Supporting Information to the paper:

5'-Modified G-quadruplex forming oligonucleotides endowed with anti-HIV activity: synthesis and biophysical properties.

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5'-*O*-*tert*-butyldiphenylsilyl-thymidine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite (6)

5'-O-*tert*-butyldiphenylsilyl-thymidine **5** (1.0 g, 2.08 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (780 μL, 3.33 mmol) and *N*,*N*-diisopropylethylamine (1 mL, 6.24 mmol) in the presence of molecular sieves 4Å (2 mL). After 30 min the reaction mixture was diluted with CH_2Cl_2 and washed twice with 5 % $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude was purified by silica gel chromatography [eluent system: 30 % AcOEt in *n*-hexane containing 1 % Et_3N] affording the pure target compound as an oil (1.25 g, 1.91 mmol, 92 %).

R_f = 0.8 in $CHCl_3/CH_3OH$ 99:1 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz) as a mixture of diasteroisomers: 7.68-7.26 (22H, complex signals, aromatic protons and H-6), 6.39 (2H, dd, H-1', J = 7.2 and 6.4 Hz), 4.66 (2H, broad signals, H-4'), 4.15-3.57 (12H, overlapped signals, H_2-5, C<u>H</u>$(CH_3)_2$ and OC<u>H</u>$_2$CH$_2$CN), 2.64 (2H, t, C<u>H</u>$_a$CN, J = 6.4 and 6.4 Hz), 2.52 (2H, m, H-2'$_a$), 2.47 (2H, t, C<u>H</u>$_b$CN, J = 6.4 and 6.4 Hz), 2.19 (2H, m, H-2'$_b$), 1.62 and 1.61 (3H each, s's, CH_3 T), 1.28 and 1.18 [9H each, s's, (C<u>H</u>$_3$)$_3$C], 1.14, 1.12, 1.10, 1.09 [6H each, s's, (NCH(C<u>H</u>$_3$)$_2$]. **^{31}P NMR** ($CDCl_3$, 161.98 MHz): 151.9 and 151.7. **ESI-MS** (positive ions): m/z 657.89 [M+H$^+$]; 679.92 [M+Na$^+$]; 695.20 [M+K$^+$]. Mass calculated for $C_{33}H_{49}N_4O_6PSi$: 656.3159.

Chemical synthesis of phosphoramidite building block 13.

5'-*O*-*tert*-butyldimethylsilyl-3'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine (7).

5'-*O*-*tert*-butyldimethylsilyl-thymidine (1.0 g, 2.81 mmol), dissolved in anhydrous pyridine (10 mL), was treated with DMTCl (1.0 g, 2.95 mmol) and DMAP (19 mg, 0.15 mmol). After 18 h the reaction was quenched by addition of CH_3OH and the resulting mixture dried under reduced pressure. The crude was redissolved in $CHCl_3$ (100 mL), washed twice with water and then purified

by silica gel chromatography [eluent system: 1% acetone in $CHCl_3$/Py (1:0.05, v/v)] affording the pure target compound as a glassy solid (1.76 g, 2.67 mmol, 95 %).

R_f = 0.5 in $CHCl_3/CH_3OH$ 98:2 (v/v); **^{1}H NMR** ($CDCl_3$, 300 MHz): 7.45-6.81 (14H, complex signals, aromatic protons of the DMT group and H-6), 6.39 (1H, dd, H-1', J = 5.1 and 9.3 Hz), 4.26 (1H, d, H-3', J = 6.0 Hz), 4.02 (1H, s, H-4'), 3.79 (6H, s, OCH_3 of the DMT group), 3.67 (1H, d, H-5'$_a$, J = 11.4 Hz), 3.29 (1H, A part of an AA'X system, H-5'$_b$, J = 11.4 and 2.1 Hz), 1.85 (3H, s, CH_3), 1.71-1.51 (2H, m, H_2-2'), 0.80 {9H, s, [(C$\underline{H}_3$)$_3$C]Si}, -0.05 and -0.10 [3H each, s's, Si(C$\underline{H}_3$)$_2$]. **^{13}C NMR** ($CDCl_3$, 75 MHz): 163.46 (C-4), 150.12 (C-2), 135.49 (C-6), 158.65, 145.06, 136.30, 130.27, 128.24, 127.94, 127.07, 113.25 (aromatic carbons), 110.81 (C-5), 87.25 (C-1'), 86.66 (quaternary C of the DMT group), 84.92 (C-4'), 74.98 (C-3'), 63.58 (C-5'), 55.24 (OCH_3 of the DMT group), 39.96 (C-2'), 25.78 {[($\underline{C}H_3$)$_3$C]Si}; 18.23 [(CH_3)$_3\underline{C}$]; 12.42 (CH_3 T), -5.39 and -5.72 [Si($\underline{C}H_3$)$_2$]. **ESI-MS** (positive ions): m/z 681.43 [M+Na$^+$]; 697.38 [M+K$^+$]. Mass calculated for $C_{37}H_{46}N_2O_7Si$: 658.3074.

_N_-3-(2-phenylthioethyl)-5'-_O_-_tert_-butyldimethylsilyl-3'-_O_-(4,4'-dimethoxytriphenylmethyl)-thymidine (8).

5'-*O*-*tert*-butyldimethylsilyl-3'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine (900 mg, 1.37 mmol) was dissolved in benzene (10 mL) at 0 °C and treated with 2-(phenylthio)-ethanol (38 µL, 1.02 mmol) and *n*-tributylphosphine (422 µL, 1.71 mmol). After 10 min the reaction mixture was taken to r.t., treated with ADDP (431 mg, 1.71 mmol) and left at r.t. for 18 h. The crude was then taken to dryness, redissolved in AcOEt (100 mL) and washed twice with water. The organic layer was concentrated under reduced pressure and purified by silica gel chromatography [eluent system: 2 % acetone in $CHCl_3$/Py (1:0.05, v/v)], affording the pure target compound as an oil (980 mg, 1.23 mmol, 90 %).

R_f = 0.3 in ethyl acetate/*n*-hexane 4:1 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz): 7.49-6.85 (19H, complex signals, aromatic protons and H-6), 6.48 (1H, dd, H-1', J = 5.6 and 5.2 Hz), 4.27 (1H, d,

H-3', J = 4.0 Hz), 4.22 (2H, m, C$\underline{H_2}$-N), 4.01 (1H, s, H-4'), 3.82 (6H, s, OCH_3 of the DMT group), 3.68 (1H, dd, H-5'$_a$, J = 4.0 and 12.0 Hz), 3.29 (1H, dd, H-5'$_b$, J = 4.0 and 12.0 Hz), 3.19 (2H, t, C$\underline{H_2}$-S, J = 6.0 and 6.0 Hz), 1.87 (3H, s, CH_3), 1.76 (1H, dd, H-2'$_a$, J = 4.0 and 12.0 Hz), 1.57 (1H, complex signal, H-2'$_b$), 0.83 {9H, s, [(C$\underline{H_3}$)$_3$C]Si}, -0.02 e -0.08 [3H each, s's, Si(C$\underline{H_3}$)$_2$]. **^{13}C NMR** ($CDCl_3$, 100 MHz): 162.83 (C-4), 150.37 (C-2), 135.86 (C-6), 158.29, 144.70, 135.95, 135.28, 133.27, 129.87, 129.75, 128.51, 128.00, 127.86, 127.51, 126.63, 125.35, 112.83 (aromatic carbons), 109.62 (C-5), 86.81 (quaternary C of the DMT group), 86.16 (C-1'), 85.12 (C-4'), 74.55 (C-3'), 63.12 (C-5'), 54.80 (OCH_3 of the DMT group), 40.59 ($\underline{C}H_2$-N3), 39.55 (C-2'), 29.22 ($\underline{C}H_2$-S), 25.34 {[($\underline{C}H_3$)$_3$C]Si}, 17.77 [(CH_3)$_3\underline{C}$], 12.68 (CH_3 T), -5.86 and -6.18 [Si($\underline{C}H_3$)$_2$]. **ESI-MS** (positive ions): m/z 795.82 [M+H$^+$]; 833.75 [M+K$^+$]. Mass calculated for $C_{45}H_{54}N_2O_7SSi$: 794.3421.

***N*-3-(2-phenylthioethyl)-3'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine (9).**

N-3-(2-phenylthioethyl)-5'-*O*-*tert*-butyldimethylsilyl-3'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine (810 mg, 1.02 mmol) was treated with 1 M TBAF in THF (1.5 mL). After 30 min the crude was diluted with AcOEt and washed twice with water. The organic layers were collected, dried under reduced pressure and the residue was purified by silica gel chromatography [eluent system: $CHCl_3$/Py (1:0.05, v/v)] affording the pure target compound as a yellow, amorphous powder (680 mg, 1.00 mmol, 98 %).

R$_f$ = 0.5 in $CHCl_3$/CH_3OH 98:2 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz): 7.49-6.85 (19H, complex signals, aromatic protons and H-6), 6.16 (1H, dd, H-1', J = 6.0 and 6.0 Hz), 4.41 (1H, d, H-3', J = 6.4 Hz), 4.19 (2H, t, C$\underline{H_2}$-N, J = 5.6 and 7.2 Hz), 4.00 (1H, d, H-4', J = 2.0 Hz), 3.82 (6H, s, OCH_3 of the DMT group), 3.68 (1H, d, H-5'$_a$, J = 11.6 Hz), 3.34 (1H, m, H-5'$_b$), 3.16 (2H, t, C$\underline{H_2}$-S, J = 7.6 and 7.6 Hz), 2.62 (1H, broad t, OH), 1.97 (1H, m, H-2'$_a$), 1.88 (3H, s, CH_3), 1.74 (1H, m, H-2'$_b$). **^{13}C NMR** ($CDCl_3$, 100 MHz): 162.99 (C-4), 150.68 (C-2), 136.18 (C-6), 158.62, 149.65, 144.98, 135.89, 135.50, 135.24, 130.13, 128.83, 128.28, 128.15, 127.87, 126.99, 125.69, 123.64,

113.19 (aromatic carbons), 110.09 (C-5), 88.30 (C-1'), 87.09 (quaternary C of the DMT group), 86.45 (C-4'), 74.18 (C-3'), 62.54 (C-5'), 55.14 (OCH_3), 40.84 ($\underline{C}H_2$-N3), 38.49 (C-2'), 29.46 ($\underline{C}H_2$-S), 13.06 (CH_3 T). **ESI-MS** (positive ions): m/z 681.57 [$M+H^+$]; m/z 703.61 [$M+Na^+$]; m/z 719.62 [$M+K^+$]. Mass calculated for $C_{39}H_{40}N_2O_7S$: 680.2556.

***N*-3-(2-phenylthioethyl)-5'-*O*-(3,4-dibenzyloxy)-benzyl-3'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine (10).**

3'-*O*-(4,4'-dimethoxytriphenylmethyl)-*N*-3-(2-phenylthioethyl)-thymidine (600 mg, 0.88 mmol) was dissolved in anhydrous DMF (5 mL) and treated with NaH (60% in mineral oil, 100 mg, 2.6 mmol). After 20 min, DBBCl (1.5 g, 4.41 mmol) and NaI (66 mg, 0.44 mmol) were added. After 18 h, the reaction was quenched by addition of water, diluted with AcOEt and washed twice with water. The organic layers were collected, dried under reduced pressure and purified by silica gel chromatography [eluent system: 40 % AcOEt in *n*-hexane/Py (1:0.05, v/v)] affording 605 mg (0.62 mmol, 70 %) of the pure target compound as an oil.

R_f = 0.3 in ethyl acetate/*n*-hexane 7:3 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz): 7.47-6.66 (32H, complex signals, aromatic protons and H-6), 6.49 (1H, dd, H-1', J = 5.6 and 6.0 Hz), 5.16 and 5.15 (2H each, s's, Ph-O-$\underline{C}H_2$), 4.58 (2H, d, Ph-$\underline{C}H_2$-O-C5', J = 5.6 Hz), 4.29 (1H, m, H-3'), 4.17 (2H, m, $\underline{C}H_2$-N), 3.82 (1H, s, H-4'), 3.76 (6H, s, OCH_3 of the DMT group), 3.38 (1H, dd, H-5'$_a$, J = 2.0 and 10.4 Hz), 3.14 (2H, t, $\underline{C}H_2$-S, J = 8.0 and 7.6 Hz), 2.96 (1H, dd, H-5'$_b$, J = 2.4 and 10.8 Hz), 1.96 (1H, dd, H-2'$_a$, J = 6.0 and 13.6 Hz), 1.69 (1H, m, H-2'$_b$), 1.53 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100 MHz): 163.09 (C-4), 150.77 (C-2), 136.93 (C-6), 158.60, 148.83, 148.64, 144.98, 137.09, 136.19, 135.61, 134.26, 134.03, 130.67, 130.11, 128.66, 128.37, 128.27, 128.20, 127.86, 127.77, 127.69, 127.22, 127.16, 127.09, 126.98, 125.68, 120.53, 120.07, 115.03, 114.66, 114.29, 113.95, 113.16 (aromatic carbons), 109.95 (C-5), 87.11 (quaternary C of the DMT group), 85.59 (C-1'), 85.21 (C-4'), 74.95 (C-3'), 71.28 and 71.05 (2 x Ph-$\underline{C}H_2$-O), 69.91 (Ph-$\underline{C}H_2$O-C5'), 65.10 (C-5'), 55.12 (OCH_3), 40.79 ($\underline{C}H_2$-N3), 39.66 (C-2'), 29.51 ($\underline{C}H_2$-S), 12.77 (CH_3 T). **ESI-MS**

(positive ions): m/z 1005.93 [M+Na^+]; 1021.88 [M+K^+]. Mass calculated for $C_{60}H_{58}N_2O_9S$: 982.3863.

_N_-3-(2-phenylsulfonylethyl)-5'-_O_-(3,4-dibenzyloxy)-benzyl-3'-_O_-(4,4'-dimethoxytriphenyl-methyl)-thymidine (11)

_N_-3-(2-phenylthioethyl)-5'-_O_-(3,4-dibenzyloxy)-benzyl-3'-_O_-(4,4'-dimethoxytriphenylmethyl)-thymidine (600 mg, 0.61 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with _m_-CPBA (309 mg, 1.8 mmol) at r.t. After 1 h the crude was diluted with CH_2Cl_2 and washed twice with 5 % $NaHCO_3$. The organic layers were collected and purified by silica gel chromatography [eluent system: CH_3OH in $CHCl_3$/Py (1:0.05, v/v) from 0 % to 5 %] affording 612 mg (0.60 mmol, 98 %) of the pure target compound as an oil.

R_f = 0.6 in $CHCl_3$/CH_3OH 98:2 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz): 7.96-6.65 (32H, complex signals, aromatic protons and H-6), 6.42 (1H, dd, H-1', J = 5.6 and 5.8 Hz), 5.16, 5.14 and 5.04 (2H each, s's, Ph-C$\underline{H_2}$-O), 4.27 (3H, overlapped signals, H-3' and C$\underline{H_2}$-N), 3.80 (1H, s, H-4'), 3.75 (3H, s, OCH_3 of the DMT group), 3.46 (2H, t, C$\underline{H_2}$-S, J = 7.2 and 7.6 Hz), 3.38 (1H, dd, H-5'$_a$, J = 1.6 and 10.4 Hz), 2.96 (1H, dd, H-5'$_b$, J = 2.0 and 10.4 Hz), 1.96 (1H, dd, H-2'$_a$, J = 5.6 and 13.2 Hz), 1.68 (1H, m, H-2'$_b$), 1.50 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 100 MHz): 162.74 (C-4), 150.38 (C-2), 136.17 (C-6), 158.63, 149.72, 148.88, 148.71, 144.97, 138.87, 137.22, 136.95, 136.94, 135.85, 134.31, 133.73, 130.65, 130.11, 129.19, 128.37, 128.20, 128.00, 127.86, 127.70, 127.18, 127.10, 127.03, 123.62, 120.58, 120.09, 115.12, 114.74, 114.43, 114.03, 113.19 (aromatic carbons), 109.76 (C-5), 87.14 (quaternary C of the DMT group), 85.73 (C-4'), 85.29 (C-1'), 74.90 (C-3'), 72.90 and 71.33 (2 x $\underline{C}H_2OPh$), 69.84 (Ph-$\underline{C}H_2$-O-C5'), 65.09 (C-5'), 55.13 (O$\underline{C}H_3$), 52.44 ($\underline{C}H_2$-S), 39.71 (C-2'), 35.09 ($\underline{C}H_2$-N3), 12.64 (CH_3 T). **ESI-MS** (positive ions): m/z 1037.93 [M+Na^+]; 1053.86 [M+K^+]. Mass calculated for $C_{60}H_{58}N_2O_{11}S$: 1014.3761.

***N*-3-(2-phenylsulfonylethyl)-5'-*O*-(3,4-dibenzyloxy)-benzyl-thymidine (12)**

N-3-(2-phenylsulfonylethyl)-5'-*O*-(3,4-dibenzyloxy)-benzyl-3'-*O*-(4,4'-dimethoxytriphenyl-methyl)-thymidine (600 mg, 0.59 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated with 3 % TFA in CH_2Cl_2 (1 mL). After 30 min the crude was diluted with CH_2Cl_2 and washed twice with 5 % $NaHCO_3$. The organic layers were dried under reduced pressure and purified by silica gel chromatography [eluent system: CH_3OH in $CHCl_3$/Py (1:0.05, v/v) from 0 % to 5 %] affording 395 mg (0.56 mmol, 94 %) of pure **12** as an oil.

R_f = 0.2 in $CHCl_3/CH_3OH$ 98:2 (v/v); **^{1}H NMR** ($CDCl_3$, 500 MHz): 7.86-6.80 (19H, complex signals, aromatic protons and H-6), 6.32 (1H, t, H-1', J = 6.5 and 6.5 Hz), 5.16 and 5.15 (2H each, s's, 2 x Ph-O-C$\underline{H}_2$), 4.54 (2H, m, Ph-C$\underline{H}_2$-O-C5'), 4.38 (1H, m, H-3'), 4.27 (2H, m, C$\underline{H}_2$-N), 4.02 (1H, d, H-4', J = 2.5 Hz), 3.69 (1H, dd, H-5'$_a$, J = 2.5 and 13.0 Hz), 3.59 (1H, d, H-5'$_b$, J = 10.0 Hz), 3.47 (2H, t, C$\underline{H}_2$-S, J = 7.5 and 7.5 Hz), 2.28 (1H, m, H-2'$_a$), 2.18 (1H, d, OH, J = 4.0 Hz), 2.10 (1H, m, H-2'$_b$), 1.60 (3H, s, CH_3). **^{13}C NMR** ($CDCl_3$, 125 MHz): 162.79 (C-4), 150.28 (C-2), 136.96 (C-6), 148.86, 148.81, 138.76, 137.05, 134.20, 133.77, 130.51, 129.20, 128.38, 127.98, 127.76, 127.65, 127.12, 127.03, 120.92, 114.73 (aromatic carbons), 109.70 (C-5), 85.63 (C-4'), 85.48 (C-1'), 73.17 (C-3'), 72.05 (Ph-$\underline{C}H_2$-O-C5'), 71.19 (2 x $\underline{C}H_2$OPh), 69.54 (C-5'), 52.41 ($\underline{C}H_2$-S), 40.84 (C-2'), 35.02 ($\underline{C}H_2$-N3), 12.73 (CH_3 T). **ESI-MS** (positive ions): m/z 712.89 [M+H$^+$]; 735.57 [M+Na$^+$]; 751.64 [M+K$^+$]. Mass calculated for $C_{39}H_{40}N_2O_9S$: 712.2454.

***N*-3-(2-phenylsulfonylethyl)-5'-*O*-(3,4-dibenzyloxy)-benzyl-thymidine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite (13)**

N-3-(2-phenylsulfonylethyl)-5'-*O*-(3,4-dibenzyloxy)-benzyl-thymidine (350 mg, 0.49 mmol), was dissolved in CH_2Cl_2 (5 mL) and treated, under nitrogen atmosphere, with *N*,*N*-diisopropylethylamine (257 μL, 1.47 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (183 μL, 0.78 mmol) in the presence of molecular sieves 4Å (2 mL) and the reaction mixture left at r.t. After 30 min this was diluted with CH_2Cl_2 and washed

twice with 5 % $NaHCO_3$. The collected organic layers were dried over anhydrous $MgSO_4$ and purified by silica gel chromatography [eluent system: AcOEt/*n*-hexane 1:1 (v/v) with 1 % Et_3N] affording the target compound in a pure form as a white, amorphous powder (380 mg, 0.42 mmol, 85 %, unoptimized yields).

R_f = 0.3 in ethyl acetate/*n*-hexane 1:1 (v/v); **^{1}H NMR** ($CDCl_3$, 400 MHz) as a mixture of diasteroisomers: 7.96-6.89 (37H, complex signals, aromatic protons and H-6), 6.33 (2H, t, H-1', J = 7.2 and 6.4 Hz), 5.14 and 5.13 (4H each, s's, Ph-O-C$\underline{H}_2$), 4.56 (2H, m, H-3'), 4.46 (4H, m, Ph-C$\underline{H}_2$-O-C5'), 4.26 (4H, m, C$\underline{H}_2$-N), 4.20 and 4.13 (1H each, s's, H-4'), 3.84-3.55 [12H, overlapped signals, H_2-5', C$\underline{H}$(CH_3)$_2$ and OC$\underline{H}_2$$CH_2$CN], 3.47 (4H, t, C$\underline{H}_2$-S, J = 6.4 and 8.0 Hz), 2.61 (2H, t, CH_aCN, J = 6.0 and 6.0 Hz), 2.55 (2H, t, CH_bCN, J = 6.0 and 6.4 Hz), 2.41 (1H, m, H-2'$_a$), 2.37 (1H, m, H-2'$_a$), 2.14 (2H, m, H-2'$_b$), 1.55 (6H, s, CH_3), 1.19, 1.17, 1.16, 1.14 [6H each, d's, (NCH(C$\underline{H}_3$)$_2$]. **^{13}C NMR** ($CDCl_3$, 100 MHz): 162.77 (C-4), 150.34 (C-2), 137.05 (C-6), 148.99, 138.94, 134.27, 133.71, 130.74, 129.18, 128.38, 128.01, 127.72, 127.14, 120.96, 114.95 (aromatic carbons), 117.45 (C$\underline{N}$), 109.80 (C-5), 85.61 (C-4'), 85.58 (C-1'), 74.09 (C-3'), 73.24 (Ph-C$\underline{H}_2$-O-C5'), 71.35 and 71.28 (2 *x* Ph-C$\underline{H}_2$-O), 69.67 and 69.55 (C-5'), 58.03 and 58.01 (OC$\underline{H}_2$$CH_2$CN), 52.48 (C$\underline{H}_2$-S), 43.31 and 43.19 [C$\underline{H}$($CH_3$)$_2$], 39.92 (C-2'), 35.07 (C$\underline{H}_2$-N3), 24.45 [NCH(C$\underline{H}_3$)$_2$], 20.29 (C$\underline{H}_2$CN), 12.68 (CH_3 T). **^{31}P NMR** ($CDCl_3$, 161.98): 149.3. **ESI-MS** (positive ions): m/z 913.27 [M+H$^+$]; 935.89 [M+Na$^+$]; 952.90 [M+K$^+$]. Mass calculated for $C_{48}H_{57}N_4O_{10}PS$: 912.3533.

General Experimental Methods.

NMR spectra were recorded on Bruker WM-400 and Varian INOVA 500 spectrometers. All chemical shifts are expressed in ppm with respect to the residual solvent signal. Peak assignments have been carried out on the basis of standard ^{1}H-^{1}H COSY and HSQC experiments. ^{31}P NMR spectra were recorded on a Bruker 400-WM spectrometer at 161.98 MHz using 85% H_3PO_4 as external standard. The oligonucleotides were assembled on an Expedite PerSeptive Biosystems

DNA synthesizer, using standard commercially available 5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-O-[(2-cyanoethyl)-N,N-diisopropyl] phosphoramidite 2'-deoxyribonucleosides as buildings blocks. HPLC analyses and purifications were performed on a Beckman System Gold instrument equipped with a UV detector module 166 and a Shimadzu Chromatopac C-R6A integrator. ESI mass spectrometric analyses were carried out on a Waters Micromass ZQ mass spectrometer, equipped with an Electrospray source used in the positive and/or negative mode. The reported mass obtained in the ESI-MS characterization of ODNs **1**, **2** and **4** is the calculated value on the basis of the combination of the found multiple charged ions. MALDI TOF mass spectrometric analyses were performed on a PerSeptive Biosystems Voyager-De Pro MALDI mass spectrometer in the Linear mode using a picolinic/3-hydroxypicolinic acids mixture as the matrix. UV measurements were carried out on a Jasco V-530 UV spectrophotometer equipped with a Jasco ETC-505T temperature controller unit. CD spectra were registered on a JASCO J-715 spectrophotometer equipped with a thermoelectrically controlled couvette holder (JASCO PTC-348), in a 0.2 cm path length couvette.

General procedure for the synthesis of 5'-*O*-substituted thymidine-3'-phosphate and determination of the corresponding ε values.

100 mg (0.13 mmol) of 5'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite, 100 mg (0.14 mmol) of 5'-*O*-*tert*-butyldiphenylsilyl-thymidine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite, or 100 mg (0.11 mmol) of *N*-3-(2-phenylsulfenylethyl)-5'-*O*-(3,4-dibenzyloxybenzyl)-thymidine-3'-*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite were respectively treated with an excess of the activator solution (1.5 mL, 0.45 M tetrazole in acetonitrile) and with 3-hydroxypropionitrile (50 μL, 0.65 mmol). After 1 h, the resulting mixtures were reacted with 1 mL of the oxidizer solution, left at r.t. for an additional hour and then dried under reduced pressure. The crudes were redissolved in 100 mL of $CHCl_3$, washed twice with water and then purified by silica gel chromatography [eluent system: 5 %

CH_3OH in $CHCl_3$/pyridine (1:0.05, v/v)] affording 87 mg (0.12 mmol, 92 %) of 5'-*O*-(4,4'-dimethoxytriphenylmethyl)-thymidine-3'-*O*-bis-(2-cyanoethyl)-phosphate, 76 mg (0.11 mmol, 86 %) of 5'-*O*-*tert*-butyldiphenylsilyl-thymidine-3'-*O*-bis-(2-cyanoethyl)-phosphate, or 75 mg (0.082 mmol, 75 %) of *N*-3-(2-phenylsulfenylethyl)-5'-*O*-(3,4-dibenzyloxybenzyl)-thymidine-3'-*O*-bis-(2-cyanoethyl)-phosphate, all pure by TLC and ^{1}H NMR control. 50 mg (0.054-0.073 mmol) of the obtained compounds were treated with an excess of Et_3N (1 mL) for 18 h at 50 °C and then with 17 M NH_4OH (1 mL) for 18 h at 50 °C for the full deprotection of the phosphate moiety. The crudes were then applied onto a Sephadex G25 column eluted with CH_3OH/H_2O 1:1 (v/v). The fractions containing the desired 5'-substituted-3'-phosphate nucleosides, monitored by UV measurements at λ = 260 nm, were collected and taken to dryness, yielding 15-20 mg of the pure target compounds (45-50 % yields, unoptimized procedure).

The 3'-monophosphate nucleosides, checked for purity by RP-HPLC, were then characterized on the basis of their ^{1}H, ^{13}C and ^{31}P NMR and ESI-MS spectra (data not shown) which fully confirmed the identity of the target compounds. Determination of the ε values at 25 °C for these compounds was carried out by averaging at least five independent A^{260} measurements of the original samples at different, known concentrations, allowing to determine the following ε data: 9100, 8900 and 9000 cm^{-1} M^{-1}, respectively for 5'-DMT, 5'-TBDPS and 5'-DBB-thymidine-3'-monophosphate.

Captions to Supplementary Figures.

Figure S1 Association profiles for **3** at three different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All the experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

Figure S2 Association profiles for **2** at three different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All the experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

Figure S3 Association profiles for **4** at three different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All the experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

Figure S4 Dissociation profiles for Q-TGGGAG at four different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All the experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

Figure S5 Dissociation profiles for Q-TBDPS at four different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

Figure S6 Dissociation profiles for Q-DBB at four different temperatures and at 4.0×10^{-5} M single strand concentration. Mathematical fits are shown in solid line. All the experiments were performed in 10 mM potassium phosphate buffer (pH 7.0) supplemented with 200 mM KCl.

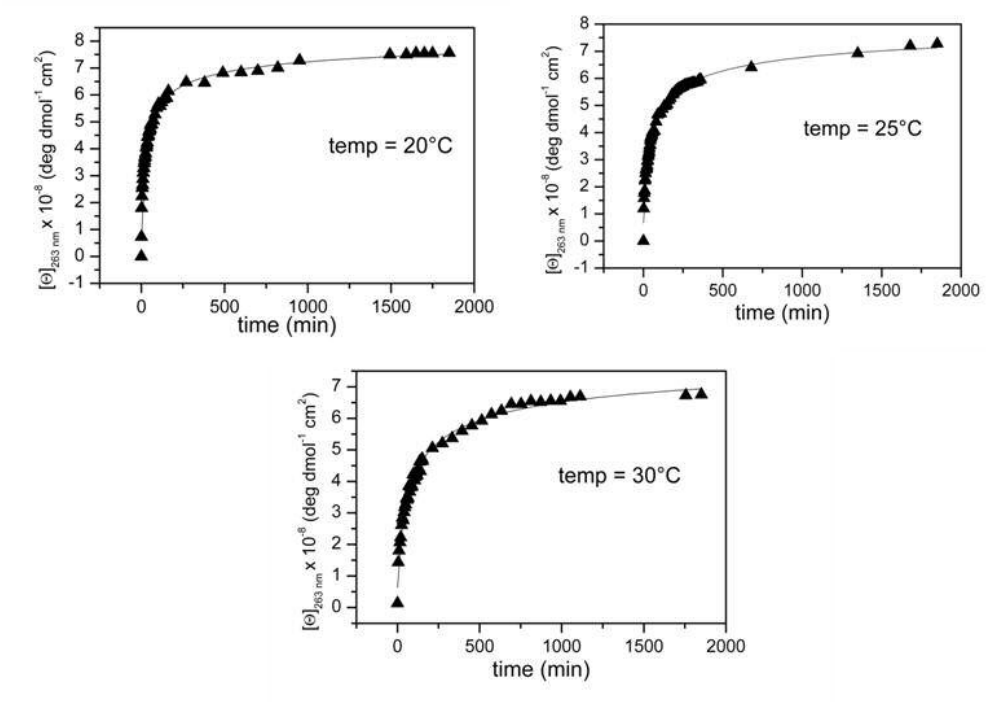

temp = 20°C
temp = 25°C
temp = 30°C
time (min)
$[\Theta]_{253\ nm}$ x 10^{-8} (deg $dmol^{-1}$ cm^2)

Figure S1

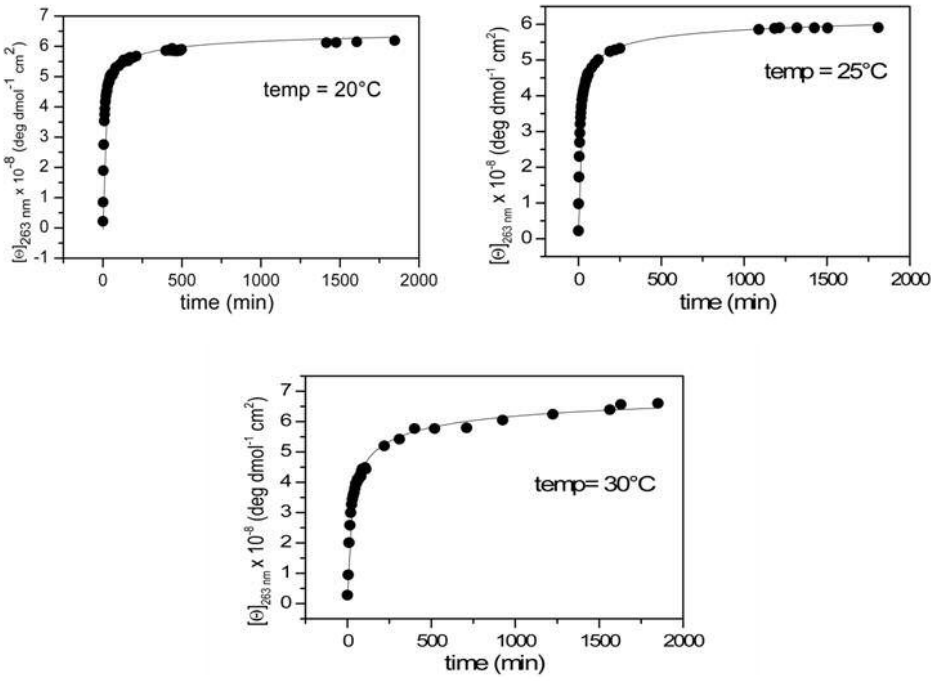


Figure S2

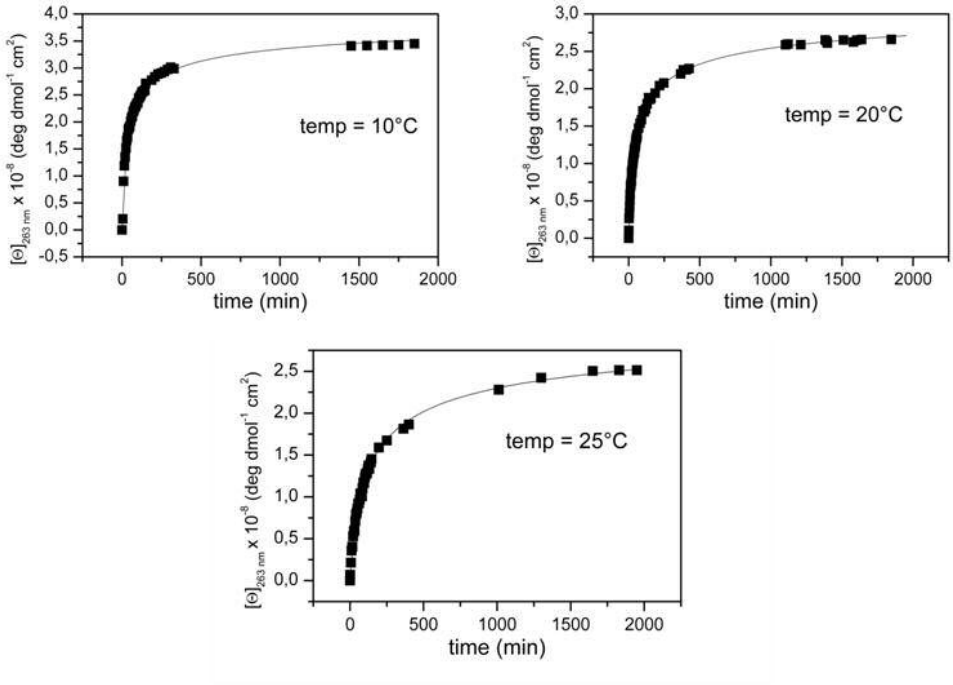
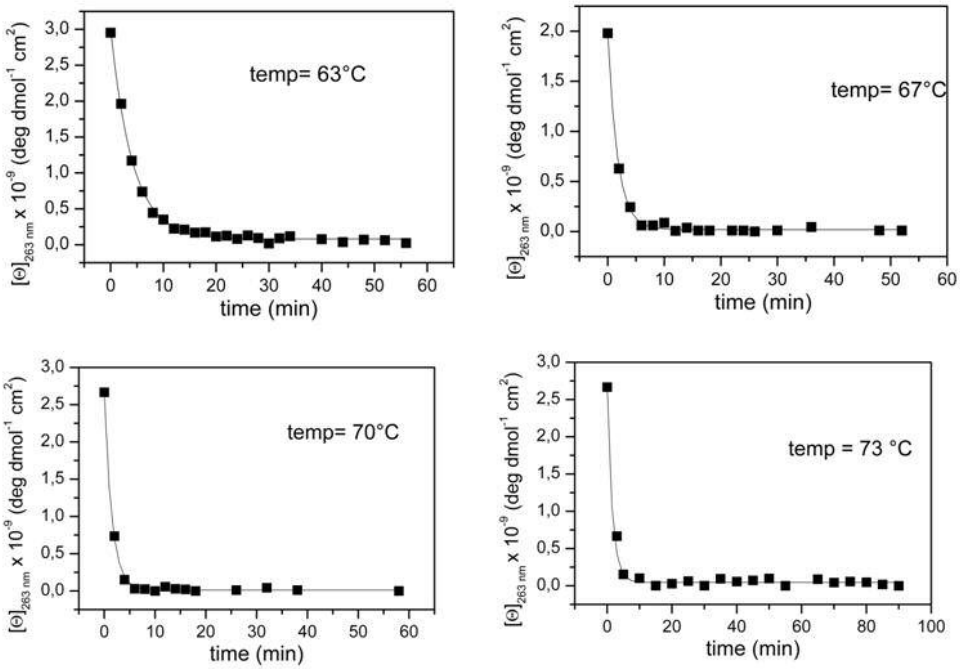


Figure S3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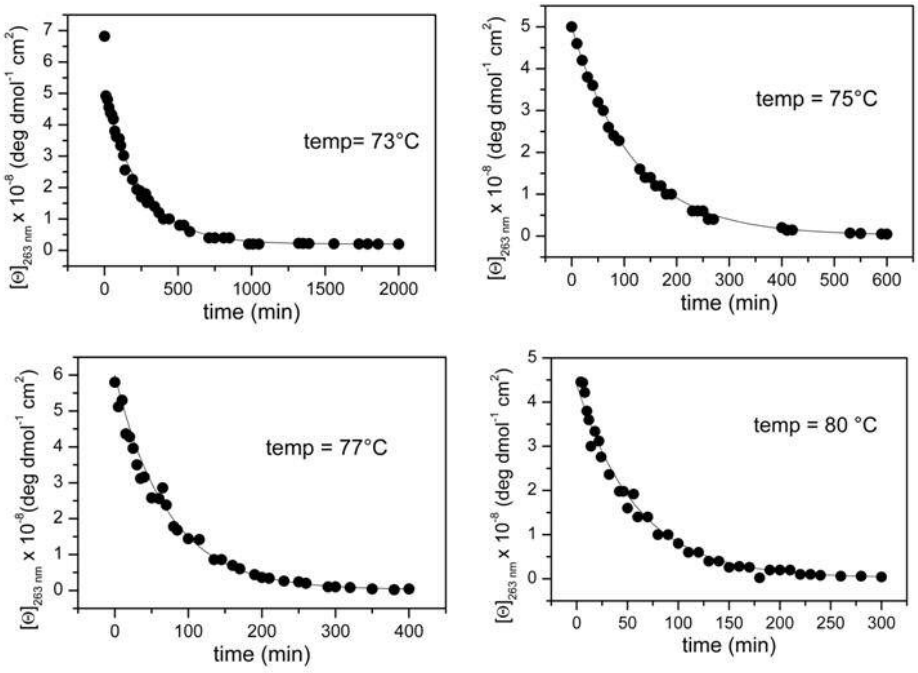
temp= 67°C
temp= 70°C
temp = 73 °C
time (min)
[Θ]263 nm x 10-9 (deg dmol-1 cm2)

Figure S4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temp = 75°C
temp = 77°C
temp = 80 °C
time (min)
[Θ]263 nm x 10-8 (deg dmol-1 cm2)

Figure S5

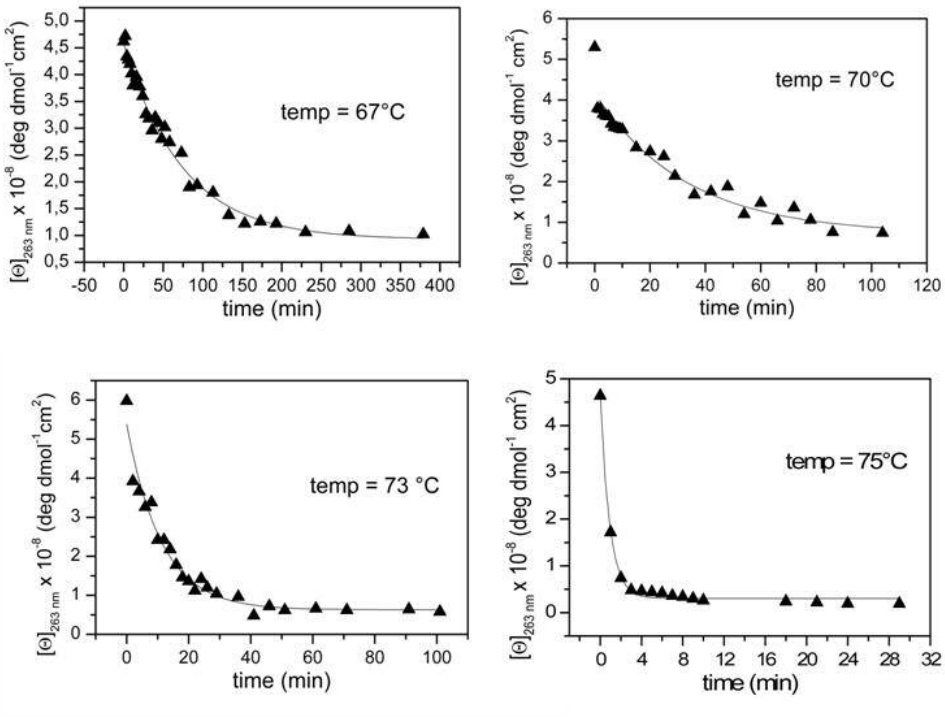
temp = 67°C
temp = 70°C
temp = 73 °C
temp = 75°C
time (min)
[Θ]263 nm x 10-8 (deg dmol-1cm2)

Figure S6