetraoxatetradecan-1-oate (5)



To a stirred solution of **(4)** (1.0 g, 2.67 mmol) and 35 µl (0.28 mmol) of borontrifluoride ether adduct in 20 ml of dry DCM, 0.3 ml (2.85 mmol) of ethyl diazoacetate was added dropwise at 0^o C. After the addition was completed, the reaction mixture was allowed to stir at room temperature for 1.5 h. The reaction mixture was quenched by the addition of 15 ml of saturated NH_4Cl. The mixture product was extracted twice with 30 ml of DCM and the combined organic phases were dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc / *n*-pentane 1:2 followed by 2:1) to afford title compound.

Molecular formula: $C_{24}H_{32}N_2O_7$ (orange oil).

Yield: 0.49 g (1.11 mmol, 40 %).

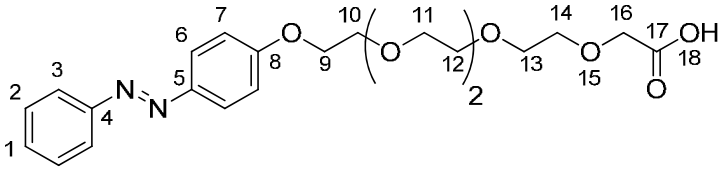
^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.09 – 1.16 (m, 3H, 19-H), 3.47-3.67 (m, 12H, 11,12,13,14-H), 3.71 (m, 2H, 10-H), 3.95 – 4.11 (m, 6H, 9,16,18-H), 6.84 – 6.95 (m, 2H, 7-H), 7.21 – 7.43 (m, 3H, 1,2-H), 7.70 – 7.87 (m, 4H, 3,6-H).

^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 170.10 (Cq, 17-C), 161.07 (C_q, 8-C), 152.38 (C_q, 4-C), 146.68 (C_q, 5-C), 130.17 (CH, 1-C), 128.75 (CH, 2-C), 124.44 (CH, 6-C), 122.29 (CH, 3-C), 114.54 (CH, 7-C), 70.52 (CH_2, 10-C), 70.27, 70.28 (4 CH_2, 11,12-C), 70.25 (CH_2, 13-C), 69.25 (CH_2, 14-C), 68.33 (CH_2, 9-C), 67.42 (CH_2, 16-C), 60.43 (CH_2, 18-C), 13.93 (CH_3, 19-C).

IR (neat): ν = 559 (m), 691 (m), 731 (w), 771 (m), 778 (w), 844 (s), 920 (m), 1065 (m), 1091 (s), 1166 (s), 1233 (s), 1347 (w), 1503 (m), 1579 (m), 1721 (s), 2876 (m).

ESI-HRMS (m/z): Calculated for $[C_{24}H_{32}N_2O_7Na]^+$: 483.2102, Found: 483.2101.

(*E*)-14-(4-(phenyldiazenyl)phenoxy)-3,6,9,12-tetraoxatetradecan-1-oic acid (6)



To a solution of **(5)** (0.27 g, 0.59 mmol) in 10 ml of methanol was added 2 ml of sodium hydroxide (1 N solution) at 0^o C and the reaction mixture was allowed to stir at room temperature for 2 h. Then 30 ml of ethyl acetate was added and the aqueous layer was acidified to a pH of 2 with 1 N HCl. The organic phase was collected and the aqueous layer was extracted with two portions of 10 ml of ethyl acetate. The combined organic layers were washed with 10 ml of water and 10 ml of brine, dried over $MgSO_4$ and concentrated to afford the title compound.

Molecular formula: $C_{22}H_{28}N_2O_7$ (orange oil).

Yield: 0.22 g (0.52 mmol, 87 %).

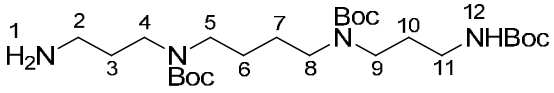
^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 3.77 – 3.57 (m, 12H, 11,12,13,14-H), 3.91 - 3.85 (m, 2H, 10-H), 4.17 – 4.11 (s, 2H, 14-H), 4.24 - 4.17 (m, 2H, 9-H), 7.07 – 6.97 (m, 2H, 7-H), 7.53-7.37 (m, 3H, 1,2-H), 7.95 – 7.82 (m, 4H, 3,6-H), 8.72 (s, 1H, 18-H).

^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ = 67.67 (CH_2, 16-C), 68.82 (CH_2, 9-C), 69.63 (CH_2, 14-C), 70.36 (CH_2, 13-C), 70.43 ,70.53, 70.64 ,70.91 (CH_2, 11,12-C), 71.28 (CH_2, 10-C), 114.89 (CH, 7-C), 122.61 (CH, 3-C), 124.77 (CH, 6-C), 129.09 (CH, 2-C), 130.45 (CH, 1-C), 147.09 (C_q, 10-C), 152.75 (C_q, 4-C), 161.29 (C_q, 8-C), 172.93 (C_q, 17-C).

IR (neat): ν = 549 (m), 689 (m), 722 (w), 768 (m), 839 (s), 926 (m), 1057 (m), 1105 (s), 1138 (s), 1250 (s), 1351 (w), 1500 (m), 1599 (m), 1735 (m), 2876 (m).

ESI-HRMS (m/z): Calculated for $[C_{22}H_{28}N_2O_7Na]^+$: 455.1789, Found: 455.1793.

Tert-butyl-(3-aminopropyl)(4-((tert-butoxycarbonyl)(3-((tert-butoxycarbonyl)amino)-propyl)amino)butyl)carbamate (7)



The synthesis was carried out as described in the literature.[S2] To a stirred solution of spermine (2.00 g, 10.2 mmol) in MeOH at -78° C was added ethyl-trifluoroacetate (1.16 g, 10.2 mmol). The mixture was stirred for one hour at -78°C followed by stirring for one hour at 0° C. After that di-tert-butyldicarbonate (8.89 g, 40.8 mmol) was added to the solution and stirring was continued for an additional hour at room temperature. Increasing the pH to 11 with aqueous ammonia and stirring for 15 h at room temperature lead to the desired compound which was further purified via column chromatography (CH_2Cl_2 / MeOH / NH_4OH 5:1:0.1, R_f = 0.43) to afford the title compound.

Molecular formula: $C_{25}H_{50}N_4O_6$ (colorless oil)

Yield: 1.93 g (3.83 mmol, 38 %).

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.38 (bs, 27H, 3 Boc), 1.42 (bs, 2H, 2-H), 1.60 (bs, 4H, 6,7-H), 2.67 (bs, 4H), 2.92-3.33 (m, 10H, 4,5,8,9,11-H), 4.82 (s, 2H, 1-H), 5.32 (s, 1H, 12-H).

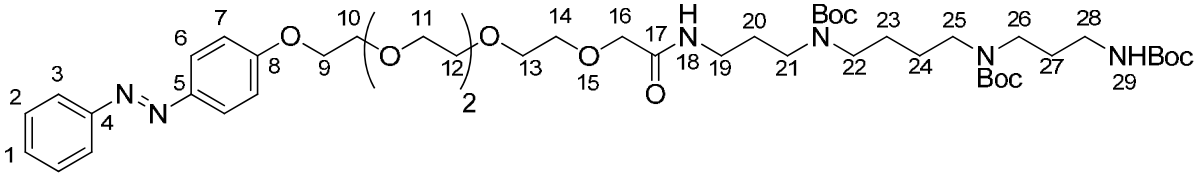
[S2] I. S. Blagbrough, A. J. Geall, *Tetrahedron Lett.* **1998**, *39,* 439-442.

^{13}C-NMR (75 MHz, $CDCl_3$, 298 K): δ = 28.28 (CH_3, Boc), 30.48, 32.09, 37.23, 37.55 (CH_2, 3,6,7,10-C), 38.43, 39.16, 43.58, 44.07, 44.30, 46.19 (CH_2, 2,4,5,8,9,11-C), 46.64 (CH_2, 5,8-C), 79.40 (C_q, Boc), 155.92, 155.86, 155.82 (C_q, Boc).

IR (neat): ν = 721 (m), 773 (w), 799 (w), 834 (w), 869 (w), 1049 (w), 1133 (s), 1157 (s), 1201 (m), 1250 (m), 1296 (w), 1367 (m), 1392 (w), 1420 (m), 1480 (w), 1670 (s), 2933 (br), 2976 (br).

ESI-HRMS (m/z): Calculated for $[C_{25}H_{50}N_4O_6H]^+$: 503.3803, found: 503.3803.

(E)-tert-butyl (4-((tert-butoxycarbonyl)(3-((tert- butoxycarbonyl)amino)propyl)amino)-butyl)(14-oxo-1-(4-(phenyldiazenyl)phenoxy)-3,6,9,12-tetraoxa-15-azaoctadecan-18-yl)-carbamate (8)



To a stirred solution of tri-BOC-spermine **(7)** (0.14 g, 0.28 mmol) and (0.14 g, 0.34 mmol) of **(6)** in 40 ml of EtOAc were added 1.79 g (8.66 mmol) of DCC, 1.33 g (8.66 mmol) of HOBt and 1.2 ml (8.67 mmol) of Et_3N and the the mixture was stirred under argon at 0^o C for 1 h and then left for stirring at room temperature for 2 days. After removing dicyclohexylurea (DCU) by filtration, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2 / MeOH 98:2) to afford the title compound.

Molecular formula: $C_{47}H_{76}N_6O_{12}$ (orange oil).

Yield: 0.06 g (0.07 mmol, 24%).

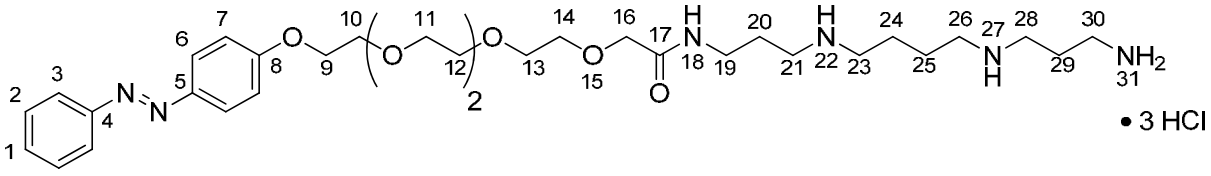
^{1}H-NMR (500 MHz, $CDCl_3$, 298 K): δ = 1.36 – 1.52 (m, 31H, 23,24,3Boc), 1.57 – 1.74 (*br* m, 4H, 20,27-H), 3.03 – 3.28 (*br* m, 12H, 19,21,22,25,26,28-H), 3.61-3.75 (m, 12H, 11,12,13,14-H), 3.85-3.90 (m, 2H, 10-H), 3.96 (s, 2H, 16-H), 4.17-4.22 (m, 2H, 9-H), 5.28 (s, 1H, 29-H), 6.99-7.03 (m, 2H, 7-H), 7.39-7.52 (m, 4H, 1,2,18-H), 7.81-7.94 (m, 4H, 3,6-H).

^{13}C-NMR (125 MHz, $CDCl_3$, 298 K): δ = 26.14, 25.61 (CH_2, 20,23,24,27-C), 28.54 (CH_3, 3Boc), 35.77, 36.68, 37.47, 37.77, 43.96, 44.31, 44.86, 46.58, 46.93, (CH_2, 19,21,22,25,26,28-C), 67.82 (CH_2, 9,14-C), 69.73 (CH_2, 13-C), 70.42 (CH2, 10-C), 70.70, 70.98 (CH_2, 11,12-C), 71.15 (CH_2, 16-C), 78.97, 79.53 (C_q, 3Boc), 114.91 (CH, 7-C), 122.63 (CH, 3-C), 124.78 (CH, 6-C), 129.10 (CH, 2-C), 130.47 (CH, 1-C), 147.17 (C_q, 5-C), 152.81 (C_q, 4-C), 155.57 (C_q, Boc), 156.06 (C_q, 2Boc), 161.33 (C_q, 8-C), 170.01 (C_q, 17-C)

IR (neat): ν = 579 (w), 604 (w), 687 (m), 763 (m), 840 (m), 1058 (m), 1109 (s), 1140 (m), 1251 (m)s), 1297 (w), 1344 (w), 1459 (w), 1527 (w), 1603 (w), 1654 (m), 2751 (br), 2953 (br).

ESI-HRMS (m/z): Calculated for $[C_{47}H_{76}N_6O_{12}Na]^+$: 939.5413, found: 939.5418.

(E)-N-(3-((4-((3-aminopropyl)amino)butyl)amino)propyl)-2-(2-(2-(2-(4-(phenyldiazenyl)-phenoxy)ethoxy)ethoxy)ethoxy)acetamide (2)



To a stirred solution of **(8)** in MeOH (60 mg, 0.07 mmol) was added 0.1 mL of acetylchloride at 0°C. After 1h, stirring was stopped and the resulting precipitate was filtered off. Afterwashing with acetonitrile followed by drying in vacuum affords the title compound.

Molecular formula: $C_{32}H_{52}N_6O_6$ (yellow solid).

Yield: 36 mg, (0.06 mmol, 83 %).

^{1}H NMR (300 MHz, D_2O, 298 K): δ = 1.78 – 1.69 (m, 4H, 24,25-H), 1.89-1.80 (m, 2H, 29-H), 2.01-2.13 (m, 2H, 20-H), 2.95 – 3.16 (m, 10H, 19,21,23,26,28,30-H), 3.22 (t, J = 6.4 Hz, 2H, 19-H), 3.43-3.60 (m, 12H, 11,12,13,14-H), 3.62 (s, 2H, 10-H), 3.84 (bs, 2H, 9-H), 3.92 (s, 2H, 16-H), 6.71 (d, J = 7.3 Hz, 2H, 7-H), 7.27 (bs, 4H, 1,2-H), 7.54 (bs, 4H, 3,6-H).

^{13}C-NMR (75 MHz, D_2O, 298 K): δ = 22.71, 22.75, 23.68, 25.59 (CH_2, 20,24,25,29-C), 35.57, 36.47, 44.48, 45.02, 46.86, 46.95, (CH_2, 19,21,22,25,26,28-C), 67.24 (CH_2, 9,14-C), 68.73 (CH_2, 13-C), 69.32 (CH_2, 10-C), 69.47, 69.77 (CH_2, 11,12-C), 70.14 (CH_2, 16-C), 114.83 (CH, 7-C), 122.23 (CH, 3-C), 124.56 (CH, 6-C), 129.21 (CH, 2-C), 130.72 (CH, 1-C), 146.21 (C_q, 5-C), 151.87 (C_q, 4-C), 160.84 (C_q, 8-C), 172.61 (C_q, 17-C).

IR (neat): ν = 579 (w), 604 (w), 687 (m), 763 (m), 840 (m), 1058 (m), 1109 (s), 1140 (m), 1251 (m)s), 1297 (w), 1344 (w), 1459 (w), 1527 (w), 1603 (w), 1654 (m), 2751 (br), 2953 (br).

ESI-HRMS (m/z): Calculated for $[C_{32}H_{52}N_6O_6H_2]^{2+}$: 309.20469, found: 309.20498.

Melting point: >300°C decomp. (CH_2Cl_2).

UV-Vis spectrum of bifunctional conjugate 2:

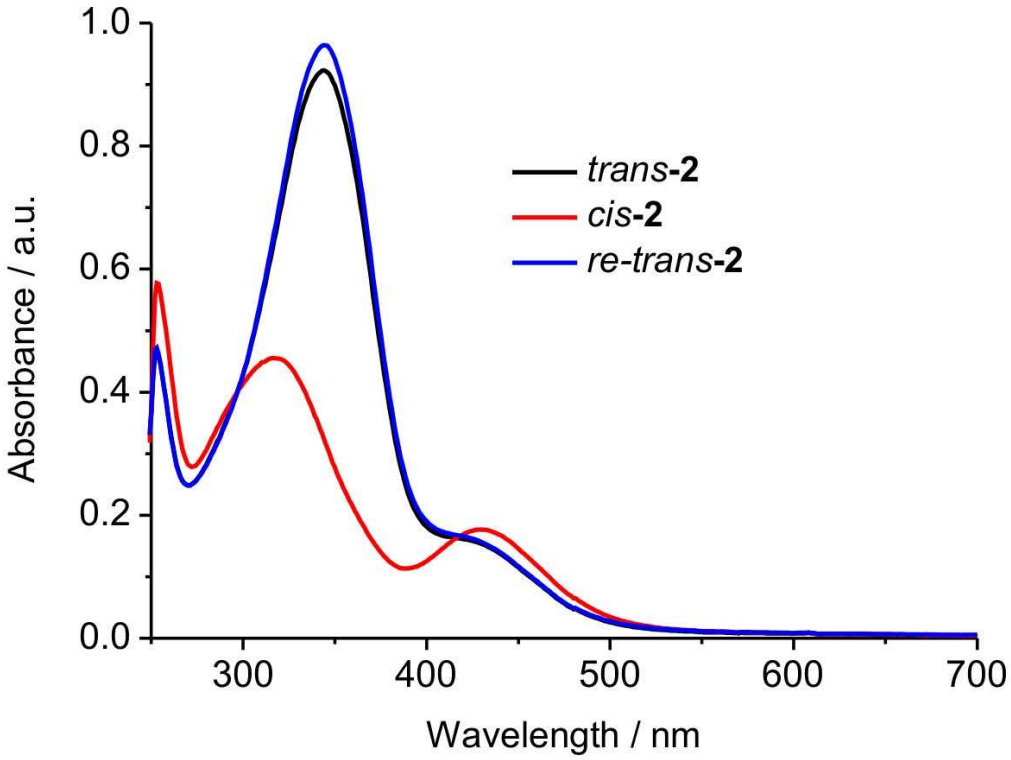


Figure S1: UV – Vis spectra of bifunctional conjugate **2** (c = 5 × 10^{-5} M) in water: Before (*trans*-**2**, black), after UV light irradiation at λ = 350 nm for 4 min (*cis*-**2**, red), and after subsequent visible light irradiation at λ = 455 nm for 8 min (*re-trans*-**2**, blue).

^{1}H NMR spectrum of bifunctional conjugate 2:

The percentages of both *trans* and *cis* isomers of conjugate **2** under UV and subsequent visible light irradiations are calculated by ^{1}H NMR.

A) (Green) Before UV light irradiation (80% *trans* and 20% *cis*),

B) (Red) After UV light irradiation (20% *trans* and 80% *cis*),

C) (Blue) After subsequent visible light irradiation (70% *trans* and 30% *cis*).

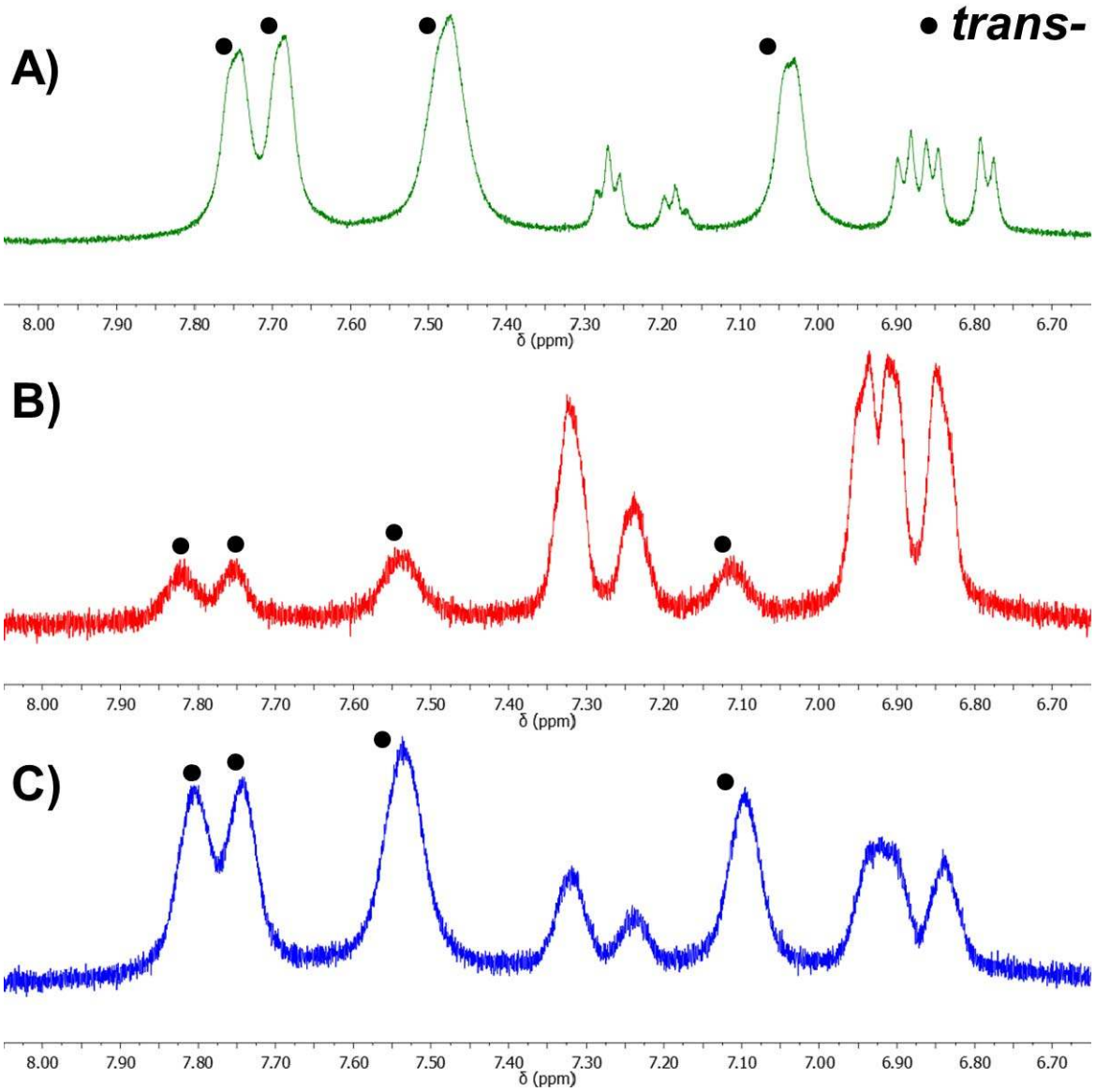


Figure S2: Partial ^{1}H NMR (500 MHz) spectra of bifunctional conjugate **2** before UV (A), after UV (B) and after subsequent visible (C) light irradiation in D_2O.

Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetry (ITC) was performed by using a Nano-Isothermal Titration calorimeter III (model CSC 5300, Calorimetry Sciences Corporation, London, Utah, USA). ITC measurements were performed in milli-Q water. 20 injections of bifunctional conjugates *trans*-**2** (10 mM) with a volume of 10 µL were titrated into a solution of α-CD or β-CD (1 mM). The solution was stirred with 300 rpm at 23 °C.

Table S1: Thermodynamic data for the interaction of α-CD and β-CD, respectively, with bifunctional conjugates *trans*-**2.**

Entry	**Guest**	**Host**	**K** (M^{-1})	**ΔS** (J/Kmol)	**ΔG** (kJ /mol)	**ΔH** (kJ/mol)
A	(*trans*-**2**)	α-CD	5980	4.19	-21.54	-20.29
B	(*trans*-**2**) [a]	β-CD	2440	38.91	-19.32	-7.73

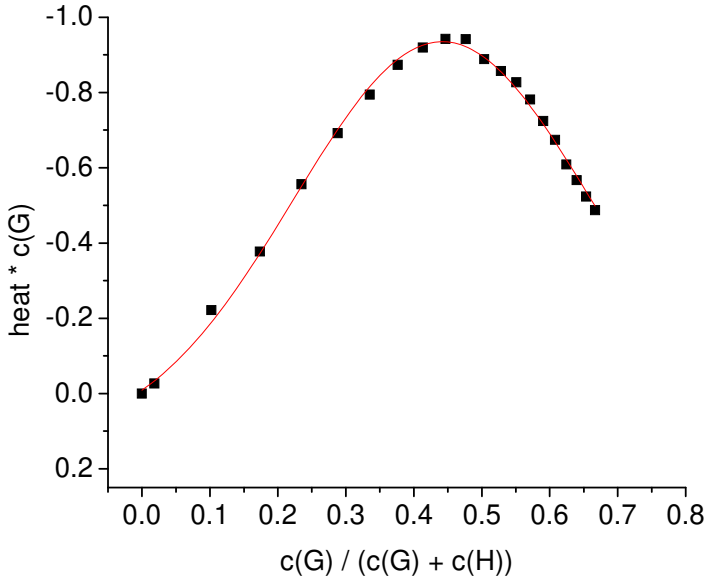
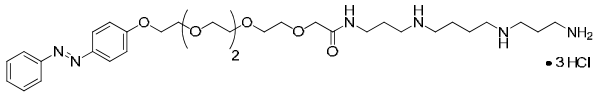
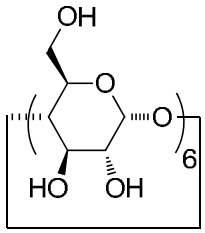
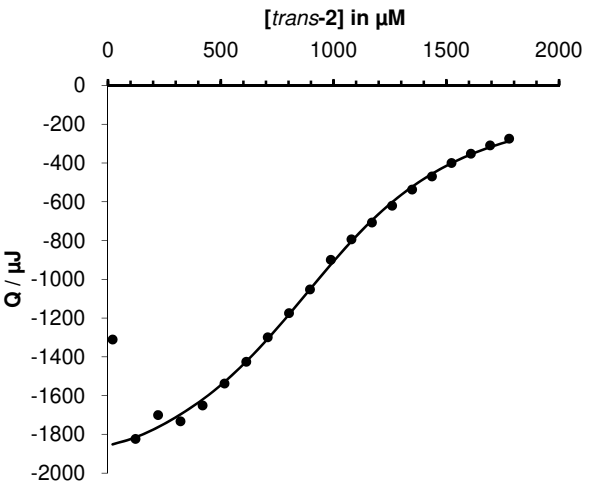
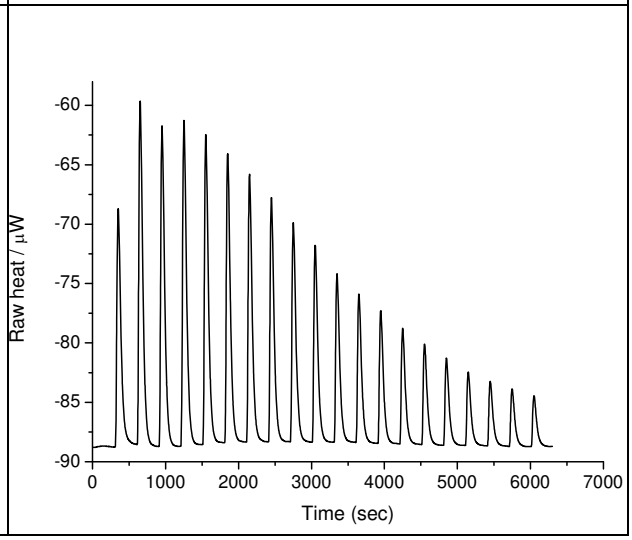
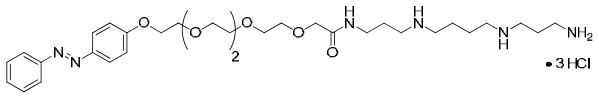
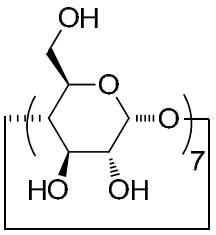
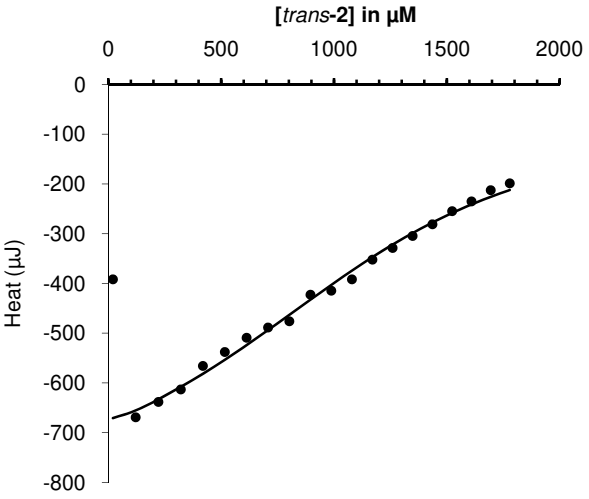
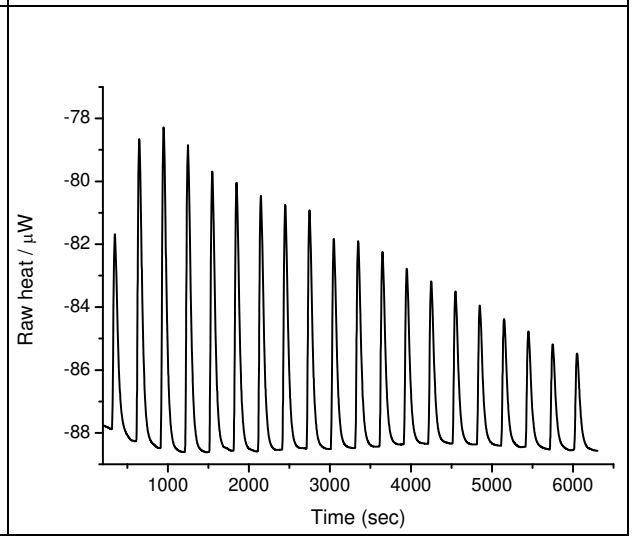


Figure S3: Job´s Plot for the titration of α-CD with *trans*-**2**.

C (*trans*-**2**) : 10 mM, C(α-CD): 1 mM	T = 23 °C, Rpm = 300, 20 x 10 µL injections
	
	

C (*trans*-**2**) : 10 mM, C(β-CD): 1 mM	T = 23 °C, Rpm = 300, 20 x 10 µL injections
	
	

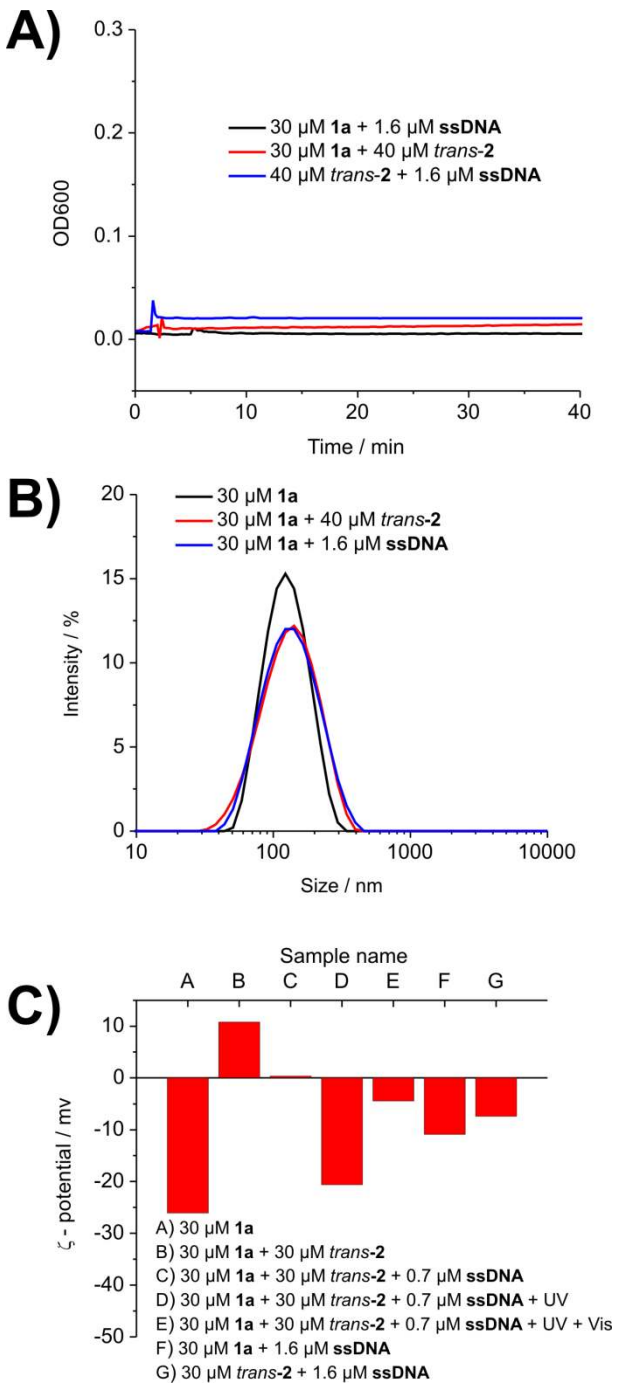


Figure S4: Ternary complex formation between vesicles of **1a,** conjugate *trans*-**2** and 50-mer **ssDNA**. (A) Time-dependent measurement of OD600. (B) Size distribution according to DLS. (C) Zeta potential determined by DLS.

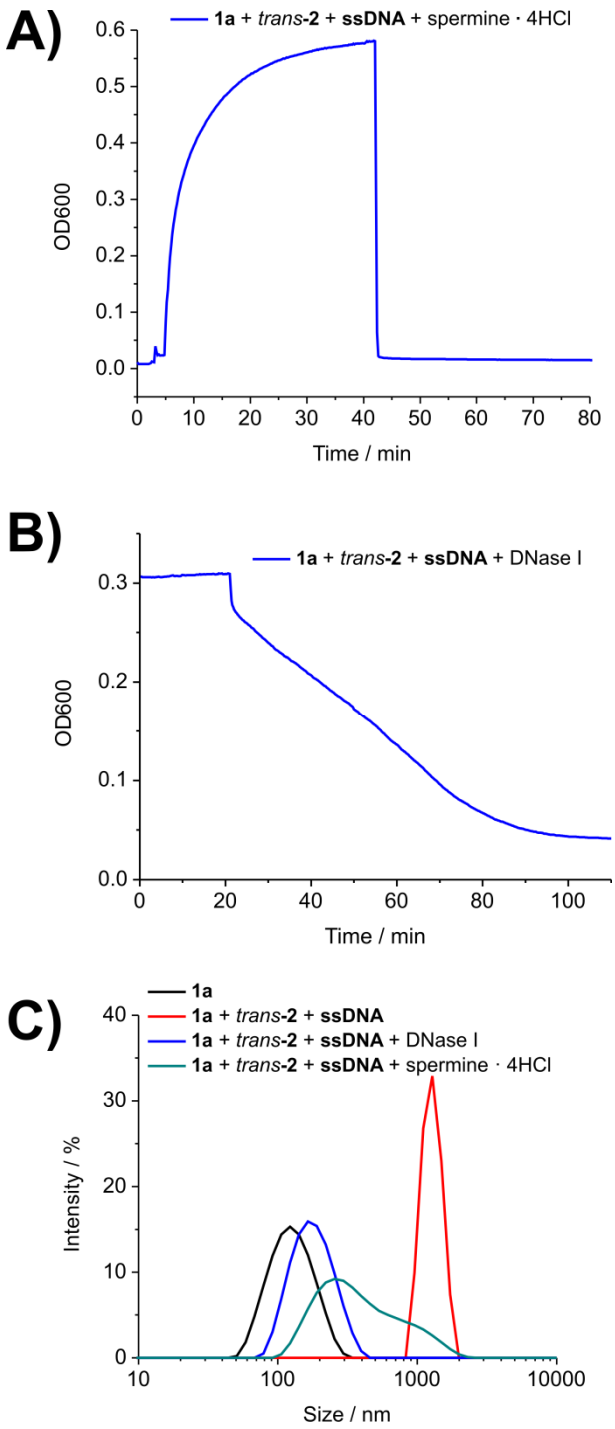


Figure S5: Dissociation of a ternary complex of vesicles of α-CD **1a,** conjugate **2** and 50-mer **ssDNA**. (A) Addition of excess spermine: time-dependent measurement of OD600. (B) Addition of DNase I: time-dependent measurement of OD600. (C) Size distribution according to DLS. Concentrations: [**1a**] = 30 µM, [*trans*-**2**] = 35 µM, [**ssDNA**] = 0.7 µM, [spermine.4HCl] = 50 mM and [DNase I] = 30 U ml^{-1} in aqueous buffer (2 mM HEPES and 10 µM EDTA at pH 7.2).

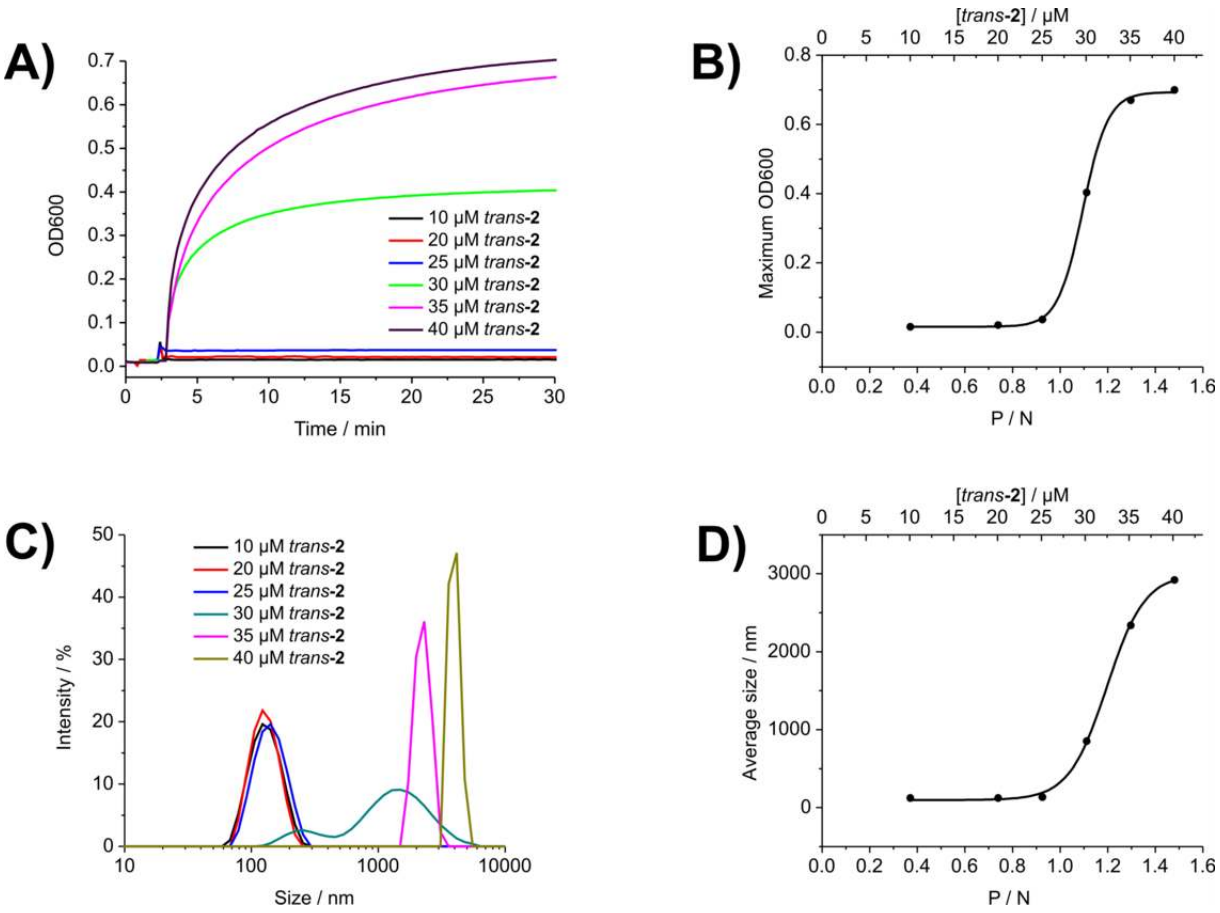


Figure S6: Ternary complex formation between vesicles of **1b,** conjugate *trans*-**2** and 50-mer **ssDNA**. (A) Time-dependent measurement of OD600. (B) Maximum OD600 versus concentration of *trans*-**2** and positive (P) / negative (N) charge ratio. (C) Concentration dependent size distribution according to DLS. (D) Average size versus concentration of *trans*-**2** and positive (P) / negative (N) charge ratio. Concentrations: [**1b**] = 30 µM and [**ssDNA**] = 1.6 µM in aqueous buffer (2 mM HEPES and 10 µM EDTA, pH 7.2).

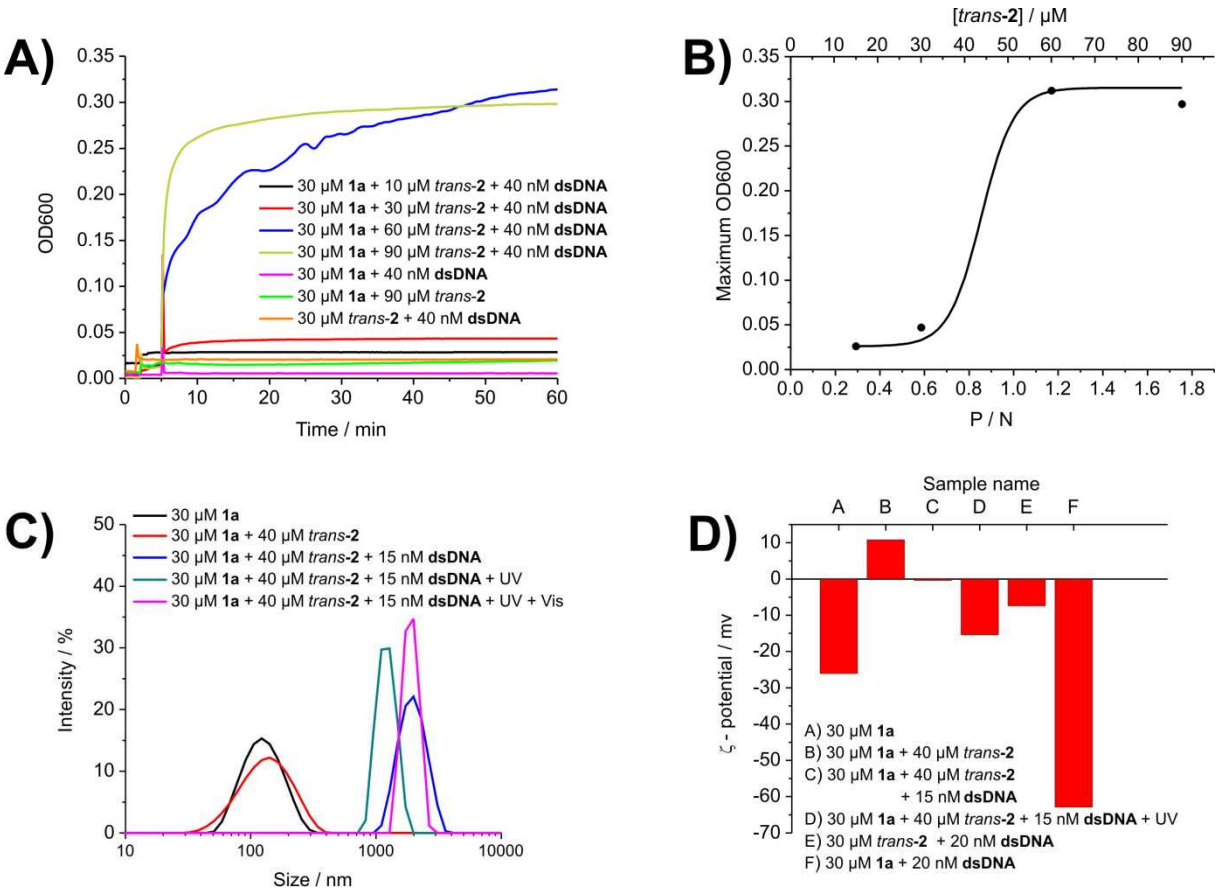


Figure S7: Ternary complex formation between vesicles of **1a,** conjugate *trans*-**2** and 2 kbp **dsDNA**. (A) Time-dependent measurement of OD600. (B) Maximum OD600 versus concentration of *trans*-**2** and positive (P) / negative (N) charge ratio. (C) Effect of light irradiation on the size distribution according to DLS. (D) Zeta potential determined by DLS.

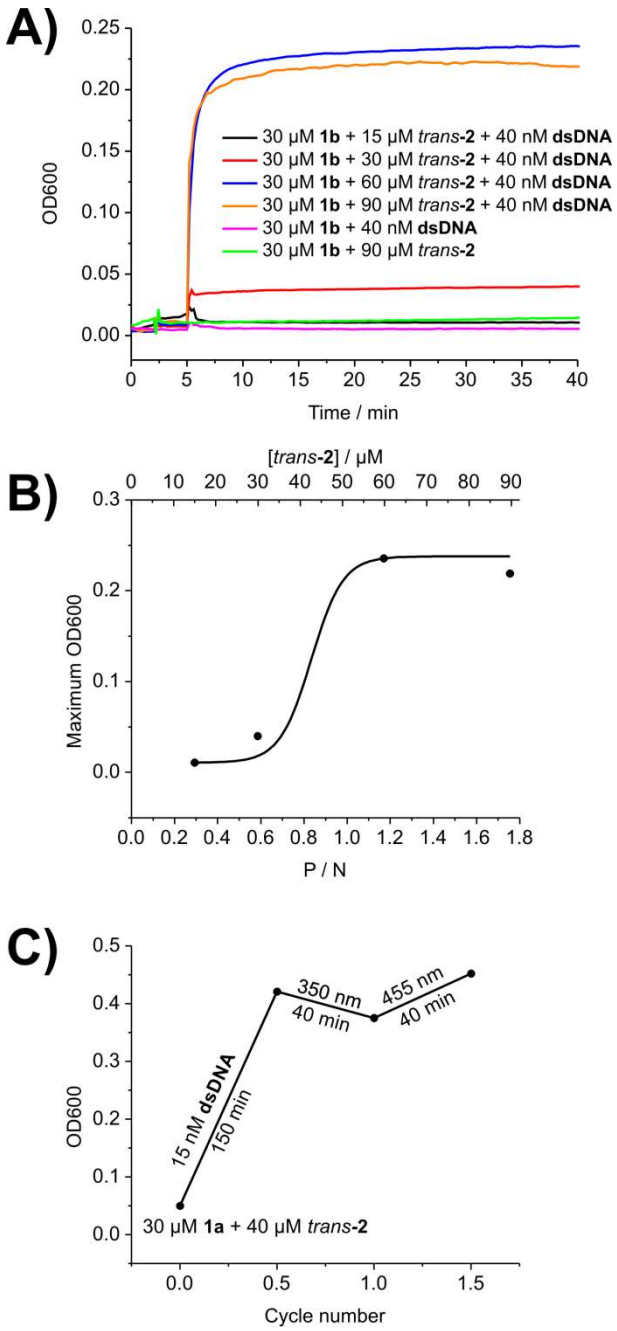


Figure S8: Ternary complex formation between vesicles of **1b,** conjugate *trans*-**2** and 2 kbp **dsDNA**. (A) Time-dependent measurement of OD600. (B) Maximum OD600 versus concentration of *trans*-**2** and positive (P) - negative (N) charge ratio. (C) Changes in the optical density (OD600) of the ternary complex of vesicles of **1a,** conjugate *trans*-**2** and **dsDNA** upon UV and subsequent visible light irradiations at 350 nm and 455 nm.

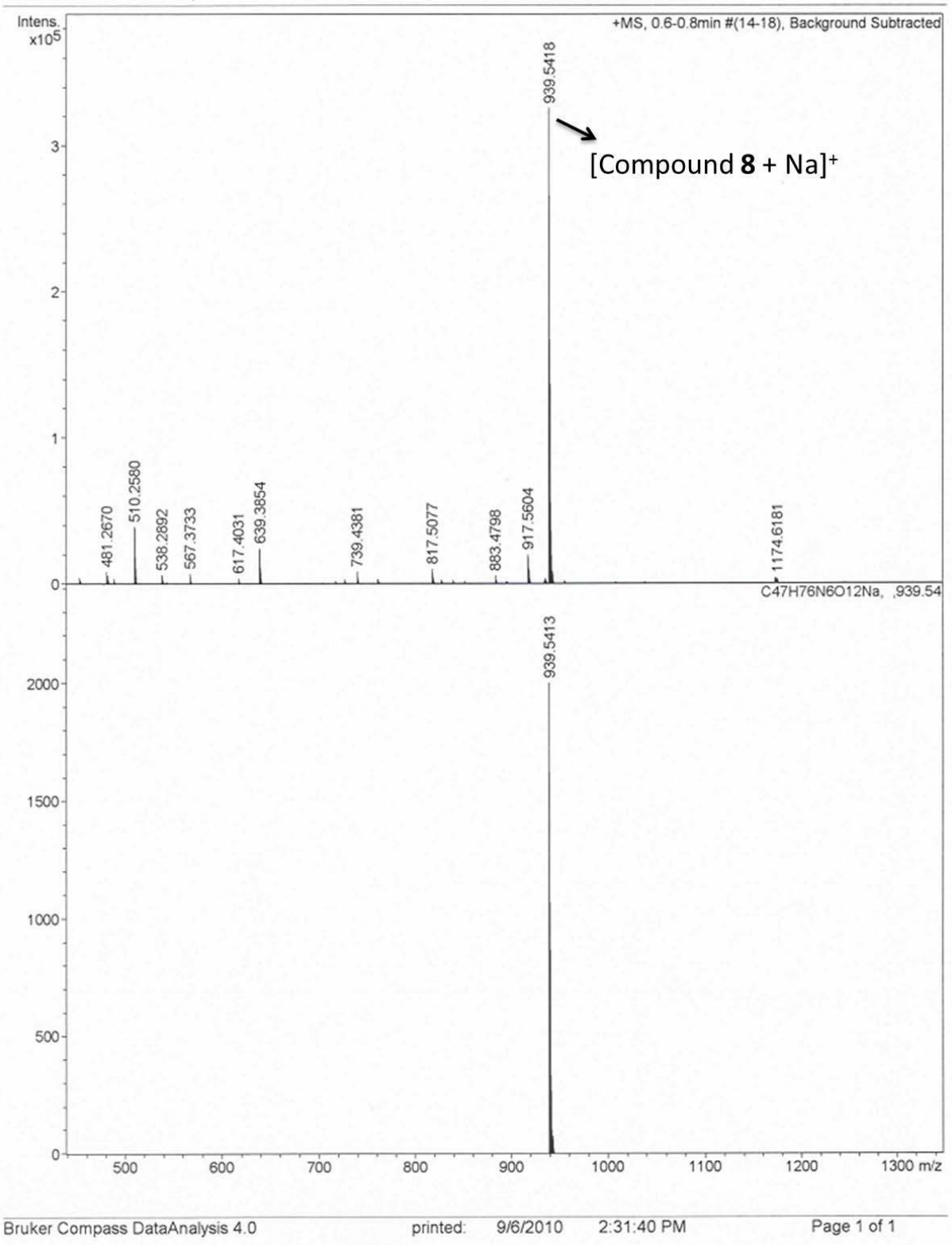


Figure S9: Mass spectrum of compound **8**.

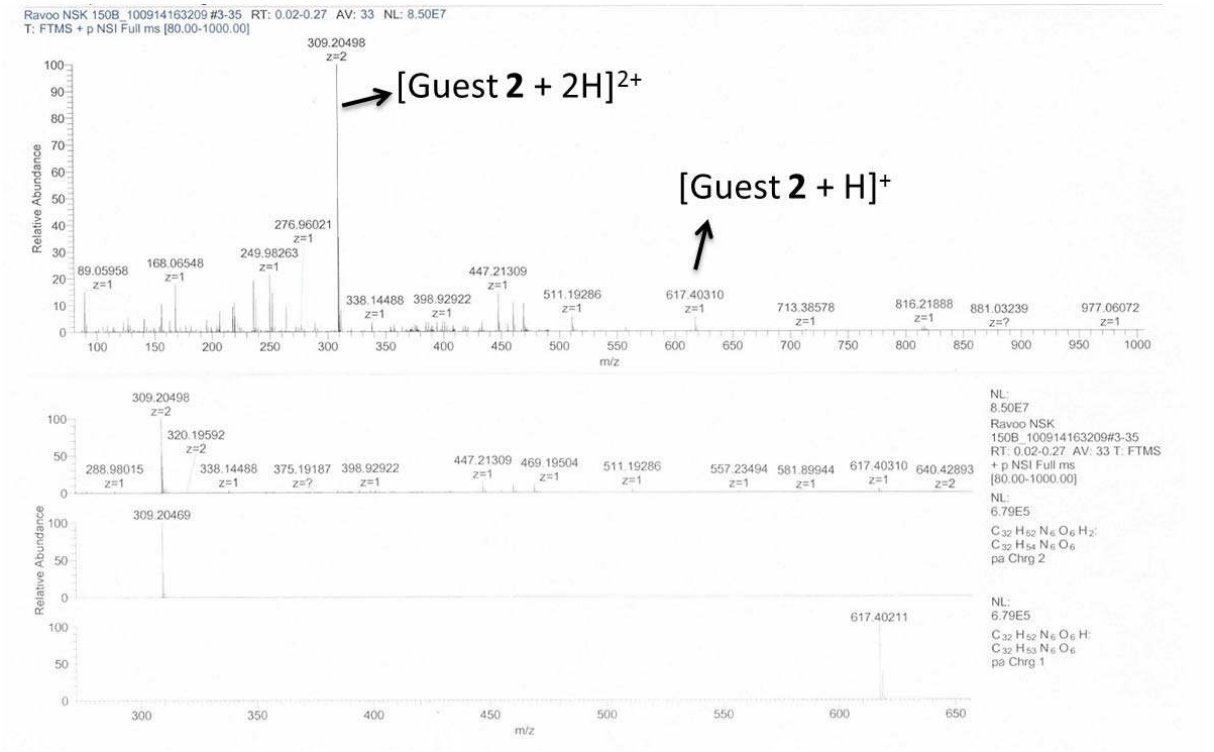


Figure S10: Mass spectrum of bifunctional conjugate **2**.

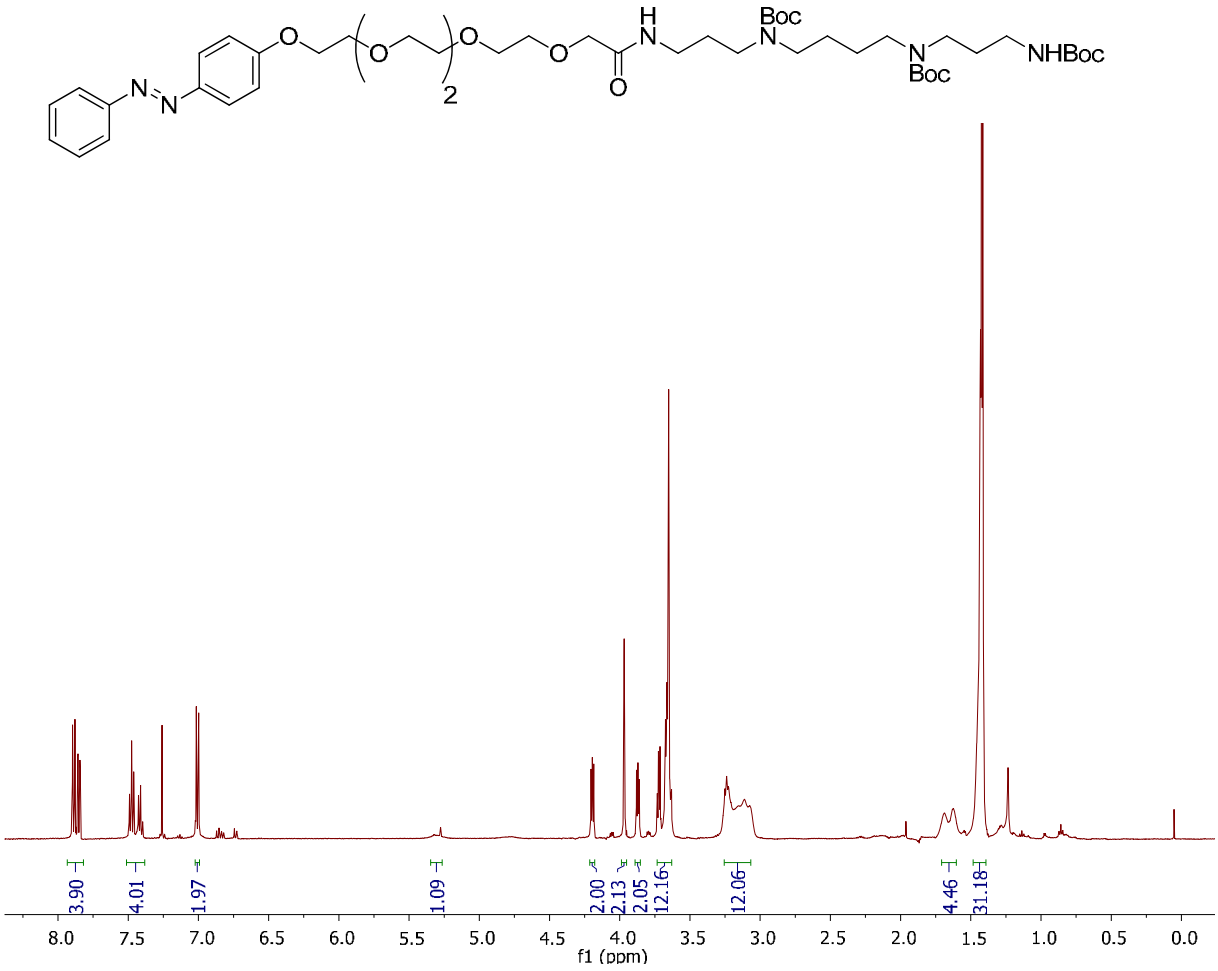


Figure S11: ^{1}H-NMR of compound **8** in $CDCl_3$ at 298 K.

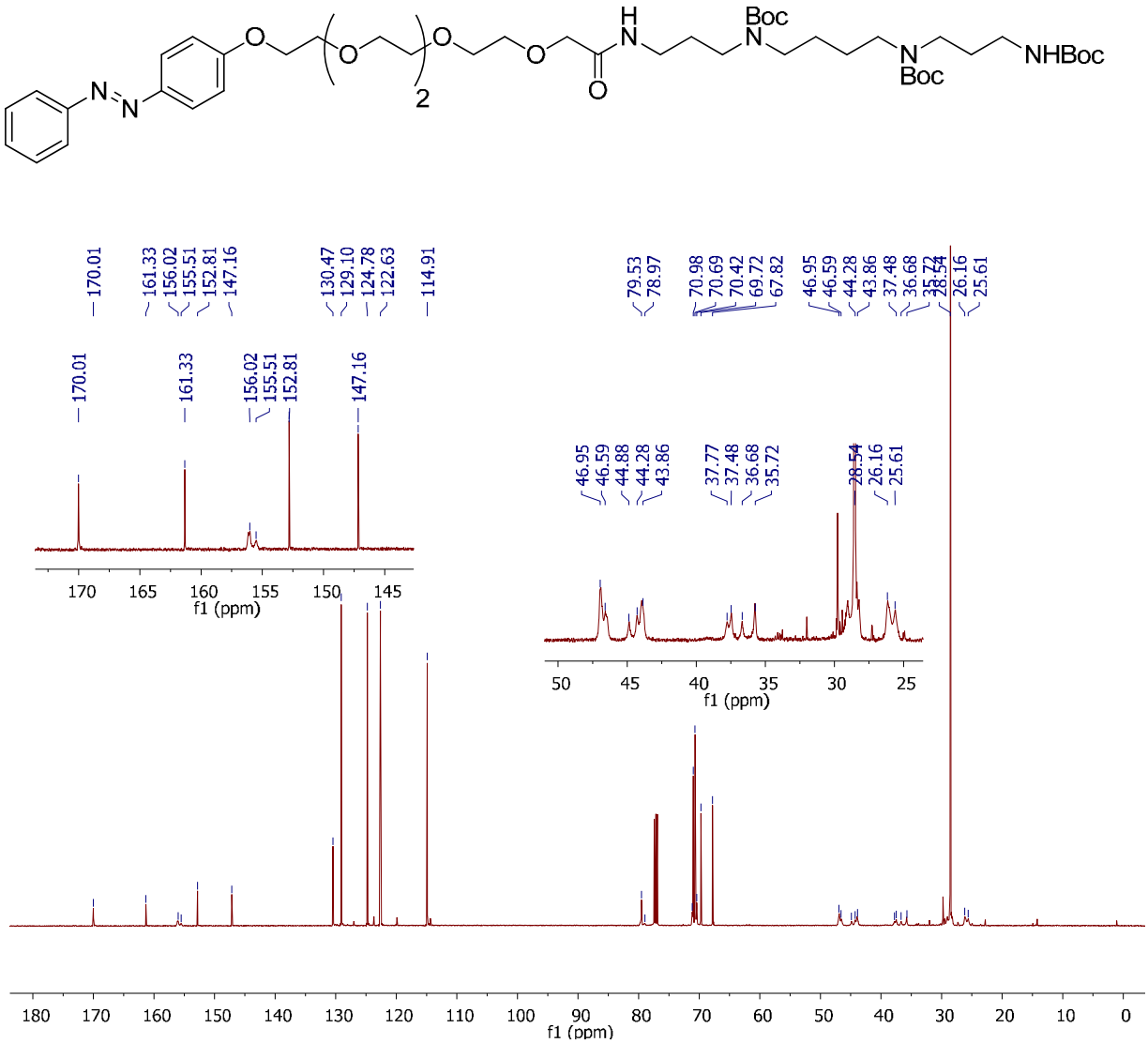


Figure S12: ^{13}C-NMR of compound **8** in $CDCl_3$ at 298 K.

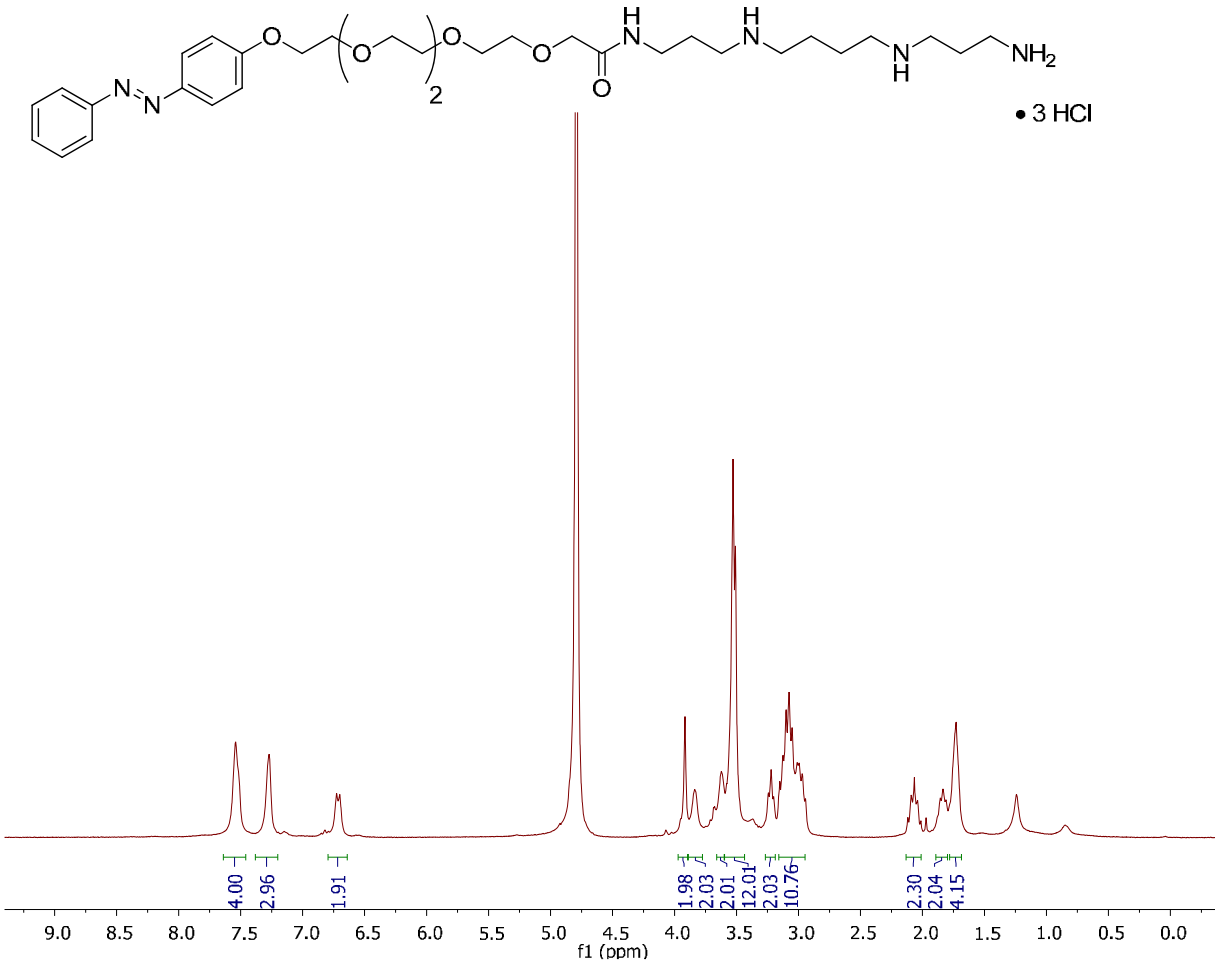


Figure S13: ^{1}H-NMR of compound **2** in D_2O at 298 K.

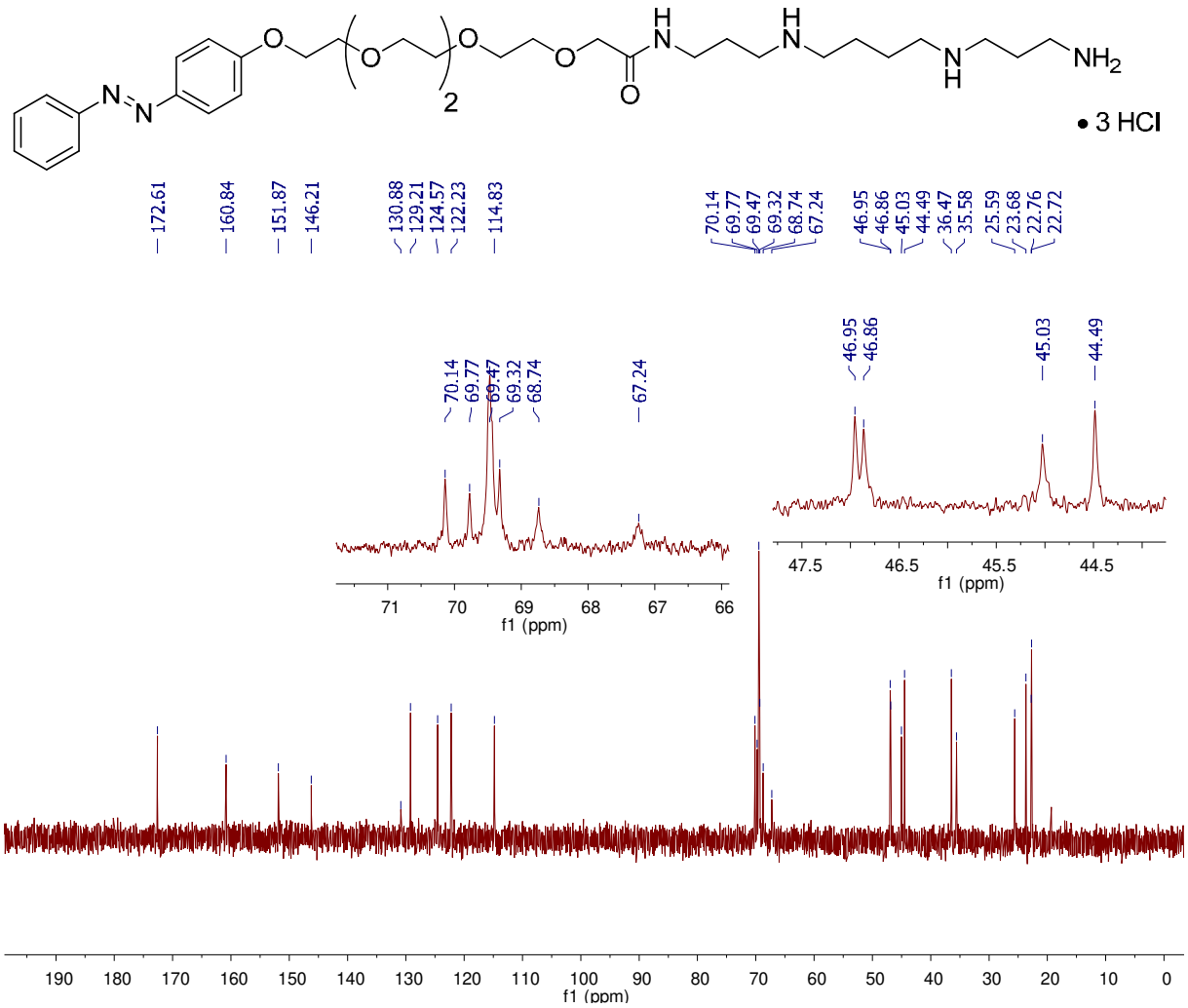


Figure S14: ^{13}C-NMR of compound **2** in D_2O at 298 K.