Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

for manuscript entitled

"Stabilizing DNA Nanostructures through Reversible Disulfide Crosslinking"

by

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1. Materials and Methods

General. Chemicals and anhydrous solvents were from Sigma-Aldrich (Munich, Germany), Alfa Aesar (Karlsruhe, Germany), Acros Organics (Geel, Belgium), Carbosynth (Berkshire, UK), or Carl Roth (Karlsruhe, Germany). Solvents were stored over molecular sieves or dried in house. To perform reactions under nitrogen atmosphere, a flask was first heat-dried, activated molecular sieves (3 Å or 4 Å) and water-free solvents were employed. Solvents that were used for HPLC were purchased from VWR (Darmstadt) in HPLC quality. The 5-iodo-2'-deoxycytidine and 5-iodo-2'-deoxyuridine were from Carbosynth (Berkshire, UK), and 4,4'-dimethoxytriphenylmethyl chloride (DMT-Cl) was from Sigma Aldrich (Munich, Germany). Oligonucleotides were purchased from Ella Biotech (Munich, Germany) in HPLC-purified form and were used without modification. Amicon Ultra centrifugal filters with a molecular weight cut-off (MWCO) of 3 kDa, 30 kDa or 100 kDa were from Merck/Millipore (Darmstadt, Germany). Chelex 100 was from Bio-Rad (München, Germany). The reagents for oligonucleotide synthesis, including deblock solution (3 % tricholoracetic acid in CH_2Cl_2), cap A (10 % *tert*-butylphenoxyacteyl anhydride in THF), cap B (16 % 1-methylimidazole in THF), activator (0.25 M 4,5-dicyanoimidazole in MeCN), and oxidizer (0.1 M iodine in THF/pyridine/H_2O; 77/21/2 *v*/*v*/*v*) were from Sigma Aldrich (Munich, Germany). The phosphorylation reagent 2-cyanoethyl(*N*,*N*,*N*',*N*'-tetraisopropyl) phosphorodiamidite was from Glen Research (Sterling, USA), or was synthesized in-house following a published procedure.[S1] Deuterated acetonitrile and chloroform were from Euriso-Top (Saclay Gif/Yvette, France). The 5-iodo-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-cytidine (5) was prepared from **4** following a literature protocol[S2] on a 2.0 g (5.6 mmol) scale and was obtained as a colorless solid (2.5 g, 4.2 mmol, 75 %). Long-chain alkylamine controlled pore glass (lcaa cpg) with 1000 Å pore size was from Prime Synthesis (Aston, USA).

2. Synthesis of modified nucleosides

Elaboration of side chain

3,6,9,12-Tetraoxapentadec-14-in-1-ol (2). The following protocol is a slight modification of a literature protocol.[S3] The synthesis should be performed in a well-ventilated fume hood to avoid combustion of the mixture of hydrogen gas and air, as well as exposure to propargyl bromide. We note that the protocol does not contain a quenching step prior to work-up, which may lead to uncontrolled reactions of residual reagents when performed on a large scale. Tetraethylene glycol (10.0 g, 51.6 mmol) was dissolved in THF (100 mL) and cooled to 0 °C in an ice bath. Then, NaH (60 % in mineral oil, 2.4 g, 56.7 mmol, 1.1 eq) was added in three portions under stirring and continued cooling over the course of 10 min, leading to the evolution of gas. After stirring for 15 min, propargyl bromide (80 % in toluene, 5.7 mL, 56.7 mmol, 1.1 eq) was added under continued cooling over the course of 5 min. The reaction mixture was stirred for 40 h, while the ice cooling was not renewed to allow for warming up to room temperature. The suspension was then filtered over celite, the solvent was evaporated, and the residue was purified by column chromatography (silica, 500 g; ethyl acetate/MeOH 0-15 %). The title compound showed an R_f of 0.4, whereas the starting material eluted at an R_f of 0.2 (MeOH/ethyl acetate, 10:90). Compound **2** was obtained as yellow oil (5.2 g, 22.4 mmol) in 43 % yield (55 % yield, based on recovered starting material). The analytical data (^{1}H-NMR) were in agreement with the literature.[S3]

(3,6,9,12-Tetraoxapentadec-14-in-1-*S*-yl-thiobenzoic acid (3). Triphenylphosphine (4.5 g, 17.2 mmol, 2 eq) was dissolved at 0 °C in THF (10 mL) under argon atmosphere. Subsequently DIAD (3.5 g, 17.2 mmol, 2 eq) was added in four portions. After 10 min thiobenzoic acid (2.4 g, 17.2 mmol, 2 eq) was added slowly over a period of 10 min. The solution was stirred for 10 min and compound **2** (2.0 g, 8.6 mmol, 1 eq) was added to the mixture. The reaction mixture was cooled for 30 min to 0 °C and stirred for further 20 h at room temperature. The solvent was evaporated and the solid residue was purified by column chromatography (silica 200 g, petroleum ether (PE)/ethyl acetate (EtOAc) 3-33 %). Compound **3** was obtained as a yellow liquid (2.9 g, 8.3 mmol, 95 %). R_f = 0.4 (silica, petroleum ether / ethyl acetate 97:3 *v*/*v*). ^{1}H NMR (500 MHz, CD_3CN): δ = 7.86 – 7.92 (m, 2H), 7.54 - 7.60 (m, 1H), 7.43 (t, 2H), 4.06 (d, 2H), 3.57 (t, 2H), 3.46 - 3.52 (m, 14H), 3.18 (t, 2H*)*, 2.61 (t, 1H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 190.9, 136.6, 133.3, 128.6, 126.6, 79.6, 74.3, 69.9, 69.8, 69.7, 69.6, 68.9, 68.6, 57.4, 28.2 ppm. HRMS (ESI^+): *m/z* calcd. for $C_{18}H_{24}O_5S$ $[M+Na]^+$ 375.124 ; found: 375.124 correct isotope distribution.

Synthesis of a modified 2'-deoxycytidines

5-[15-Benzoylthio-(4,7,10,13-tetraoxapentadec-1-in-1-yl)]-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-cytidine (6). The DMT protected nucleoside **5** (0.10 g, 0.15 mmol) was dissolved in DMF (10 mL). The alkyne **3** (0.16 g, 0.45 mmol, 3 eq) was dissolved in DMF (5 mL) and added to the reaction mixture. After 5 min NEt_3 (0.5 mL, 0.38 mmol, 2.5 eq) and CuI (0.028 g, 0.15 mmol) were added to the solution. Following the addition of $Pd(PPh_3)_4$ (0.08 g, 0.075 mmol, 0.5 eq) the mixture was stirred for 2 d at 60 °C. The reaction was quenched with saturated aqueous solution of $NaHCO_3$ (25 mL) and ethyl acetate (25 mL) was added. The organic phase was washed with EDTA-solution (pH 8, 200 mM, 3 x 25 mL) and brine (3 x 25 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent was evaporated. Column chromatography (silica 20 g; dichloromethane/MeOH 0-15 %) yielded **6** as a brown solid (79 mg, 0.09 mmol, 60 %). R_f = 0.4 (silica, dichloromethane/methanol/triethylamine 97:3:1 *v*/*v*/*v*). ^{1}H NMR (500 MHz, CD_3CN): δ = 8.58 (s, 1H), 8.09 (m, 4H), 7,52 (s, 1H), 7.34 (m, 4H), 7.32 (d, 4H), 7.26 (t, 2H), 6.26 (dd, 4H), 6.16 (s, 1H), 4.46 (m, 1H), 4.17 (m, 2H), 4.10 (d, 6H), 3.64-3.60 (m, 14H), 3.59 (d, 1H), 3.24 (t, 2H), 2.45 (m, 3H), 2.25 (m, 3H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 190.9, 170.2, 164.3, 158.2, 153.9, 144.4, 144.1, 137.5, 136.5, 135.9, 135.6, 135.5, 135.3, 135.1, 133.1, 129.6, 129.5, 128.5, 127.6, 127.4, 126.5, 116.9, 112.8, 98.9, 91.2, 87.1, 86.1, 85.8, 76.7, 70.6, 69.8, 68.9, 68.4, 68.1, 64.8, 62.9, 62.3, 58.0, 54.6, 45.2, 41.1, 40.9, 28.2, 19.7, 7.6 ppm. HRMS (ESI^+): *m/z* calcd. for $C_{48}H_{53}N_3O_{11}S$ $[M+H]^+$ 880.34, found: 880.34, correct isotope distribution.

5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-cytidine (7). Compound **6** (2.0 g, 2.3 mmol) was dissolved in a mixture of MeOH and THF (60 mL, MeOH:THF, 1:2 *v*/*v*). To the reaction mixture LiOH (82 mg, 3.4 mmol, 1.5 eq) was added and stirred for 30 min. After addition of di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazine dicarboxylate (1.0 g, 3.4 mmol, 1.5 eq) stirring was continued for 18 h at r.t. and for 3.5 h at 50 °C. The solvent was evaporated and the crude was purified by column chromatography (silica 200 g; dichloromethane/MeOH 0-15 %) yielding a brown solid **7** (1.2 g, 1.3 mmol, 60 %). R_f = 0.3 (silica, dichloromethane/methanol/triethylamine 97:3:1 *v*/*v*/*v*). ^{1}H NMR (500 MHz, CD_3CN): δ = 8.09 (s, 1H), 7.50 (d, 2H), 7.38 (d, 4H), 7.35 (t, 2H), 7.27 (t, 1H), 6.90 (dd, 4H), 6.16 (t, 1H), 4.24 (m, 1H), 4.04 (s, 1H), 3.80 (d, 6H), 3.68-3.48 (m, 14H), 3.43 (s, 1H), 3.34-3.33 (dd, 1H), 3.27-3.25 (dd, 1H), 2.90 (t, 2H), 2.00 (m, 3H), 2.19 (m, 3H), 1.30 (s, 9H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 164.2, 158.3, 153.6, 144.6, 144.2, 135.5, 129.3, 127.5, 126.3, 112.6, 91.2, 88.9, 86.1, 77.0, 70.8, 69.7, 68.6, 68.2, 63.1, 58.0, 54.4, 47.1, 41.4, 39.5, 29.0 ppm. HRMS (ESI^+): *m/z* calcd. for $C_{45}H_{57}N_3O_{10}S_2$ $[M+H]^+$: 864.35; found: 864.35 correct isotope distribution.

5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-*N*-dimethylformamid-5'-*O*-(4,4'-dimethoxytrityl)-cytidine (8). Compound **7** (2.1 g, 2.5 mmol) was dissolved in DMF (40 mL) and *N*,*N*-dimethylformamide dimethyl acetal (5.4 g, 36.7 mmol, 15 eq) was added. The reaction mixture was heated to 50 °C for 48 h. The solvent was evaporated and the residue was dissolved in dichloromethane (25 mL). The organic phase was washed with saturated aqueous solution of $NaHCO_3$ (3 x 15 mL) and brine (3 x 25 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent was evaporated. Column chromatography (silica 200 g; dichloromethane/MeOH, 0-3 % MeOH) yielded a brown solid of **6** (1.8 g, 1.95 mmol, 78 %). R_f = 0.2 (silica, dichloromethane/methanol/triethylamine 97:3:1 *v*/*v*/*v*). ^{1}H NMR (500 MHz, CD_3CN): δ = 8.71 (s, 1H), 8.16 (s, 1H), 7.50 (d, 2H), 7.38 (d, 4H), 7.34 (t, 2H), 7.27 (t, 1H), 6.90 (dd, 4H), 6.16 (t, 1H), 4.46 (m, 1H), 4.12 (m, 2H), 4.04 (m, 1H), 3.80 (d, 6H), 3.68-3.50 (m, 14H), 3.43 (d, 1H), 3.35-3.33 (dd, 1H), 3.28-3.26 (dd, 1H), 3.20 (s, 3H), 3.15 (s, 3H), 2.90 (t, 2H), 2.45 (m, 3H), 2.25 (m, 3 H), 1.32 (s, 9H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 170.1, 158.1, 157.5, 153.6, 144.5, 135.5, 135.2, 129.5, 129.4, 127.4, 126.3, 116.8, 112.6, 98.4, 97.1, 87.6, 86.1, 85.9, 85.8, 79.7, 70.3, 69.5, 68.6, 68.0, 62.9, 57.8, 54.3, 46.8, 40.8, 40.2, 39.6, 33.9, 28.7. ppm. HRMS (ESI^+): *m/z* calcd. for $C_{48}H_{62}N_4O_{10}S_2$ $[M+H]^+$: 919.40, found: 919.37 correct isotope distribution.

[5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-cytidin-3'-*O*-yl]-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (9). The nucleoside **8** (0.10 g, 0.12 mmol) was dried under reduced pressure for 1.5 h and dissolved in absolute acetonitrile (5 mL). To the solution DIPAT (0.03 g, 0.17 mmol, 1.5 eq) and the phosphorylation reagent 2-cyanoethyl(*N*,*N*,*N*',*N*'-tetraisopropyl) phosphorodiamidite (0.05 g, 0.17 mmol, 1.5 eq) was added. After 4 h the reaction mixture was poured on an ice cooled saturated aqueous $NaHCO_3$ solution (50 mL) and washed with saturated aqueous $NaHCO_3$ (3 x 25 mL). The aqueous phase was extracted with ethyl acetate, dried over Na_2SO_4, filtered and the solvent was evaporated. The crude liquid was purified by column chromatography (silica 20 g, dichloromethane/ethyl acetate/triethylamine 45:45:10 *v*/*v*/*v*) and yielded a yellow solid: 0.07 g, 0.07 mmol, 60 %. R_f = 0.75 (silica, dichloromethane/ethyl acetate/triethylamine 45:45:10 *v*/*v*/*v*). ^{31}P NMR (161 MHz, CD_3CN): δ = 147.93, 147.84 ppm.

5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-cytidine on solid support CPG (11). The nucleoside **8** (0.10 g, 0.12 mmol) was dried together with 4-dimethylamino pyridine (7.1 mg, 0.06 mmol, 0.5 eq) under reduced pressure for 3 h at 40 °C. The solid mixture was dissolved in absolute pyridine (5 mL) and succinic anhydride (11 mg, 0.11 mmol, 0.9 eq) was added. The reaction mixture was stirred for 72 h and the solvent was coevaporated with toluene (3 x 10 mL). The solid residue was dissolved in DCM (10 mL) and washed with ice cold citric acid (5 mL, 3.5 % in water *w*/*v*). The organic phase was dried over Na_2SO_4 and the

solvent removed *in* vacuo. The residue was dissolved in DCM (0.5 mL) and precipitated from ice cooled hexane (15 mL). Product **10** could be obtained as a colorless foam (0.10 g, 0.10 mmol, 85%). Without further purification compound **10** (190 mg, 0.2mmol, 1 eq) was added to HOBT (51.2 mg, 0.2 mmol, 1 eq), HBTU (6.6 mg, 0.18 mmol, 0.9 eq), diisopropylethylamine (75 µl, 0.44 mmol, 2.2 eq), DMF (1 mL) and the mixture was shaken for 10 min. Subsequently amino functionalized cpg (700 mg) was added and shaken for 2 h. The supernatant was removed and the cpg was washed with DMF and acetonitrile (both 2 x 1 mL). The cpg was dried under vacuum and the loading was determined. Therefore an analytical sample was mixed with TCA deblock solution (200 µl) diluted with DCM (800 µl) and the extinction was measured at 498 nm. Using the extinction coefficient ε_{498} = 71 000 $M^{-1}cm^{-1}$a loading of 25 µmol/g in compound **11** was found.

Synthesis of modified 2'-deoxyuridines

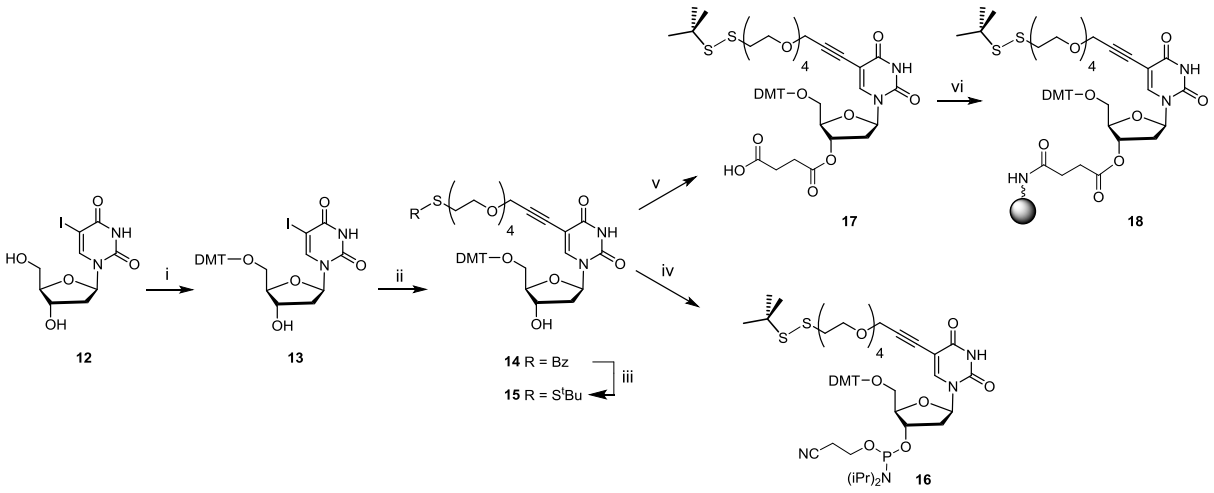

Figure S1. Overview of the synthetic routes to the phosphoramidite building block and controlled-pore glass of the modified 2'-deoxyuridine employed in this study. Conditions: (i) DMT-Cl, pyridine, 73 %; (ii) **3**, $Pd(PPh_3)_4$, CuI, DMF, 20 h, 40 °C, 65 %; (iii) di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazine dicarboxylate, LiOH, MeOH/THF, 2 h, 66 %; (iv) 2-cyanoethyl (*N*,*N*,*N*',*N*'-tetraisopropyl) phosphorodiamidite, DIPAT, MeCN, 60 %; (v) succinic acid anhydride, DMAP, pyridine, 84 %; (vi) HOBT, HBTU, DMF, DIEA; loading of nucleoside on cpg: 35 µmol/g.

5-Iodo-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-uridine (13). The uridine compound **13** was synthesized from **12** using a literature protocol on a 2.0 g (6.7 mmol) scale and was obtained as a colorless solid (2.7 g, 4.2 mmol, 73 %).[S4]

5-[15-Benzoylthio-(4,7,10,13-tetraoxapentadec-1-in-1-yl)]-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-uridine (14). Analogous the procedure of the synthesis for compound **6** the nucleoside **13** (0.2 g, 0.3 mmol) was dissolved in DMF (5 mL). The alkyne **3** (0.32 g, 0.9 mmol, 3 eq) was also dissolved in DMF (2.5 mL) and added to the prepared solution of the nucleoside. The reaction mixture was stirred for 5 min and NEt_3 (0.5 mL, 0.38 mmol, 2.5 eq) and CuI (0.028 g, 0.15 mmol) were added to the solution. After 5 more min $Pd(PPh_3)_4$ (0.08 g, 0.075 mmol, 0.5 eq) was added and the mixture was stirred for at 40 °C for 20 h. An aqueous solution of $NaHCO_3$ (25 mL) and ethyl acetate (25 mL) was added to the reaction mixture. The organic phase was washed with EDTA-solution (pH 8, 200 mM, 3 x 25 mL) and brine (3 x 25 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvent was evaporated. Purification *via* column chromatography (silica 20 g; dichloromethane/MeOH 0-3 %) yielded a brown solid of **14** (171 mg, 0.19 mmol, 65 %). R_f = 0.2 (silica, dichloromethane/methanol/triethylamine 97:3:1 *v*/*v*/*v*). ^{1}H NMR (300 MHz, CD_3CN): δ = 8.08-7.94 (m, 4H), 7.84-7.62 (m, 3H),7.58-7.19 (m, 12H), 6.98-6.80 (m, 4H), 6.15 (t, *J* = 6.6 Hz, 1H), 3.79 (s, 6H), 3.73-3.37 (m, 16H), 3.35-3.18 (m, 4H), 2.37-2.28 (m, 2H,) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 137.6, 135.8, 135.7, 134.6, 134.5, 133.5, 133.4, 133.3, 131.8, 130.9, 130.1, 129.1, 129.0, 128.6, 128.5, 128.2, 127.7, 127.6, 126.9, 126.6, 116.9, 115.8, 73.7, 69.9, 69.8, 69.7, 68.90, 68.7, 68.5, 63.4, 28.2, 20.9 ppm. HRMS (ESI^+): *m/z* calcd. for $C_{48}H_{52}N_2O_{11}S$ $[M+Na]^+$: 903.313; found: 903.313 correct isotope distribution.

5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-uridine (15). Analogous to the procedure for the synthesis for compound **7**, nucleoside **14** (0.4 g, 0.5 mmol) was dissolved in a mixture of MeOH and THF (6 mL, MeOH:THF, 1:2 *v*/*v*). Then, LiOH (18 mg, 0.7 mmol, 1.5 eq) was added, and the mixture was stirred for 30 min, followed by the addition of di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazinedicarboxylate (0.24 g, 0.7 mmol, 1.5 eq) and stirring at r.t. for 2 h. The solvent was evaporated and the residue was purified *via* column chromatography (silica 20 g; dichloromethane/MeOH 0-3 %) yielding a brown solid of **15** (0.28 g, 0.33 mmol, 66 %). R_f = 0.3 (silica, dichloromethane/methanol/triethylamine 97:3:1 *v*/*v*/*v*). ^{1}H NMR (500 MHz, CD_3CN): δ = 8.00 (s, 1H), 7.51-7.46 (m, 2H), 7,44-7.30 (m, 6H), 6.95-6.86 (m,4H, *Ar-H*), 6.16 (t, *J* = 6.6 Hz, 1H), 5.47 (s, 1H), 3.79 (d, *J* = 0.7 Hz, 6H), 3.75-3.61 (m, 2H), 3.60-3.40 (m, 12H), 3.38-3.18 (m, 2H), 1.94-1.89 (m, 2H), 1.33 (s, 9H) ppm. ^{13}C NMR (126 MHz, CD_3CN): δ = 158.3, 143.0, 129.7, 129.6, 127.6, 127.5, 126.5, 116.9, 115.8, 112.8, 86.08, 85.24, 70.6, 69.8, 69.7, 69.6, 69.5, 68.9, 68.6, 68.5, 63.0, 58.0, 54.6, 45.6, 40.4, 39.8, 28.7, 7.66 ppm. HRMS (ESI^+): *m/z* calcd. for $C_{45}H_{56}N_2O_{11}S_2$ $[M+Na]^+$: 887.32; found: 887.32 correct isotope distribution.

[5-(18,18-Dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-in-1-yl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-uridin-3'-*O*-yl]-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (16). Following the procedure for the synthesis of **9**, nucleoside **15** (0.10 g, 0.12 mmol) was dissolved in acetonitrile (5 mL), and DIPAT (0.03 g, 0.17 mmol, 1.5 eq) and 2-cyanoethyl(*N*,*N*,*N*',*N*'-tetraisopropyl) phosphorodiamidite (0.03 g, 0.17 mmol, 1.5 eq) were added to the solution. After 4 h, the reaction mixture was poured on an ice-cooled saturated aqueous $NaHCO_3$ solution and washed with $NaHCO_3$ solution (3 x 25 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate. The solvent of the combined organic phases was evaporated. The crude was purified by column chromatography (silica 200 g, dichloromethane/ethyl acetate/triethylamine 45:45:10 *v*/*v*/*v*) and yielded a yellow solid **16** (0.07 g, 0.07 mmol, 60 %). R_f = 0.75 (SiO_2, dichloromethane/ethyl acetate/triethylamine 45:45:10 *v*/*v*/*v*). ^{31}P NMR (121 MHz, CD_3CN): δ = 147.97, 147.91 ppm.

Controlled pore glass loaded with 5-(18,18-dimethyl-4,7,10,13-tetraoxa-16,17-dithianonadec-1-inyl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-uridine (18). The following procedure is similar to that reported above for support **10**. Briefly, nucleoside **15** (100 mg, 0.12 mmol) and 4-dimethylaminopyridine (7.1 mg, 0.06 mmol, 0.5 eq) were dissolved in absolute pyridine (5 mL) and treated with succinic anhydride (11 mg, 11 mmol, 0.9 eq) for 72 h. After coevaporation with toluene (3 x 10 mL) the solid residue was dissolved in DCM (10 mL) and washed with ice cold citric acid (5 mL, 3.5 % in water *w*/*v*). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was dissolved in DCM (0.5 mL), followed by precipitation from ice-cold hexanea (15 mL). Intermediate **17** was obtained as a colorless foam (100 mg, 0.10 mmol, 85%). Compound **17** (190 mg, 0.2 mmol) was coupled to long-chain alkylamine controlled pore glass as described in the synthesis of **11**, above. For this, compound **17** (190 mg, 0.2mmol, 1 eq) was dissolved in DMF (1 mL) and HOBT (51.2 mg, 0.2 mmol, 1 eq), HBTU (6.6 mg, 0.18 mmol, 0.9 eq), and diisopropylethylamine (75 µl, 0.44 mmol, 2.2 eq) were added. The resulting mixture was shaken for 10 min. Then, the cpg (700 mg) was added, and the suspension was shaken for 2 h. The supernatant was removed and the cpg was washed with DMF and acetonitrile (both 2 x 1 mL) to yield **18** (690 mg) with a loading of 35 µmol/g, as determined by DMT deprotection, using an extinction coefficient ε_{498} = 71 000 $M^{-1}cm^{-1}$.

3. NMR-Spectra

Shown below are NMR spectra of the new compounds synthesized.

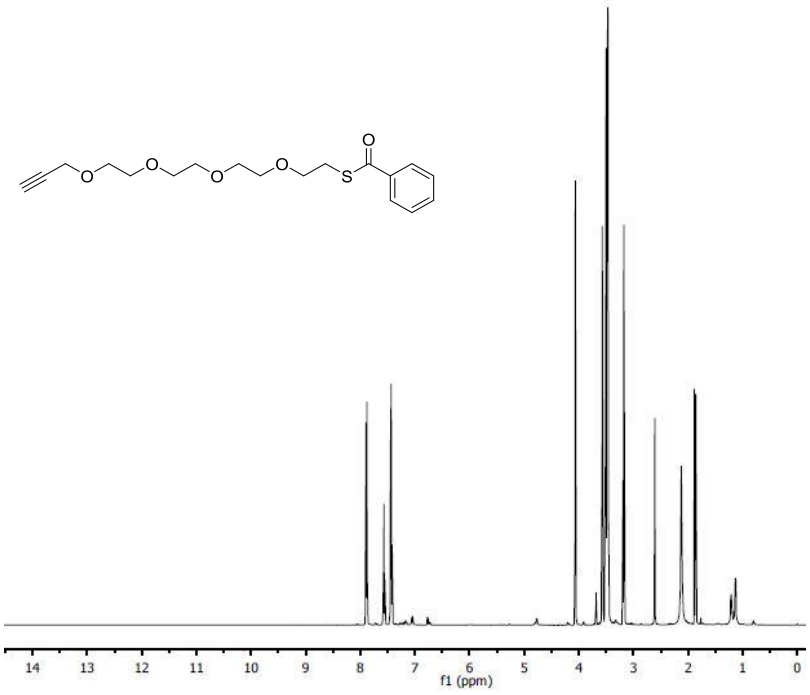

Figure S2. ^{1}H-NMR spectrum of compound **3** (500 MHz, CD_3CN).

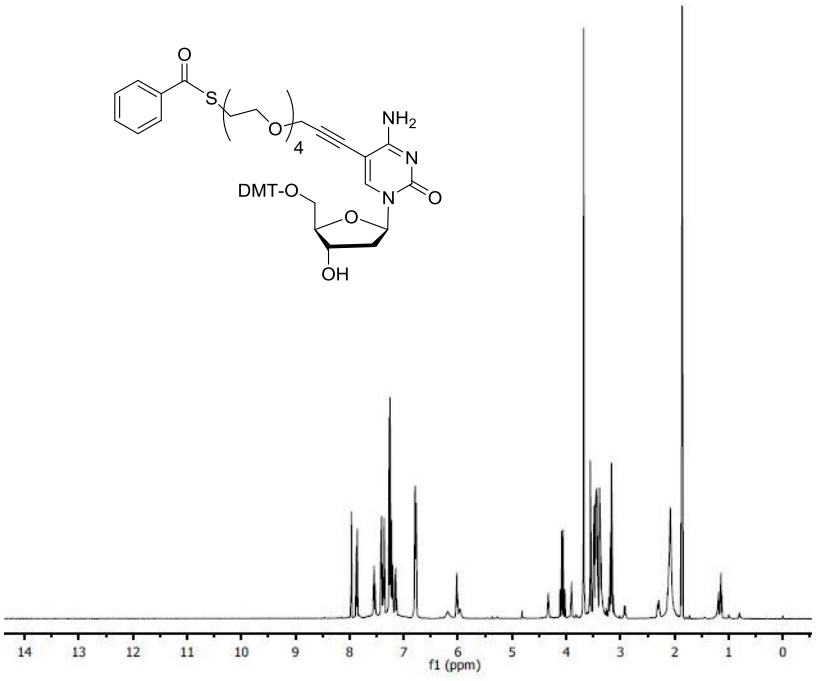

Figure S3. ^{1}H-NMR spectrum of compound **6** (500 MHz,CD_3CN).

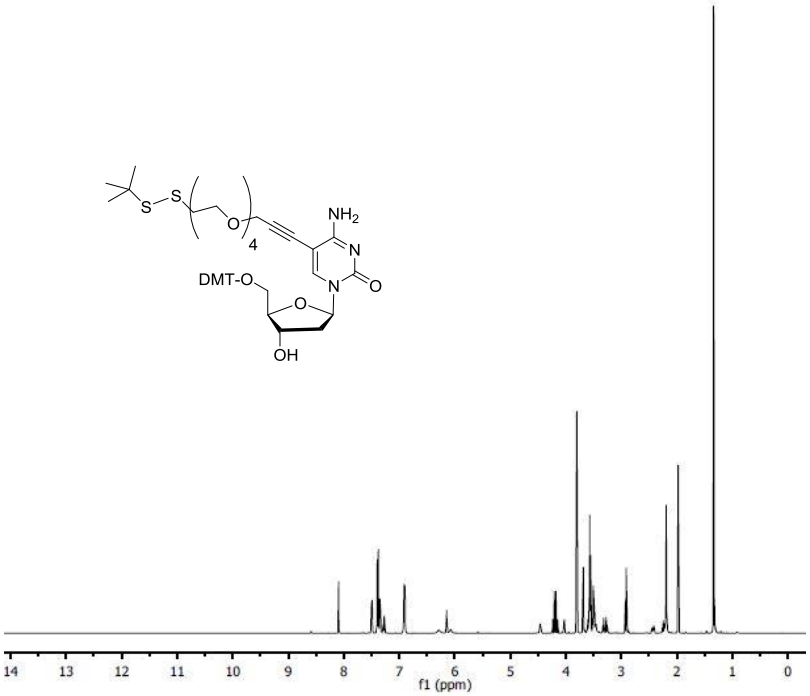

Figure S4. ^{1}H-NMR spectrum of compound **7** (500 MHz,CD_3CN).

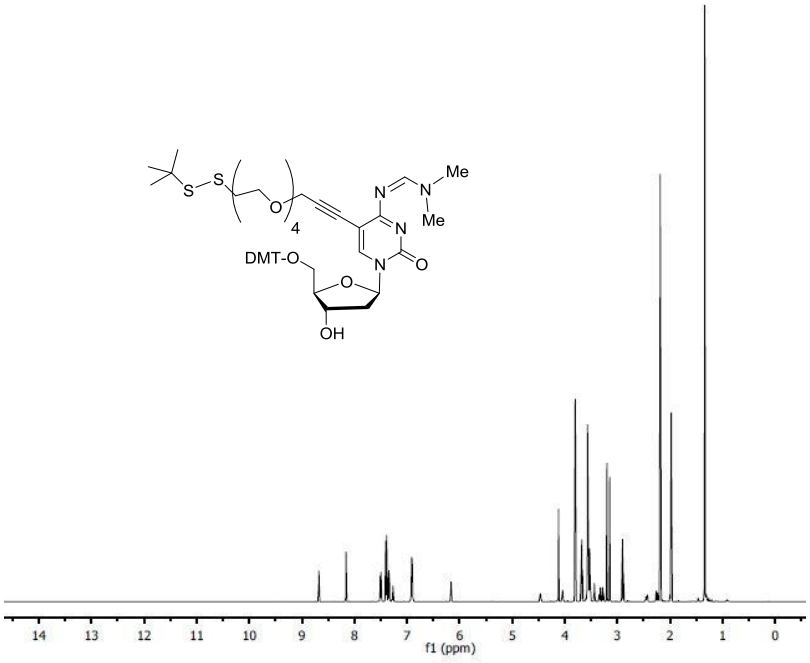

Figure S5. ^{1}H-NMR spectrum of compound **8** (500 MHz,CD_3CN).

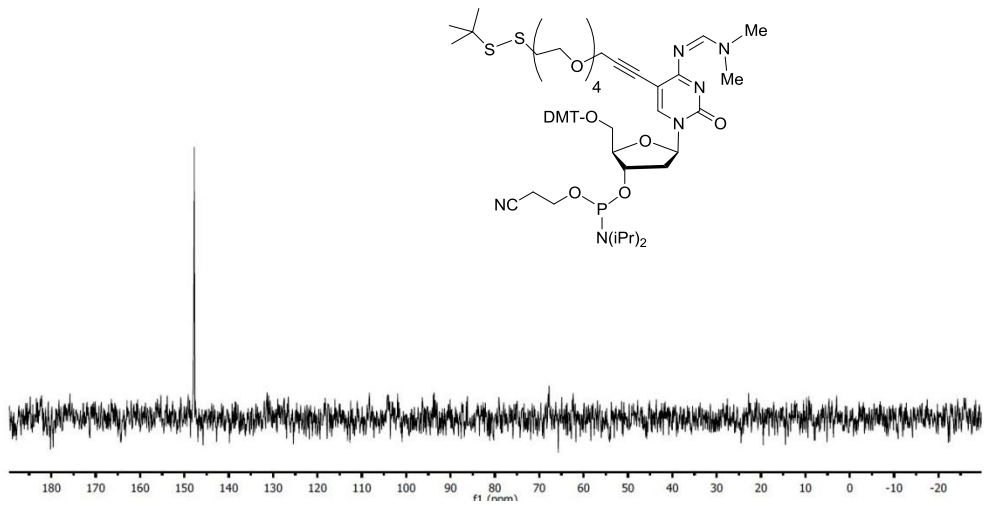

Figure S6. ^{31}P-NMR spectrum of compound **9** (161 MHz,CD_3CN).

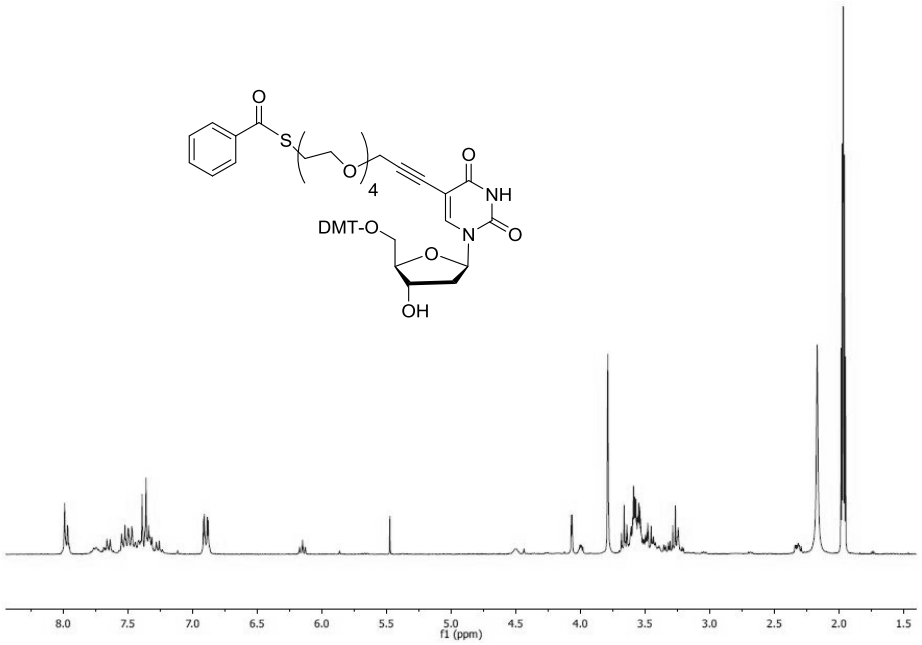

Figure S7. ^{1}H-NMR spectrum of compound **14** (300 MHz,CD_3CN).

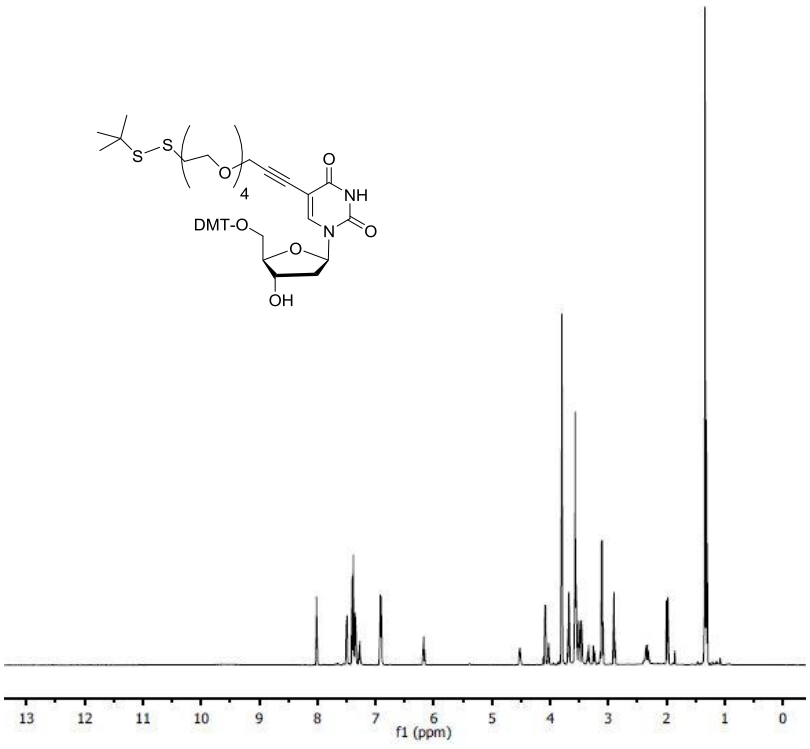

Figure S8. ^{1}H-NMR spectrum of compound **15** (500 MHz,CD_3CN).

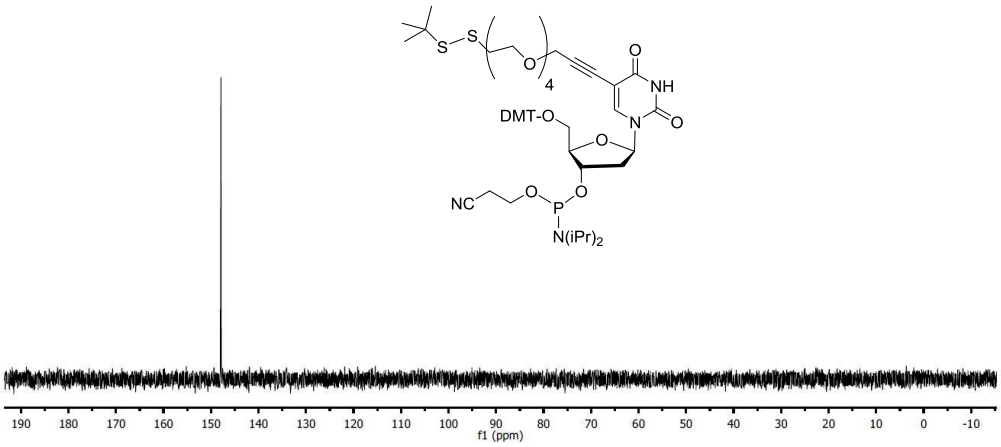

Figure S9. ^{31}P-NMR spectrum of compound **16** (121 MHz,CD_3CN).

4. Sequences and Structural Details

Sequences

Motiv I:
5'-TTTACGGTAGCTCAAGTCGGCTCATTTTTTTT-3'
5'-TGAGCCGACU*-3'
5'-U*GAGCTACCGTAAA-3'

Motiv II:
5'-TTCCCATTTTTTTTTTCGGAGAATCCGACGGAU*TTTTTTTTTTTTTTTTATAACTCATACA
CTTGTU*GCGATGAAGATGAAAGATTTTTU*AGCTACAGGA-3'
5'- AATGTGTAATTCTCCGTGGGAATCCTGTAGCU*-3'
5'- AU*CCGTCGGTGAGTTATTCTTTCATC -3'
5'- TCATCGCAU*-3'

Motiv III:
5'-CGTTGCAGTTTTTTTTCCAAC*GGAAAATATAGTTC*GCGATCCAGATGAC*GGATTT
TTTGAAGC*CAGGA-3'
5'-TTCAC*GTTTTTTTTTTCGAC*GAATCCGAC*GGG-3'
5'-CTATATTTATTC*GTCGC*GTGAATCCTGGC*TTC -3'
5'-CTGCAACGCCC*GTCGGTCC*GTTGGTCC*GTCATCTG-3'
5'-ATCGCGC*A -3'

Motiv IV:
5'-
AATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCA
CTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGC
CTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGC
CCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCA
GAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTC
CCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCC
ATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACAT
TTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTC
CTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAATGCGAATTTTAACAAAATATT
AACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTAT
CAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGATTCTCTTGT
TTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGTA-3'

5'-C*TGCCAGTU*GATCGCACCTGTTGGGAAGGGC*GATCGGTTTT-3'

5'-TTTTCAACATTAAATGTGAGCGAGTAACAAGTCTGGCCU*-3'

5'-U*CGCCCCGTCGGCGTGCAU*-3'

5'-C*ATTTTTTATTGACCGTAATGTAAAATTCGCATTATTT-3'

5'-C*GACTCTAGACAGGGTTTTCCCAGTCCTAU*-3'

5'-TTTGTATCGGCCTCAGGAAU*GAGGGGACGACGACATTT-3'

5'-U*TAAATTGTATTT-3'

5'-C*AATCATATGACAGGAAGATTACCAGCGGAAGTATAAGCAAATAU*-3'

5'-C*GATGAACGGGGCTATCAAATCAGCU*-3'

5'-U*AAGTTGGGTAACGCGGATCCCCATCAAAAATAAU*-3'

5'-TTTGGAGCAAAC*AATTGATAATTTT-3'

5'-U*ACGCCAGCTGGCGAAAGGGCATTCAGGCTGCGCAATCCAGCCAGCU*-3'

5'-TTTCAGTGCCAAGCU*TGCCAGCTTTCATTT-3'

5'-TTTGCGGGCCU*CTTCGACGACGTTGU*AAAACGACGGCTTT-3'

5'-TTTAACGTTAATATTTTGTGGATAGGTCACGU*-3'

5'-TTTAATTTTTGTTAGGTCATTGCCU*GAGAGTCTTTT-3'

5'-TTTCAGAAAAGCCCCAAAATACCCCGGGAGAAU*-3'

5'-C*GAATTTACAAATAATCGTAAAAGGCGAU*-3'

5'-U*CCTGTAGCATGCCTGCAGGU*-3'

5'-U*CGCGGATGTGCTGCAACTAGCATGU*-3'

5'-U*GGTGTAGTCTGGTGCGCAAAGCGCCAU*-3'

5'-C*CGTGGGAACAAACGGCGGAACCAATAGGAACGCCGGGTACCGAGCT-3'

5'-U*TCCGGCACCGCTATGGGCGCATCGTAACATTCU*-3'

Structural Details of Designs

The following drawings show the position of the DNA strands in the structures of the motifs, with cross-linked positions highlighted with boxes. In caDNAno designs, the deoxyuridine residues used for crosslinking are given as "T's" because the program calculates structures for unmodified DNA only.

Figure S10. Sequence of nicked duplex **I** with the cross-linking site highlighted with a black box. The two bases given in the box as T's are the disulfide-forming ones 2'-deoxyuridine residues in our crosslinking study.

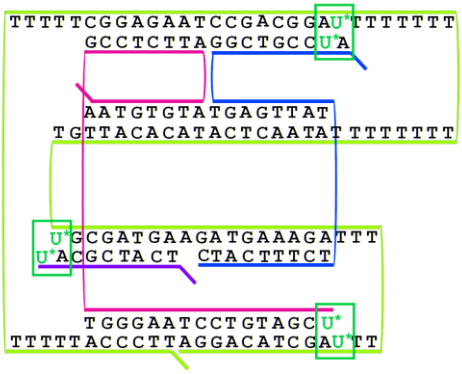

Figure S11. Structural details of motiv **II** with cross-linking sites highlighted with boxes. The bases marked with an asterisk are the disulfide-forming ones (U*).

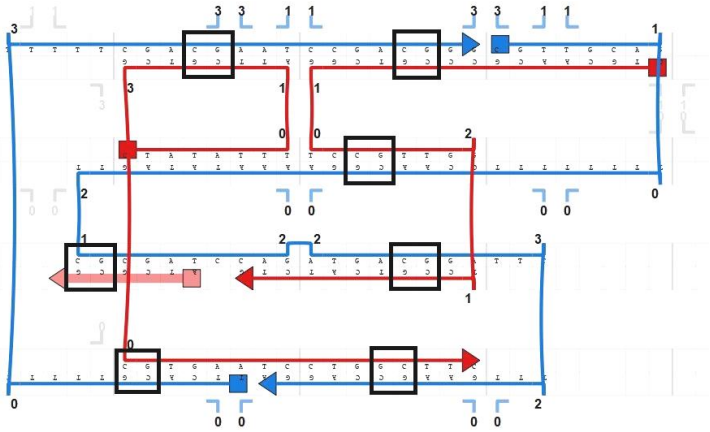

Figure S12. Structural details of motiv **III** with cross-linking sites highlighted with boxes. The boxes, the cytosine bases (C's) are the ones involved in disulfide cross-linking.

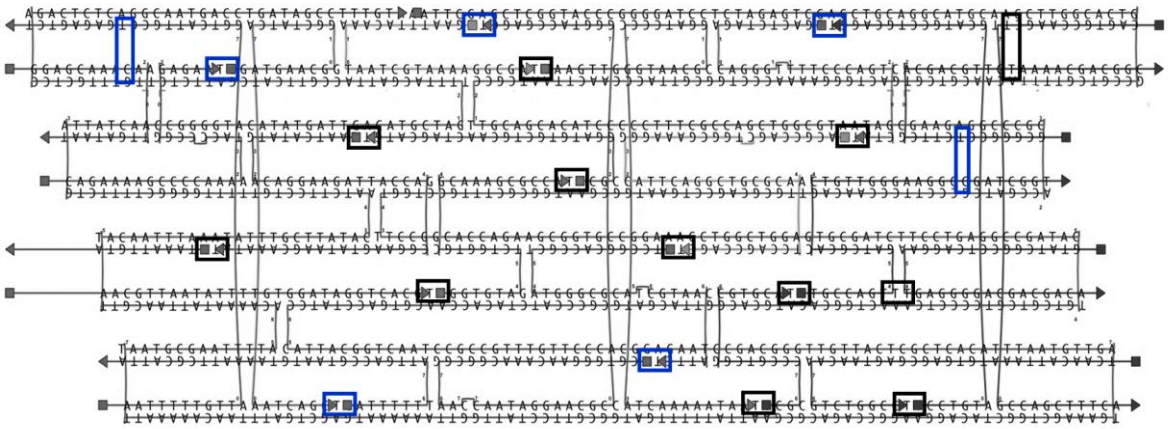

Figure S13. The caDNAno-based design of motif **IV**. Overhangs on both sides of the origami, consisting of stretches of oligo-dT, were used to avoid aggregation of the nanostructures in solution. Highlighted in blue are disulfide links between nucleotide combinations U/C, disulfides between U/U bases (shown as T/T in caDNAno) are highlighted in black.

5. References for Supplementary Information

S1. Hamamoto, S.; Takaku, H., *Chem. Lett.*, 1986, **8**, 1401-1404.

S2. M. Reddy, F. Farooqui and N. B. Hanna, *Tetrahedron Lett.*, 1995, **36**, 8929–8932.

S3. K.-L. Dao, R. R. Sawant, J. A. Hendricks, V. Ronga, V. P. Torchilin and R. N. Hanson, *Bioconjugate Chem.*, 2012, **23**, 785–795.

S4. L. J. Brown, J. P. May and T. Brown, *Tetrahedron Lett.*, 2001, **42**, 2587–2591.