EPR STUDY OF SPIN-LABELED CAMPTOTHECIN DERIVATIVES: A DIFFERENT LOOK OF THE TERNARY COMPLEX

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SUPPORTING INFORMATION

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General Information.

All water and/or air sensitive reactions were carried out in oven-dried glassware under a nitrogen atmosphere with dry solvents, unless otherwise noted. All commercial reagents were purchased from Sigma-Aldrich and were used without further purification. All the phosphoramidites were purchased from Glen Research (22825 Davis Drive, Sterling, Virginia, 20164) and were used without further purifications. The bottles containing the desired phosphoramidites were open and left under vacuum (14 mmHg) overnight before preparing the coupling solution (as to prevent moisture interference). The solvents were purchased from Fluka, Carlo Erba and Glen Research; anhydrous solvents were therefore obtained as follow: tetrahydrofuran (THF) was distilled from sodium with benzophenone, pyridine was distilled over KOH dichloromethane (CH_2Cl_2) and acetonitrile (ACN) were distilled from calcium hydride. Reactions were usually monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck glass plates (60 F_{254}) using UV light at 254 nm as visualizing agent and an ethanolic solution of phosphomolybdic acid or ninhydrin as developing agents when required. Merck silica gel (230-400 mesh) was used for silica gel chromatography. ^{1}HNMR and ^{31}PNMR spectra were recorded on a Bruker Avance AMX 300 spectrometer. Chemical shifts (δ) were expressed in parts per million relative to tetramethylsilane (ppm) and the following abbreviations were used to explain the multiplicities: s=singlet, d=doublet, dd=doublet doublets, t=triplet, dt=doublet triplets, tt=triplet triplets, q=quartet, m=multiplet, bm=broad multiplet bs=broad singolet. IR spectra were recorded on a Perkin-Elmer 1760 Infrared Fourier Trasform Spectrometer. Mass spectrometric data were obtained using a Mariner^{TM} API-TOF (Perseptive Biosystems Inc.- Framingham MA 01701 USA) while mass spectra of oligonucleotides were obtained with an Applied Biosystem Voyager System 2081 MALDI-TOF Mass Spectrometer. Purities (>97%) of all tested compounds were established by HPLC using a Varian pro star equipped with a double pump, an UV-VIS detector (BioRad) and a Phenomenex

C18 (4.6 mm _ 250 mm, 5 μm) column, and H_2O and CH_3CN as eluent. A gradient from 5% to 95% of CH_3CN over 30 min followed by 5 min more at 95% CH_3CN percentage was used at a flow rate of 1.00 mL/min. The signal was detected with an UV-VIS detector fixed at 254 nm.

Experimental Part.

Synthesis of the TEMPO-amine probe.

Synthesis of 2,2,5,5-tetramethylpiperidin-4-oxime (2). 6.71 g (96.53 mmol) of hydroxylamine hydrochloride were added to a solution of 15.84 g (193.06 mmol) of sodium acetate in H_2O (50 ml) and the whole was heated up to 60 °C. 10 grams (64.52 mmol) of triacetonamine (**A**) were subsequently added to the solution and the mixture was kept at this temperature for 30 minutes. The reaction was cooled to RT, the precipitate filtered and solved again in water (50 ml). The solution was saturated with Na_2CO_3 and extracted with EtOAc (50 ml for three times); the organic layer were collected, dried with Na_2SO_4 and evaporated in vacuo to obtain 10.43 g (61.29 mmol) of **2**. Yield 95%. ^{1}HNMR (Acetone-*d*6, 300MHz,) δ (ppm): 9.51 (s, 1H), 2.96 (s, 1H), 2.34 (s, 2H), 2.15 (s, 2H), 1.113 (s, 6H), 1.11 (s, 6H). HRMS (ESI): calcd for $C_9H_{19}N_2O$ ($M+H^+$) 171.1570, found 171.1581.

Synthesis of 2,2,5,5-tetramethylpiperidin-4-amine (3). A three neck round bottom flask fitted with a bubble condenser was charged with a suspension of 2 grams (11.76 mmol) of **2** in 25 ml of freshly distilled THF; nitrogen was then bubbled inside the suspension. The mixture was cooled to 0 °C and 35.3 ml (35.30 mmol) of a 1,0 M solution of $LiAlH_4$ in THF were slowly added to the suspension. After the initial formation of hydrogen, the suspension was heated to reflux for 3h, during which the reaction was followed by TLC (eluent EtOAc:MeOH 1:1; the product could be observed performing the ninhydrin test). The reaction was subsequently cooled to RT, quenched with 1 ml of H_2O (slowly added to the mixture), 1 ml of a 5% NaOH watery solution and filtered. The organic solution was dried (Na_2SO_4) and stored under reduced pressure, giving an oil (1.56 g, 10 mmol), which was used in the next step without further purifications. Yield 98%. ^{1}HNMR (Acetone-*d*6; 300MHz,) δ (ppm): 4.30-4.15 (tt, 1H, J_1 = 12.5 Hz, J_2 = 3.7 Hz), 3.05 (s, 1H), 2.50 (s,

2H), 2.10 (s, 2H, *J* = 3.7 Hz), 2.06 (s, 2H, *J* = 3.7 Hz), 1.20 (s, 6H), 1.15 (s, 6H); HRMS (ESI): calcd for $C_9H_{21}N_2$ ($M+H^+$) 157.1699, found 157.1703.

Synthesis of 2,2,5,5-tetramethylpiperidin-4-acetamide acetate (4). A round bottomed flask was charged with 1.56 g (10 mmol) of crude **3** and 10 ml of $(Et)_2O$. The solution was cooled to 0 °C and 2.25 ml (23.84 mmol) of acetic anhydride was then slowly dropped inside, taking care not to exceed 20 °C. The mixture was allowed to warm to room temperature for 2h, during which a white precipitate was produced. The solvent was removed, the precipitate washed with acetone and dried to obtain 2 g (7.75 mmol) of pure **4**. Yield 75%. ^{1}HNMR (D_2O, 300MHz) δ (ppm): 4.29-4.18 (tt, 1H, J_1 = 12.35 Hz, J_2 = 3.60 Hz), 2.06-2.05 (d, 2H, *J* = 3.90 Hz), 2.02-2.00 (d, 2H, *J* = 3.60 Hz), 1.96 (s, 3H), 1.89 (s, 3H), 1.50 (s, 6H), 1.40 (s, 6H); HRMS (ESI): calcd for $C_{11}H_{22}N_2O$ ($M+H^+$) 199.1805, found 199.1831.

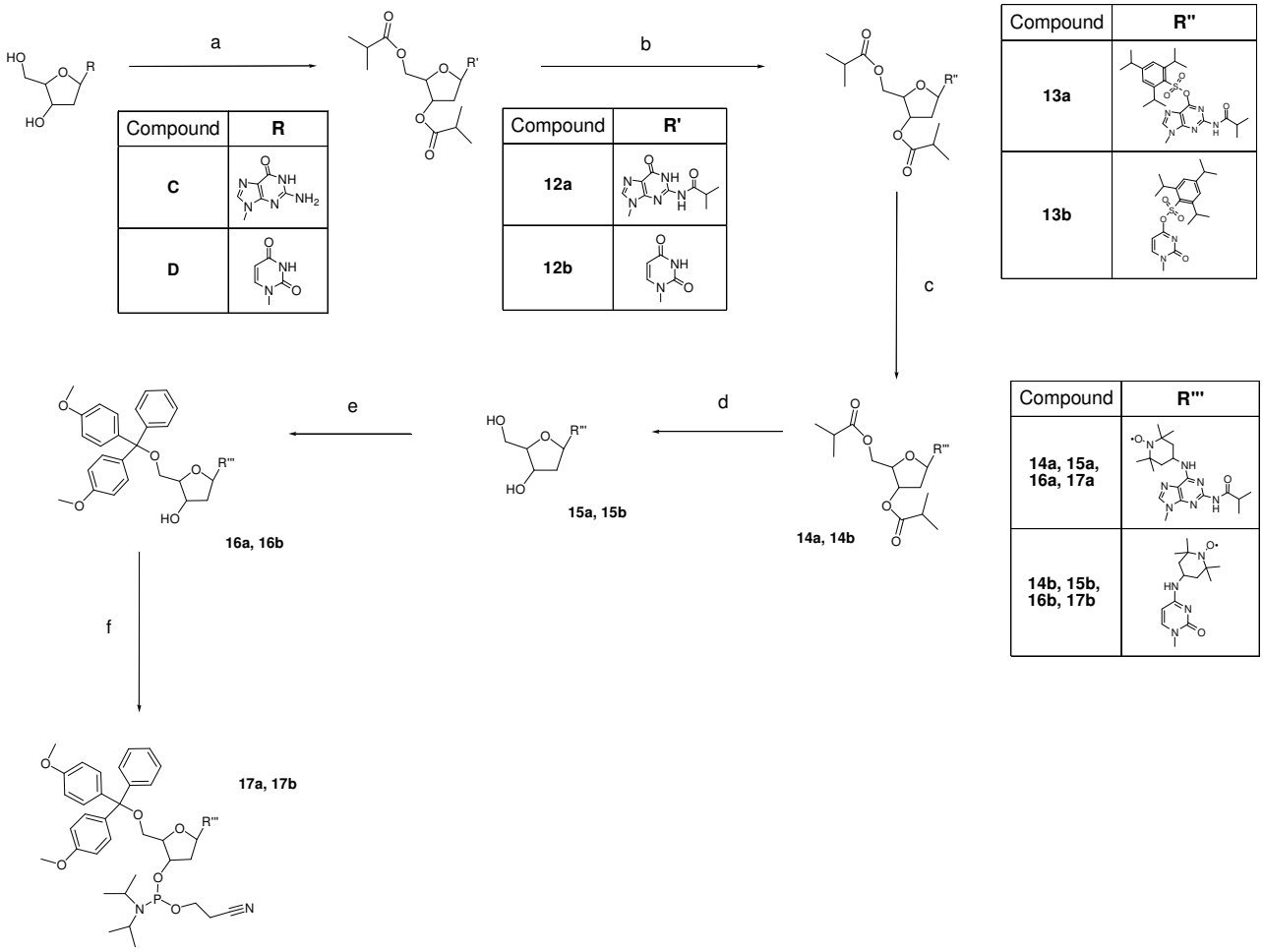
Synthesis of 1-oxyl-2,2,5,5-tetramethylpiperidin-4-acetamide (5). In a 250 ml round-bottomed flask 500 mg (1.92 mmol) of **4** were mixed with 20 ml of a 5% sodium carbonate aqueous solution. 79 mg (0.219 mmol) of EDTA-4Na^+ and 79 mg (0.24 mmol) of sodium tungstate were further added before cooling the solution to 0-4 °C (ice bath). 1.58 ml (51.58 mmol) of cold 30% aqueous solution of hydrogen peroxide were slowly dropped into the clear solution; the mixture was then allowed to warm at RT and leaved at this temperature for 72 h. The orange suspension obtained was filtered off and the solution filtered was saturated with sodium carbonate. A new orange precipitate formed and it was filtered, collected with the previous one and dried to obtain 370 mg (1.73 mmol) of **5** as bright orange crystals. Yield 89.5%. ^{1}HNMR (Acetone-*d*6, 300MHz) δ (ppm): 1.90 (bs, 3H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{11}H_{22}N_2O_2$ ($M+H^+$) 214.1676, found 214.1680.

Synthesis of 1-oxyl-2,2,5,5-tetramethylpiperidin-4-amine (6). 200 mg (0.94 mmol) of **5** were suspended into 12.5 ml of a 15% KOH aqueous solution and the whole was heated to reflux. The reaction was monitored by TLC (eluent EtOAc) until complete disappearance of the starting

material. After 36 h the solution obtained was cooled to RT, saturated with K_2CO_4 and extracted with $(Et)_2O$. The organic phase was collected, dried (Na_2SO_4) and evaporated under reduced pressure to obtain 147 mg (0.86 mmol) of **6** as a red liquid that crystallize at 14 °C. Yield: 92.0 %. ^{1}HNMR (Acetone-*d*6, 300MHz) δ (ppm): from -15 to -35 (bm, 16H); IR (KBr): 2973 (ν_{as} $^{TEMPO}CH_3$), 2936 (ν_s $^{TEMPO}CH_3$), 1459-1363 (δ $^{TEMPO}CH_3$); HRMS (ESI): calcd for $C_9H_{20}N_2O$ ($M+H^+$) 172.1570, found 172.1578.

Synthesis of the TEMPO-labeled nucleotides.

Scheme S-1. Route for the synthesis of the TEMPO substituted nucleotides



[a] Isobuthyric anhydride, pyridine, DMAP, 50 °C, 24 h; [b] TPS-Cl, triethylamine, DMAP, RT, 16 h; [c] TEMPOamine, CH_2Cl_2, reflux, 16 h; [d] NH_4OH 25%, MeOH, RT, 4 h; [e] DMT-Cl, DMAP, CH_2Cl_2, RT, 16 h; [f] Bisphosphoramidite, tetrazole, acetonitrile, RT, 2 h

General procedure for the 3', 5' hydroxyl esterification of the nucleosides. A mixture of 1.12 mmol of the required nucleoside, 0.22 mmol of dimethylaminopyridine (DMAP) and 8.98 mmol of isobutyric anhydride in 8,5 ml of anhydrous pyridine was heated up to 50 °C until no more starting reagent was observed by TLC (eluent EtOAc). The reaction was firstly quenched with MeOH (2 ml) and then cooled to RT, concentrated to small volume and partitioned between EtOAc and H_2O. The organic phase was collected, washed with water (two times), anhydrified with Na_2SO_4 and lastly dried to obtain the desired product.

2,3',5'-triisobutyryl-2'-deoxyguanidine (12a). Obtained from 2'-deoxyguanidine (**C**). Yield 89%. ^{1}HNMR (Acetone- *d*6, 300MHz) δ (ppm): 8.01 (s, 1H), 6.32-6.27 (dd, 1H, J_1 = 8.39 Hz, J_2 = 5.99), 5.45-5.41 (dt, 1H, J_1 = 6.26 Hz, J_2 = 1.76), 4.37-4.30 (m, 3H), 3.08-2.89 (m, 3H), 2.69-2.55 (m, 3H), 1.25-1.24 (d, 3H, J_1 = 1.83 Hz), 1.23-1.22 (d, 3H, J_1 = 1.83 Hz), 1.206-1.204 (d, 3H, J_1 = 0.60 Hz), 1.183-1.181 (d, 3H, J_1 = 0.60 Hz), 1.18 (d, 3H), 1.11 (d, 3H); HRMS (ESI): calcd for $C_{22}H_{32}N_5O_7$ ($M+H^+$) 478.2296, found 478.2302

2,3',5'-diisobutyryl-2'-deoxyuridine (12b). Obtained from 2'-deoxyuridine (**D**). Yield 91%. ^{1}HNMR (Acetone-*d*6 300MHz) δ (ppm): 7.85 (s, 1H), 6.28-6.24 (dd, 1H, J_1 = 8.25 Hz, J_2 = 5.30), 5.58 (s, 1H) 5.45-5.41 (dt, 1H, J_1 = 6.26 Hz, J_2 = 1.76), 4.34-4.30 (m, 1H), 3.08-2.89 (m, 2H), 2.69-2.55 (m, 4H) 1.25-1.24 (d, 3H, J_1 = 1.83 Hz), 1.23-1.22 (d, 3H, J_1 = 1.83 Hz), 1.206-1.204 (d, 3H, J_1 = 0.60 Hz), 1.183-1.181 (d, 3H, J_1 = 0.60 Hz) ; HRMS (ESI): calcd for $C_{17}H_{25}N_2O_7$ ($M+H^+$) 369.1656, found 369.1662

General procedure for TPS-activation of the nucleosides. In a round bottomed flask were solved 0.98 mmol of protected nucleoside in 5 ml of anhydrous CH_2Cl_2. 1.96 mmol of triisopropylphenylsulphonyl chloride (TPS-Cl), 1.96 mmol of triethylamine and 0.10 mmol of DMAP were added to the solution and the whole was stirred overnight at RT under N_2 atmosphere.

The mixture was then evaporated and the residue was purified by silica gel chromatography (eluent hexane:CH_3Cl gradient from 1:1 to 0:1) to obtain the pure product.

2,3',5'-triisobutyryl-O^6-[(2,4,6-triisopropylphenyl)sulphonyl]-2'-deoxyguanosine (13a). Obtained from **12a**; Yield 78%. ^{1}HNMR (Acetone- *d*6, 300MHz) δ (ppm): 7.95 (s, 1H), 7.05 (s, 1H), 7.01 (s, 1H), 6.30-6.25 (dd, 1H, J_1 = 8.5 Hz, J_2 = 6.2 Hz), 5.47-5.41 (dt, 1H, J_1 = 6.26 Hz, J_2 = 1.76), 4.30-4.30 (m, 2H), 3.15-2.93 (m, 4H), 2.69-2.52 (m, 6H), 1.25-1.23 (d, 3H, J_1 = 1.95 Hz), 1.23-1.21 (d, 3H, J_1 = 1.95 Hz), 1.202-1.201 (d, 3H, J_1 = 0.55 Hz), 1.183-1.182 (d, 3H, J_1 = 0.50 Hz), 1.18 (d, 3H), 1.11 (d, 3H), 1.05-0.75 (m, 18H); HRMS (ESI): calcd for $C_{37}H_{54}N_5O_9S$ ($M+H^+$) 744.3637, found 744.3638

3',5'-diisobutyryl-O^4-[(2,4,6-triisopropylphenyl)sulphonyl]-2'-deoxyuracile (13b). Obtained from **12b**; Yield 81%. ^{1}HNMR (Acetone-*d*6 300MHz) δ (ppm): 7.90 (s, 1H), 7.10 (s, 1H), 7.05 (s, 1H), 6.26-6.20 (dd, 1H, J_1 = 8.25 Hz, J_2 = 5.30 Hz), 5.64 (s, 1H), 5.50-5.46 (dt, 1H, J_1 = 6.26 Hz, J_2 = 1.76 Hz), 4.33-4.28 (m, 1H), 3.08-2.89 (m, 2H), 2.69-2.55 (m, 7H), 1.23-1.22 (d, 3H, J_1 = 1.7 Hz), 1.20-1.19 (d, 3H, J_1 = 1.7 Hz), 1.202-1.20 (d, 3H, J_1 = 0.6 Hz), 1.183-1.181 (d, 3H, J_1 = 0.6 Hz), 1.10-0.75 (m, 18H); HRMS (ESI): calcd for $C_{32}H_{47}N_2O_9S$ ($M+H^+$) 635.2997, found 635.2998; ($M+Na^+$) 653.3507, found 653.3506.

General procedure for the synthesis of the spin-labeled nucleosides. The proper sulphonylated nucleoside (0.65 mmol) was placed in a round bottomed flask with **6** (1.30 mmol) and 3 ml of CH_2Cl_2. The solution was refluxed overnight after which it was diluted with other 20 ml of CH_2Cl_2, washed with a saturated aqueous solution of $NaHCO_3$ and with brine. The organic layer was thus dried with Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (eluent EtOAc:hexane 70:30).

3',5'-diisobutyryl-2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (14a). Obtained from **13a**. Yield 55%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 7.85 (bs, 1H), 6.41 (bs, 1H), 4.78 (bs, 1H), 4.17 (bs, 1H), 3.90 (m, 2H), 2.80-2.45 (m, 5H), 1.40-1.20 (m, 18H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{31}H_{49}N_7O_7$ ($M+H^+$) 631.3687, found 631.3689; ($M+Na^+$) 653.3507, found 653.3506.

3',5'-diisobutyryl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (14b). Obtained from **13b**. Yield 59%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 7.99 (bs, 1H), 6.26 (bs, 1H), 5.82 (bs, 1H), 4.36 (bs, 1H), 3.93 (bs, 1H), 3.74 (m, 2H), 2.13-2.35 (m, 4H), 1.20-0.95 (2 bs, 12H) from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{26}H_{42}N_4O_7$ ($M+H^+$) 522.3048, found 522.3052.

General procedure for deprotection of the 3' and 5' hydroxyl function. In a round bottomed flask were placed 0.23 mmol of the chosen TEMPO-nucleoside with a mixture of 1.1 ml of NH_4OH 25% and 3.3ml of MeOH. The solution was stirred at RT for 4 h after which the solvent was removed and the residue was purified by column chromatography with silica gel (eluent EtOAc:MeOH 98:2).

2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (15a). Obtained from **14a**. Yield 84%. ^{1}HNMR (CD_3OD, 300MHz) δ (ppm): 7.90 (bs, 1H), 6.35 (bs, 1H), 4.78 (bs, 1H), 4.21 (bs, 1H), 3.92 (m, 2H), 2.50-2.15 (m, 3H), 1.32-1.15 (2 bs, 6H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{23}H_{37}N_7O_5$ ($M+H^+$) 491.2851, found 491.2873.

N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (15b). Obtained from **14b**. Yield 87%. ^{1}HNMR (CD_3OD, 300MHz) δ (ppm): 7.99 (bs, 1H), 6.26 (bs, 1H), 5.82 (bs, 1H), 4.36 (bs, 1H), 3.93 (bs, 1H), 3.74 (m, 2H), 2.35-2.13 (m, 2H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{18}H_{30}N_4O_5$ ($M+H^+$) 382.2211, found 382.2274.

General procedure for 5' DMT protection. 0.45 mmol of the desired TEMPO-nucleoside were coevaporated three times with a small volume of anhydrous pyridine (~1 ml). The film thus obtained was solved into 3 ml of anhydrous pyridine and added with 0.54 mmol of dimethoxytrityl chloride (DMT-Cl) and 0.05 mmol of DMAP. The reaction is stirred overnight at RT under N_2 atmosphere after which the solvent was removed under vacuum. The residue was diluted with CH_2Cl_2, washed with a 5% $NaHCO_3$ watery solution and finally with brine. The organic layer was dried and the crude product was purified by silica gel chromatography (eluent EtOAc:Methanol:TEA 97:2:1). The purified product was lastly solved into a small amount of CH_2Cl_2 and dropped into cold hexane. The final product was recovered by centrifugation (2400 rpm, 3 min.).

5'-dimethoxytrityl-2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (16a). Obtained from **15a**. Yield 83%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 7.85 (bs, 1H), 7.43 (bs, 4H), 7.30 (bs, 5H), 6.80 (bs, 4H), 6.32 (bs, 1H; H^2), 4.71 (bs, 1H), 4.51 (bs, 1H), 3.79 (bs, 6H), 3.75-3.56 (m, 2H), 2.64-2.43 (m, 2H), 1.20-1.17 (2 bs, 6H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{44}H_{55}N_7O_7$ ($M+H^+$) 793.4157, found 793.4160

5'-dimethoxytrityl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (16b). Obtained from **15b**. Yield 81%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 7.91 (bs, 1H), 7.40 (bs, 4H), 7.30 (bs, 5H), 6.84 (bs, 4H), 6.30 (bs, 1H), 5.43 (bs, 1H), 4.53 (bs, 1H), 4.06 (bs, 1H), 3.80 (bs, 6H), 3.61 (m, 2H), 3.48-3.39 (m, 2H), from -15 to -35 (bm, 16H); HRMS (ESI): calcd for $C_{39}H_{48}N_4O_7$ ($M+H^+$) 684.3517, found 684.3528; ($M+Na^+$) 706.3336, found 706.3337; ($M+K^+$) 722.3076, found 722.3337.

General procedure for the 3' phosphoramidation. 0.22 mmol of the required DMT protected TEMPO-nucleoside is firstly coevaporated three times with anhydrous acetonitrile. The amorphous

solid was dissolved into 2 ml of anhydrous acetonitrile and to the solution were firstly added 0.44 mmol of bisphosphoramidite and, then, 0.27 mmol of sublimated tetrazole. The whole was left under Ar pressure at RT for 2 h during which the organic salts precipitated out of the solution. Work-up of the reaction consisted in a first filtration of the suspension, as to remove the salts, followed by the removal of the solvent under vacuum. The crude mixture was dissolved in CH_2Cl_2 and washed (two times) with brine. The organic layer was dried and purified by silica gel chromatography (eluent EtOAc:Methanol:TEA gradient from 80:15:5 to 70:23:7). The purified product was solved again into a small amount of CH_2Cl_2 and dropped into cold hexane. The final product is recovered by centrifugation (2400 rpm, 3 min.)

5'-dimethoxytrityl-2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine-3'-(2-cyanoethyl-N,Ndiisopropyl) phosphoramidite (17a). Obtained from **16a**. Yield 72%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 7.88 (bs, 1H), 7.41 (bs, 4H), 7.29 (bs, 5H), 6.80 (bs, 4H), 6.36 (bs, 1H), 4.72 (bs, 1H), 4.27 (bs, 1H), 3.79 (bs, 6H), 3.77-3.56 (m, 6H), 2.82-2.43 (m, 5H), 1.20-1.17 (m, 18H) from -15 to -35 (bm, 16H); ^{31}PNMR (CD_3Cl, 300MHz) δ (ppm): 148.94, 148.81; HRMS (ESI): calcd for $C_{53}H_{72}N_9O_8P$ ($M+H^+$) 993.5236, found 993.5236; ($M+Na^+$) 1015.5055, found 1015.5048; ($2M+H^+$) 1986.0399, found 1986.0414.

5'-dimethoxytrityl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine-3'-(2-cyanoethyl-N,N-diisopropyl) phosphoramidite (17b). Obtained from **16b**. Yield 75%. ^{1}HNMR (CD_3Cl, 300MHz) δ (ppm): 8.01 (bs, 1H), 7.43 (bs, 4H), 7.32 (bs, 5H), 6.87 (bs, 4H), 6.35 (bs, 1H), 5.49 (bs, 1H), 4.53 (bs, 1H), 4.15 (bs, 1H), 3.83 (bs, 6H), 3.60-3.39 (m, 6H), 2.78-2.40 (m, 4H), 1.29-1.19 (2 bs, 12H) from -15 to -35 (bm, 16H); ^{31}PNMR (CD_3Cl, 300MHz) δ (ppm): 149.25, 148.66; HRMS (ESI): calcd for $C_{48}H_{65}N_6O_8P$ ($M+H^+$) 884.4596, found 884.4592; ($M+Na^+$) 906.4409, found 906.4409; ($2M+H^+$) 1767.9116, found 1767.9116.

Synthesis of the oligonucleotide strands.

Synthesis of the unmodified oligonucleotides. Unmodified oligonucleotides were automatically synthesized on 1.0 μmol scale (1000 Å CPG columns) with an Applied Biosystem 3400 DNA synthesizer using standard protected phosphoramidites; the final trityl protective group was untouched in order to facilitate the purification step. All the nucleotides solutions were constituted of a 0.1 M desired phosphoramidite into anhydrous THF and were prepared immediately before the use. The coupling solution consisted of a 0.3 M tetrazole solution into anhydrous THF. The capping solution was composed by two different solution, a 10% acetic anhydride solution into anhydrous THF and a 10% N-methylimidazole solution into a THF/pyridine (90/10) mixture. The oxidation mixture was prepared mixing iodine with a mixture of pyridine, THF and H_2O with a final 0.02 M iodine concentration. The deprotection mixture was a 3% trichloroacetic acid solution into anhydrous CH_2Cl_2. The DNA synthesizer was initially pressurized with a 15 second flux of Ar in order to guarantee a constant flow of 30 μl/sec for each solution. The nucleotide-coupling mixture delivery time was set at 7 sec, followed by an additional 3.9 sec coupling solution delivery and a sleeping time of 120 sec. The capping solution delivery time was set at 12 sec, followed by 6 sec of sleeping time; the oxidation solution delivery time was set at 12 sec, followed by further 12 sec of sleeping time, and the deprotection solution was delivered to the column for 110 sec. The coupling yield was measured during the deprotection step, recording the trityl carbocation passage (freed during the acidic cleavage) into a potentiometric detector. The average coupling yield was >98.5%. Cleavage of the oligonucleotides from the solid glass support and deprotection of the reactive nucleobases functions were obtained by reaction carried with 35% ammonia solution at 55°C for 18 hours. Purification was performed by medium pressure liquid chromatography (MPLC) using these two solutions as the mobile phase: A) a mix composed by 95% of a 0.05 M triethylamonium acetate (TEAA) aqueous solution and 5% of a 50/50 ACN/H_2O solution; the second eluent (B) was a mix

composed by 50% of a 0.05 M triethylamine acetate (TEAA) aqueous solution and 50% of a 50/50 ACN/H_2O solution. A first gradient starting from A_100% to A/B_50/50% was applied for 3.5 h, until the exit of the truncated oligo sequences, followed by an isocratic A/B_50/50 ratio until the exit of the desired DMT-oligonucleotide strand. The last DMT group was removed placing 1 µmol of each oligonucleotide in 1 ml of an 80% acetic acid watery solution for 30 min at 0-4°C and washing the water layer with $(Et)_2O$ (4 ml for four times). The purity of each oligonucleotide was lastly checked by HPLC, showing a purity ≥98% for all the oligonucleotides. Concentration was calculated by UV spectroscopy (wavelength set at 260 nm) using the Lambert-Beer equation; molar extinction coefficient ε were calculated with the nearest neighbour method. The characterization of all the oligonucleotides was determined using an Applied Biosystem Voyager System 2081 MALDI-TOF Mass Spectrometer. The samples were prepared mixing 1 µl of the oligo solution with 1 µl of a 2-hydroxypicolinic acid (50 mg in 1 ml of a H_2O/CAN 50/50 mixture) solution and 1 µl of a 0.1 M citric acid aqueous solution The following results were obtained:

calcd for the 5'-AAAAATTTTTCCAAGTCAAAAA-3' sequence ($M+H^+$) 6673.4, found 6668.2

calcd for the 5'-AAAAAGACTTGGAAAAATTTTT-3' sequence ($M+H^+$) 6789.4, found 6780.2

Synthesis of the TEMPO-modified oligonucleotides. The 1.0 µmol scale TEMPO-modified oligonucleotides synthesis was divided into three steps: 1) A first automatic synthesis was performed using the same condition described in the experimental part. 2)TEMPO-phosphoramidites **24** and **25** were then specifically incorporated into the single modified and double modified oligonucleotides by manual coupling. The yield of coupling (~93% for all the substitutions) was determined placing a small quantity of CPG resin in a 70% perchloric acid ethanolic solution and reading the absorption value of the trityl cation released by UV-VIS spectroscopy (ε_{499}= 71770). 3) The oligonucleotide strands were completed via automatic synthesis

changing the oxidation solution, which was substituted with a 1M ^{tert}butylhydroperoxide solution in CH_2Cl_2 and the deprotection solution, which was replaced with a 3% dichloroacetic acid solution in CH_2Cl_2. Cleavage and purification processes were performed according to the procedure described above. Once again, these oligonucleotides were characterized via MALDI-TOF Mass Spectrometry giving the following results:

calcd for the 5'-AAAAAGACTTGG**tA**AAAATTTTT-3' sequence ($M+H^+$) 6984.4, found 7003.3

calcd for the 5'-AAAAAGA**tC**TTGGAAAAATTTTT-3' sequence ($M+H^+$) 6969.4, found 6780.2

calcd for the 5'-AAAAAGA**tC**TTGG**tA**AAAATTTTT-3' sequence ($M+H^+$) 7146.4, found 7156.3

EPR experiments at 298 K

We report in Figure.S1 the spectrum of the ternary complex recorded at 298 K. The conditions are the same as those reported in the text for the spectra at 277 K. All the parameters used in the simulations are the same as those reported in the text for the ternary complex at 277 K, aside from the faster correlation time of **9** free in solution, as expected at an higher temperature. The ratio of the free and bound components is the same within the experimental error.

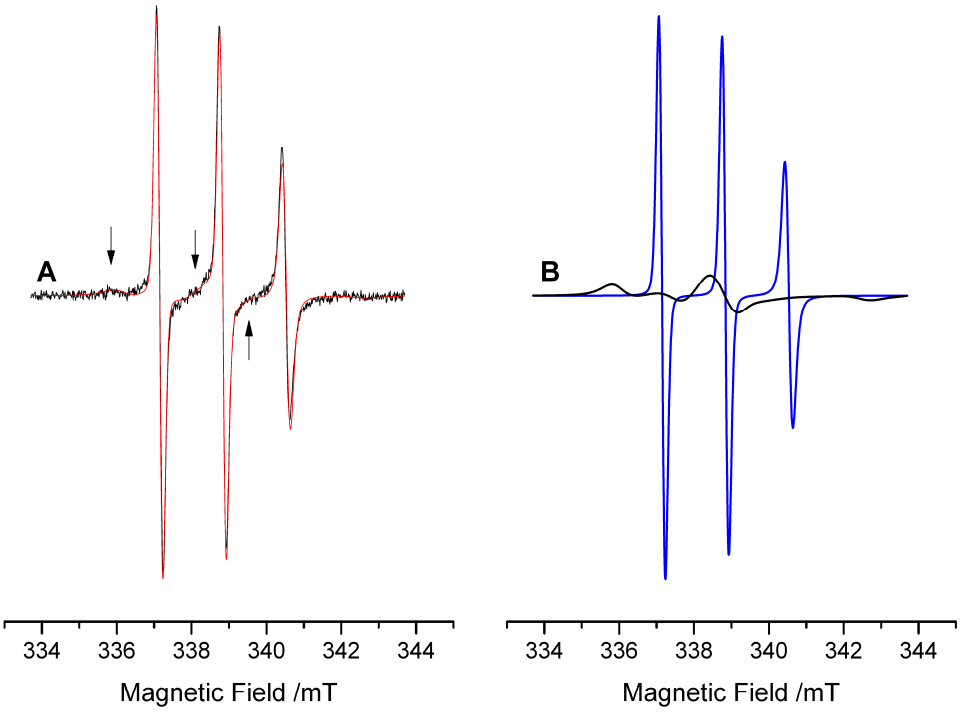


Figure S1. A) EPR spectrum of the ternary complex recorded at 298 K (black line), together with its simulation (thin red line) obtained as a sum of a component arising from **9** in the ternary complex (~40%), and a component arising from **9** free in solution (~60%). B) Individual components of the simulation in A: **9** free in solution (thin blue line) and in the ternary complex (thick black line).