Photocleavable Ligands for Protein-Decoration of DNA Nanostructures

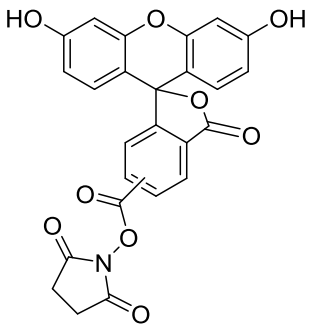
Josipa Brglez, Ishtiaq Ahmed, and Christof M. Niemeyer*

Supporting information

Synthetic methods

Synthesis of Fluorescein-Photocleavable linker (Fsc-PCL 1)

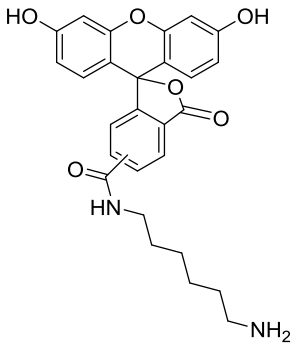
Preparation of N-hydroxysuccinimidyl active ester of 5,6-carboxyfluorescein



Preparation of 5,6-carboxyfluorescein-succinimidyl ester was adopted from the literature.[1] To a solution of 5(6)-carboxyfluorescein (1.0 g, 2.6 mmol) in dry DMF (15 mL), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCxHCl 609 mg, 3.18 mmol) was added, followed by N-hydroxysuccinimide (NHS, 366 mg, 3.18 mmol). The reaction mixture was covered with foil, stirred under Ar and monitored by TLC using a DCM:MeOH 9:1 solvent system. After 4-5 h, additional EDC (101 mg, 0.2 equiv) was added and the reaction mixture was stirred o.n at rt. The reaction mixture was poured in DMF (2 mL) in an extraction funnel and diluted with acetone (40 mL). Buffer (0.05 M, pH 6 phosphate buffer, 50 mL) was added, and the mixture was extracted with diethylether (Et_2O)/ethyl acetate (EtOAc), 2:1, 60 mL). The organic layer was separated, and the aqueous layer was extracted two times with 50 ml of Et_2O/EtOAc, 2:1. The combined organic extracts were washed with water (3 x 20 mL) and brine, dried over Na_2SO_4 (anhyd) and filtered. Solvents were removed *in vacuo*. The final product, 5(6)-carboxyfluorescein succinimidyl active ester, was obtained in 73 % yield and used in further steps without purification.

ESI-MS calcd for $C_{25}H_{15}NO_9$ $[M+H]^+$ 474.03, found 474.13,
^{1}H NMR (DMSO-*d6*): δ2.87-2.89 (m, 4H, 2xCH_2, NHS), 6.53-6.69 (m, 4H, Ar-H), 7.56 (d, *J* = 8.1 Hz 1H, Ar-H), 7.95 (d, *J* = 8.1 Hz 1H, Ar-H), 8.38-8.54 (m, 2H, Ar-H), 10.20 (s, 2H, Ph-OH)

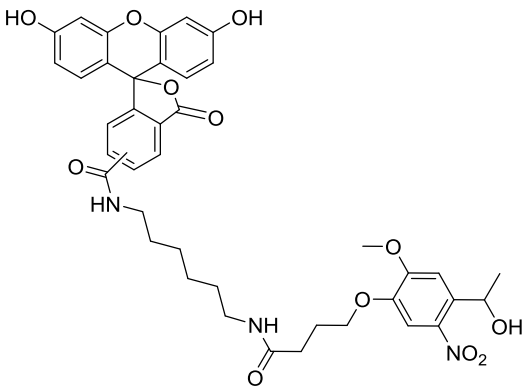
Preparation of amine 3



N-boc-1,6-hexanediamine (AlfaAesar, 301 mg, 1.2 mmol) was dissolved in DMF (7 mL) under Ar and DIPEA (522 µL, 3 eq) was added. The solution was stirred for 30 min and then 5(6)-carboxyfluorescein succinimidyl active ester (470 mg, 1 mmol) was slowly added under Ar. The reaction was monitored by TLC using DCM:MeOH (+0,05%HOAc) 9:1 solvent system. After overnight stirring, the formed precipitate was filtered and the solvent was removed *in vacuo*. The obtained crude product was dissolved in DCM (50 mL), extracted with 10% citric acid (2 x 10 mL) and washed with brine. The Boc protection group was then removed by dissolving the crude product in DCM (10 mL) and TFA (1 mL).[2] The reaction mixture was stirred until TLC showed completion of the reaction (1-2h). DCM and TFA were co- evaporated with toluene and the obtained product was used without purification for the next step. For spectra see Fig. S5.

HRMS: calcd for $C_{27}H_{26}N_2O_6$ $[M+H]^+$ 575.24, found 575.23,
^{1}H NMR: (500 MHz, CD_3OD, mixture of isomers) : δ 1.21-1.27 (m, 4H, 2xCH_2), 1.30-1.35 (m, 4H, 2xCH_2), 1.42-1.47 (m, 4H, 2xCH_2), 1.61-1.64 (m, 4H, 2xCH_2), 3.14 (t, *J* = 7.1 Hz, 2H, *CH_2*NH_2), 3.21 (t, *J* = 7.1 Hz, 2H, *CH_2*NHCO), 6.51-6.53 (m, 4H, Ar-H) 6.59-6.65 (m, 4H, Ar-H), 6.72-6.76 (m, 4H, Ar-H), 7.29 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.39-7.48 (m, 2H, Ar-H), 7.66 (s, 1H, Ar-H), 7.72 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.82 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.05 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.13 (dd, *J* = 1.69, 8.2 Hz, 1H, Ar-H), 8.22 (dd, *J* = 1.69, 8.2 Hz, 1H, Ar-H), 8.45 (br s, 1H, NH).
^{13}CNMR: (125 MHz, CD_3OD, mixture of isomers) : δ 29.9, 31.5, 32.7, 32.9, 33.4, 34.4, 39.7, 43.7, 43.8, 61.0, 82.4, 106.3, 114.3, 116.4, 120.9, 126.6, 127.5, 128.3, 128.8, 130.0, 130.7, 131.1, 132.1, 132.7, 132.8, 138.1, 140.4, 144.9, 156.6, 156.9, 161.0, 161.1, 163.9, 164.0, 167.4, 170.5, 170.8, 173.2.

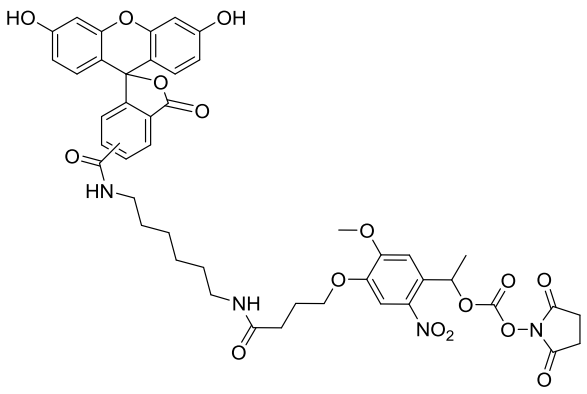
Preparation of N-[Fluorescein-5(6)-carboxamido]-N´-(α-methyl-nitroveratryl)-butyryl-1,6-diaminohexane 6



Synthesis of **6** was adopted from protocols given in the literature.[3,4] α-methyl-nitroveratryl-butyric acid **5** (Sigma,Aldrich, 80 mg, 0.27 mmol) was dissolved in dry DMF (5 mL). Subsequently, HOBt (39 mg, 0.29 mmol)and DIPEA (366 µL, 8eq) was added and the solution was cooled down to 0°C. Then EDC x HCl (69 mg, 0.36 mmol) was slowly added. The reaction mixture was stirred for 15-20 min at 0°C and then slowly warmed to rt and stirred for additional 30 min. Amine **3** (230 mg, 0.4 mmol, dissolved in DMF (1 mL) was then added slowly into the solution. After 4h, TLC showed consumption of the amine. The crude product was purified by silica gel flash chromatography using 2-20 % gradient of MeOH (+ 0.05%HOAc) in DCM, 30 column volumes (CV) to obtain the final product **6** in 15% yield. For spectra see Fig.S6.

HRMS: calcd for $C_{40}H_{41}N_3O_{12}$ $[M+H]^+$ 756.77, found $[M+H]^+$ 756.27
^{1}H NMR (500 MHz, CD_3OD, mixture of isomers): δ 1.27-1.35 (m, 14H, 7xCH_2), 1.46 (d, *J* = 6.4 Hz, 3H, CH_3), 1.52(t, *J* = 6.3 Hz, 2H, CH_2), 2.03-2.12 (m, 2H, CH_2), 2.36-2.42 (m, 2H, CH2CO), 3.17-3.21 (m, 2H, CH_2NHCO), 3.41 (t, *J* = 6.9 Hz, 2H, CH_2NHCO), 3.66-3.72 (m, 1H, CH_2), 3.93 (s, 3H, OCH_3), 4.01-4.06 (m, 2H, CH_2O), 5.45 (q, *J* = 6.4 Hz, 1H, *CH*-OH), 6.51-6.56 (m, 2H, Ar-H), 6.65-6.69 (m, 4H, Ar-H), 7.11-7.21 (m, 4H, Ar-H), 7.31-7.37 (m, 4H, Ar-H), 7.54 (s, 1H, Ar-H), 7.70 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.76 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.45 (s, 1H, NH).
^{13}CNMR (125 MHz, CD_3OD, mixture of isomers): δ 17.9, 20.1, 20.4, 23.8, 25.1, 26.1, 26.2, 28.9, 32.1, 38.9, 39.7, 42.3, 54.3, 55.3, 64.8, 68.2, 102.3, 108.5, 108.7, 110.7, 117.2, 124.4, 124.7, 124.9, 127.8, 128.5, 129.1, 133.2, 136.4,137.5, 137.6, 139.3, 142.5, 146.8, 153.3, 154.0, 166.7, 167.0, 168.7, 173.8, 176.5.

Preparation of Fsc-PCL 1

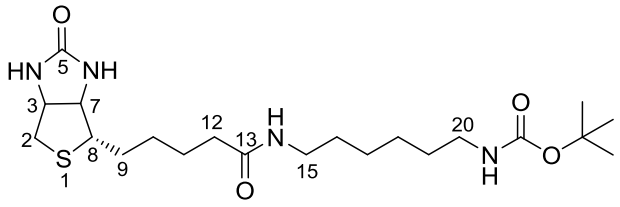


Compound **6** (20 mg, 0.026mmol) was dissolved in dry DMF (250 µL) and DIPEA (18 µL, 4eq) was added followed by N,N´-disuccinimidyl carbonate (DSC, 34mg, 5eq) under Ar. The reaction mixture was stirred overnight at rt. The solvent was removed *in vacuo* and the crude product was purified by silica gel flash chromatography using 2-20 % gradient of MeOH in DCM. The final product **1** was obtained in 31 % yield. For spectra see Fig. S7.

ESI-MS: calcd for $C_{45}H_{44}N_4O_{16}$ $[M+H]^+$ 896.28, found $[M+H]^+$ 896.93, $[M-H]^-$ 895
^{1}H NMR (500 MHz, CD_3OD: $CDCl_3$, mixture of isomers): δ 1.35-1.30 (m, 12H, 6xCH_2), 1.32-1.38 (m, 4H, 2xCH_2),1.50-1.54 (m, 2H, CH_2), 1.62-1.66 (m, 2H, CH_2), 1.74 (d, *J* = 6.4 Hz, 3H, CH_3), 1.78 (t, *J* = 6.3 Hz, 2H, CH_2), 2.08-2.15 (m, 2H, CH_2CO), 2.33 (br s, 2H, CH_2), 2.36-2.43 (m, 2H, CH_2), 2.70 (br s, 2H, CH_2), 2.80-2.84 (m, 8H, CH_2-NHS), 3.42 (t, *J* = 7.0 Hz, 2H, CH_2NHCO), 3.90-3.96 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 4.06-4.10 (m, 4H, OCH_2), 6.43 (q, *J* = 6.3 Hz, 1H, C*H*OH), 6.54-6.66 (m, 2H, Ar-H), 6.68-6.75 (m, 2H, Ar-H), 6.80-6.90 (m, 3H, Ar-H), 7.06-7.15 (m, 3H, Ar-H), 7.51-7.56 (m, 1H, Ar-H), 7.61-7.67 (m, 1H, Ar-H), 7.97 (br s, 2H, Ar-H), 8.04-8.22 (m, 3H, Ar-H), 8.45 (s, 1H, NH).
^{13}CNMR (125 MHz, CD_3OD, $CDCl_3$, mixture of isomers): δ 16.5, 21.0, 25.0, 25.1, 25.2, 26.2, 28.9, 29.3, 29.4, 30.8, 32.2, 36.1, 39.0, 39.8, 55.9, 68.3, 76.2, 102.5, 107.4, 108.8, 112.7, 112.8, 122.7, 124.2, 124.9, 126.7, 128.3, 128.6, 128.8, 128.9, 130.8, 139.4, 147.8, 150.7, 151.9, 152.0, 152.2, 154.1, 154.5, 159.8, 163.3, 165.9, 166.6, 167.3, 169.2, 169.6, 169.8, 170.0, 170.1, 170.3, 173.3, 173.6.

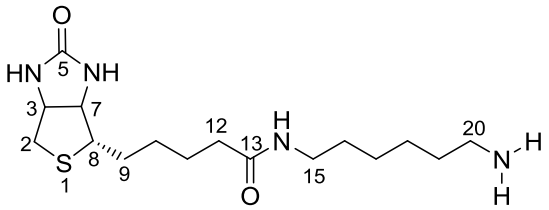
Synthesis of Biotin - photocleavable linker (Biotin-PCL 2)

Preparation of amine 4



To a solution of biotin-NHS (500 mg, 1.46 mmol, in 15 mL dry DMF), prepared as described in the literature,[5] N-boc-1,5-pentanediamine (384 mg, 1.61 mmol) was added followed by TEA (407 µL, 2eq). The reaction mixture was stirred under Ar at 50°C for 22h. The solvent was removed in vacuo and the residue was diluted in 40 ml of DCM, then extracted with 10% citric acid (2 x 15 mL), washed with brine and evaporated to give the boc-protected product in 40 % yield.

ESI-MS: calcd for $C_{21}H_{38}N_4O_4S$ [M+H]+ 443.26, found [M+H]+ 443.07, [M+Na]+ 465.20
^{1}H NMR (500 MHz, DMSO-d_6): δ 7.74 (t, *J* = 5.2 Hz, 1H, $N_{14}H$), 6.76 (t, *J* = 5.2 Hz, 1H, $N_{21}H$), 6.43 (br s, 1H, N_6H), 6.37 (br s, 1H, N_4H), 4.32-4.27 (m, 1H, C_3H), 4.13-4.09 (m, 1H, C_7H), 3.11-3.07 (m, 1H, C_8H), 2.99 (d, *J* = 6.0 Hz, 2H, $C_{15}H_2$), 2.87 (br q, *J* = 6.0 Hz, 2H, $C_{20}H_2$), 2.81 (dd, *J* = 5.0, 12.3 Hz, 1H, $C_2H_AH_B$), 2.55 (d, *J* = 12.3 Hz, 1H, $C_2H_AH_B$), 2.01 (t, *J* = 7.3 Hz, 2H, $C_{12}H_2$), 1.64-1.20 (m, 14H, C_9H_2, $C_{10}H_2$, $C_{11}H_2$, $C_{16}H_2$, $C_{17}H_2$, $C_{18}H_2$, $C_{19}H_2$), 1.35 (s, 9H, ^{t}BuO).
^{13}CNMR (125 MHz, DMSO-d_6): δ 175.3, 173.2, 164.5, 78.8, 62.3, 60.9, 56.7, 41.5, 41.3, 39.7, 36.7, 29.8, 30.8, 30.6, 29.6, 27.5, 27.3, 26.8, 26.6.

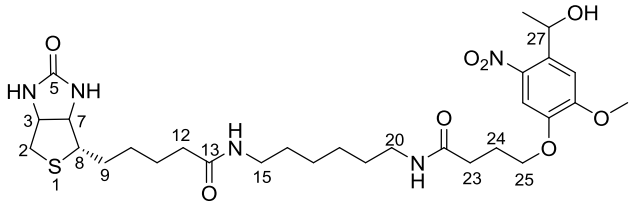


To remove the Boc protection group,[6] N-Boc-Biotinylhexanediamine (260 mg, 0.59 mmol) was dissolved in DCM (12 mL) with addition of TFA (3 mL) and stirred until TLC (DCM:MeOH 9:1) showed no starting material. The solvent was evaporated in vacuo, washed with DCM and evaporated to obtain the oil-like crude product which was redissolved in 4mL DCM:MeOH 1:1 and mixed with ~10 eq of Amberlyst A-21 (Sigma) for 30 min. After filteration, the volatiles were removed in vacuo to obtain final product in 82 % yield. For spectra see Fig.S8.

ESI-MS: calcd for $C_{16}H_{30}N_4O_2S$ [M+H]+ 343.21, found [M+H]+ 343.20,

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.74 (t, *J* = 5.2 Hz, 1H, $N_{14}H$), 6.76 (t, *J* = 5.2 Hz, 1H, N_6H), 6.43 (br s, 1H), 6.36 (br s, 1H, N_4H), 4.30-4.25 (m, 1H, C_3H), 4.12-4.07 (m, 1H, C_7H), 3.10 (br t, *J* = 7.3 Hz, 2H, $C_{20}H_2$), 3.08-3.05 (m, 1H, C_8H), 3.01 (d, *J* = 6.0 Hz, 2H, $C_{15}H_2$), 2.79 (dd, *J* = 5.0, 12.5 Hz, 1H, $C_2H_AH_B$), 2.54 (d, *J* = 12.5 Hz, 1H, $C_2H_AH_B$), 2.01 (t, *J* = 7.3 Hz, 2H, $C_{12}H_2$), 1.57-1.22 (m, 14H, C_9H_2, $C_{10}H_2$, $C_{11}H_2$, $C_{16}H_2$, $C_{17}H_2$, $C_{18}H_2$, $C_{19}H_2$).
^{13}CNMR (125 MHz, DMSO-d_6): δ 173.5, 164.4, 62.3, 60.9, 56.8, 41.3, 41.1, 39.7, 36.7, 30.8, 30.7, 29.6, 27.5, 27.4, 26.8, 26.6.

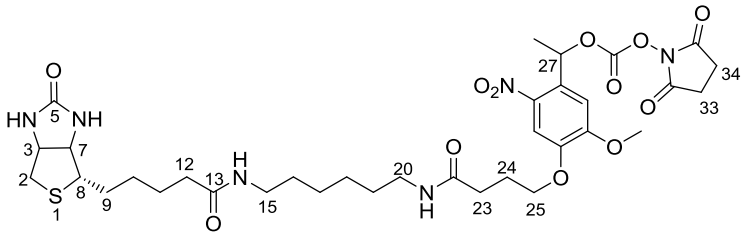
Preparation N-biotinamido-N´-(α-methyl-nitroveratryl)-butyryl-1,6-diaminohexane 7



α-Methyl-nitroveratryl-butyric acid (Sigma Aldrich,192 mg, 0.64 mmol) was dissolved in DMF/DCM (1:1, 6 mL) under Ar. HOBt (95 mg, 0.7 mmol) and TEA (357 µL, 4eq) were added subsequently. This solution was cooled down to 0°C and and EDC x HCl (135 mg, 0.7 mmol) was slowly added. The reaction mixture was initially stirred for 15-20 min at 0°C and then slowly warmed to r.t. and stirred for another 30 min. Amine **4** (219 mg, 0.64 mmol) was then slowly added into the reaction mixture, which was then stirred for 6h. Reaction was monitored by TLC (DCM/MeoH 9:1). The solvent was removed *in vacuo* and the crude product was diluted with DCM (40 mL), extracted with 2 x 10 mL 10 % citric acid and washed with brine. The solution was dried over anhyd. Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by silica gel flash chromatography using 2-20 % gradient of MeOH in DCM. The final product was obtained in 21 % yield. For spectra see Fig.S9.

HRMS: calcd for $C_{29}H_{45}N_5O_8S$ $[M+H]^+$ 624.76; found $[M+H]^+$ 624.30
^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD): δ 7.47 (s, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.23-7.20 (br s, 2H, NH), 5.44 (br q, *J* = 5.9 Hz, 1H, $C_{27}H$), 4.42 (t, *J* = 5.3 Hz, 1H, C_3H), 4.22 (m, 1H, C_7H), 4.01 (t, *J* = 5.3 Hz, 2H, $C_{25}H_2$), 3.90 (s, 3H, OCH_3), 3.65-3.54 (m, 1H, C_8H), 3.10-3.02 (m, 6H, $C_{12}H_2$, $C_{11}H_2$, $C_{18}H_2$), 2.84-2.79 (m, 1H, $C_2H_AH_B$), 2.63 (d, *J* = 12.0, 1H, $C_2H_AH_B$), 2.35-2.29 (m, 2H, $C_{15}H_2$), 2.12-2.06 (m, 4H, C_9H_2, $C_{23}H_2$), 1.56-1.52 (m, 4H, $C_{10}H_2$, $C_{24}H_2$), 1.41 (d, *J* = 5.9 Hz, 3H, CH_3), 1.35-1.31 (m, 6H, $C_{16}H_2$, $C_{17}H_2$, $C1_9H_2$)
^{13}CNMR (125 MHz, $CDCl_3$:CD_3OD): δ 178.1, 177.2, 168.1, 157.9, 150.5, 143.0, 142.2, 112, 72.4, 69.03, 65.8, 64.1, 61.4, 60.1, 59.5, 58.2, 46.4, 44.2, 43.1, 43.0, 39.6, 36.4, 32.9, 32.2, 32.0, 29.5, 29.0, 28.5, 21.7

Preparation of Biotin-PCL 2



Modifier **2** was prepared with a protocol adopted from literature.[7] Compound **7** was dissolved in DMF (1.5 mL) and TEA (35 µL, 3 eq) was added followed by DSC (28.3 mg, 0.11 mmol). The reaction mixture was stirred o.n. at rt. under Ar. The solvent was evaporated *in vacuo*, and the residue was dissolved in 10 mL 1M $NaHCO_3$ and extracted with EtOAc (3 x 4 mL), washed with brine, dried over anhyd. Na_2SO_4, filtered and dried *in vacuo*. The final t Biotin-PCL **2** was obtained in 62 % yield. For spectra see Fig.S10.

HRMS: calcd for $C_{34}H_{48}N_6O_{12}S$ $[M+H]^+$ 765.31 found $[M+H]^+$ 765.31
^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD): δ 7.68 (t, *J* = 5.4 Hz, 1H, $N_{14}H$), 7.63 (s, 1H, Ar-H), 7.61-7.58 (m, 1H, NH), 7.11 (s, 1H, Ar-H), 6.42 (q, *J* = 6.3, 12.7 Hz, 1H, $C_{27}H$), 4.48-4.41 (m, 1H, C_3H), 4.31-4.28 (m, 1H, C_7H), 4.11 (t, *J* = 6.3 Hz, 2H, $C_{25}H$), 4.04 (s, 3H, OCH_3), 3.65-3.60 (m, 1H, C_8H), 3.19-3.13 (m, 4H, $C_{12}H_2$, $C_{15}H_2$), 2.90 (dd, *J* = 4.9, 12.7 Hz 1H, $C_2H_AH_B$), 2.82 (br s, 4H, $C_{33}H_2$, $C_{34}H_2$), 2.41 (t, *J* = 6.3 Hz, 2H, $C_{23}H_2$), 2.20-2.14 (m, 4H, $C_{20}H_2$, $C_{24}H_2$), 1.76 (d, *J* = 6.4, 3H, CH_3), 1.68-1.57 (m, 4H, $C_{10}H_2$, $C_{11}H_2$), 1.49-1.46 (m, 4H, C_9H_2, $C_{16}H_2$), 1.36-1.32 (m, 6H, $C_{17}H_2$, $C_{18}H_2$, $C_{19}H_2$).
^{13}CNMR (125 MHz, $CDCl_3$:CD_3OD): δ 174.4, 173.5, 173.3, 169.5, 164.4, 154.5, 150.7, 147.7, 139.3, 130.9, 108.9, 107.4, 76.3, 68.5, 61.9, 60.1, 57.3, 56.1, 55.6, 40.1, 39.1, 39.0, 35.6, 32.3, 29.0, 28.4, 28.1, 26.3, 25.6, 25.2, 25.1, 25.0, 21.3, 17.5

General Procedures

Assembly and characterization of DNA origami

Assembly of DNA Origami

DNA origami was assembled from solutions containing the 109Z5 ssDNA scaffold strand [700-1200 nM, in TE 1x, pH 8,2] and each of the staple strands [100 µM, in water] in 1X TEMg in a total volume of 0.5-1 ml, as priviously described.[8] The sequence of the 109Z5 ssDNA scaffold strand and the full list of staple strands are given in Tables S1 and S2, respectively. The assembly of origami structures bearing linker **1** was achieved by modifying 6 amino-modified staples(Fig. 2A and for the sequences see Table S3) with respective linker. The annealing was performed by decreasing the temperature from 75° C to 25° at -6°C/min on a PCR cycler (Mastercycler Pro, Eppendorf). Quality control was performed with AFM and agarose gel analysis using a 1.5% agarose gel in 1X TBEMg buffer (40 mM Tris, 20 mM boric acid, 2 mM EDTA, 12.5 mM Mg acetate, pH 8.00) and running the gel at 80 V for 2 hrs at 4°C.

AFM characterisation of DNA origami

For AFM analysis the DNA samples were deposited on freshly cleaved MICA surface (Plano GmbH) and adsorbed for 3 min at room temperature. After addition of 15 µL 1X TAEMg the sample was scanned in ScanAsyst mode with use of scan-Asyst-Fluid+ tips (Bruker).During scanning, peak force set point was set between 0.15-0.4 V and speed between 1-2 Hz. Image analysis was performed using Nanoscope 1.14 Software.

Modification of oligonucleotides

Prior to use, Am-DNA (SigmaAldrich) was desalted and purified by gel filtration, using NAP5 and NAP10 columns (GE Healthcare, Sephadex G-25 DNA grade). NAP5/NAP10 columns were used according to manufacturer's instructions. PBS 1x, pH 7.3 buffer was used as an eluent. Subsequently, eluate was concentrated using Vivaspin 500, MWCO 3000 (Sartorius) centrifugal concentrators. Concentration of final solution was determined by UV/VIS spectroscopy.

5µL [280 µM] of Am-DNA in PBS 1x was mixed with Fsc-PC **1** or Bt-PC **2** active ester [10 mM in DMSO]. Different molar eq (15 and 30 eq) of **1** or **2** were used to optimize the coupling reaction. The reaction was performed o.n. at rt. Analysis of coupling products was performed by gel mobility shift assays on 20% urea gel, 1x TBE, 220V, 50 min, rt. The same reaction conditions were used for modification of selected staple stands for DNA nanostructure assembly. The staple strands were purified using NAP5 / NAP10 columns and TE 1x buffer as eluent to remove DMSO and excess of **1**. Staples were then concentrated using Vivaspin 500, MWCO 3 kDa centrifugal concentrators.

Photolytic cleavage of 2-nitrobenzyl linker

Photolysis of the 2-nitrobenzyl linker moiety was performed in thin-walled, polypropylene tubes in TE 1x buffer, pH 7,6 using near-UV light of λ=366 nm (Bio-Link 254 microprocessor controlled UV irradioation system). Samples were irradiated for 5, 10 and 15 min. Aliquots were taken after different irradiation times and analyzed by Urea-PAGE.

Coupling of proteins to PC-DNA oligomers

To optimize coupling between **1**- and **2**-DNA and their respective cognate proteins, anti-fluorescein single-chain Fv fragment FITC-E2 or STV were used. To this end, reactions were performed by mixing 2, 5, 10 and 30 eq of FITC-E2 and **1**-DNA in PBS pH 7.3 Samples were incubated for 1h or o.n at 4°C. To obtain STV-**2**-DNA conjugate, 30ul [4uM] of **2**-DNA were incubated with a 5, 10, 15, or 100 molar excess of STV [100uM]. Incubation was performed for 30 min at rt. After completion of the reactions, samples were analysed by native-PAGE.

For binding of anti-fluorescein IgG to 1-origami and Fsc-origami, an assembled and purified origami solution (20 µL, ~10 nM) was incubated with 5µl of anti-Fsc-IgG (0.1 µg/µl, Thermo Scientific). The mixture was incubated at r.t. for 4h and then analysed by AFM.

Supplementary Figures

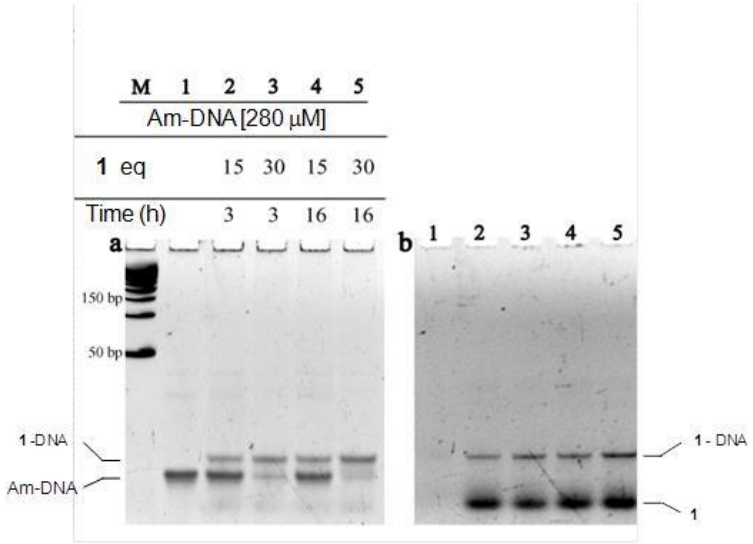


Figure S1: Optimization of coupling reaction between linker **1** and amino-modified DNA oligomers (Am-DNA). Reaction was performed for 3h or overnight using different ratios of **1**/Am-DNA. Bands were visualised with Sybr Gold staining for DNA (a) or fluorescein detection (Ex./Em. 475/542) (b). Running conditions: 20% urea gel, 1x TBE, 220V, 50 min, rt.

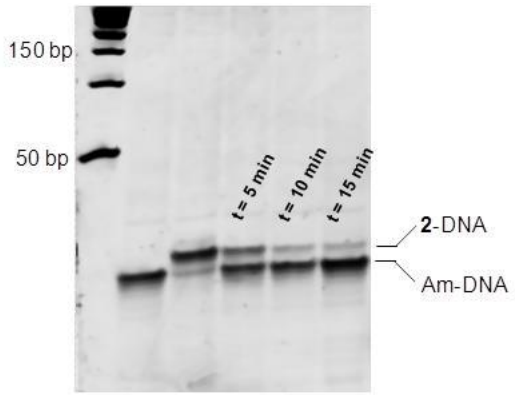


Figure S2: Time course of the photolytic cleavage reaction of **1**-DNA. Note that 15 min irradiation with near UV light (350-370 nm) led to efficient cleavage of the ligand from the oligonucleotide. Running conditions: 20% urea gel, 1x TBE, 220V, 50 min, rt.

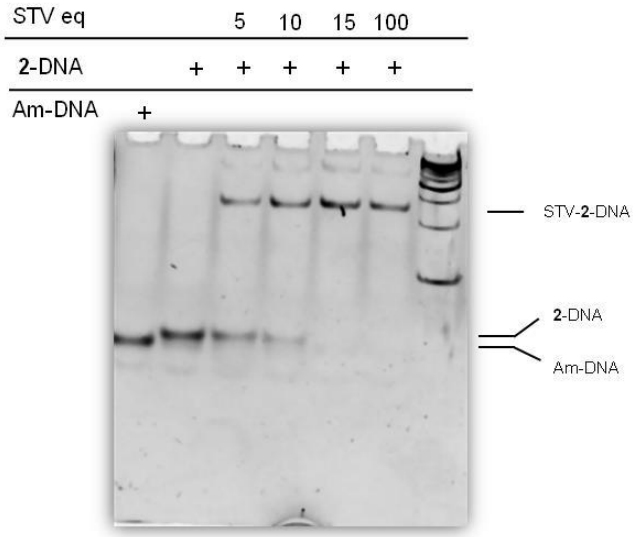


Figure S3: Binding of **2**-DNA by STV. Running conditions: 10% native gel, TBE 1x, 220V, 20 min, rt.

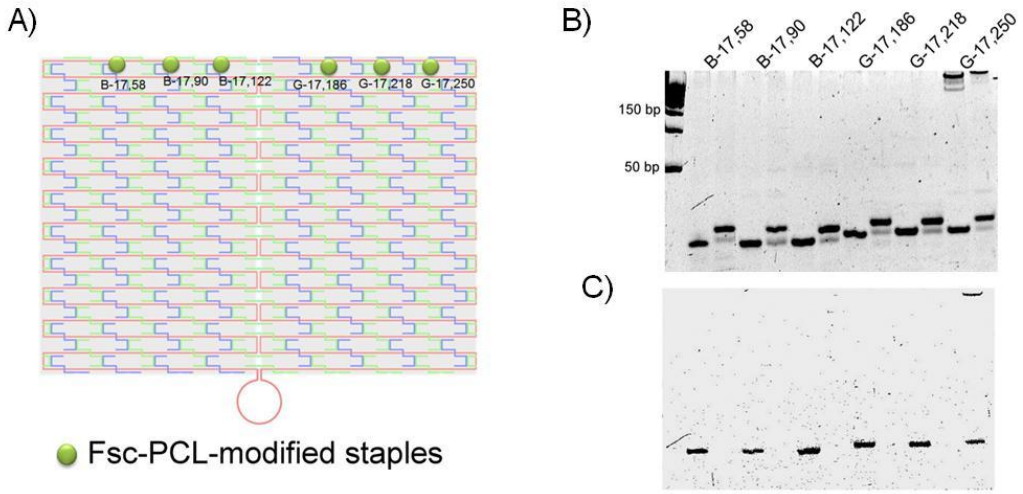
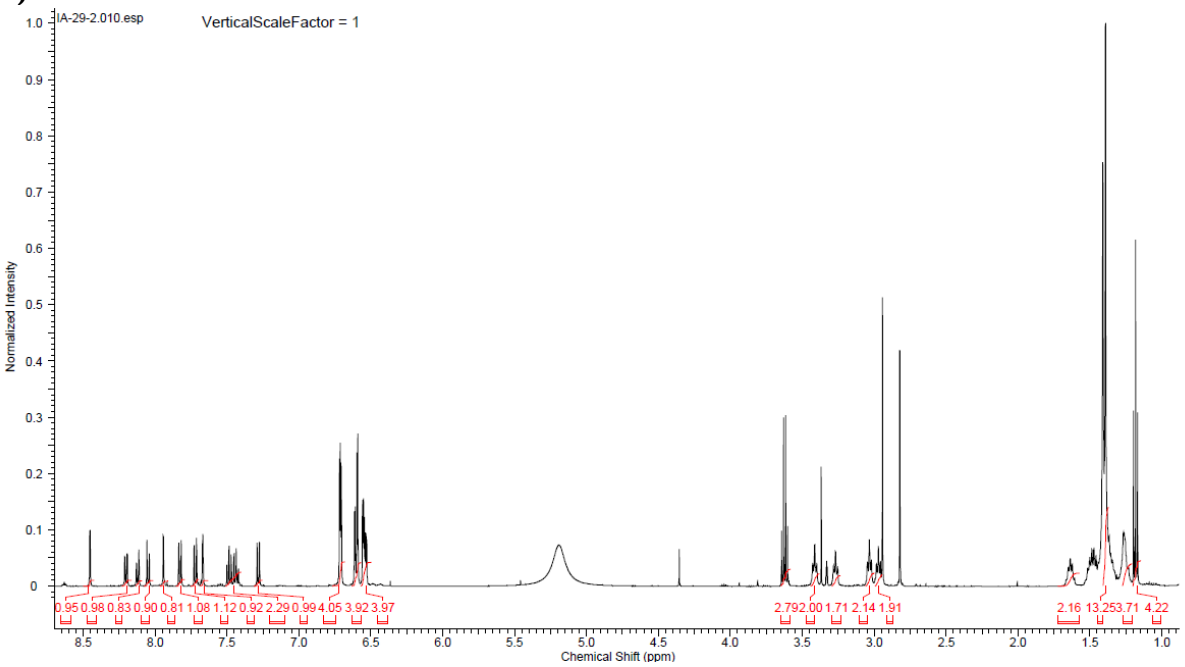


Figure S4. Labelling of DNA origami and staple strands with linker **1**. A: Schematic representation of DNA origami nanostructure depcting the position of **1**-modified staple strands as green dots. B, C: Electrophoretic analysis of labeling reactions. Staple strands were purified and analysed by urea PAGE. Bands were visualised with Sybr Gold staining for DNA (B) and fluorescein detection with specific filters (Ex./Em. 475/542). Running conditions: 20% urea gel, 1x TBE, 220V, 45 min, rt.

A)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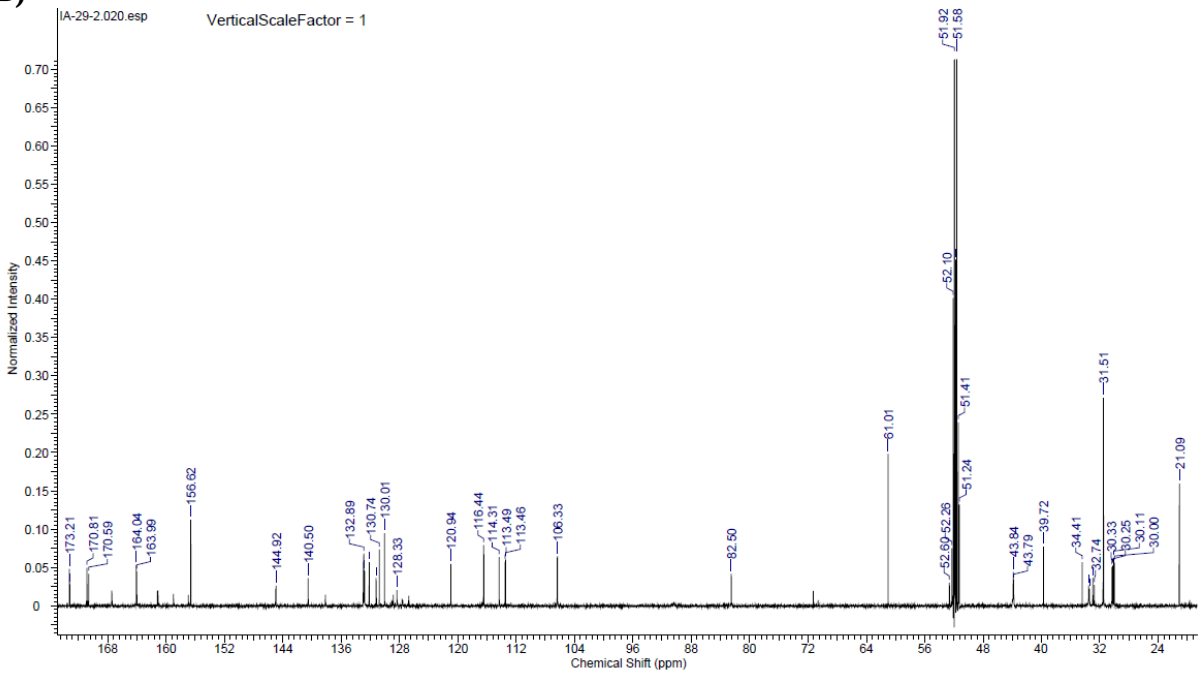
2.16 13.25 3.71 4.22

B)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116.44
114.31
113.49
113.46
106.33
82.50
61.01
51.92
51.58
52.10
51.41
51.24
52.60
52.26
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30.33
30.25
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30.00
21.09

C)

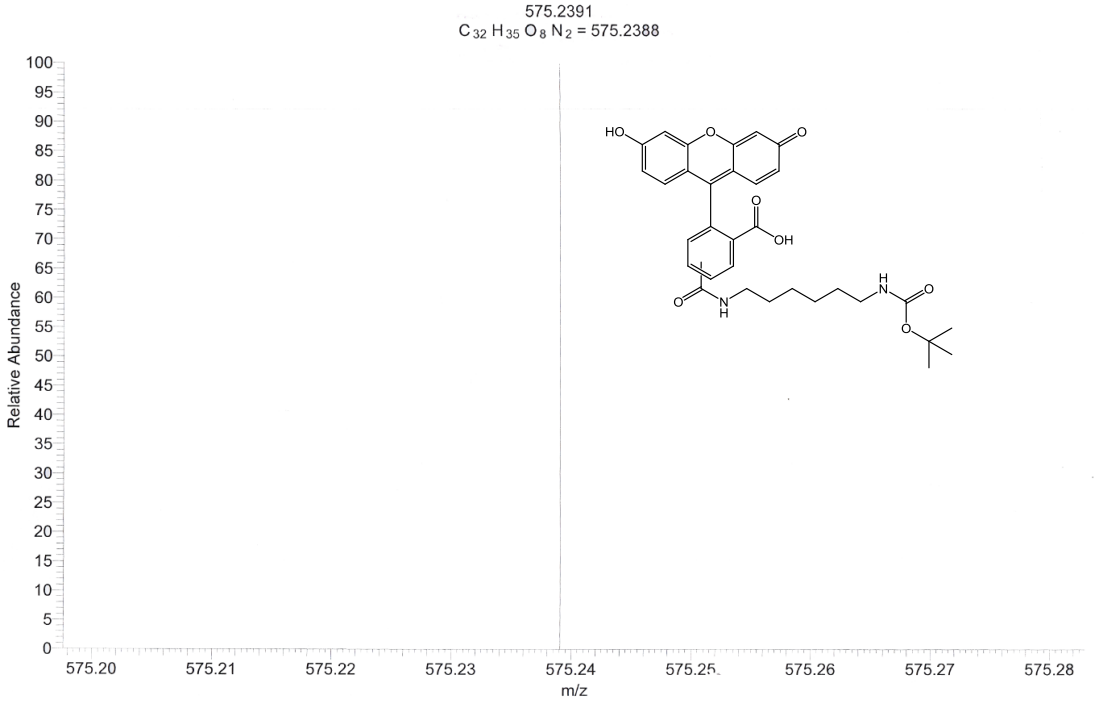
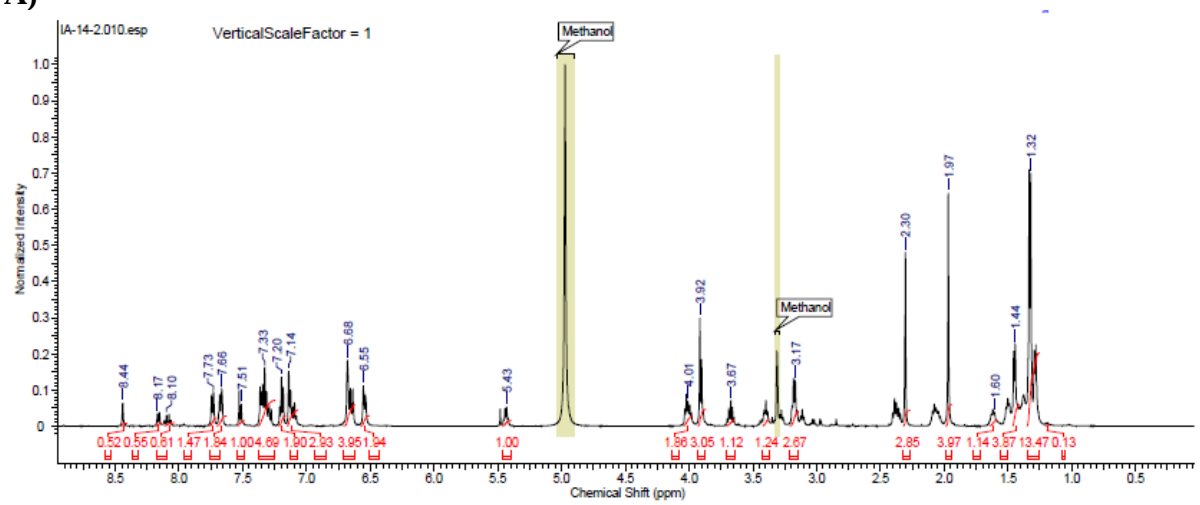


Figure S5. ^{1}H (A), ^{13}C NMR (B) and HRMS (C) analysis of the Boc-protected amine **3.**

A)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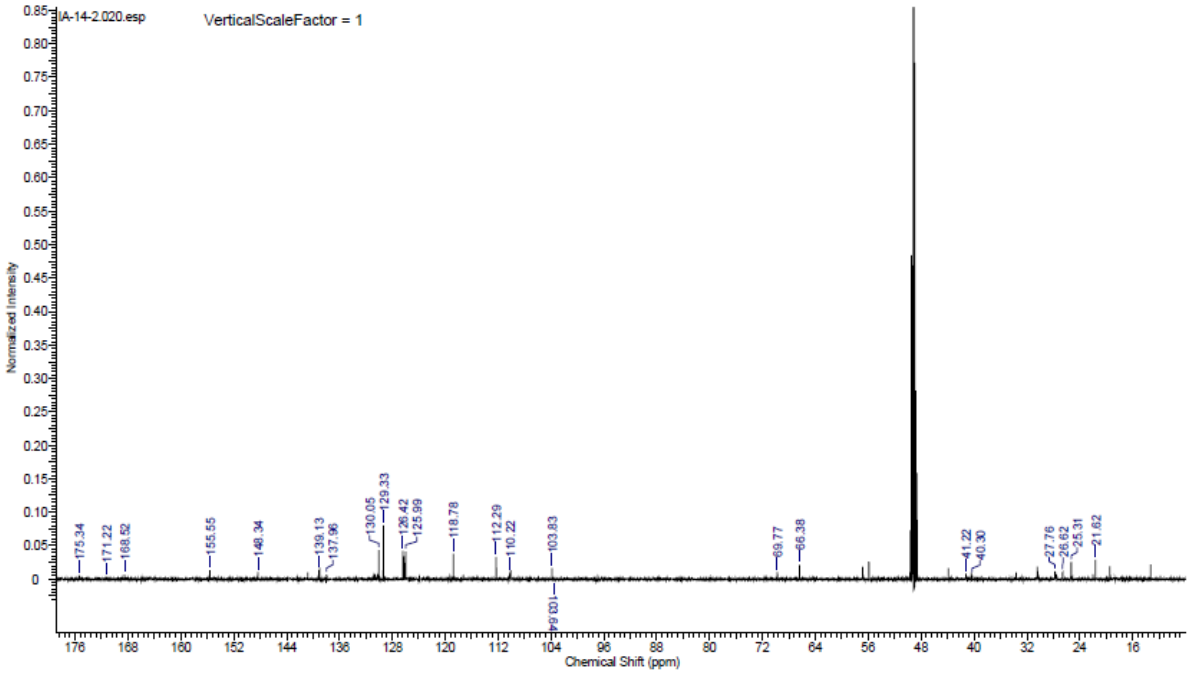
Chemical Shift (ppm)

B)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Chemical Shift (ppm)

C)

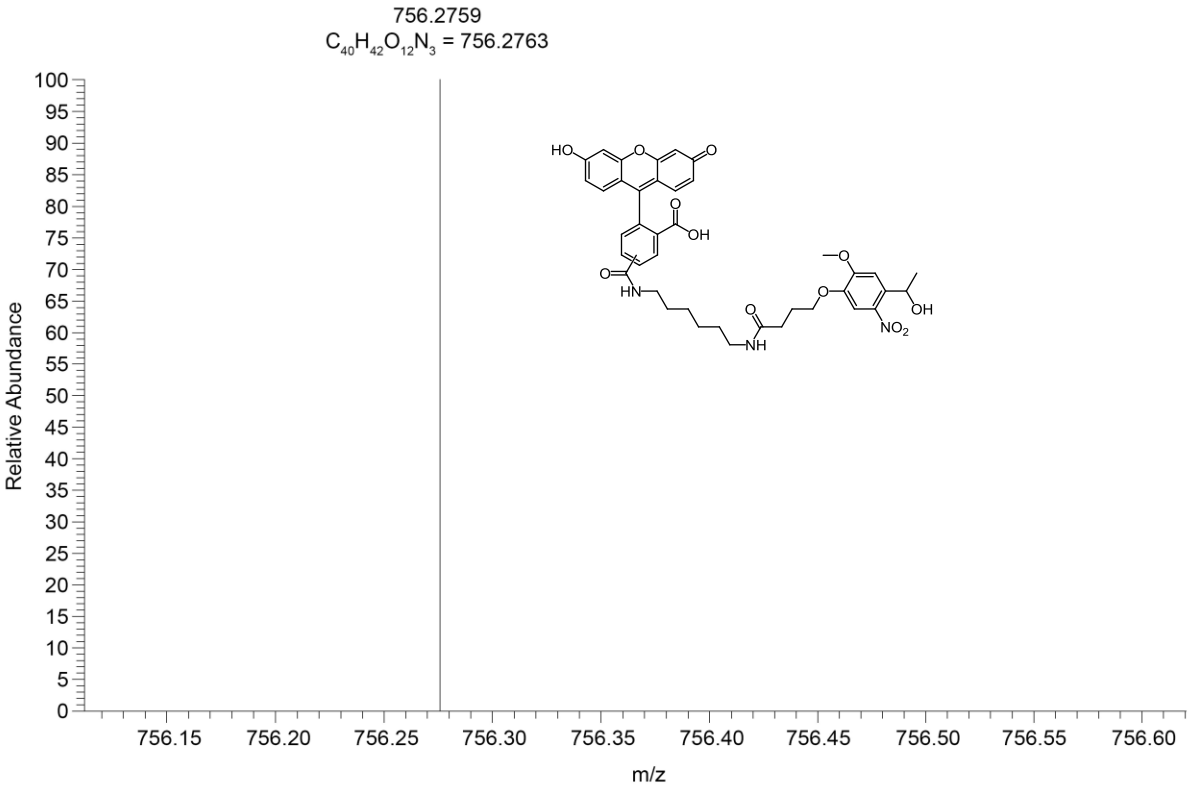
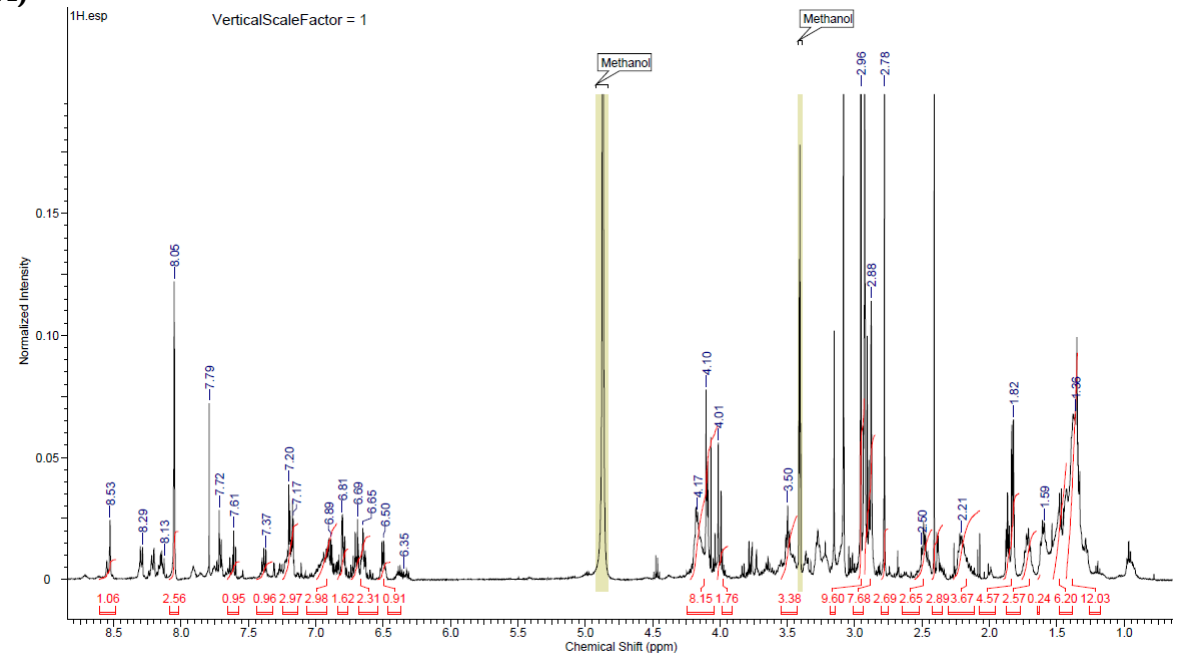


Figure S6. ^{1}H (A) ,^{13}C NMR (B) and HRMS (C) analysis of the compound **6.**

A)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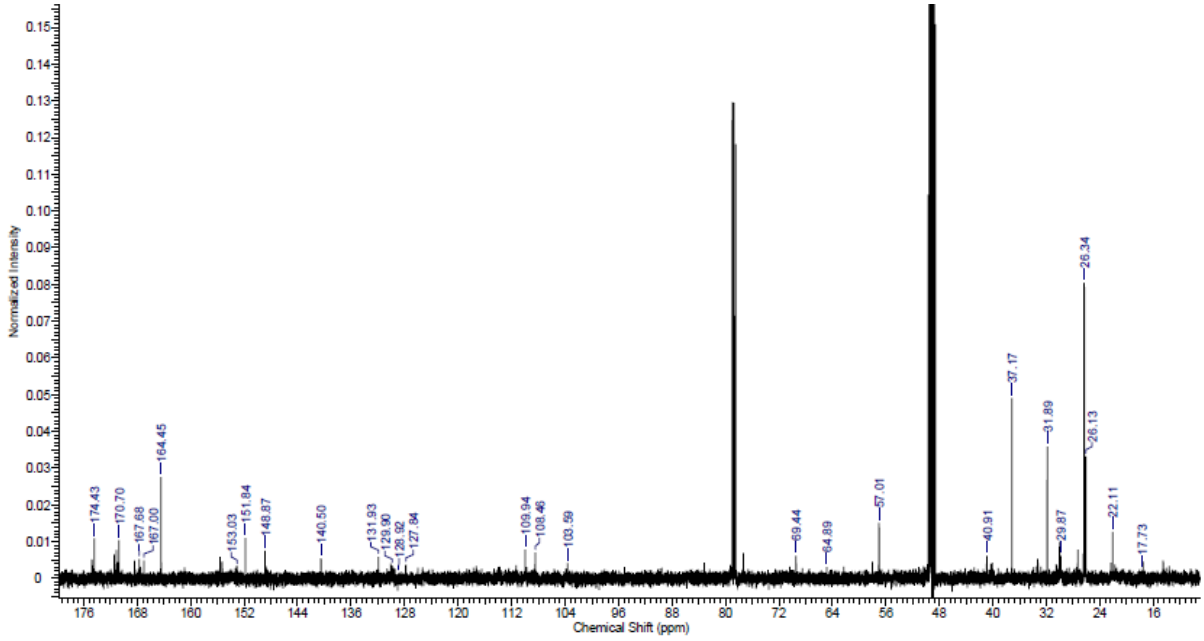
Normalized Intensity
Chemical Shift (ppm)

B)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Chemical Shift (ppm)

C)

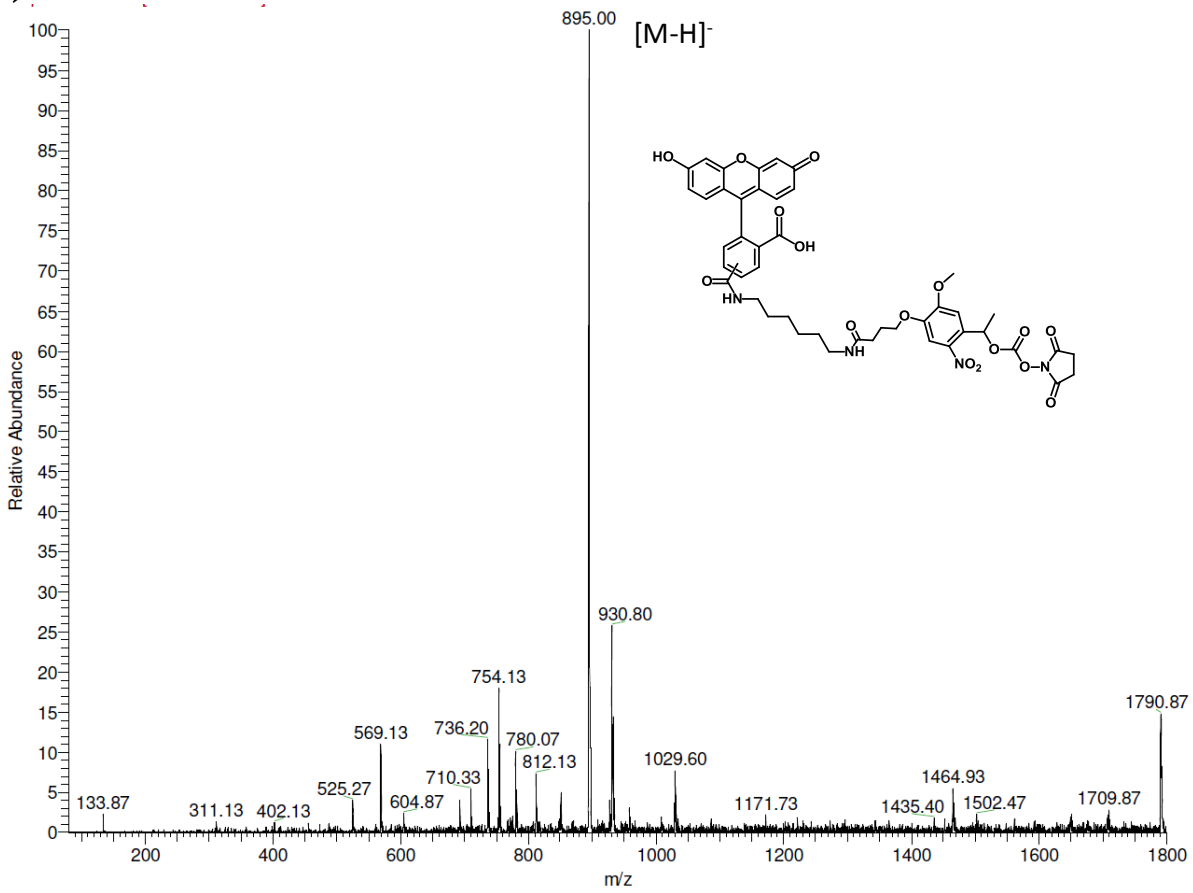

Figure S7. ^{1}H (A), ^{13}C NMR (B) and ESI-MS (C) analysis of the Fsc-PCL **1.**

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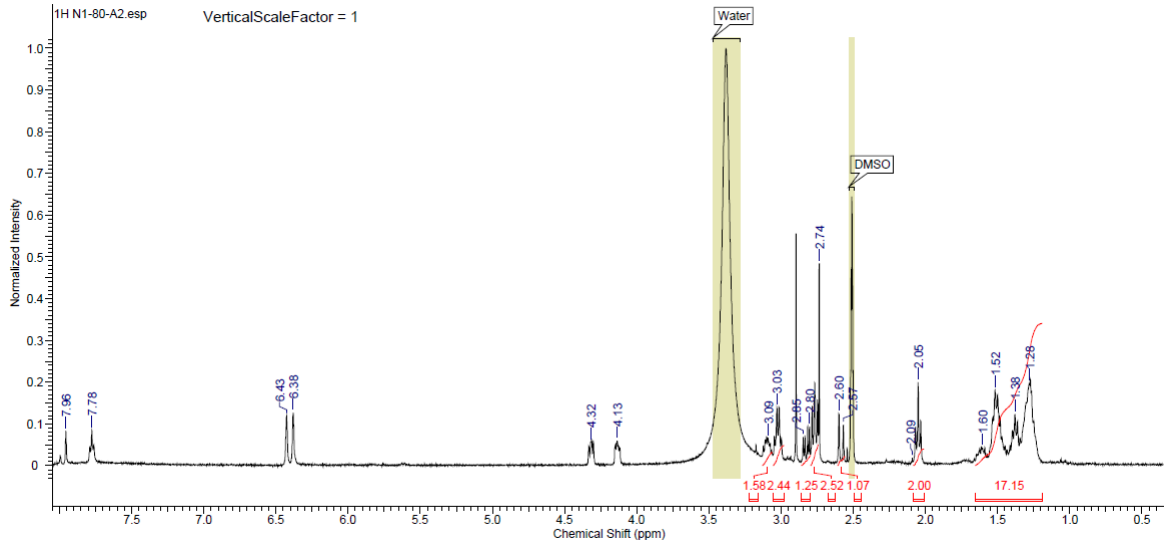
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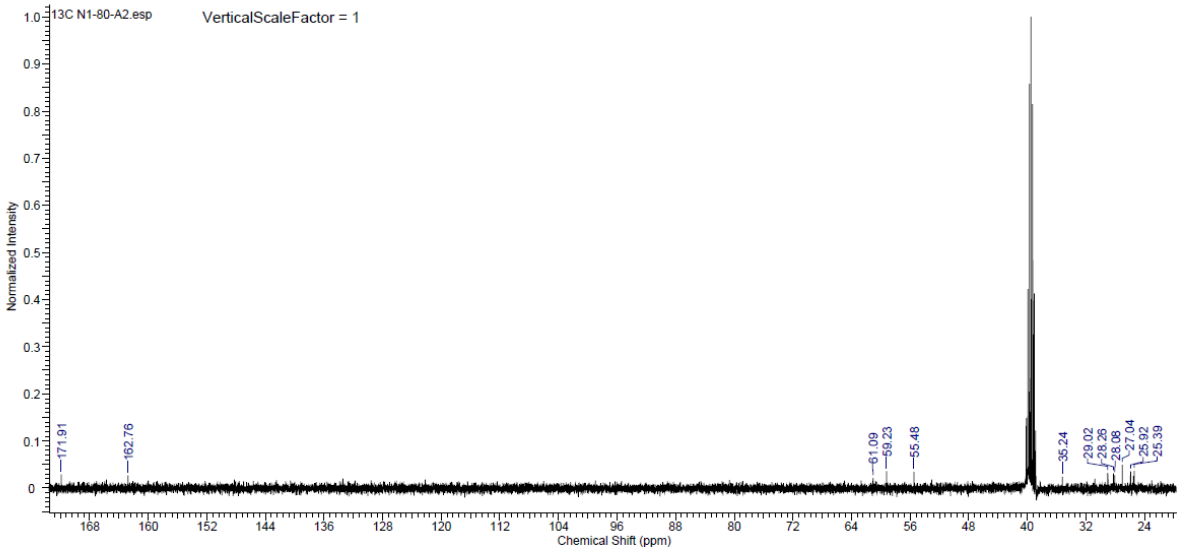
1.58 2.44 1.25 2.52 1.07
2.00
17.15

B)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25.39

C)

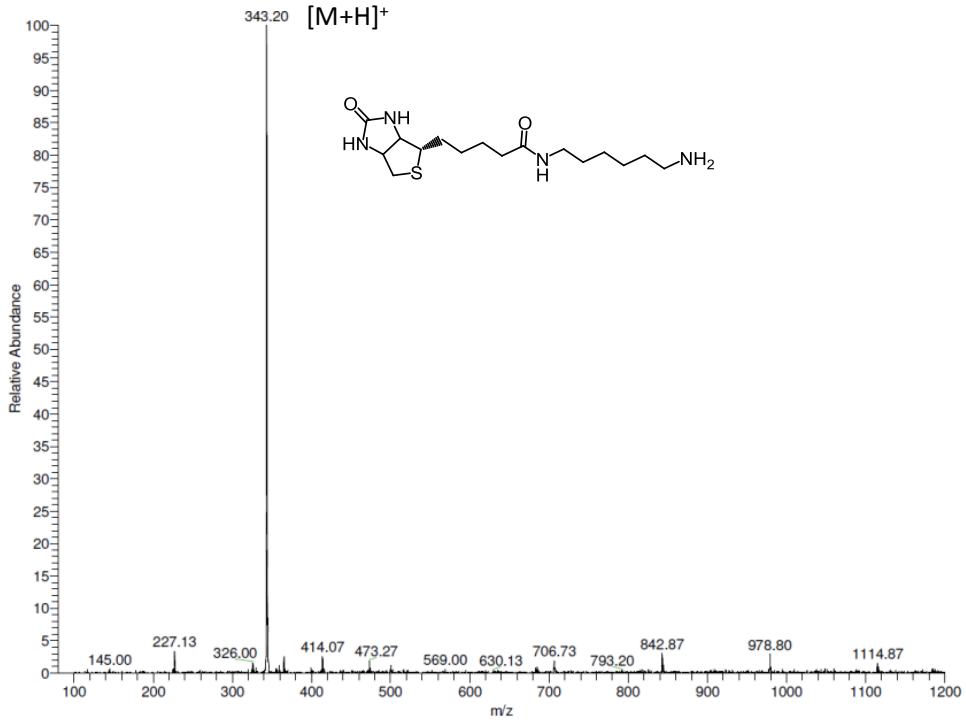
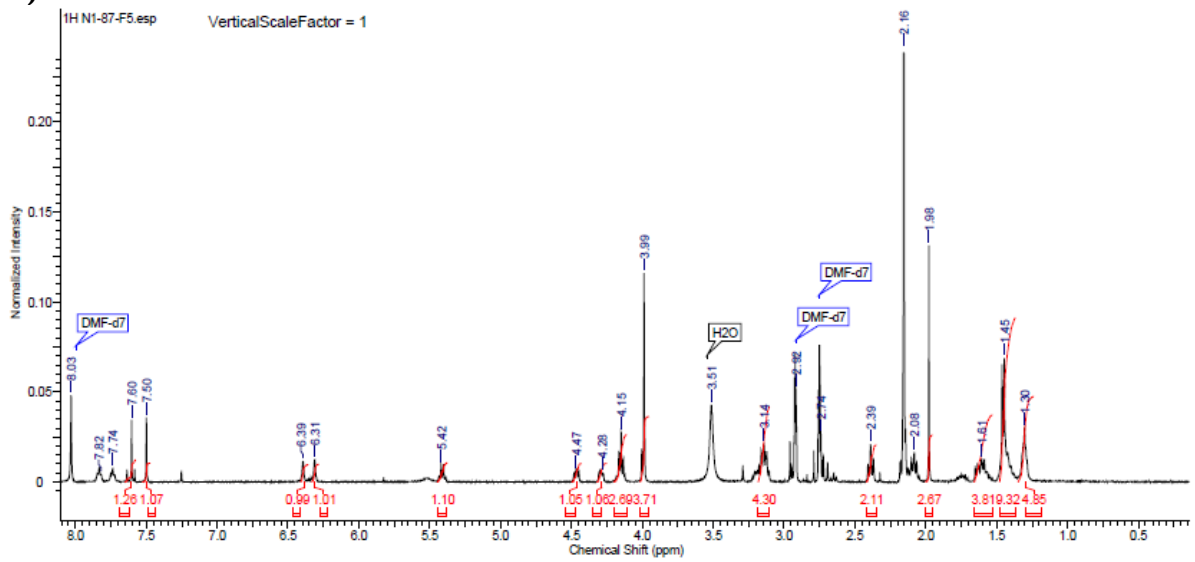


Figure S8. ^{1}H (A), ^{13}C NMR (B) and ESI-MS (C) analysis of the amine **4.**

A)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DMF-d7
DMF-d7
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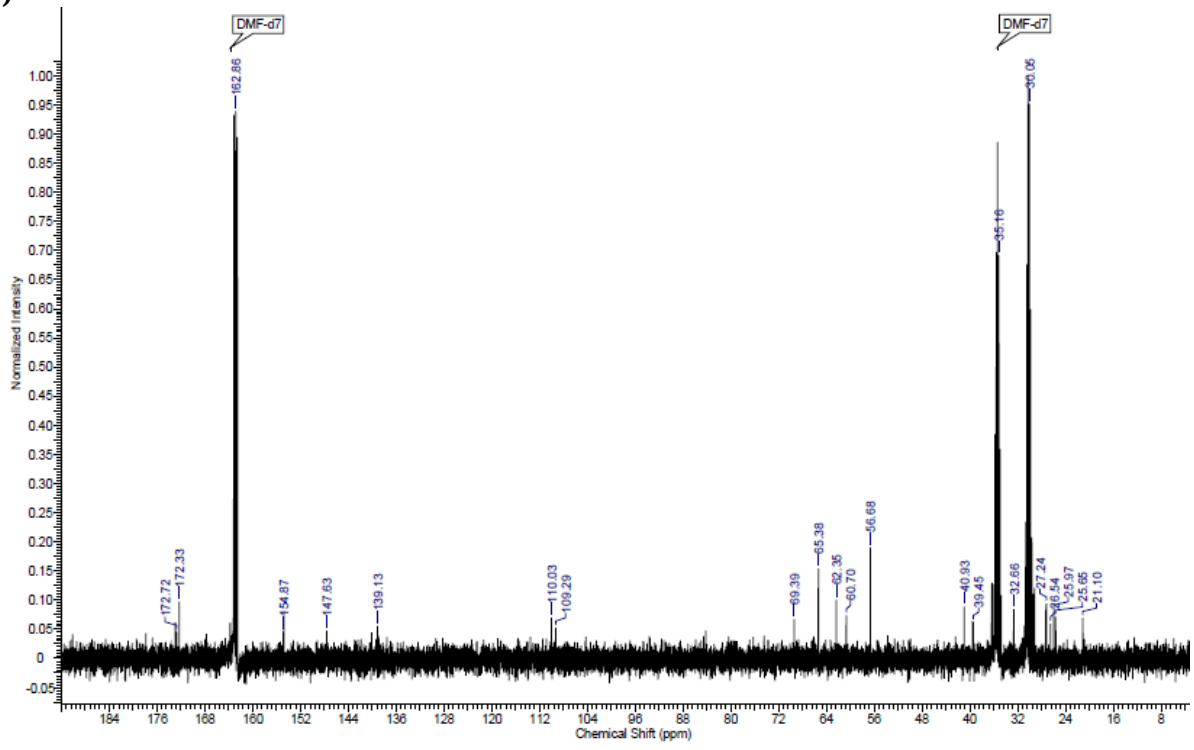
B)


Normalized Intensity
Chemical Shift (ppm)
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DMF-d7

C)

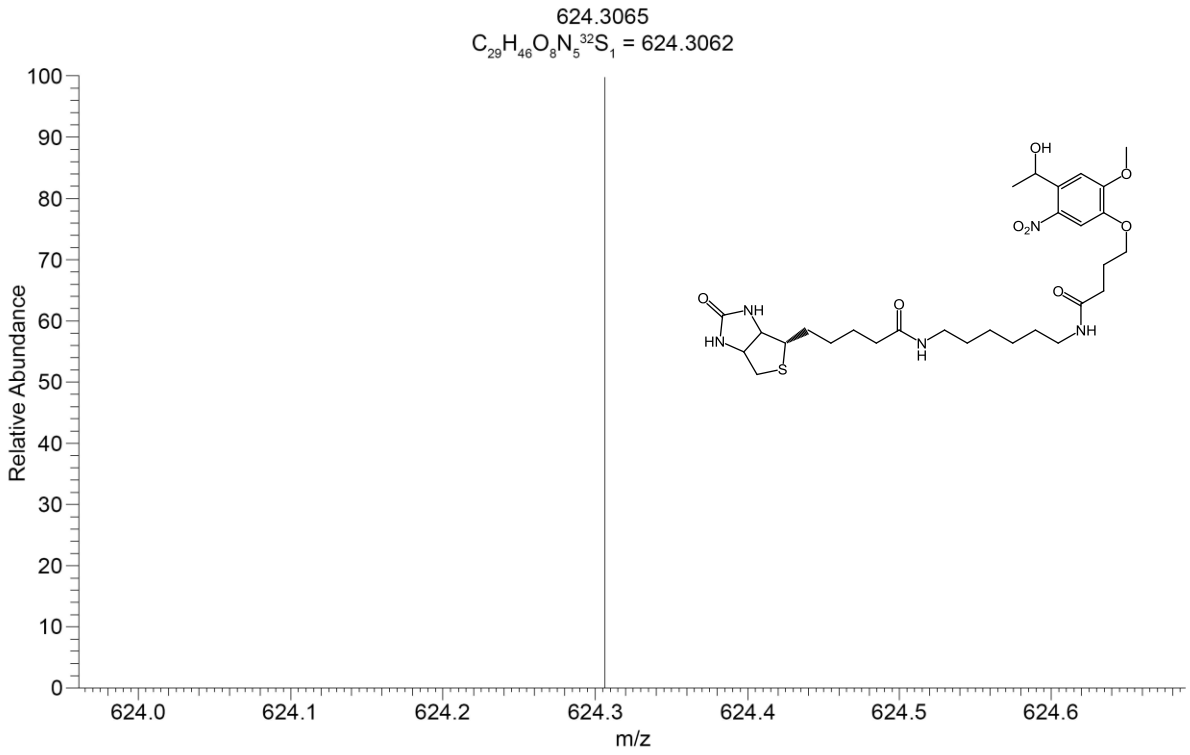

Figure S9. ^{1}H (A), ^{13}C NMR (B) and HRMS (C) analysis of the compound **7.**

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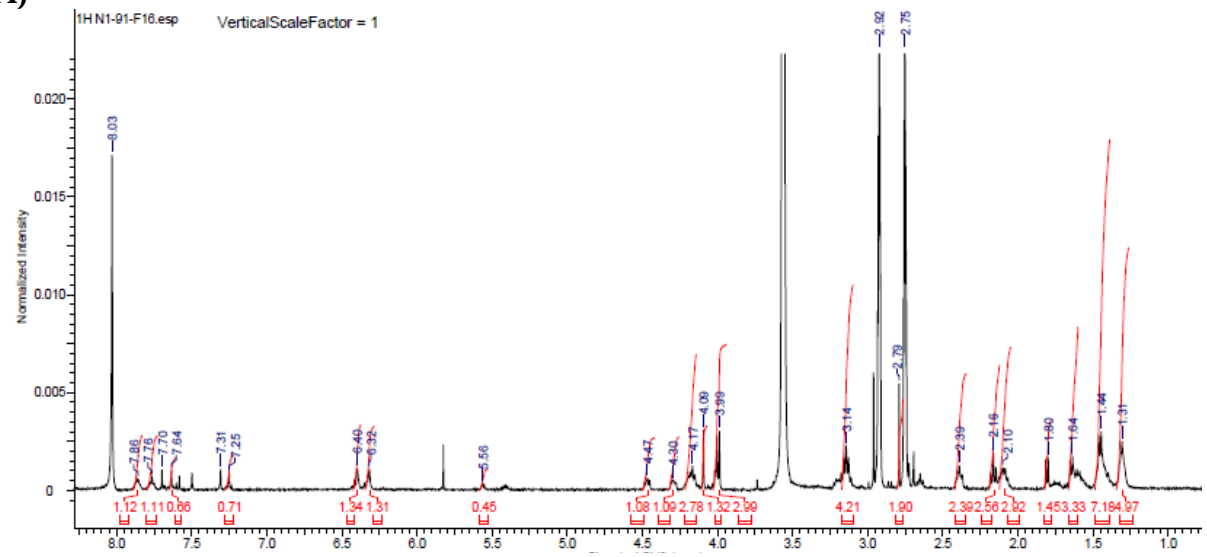
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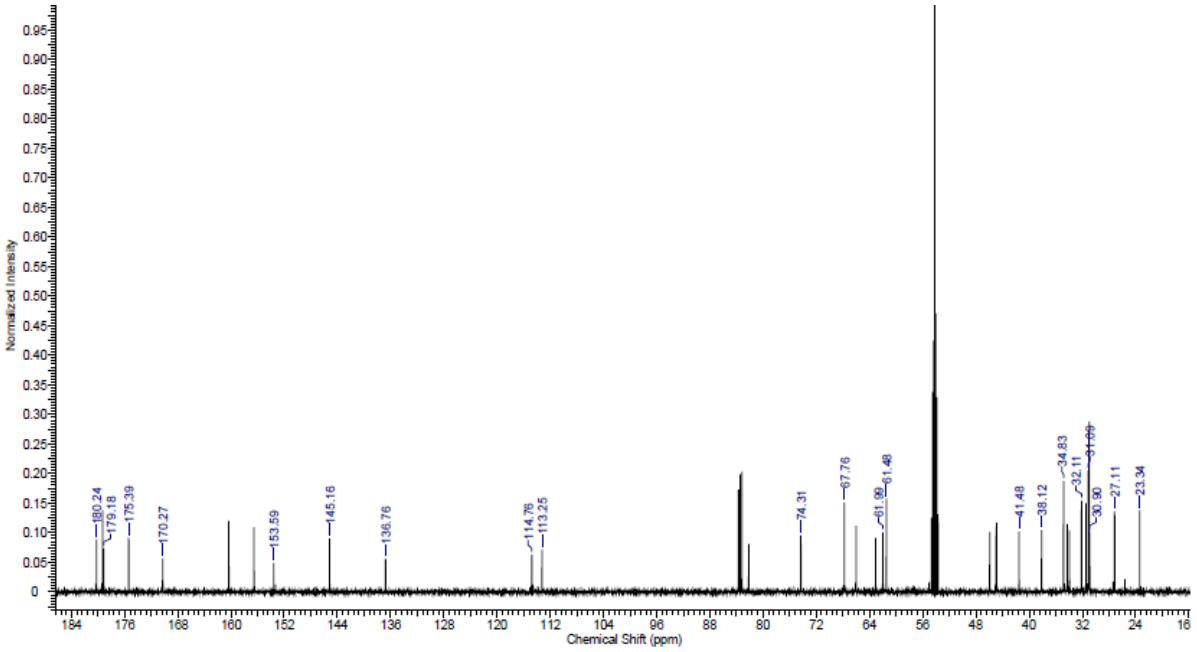
2.79
2.39
2.16
2.10
1.80
1.64
1.44
1.31
1.12 1.11 0.86 0.71 1.34 1.31 0.45 1.08 1.09 2.78 1.32 2.99 4.21 1.90 2.39 2.56 2.92 1.45 3.33 7.18 4.97
Chemical Shift (ppm)

B)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32.11
31.09
30.90
27.11
23.34
Chemical Shift (ppm)

C)

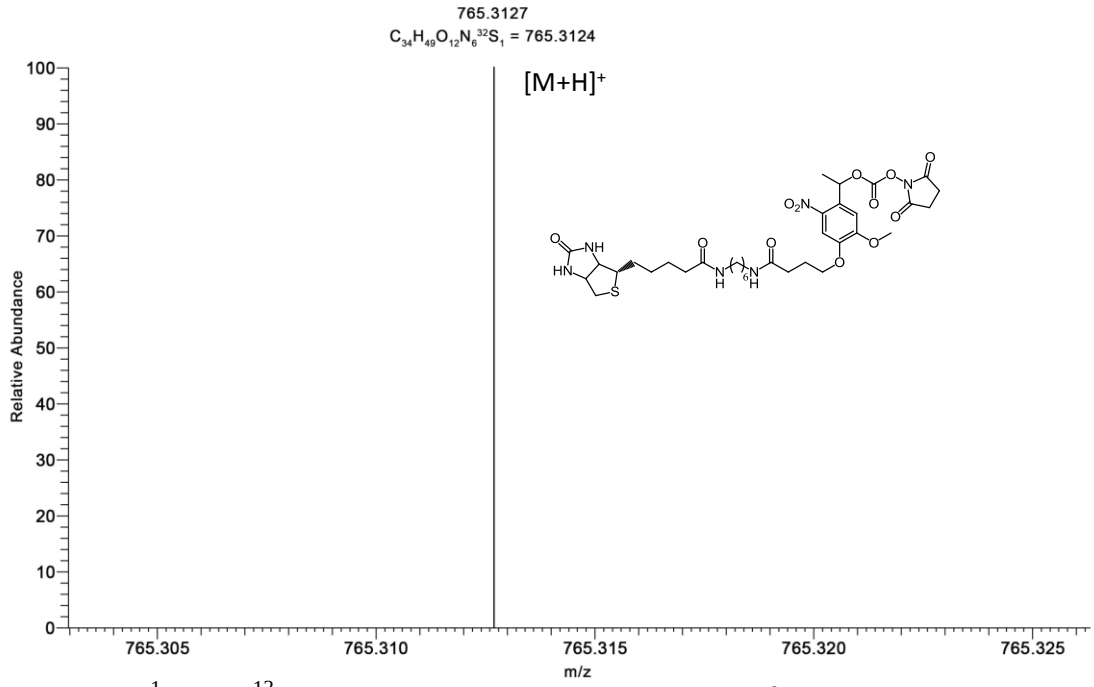

Figure S10. ^{1}H (A), ^{13}C NMR (B) and HRMS (C) analysis of the Biotin-PCL **2.**

Supplementary Tables

Table S1: DNA sequence of ss109Z5 template used for assembly of rectangular -shaped DNA origami

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61 GCACTTGAAG TGGTGGTTGT TCACGGTGCC CTCCATGTAC AGCTTCATGT GCATGTTCTC
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181 GTACAAACTT GTGATATTCC TTCTTAAAGT TAAACAAAAT TATTTCTAGA GGGGAATTGT
241 TATCCGCTCA CAATTCCCCT ATAGTGAGTC GTATTAATTT CGCGGGATCG AGATCTCGAT
301 CCTCTACGCC GGACGCATCG TGGCCGGCAT CACCGGCGCC ACAGGTGCGG TTGCTGGCGC
361 CTATATCGCC GACATCACCG ATGGGGAAGA TCGGGCTCGC CACTTCGGGC TCATGAGCGC
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481 CTTGCATGCA CCATTCCTTG CGGCGGCGGT GCTCAACGGC CTCAACCTAC TACTGGGCTG
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841 AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCCG TCGCAAATTG
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3181 ACGACCACGC TGATGAGCTT TACCGCAGCT GCCTCGCGCG TTTCGGTGAT GACGGTGAAA
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3301 GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC GCAGCCATGA
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GACACGGAAA
5281 TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT
5341 CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC
5401 ACATTTCCCC GAACCTCAGC CATATGGAAG AAGAAGAA

Table S2: Staple strands used for the assembly of the rectangular DNA origami nanostructure.

Left side

B-17,26 GGCGGACGGTTCTGCCAAGGGTTGGGTCCAAT
B-17,58 CCCGAGATTTCTCCGCAAGAATTGCCGAGGCG
B-17,90 TCATGCGCGGAGTGGTGAATCCGTTACAATCC
B-17,122 GCCTAGCCGCTTCCATTCAGGTCGGCGGGGAG
B-37,26 GATCGAAGAAGAGCCGCGAGCGATTTGAGCGA
B-37,58 GCATAAATGATGGTCGTCATCTACAAACGTTT
B-37,90 ATGCCAACGCCTGCAACGCGGGCACCATGCCG
B-37,122 GCAGACAAAGCGAGAAGAATCATACGCCAGCA
B-57,26 GGGCGTGCATACCGCAAGCGACAGTGCGCTCA
B-57,58 GGTGGCGGCTCCAGCGAAAGCGGTCTAATGAG
B-57,90 GCGATAATCCAGAGCGCTGCCGGCGCTCTCAA
B-57,122 AGACGTAGGCATGATAAAGAAGACGCGCCCAC
B-77,26 CTGCCCGCGGAAACCTGTCGTGCCGTCGTATC
B-77,58 TGAGCTAACGGCCAACGCGCGGGGGGCGGGAT
B-77,90 GGGCATCGTTGGGCGCCAGGGTGGGCAGGCGA
B-77,122 CGGAAGGATGAGACGGGCAACAGCTTGCAGCA
B-97,26 CCACTACCCACCAACGCGCAGCCCCTAACAGC

B-97,58 ATAACATGCGCATTGCGCCCAGCGCAGACGCG
B-97,90 AAATCCTGAACCAGCATCGCAGTGCGAGTGAG
B-97,122 AGCGGTCCTCAGCATTTGCATGGTCTTCCCGT
B-117,26 GCGATTTGAATGCGACCAGATGCTGACACCAC
B-117,58 CCGAGACAACCGTCTTCATGGGAGTACAGGCT
B-117,90 ATATTTATTGGGTGTCTGGTCAGAGCGTTGCG
B-117,122 TCCGCTATGCCGGAACATTAGTGCTCCAGCGG
B-137,26 CACGCTGGATCGGCGCGAGATTTAATCGTATA
B-137,58 TCGACGCCGCGACGGCGCGTGCAGGTCTGATA
B-137,90 CGAGAAGAGCAACGCCAATCAGCACGTGGCTG
B-137,122 ATAGTTAACAGTTGTTGTGCCACGCGCTTCCA
B-157,26 ACGTTACTTCACCACCCTGAATTGGCGCTCAT
B-157,58 AGAGACACTATCATGCCATACCGCCGGGGCCT
B-157,90 GCCTGGTTTTCGATGGTGTCCGGGCAAGGAGA
B-157,122 CTTTTTCCTTATGCGACTCCTGCATTGAGCAC
B-177,26 GAGCCCGACCGATCTTCCCCATCGAACCATGG
B-177,58 GCCACCATAGGCGCCAGCAACCGCTTGTACAA
B-177,90 TGGCGCCCATGCCGGCCACGATGCTTTGTTTA
B-177,122 CGCCGCCGCGAGATCTCGATCCCGTGAGCGGA
B-197,26 TGTCTAAGTGATTAAGGAGAACATGCACATGA
B-197,58 AAAAGCAGATGGAGGGCACCGTGAACAACCAC
B-197,90 ACTTTAAGGTGCACATCCGAGGGCGAAGGCAA
B-197,122 TAACAATTAGGGCACCCAGACCATGAGAATCA
G-23,14 TTGATATCGATGTT
G-23,43 ATTCACAGGCGCCGCGTGCGGCTGCTGGAGAT
G-23,75 CAATTCTTACCCGTGGCCAGGACCCAACGCTG
G-23,107 TGCCGCCGGGGTCCTCAACGACAGGAGCACGA
G-43,14 TTCTGGTTTAGGTT
G-43,43 CTGTCCCTCGCCGTGACGATCAGCGTTTGCGC
G-43,75 ACAGCATGCCGTTCCATGTGCTCGATTGGCTC
G-43,107 CCGCCGGAGGTATAGGGCGGCGCCTAGCGAGG
G-63,14 TTTCCGAAAGATTT
G-63,43 TCGTCGCGGACCAGTGACGAAGGCCCTTGAAG
G-63,75 AAAATGACGGCCTGCTTCTCGCCGCTGCCTGG
G-63,107 TACGAGTTCCCAGCGCGTCGGCCGTCCCGATG
G-83,14 TTAGTCGTTTCCTT
G-83,43 TAATGAATCTTACATTAATTGCGTGCCGATCA
G-83,75 TTTGCGTAGTCGAGATCCCGGTGCCCTCGCCG
G-83,107 TTCACCAGGCTGACTGGGTTGAAGACCTGTCC
G-103,14 TTATCCGGAGATTT
G-103,43 TAATGGCGAGCTGTCTTCGGTATCAGCTGCAT
G-103,75 TCGTTGGCTTTGATGGTGGTTAACAGAGGCGG
G-103,107 GCCCTCATACGCTGGTTTGCCCCATTTTTCTT
G-123,14 TTGACCCCTGGTTT
G-123,43 AGTCGCGTGAACTTAATGGGCCCGGGACTCGG
G-123,75 ACTGTTGAGCCAGCCAGCCAGACGCCATCTGA
G-123,107 GAAATAACCGGCTGAATTTGATTGGGAACGAT
G-143,14 TTAGTTGCACCCTT
G-143,43 GACAATTTGCTTCGTTCTACCATCCCACGCCC
G-143,75 TGGAGGTGTTGTGCACCGCCGCTTAAAATAAT
G-143,107 TTGCCCGCTGATCAGCCCACTGACGACATCAA
G-163,14 TTCACATGGTTTTT
G-163,43 CCGGGCGCCGGCATACTCTGCGACATCGCCGC
G-163,75 TTGCGCCACACCACGCGGGAAACGGGCCAGAC
G-163,107 GCTCTCCCCGCGTTTTCGCAGAAAACGACTGT
G-183,14 TTCGAGCAGTGGTT
G-183,43 GGCGATATACCCACGCCGAAACAAACTCTCTT
G-183,75 CGCCGGTGAACAGTCCCCCGGCCAGAAAGGTT
G-183,107 TAGAGGATCAAGGAATGGTGCATGATCTCGAC
G-203,14 TTAGAGCGGCGATT
G-203,43 AGCTGTACGCTCTAAGGAGGATAGGTGATGTC
G-203,75 CACTTCAAAAGGAATATCACAAGTACCTGTGG

G-203,107 GCCCTACGCCCCTCTAGAAATAATGTCCGGCG

Right side

B-23,171 ACCAGAGACACGTTCGCTCGCGTATCGGTGAT
B-23,203 CGCTTCGTAGGTCGCAGACGTTTTGCAGCAGC
B-23,235 GGGTAGCCACGGAAACCGAAGACCATTCATGT
B-23,267 GGAACATAGCTGACTTCCGCGTTTCCAGACTT
B-43,171 TCACAGATTGCCCGGTTACTGGAAGCGGCGGG
B-43,203 CTCGTTGAATGCTCACGATACGGGAATGCCAG
B-43,235 GGCTTCTGTGGGGGTAATGATACCGTTCCACA
B-43,267 GTTTTTTCTGATGCCTCCGTGTAAGCAGATCC
B-63,171 AATGGTGCCACCGAAACGCGCGAGGAAGCGAT
B-63,203 GCCGCATAGAGCTGCATGTGTCAGGCGTCCAG
B-63,235 TCGCTACGTCCGCTTACAGACAAGTAATGTCT
B-63,267 ACACCCGCACGCGCCCTGACGGGCTAAGGGCG
B-83,171 GTGATGCTAAGAGCGCCTGATGCGCACACCGC
B-83,203 AAAACGCCCGAGCGCAGCGAGTCAGCTCTGAT
B-83,235 CTGGCCTTTGAGCTGATACCGCTCCTCCGCTA
B-83,267 CTTTCCTGATTCTGTGGATAACCGGCGCCCCG
B-103,171 AGTTACCGTGGTATCTTTATAGTCCGATTTTT
B-103,203 ACGGGGGGAGCGCACGAGGGAGCTCCTATGGA
B-103,235 GCGAACGAGTATCCGGTAAGCGGCTACGGTTC
B-103,267 AGCGTGAGGCCACGCTTCCCGAAGCACATGTT
B-123,171 CGCGTAATAGTGGCTGCTGCCAGTAAGACGAT
B-123,203 ACCGCTACCCTACATACCTCGCTCCGGGCTGA
B-123,235 AAGAGCTATAGGCCACCACTTCAAAGCTTGGA
B-123,267 GGCTTCAGACCAAATACTGTCCTTATACCTAC
B-143,171 GACGGGGAGAGCGTCAGACCCCGTTTTTTCTG
B-143,203 ATAGACAGCAAAATCCCTTAACGTAAAAAACC
B-143,235 ATTAAGCATAGGTGAAGATCCTTTGCCGGATC
B-143,267 CTCATATAATTTAAAACTTCATTTAGGTAACT
B-163,171 CAAACGACATCATTGCAGCACTGGATCTACAC
B-163,203 GCAATGGCAATCTGGAGCCGGTGATGAACGAA
B-163,235 TGGCGAACGGCCCTTCCGGCTGGCCCTCACTG
B-163,267 AATTAATAGCGGATAAAGTTGCAGCAAGTTTA
B-183,171 GACGCCGGGGGGGATCATGTAACTAGCCATAC
B-183,203 ACACTATTCCGAAGGAGCTAACCGTGCCTGCA
B-183,235 CACCAGTCCGGCCAACTTACTTCTCTATTAAC
B-183,267 ATGACAGTCAGTGCTGCCATAACCCCGGCAAC
B-203,171 ATAATATTTTCCAATGATGAGCACCCCGTGTT
B-203,203 AACATTTCGATCCTTGAGAGTTTTCGCCGCAT
B-203,235 GCGGCATTGGTTACATCGAACTGGTGAGTACT
B-203,267 AGAAACGCAAGATGCTGAAGATCACGGATGGC
G-17,186 AGTCGCTTAAAATCACTCAGGGTCTTACTGAT
G-17,218 TGTTGCTCTAATACAGATGTAGGTGATGAAAC
G-17,250 TACGAAACAGCAGCATCCTGCGATGGGGGATT
G-17,280 TTCAGGGCATGGTGTT
G-37,186 GATGAACAGTCTGCCTGTTCATCCAGGTTTTC
G-37,218 GAGAGAGGGTTTCTCCAGAAGCGTCTGTGACC
G-37,250 TCTGTTCAATAAAGCGGGCCATGTTTGTCTGC
G-37,280 TTGGTCACCTGTTTTT
G-57,186 ACCGTCATACTCTCAGTACAATCTGTGAGCGA
G-57,218 GTCTCCGGGTTAAGCCAGTATACAGCCGCAGC
G-57,250 TCCCGGCATGACTGGGTCATGGCTTATTACCG
G-57,280 TTCCGCTGCAACACTT
G-77,186 GGAAGCGGCGTCAGGGGGGCGGAGTCCAGGGG
G-77,218 CGAACGACAGCAACGCGGCCTTTTAGGGTCGG
G-77,250 CCTTTGAGTTGCTGGCCTTTTGCTGGAGAAAG
G-77,280 TTCCCCTGCGTTATTT
G-97,186 GAAACGCCGATAAGGCGCAGCGGTTGCTAATC
G-97,218 AACAGGAGTTCGTGCACACAGCCCGAACTCTG
G-97,250 GCGGACAGCCTACACCGAACTGAGCTAGTGTA
G-97,280 TTGAAAGCCTATGATT

G-117,186 CTGTTACCCTGCTGCTTGCAAACAGAGTTTTC
G-117,218 TAGCACCGCAGCGGTGGTTTGTTTTTGATAAT
G-117,250 GCCGTAGTCCAACTCTTTTTCCGATTAATTTA
G-117,280 TTGCAGATCAGAGCTT
G-137,186 GTTCCACTGTCAGGCAACTATGGAGCGTGGGT
G-137,218 CTCATGACATCGCTGAGATAGGTGTGGTTTAT
G-137,250 AAAGGATCTTGGTAACTGTCAGACGACCACTT
G-137,280 TTAGATTGTACTTTTT
G-157,186 CTCGCGGTGAGCGTGACACCACGACTTTTTTG
G-157,218 TGCTGATAAACAACGTTGCGCAAAGACAACGA
G-157,250 CTGCGCTCTACTTACTCTAGCTTCATGAGTGA
G-157,280 TTATGGAGGACTGGTT
G-177,186 CACAACATGCAAGAGCAACTCGGTCGCCCCGA
G-177,218 TCGGAGGACTCAGAATGACTTGGTATCTCAAC
G-177,250 TAACACTGACAGAAAAGCATCTTAGTTGGGTG
G-177,280 TTATTATGAAGAGATT
G-197,186 AGAACGTTGAAAAAGGAAGAGTATGAGTATTC
G-197,218 AGCGGTAACGTGTCGCCCTTATTCCCTTTTTT
G-197,250 CACGAGTGTTGCCTTCCTGTTTTTGCTCACCC
G-197,280 TTAAGTAATGGTGATT

Central seam

G-17,154 TCATTCTGCTAACCAGTAAGGCAACCCCGCCA
G-23,139 CGGCTCCAAACTGGCGGTATGGATCGTTGTGA
G-37,154 GGGTAAACTGCACCGCGACGCAACAGGTGGCC
G-43,139 GGCCATCCTCATCAGCGTGGTCGTGCAGCTGC
G-57,154 GGTAAAGCAGCCTCGCGTCGCGAAATGGGGAA
G-63,139 GTGCGGCGCATCTGTGCGGTATTTGTATTTTC
G-77,154 TCCTTACGACGATAGTCATGCCCCAGTCATAA
G-83,139 CTTCACCGCCTCTGACTTGAGCGTCTGTCGGG
G-97,154 TTTCGCCACCTGGCCCTGAGAGAGTGATTGCC
G-103,139 AACCGGACTTACCGGGTTGGACTCGGCGATAA
G-117,154 GTCGTGTCATGGCACTCCAGTCGCTTGTTGAA
G-123,139 TCCACAGCTCTTCTTGAGATCCTTAGAAAAGA
G-137,154 TCAAAGGAAATGGCATCCTGGTCAAGGCAGCT
G-143,139 AATGTAATCTCCCGTATCGTAGTTGGCCAGAT
G-157,154 GGTAAGCCTCAGCTCCGCCATCGCCGGTTGGG
G-163,139 CAGCCCAGAACCGGAGCTGAATGACGCCTTGA
G-177,154 TCGTTGGGTAGTAGGTTGAGGCCGTTAGGAAG
G-183,139 ATACGACTTGTGGCGCGGTATTATTTTTAAAG
G-197,154 TTCTGCTACACTATAGGGGAATTGCGAAATTA
G-203,139 AGGTGGTCACAATAACCCTGATAAATGCTTCA

Table S3: Amino-modified staple strands used firstly in coupling reaction with **1** and further in assembly of **1**-origami.

amC7-B-17,26	amC7-GGCGGACGGTTCTGCCAAGGGTTGGGTCCAAT
amC7-B-17,58	amC7-CCCGAGATTTCTCCGCAAGAATTGCCGAGGCG
amC7-B-17,90	amC7-TCATGCGCGGAGTGGTGAATCCGTTACAATCC
amC7-B-17,122	amC7-GCCTAGCCGCTTCCATTCAGGTCGGCGGGGAG
amC7-G-17,186	amC7-AGTCGCTTAAAATCACTCAGGGTCTTACTGAT
amC7-G-17,218	amC7-TGTTGCTCTAATACAGATGTAGGTGATGAAAC
amC7-G-17,250	amC7-TACGAAACAGCAGCATCCTGCGATGGGGGATT

Tabel S4: Fsc- modified staples strands used in assembly of Fsc-origami.

G-23,43-Fsc C7	ATTCACAGGCGCCGCGTGCGGCTGCTGGAGAT-FlcC7
G-23,107-Fsc C7	TGCCGCCGGGGTCCTCAACGACAGGAGCACGA-FlcC7
B-23,171-Fsc C7	ACCAGAGACACGTTCGCTCGCGTATCGGTGAT-FlcC7
B-23,235-Fsc C7	GGGTAGCCACGGAAACCGAAGACCATTCATGT-FlsC7
FAM C6-B-17,122	FAM-C6-GCCTAGCCGCTTCCATTCAGGTCGGCGGGGAG
FAM C6-G-17,186	FAM-C6-AGTCGCTTAAAATCACTCAGGGTCTTACTGAT
FAM-C6-B-17,58	FAM-C6-CCCGAGATTTCTCCGCAAGAATTGCCGAGGCG

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