Supporting Information

Interstrand interactions on DNA duplexes modified by TTF units at the 3' or 5'-ends

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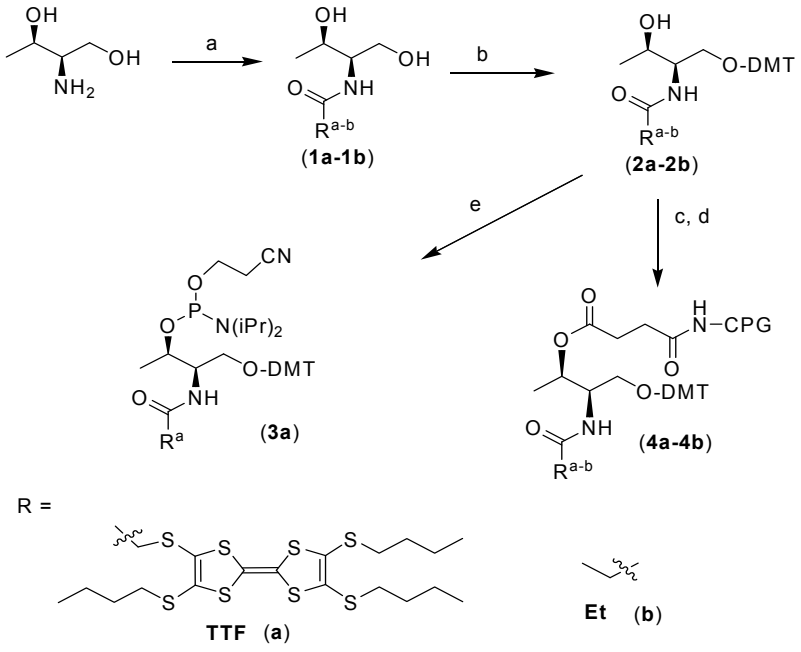
Experimental Section

General Information: All the standard phosphoroamidites and reagents for DNA synthesis were purchased from Applied Biosystems and from Link Technologies. L-threoninol and other chemicals were purchased from Sigma-Aldrich and Fluka. The DMF was degassed by three freeze-pump-thaw cycles before use. HPLC grade methanol was stored under molecular sieves. Dry solvents were purchased from Sigma-Aldrich and Fluka and used as supplied. NMR spectra were recorded at 25ºC on Varian Gemini 300 MHz, Varian Mercury 400 or Varian Inova 500 MHz spectrometers using deuterated solvents. Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^{1}H spectra recorded in $CDCl_3$ and the residual signal of the solvent (77.16 ppm) for ^{13}C- ^{31}P- and ^{19}F-spectra. For CD_3OD or d_6-acetone the residual signal of the solvent was used as a reference. Chemical shifts are reported in part per million (ppm) in the δ scale, coupling constants in Hz and multiplicity as follows: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), br (broad signal). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on a Voyager-DE^{TM}RP spectrometer (Applied Biosystems) in negative mode (2,4,6-trihidroxyacetophenone matrix with ammonium citrate as an additive). Electrospray ionization mass spectra (ESI-MS) were recorded on a Micromass ZQ instrument with single quadrupole detector coupled to an HPLC, and high-resolution (HR) ESI-MS on an Agilent 1100 LC/MS-TOF instrument.

Scheme S1. The preparation of the phosphoramidites and solid supports needed for the assembly of oligonucleotides. a) R-COOH, EDCI, 1-hydroxybenzotriazol, DIEA; b) dimethoxytrityl chloride, DMAP; c) succinic anhydride, DMAP; d) hemisuccinate, NH_2-controlled pore glass (CPG), TBTU, triethylamine; e) *O*-2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite.



{[4',5,5'-Tris(butylsulfanyl)-2,2'-bi-1,3-dithiol-4-yl]sulfanyl}acetic acid: (2-Cyanoethyl)sulfanyl TTF derivative (Rybáček, J.; Rybáčková, M.; Høj, M.; Bělohradský, M.; Holý, P.; Kilså, K.; Nielsen, M.B. *Tetrahedron* **2007**, *63*, 8840)(1.23 g, 2.22 mmol) was dissolved in anhydrous DMF (50 mL) and degassed. A solution of $CsOH•H_2O$ (0.43 g, 2.58 mmol) in anhydrous MeOH (5 mL) was added. After stirring the reaction mixture at room temperature for 30 min, a suspension of 3-bromoacetic acid (0.55 g, 3.94 mmol) and freshly annealed K_2CO_3 (1.8 g, 13 mmol) in anhydrous DMF (60 mL) were added. The reaction mixture was stirred at room temperature for 2 h. A solution of 1 M HCl (20 mL) was added and the mixture was extracted with EtOAc (3×100 mL). The combined organic phases were washed with water (3×100 mL), dried over anhydrous magnesium sulphate and concentrated to dryness under reduced pressure. The crude residue was purified by chromatography on silica gel (EtOAc-Me_2CO-EtOH-H_2O 80:12:5:3) providing a red amorphous solid (1.01 g, 81%). TLC (EtOAc-Me_2CO-EtOH-H_2O 72:12:10:6) R_f=0.61. ^{1}H NMR, δ_H (500 MHz, d_6-acetone): 3.74 (bs, 2H), 2.90 (m, 6H), 1.64 (m, 6H), 1.46 (m, 6H), 0.930, 0.932 (2 x t, J = 7.3 Hz, 9H).^{13}C NMR, δ_H (126 MHz $CDCl_3$): 169.7 (C), 130.3 (C), 128.6 (C), 128.5 (C), 127.3 (C), 110.6 (C), 110.4 (C), 38.0 (CH_2), 36.5 (CH_2), 36.3 (CH_2), 32.5 (CH_2), 32.5 (CH_2), 22.1 (CH_2), 13.8 (CH_3). IR ($CHCl_3$): ν = 3503, 3093, 2961, 2931, 2875, 2863, 2673, 2562, 1714, 1603, 1486, 1465, 1459, 1433, 1418, 1381, 1291, 1195, 1101, 914, 886, 458 cm^{-1}. UV/Vis: λ_{max} (log ε) = 264 nm (4.12), 334 nm (4.11). MS (ESI+): *m/z*: 581 ($[M+Na]^+$), 558 ($[M]^+$). HRMS (ESI+): *m/z*: calc for $C_{20}H_{30}O_2NaS_8$ 580.9904, found 580.9902.

{[4',5,5'-Tris(butylsulfanyl)-2,2'-bi-1,3-dithiol-4-yl]sulfanyl}-*N*-[(2*R*,3*R*)-1,3-dihydroxybutan-2-yl)acetamide (1a): A solution of **TTF** carboxylic acid (0.54 mmol), L-threoninol (0.54 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.81 mmol), hydroxybenzotriazole (HOBt) (0.81 mmol) and diisopropylethylamine (0.81 mmol) in anhydrous DMF (10 mL) was prepared under argon. After stirring the reaction mixture at room temperature overnight, the mixture was concentrated to dryness under reduced pressure. The residue was dissolved in toluene and concentrated to dryness under reduced pressure (3x). The residue was dissolved in dichloromethane (100 mL) and the organic phase was washed with 10% aqueous $NaHCO_3$ (2x 50 mL) and saturated aqueous NaCl (50 mL). The organic phase was dried and concentrated to dryness under reduced pressure. The crude product was purified by chromatography on silica gel under elution with a gradient of methanol from 0 to 10% in DCM. The desired compound was obtained as orange oil (255 mg, 73%). TLC (2% methanol-dichloromethane) R_f=0.34. ^{1}H NMR, δ_H (,400 MHz, $CDCl_3$): 4.24-4.22 (qd, J = 6.4 and 1.6 Hz, 1H), 3.88-3.80 (m, 3H), 3.59 (d, J = 16.4 Hz, 1H), 3.57 (d, J = 16.4 Hz, 1H), 2.82-2.73 (m, 6H), 1.59-1.53 (m, 6H), 1.41-1.35 (m, 6H), 1.22 (d, J = 6.4 Hz 3H), 0.86 (t, J = 7.8 Hz, 9H). ^{13}C NMR, δ_C (100 MHz, $CDCl_3$):168.3 (C), 130.1 (C), 129.3 (C), 128.2 (C), 124.4 (C), 113.4 (C), 106.9 (C), 68.8 (CH), 64.8 (CH_2), 55.6 (CH), 39.5 (CH_2), 36.4 (CH_2), 36.2 (CH_2), 32.0 (CH_2), 21.9 (CH_2), 20.7 (CH_3), 13.8 (CH_3). MS (ESI-): *m/z*: calc for $C_{24}H_{39}NO_3S_8$ 646.08; found 645.07 ($[M-H]^-$).

***N*-[(2*R*,3*R*)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)-{[4',5,5'-tris(butylsulfanyl)-2,2'-bi-1,3-dithiol-4-yl]sulfanyl}acetamide (2a)**: The compound 1a (0.33 mmol) was dried by evaporation of anhydrous acetonitrile (ACN) under reduced pressure. The product was dissolved in anhydrous pyridine (5 mL) and reacted with 4,4'-dimethoxytriphenylmethyl chloride (0.36 mmol) and 4-dimethylaminopyridine (0.33 mmol, DMAP). After stirring for 6 h at room temperature, 4,4´-dimethoxyltritylchloride (0.33 mmol) and DMAP (0.33 mmol) dissolved in dry pyridine (5 mL) were added and the mixture was stirred at room temperature overnight. The reaction was quenched with methanol (0.5 mL) and the solvents were removed under reduced pressure. The residue was dissolved in toluene (3×10 mL) and concentrated to dryness. Then, the resulting material was dissolved in dichloromethane (100 mL) and the organic phase was washed with 5% aqueous $NaHCO_3$ (50 mL) and with saturated aqueous NaCl (50 mL). The solvent was evaporated and the residue was purified by chromatography on

neutral aluminium oxide. The product was eluted with a slow gradient of ethyl acetate from 50 to 100% in hexane and then 0 to 10% of methanol in ethyl acetate. The pure compound was obtained as an intense orange solid (294 mg, 50%). TLC (aluminium oxide) (ethyl acetate-hexane 1:1) R_f=0.20. ^{1}H NMR, δ_H (400 MHz, $CDCl_3$): 7.40-6.82 (m, 13H), 4.12 (qd, *J* = 6.2 and 2.8 Hz, 1H), 3.97-3.91 (m, 1H), 3.79 (s, 6H), 3.61 (d, *J* = 16.4 Hz, 1H), 3.52 (d, *J* = 16.4 Hz, 1H), 3.41 (dd, *J* = 9.6 and 4.8 Hz, 1H), 3.30 (dd, *J* = 9.6 and 4.0 Hz, 1H), 2.83-2.78 (m, 6H), 1.65-1.55 (m, 6H), 1.48-1.35 (m, 6H), 1.13 (d, *J* = 6.4 Hz, 3H), 0.94-0.88 (m, 9H). ^{13}C NMR, δ_C (100 MHz, $CDCl_3$): 167.4 (C), 158.8 (CH), 144.5 (C), 135.7 (C), 135.5 (C), 130.8 (C), 130.2 (CH), 129.3 (C), 128.4 (C), 128.2 (CH), 127.6 (C), 127.2 (C), 124.5 (C), 113.5 (CH), 112.7 (C), 108.8 (C), 87.0 (C), 68.5 (CH), 64.9 (CH_2), 55.5 (CH_3), 54.5 (CH), 39.5 (CH_2), 36.5 (CH_2), 36.2 (CH_2), 32.0 (CH_2), 31.9 (CH_2), 21.9 (CH_2), 21.8 (CH_2), 20.2 (CH_3), 13.8 (CH_3), 13.8 (CH_3), 13.8 (CH_3). MS (ESI-): *m/z*: calc for $C_{45}H_{57}NO_5S_8$ 948.46; found 947.20 ([M-H]$^-$).

Functionalization of CPG solid supports (4a-b): The DMT derivatives (**2a-b**) were reacted with succinic anhydride and then they were incorporated on a long-chain alkylamine-controlled pore glass support (LCAA-CPG) as follows. The DMT derivative (0.09 mmol) was dried by evaporation with anhydrous ACN under reduced pressure. The residue was dissolved in anhydrous pyridine (5 mL) under argon. Succinic anhydride (0.22 mmol) and DMAP (0.05 mmol) were dissolved in 1 mL of pyridine and added to the solution. After 4 h of stirring at room temperature, succinic anhydride (0.22 mmol) and DMAP (0.05 mmol) were dissolved in 1 mL of pyridine and added to the solution. The reaction mixture was allowed to react overnight. The solvent was removed under reduced pressure and the residue was dissolved in toluene (3×10 mL) and concentrated to dryness. The resulting material was dissolved in dichloromethane (20 mL). The solution was washed with 0.1 M sodium monophosphate (15 mL) and saturated aqueous NaCl (15 mL). The solvent was evaporated under reduced pressure. The resulting monosuccinate derivative was used in the next step without further purification.

Amino-LCAA-CPG (CPG New Jersey, 73 μmol amino/g, 300 mg) was placed into a polypropylene syringe fitted with a polypropylene disc and washed sequentially with DMF, methanol, THF, DCM and ACN (2x 5 mL). Then, a solution of the appropriate homosuccinate (44 μmol) and triethylamine (175 μmol) in anhydrous ACN (0.5 mL) was prepared. The solution was added to the support and then a solution of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (175 μmol) in anhydrous ACN (300 μL) was added. The mixture was left to react for 1 h. The support was washed with DMF, methanol, DCM and ACN (2x 5 mL). The degree of functionalization was determined by analysis of the DMT released upon the treatment of an aliquot of the support with the dichloroacetic acid. The coupling procedure was repeated once more if needed and the functionality of the resin was determined by DMT quantification (f=20 μmol/g). Finally, the solid support was treated with the mixture of Ac_2O/DMF (1:1, 500 μL) to cap free amino groups.

***N*-[(2*R*,3*R*)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-O^3-(2-cyanoethyl-*N*,*N*-diisopropylaminophosphinyl)-3-hydroxybutan-2-yl)-{[4',5,5'-tris(butylsulfanyl)-2,2'-bi-1,3-dithiol-4-yl]sulfanyl}acetamide (3a)**: The compound 2a (0.21 mmol) was dried by evaporation with anhydrous ACN under reduced pressure. The residue was dissolved in anhydrous DCM (10 mL) and diisopropylethylamine (DIEA) (0.84 mmol) was added under argon. The solution was cooled to 0 ºC in a ice bath and 2-cyanoethoxy-*N,N'*-diisopropylaminochlorophosphine (0.32 mmol) was added dropwise with a syringe. Afterward, the solution was stirred at room temperature for 1.5 h. Then, the solution was cooled to 0 ºC and DIEA (0.32 mmol) and 2-cyanoethoxy-*N,N'*-diisopropylaminochlorophosphine (0.10 mmol) were added. The reaction mixture was allowed to react at room temperature for another 1.5 h. DCM (10 mL) was added and the organic layer was washed with saturated aqueous NaCl (20 mL). The solvent was evaporated under reduced pressure

and the residue was purified by column chromatography on neutral aluminium oxide. The product was eluted with hexane/ethyl acetate 2:1 and then ethyl acetate. The desired compound was obtained as orange foam (56 mg 23%). TLC (aluminium oxide) (ethyl acetate-hexane 1:1) R_f=0.48 and 0.44. ^{1}H NMR, δ_H (400 MHz $CDCl_3$): 7.43-6.79 (m, 13H), 6.10 and 6.07 (2xd, J = 6.0 and 5.7 Hz, respectively, two isomers, 1H), 4.33-4.08 (m, 2H), 3.79 (s, 6H), 3.47-3.37 (m, 6H), 3.26-3.08 (m, 2H), 2.76-2.68 (m, 6H), 2.34-2.29 (m, 2H), 1.56-1.47 (m, 6H), 1.41-1.30 (m, 6H), 1.22-0.93 (m, 15H), 0.87-0.81 (m, 12H). ^{31}P NMR, δ_P (81 MHz, $CDCl_3$): 150.87, 148.40 and 147.92 (mixture of isomers including amide *cis* and *trans* conformers). ^{13}C NMR, δ_C (100 MHz, $CDCl_3$): 167.0 (C), 158.7 (CH), 145.0 and 144.9 (C, two isomers), 136.13 (C), 130.8 (C), 130.4 (CH), 130.3 and 130.2 (CH, two isomers), 129.9 (C), 128.5 and 128.4 (CH, two isomers), 128.3 (C), 128.2 and 128.0 (CH, two isomers), 127.3 (C), 127.0 (C), 125.2 and 125.1 (C, two isomers), 118.1 (C), 113.5 and 113.3 (CH, two isomers), 113.3 (CH), 112.0 (C), 109.0 (C), 86.5 and 86.4 (C, two isomers), 68.8 (d, J = 15.9 Hz, CH), 62.6 (CH_2), 58.6 (d, J = 19.4 Hz, CH_2), 55.5 and 55.4 (CH_3, two isomers), 55.0 (d, J = 4.8 Hz, CH), 43.5-43.3 (m, CH), 39.7 and 39.7 (CH_2, two isomers), 36.4 (CH_2), 36.2 (CH_2), 36.2 (CH_2), 32.0 (CH_2), 31.9 (CH_2), 31.9 (CH_2), 25.0-24.7 (m, CH_3), 21.8 (CH_3), 20.7 and 20.5 (CH_3, 2xd, J = 6.8 and 6.8 Hz, respectively, two isomers), 19.9-19.8 (m, CH_2), 13.83 (CH_3), 13.81 (CH_3), 13.78 (CH_3). MS (ESI+): *m/z*: calc for $C_{54}H_{74}N_3O_6PS_8$ 1146.70; found 1147.31 ($[M+H]^+$).

***N*-[(2*R*,3*R*)-1,3-Dihydroxybutan-2-yl)-propionamide (1b):** To a solution of propionic acid (1.7 mmol) in THF (6 mL) at room temperature, *N*-hydroxybenzotriazole (1.57 mmol) and diisopropylcarbodiimide (1.57 mmol) were added. After stirring the mixture for 5 min, threoninol (1.43 mmol) was added. The mixture was stirred at room temperature for 24 h and then quenched with methanol. The solvent was evaporated under vacuum and the residue purified by flash chromatography (hexane-CH_2Cl_2- MeOH 3:3:0.5). The compound **1b** was isolated as a white solid (198 mg, 86%). ^{1}H NMR, δ_H (300 MHz, acetone-d_6): 6.72 (bs, 1H), 4.06 (dq, J = 6.4 and 2.9 Hz, 1H), 3.98 (bs, 1H), 3.76 (dtd, J = 8.5, 5.7 and 2.9 Hz, 1H), 3.61 (dd, J = 5.7 and 2.3 Hz, 2H), 2.93 (bs, 1H), 2.25 (dq, J = 7.6 and 1.6 Hz, 2H), 1.10 (d, J = 6.3 Hz, 3H), 1.08 (t, J = 7.6 Hz, 3H). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 175.7 (C), 68.1 (CH), 64.4 (CH_2), 57.7 ((CH), 30.8 (CH_2), 21.7 (CH_3), 11.4 (CH_3). HRMS (ESI+): *m/z*: calc for $C_7H_{15}NO_3Na$ ($[M + Na]^+$) 184.0944, found 184.0957.

***N*-[(2*R*,3*R*)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)- propionamide (2b):** To a solution of diol **1b** (0.95 mmol) in pyridine (4.7 mL) at 0º C, diisopropylethylamine (1.42 mmol) and 4,4´-dimethoxyltritylchloride (1.42 mmol) were added. After 15 min the mixture was allowed to reach room temperature, then it was stirred for 24 h and finally the reaction was quenched with methanol. The solvent was evaporated under vacuum and the residue purified by flash chromatography (hexane- EtOAc 1:3). The compound **2d** was isolated as a yellowish solid (232 mg, 52%). ^{1}H NMR, δ_H (400 MHz, $CDCl_3$): 7.33 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 8.8 Hz, 6H), 7.27-7.15 (m, 1H), 6.79 (d, J = 8.8 Hz, 4H) 6.02 (d, J = 8.7 Hz, 1H), 4.05 (d, J = 6.3 Hz, 1H), 3.92-3.85 (m, 1H), 3.75 (s, 6H), 3.31 (System ABX, J_{AB} = 9.6 Hz, J_{AX} = 4.3 Hz, J_{BX} = 3.5 Hz, 2H), 3.02 (s, 1H), 2.22 (dq, J = 7.7 and 1.4 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H), 1.09 (d, J = 6.4 Hz, 3H). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 174.1 (C), 158.6 (2xC, C), 144.3 (C), 135.5 (C), 135.3 (C), 129.9 (2xC, CH), 129.8 (2xC, CH) , 127.9 (2xC, CH), 127.8 (2xC, CH), 127.0 (CH), 113.3 (4xC, CH), 86.8 (C), 68.8 (CH_2), 65.4 (CH), 55.2 (2xC, CH_3), 53.2 (CH), 29.8 (CH_2), 19.9 (CH_3), 9.9 (CH_3). HRMS (ESI+): *m/z*: calc for $C_{28}H_{33}NO_5Na$ ($[M + Na]^+$) 486.2250, found 486.2250.

Oligonucleotide synthesis: The unmodified oligonucleotide sequnces **1** ($^{5'}$CCAATTGG$^{3'}$), **3** ($^{5'}$TAGAGGCTCCATTGC$^{3'}$) and **4** ($^{5'}$GCAATGGAGCCTCTA$^{3'}$) were synthesized on a DNA synthesizer (*Applied Biosystems 3400*) using 200-nmol scale LV200® polystyrene supports and commercially available chemicals. The benzoyl (Bz) group was used for the protection of the amino group of C and A, and the isobutyryl (^{i}Bu) group for the protection of G. The coupling yields were >97%. The last DMT group was removed at the end of the synthesis. Each solid support was treated

with aqueous concentrated ammonia at 55 ºC for 12 h to cleave the products from the supports and remove the benzoyl and isobutyryl groups. The mixtures were filtered and ammonia solutions were concentrated to dryness. Unmodified oligonucleotides were desalted with with *Sephadex G-25* (*NAP-10* column) and used without further purification. TTF- and Et-modified oligonucleotides were purified by HPLC on an XBridge™ OST C_{18} semipreparative column (10x50 mm, 2.5 µm). Solvent A: 5% ACN in 100 mM of triethylammonium acetate (pH=7) and solvent B: 70% ACN in 100 mM of triethylammonium acetate (pH=7). The resulting products were desalted with *Sephadex G-25* (*NAP-10* column) and analyzed by HPLC. Column: XBridge™ OST C_{18} (4.6x50 mm, 2.5 µm). Solvent A: 5% ACN in 100 mM triethylammonium acetate (pH=7) and solvent B: 70% ACN in 100 mM triethylammonium acetate (pH=7). Flow rate: 1 mL/min. Conditions: 20 min of linear gradient from 0 to 20% B. The resulting oligomers were analyzed by mass spectrometry (MALDI-TOF) and UV/Vis spectroscopy.

The oligonucleotide sequences containing the **TTF** modification at the 3'-end: **2** ($^{5'}$CCAATTGG$^{3'}$-**TTF**), **5** ($^{5'}$TAGAGGCTCCATTGC$^{3'}$-**TTF**) and **6** ($^{5'}$GCAATGGAGCCTCTA$^{3'}$-**TTF**) were synthesized on a 0.5 µmol scale using the solid support functionalized with the TTF derivative as described above. The last DMT group was removed at the end of the synthesis. Each resin was treated with aqueous concentrated ammonia at 55 ºC for 4 h to cleave the products from the supports and to remove the Bz and iBu groups. In addition the dimethylformamidino (dmf) group for the protection of G was used with similar results but, in this case, the ammonia treatment was run at room temperature for 4 hours (data not shown). Work-up was similar as described above. Products were purified by HPLC on an XBridge™ OST C_{18} semipreparative column (10x50 mm, 2.5 µm). Solvent A: 5% ACN in 100 mM of triethylammonium acetate (pH=7) and solvent B: 70% ACN in 100 mM triethylammonium acetate (pH=7). Flow rate: 3 mL/min. Conditions: 4 min of linear gradient from 0 to 12% B, then 3 min of linear gradient from 12 to 70% B, then 5 min of linear gradient from 70 to 75% B, then 5 min of linear gradient from 75 to 100% (at 60 ºC for sequence **2**). The purified oligomers were analyzed by mass spectrometry (MALDI-TOF), UV/Vis and HPLC. Column: XBridge™ OST C_{18} (4.6x50 mm, 2.5 µm) using the conditions described above except the flow rate that was 1 mL/ min.

The oligonucleotide sequences **7** (**TTF**-$^{5'}$TAGAGGCTCCATTGC$^{3'}$) and **8** (**TTF**-$^{5'}$GCAATGGAGCCTCTA$^{3'}$) were synthesized on a 200 nmol scale employing LV200® polystyrene supports as described above. **TTF**-phosphoramidite was used to incorporate **TTF** at the 5'-end. In this case, the protected phosphoramidite was dissolved in anhydrous DCM instead of ACN (to obtain a 0.1 M solution) and was allowed to react for 300 s instead of 30 s used for an unmodified phosphoramidite. The DMT determination showed that the efficiency of coupling of the TTF-phosphoramidite was 87%. For the synthesis of the sequence **8**, the coupling time for the modified phosphoramidite was reduced to 30 s.

The oligonucleotide **9**, ($^{5'}$TAGAGGCTCCATTGCX$^{3'}$, X being the ethyl (**Et**) modification) was synthesized on a 1 µmol scale using the corresponding solid support functionalized with the appropriate threoninol derivative. The synthesis and purification was carried out as described above.

Melting experiments on self-complementary oligonucleotides: An effect of the salt concentration was investigated on the modified self-complementary oligonucleotide **2** and the unmodified DNA duplex **1** (Figure 5). Melting experiments were performed in duplicate at 6.6 µM concentration of duplex. The solutions of oligonucleotides were prepared at concentrations ranging from 50 mM to 1 M NaCl containing 10 mM sodium phosphate buffer (pH=7). The samples were heated at 90 ºC for 5 min, allowed to cool slowly to room temperature to induce annealing and then kept overnight in a refrigerator (4 ºC). The melting curves were recorded by heating the samples with a temperature controller from 10 to 80 ºC at a constant rate of 1 ºC/min when monitoring the absorbance at 260

nm. During the experiment, when the temperature was below 25 ºC, argon was flushed to prevent water condensation on the cuvettes. The absorption spectra and melting experiments (absorbance vs. temperature) were recorded in 1 cm path-length cells. The T_m values were calculated with the *Meltwin 3.2* software.

At the low NaCl concentration (50 mM-200 mM), the standard denaturation curve of a DNA duplex was observed. The melting temperature (T_m) increased with the salt concentration. At the high salt concentration (500 mM and 1 M) no transition was observed. The results are summarized in Table S1.

Melting experiments performed with non self-complementary oligonucleotides: The melting experiments were performed in duplicate at 3.3 μM concentration of oligonucleotide. The solutions were prepared at concentrations either 50 mM or 1 M NaCl and 10 mM sodium phosphate buffer (pH=7). The samples were heated at 90 ºC for 5 min, allowed to cool slowly to room temperature to induce annealing and then kept overnight in a refrigerator at 4 ºC. The melting curves were recorded by heating the samples with a temperature controller from 10 to 80 ºC or from 0 to 95 ºC at a constant rate of 1 ºC/min and when monitoring the absorbance at 260 nm or 300 nm (depending on the experiment). During the experiment, when the temperature was below 25 ºC, argon was flushed to prevent water condensation on the cuvettes. The absorption spectra and melting experiments (absorbance vs. temperature) were recorded in 1 cm path-length cells. The T_m values were calculated with the *Meltwin 3.2* software.

Dynamic Light Scattering (DLS) experiments. DLS measurements were performed using a Zetasizer Nano S instrument (4 mW He-Ne red laser (633 nm), Malvern Instruments). The detector was located at 173º relative to the laser soured, so backscatter detection was used. An auto attenuator fitted inside the instrument ensured that the sample count rate was suitable for the requirements of the detector (avalanche photodiode, Q.E.>50% at 633 nm). A cumulants analysis of the correlation functions of scattering data was performed to obtain information of the z-average diffusion coefficient and the polydispersity index (PDI). The cumulants analysis was actually the fit of a polynomial to the logarithm of the correlation function. For monomodal particle distribution, the diffusion coefficient was obtained from the second order term of the polynomial fit. This was converted to size via the Stokes-Einstein equation using the dispersant viscosity and some instrumental constants. All these data (hydrodynamic diameter and polydispersity index) were obtained using the software provided by the manufacturer. As the intensity of scattered light for large particles is bigger than for small particles, the software of the Zetasizer Nano S transforms the intensity distribution into volume and number distributions based on the Mie theory. For that reason once the measurements were performed, the results were presented as a size distribution by number of particles.

Solutions of oligonucleotide **2** (6.6 μM of duplex) were prepared using 50 mM or 1M sodium chloride and 10 mM sodium phosphate buffer (pH=7). Buffers were filtered trough 0.2 μm nylon membrane filter (Acrodisc®, PALL) into dust-free microtubes. Samples were heated at 90ºC for 5 minutes, allowed to cool down slowly to room temperature to induce annealing and kept in a refrigerator (4ºC) for 24 h or 48 h. The solutions were pre-equilibrated for at least 5 minutes at the required temperature before the measurement. The experiments were performed in low volume glass cuvettes (45 μL). DLS measurements were performed in duplicate and at least three measures from each sample were recorded. Each measure was presented as the average value of 20 runs. The final value was presented as the average of all measures recorded. All the measurements were performed in automatic mode and with the following specifications: medium viscosity, 1.1442 cP (water); refractive index (RI) dispersant, 1.33 (water) and refractive index material, 1.45.

Transmission Electron Microscopy (TEM) experiments. Solutions of oligonucleotide **2** (6.6 μM of duplex) were prepared using 1 M sodium chloride and 10 mM sodium phosphate buffer (pH=7). The buffer was filtered trough 0.2 μm nylon membrane filter (Acrodisc®, PALL) into dust-free microtubes. Samples were heated at 90ºC for 5 min, allowed to cool down slowly to room temperature and kept for 24 h or 48 h at 4 ºC. Prior to the spreading of the sample, carbon-coated grids (400-mesh, Ted Pella Inc.) were glow discharged and activated with a 5 μL drop of 0.01% of polylysine (10 seconds). Then the grid was placed on a screw cap of an inverted microtube containing 70 μL of the sample. The inverted tubes were centrifuged at 4ºC for 10 minutes at 1500 *g* on a swinging bucket rotor. Then stained with a 2 μL drop of 2% uranyl acetate (1 minute) and rinsed with MilliQ water (5 μL drop, 30 seconds). Some samples were spread by direct adsorption onto the grid (5 μL drop, 5 min) after polylysine activation. The best yield of deposition of the DNA spheres was obtained by centrifugation spreading. Finally the sample was dried in a desiccator overnight. After every treatment, the excess of liquid was removed with the help of a filter paper. The grid was held with tweezers for the deposition of the drops. Imaging was performed using a Tecnai Spirit TWIN (FEI) operated at 120 kV. The size of the spheres was obtained analyzing the digital micrographs with the Analysis program and then statistically processed and fitted to a Gauss curve with Origin 8.0.

Figure S1. HPLC profiles of A) crude and purified oligonucleotide **2**, B) crude and purified oligonucleotide **5**, and C) crude and purified oligonucleotide **6**, (black lines correspond to crudes and blue lines correspond to purified oligonucleotides).

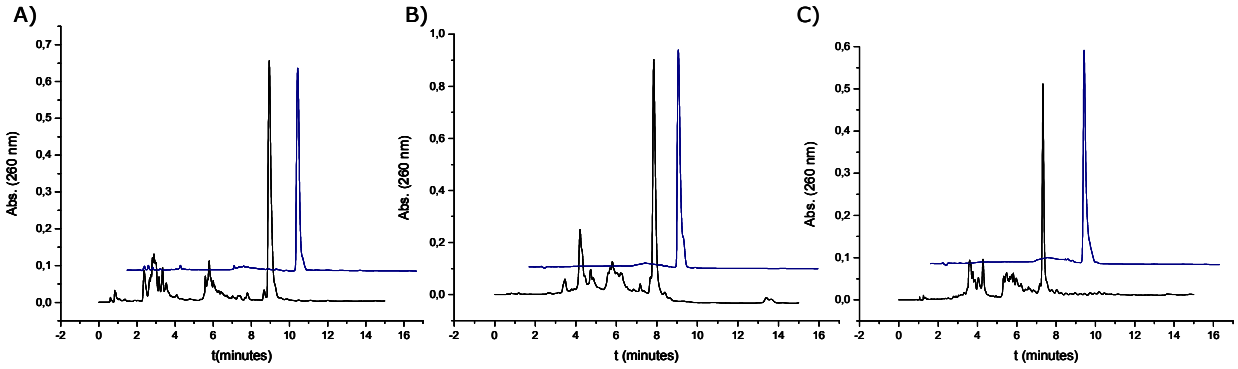
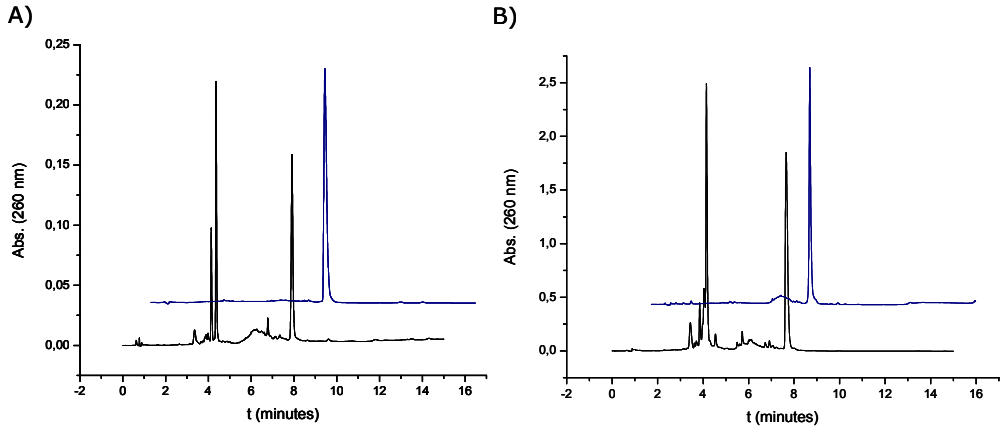


Figure S2. HPLC profiles of A) crude and purified sequence **7** and B) sequence **8** (black lines correspond to crudes and blue lines correspond to purified oligonucleotides).



Characterization data of oligonucleotides

Unmodified (desalted by Sephadex G-25)

Sequence 1: t_R=5.3 min. λ=259 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, $[M-H]^-$) calculated for $C_{78}H_{99}N_{30}O_{46}P_7$ 2409.6, found 2407.0). Yield was 11 OD units at 260 nm (73%).
Sequence 3: t_R=7.8 min. λ=260 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, $[M-H]^-$) calculated for $C_{146}H_{185}N_{55}O_{89}P_{14}$ 4568.0, found 4563.8). Yield was 24 OD units at 260 nm (85%).
Sequence 4: t_R=7.7 min. λ=259 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, $[M-H]^-$) calculated for $C_{146}H_{184}N_{58}O_{87}P_{14}$ 4577.0, found 4572.8). Yield was 23 OD units at 260 nm (79%).

TTF-modified (HPLC purified and desalted by Sephadex G-25)

Sequence 2: t_R=8.9 min. λ=260 and 332 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, $[M-H]^-$) calculated for $C_{102}H_{137}N_{31}O_{51}S_8P_8$ 3116.9, found 3112.6). Yield was 8 OD units at 260 nm (21%).

Sequence 5: t_R=7.8 min. λ=257 and 332 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, [M-H]$^-$) calculated for $C_{170}H_{223}N_{56}O_{94}S_8P_{15}$ 5275.3 found 5272.6). Yield was 13 OD units at 260 nm (19%).

Sequence 6: t_R=7.3 min. λ=258 and 332 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, [M-H]$^-$) calculated for $C_{170}H_{222}N_{59}O_{92}S_8P_{15}$ 5284.3, found 5279.7). Yield was 18 OD units at 260 nm (25%).

Sequence 7: t_R=7.9 min. λ=259 and 332 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, [M-H]$^-$) calculated for $C_{170}H_{223}N_{56}O_{94}S_8P_{15}$ 5275.3, found 5274.2). Yield was 7 OD units at 260 nm (24%).

Sequence 8: t_R=7.6 min. λ=258 and 332 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, [M-H]$^-$) calculated for $C_{170}H_{222}N_{59}O_{92}S_8P_{15}$ 5284.3, found 5287.0). Yield was 5 OD units at 260 nm (17%).

Et-modified (HPLC purified and desalted by Sephadex G-25)

Sequence 9: t_R=4.1 min. λ=260 nm. MALDI-TOF MS *m/z* (negative mode, THAP-CA, [M-H]$^-$) calculated for $C_{155}H_{199}N_{56}O_{94}P_{15}$ 4791.2, found 4788.8). Yield was 9 OD units at 260 nm (32%).

Figure S3. Heating and cooling profiles of the octamer **2** registered in a A) 1 M NaCl and 10 mM sodium phosphate buffer using 1 ºC/min and 0.1 ºC/min and B) 50 mM NaCl, 10 mM sodium cacodylate and 10 mM magnesium chloride buffer using 1 ºC/min.

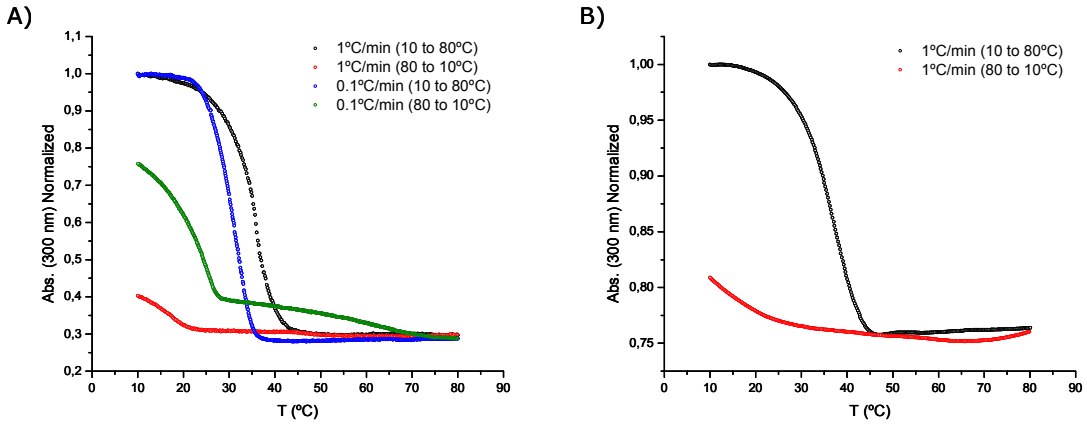


Figure S4. A) Melting profiles of sequence **2** registered under different conditions: (o50 mM NaCl and 10 mM sodium cacodylate; o50 mM NaCl, 10 mM $MgCl_2$, and 10 mM sodium cacodylate; oTAE 1x and 10 mM $Mg(OAc)_2$. B) Absorption spectra of sequence **2** in a 50 mM NaCl, 10 mM $MgCl_2$, and 10 mM sodium cacodylate buffer registered at 10 ºC and 80 ºC.

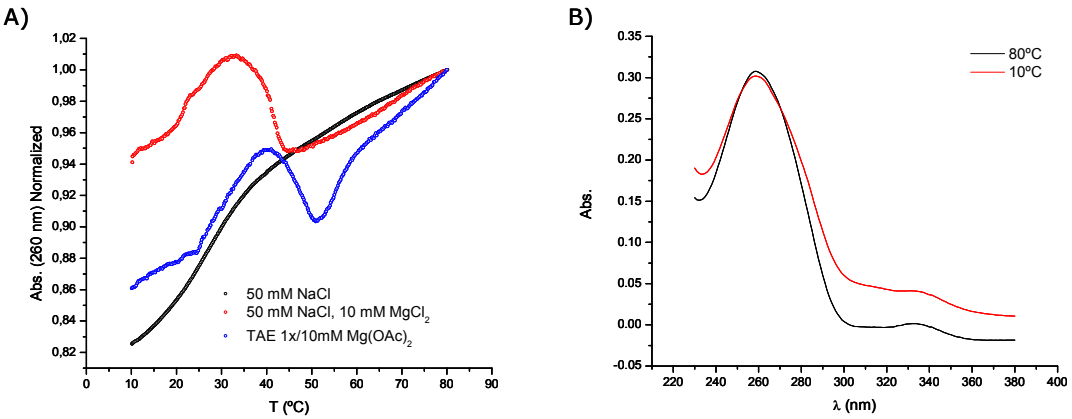


Table S1. Melting experiments with octamers **1** and **2** under different conditions.

Octamer	Buffer composition	Conditions	Result	T_m
1	10 mM P, 1 M NaCl	A5	duplex	36.1 ºC
2	10 mM P, 50 mM NaCl	A1	duplex	27.3 ºC
2	10 mM P, 100 mM NaCl	A2	duplex	33.8 ºC
2	10 mM P, 200 mM NaCl	A3	duplex	35.2 ºC
2	10 mM P, 500 mM NaCl	A4	aggregate	-------
2	10 mM P, 1 M NaCl	A5	aggregate	-------
2	10 mM cac, 50 mM NaCl	B1	duplex	27.2 ºC
2	10 mM cac, 50 mM NaCl, 10 mM $MgCl_2$	B2	aggregate	-------
2	TAE 1x, 10 mM $Mg(OAc)_2$	C	aggregate	-------

Figure S5. Absorption spectra of sequence **5** at 10 ºC and 80 ºC. B) Heating and cooling profiles of sequence **5**.

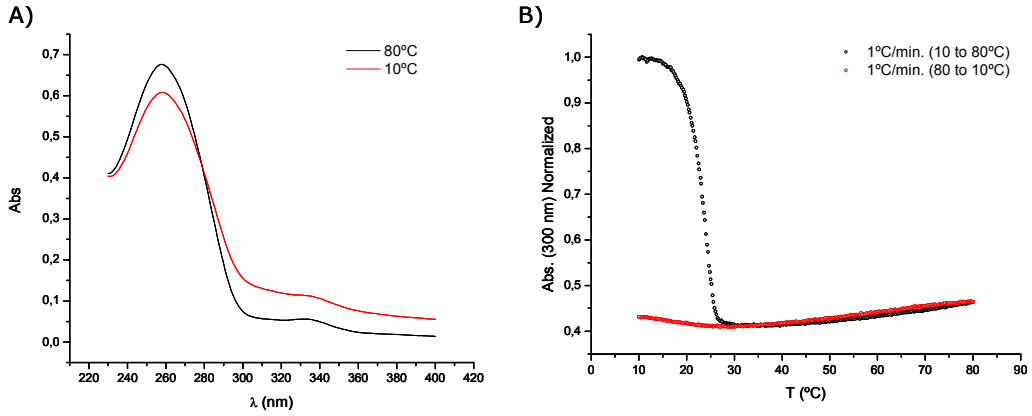


Figure S6. Melting curves of unmodified duplex **3**+**4** and duplexes carrying one TTF unit formed by sequences **3**+**6** and **5**+**4** at A) low and B) high salt concentration.

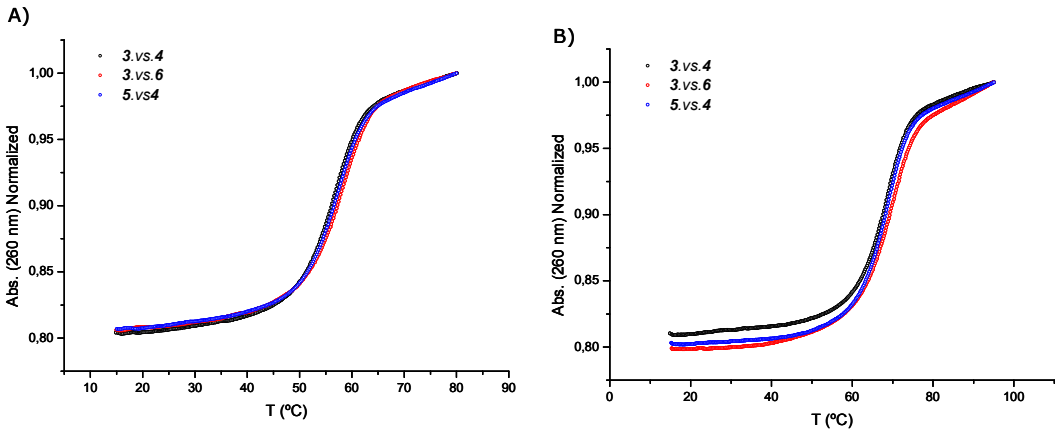


Figure S7. Melting curves of unmodified duplex **3**+**4** and duplexes having two TTF units at opposite ends **5**+**6** or **7**+**8** at A) low and B) high salt concentration. Absorption spectra C) of duplex formed by **5**+**6** and D) of duplex formed by **7**+**8** at 10 ºC and 95 ºC at high salt concentration.

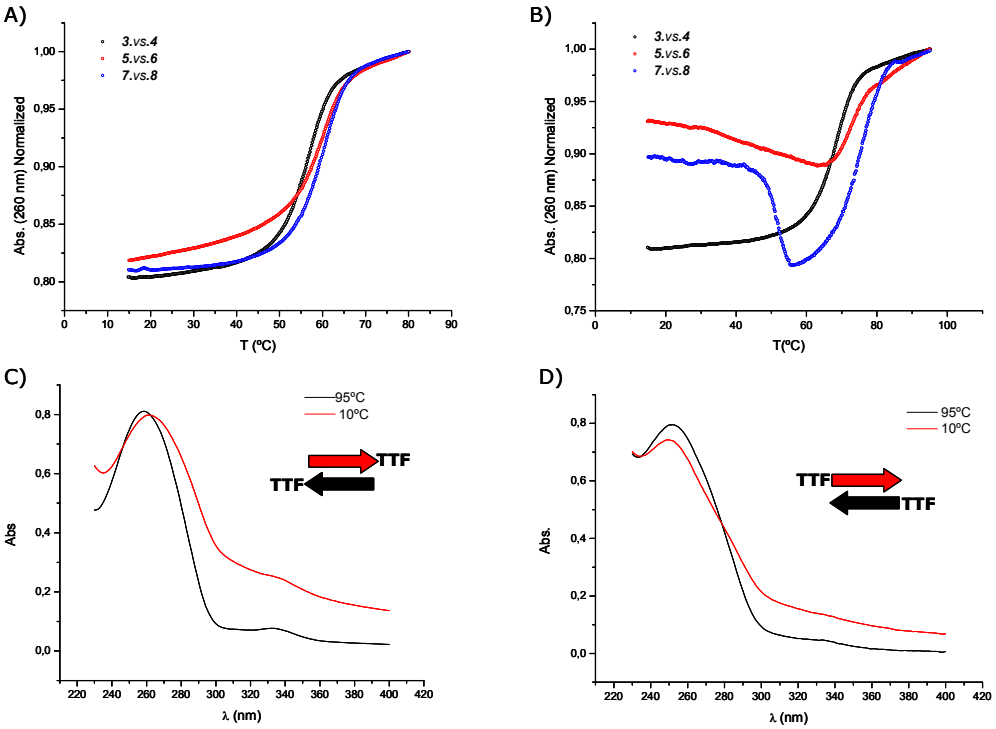


Figure S8. Melting curves of unmodified duplex **3**+**4** and duplexes having two TTF units at the same site **7**+**6** and **5**+**8** at A) low and B) high salt concentration. Absorption spectra C) of duplex formed by sequences **7**+**6** and D) of duplex formed by sequences **5**+**8** at high salt concentration at different temperature.

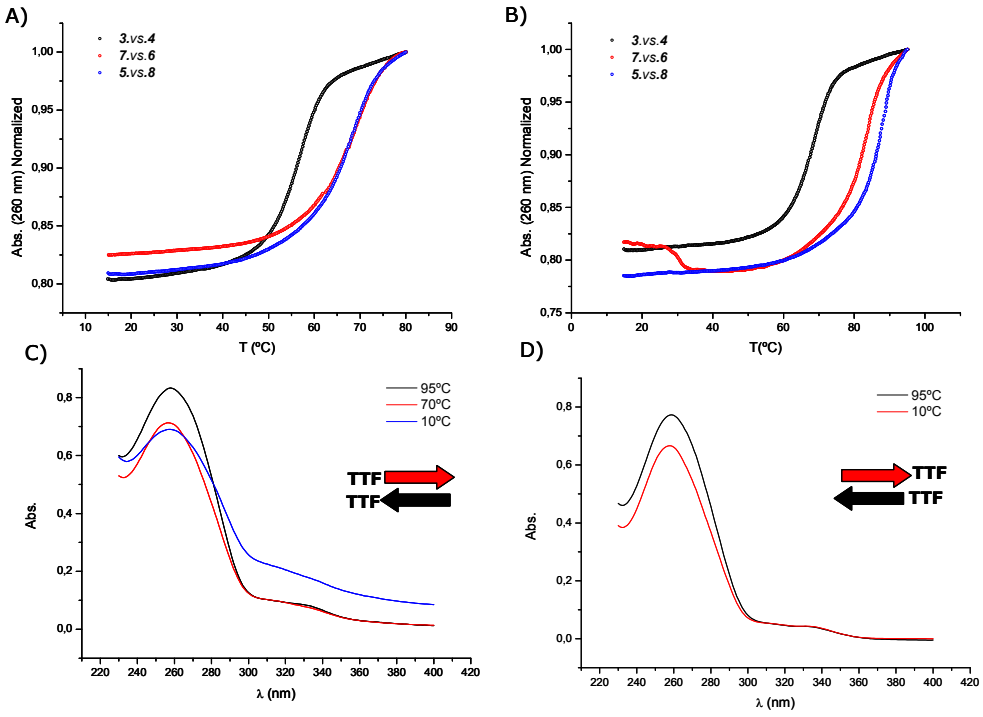


Figure S9. Heating and cooling profiles A) of duplex formed by sequences **5**+**6** and B) duplex formed by sequences **7**+**6** registered in 1 M NaCl and 10 mM sodium phosphate buffer using 1 ºC/min.

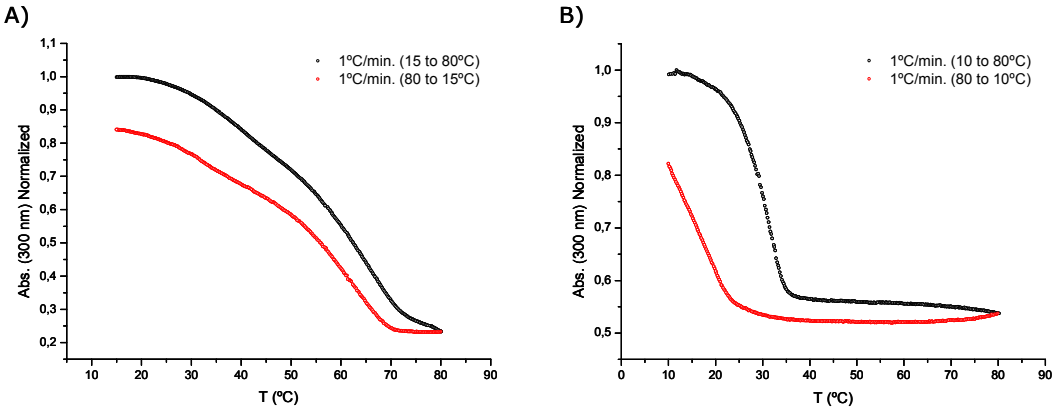


Figure S10. DLS analysis of octamer **2** at A) 15 ºC and high salt concentration, B) 80 ºC and high salt concentration, and C) 15 ºC and low salt concentration.**.** Samples incubated at 4ºC for 24 h (in black) and 48 h (in red).

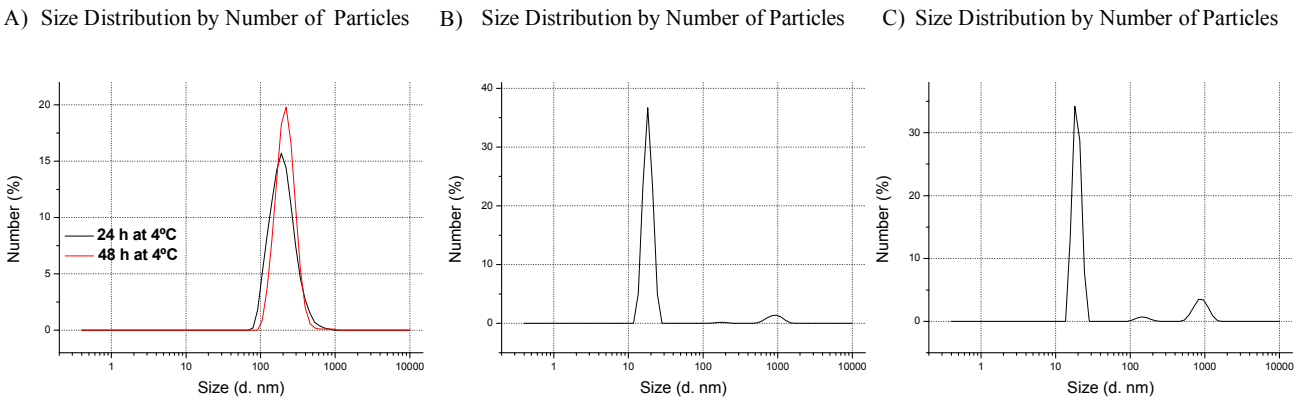
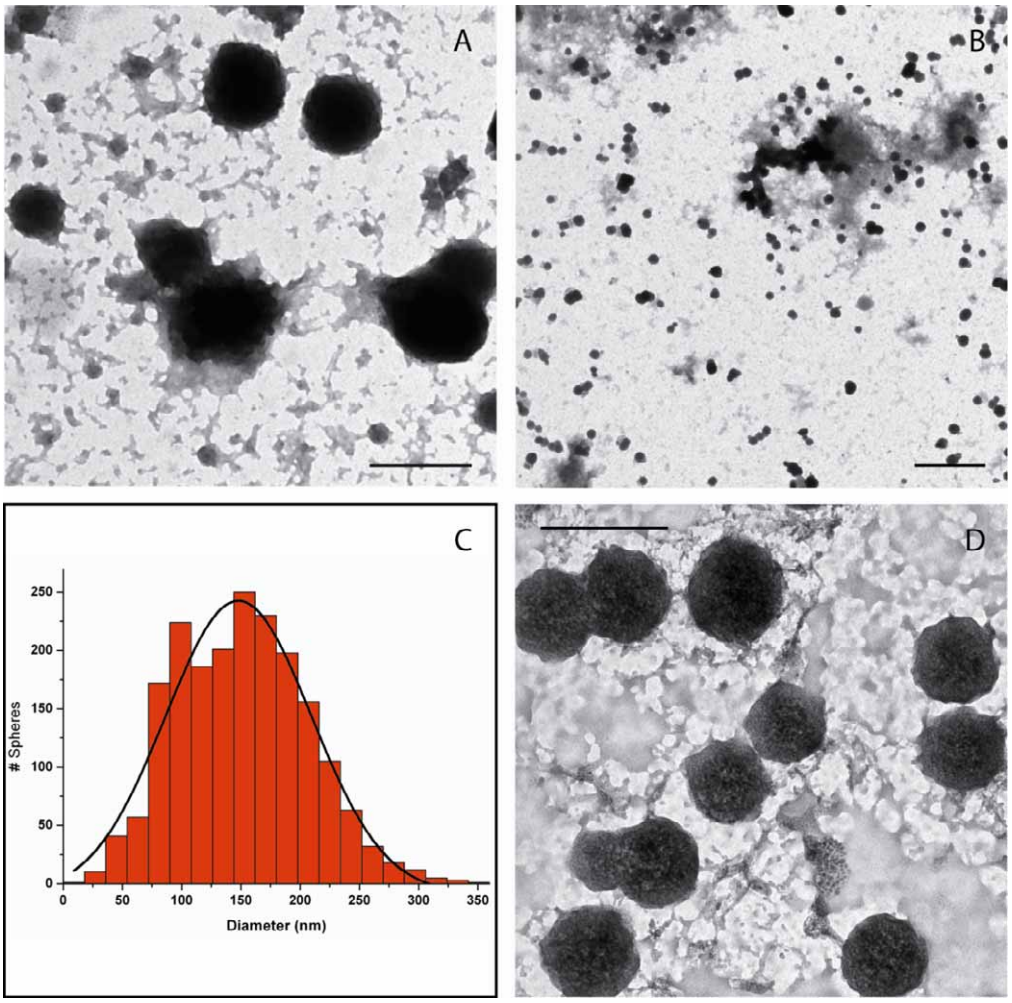
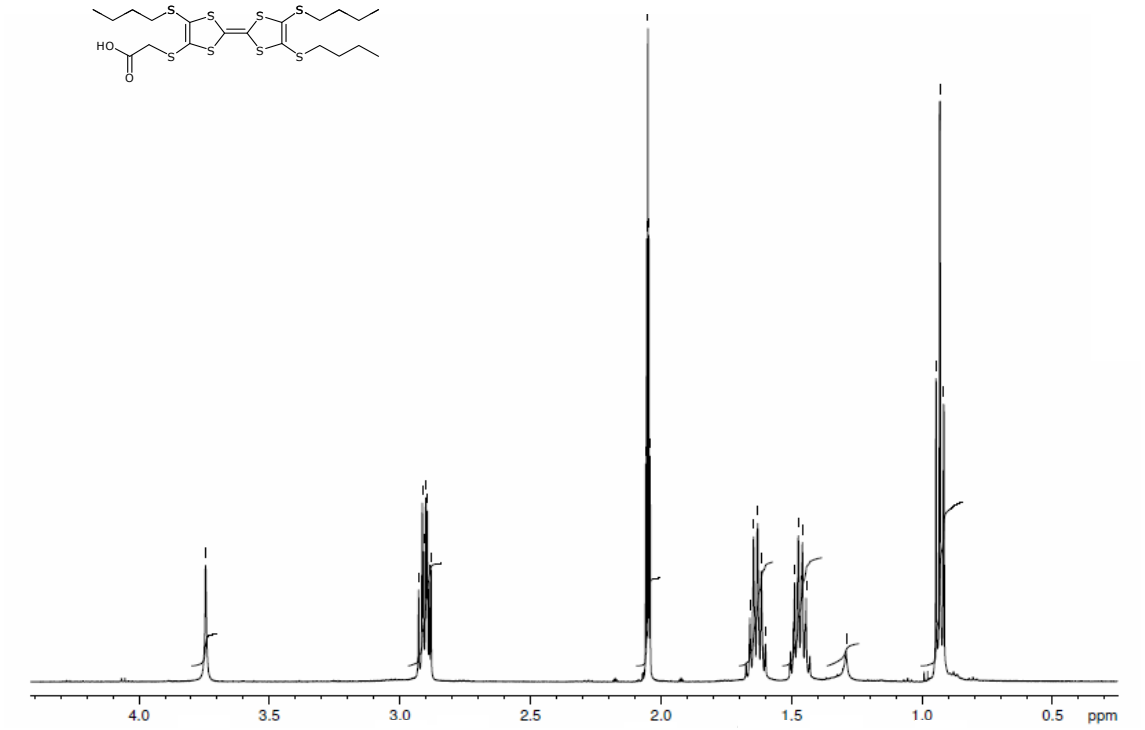


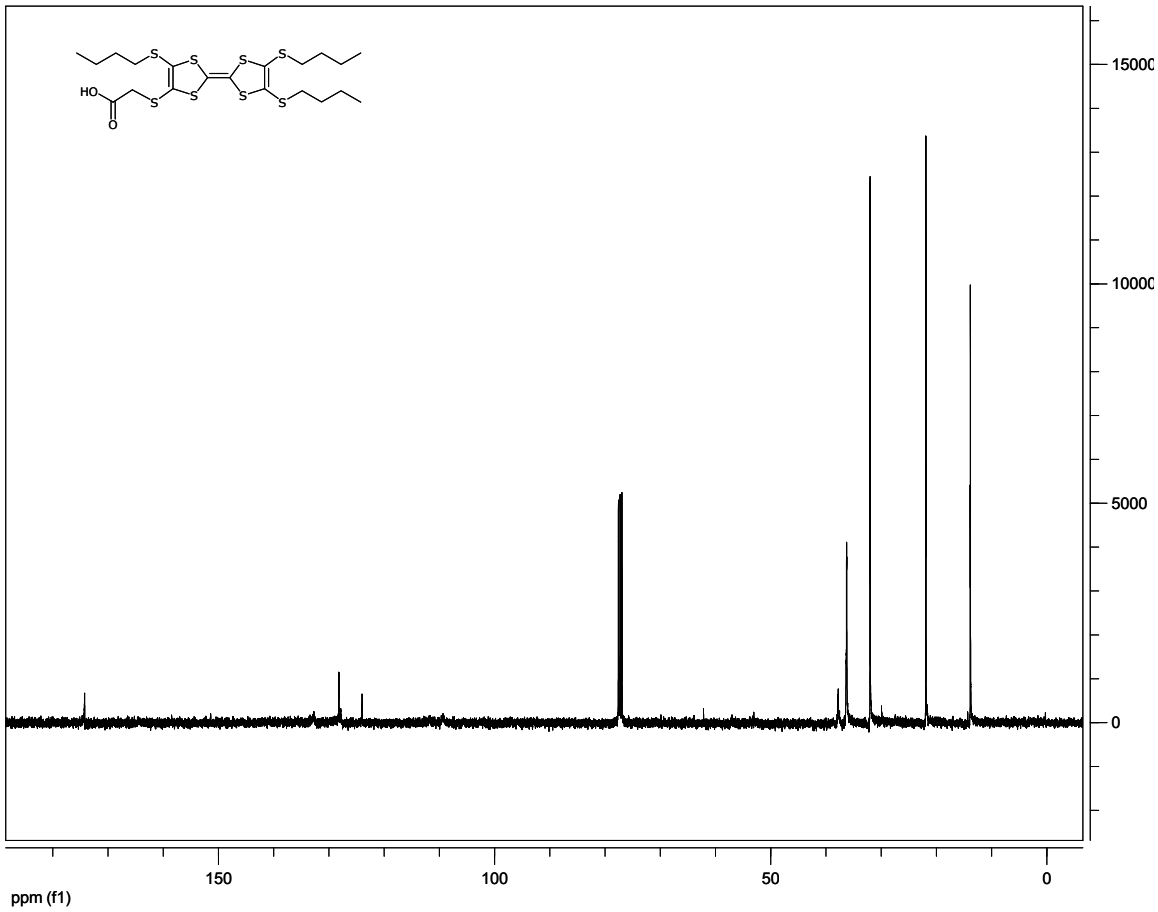
Figure S11. TEM micrographs and statistical analysis of the DNA spherical structures obtained with the octamer **2** in 1 M NaCl buffer. Micrographs of the samples after A) 24 h or B and D) 48 h of incubation at 4 ºC. The DNA spheres were observed after the spreading method A) and B) by centrifugation or D) direct adsorption onto the grid. C) Histogram of the diameter distribution of DNA spheres showing the range of sizes (from 90 to 210 nm). The Gauss fitting of the data (black line) revealed that the mean diameter of the DNA spheres is 147.97 ± 4.89 nm (n=1965). The statistical analysis was performed using the samples incubated at 24 h and 48 h. Bars: 500 nm.



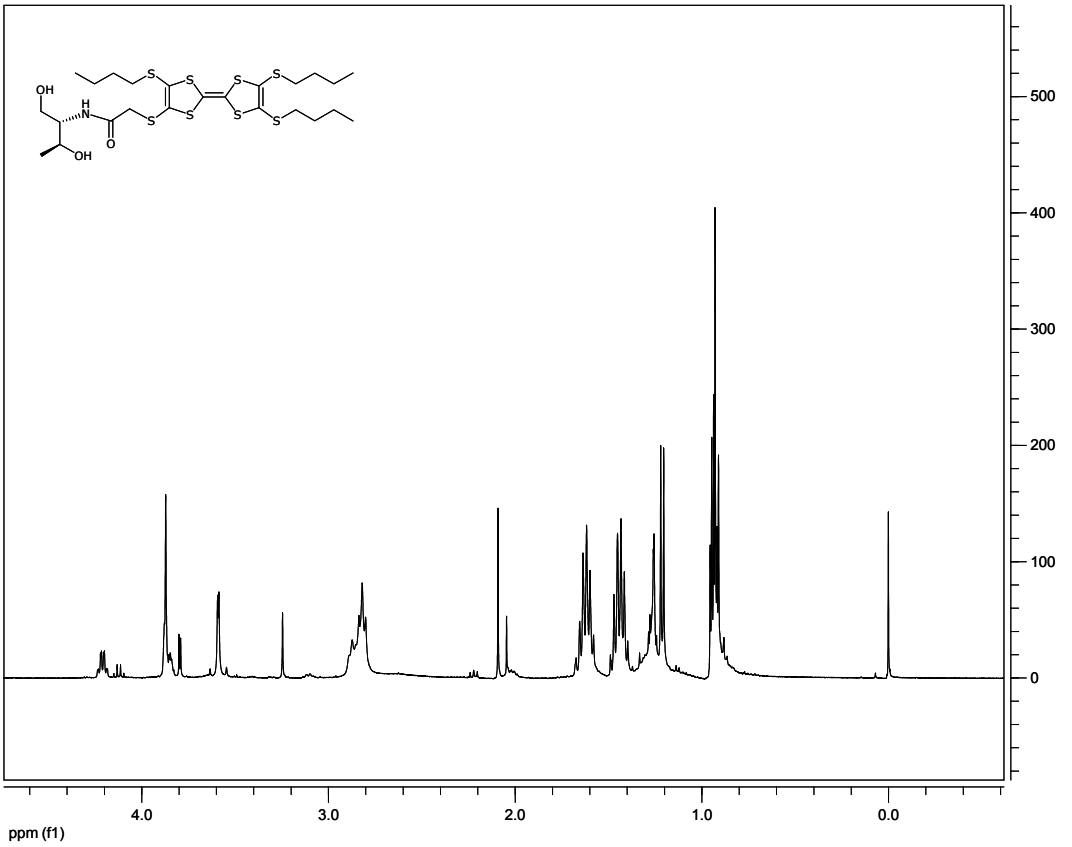
^{1}H-NMR spectrum of **TTF** carboxylic acid.



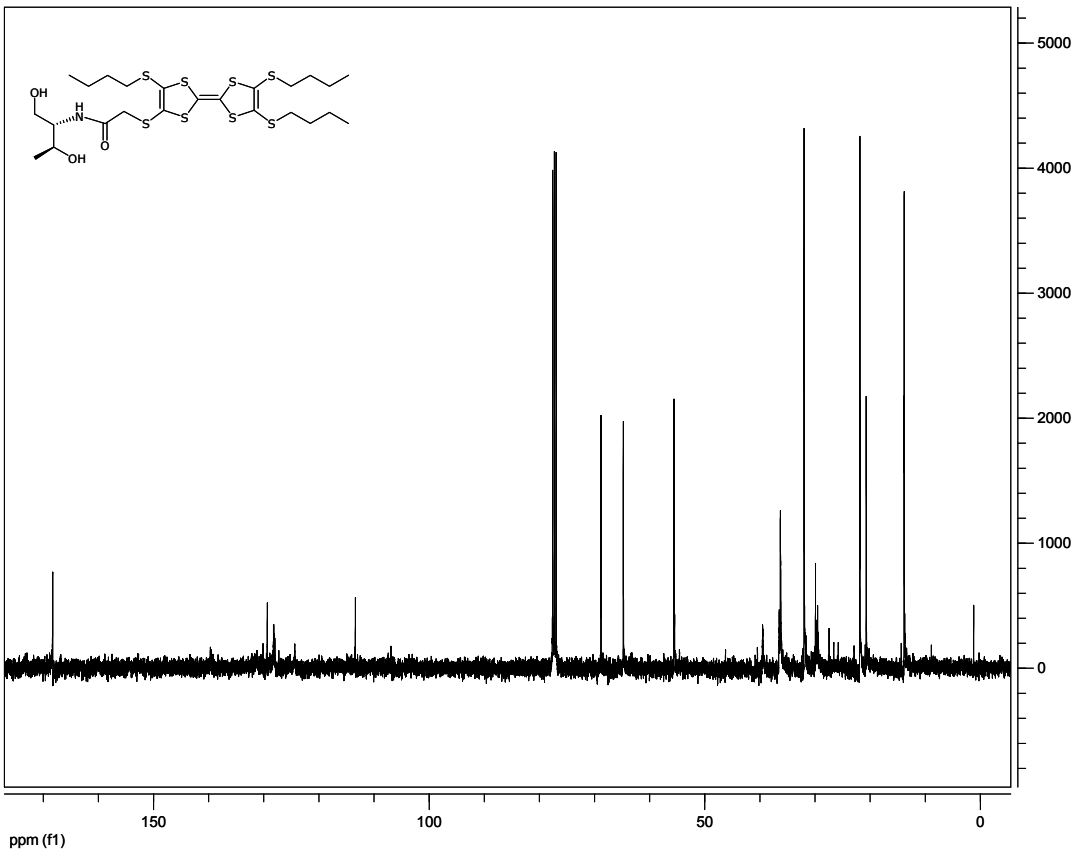
^{13}C-NMR spectrum of **TTF** carboxylic acid.



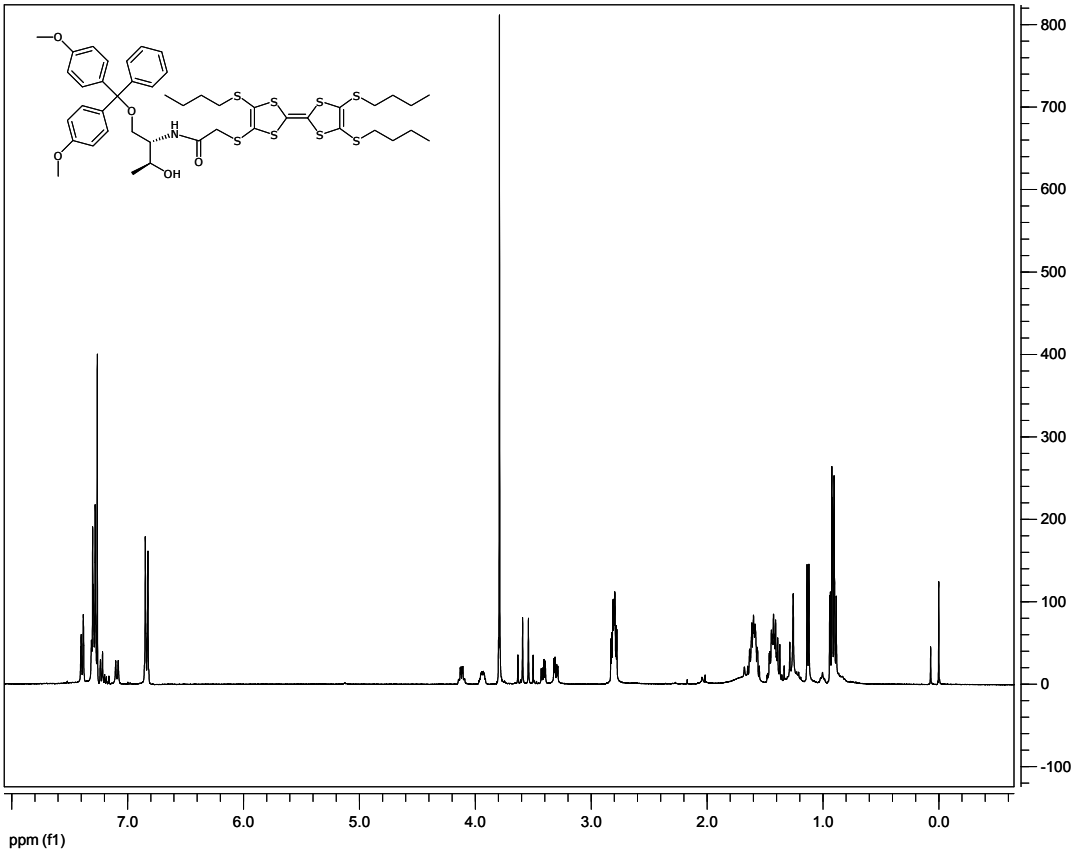
^{1}H-NMR spectrum of compound **1a**.



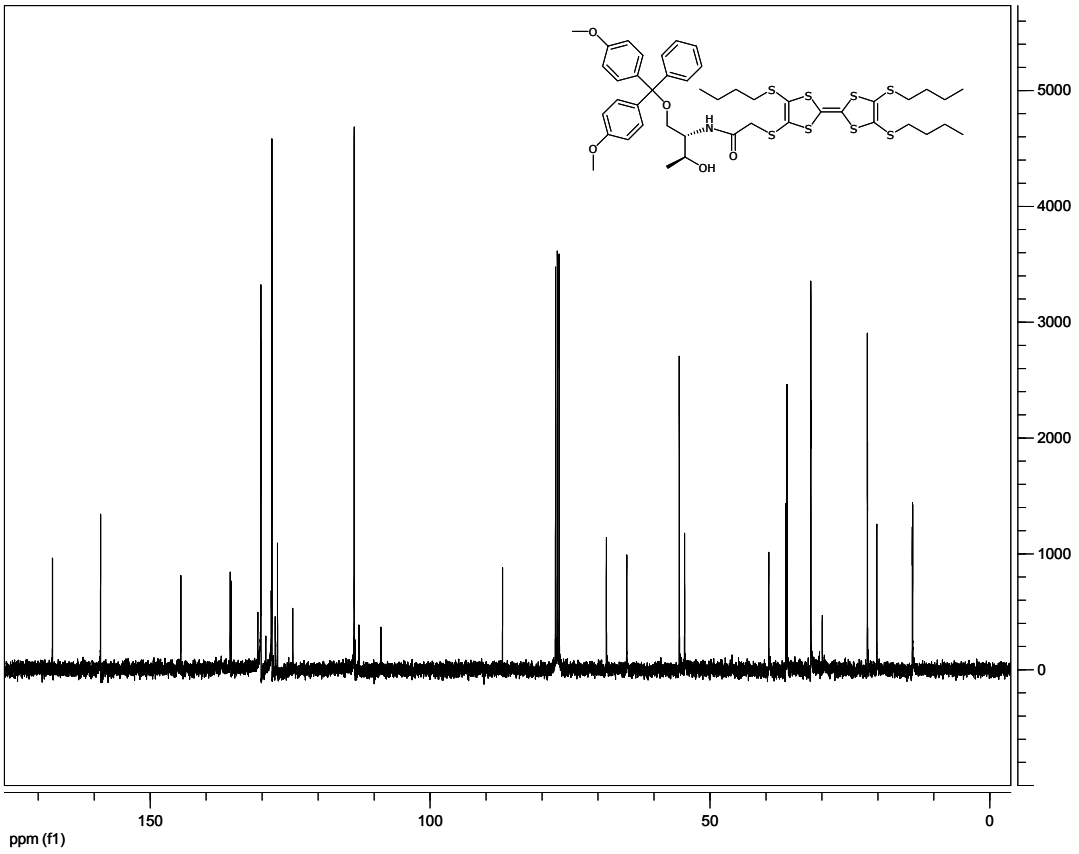
^{13}C-NMR spectrum of compound **1a**.



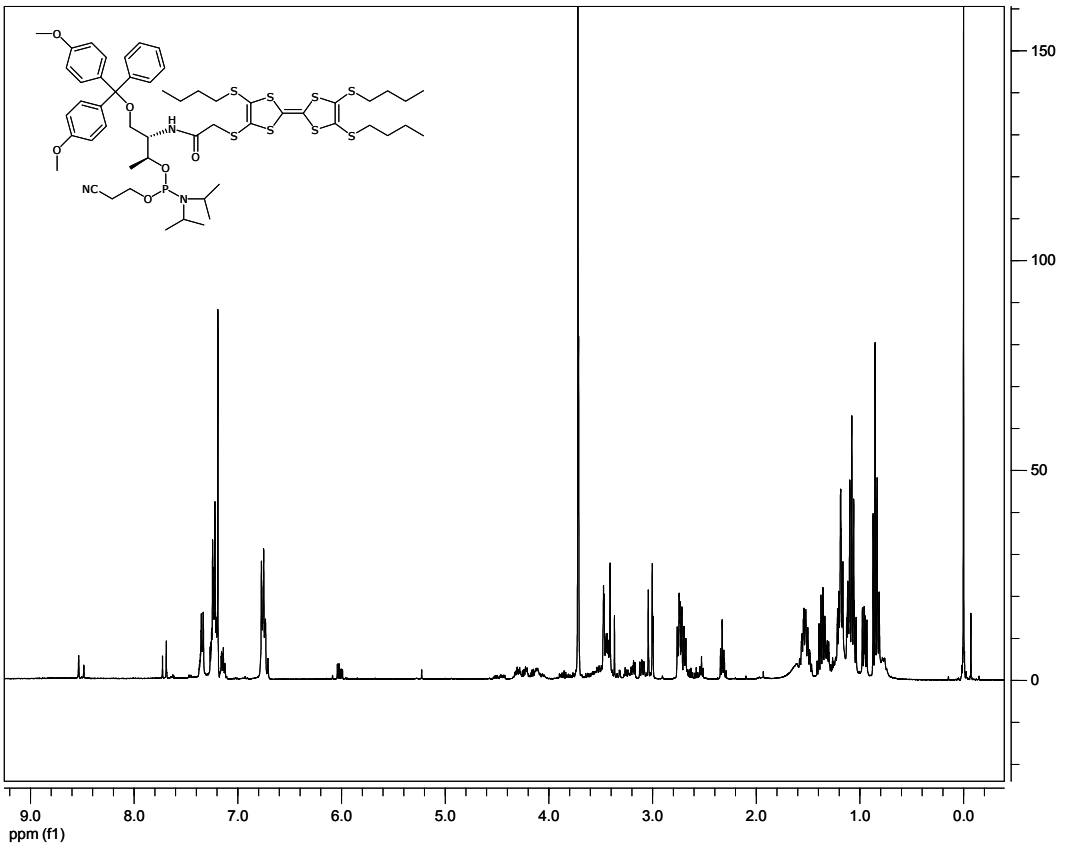
^{1}H-NMR spectrum of compound **2a**.



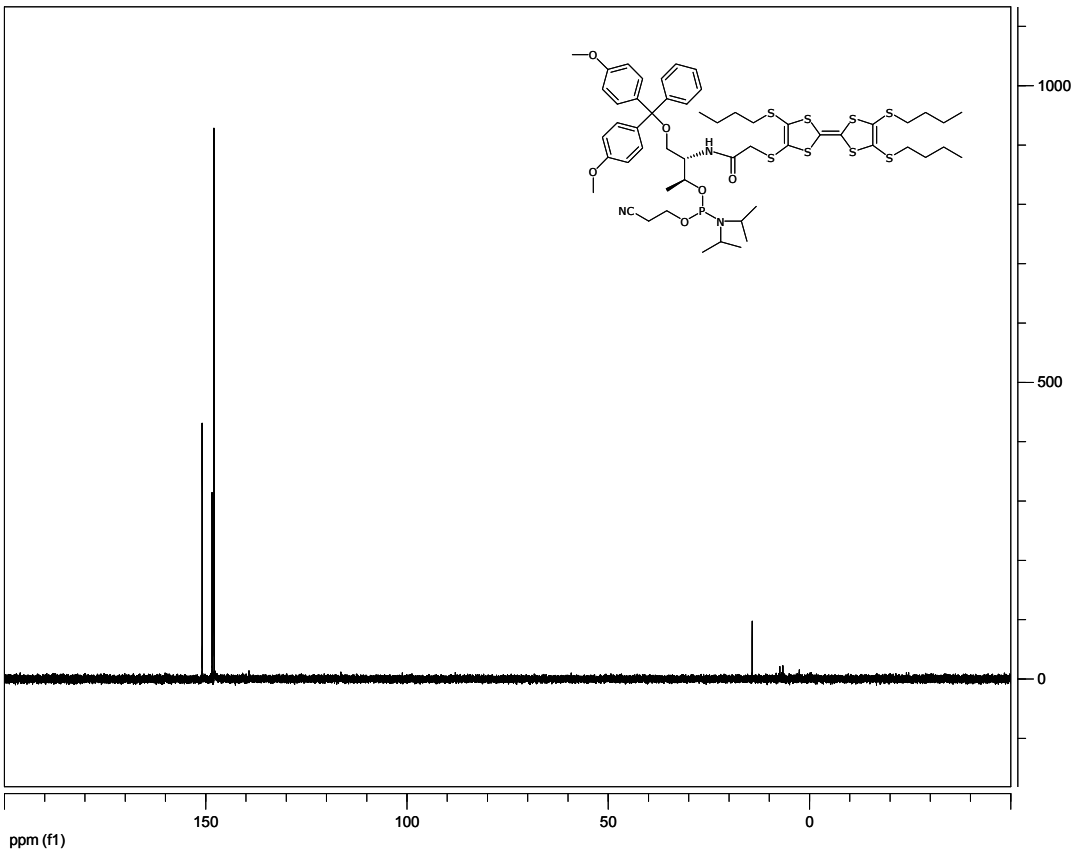
^{13}C-NMR spectrum of compound **2a**.



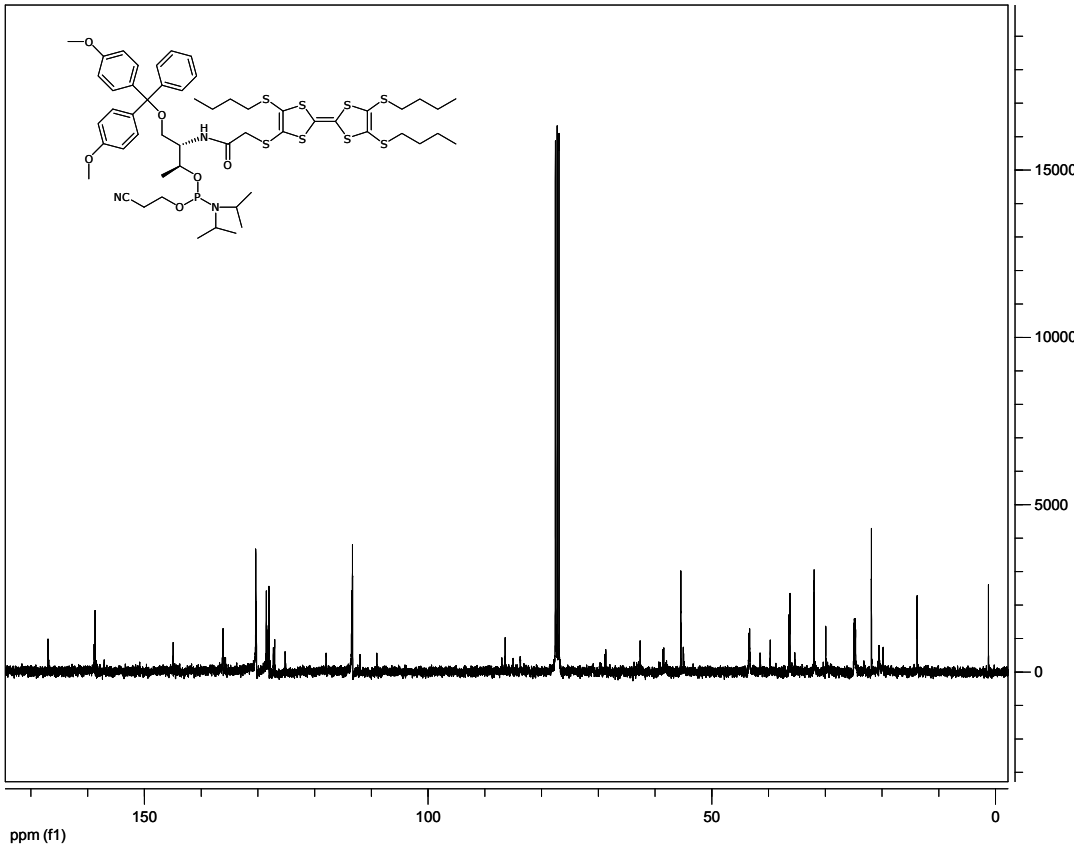
^{1}H-NMR spectrum of compound **3a**.



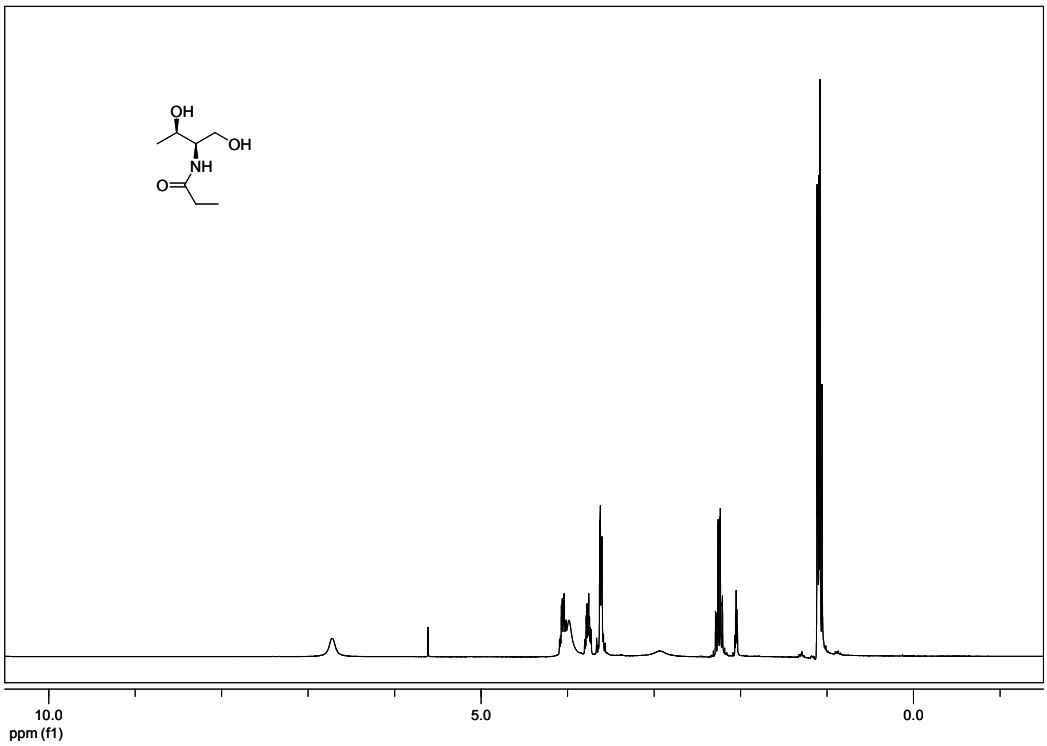
^{31}P-NMR spectrum of compound **3a**.



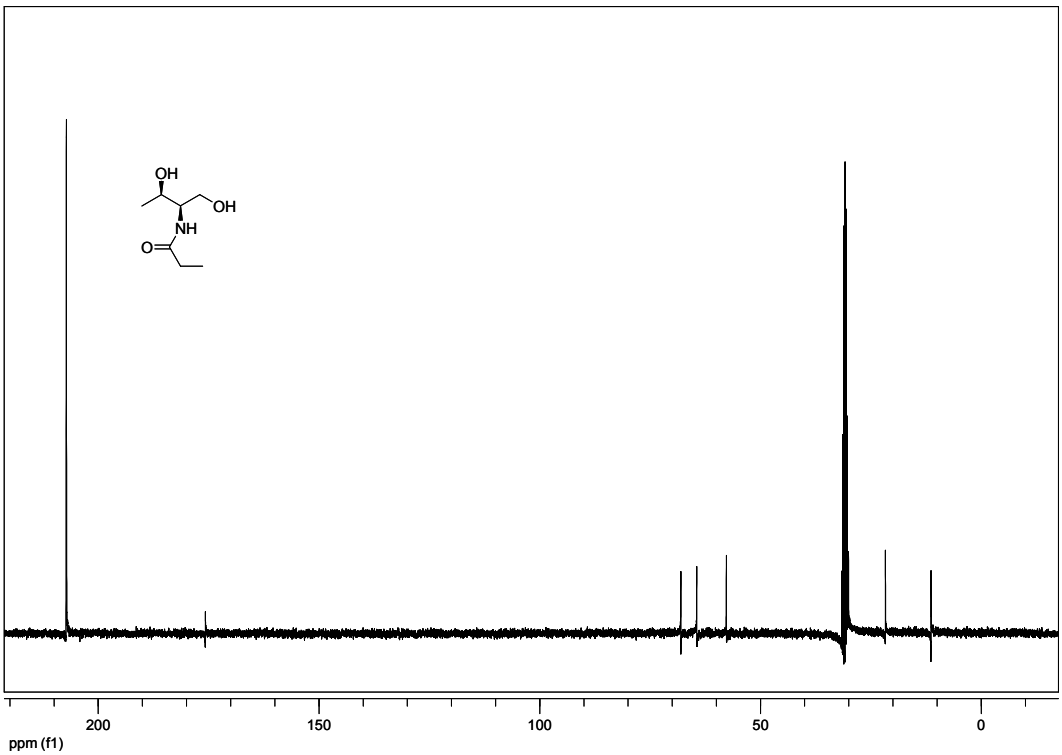
^{13}C-NMR spectrum of compound **3a**.



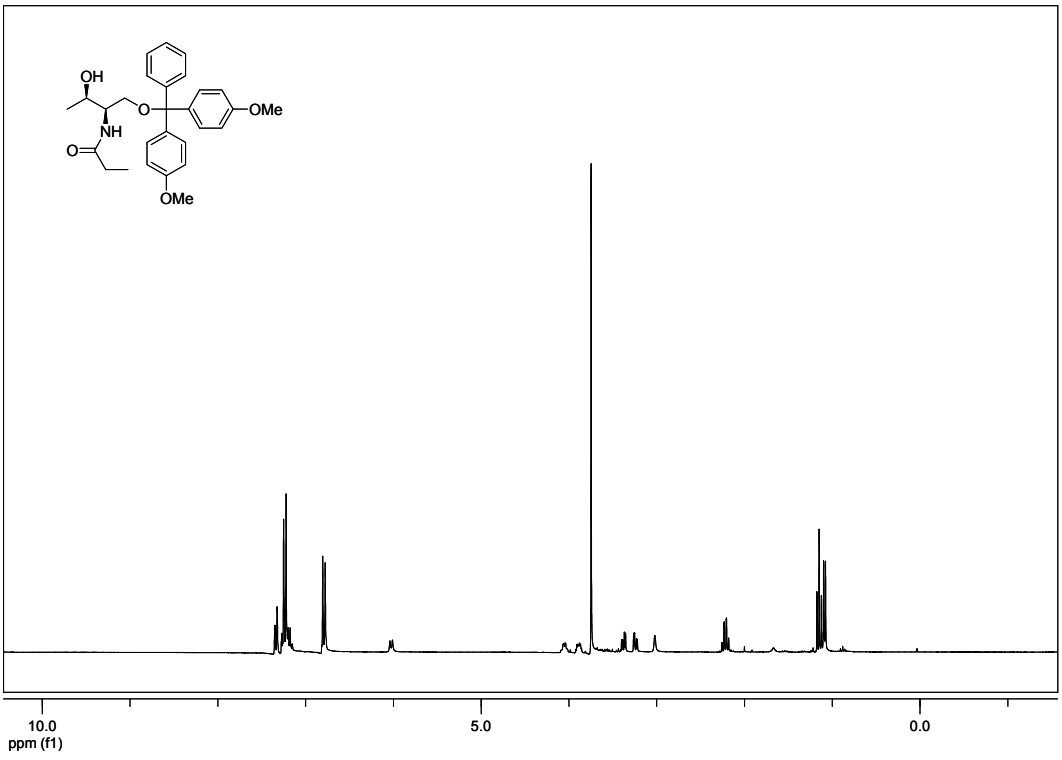
^{1}H-NMR spectrum of compound **1b**.



^{13}C-NMR spectrum of compound **1b**.



^{1}H-NMR spectrum of compound **2b**.



^{13}C-NMR spectrum of compound **2b**.