Supporting Information

for

Phosphoramidite building blocks with protected nitroxides for the
synthesis of spin-labeled DNA and RNA

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Synthesis, purification and photochemical deprotection of oligonucleotides,
mass spectra and HPLC plots. ¹H and ¹³C NMR spectra

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General information

Compounds containing 2-nitrobenzyl groups should be handled in dim light only! Anhydrous pyridine, dichloromethane and methanol were purchased from Sigma-Aldrich. Flash column chromatography: silica gel (60 Å pore size, 0.04–0.063 mm particle size). Analytical thin layer chromatography: aluminum plates pre-coated with silica gel (0.2 mm, 60 Å pore size, Merck) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV). After purification via silica gel chromatography every compound was lyophilized with benzene. Proton nuclear magnetic resonance (\(^1\)H NMR) spectra, carbon nuclear magnetic resonance (\(^{13}\)C NMR) and phosphorus nuclear magnetic resonance (\(^{31}\)P NMR) were recorded at 300 K with Bruker AV 300 (\(^1\)H: 300 MHz; \(^{13}\)C: 75.5 MHz; \(^{31}\)P: 121.5 MHz) or Bruker AV 500 (\(^1\)H: 500 MHz; \(^{13}\)C: 125.8 MHz) NMR spectrometers. Chemical shifts for protons are reported in parts per million (\(\delta\) scale) and internally referenced to the proton resonances of the solvent (CDCl\(_3\): \(\delta\) 7.26, DMSO-\(d_6\): \(\delta\) 2.50). Chemical shifts for carbon are reported in parts per million (\(\delta\) scale) and referenced to the carbon resonances of the solvent (CDCl\(_3\): \(\delta\) 77.00, DMSO-\(d_6\): \(\delta\) 39.51). Data are represented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, dt = doublet of triplets, bt = broad triplet, q = quartet, quin = quintet, m = multiplet), coupling constants in Hz, and integration. ESI MS spectra were obtained on a Fisons VG Plattform II. HRMS spectra were recorded on a MALDI LTQ Orbitrap mass spectrometer from Thermo Scientific.

Synthesis of phosphoramidites

1-(3’-O-Acetyl-5’-O-DMT-2’-deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzyl-oxy)methoxy)piperidin-4-ylamino)pyrimidin-2(1H)-one (11): A solution of 3’-O-acetyl-5’-O-DMT-deoxyuridine 9 [1] (4.86 g, 8.48 mmol, 1.00 equiv), 4-dimethylaminopyridine (0.16 g, 1.27 mmol, 0.15 equiv) and Et\(_3\)N (10.7 mL, 9.75 mmol, 9.00 equiv) in 80 mL CH\(_2\)Cl\(_2\) was cooled to 0 °C, treated with 2,4,6-triisopropylbenzenesulfonyl chloride (2.95 g, 9.75 mmol, 1.15 equiv) and stirred for 10 min at 0 °C. The solution was allowed to warm up and was stirred for 19 h at ambient temperature. Subsequently, the reaction mixture was quenched with conc. NaHCO\(_3\) solution, the organic layer was separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\). The combined organic layers were dried with MgSO\(_4\) and the solvent was evaporated under reduced pressure (waterbath 30 °C). The O-sulfonylated intermediate was resolved in 40 mL DMF and diisopropylethylamine (2.8 mL, 16.53 mmol, 2.60 equiv) and 10 [2] (2.79 g, 8.26 mmol, 1.30 equiv) was added. The reaction mixture was heated to 90 °C and stirred for 24 h at the same temperature. Afterwards the solvent was removed under reduced pressure (waterbath at 60 °C to remove DMF). Purification by silica gel
chromatography (EtOAc/Et$_3$N 100:1) gave nucleoside 11 as a colourless foam (5.18 g, 69%). $R_t = 0.60$ (EtOAc). $^1$H-NMR (500 MHz, d$_2$-DMSO): 8.08 (d, $J = 8.5$ Hz, 1 H, Ar-H), 7.78-7.77 (m, 2 H, Ar-H), 7.59-7.55 (m, 3 H, Ar-H, NH, H-6), 7.36 (d, $J = 7.0$ Hz, 2 H, Ar-H), 7.31 (t, $J = 7.6$ Hz, 2 H, Ar-H), 7.25-7.22 (m, 5 H, Ar-H), 6.90-6.88 (m, 4 H, Ar-H), 6.17 (t, $J = 7.1$ Hz, 1 H, 1’H), 5.55 (d, $J = 7.8$ Hz, 1 H, H-5), 5.22-5.21 (m, 1 H, 3’H), 5.00 (s, 2 H, OCH$_2$O), 4.97 (s, 2 H, ArCH$_2$O), 4.24-4.17 (m, 1 H, CH$_2$NH), 4.06 (q, $J = 4.5$ Hz, 1 H, 4’H), 3.74 (s, 6 H, OCH$_3$), 3.31-3.29 (m, 1 H, 5’H), 3.22 (dd, $J = 10.0$, 3.0 Hz, 1 H, 5’’H), 2.34-2.22 (m, 2 H, 2’H, 2’’H), 2.04 (s, 3 H, COCH$_3$). 17$^5$C-NMR: (125.8 MHz, d$_2$-DMSO): 170.0, 162.6, 158.1, 154.8, 147.3, 144.6, 139.5, 135.3, 135.2, 133.9, 133.7, 129.7, 128.9, 127.9, 126.8, 124.6, 113.2, 94.9, 85.9, 84.9, 82.8, 74.2, 67.2, 63.4, 59.2, 55.1, 45.7, 44.7, 40.8, 37.2, 32.8, 20.8, 20.5 ppm. MS (ESI): $m/z = 892.70$ [M + H$^+$].

HRMS (MALDI): calcd. for C$_{49}$H$_{57}$N$_5$O$_{11}$Na [M + Na$^+$]: 914.39468 found 914.39641.

**1-(5’-O-DMT-2’-Deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzoyl oxy)methoxy)-piperidin-4-ylamino)pyrimidin-2(1H)-one (12):** Compound 11 (5.06 g, 5.67 mmol, 1.00 equiv) was dissolved in 100 mL MeOH and NaHCO$_3$ (5.95 g, 70.90 mmol, 12.50 equiv) was added. After stirring for 2.5 h at ambient temperature, 200 mL of a CH$_2$Cl$_2$/MeOH/Et$_3$N-mixture (96:4:1) was added and the reaction mixture was stirred for 20 h at ambient temperature. Subsequently conc. NaHCO$_3$ solution was added, the organic layer was separated and the aqueous layer was extracted with CH$_2$Cl$_2$. After evaporation of solvents under reduced pressure, 12 was obtained as a colourless foam (4.81 g, quant.). $R_t = 0.14$ (CH$_2$Cl$_2$/MeOH 19:1). $^1$H-NMR (500 MHz, d$_2$-DMSO): 8.08 (d, $J = 7.8$ Hz, 1 H, Ar-H), 7.78-7.77 (m, 2 H, Ar-H), 7.60-7.57 (m, 2 H, Ar-H, NH), 7.52 (d, $J = 7.5$ Hz, 1 H, H-6), 7.38-7.36 (m, 2 H, Ar-H), 7.31 (t, $J = 7.5$ Hz, 2 H, Ar-H), 7.26-7.23 (m, 5 H, Ar-H), 6.90-6.88 (m, 4 H, Ar-H), 6.15 (t, $J = 6.5$ Hz, 1 H, 1’H), 5.52 (d, $J = 7.5$ Hz, 1 H, H-5), 5.28 (d, $J = 4.5$ Hz, 1 H, 3’H), 5.00 (s, 2 H, OCH$_2$O), 4.97 (s, 2 H, ArCH$_2$O), 4.26-4.17 (m, 2 H, CH$_2$NH, 3’-OH), 3.86 (q, $J = 3.5$ Hz, 1 H, 4’H), 3.74 (s, 6 H, OCH$_3$), 3.22-3.16 (m, 2 H, 5’H, 5’’H), 2.19-2.14 (m, 1 H, 2’H), 2.05-1.99 (m, 1 H, 2’’H), 1.77 (d, $J = 10.0$ Hz, 2 H, CH$_2$CH), 1.32 (t, $J = 12.5$ Hz, 2 H, CH$_2$CHCH), 1.13-1.12 (m, 12 H, CH$_3$) ppm. $^{13}$C-NMR: (125.8 MHz, d$_2$-DMSO): 162.6, 158.1, 154.9, 147.3, 144.7, 139.5, 135.4, 135.3, 133.9, 133.7, 129.7, 128.9, 128.7, 127.9, 127.7, 126.7, 124.6, 113.2, 101.1, 94.5, 85.7, 85.1, 84.7, 70.0, 67.2, 63.4, 59.2, 55.0, 45.7, 44.7, 40.7, 40.4, 32.8, 20.5 ppm. MS (ESI): $m/z = 850.64$ [M + H$^+$]. HRMS (MALDI): calcd. for C$_{75}$H$_{83}$N$_5$O$_{10}$ [M + H$^+$]: 850.40217 found 850.40268.

**Deoxycytidine phosphoramidite with protected spin label (5):** To a solution of 12 (4.81 g, 5.67 mmol, 1.00 equiv) and Et$_3$N (4.0 mL, 28.29 mmol, 5.00 equiv) in 50 mL CH$_2$Cl$_2$ N,N-diisopropylamino(2-cyanoethyl)phosphoramidic chloride (2.68 g, 11.31 mmol, 2.00 equiv) was added. The reaction mixture was stirred for 20 h at ambient temperature. Subsequently conc. NaHCO$_3$ solution was added, the organic layer was separated and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried with MgSO$_4$ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc/Et$_3$N 100:1) 5 was obtained as a
colourless foam (4.83 g, 81%). $R_f = 0.75$, 0.60 (EtOAc (mixture of 2 diastereomers)). $^1$H-NMR (300 MHz, $\text{d}_6$-DMSO): 8.08 (d, $J = 8.7$ Hz, 1 H, Ar-H), 7.78-7.77 (m, 2 H, Ar-H), 7.64-7.52 (m, 3 H, Ar-H, H-6, NH), 7.40-7.36 (m, 2 H, Ar-H), 7.34-7.22 (m, 7 H, Ar-H), 6.90-6.85 (m, 4 H, Ar-H), 6.19-6.13 (m, 1 H, 1’H), 5.56-5.53 (m, 1 H, H-5), 5.00 (s, 2 H, OCH$_2$O), 4.97 (s, 2 H, ArCH$_2$O), 4.52-4.44 (m, 1 H, CHNH), 4.26-4.15 (m, 1 H, 3’H), 4.02-3.96 (m, 1 H, 4’H), 3.74, 3.73 (2 x s, 6 H, OCH$_3$), 3.67-3.43 (m, 4 H, POCH$_2$, NCH(CH$_3$)$_2$), 3.29-3.19 (m, 2 H, 5’H, 5’’H), 2.75 (t, $J = 5.7$ Hz, 1 H, CHHCN), 2.64 (t, $J = 6.3$ Hz, 1 H, CHHCN), 2.36-2.15 (m, 2 H, 2’H, 2’’H), 1.77 (dd, $J = 12.6$, 3.0 Hz, 2 H, CHCH), 1.32 (t, $J = 12.6$ Hz, 2 H, CHHC), 1.15-1.08 (m, 21 H, NCH(CH$_3$)$_2$, CH$_3$), 0.98 (d, $J = 6.9$ Hz, 3 H, NCH(CH$_3$)$_2$) ppm. (mixture of 2 diastereomers). $^{13}$C-NMR (75.5 MHz, $\text{d}_6$-DMSO): 162.6, 158.1, 154.8, 147.3, 144.6, 139.8, 139.5, 135.3, 135.2, 133.8, 133.7, 129.7, 128.9, 128.6, 127.8, 127.7, 126.8, 124.5, 118.9, 118.7, 113.2, 101.1, 94.74, 94.65, 85.9, 85.8, 84.9, 72.9, 72.6, 67.2, 59.2, 58.5, 58.3, 58.2, 58.1, 55.0, 44.7, 42.6, 42.5, 40.8, 32.8, 24.4, 24.3, 24.2, 24.1, 20.5, 19.81, 19.76, 19.72, 19.67, 14.0 ppm. $^{31}$P-NMR (121.5 MHz, $\text{d}_6$-DMSO): 147.7, 147.3 ppm. MS (ESI): $m/z$ = 1050.88 [M + H$^+$]; calcd. for C$_{56}$H$_{72}$N$_{10}$O$_7$P [M + H$^+$]: 1050.51.

9-(3’,5’-Di-O-acetyl-2’-deoxyribofuranosyl)-6-chloropurine (13): 3’,5’-Di-O-acetyldideoxyinosine [3] (1.52 g, 4.52 mmol, 1.00 equiv), benzyltriethylammonium chloride (2.06 g, 9.04 mmol, 2.00 equiv) and N,N-dimethylaniline (0.6 mL, 4.97 mmol, 1.10 equiv) were dissolved in 18 mL dry acetonitrile. The flask was placed on a preheated oil bath (70 °C), POCl$_3$ (2.1 mL, 22.60 mmol, 5.00 equiv) was added slowly and the reaction mixture was stirred for 1 h at the same temperature. After that, the solvent and excess POCl$_3$ were removed under reduced pressure (high vacuum, 70 °C). The residue was poured on a CHCl$_3$/ice-mixture and the solution was stirred for 20 min. The organic phase was separated and the aqueous phase was extracted 3 times with CHCl$_3$. Organic phases were combined and washed with a 5% NaHCO$_3$ solution until the aqueous layer showed a slightly basic reaction. Subsequently the organic phase was separated, dried with MgSO$_4$ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc) gave compound 13 as a yellow oil (1.39 g, 87%). $R_f = 0.49$ (EtOAc). $^1$H-NMR (500 MHz, $\text{d}_6$-DMSO): 8.88 (s, 1 H, H-2), 8.80 (s, 1 H, H-H-8), 6.51 (t, $J = 7.0$ Hz, 1 H, 1’H), 5.46-5.44 (m, 1 H, 3’H), 4.33-4.29 (m, 2 H, 4’H, 5’H), 4.23 (dd, $J = 12.5$, 7.7 Hz, 1 H, 5’’H), 3.19 (quintet, $J = 7.0$ Hz, 1 H, 2’H), 2.63 (dddd, $J = 14.3$, 6.4, 3.0 Hz, 1 H, 2’’H), 2.10 (s, 3 H, COCH$_3$), 1.99 (s, 3 H, COCH$_3$) ppm. $^{13}$C-NMR (128.5 MHz, $\text{d}_6$-DMSO): 170.10, 170.05, 151.7, 151.4, 149.4, 146.0, 131.5, 84.3, 82.0, 74.1, 63.4, 35.5, 20.8, 20.5 ppm. MS (ESI): $m/z$ = 355.14 [M + H$^+$]. HRMS (MALDI): calcd. for C$_{44}$H$_{56}$ClN$_{10}$O$_5$ [M + H$^+$]: 355.0837 found 355.0862.

9-(3’,5’-Di-O-acetyl-2’-deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzylxylo)methoxy)piperidin-4-ylamino)purine (14): Compound 13 (2.40 g, 6.76 mmol, 1.00 equiv) and disopropylethylamine (2.3 mL, 13.53 mmol, 2.00 equiv) were dissolved in 40 mL 1-propanol. After that 10 [2] (2.51 g, 7.44 mmol, 1.10 equiv) was added and the reaction mixture was stirred for 8 h at
75 °C, cooled down to ambient temperature and stirred for another 14 h. The reaction was quenched with conc. NaHCO₃ solution. After extraction with CH₂Cl₂, the combined organic layers were dried with MgSO₄ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (CH₂Cl₂/EtOAc 9:1 → 0:1) gave title compound 14 as a light yellow foam (2.96 g, 67%). ¹H-NMR (500 MHz, d₆-DMSO): 8.38 (bs, 0.30 H, H-2), 8.35 (bs, 0.70 H, H-2), 8.25 (bs, 0.70 H, H-8), 8.12 (bs, 0.30 H, H-8), 8.08 (d, J = 8.0 Hz, 1 H, Ar-H), 7.78 (d, J = 4.5 Hz, 2 H, Ar-H), 7.70-7.64 (m, 0.70 H, NH), 7.62-7.56 (m, 1.30 H, Ar-H, NH), 6.37 (dd, J = 8.5, 6.2 Hz, 1 H, 1'H), 5.41-5.38 (m, 1 H, 3'H), 5.23 (bs, 0.30 H, CHNH), 5.00 (s, 2 H, OCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.57 (bs, 0.70 H, CHNH), 4.31 (dd, J = 10.8, 3.8 Hz, 1 H, 5'H), 4.25-4.18 (m, 2 H, 4'H, 5'H), 3.21-3.11 (m, 1 H, 2'H), 2.53 (dd, J = 6.3, 2.5 Hz, 1 H, 2''H), 2.09 (s, 3 H, COCH₃), 2.01 (s, 3 H, COCH₃), 1.74 (d, J = 11.0 Hz, 2 H, CHHCH), 1.66-1.53 (m, 2 H, CHHCH), 1.17 (s, 6 H, CH₃), 1.12 (s, 6 H, CH₃) ppm (mixture of 2 rotamers at CN-bonds). ¹³C-NMR (125.8 MHz, d₆-DMSO): 170.14, 170.05, 152.8, 148.5, 147.2, 139.3, 133.9, 133.8, 128.9, 128.7, 124.6, 101.0, 83.5, 81.6, 74.4, 67.2, 63.6, 59.4, 44.5, 40.8, 35.2, 32.8, 20.8, 20.6, 20.5 ppm. MS (ESI): m/z = 656.52 [M + H⁺]. HRMS (MALDI): calcd. for C₃₁H₄₂N₇O₉ [M + H⁺]: 656.30385 found 656.30339.

9-(2’-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzoyloxy)-methoxy)piperidin-4-ylamino)purine (15): Compound 14 (3.12 g, 4.76 mmol, 1.00 equiv) was dissolved in 50 mL MeOH at 0 °C and 7 N NH₃ in MeOH (35 mL) was added. After stirring for 15 min at 0 °C the reaction mixture was allowed to warm up to ambient temperature and stirred for another 3 h. Afterwards the solution was cooled down again to 0 °C and neutralized with an ice-cold 6 M HCl-solution. Subsequently conc. NaHCO₃ solution was added and the mixture was extracted with CH₂Cl₂. The combined organic layers were dried with MgSO₄ and the solvent was evaporated under reduced pressure. After purification by silica gel chromatography (CH₂Cl₂/MeOH 9:1) 15 could be obtained as a light yellow foam (2.45 g, 90%). Rf = 0.46 (CH₂Cl₂/MeOH 9:1). ¹H-NMR (500 MHz, d₆-DMSO): 8.37 (bs, 0.30 H, H-2), 8.34 (bs, 0.70 H, H-2), 8.22 (bs, 0.70 H, H-8), 8.14 (bs, 0.30 H, H-8), 8.08 (d, J = 8.2 Hz, 1 H, Ar-H), 7.78 (d, J = 4.5 Hz, 2 H, Ar-H), 7.70-7.63 (m, 0.70 H, NH), 7.61-7.56 (m, 1.30 H, Ar-H, NH), 6.34 (dd, J = 8.5, 6.5 Hz, 1 H, 1'H), 5.43-5.05 (m, 2.30 H, 3'H, 5'-OH, CHNH), 5.00 (s, 2 H, OCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.56 (bs, 0.70 H, CHNH), 4.42-4.38 (m, 1 H, 3'-OH), 3.89-3.86 (m, 1 H, 4'H), 3.62 (dd, J = 11.8, 4.2 Hz, 1 H, 5'H), 3.51 (dd, J = 11.8, 4.3 Hz, 1 H, 5'H), 2.75-2.66 (m, 1 H, 2'H), 2.25 (dddd, J = 13.0, 6.5, 2.5 Hz, 1 H, 2''H), 1.74 (d, J = 10.5 Hz, 2 H, CHHCH), 1.66-1.51 (m, 2 H, CHHCH), 1.17 (s, 6 H, CH₃), 1.12 (s, 6 H, CH₃) ppm (mixture of 2 rotamers at CN-bonds). ¹³C-NMR (125.8 MHz, d₆-DMSO): 154.0, 152.5, 148.2, 147.2, 139.3, 133.9, 133.8, 128.9, 128.7, 124.6, 119.6, 101.1, 88.0, 83.9, 70.9, 67.2, 61.9, 59.4, 44.5, 40.8, 32.8, 20.6 ppm. MS (ESI): m/z = 572.30 [M + H⁺]. HRMS (MALDI): calcd. for C₂₇H₃₈N₇O₇ [M + H⁺]: 572.2872 found 572.28157.
9-(5’-O-DMT-2’-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzylxoy)-methoxy)piperidin-4-ylamino)purine (16): To an ice-cold solution of 15 (2.36 g, 4.12 mmol, 1.00 equiv) in 100 mL dry pyridine dimethoxytrityl chloride (1.67 g, 4.94 mmol, 1.20 equiv) was added. After the reaction mixture was allowed to warm up to ambient temperature, it was stirred for 23 h and cooled down to 0 °C again. The reaction was quenched with MeOH and stirred for 15 min at 0 °C. Subsequently the solvent was removed under reduced pressure and the residue was coevaporated with tolune. Purification by silica gel chromatography (1st CH₂Cl₂/MeOH/Et₂N 96:4:1; 2nd EtOAc/MeOH 100:0 → 90:10) gave title compound 16 as a light yellow foam (2.40 g, 67%). Rᵣ = 0.38 (EtOAc). ¹H-NMR (500 MHz, d₆-DMSO): 8.25 (s, 1 H, H-2), 8.17 (bs, 0.70 H, H-8), 8.08 (d, J = 8.5 Hz, 1 H, Ar-H), 8.06 (bs, 0.30 H, H-8), 7.78 (d, J = 4.0 Hz, 2 H, Ar-H), 7.64-7.51 (m, 2 H, Ar-H, NH), 7.33-7.31 (m, 2 H, Ar-H), 7.23-7.17 (m, 7 H, Ar-H), 6.81-6.76 (m, 4 H, Ar-H), 6.36 (t, J = 6.5 Hz, 1 H, 1’H), 5.35 (d, J = 4.5 Hz, 1 H, 3’H), 5.21 (bs, 0.30 H, CHNH), 5.00 (s, 2 H, OCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.56 (bs, 0.70 H, CHNH), 4.50 (bs, 1 H, 3’-OH), 3.98-3.95 (m, 1 H, 4’H), 3.715 (s, 3 H, OCH₃), 3.705 (s, 3 H, OCH₃), 3.19-3.12 (m, 2 H, 5’H, 5’´H), 3.02 (m, 1 H, 2’H), 2.93-2.81 (m, 1 H, 2’H), 2.35-2.30 (m, 1 H, 2’”H), 1.74 (d, J = 10.5 Hz, 2 H, CHHCH), 1.66-1.50 (m, 2 H, CHHCH), 1.17 (s, 6 H, CH₃), 1.12 (s, 6 H, CH₃) ppm (mixture of 2 rotamers at CN-bonds). ¹³C-NMR (125.8 MHz, d₆-DMSO): 157.98, 157.95, 153.9, 152.6, 147.2, 144.9, 139.2, 135.9, 135.6, 135.5, 133.9, 133.8, 129.7, 129.6, 128.9, 128.7, 127.71, 127.65, 126.5, 124.6, 113.05, 113.03, 101.1, 85.8, 85.4, 70.6, 67.2, 64.0, 59.4, 55.0, 44.5, 38.7, 32.8, 20.6 ppm. MS (ESI): m/z = 874.46 [M + H⁺]. HRMS (MALDI): calcd. for C₄₈H₆₅N₇O₉K [M + K⁺]: 912.36928 found 912.37126.

Deoxyadenosine phosphoramidite with protected spin label (7): To a solution of 16 (2.03 g, 2.32 mmol, 1.00 equiv) and Et₃N (1.6 mL, 11.61 mmol, 5.00 equiv) in 40 mL CH₂Cl₂ N,N-diisopropylamino(2-cyanoethyl)phosphoramidic chloride (1.10 g, 4.65 mmol, 2.00 equiv) was added dropwise. After stirring for 4.5 h at ambient temperature, conc. NaHCO₃ solution was added and the organic layer was separated. The aqueous layer was extracted with CH₂Cl₂ and the combined organic layers were dried with MgSO₄. The solvent was removed under reduced pressure. Purification by silica gel chromatography (CH₂Cl₂/EtOAc/Et₃N 50:50:1) gave amidite 7 as a colourless foam (2.02 g, 81%). Rᵣ = 0.82, 0.70 (CH₂Cl₂/EtOAc 1:1 (mixture of 2 diastereomers)). ¹H-NMR (500 MHz, d₆-DMSO): 8.27 (s, 1 H, H-2), 8.15 (bs, 0.70 H, H-8), 8.08 (d, J = 8.2 Hz, 1 H, Ar-H), 8.04 (bs, 0.30 H, H-8), 7.81-7.75 (m, 2 H, Ar-H), 7.67-7.52 (m, 2 H, Ar-H, NH), 7.34-7.29 (m, 2 H, Ar-H), 7.24-7.17 (m, 7 H, Ar-H), 6.81-6.74 (m, 4 H, Ar-H), 6.42-6.34 (m, 1 H, 1’H), 5.20 (bs, 0.30 H, CHNH), 5.00 (s, 2 H, OCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.80 (bs, 1 H, 3’H), 4.56 (bs, 0.70 H, CHNH), 4.15-4.06 (m, 1 H, 4’H), 3.80-3.73 (m, 1 H, POCHH), 3.71 (s, 3 H, OCH₃), 3.70 (s, 3 H, OCH₃), 3.67-3.63 (m, 1 H, POCHH), 3.59-3.50 (m, 2 H, NCH(CH₃)₂), 3.27-3.17 (m, 2 H, 5’H, 5’´H), 3.13-3.02 (m, 1 H, 2’H), 2.77 (t, J = 6.0 Hz, 1 H, CHHCH), 2.66 (t, J = 6.0 Hz, 1 H, CHHCH), 1.74 (d, J = 10.5 Hz, 2 H, CHHCH), 1.66-1.50 (m, 2 H, CHHCH), 1.18-1.07 (m, 21 H, NCH(CH₃)₂, CH₃), 1.03 (d, J = 6.8 Hz, 3
H, NCH(CH₃)₂) ppm (mixture of 2 rotamers at CN-bonds and 2 diastereomers). ¹³C-NMR (125.8 MHz, d₆-DMSO): 157.98, 157.95, 153.9, 152.6, 148.3, 147.2, 144.8, 139.5, 135.6, 135.5, 133.9, 133.8, 129.7, 129.6, 128.9, 128.7, 127.71, 127.65, 126.5, 124.6, 119.6, 119.0, 118.8, 113.03, 113.0, 101.0, 85.5, 84.7, 84.5, 83.5, 73.3, 73.2, 72.7, 67.2, 63.4, 63.3, 59.4, 58.4, 55.0, 44.5, 42.6, 42.5, 40.7, 37.5, 37.2, 32.8, 24.4, 24.3, 24.2, 24.1, 20.6, 19.8, 19.7 ppm. MS (ESI): m/z = 1074.69 [M + H⁺]; calcd. for C₅₅H₇₃N₉O₁₀P [M + H⁺]: 1074.52.

9-(2‘,3‘,5‘-Tri-O-acetylriburifuranosyl)-6-chloropurine (17): 2‘,3‘,5‘-Tri-O-acetyliminosine [4] (5.00 g, 12.68 mmol, 1.00 equiv), benzyltriethylammonium chloride (5.77 g, 25.36 mmol, 2.00 equiv) and N,N-dimethylaniline (1.8 mL, 13.94 mmol, 1.10 equiv) were dissolved in 50 mL dry acetonitrile. The flask was placed on a preheated oil bath (70 °C), POCl₃ (5.9 mL, 63.40 mmol, 5.00 equiv) was added slowly and the reaction mixture was stirred for 2 h at the same temperature. After that, the solvent and excess POCl₃ were removed under reduced pressure (high vacuum, 70 °C). The residue was poured on a CHCl₃/ice-mixture and the solution was stirred for 20 min. The organic phase was separated and the aqueous phase was extracted 3 times with CHCl₃. The organic phases were combined and washed with a 5% NaHCO₃ solution until the aqueous layer showed a slightly basic reaction. Subsequently the organic phase was separated, dried with MgSO₄ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc) gave the title compound for another 14 h and the product was dried with diisopropylethylamine (EtOAc) chromato- graphy (EtOAc) gave the title compound 17 (0.59 g, 12.68 mmol, 1.00 equiv) as a yellow oil (5.23 g, quant.). Rₐ = 0.65 (EtOAc). ¹H-NMR (500 MHz, d₆-DMSO): 8.90 (s, 1 H, H-2), 8.85 (s, 1 H, H-8), 6.37 (d, J = 5.0 Hz, 1 H, H-1), 6.03 (t, J = 5.5 Hz, 1 H, 2´H), 5.65 (t, J = 5.4 Hz, 1 H, 3´H), 4.45-4.40 (m, 2 H, 4´H, 5´H), 4.30-4.26 (m, 1 H, 5´´H), 2.12 (s, 3 H, COCH₃), 2.04 (s, 3 H, COCH₃), 2.01 (s, 3 H, COCH₃) ppm. ¹³C-NMR (125.8 MHz, d₆-DMSO): 170.0, 169.4, 169.3, 152.0, 151.3, 149.7, 146.4, 131.6, 86.2, 79.7, 72.1, 69.9, 62.7, 20.5, 20.4, 20.2 ppm. MS (ESI): m/z = 413.11 [M + H⁺]. HRMS (MALDI): calcd. for C₅₅H₇₃N₉O₁₀PNa [M + Na⁺]: 435.06780 found 435.06727.

9-(2‘,3‘,5‘-Tri-O-acetylriburifuranosyl)-6-(2,2,6,6-tetramethyl-1-(2-nitrobenzylolxy)methoxy)piperidin-4-ylamino)purine (18): A solution of 17 (0.55 g, 1.33 mmol, 1.00 equiv), diisopropylethylamine (0.5 mL, 2.93 mmol, 2.20 equiv) and 10 [2] (0.59 g, 1.74 mmol, 1.30 equiv) in 1-propanol was stirred for 7 h at 75 °C. The solution was cooled down to ambient temperature, stirred for another 14 h and the solvent was removed under reduced pressure. Purification by silica gel chromatography (EtOAc) gave the title compound 18 as a light yellow foam (0.74 g, 78%). Rₐ = 0.56 (EtOAc). ¹H-NMR (500 MHz, d₆-DMSO): 8.36 (bs, 1 H, H-2), 8.26 (bs, 0.70 H, H-8), 8.14 (bs, 0.30 H, H-8), 8.08 (d, J = 8.0 Hz, 1 H, Ar-H), 7.78 (d, J = 4.5 Hz, 2 H, Ar-H), 7.75-7.65 (m, 1 H, NH), 7.60-7.56 (m, 1 H, Ar-H), 6.21 (d, J = 5.8 Hz, 1 H, 1 H), 6.03 (t, J = 5.8 Hz, 1 H, 2´H), 5.62 (t, J = 5.0 Hz, 1 H, 3´H), 5.19 (bs, 0.30 H, CHNH), 5.00 (s, 2 H, OCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.57 (bs, 0.70 H, CHNH), 4.41 (dd, J = 12.5, 3.0 Hz, 1 H, 5´H), 4.36 (q, J = 4.8 Hz, 1 H, 4´H), 4.24 (dd, J = 12.0, 5.5 Hz, 1 H, 5´´H), 2.12 (s, 3 H, COCH₃), 2.03 (s, 3 H, COCH₃), 2.01 (s, 3 H, COCH₃), 1.81-1.72 (m, 2 H, CHHCH), 1.67-1.53 (m, 2 H, CHHCH), 1.17 (s, 6 H, CH₃), 1.12 (s, 6 H, CH₃) ppm (mixture of 2
rotamers at CN-bonds). $^{13}$C-NMR (125.8 MHz, $d_{6}$-DMSO): 170.0, 169.5, 169.3, 154.0, 153.0, 148.4, 147.2, 139.8, 133.9, 133.8, 128.9, 128.7, 124.6, 119.5, 101.1, 85.6, 79.4, 71.9, 70.1, 67.2, 62.8, 59.4, 44.5, 40.8, 32.8, 20.6, 20.5, 20.4, 20.2 ppm. MS (ESI): $m/z$ = 714.47 [M + H$^+$]. HRMS (MALDI): calcd. for C$_{33}$H$_{44}$N$_5$O$_{11}$ [M + H$^+$]: 714.30933 found 714.30886.

9-(Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyl)oxy)methoxy)piperidin-4-ylamino)-purine (19): Compound 18 (1.54 g, 2.15 mmol, 1.00 equiv) was dissolved in 45 mL 7 N NH$_3$ in MeOH at 0 °C. After stirring for 10 min the reaction mixture was allowed to warm up to ambient temperature and stirred for another 3 h. Subsequently the reaction mixture was neutralized with an ice-cold 6 M HCl solution. After adding conc. NaHCO$_3$ solution, the reaction mixture was extracted with CH$_2$Cl$_2$. The combined organic layers were dried with MgSO$_4$ whereupon the solvent was removed under reduced pressure. After purification by silica gel chromatography (CH$_2$Cl$_2$/MeOH 9:1) 19 was obtained as a light yellow foam (1.26 g, 93%). $R_f$ = 0.36 (CH$_2$Cl$_2$/MeOH 9:1). $^1$H-NMR (300 MHz, $d_6$-DMSO): 8.36 (bs, 1 H, H-2), 8.22 (bs, 1 H, H-8), 8.08 (d, $J$ = 7.8 Hz, 1 H, Ar-H), 7.78 (d, $J$ = 4.1 Hz, 2 H, Ar-H), 7.70-7.54 (m, 2 H, Ar-H, NH), 5.89 (d, $J$ = 6.0 Hz, 1 H, 1'H), 5.42 (d, $J$ = 6.3 Hz, 1 H, 2'-'OH), 5.36 (dd, $J$ = 6.8, 4.8 Hz, 1 H, 5'-'OH), 5.25 (bs, 0.30 H, CHNH), 5.18 (d, $J$ = 4.7 Hz, 1 H, 3'-'OH), 5.00 (s, 2 H, OCH$_2$O), 4.98 (s, 2 H, ArCH$_2$O), 4.65-4.47 (q, bs, $J$ = 6.3 Hz, 1.70 H, 2'H, CHNH), 4.17-4.12 (m, 1 H, 3'H), 3.96 (q, $J$ = 3.3 Hz, 1 H, 4'H), 3.67 (dt, $J$ = 12.0, 3.9 Hz, 1 H, 5'H), 3.59-3.50 (m, 1 H, 5'-'H), 1.76 (bd, $J$ = 12.8 Hz, 2 H, CHHCH), 1.63 (bt, $J$ = 10.8 Hz, 2 H, CHHCH), 1.18 (s, 6 H, CH$_3$), 1.13 (s, 6 H, CH$_3$) ppm (mixture of 2 rotamers at CN-bonds). $^{13}$C-NMR (75.5 MHz, $d_6$-DMSO): 154.0, 152.5, 148.4, 147.2, 147.2, 139.6, 133.9, 133.8, 128.9, 128.7, 124.6, 119.7, 101.1, 87.9, 85.8, 73.5, 70.6, 67.2, 61.6, 59.4, 45.6, 44.5, 40.8, 32.8, 20.6 pm. MS (ESI): $m/z$ = 588.37 [M + H$^+$]. HRMS (MALDI): calcd. for C$_{37}$H$_{38}$N$_7$O$_8$ [M + H$^+$]: 588.27764 found 588.27681.

9-(5'-O-DMT-Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyl)oxy)methoxy)piperidin-4-ylamino)purine (20): To a solution of 19 (1.13 g, 1.92 mmol, 1.00 equiv) in 50 mL dry pyridine, dimethoxytrityl chloride (0.78 g, 2.30 mmol, 1.20 equiv) was added at 0 °C. After stirring for 10 min at 0 °C the reaction mixture was allowed to warm up at ambient temperature and was stirred for another 22 h. The reaction was quenched with MeOH at 0 °C, stirred for 10 min at the same temperature whereupon the solvent was removed under reduced pressure. After coevaporation with toluene the residue was purified by silica gel chromatography ($^1$st CH$_2$Cl$_2$/MeOH/Et$_3$N 96:4:1; $^{2}$nd EtOAc/MeOH/Et$_3$N 100:0:1 → 90:10:1). Title compound 20 was obtained as a light yellow foam (1.43 g, 84%). $R_f$ = 0.46 (CH$_2$Cl$_2$/MeOH 9:1). $^1$H-NMR (500 MHz, $d_6$-DMSO): 8.26 (bs, 1 H, H-2), 8.20 (bs, 1 H, H-8), 8.08 (d, $J$ = 8.5 Hz, 1 H, Ar-H), 7.78 (d, $J$ = 4.0 Hz, 2 H, Ar-H), 7.68-7.54 (m, 2 H, Ar-H, NH), 7.35-7.33 (m, 2 H, Ar-H), 7.26-7.17 (m, 7 H, Ar-H), 6.83-6.78 (m, 4 H, Ar-H), 5.93 (d, $J$ = 4.0 Hz, 1 H, 1'H), 5.53 (d, $J$ = 6.0 Hz, 1 H, 2'-'OH), 5.25-5.17 (bs, d, $J$ = 5.5 Hz, 1.30 H, CHNH, 3'-'OH), 5.01 (s, 2 H, OCH$_2$O), 4.98 (s, 2 H, ArCH$_2$O), 4.73-4.21 (m, 1.70 H, 2'H, CHNH), 4.37-4.27 (m, 1 H, 3'-'H), 4.05 (q, $J$ = 5.0 Hz, 1 H, 4'H), 3.72, 3.71 (2 x s, 6 H, OCH$_3$), 3.23-3.15 (m, 2 H, 5'-'H).
As added dropwise. After stirring for 23 h at ambient temperature conc. NaHCO$_3$ solution was added, the organic layer was separated and the aqueous phase was extracted with CH$_2$Cl$_2$. The combined organic layers were dried with MgSO$_4$ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (CH$_2$Cl$_2$/EtOAc/Et$_3$N 30:70:1) gave the title compound 21 as a colourless foam (0.20 g, 35%) (The 3’-O-TBDMS regioisomer and the bisilylated product could be deprotected with 1 M tetraethylammonium fluoride (THF) and used again for the reaction after a silica gel chromatography (CH$_2$Cl$_2$/MeOH/Et$_3$N 90:10:1)). $R_t$ = 0.82 (CH$_2$Cl$_2$/EtOAc 3:7). $^1$H-NMR (500 MHz, d$_6$-DMSO): 8.27 (bs, 1 H, H-2), 8.17 (bs, 1 H, H-8), 8.08 (d, $J$ = 8.0 Hz, 1 H, Ar-H), 7.78 (d, $J$ = 4.5 Hz, 2 H, Ar-H), 7.70-7.52 (m, 2 H, Ar-H, NH), 7.38-7.37 (m, 2 H, Ar-H), 7.28-7.19 (m, 7 H, Ar-H), 6.85-6.82 (m, 4 H, Ar-H), 5.94 (d, $J$ = 5.0 Hz, 1 H, H-1’), 5.60 (m, 1 H, CHNH, 3’-OH), 5.00 (s, 2 H, OCH$_2$O), 4.98 (s, 2 H, ArCH$_2$O), 4.87-4.75 (m, 1 H, 2’H), 4.61-4.51 (m, 0.70 H, CHNH), 4.31-4.21 (m, 1 H, 3’H), 4.08 (q, $J$ = 4.5 Hz, 1 H, 4’H), 3.72 (s, 6 H, OCH$_3$), 3.27-3.22 (m, 2 H, 5’H, 5’’H), 1.75 (bd, $J$ = 10.5 Hz, 2 H, CHHCH), 1.67-1.55 (m, 2 H, CHHCH), 1.17 (s, 6 H, CH$_3$), 1.12 (s, 6 H, CH$_3$), 0.76 (s, 9 H, Si(C$_2$H$_5$)$_3$), (-0.03) – (-0.04) (m, 3 H, SiCH$_2$), (-0.13) (s, 3 H, SiCH$_3$) ppm (mixture of 2 rotamers at CN-bonds). $^{13}$C-NMR (125.8 MHz, d$_6$-DMSO): 158.0, 153.9, 152.8, 148.6, 147.2, 144.9, 139.3, 135.5, 135.4, 133.9, 133.8, 129.7, 128.9, 128.7, 127.8, 126.6, 124.6, 119.4, 113.1, 101.0, 87.9, 85.5, 83.2, 75.0, 70.2, 67.2, 63.4, 59.4, 55.0, 44.6, 40.8, 32.8, 25.8, 25.6, 25.5, 20.6, 17.9, -3.2, -4.8, -5.2 ppm. MS (ESI): $m/z$ = 1004.70 [M + H$^+$]. HRMS (MALDI): calcd. for C$_{98}$H$_{55}$N$_7$O$_{16}$Si [M + H$^+$]: 1004.4974 found 1004.49743.

**Adenosine phosphoramidite with protected spin label (8):** To a solution of 21 (0.54 g, 0.53 mmol, 1.00 equiv) and Et$_3$N (0.4 mL, 2.68 mmol, 5.00 equiv) in 20 mL CH$_2$Cl$_2$ N,N-diisopropylamino(2-cyanoethyl)phosphoramidic chloride (0.25 g, 1.07 mmol, 2.00 equiv) was added dropwise. After stirring for 23 h at ambient temperature conc. NaHCO$_3$ solution was added, the organic layer was separated and the aqueous phase was extracted with CH$_2$Cl$_2$. The combined organic layers were dried with MgSO$_4$ and the solvent was removed under reduced pressure. Purification by silica gel chromatography (CH$_2$Cl$_2$/EtOAc/Et$_3$N 80:20:1) gave amidite 8 as a colourless foam (0.54 g, 86%). $R_t$
1H-NMR (300 MHz, d$_6$-DMSO): 8.32-8.26 (m, 1 H, H-2), 8.18-8.02 (m, 1 H, H-8), 8.03 (d, J = 8.7 Hz, 1 H, Ar-H), 7.80-7.75 (m, 2 H, Ar-H), 7.42-7.36 (m, 2 H, Ar-H), 7.30-7.25 (m, 2 H, Ar-H), 6.86-6.80 (m, 4 H, Ar-H), 5.96-5.88 (m, 1 H, 1H), 5.17-5.09 (m, 1 H, 2H), 5.00 (s, 2 H, OCH$_2$O), 4.97 (s, 2 H, ArCH$_2$O), 4.69-4.44 (m, 1 H, CH$_2$H), 4.50 (s, 2 H, ArC$_2$H$_3$O), 4.32-4.19 (m, 1 H, 4H), 3.89-3.76 (m, 1.30 H, POC$_2$H, NC$_2$H), 3.72 (s, 6 H, OCH$_3$), 3.69-3.50 (m, 3 H, POC$_3$H, NC$_2$H(CH$_3$)$_2$), 3.42-3.37 (m, 1 H, 5H), 3.29-3.20 (m, 1 H, 5´H), 0.75-0.64 (m, 9 H, SiC(CH$_3$)$_3$), 0.70-0.50 ppm (mixture of 2 rotamers at CN-bonds and 2 diastereomers).

13C-NMR (75.5 MHz, d$_6$-DMSO): 158.1, 153.9, 152.7, 147.2, 144.8, 144.7, 139.8, 139.5, 135.4, 135.3, 135.2, 133.9, 133.8, 129.7, 128.9, 128.6, 127.7, 127.6, 126.7, 126.5, 118.8, 118.6, 113.1, 101.0, 87.5, 85.8, 85.7, 83.0, 82.8, 73.5, 72.3, 67.1, 63.2, 59.4, 58.9, 58.7, 57.7, 55.0, 44.4, 42.8, 42.7, 42.4, 42.3, 32.8, 29.0, 25.8, 25.4, 24.5, 24.3, 24.2, 24.1, 20.6, 20.0, 19.9, 19.8, 19.7, 17.6, -3.2, -5.0, -5.4 ppm. 31P-NMR (121.5 MHz, d$_6$-DMSO): 149.5, 148.2 ppm. MS (ESI): m/z = 1204.37 [M + H$^+$]; calcd. for C$_{63}$H$_{87}$N$_9$O$_{11}$P$_3$Si [M + H$^+$]: 1204.60.

**Synthesis, purification and quantification of oligonucleotides**

**General.** DNA and RNA synthesis was executed on an Expedite Nucleic Acid Synthesis System from PerSeptive Biosystems. Anion-exchange (AE) and Reversed Phase (RP) HPLC was performed on a Jasco LC-900 HPLC system mounted with a Jasco UV-975 detector (detection at 254 nm). For AE-HPLC a Dionex BioLC® DNAPac® PA-100 (250 × 9 mm) column and for RP-HPLC a preparative column Phenomenex Jupiter 4 μm Proteo 90 Å (250 × 10 mm) was used. All DNA and RNA samples were concentrated in a SpeedVac (Christ). Water was treated with DEPC and autoclaved.

**Oligonucleotide synthesis.** For DNA and RNA synthesis the standard synthesis protocols on a PerSeptive Expedite Synthezier at 1.0 μmol scale were used. Trichloroacetic acid in CH$_2$Cl$_2$ (deblock solution), acetic anhydride in THF (Cap A), N-methylimidazole in THF/pyridine (Cap B) and iodine in pyridine/H$_2$O/THF (oxidizer) were acquired from SAFC-Proligo (Sigma-Aldrich). Activator (0.35 M ETT in acetonitrile (molecular sieve)) was freshly prepared. Columns, which were purchased from Link Technologies, were self-packed with cpg-solid support. For DNA synthesis fast deprotecting amidites and for RNA synthesis fast deprotecting 2´-O-TBS amidites were used.

**Isolation and purification.** For deprotection and cleavage from solid support, cpg was removed from the column and treated for 20 h at 37 °C with 2 mL of a mixture of 32% aq ammonia/ethanol (3:1). Afterwards the supernatant was separated and the cpg material was washed two times with DEPC-H$_2$O. The combined fractions were evaporated to dryness. The crude DNA oligonucleotide was
purified by AE-HPLC. In case of RNA oligonucleotides, the residue was treated with 300 µL of a NMP/Et₃N/Et₃N·3HF (97%) mixture (6:3:4) for 90 min at 65 °C. For precipitation of the oligonucleotide, 1.2 mL n-butanol was added and the suspension was stored at −40 °C for 72 h. Afterwards it was centrifuged at 10000 rpm at 4 °C for 90 min. The supernatant was discarded and the crude RNA was purified by AE-HPLC. AE-HPLC conditions: (A: water, B: 1 M LiCl; gradient: 0–56% B within 32.00 min; flow: 5 mL/min) for 12mer DNAs (22a, 24a) and 12mer RNA (26a) oligonucleotides; (A: water, B: 1 M LiCl; gradient: 0–10% from 0.00–2.50 min, 10–70% from 2.50–32.00 min; flow: 5 mL/min) for 18mer DNAs (23a, 25a) and 18mer RNA (27a). An additional purification and desalting was done by RP-HPLC. RP-HPLC conditions: (A: 1 M TEAA buffer pH 7.0, B: acetonitrile, C: DEPC-H₂O; gradient: constant 10% A, 5% B from 0.00–3.00 min, 5–40% from 3.00–25.00 min; flow 4 mL/min) for all DNA (22a–25a) and RNA (26a, 27a) oligonucleotides. The column was heated to 55 °C in most cases (55 °C and 20 °C for 27c).

Quantification. Oligonucleotide concentrations were determined via UV spectrometry on a nanodrop2000 (Thermo Scientific) using Lambert-Beer’s law. Extinction coefficients were calculated by a nearest neighbor model according to literature [5]. For modified bases identical increments were used as for their natural counterparts.

Following sequences were prepared:

DNA: dC TEMPO amidite 5: 22a 5´-GCT GAT ATX AGC-3´
23a 5´-GCT GAT GCA TGC ATX AGC-3´
dA TEMPO amidite 7: 24a 5´-GCT GAT ATC XGC-3´
25a 5´-GCT GAT GCA TGC ATC XGC-3´

RNA: A TEMPO amidite 8: 26a 5´-GCU GAU AUC XGC-3´
27a 5´-GCU GAU GCA UGC AUC XGC-3´

Good yields of all oligonucleotides were obtained after HPLC purification.

Figure S1: Trityl protocols of DNA synthesis: 22a (left) and 24a (right).
Mass spectrometry. Oligonucleotides were analyzed via ESI mass spectrometry using a LC–MS instrument with microTOF-Q II analyser (Bruker). An Agilent 1200 Series HPLC using methanol/0.005 M TEAA buffer (gradient 0–60%) was applied as LC system.

DNA **22a** calculated exact mass: 3965.8; found 3965.6; 7930.6 (duplex)

![Figure S4: LC–MS analysis of protected DNA 22a.](image)
DNA 24a calculated exact mass: 3965.8; found 3965.6; 7930.6 (duplex)

Figure S5: LC–MS analysis of protected DNA 24a.

DNA 23a calculated exact mass: 5819.9; found 5820.6

Figure S6: LC–MS analysis of protected DNA 23a.
DNA **25a** calculated exact mass: 5819.9; found 5819.7

RNA **26a** calculated exact mass: 4115.7; found 4115.6, 8230.5 (duplex)

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**Figure S7:** LC–MS analysis of protected DNA **25a**.

**Figure S8:** LC–MS analysis of protected RNA **26a**.

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S14
RNA 27a calculated exact mass: 6051.6; found 6051.6, 6077.5 (+ Na⁺)

Photochemical deprotection of oligonucleotides

Conditions for deprotection. Sample (100 µL; 100 µM, 100 mM NaCl; 10 mM NaH₂PO₄/Na₂HPO₄ pH 7.4) was irradiated in a custom built apparatus containing LEDs (Nichia NCCU033, 365 nm, each with 100 mW optical output power) [6] for 20 min in a round glass cuvette (Carl Roth 50 × ø 10 mm) and subsequently annealed (see Table S1). Conditions for RP-HPLC: A: 1 M TEAA buffer pH 7.0, B: acetonitrile, C: DEPC-H₂O; gradient: constant 10% A, 5% B from 0.00–3.00 min, 5–20% from 3.00–25.00 min; flow 4 mL/min; column temperature 55 °C. A semipreparative Phenomenex Jupiter 4 µm Proteo 90 Å column (250 × 10.0 mm) was used.
RNA 26b calculated mass: 3980.6; found 3950.4, 7900.2 (duplex) (Hemiacetal decomposes during measurement). RNA 26c calculated mass: 3949.5; found 3949.4

![Image of hemiacetal 26b and radical 26c]

Figure S10: Deprotection of RNA 26a with annealing (red) and without annealing after irradiation (black). Annealing procedure is shown in Table S1 below. This reaction is shown as an example. Insets: LC–MS analysis of hemiacetal 26b and of spin-labeled RNA 26c.

| t (min) | T (°C)       | (°C/s) |
|---------|--------------|--------|
| 70.00   | 90.0         |        |
| 1.00    | 20.00        |        |
| 20.0 → 90.0 | 3.0     |        |
| 90.0 → 20.0 | 0.1     |        |

Table S1: Annealing procedure performed in a Biometra T-Personal Thermocycler.
DNA 22c calculated mass: 3799.6; found 3799.5

Figure S11: Deprotection of DNA 22a after annealing procedure. Insets: LC–MS analysis of spin labeled DNA 22c.

DNA 24c calculated mass: 3799.6; found 3799.5

Figure S12: Deprotection of DNA 24a after annealing procedure. Insets: LC–MS analysis of spin labeled DNA 24c.
RNA 26c calculated mass: 3949.5; found 3949.4

Figure S13: Deprotection of RNA 26a after annealing procedure. Insets: LC–MS analysis of spin labeled RNA 26c.

DNA 23c calculated mass: 5653.8; found 5653.5

Figure S14: Deprotection of DNA 23a after annealing procedure. Insets: LC–MS analysis of spin labeled DNA 23c.
DNA 25c calculated mass: 5653.8; found 5653.5

RNA 27c calculated mass: 5885.7; found 5886.5, 5911.5 (+ Na⁺)

Figure S15: Deprotection of DNA 25a after annealing procedure. Insets: LC–MS analysis of spin labeled DNA 25c.

Figure S16: Deprotection of RNA 27a after annealing procedure. Insets: LC–MS analysis of spin labeled RNA 27c. The chromatogram shows three major peaks giving identical mass spectra. The sample also looks homogeneous in gel electrophoresis (Figure S19). This behaviour suggests the presence of different stable conformers under HPLC conditions. Strong support comes from HPLC separations at 20 °C: The main components are in equilibrium with each other (Figures S17, S18).
Peak 1 and 2 were separated and solvent was removed under reduced pressure. Subsequently both samples were resolved (100 µL; 100 mM NaCl; 10 mM NaH$_2$PO$_4$/Na$_2$HPO$_4$ pH 7.4), annealed again and reinjected. HPLC conditions: see above. Column was cooled to 20 °C.

Figure S17: Deprotection of RNA 27a with annealing (red) and without annealing (black). Column temperature 20 °C. Insets: LC–MS analysis of spin labeled RNA 27c. Continued heating of the sample does not further change the ratio of peaks thus ruling out that peak 1 corresponds to the hemiacetal 27b. After preparative separation, peak 1 and peak 2 form a mixture of both after standing or induced by a second annealing procedure (Figure S18).

Peak 1 and 2 were separated and solvent was removed under reduced pressure. Subsequently both samples were resolved (100 µL; 100 mM NaCl; 10 mM NaH$_2$PO$_4$/Na$_2$HPO$_4$ pH 7.4), annealed again and reinjected. HPLC conditions: see above. Column was cooled to 20 °C.

Figure S18: Overlay of peak 1 (black) and 2 (red) after clean separation and a second annealing step. The chromatograms show a conversion of peak 1 into peak 2 and vice versa suggesting a conformational equilibrium. If isolated peak 1 or 2 is kept at room temperature for several days, in both cases peak 1 dominates by far. A cautious interpretation is that the second peak might correspond to a stem-loop structure and peak 1 to the duplex, in accordance with PELDOR data (Figure S21).
EPR method part

**cw-EPR before and after annealing.** Continuous wave (cw) EPR spectra were measured at X-band (9.4 GHz) and room temperature on a Bruker EMXnano benchtop spectrometer after irradiation and after additional annealing. The experimental parameters were: 1 mW microwave power, 1.5 G modulation amplitude, 100 kHz modulation frequency, 20.48 ms time constant and 80.74 ms conversion time. The cw-EPR spectra of the DNA and RNA oligonucleotides 22c–27c after irradiation and after additional annealing are shown in Figure S20. Sole irradiation leads to a mixture of EPR-inactive hemiacetals 22b–27b and EPR-active nitroxides 22c–27c. The spin concentration is
increased after the following step of annealing, which leads to mean spin labeling efficiencies around 96%.

**Figure S20:** cw-EPR spectra of 22c-27c a) directly after irradiation (grey) and b) after additional annealing (black).

**PELDOR distance measurements.** 10 μL of the samples mixed with 20% (v/v) deuterated glycerol as a cryoprotectant were transferred into 1.6 mm outer diameter EPR tubes (Suprasil, Wilmad LabGlass) and frozen in liquid nitrogen. PELDOR experiments were conducted at Q-band (33.8 GHz) and 50 K on a Bruker ELEXSYS E580 spectrometer equipped with a continuous-flow helium cryostat (CF935, Oxford Instruments), a temperature control system (ITC502, Oxford Instruments) and a 150 W TWT (Applied Systems Engineering Inc.) amplifier with a Bruker EN5107D2 cavity resonator. For all experiments the dead-time free four pulse PELDOR sequence [7] was applied with pulse lengths of 22 ns for the detection pulses (π/2 and π) and 12 ns for the pump pulse (π). The pump pulse frequency was set to the maximum of the echo detected field sweep spectrum and the frequency of the detection pulses 70 MHz lower. The first interpulse delay was increased by 16 ns for eight steps
to avoid deuterium modulation. Figure S21 shows the results of the PELDOR measurements for the samples 22c–27c and Table S2 compares the experimentally obtained distances with the simulations.

Figure S21: PELDOR measurements of 22c–27c. After correction of the intermolecular exponential background (red) from the time traces V(t)/V(0) the form factors F(t)/F(0) were obtained. They were fitted with a model-free Tikhonov regularization (DeerAnalysis15) [8]. On the form factors the fits are superimposed (red). The distance distributions P(r) show distinct values and the asterisks indicate additional distances for the RNA sample 27c, probably due to stacking of the RNA. DNA samples show reduced levels of modulation depth caused by the presence of monomeric strands (Figure S19). For example, the modulation depths λ of the palindromic 12mers can be compared with λ of an ideal 2-spin model system (λ = 0.31) to estimate the amount of the duplex structure. Taking the spin labeling efficiencies into account, the modulation depths suggest 100% duplex structure for sample 26c (λ = 0.31) and roughly 45% and 25% duplex structure for samples 22c (λ = 0.13) and 24c (λ = 0.08), respectively. The distances predicted by molecular modeling are shown in blue.
Spin-spin-distances in palindromic duplexes

| Sample | distance [Å] | PELDOR [nm] | Sample | distance [Å] | PELDOR [nm] |
|--------|--------------|-------------|--------|--------------|-------------|
| 22c    | 25.4 (N-N)   |             | 23c    | 42.3 (N-N)   |             |
|        | 26.3 (N-O)   |             |        | 43.0 (N-O)   |             |
|        | 26.3 (O-N)   |             |        | 42.9 (O-N)   |             |
|        | 27.3 (O-O)   |             |        | 43.6 (O-O)   |             |
| average| 26.3         | 2.5 ± 0.2   | average| 42.9         | 4.1 ± 0.2   |
| 24c    | 28.5 (N-N)   |             | 25c    | 49.0 (N-N)   |             |
|        | 29.4 (N-O)   |             |        | 50.0 (N-O)   |             |
|        | 29.7 (O-N)   |             |        | 49.8 (O-N)   |             |
|        | 30.6 (O-O)   |             |        | 50.8 (O-O)   |             |
| average| 29.5         | 2.9 ± 0.2   | average| 49.9         | 4.9 ± 0.2   |
| 26c    | 24.0 (N-N)   |             | 27c    | 39.5 (N-N)   |             |
|        | 25.0 (N-O)   |             |        | 40.3 (N-O)   |             |
|        | 25.0 (O-N)   |             |        | 40.5 (O-N)   |             |
|        | 26.0 (O-O)   |             |        | 41.3 (O-O)   |             |
| average| 25.0         | 2.5 ± 0.2   | average| 40.4         | 4.1 ± 0.2   |

Table S2: Comparison of the spin-spin distances in oligonucleotides 22c–27c determined by PELDOR and by molecular modelling. The predicted distance is an average of N-N, N-O, O-N, and O-O distances.

Simulation of the spin-spin distances. All deoxyribonucleic acids (22c-25c) were generated as a B-form duplex using SPARTAN [9]. Ribonucleic acids (26c, 27c) were built as an A-form duplex. The attachment of the spin label was done with SPARTAN as well. After that, a local optimization, based on the force field MMFF94, was carried out applying AVOGADRO [10]. Optimization was executed twice for each duplex.
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1-(3'-O-Acetyl-5'-O-DMT-2'-deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzyl-oxy)methoxy) piperidin-4-ylamino)pyrimidin-2(1H)-one (11)
$1-(3'-O-Acetyl-5'-O-DMT-2'-deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzyl-oxy)methoxy)piperidin-4-ylamino)pyrimidin-2(1H)-one$ (11)
1-(5'-O-DMT-2'-Deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)methoxy)-piperidin-4-ylamino)pyrimidin-2(1H)-one (12)

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1-(5'-O-DMT-2'-Deoxyribofuranosyl)-4-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)methoxy)-piperidin-4-ylamino)pyrimidin-2(1H)-one (12)
Deoxycytidine phosphoramidite with protected spin label (5)
Deoxycytidine phosphoramidite with protected spin label (5)
Deoxycytidine phosphoramidite with protected spin label (5)
9-(3',5'-Tri-O-acetyl-2'-deoxyribofuranosyl)-6-chloro-purine (13)

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9-(3',5'-Tri-O-acetyl-2'-deoxyribofuranosyl)-6-chloro-purine (13)
9-(3',5'-Di-O-acetyl-2'-deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (14)
9-(3',5'-Di-O-acetyl-2'-deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (14)
9-(2'-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino) purine (15)
9-(2'-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzoyloxy)-methoxy)piperidin-4-ylamino)purine (15)
9-(5'-O-DMT-2'-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (16)

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9-(5'-O-DMT-2'-Deoxyribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (16)
Deoxyadenosine phosphoramidite with protected spin label (7)
9-(2',3',5'-Tri-O-acetyl-ribofuranosyl)-6-chloro-purine (17)
9-(2',3',5'-Tri-O-acetyl-ribofuranosyl)-6-chloro-purine (17)
9-((2',3',5'-Tri-O-acetyl-ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)methoxy)-piperidin-4-
ylamino)purine (18)

1H (BENZEN).ESP

Chemical Shift (ppm)

Normalized Intensity

Benzene

DMSO

H2O

Chemical Shift (ppm)
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9-(2',3',5'-Tri-O-acetyl-ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)methoxy)-piperidin-4-ylamino)purine (18)
9-(Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)-purine

(19)

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9-(Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)-purine (19)
9-(5'-O-DMT-Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (20)

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/
9-(5′-O-DMT-Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (20)
9-(2'-O-TBS-5'-O-DMT-Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (21)
9-(2'-O-TBS-5'-O-DMT-Ribofuranosyl)-6-(2,2,6,6-tetramethyl-1-((2-nitrobenzyloxy)-methoxy)piperidin-4-ylamino)purine (21)
Adenosine phosphoramidite with protected spin label (8)
Adenosine phosphoramidite with protected spin label (8)
Adenosine phosphoramidite with protected spin label (8)