Preparation of Spin-Labeled 2-Amino-dA, dA, dC and 5-Methyl-dC Phosphoramidites for the Automatic Synthesis of EPR Active Oligonucleotides

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Abstract: 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) labeled phosphoramidites of 2-amino-dA, dA, dC and 5-methyl-dC were synthesized and used for the automatic synthesis of mono-labeled oligodeoxynucleotides (ODNs), which proved active to EPR. It is now possibile to insert a paramagnetic probe into nucleic acids in a site- and type-specific manner. The combination of this synthetic approach with EPR spectroscopy can be exploited for performing studies on dynamics and local structural modifications in nucleic acids.

Key words: DNA, nucleosides, ODNs, nitroxides, EPR

Introduction

The insertion of a spin-label like TEMPO into nucleic acids allows the application of EPR spectroscopy to the study of dynamics and local structure modifications.[1] C^5-Spin-labeled dU analogues were prepared for antiviral activity evaluation[2] and for incorporation into ODNs by enzymatic[3] or automatic synthesis.[4] Furthermore, spin-labeling was obtained, on a micro scale, by treating halogenated adenylic coenzymes with a large excess of 4-amino-TEMPO (Tempamine).[5] Spin-labels were attached to different positions of nucleosides through tethers varying in length and flexibility.[6]

Here we report the synthesis of 6-TEMPO-labeled 2-amino-dA **(1a)** and dA **(1b)**, 4-TEMPO-labeled dC **(2a)** and 5-methyl-dC **(2b)** phosphoramidites (Figure 1), which carry a nitroxide spin-label directly linked to the exocyclic amino group. They were prepared with the aim of increasing the possibility of inserting a spin-label into nucleic acids by automatic synthesis, in a site- and type- specific manner. In fact the availability of labeled dA and dC allows one to insert the probe at any base pair along the sequence. The two pyrimidine derivatives have already been prepared, but by a different procedure and with lower yields.[7] We decided to attach TEMPO directly to the exocyclic amino group of the above mentioned nucleic bases for the following reasons: i) the presence of linkers connecting the reporter group to the nucleoside would greatly influence EPR spectra in dependence of the length and the rigidity of the linker;[8] ii) the present derivatives are able to give Watson–Crick pairings as normal bases do; and iii) the introduction of the label at those positions should bring about only minor structural perturbations into duplex structures, since the label protrudes into the major groove of the double helix.[9] In addition to spin-labeled dA and dC, we planned to synthesize also the analogues 2-amino-dA and 5-methyl-dC since both are known to stabilize duplexes relative to dA and dC. An enhanced binding can thus be achieved by using the modified bases and this can turn out to be particularly useful with short ODNs duplexes or if the spin-label is positioned close to the ends.

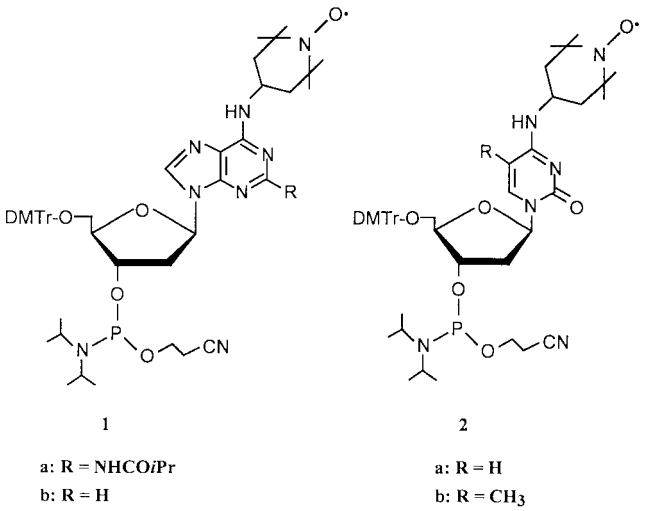
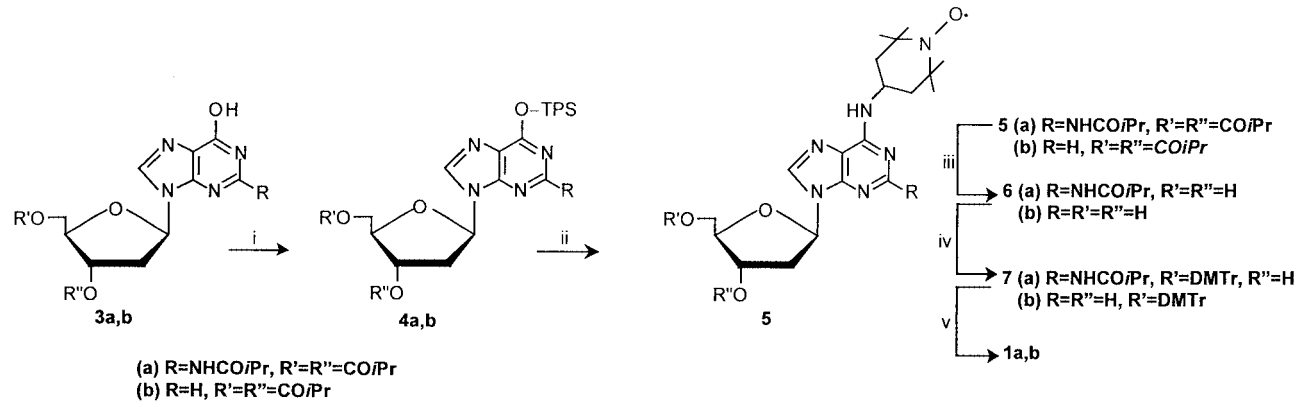


Figure 1 TEMPO-labeled 2-amino-dA, dA, dC and 5-methyl-dC phosphoramidites

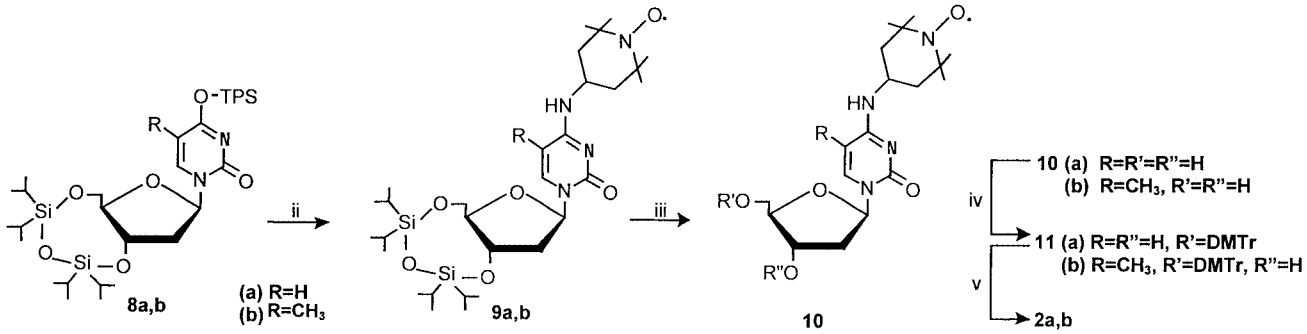
Preparation of the Spin-Labeled Phosphoramidites

The synthesis of **1a**,**b** is outlined in Scheme 1. At first, *N*^{2},3',5'-triisobutyryl-2'-deoxyguanosine (Triibu-dG)[10] (**3a**) and 3',5'-diisobutyryl-2'-deoxyinosine (Diibu-dI)[11] (**3b**) were converted to the *O*^{6}-sulfonylated derivatives **4a**,**b** by reaction with 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) in the presence of an organic base according to a known procedure.[11] Sulfonylation of **3a** gave **4a** as the only product in 90% yield; on the contrary, the reaction with **3b** produced a mixture of *N*^{1}- and *O*^{6}-sulfo-



Reagents and conditions: i) TPS-Cl/TEA/DMAP/CH_2Cl_2, r.t., 18 h, **4a**: 90%, **4b**: 50%; ii) Tempamine/CH_2Cl_2, reflux, 18 h, 54−69%; iii) **6a,b**: NH_4OH/MeOH, r.t., 4 h, 90−95%; **10a,b**: TBAF/THF, r.t., 18 h, 95%; iv) DMTr-Cl/pyridine, r.t., 3 h, 69−77%; v) $NCCH_2CH_2OP(Cl)N$*i*-Pr_2, *i*-Pr_2NEt/CH_2Cl_2, r.t., 30 min, 81−91%

Scheme 1



Reagents and conditions: See Scheme 1

Scheme 2

nylated isomers, in a ratio of nearly 1:1, which were easily separated by flash chromatography.[11]

The spin-labeled analogues were prepared by nucleophilic substitution of the respective sulfonylated compounds with two equivalents of tempamine in boiling CH_2Cl_2 overnight and gave **5a,b** in 54% and 68% yields, respectively. Deprotection of the sugar hydroxyl groups took place smoothly on treatment with methanolic ammonia at room temperature and gave **6a**,**b** in nearly quantitative yield. The regioselective protection of the 5'-position with 4,4'-dimethoxytrityl cloride (DMTr-Cl) afforded **7a**,**b** which were activated at the 3'-position by 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to give the desired phosphoramidites **1a,b**.

The synthesis of **2a**,**b** (Scheme 2) started from the sulfonylated derivatives **8a,b**, obtained from 2'-deoxy-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine and 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)thymidine,[12] respectively, and TPS-Cl by the method used for **4a,b**. This reaction occurred regioselectively and cleanly at the O^4-position to give the sulfonate esters **8a,b** in nearly quantitative yield and no O^4-N^3 isomerization was observed on standing.

Nucleophilic displacement with tempamine, by using the same procedure used for the purine derivatives, gave spin-labeled **9a,b** in 69% and 65% yields, respectively. Quantitative removal of the 3',5'-tetraisopropyldisiloxanyl group was achieved by a slight excess of fluoride ion in THF to generate **10a,b** in good yields. 5'-*O* Dimethoxytritylation and 3'-*O* activation afforded **11a,b** and the phosphoramidites **2a,b**, respectively.

Preparation of the ODNs and EPR measurements

0.1 M solutions of the TEMPO-labeled phosphoramidites **1a,b** and **2a,b** in anhydrous MeCN were used for the preparation of four mono-labeled ODNs different in length and composition. The position of insertion of the label from the 5'-terminal along the sequence was at +11 for the 40-mer 5'-d(ACT TAC AGC CAT GCA CTC CGC AAC GGC GAA TAG CCA TCC C)-3' (A), at +7 for the 20-mer 5'-d(ACA CTC ATC ACA CAT AAC GT)-3' (B), at +10 for the 20-mer 5'-d(ACA CTC GGG CAA GCG ACC GA)-3' (C) and at +15 for the 30-mer 5'-d(ACA TAC AGC CTA GCT CTC CGA CAC GGC GAA)-3' (D). The analogous non-labeled ODNs A_0, B_0, C_0 and D_0 were also synthesised as controls. The ODNs were all

checked by PAGE staining with ethidium bromide (Figure 2).

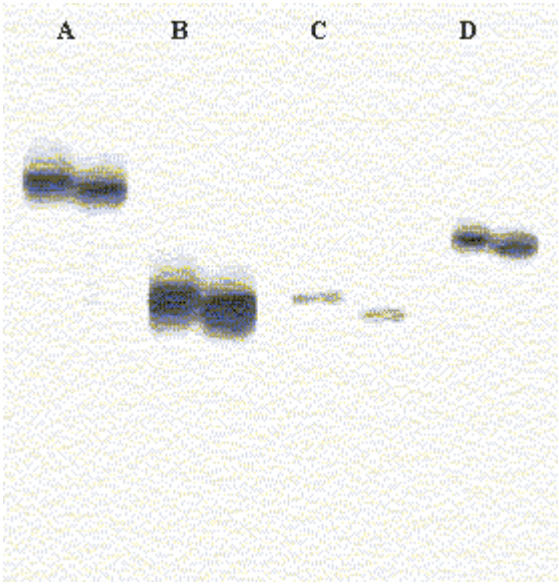


Figure 2 Comparison by PAGE (20%) of couples of spin-labeled (left spot) and control (right spot) 40-mers (A, A_0), 20-mers (B, B_0), 20-mers (C, C_0) and 30-mers (D, D_0) stained with ethidium bromide

Figure 3 shows the EPR spectra of all four A, B, C and D in comparison with the spectra of the respective labeled monomer phosphoramidites **1a,b** and **2a,b**. As expected, the EPR spectra of the ODNs are considerably broadened. Extremely marked and characteristic changes are present in the high-field line, which is somewhat broadened relative to the other two and its lineheight is decreased. All four labeled ODNs spectra display essentially the same line shapes, which are characteristic of a mobile nitroxide label.

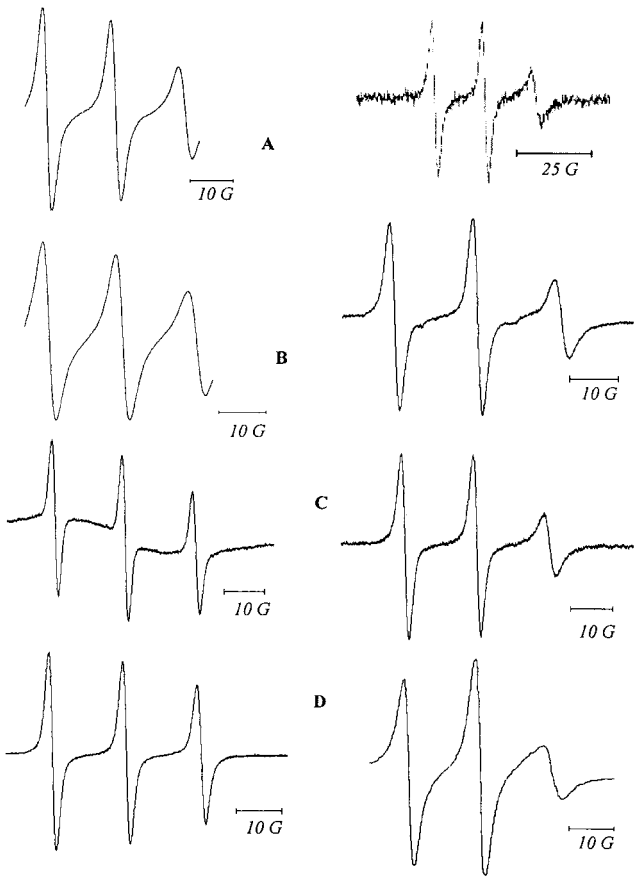


Figure 3 Comparison of the EPR spectra of 2-amino-dA (A), dA (B), dC (C) and 5-methyl-dC (D) TEMPO-labeled phosphoramidites (left side) and of mono-labeled-ODNs (right side) 40-mer (A), 20-mer (B), 20-mer (C) and 30-mer (D)

Conclusion

In conclusion, we have synthesised four spin-labeled phosphoramidites useful for the preparation of mono- and poly-labeled ODNs by automatic synthesis. These probes can be inserted in a site- and type-specific manner along the sequence in any double-strand nucleic acids. They offer a greater possibility of using EPR spectroscopy, which is a technique extremely effective not only for studies on nucleic acid dynamics and structural modifications, but also for studying the interactions of nucleic acids with other nucleic acids, or with nucleic acids binding proteins, or with small molecules, which can themselves carry a spin probe.

Melting points (Büchi oil apparatus) are uncorrected. UV and IR spectra were obtained with a Shimadzu UV-2101PC and a Perkin-Elmer 521 recording spectrophotometers, respectively. ^{1}H NMR (TMS as internal standard) and ^{31}P NMR (H_3PO_4 as external standard) spectra were recorded on a Varian XL-300 spectrometer. Before recording the NMR spectra, samples containing a nitroxide group were treated with 1.5 equiv of phenylhydrazine as reducing agent.[13] Optical rotations were determined with a Schmidt-Haensch 1604 polarimeter. Fast atom bombardment (FAB) mass spectra were recorded on a VG 70-70 EQ-MF instrument equipped with a standard FAB source (8 keV, Xe atoms, glycerol/1-thioglicerol solution). The mass spectra of **1a,b** and **2a,b** were obtained in electrospray (ES) conditions by a ZABSpec oa-TOF (Micromass Ltd. Manchester UK) instrument. Silica gel for column chromatography and TLC silica gel plates were from Merck AG (Darmstadt, Germany). The EPR spectra were performed with a Varian E109 spectrometer, X band, modulation amplitude of 0.5 gauss, time constant 300 ms, field scan time 120 s, 20 scans, microwave power 1 mV. The sample holder was a glass capillary tube and the measurements were performed on 0.1 mM solutions in MeCN for the monomers and in water for the ODNs, at 20 °C. Elemental analyses were performed by Servizio Microanalisi of CNR, Area della Ricerca di Roma, and were within ± 0.4% of the theoretical values.

Sulfonylated Nucleosides 8a,b; General Procedure

A solution of the protected nucleoside (1.0 mmol) in anhyd CH_2Cl_2 (5.0 mL) was treated overnight at r.t. with TPS-Cl (606 mg, 2.0 mmol) in the presence of Et_3N (404 mg, 4.0 mmol) and DMAP (12 mg, 0.1 mmol). The mixture was evaporated and the oily residue purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 80:20). Products were recovered as white foams and used without further purification.

4-*O*-[(2,4,6-Triisopropylphenyl)sulfonyl]-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyuridine (8a)
From 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyuridine[12] (638 mg, 1.35 mmol); yield: 780 mg (78%); R_f 0.70 ($CHCl_3$/MeOH, 98:2); $[\alpha]_D^{20}$ +47.8 (*c* = 0.5, MeCN).

UV (MeCN): λ_{max} = 288 (9800), 236 (10700) nm.

IR ($CHCl_3$): ν = 2949, 1667, 1625, 1462, 1383, 1115 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.71−1.26 [m, 42 H, $(CH_3)_2C$], 1.53−1.76 (m, 2 H, H-2' and H-2"), 2.00−2.37 (m, 4 H, Me_2CHSi), 2.46−2.95 (m, 3 H, Me_2CHPh), 3.41−4.13 (m, 4 H, H-3', H-4', H-5' and H-5"), 5.78 (d, 1 H, *J* = 8.0 Hz, H-5), 6.15 (d, 1 H, *J* = 6.0 Hz, H-1'), 7.05 (s, 2 H, Ar), 7.85 (d, 1 H, *J* = 8.0 Hz, H-6).

FAB-MS: Calcd mass for $C_{36}H_{60}N_2O_8SSi_2$: *m*/*z* = 737, found 738 $(M + H)^+$.

4-*O*-[(2,4,6-Triisopropylphenyl)sulfonyl]-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)thymidine (8b)
From 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)thymidine[12] (670 mg, 1.38 mmol); yield: 717 mg (69%); R_f 0.83 ($CHCl_3$/MeOH, 98:2); $[\alpha]_D^{20}$ +46.6 (*c* = 0.5, MeCN).

UV (MeCN): λ_{max} = 289 (6700), 233 (11000) nm.

IR ($CHCl_3$): ν = 3417, 2948, 1668, 1533, 1462, 1385, 1115 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.78−1.43 [m, 42 H, $(CH_3)_2C$], 1.62−1.98 (m, 2 H, H-2' and H-2"), 1.82 (s, 3 H, C^5-CH_3), 2.10−2.56 (m, 4 H, Me_2CHSi), 2.60−3.02 (m, 3 H, Me_2CHPh), 3.53−3.82 (m, 2 H, H-5' and H-5"), 3.90−4.06 (m, 1 H, H-4'), 4.10−4.37 (m, 1 H, H-3'), 5.67 (d, 1 H, *J* = 6.0 Hz, H-1'), 7.01 (s, 2 H, Ar), 7.69 (s, 1 H, H-6).

FAB-MS: Calcd mass for $C_{37}H_{62}N_2O_8SSi_2$: *m*/*z* = 751, found 752 $(M + H)^+$.

Spin-Labeled Nucleosides 5a,b and 9a,b; General Procedure
A solution of the appropriate sulfonylated nucleoside **4** and **8** (1 mmol) and tempamine (342 mg, 2.0 mmol) in CH_2Cl_2 (5 mL) was refluxed overnight. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with sat. aq $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), evaporated at reduced pressure and the residue purified by silica gel flash chromatography. All products were obtained as pale pink foams.

3',5'-Di-*O*-isobutyryl-2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (5a)
From **4a**[11] (800 mg, 1.1 mmol). Chromatographed with EtOAc/hexane (70:30); yield: 366 mg (54%); R_f 0.22 (EtOAc/hexane, 1:1); $[\alpha]_{Hg}^{20}$ −5.2 (*c* = 2, EtOH).

UV (MeOH): λ_{max} = 272 (22900), 232 (34900) nm.

IR ($CHCl_3$): ν = 3421, 2986, 1735, 1620, 1461, 1387, 1154 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.90−1.12 [m, 18 H, $(CH_3)_2C$], 1.20 (s, 12 H, $CH_{3piperidine}$), 1.35−1.66 (m, 4 H, $CH_{2piperidine}$), 1.70−1.94 (m, 2 H, H-2' and H-2"), 2.11−2.48 (m, 3 H, Me_2CHCO), 2.51−2.81 (m, 1 H, $CH_{piperidine}$), 4.05−4.31 (m, 3 H, H-4', H-5' and H-5"), 4.92−5.11 (m, 1 H, H-3'), 5.91 (t, 1 H, *J* = 6.0 Hz, H-1'), 7.30 (s, 1 H, H-8).

FAB-MS: Calcd mass for $C_{31}H_{47}N_7O_7$: *m*/*z* = 630, found 631 $(M + H)^+$.

3',5'-Di-*O*-isobutyryl-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (5b)
From **4b**[11] (784 mg, 1.2 mmol). Chromatographed with $CHCl_3$/EtOAc (80:20); yield: 431 mg (68%); R_f 0.20 (EtOAc/hexane, 70:30); $[\alpha]_D^{20}$ −17.3 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 267 (21000), 213 (22100) nm.

IR ($CHCl_3$): ν = 3418, 2990, 1735, 1618, 1470, 1366, 1152 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.00−1.38 [m, 24 H, $(CH_3)_2C$ and $CH_{3piperidine}$], 1.45−2.20 (m, 6 H, $CH_{2piperidine,}$ H-2' and H-2"), 2.22−2.80 (m, 3 H, Me_2CHCO and $CH_{piperidine}$), 4.00−4.20 (m, 3 H, H-4', H-5'and H-5"), 4.17 (br s, 1 H, H-3'), 5.86 (br s, 1 H, H-1'), 7.51 and 7.62 (2 s, 2 H, H-2 and H-8).

FAB-MS: Calcd mass for $C_{27}H_{40}N_6O_6$: *m*/*z* = 545, found 546 $(M + H)^+$.

N^4-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxycytidine (9a)
From **8a** (684 mg, 0.9 mmol). Chromatographed with CH_2Cl_2/*i*-PrOH (98:2); yield: 405 mg (69%); R_f 0.27 ($CHCl_3$/MeOH, 98:2); $[\alpha]_D^{20}$ +51.5 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 273 (10900), 243 (9600) nm.

IR ($CHCl_3$): ν = 3431, 2947, 1641, 1494, 1325, 1116 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.74−1.04 [m, 24 H, $(CH_3)_2C$], 1.11 (s, 12 H, $CH_{3piperidine}$), 1.28−1.60 (m, 4 H, $CH_{2piperidine}$), 1.62−2.05 (m, 2 H, H-2' and H-2"), 2.09−2.52 (m, 5 H, Me_2CHSi and $CH_{piperidine}$), 3.25−4.25 (m, 4 H, H-3', H-4', H-5' and H-5"), 5.80 (d, 1 H, *J* = 8.0 Hz, H-5), 6.18 (br s, 1 H, H-1'), 7.90 (d, 1 H, *J* = 8.0 Hz, H-6).

FAB-MS: Calcd mass for $C_{30}H_{54}N_4O_6Si_2$: *m*/*z* = 623, found 624 $(M + H)^+$.

5-Methyl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxycytidine (9b)
From **8b** (753 mg, 1.0 mmol). Chromatographed with CH_2Cl_2/MeOH (98:2); yield: 421 mg (65%); R_f 0.50 ($CHCl_3$/MeOH, 95:5); $[\alpha]_D^{20}$ +37.6 (*c* = 1, EtOH)

UV (EtOH): λ_{max} = 275 (10100), 247 (9700) nm.

IR ($CHCl_3$): ν = 3441, 2946, 1657, 1497, 1333, 1117 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.79−1.10 [m, 24 H, $(CH_3)_2C$], 1.16 (s, 12 H, $CH_{3piperidine}$), 1.20−1.46 (m, 4 H, $CH_{2piperidine}$), 1.60−1.92 (m, 2 H, H-2' and H-2"), 1.72 (s, 3 H, C^5-CH_3), 2.07−2.34 (m, 5 H, Me_2CHSi and $CH_{piperidine}$), 3.40−3.62 (m, 2 H, H-5' and H-5"), 3.70−3.86 (m, 1 H, H-4'), 4.15−4.36 (m, 1 H, H-3'), 5.64 (br s, 1 H, H-1'), 7.60 (s, 1 H, H-6).

FAB-MS: Calcd mass for $C_{31}H_{56}N_4O_6Si_2$: *m*/*z* = 637, found 638 $(M + H)^+$.

Deprotection of 3',5'-OH; General Procedures
Procedure A (for **6a,b**): The protected nucleoside **5a,b** (1.0 mmol) was treated with a mixture of MeOH (15 mL) and aq 25% NH_4OH (5 mL) for 4 h at r.t. After evaporation to dryness, the residue was purified by silica gel flash chromatography.

Procedure B (for **10a,b**): To a solution of the protected nucleoside **9a,b** (1.0 mmol) in THF (2 mL) was added TBAF (523 mg, 2.0 mmol) and the mixture was kept overnight at r.t. After removal of the solvent at reduced pressure, the residue was dissolved in H_2O (20 mL) and washed with Et_2O (2 × 15 mL). The aqueous phase was evaporated to dryness and the product purified by silica gel chromatography.

2-Isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (6a)
From **5a** (320 mg, 0.5 mmol). Chromatographed with EtOAc/*i*-PrOH (98:2); pale pink foam; yield: 220 mg (90%); R_f 0.35 (EtOAc); $[\alpha]_D^{20}$ −3.5 (*c* = 1.5, EtOH).

UV (EtOH): λ_{max} = 277 (17900), 232 (25500) nm.

IR ($CHCl_3$): ν = 3419, 2990, 1711, 1621, 1460, 1384, 1104 cm^{-1}.

^1H NMR (DMSO-d_6): δ = 0.90 and 0.94 [2 s, 6 H, $(CH_3)_2C$], 1.23 (s, 12 H, $CH_{3piperidine}$), 1.18−1.64 (m, 4 H, $CH_{2piperidine}$), 1.88−2.18 (m, 2 H, H-2' and H-2"), 2.22−2.40 (m, 1 H, Me_2CHCO), 2.65−2.88 (m, 1 H, $CH_{piperidine}$), 3.06−3.20 (m, 3 H, H-4', H-5' and H-5"), 3.99−4.28 (m, 1 H, H-3'), 5.82 (t, 1 H, *J* = 6.0 Hz, H-1'), 7.70 (s, 1 H, H-8).

FAB-MS: Calcd mass for $C_{23}H_{35}N_7O_5$: *m*/*z* = 490, found 491 (M + H)$^+$.

N^6-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (6b)

From **5b** (370 mg, 0.7 mmol). Chromatographed with EtOAc/*i*-PrOH (98:2); pale pink foam; yield: 255 mg (90%); R_f 0.20 (EtOAc); $[\alpha]_D^{20}$ −16.4 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 266 (15200), 213 (14200) nm.

IR ($CHCl_3$): ν = 3304, 2993, 1728, 1618, 1479, 1372, 1136 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 1.08 (s, 12 H, CH_3), 1.49−1.90 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 2.00−2.40 (m, 1 H, $CH_{piperidine}$), 3.31−3.47 (m, 2 H, H-5' and H-5"), 3.70 (br s, 1 H, H-4'), 4.19−4.28 (m, 1 H, H-3'), 6.01 (t, 1 H, *J* = 6.0 Hz, H-1'), 7.75 and 7.88 (2 s, 2 H, H-2 and H-8).

FAB-MS: Calcd mass for $C_{19}H_{28}N_6O_4$: *m*/*z* = 405, found 405 (M)$^+$.

N^4-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (10a)

From **9a** (371 mg, 0.6 mmol). Chromatographed with EtOAc/*i*-PrOH (80:20); light pink oil; yield: 217 mg (95%); R_f 0.27 (EtOAc/*i*-PrOH, 70:30); $[\alpha]_D^{20}$ +52.0 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 273 (11100), 242 (9500) nm.

IR ($CHCl_3$): ν = 3409, 2935, 1641, 1499, 1333, 1096 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 1.08 (s, 12 H, CH_3), 1.58−2.28 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 2.30−2.48 (m, 1 H, $CH_{piperidine}$), 3.30−3.46 (m, 2 H, H-5' and H-5"), 3.50−3.71 (m, 1 H, H-4'), 3.95−4.11 (m, 1 H, H-3'), 5.74 (d, 1 H, *J* = 8.0 Hz, H-5), 6.15 (t, 1 H, *J* = 6.0 Hz, H-1'), 7.84 (d, 1 H, *J* = 8.0 Hz, H-6).

FAB-MS: Calcd mass for $C_{18}H_{28}N_4O_5$: *m*/*z* = 380, found 381 (M + H)$^+$.

5-Methyl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (10b)

From **9b** (410 mg, 0.6 mmol). Chromatographed with EtOAc/*i*-PrOH (80:20); amorphous pink solid; yield: 225 mg (95%); mp 238−240 °C; R_f 0.17 (EtOAc/*i*-PrOH, 80:20); $[\alpha]_D^{20}$ +15.3 (*c* = 0.5, EtOH)

UV (EtOH): λ_{max} = 280 (6600), 246 (6300) nm.

IR ($CHCl_3$): ν = 3413, 2931, 1663, 1620, 1555, 1353, 1108 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 1.05 (s, 12 H, CH_3), 1.15−1.80 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 1.85 (s, 3 H, C^5-CH_3), 1.98−2.25 (m, 1 H, $CH_{piperidine}$), 3.00−3.28 (m, 3 H, H-4', H-5' and H-5"), 4.11−4.29 (m, 1 H, H-3'), 6.12 (br s, 1 H, H-1'), 7.63 (s, 1 H, H-6).

FAB-MS: Calcd mass for $C_{19}H_{30}N_4O_5$: *m*/*z* = 395, found 395 (M)$^+$.

5'-*O*-Dimethoxytritylation; General Procedure

The required derivative (1.0 mmol), previously coevaporated with anhyd pyridine and dried under high vacuum overnight, was dissolved in anhyd pyridine (12 mL) and then DMTr-Cl (678 mg, 2.0 mmol) was added at r.t. After 3 h, the reaction was quenched with MeOH (0.1 mL) and the mixture concentrated to small volume. The residue was diluted with CH_2Cl_2 (25 mL) and washed with aq sat. $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL). The organic extract was dried (Na_2SO_4), filtered, evaporated and finally coevaporated with toluene to remove the excess of pyridine. The products were isolated by silica gel chromatography.

5'-*O*-(4,4'-Dimethoxytrityl)-2-isobutyramido-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (7a)

From **6a** (200 mg, 0.47 mmol). Chromatographed with EtOAc/hexane/TEA (70:29:1); pale pink foam; yield: 223 mg (69%); R_f 0.53 (EtOAc); $[\alpha]_D^{20}$ +11.6 (*c* = 2, EtOH).

UV (EtOH): λ_{max} = 276 (19300), 232 (41100) nm.

IR ($CHCl_3$): ν = 3394, 2936, 1726, 1619, 1507, 1462, 1378, 1177 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.08 and 1.12 [2 s, 6 H, $(CH_3)_2C$], 1.26 (s, 12 H, $CH_{3piperidine}$), 1.20−1.60 (m, 4 H, $CH_{2piperidine}$), 1.70−2.06 (m, 2 H, H-2' and H-2"), 2.18−2.60 (m, 2 H, Me_2CHCO and $CH_{piperidine}$), 3.10−3.28 (m, 3 H, H-4', H-5' and H-5"), 3.55 (s, 6 H, CH_3O), 3.90−4.15 (m, 1 H, H-3'), 6.04 (br s, 1 H, H-1'), 6.31−6.60 and 6.75−7.18 (2 m, 13 H, Ar), 7.43 (s, 1 H, H-8).

FAB-MS: Calcd mass for $C_{44}H_{53}N_7O_7$: *m*/*z* = 792, found 793 (M + H)$^+$.

5'-*O*-(4,4'-Dimethoxytrityl)-N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine (7b)

From **6b** (162 mg, 0.4 mmol). Chromatographed with EtOAc/hexane/TEA (70:29:1); pale pink foam; yield: 218 mg (77%); R_f 0.36 (EtOAc/MeOH, 95:5); $[\alpha]_D^{20}$ −5.1 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 268 (19600), 235 (22400) nm.

IR ($CHCl_3$): ν = 3316, 2991, 1729, 1618, 1507, 1474, 1373, 1177 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.24 (s, 12 H, CH_3), 1.60−2.00 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 2.10−2.45 (m, 1 H, $CH_{piperidine}$), 3.18−3.39 (m, 2 H, H-5' and H-5"), 3.50 (s, 6 H, CH_3O), 3.68 (br s, 1 H, H-4'), 4.15−4.24 (m, 1 H, H-3'), 6.04 (t, 1 H, *J* = 6.0 Hz, H-1'), 6.24−6.55 and 6.64−7.20 (2 m, 13 H, Ar), 7.47 and 7.85 (2 s, 2 H, H-2 and H-8).

FAB-MS: Calcd mass for $C_{40}H_{46}N_6O_6$: *m*/*z* = 706, found 706 (M)$^+$.

5'-*O*-(4,4'-Dimethoxytrityl)-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (11a)

From **10a** (225 mg, 0.6 mmol). Chromatographed with EtOAc/*i*-PrOH/TEA (95:4:1); pale pink oil; yield: 290 mg (72%); R_f 0.27 (EtOAc/MeOH, 80:20); $[\alpha]_D^{20}$ +48.8 (*c* = 1, EtOH).

UV (EtOH): λ_{max} = 277 (13700), 235 (25300) nm.

IR ($CHCl_3$): ν = 3429, 2991, 1642, 1507, 1252, 1118 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.10 (s, 12 H, CH_3), 1.57−2.15 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 2.33−2.47 (m, 1 H, $CH_{piperidine}$), 3.15−3.33 (m, 2 H, H-5' and H-5"), 3.55 (s, 6 H, CH_3O), 3.67−3.93 (m, 1 H, H-4'), 4.03−4.36 (m, 1 H, H-3'), 5.10 (d, 1 H, *J* = 8.0 Hz, H-5), 5.95 (t, 1 H, *J* = 6.0 Hz, H-1'), 6.30−6.58 and 6.67−7.10 (2 m, 13 H, Ar), 7.95 (br s, 1 H, H-6).

FAB-MS: Calcd mass for $C_{39}H_{46}N_4O_7$: *m*/*z* = 683, found 684 (M + H)$^+$.

5'-*O*-(4,4'-Dimethoxytrityl)-5-methyl-N^4-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine (11b)

From **10b** (158 mg, 0.4 mmol). Chromatographed with EtOAc/*i*-PrOH/TEA (95:4:1); amorphous pink solid; yield: 209 mg (75%); mp 172−175 °C; R_f 0.58 (EtOAc/*i*-PrOH, 70:30); $[\alpha]_D^{20}$ +16.5 (*c* = 0.5, EtOH).

UV (EtOH): λ_{max} = 276 (14300), 234 (31300) nm.

IR ($CHCl_3$): ν = 3443, 2934, 1660, 1625, 1550, 1338, 1250, 1116 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.09 (s, 12 H, CH_3), 1.33 (s, 3 H, C^5-CH_3), 1.55−2.29 (m, 6 H, $CH_{2piperidine}$, H-2' and H-2"), 2.33−2.55 (m, 1 H, $CH_{piperidine}$), 3.09−3.29 (m, 2 H, H-5' and H-5"), 3.58 (s, 6 H, CH_3O), 3.88−4.05 (m, 1 H, H-4'), 4.24−4.54 (m, 1 H, H-3'), 6.30 (br s, 1 H, H-1'), 6.61−6.89 and 7.25−7.44 (2 m, 13 H, Ar), 7.59 (s, 1 H, H-6).

FAB-MA: Calcd mass for $C_{40}H_{48}N_4O_7$: *m*/*z* = 697, found 698 (M + H)$^+$.

Phosphoramidites 1a,b and 2a,b; General Procedure

Compounds **7a**,**b** and **11a**,**b** were dried under high vacuum overnight after coevaporation with anhyd pyridine. To a stirred solution

of the appropriate nucleoside (1.0 mmol) and *i*-Pr_2NEt (0.7 mL, 4.0 mmol) in anhyd CH_2Cl_2 (12 mL) was added 2-cyanoethyl-diisopropylchlorophosphoramidite (473 mg, 2.0 mmol) by a syringe over 2 min under N_2. The mixture was left for 30 min at r.t., filtered to remove the precipitated amine hydrochloride and diluted with CH_2Cl_2 (30 mL). The solution was washed with ice-cooled brine (2 × 15 mL) and the organic phase was dried (Na_2SO_4), filtered, concentrated to small volume at low pressure and added dropwise to stirred hexane cooled to −78 °C. Products **1a** and **2a** separated as an oil and were lyophilised from benzene; **1b** and **2b** separated as a solid and were recovered by filtration and dried. All compounds were as mixture of diastereoisomers.

5'-*O*-(4,4'-Dimethoxytrityl)-2-isobutyramido-*N*^{6}-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine-3'(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (1a)

From **7a** (120 mg, 0.15 mmol); yield: 122 mg (81%); R_f 0.54, 0.65 (EtOAc/hexane, 70:30); $[\alpha]_D^{20}$ −7.5 (*c* = 1, MeCN)

UV (MeCN): λ_{max} = 276 (18300), 233 (39900) nm.

IR ($CHCl_3$): ν = 3395, 2963, 1710, 1619, 1508, 1462, 1383, 1178 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.05−1.28 [m, 18 H, $(CH_3)_2C$], 1.42 (s, 12 H, $CH_{3piperidine}$), 1.35−1.54 (m, 4 H, $CH_{2piperidine}$), 1.85−2.20 (m, 2 H, H-2' and H-2"), 2.38−2.50 (m, 3 H, Me_2CHCO and $CH_{piperidine}$), 2.61 (br s, 2 H, CH_2CN), 3.23−3.50 (m, 2 H, Me_2CHN), 3.52−3.90 (m, 2 H, H-5' and H-5"), 3.78 (s, 6 H, CH_3O), 4.11−4.38 (m, 3 H, H-4' and CH_2OP), 4.68−4.80 (m, 1 H, H-3'), 6.37 (t, 1 H, *J* = 6.0 Hz, H-1'), 6.72−7.00 and 7.15−7.38 (2 m, 13 H, Ar), 7.85 (2 s, 1 H, H-8).

^{13}C NMR ($CDCl_3$): δ = 39.76 (C-2'), 63.57 (C-5'), 76.40 (C-3'), 84.20 (C-1'), 86.43 (C-4'), 126.90 (C-5), 137.52 (C-8), 144.55 (C-4), 158.54 (C-6), 160.95 (C-2).

^{31}P NMR ($CDCl_3$): δ = 151.30, 149.48.

HRMS: *m*/*z* Calcd for $C_{53}H_{70}N_9O_8P$ 991.508, found 991.532 (M^+), 1027.532 (+ $2H_2O$).

5'-*O*-(4,4'-Dimethoxytrityl)-*N*^{6}-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxyadenosine-3'-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (1b)

From **7b** (180 mg, 0.25 mmol); yield: 193 mg (84%); mp 82−84 °C; R_f 0.63, 0.70 (EtOAc); $[\alpha]_D^{20}$ −37.3 (*c* = 1.5, MeCN).

UV (MeCN): λ_{max} = 267 (25600), 237 (26300) nm.

IR ($CHCl_3$): ν = 3319, 2958, 1615, 1508, 1470, 1375, 1178 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.04−1.29 [m, 12 H, $(CH_3)_2C$], 1.35 (s, 12 H, $CH_{3piperidine}$), 1.53−1.89 (m, 4 H, $CH_{2piperidine}$), 1.95−2.14 (m, 2 H, H-2' and H-2"), 2.38−2.50 (m, 3 H, Me_2CHCO and $CH_{piperidine}$), 2.60 (t, 2 H, *J* = 6.0 Hz, CH_2CN), 3.25−3.49 (m, 2 H, Me_2CHN), 3.52−3.90 (m, 2 H, H-5' and H-5"), 3.75 (s, 6 H, CH_3O), 3.93−4.20 (m, 1 H, H-4'), 4.26 (br s, 2 H, CH_2OP), 4.73 (br s, 1 H, H-3'), 6.45 (br s, 1 H, H-1'), 6.70−6.95 and 7.10−7.40 (2 m, 13 H, Ar), 7.93 and 7.95 (2 s, 2 H, H-2 and H-8).

^{13}C NMR ($CDCl_3$): δ = 39.26 (C-2'), 63.48 (C-5'), 76.35 (C-3'), 84.01 (C-1'), 85.32 (C-4'), 126.52 (C-5), 138.20 (C-8), 144.56 (C-4), 151.25 (C-2), 158.51 (C-6).

^{31}P NMR ($CDCl_3$): δ = 149.97, 148.70.

HRMS: *m*/*z* Calcd for $C_{49}H_{63}N_8O_7P$ 906.455, found 906.434 (M^+), 942.372 (+ $2H_2O$).

5'-*O*-(4,4'-Dimethoxytrityl)-*N*^{4}-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine-3'-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (2a)

From **11a** (277 mg, 0.4 mmol); yield: 297 mg (83%); R_f 0.63, 0.75 (EtOAc/*i*-PrOH, 95:5); $[\alpha]_D^{20}$ +38.4 (*c* = 2, MeCN).

UV (EtOH): λ_{max} = 275 (9400), 235 (19400) nm.

IR ($CHCl_3$): ν = 3431, 2963, 1642, 1502, 1462, 1326, 1252, 1178 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.09−1.18 [m, 12 H, $(CH_3)_2C$], 1.25 (s, 12 H, $CH_{3piperidine}$), 1.74−2.20 (m, 6 H, $CH_{2piperidine,}$ H-2' and H-2"), 2.42 (t, 2 H, *J* = 6.0 Hz, CH_2CN), 2.33−2.47 (m, 1 H, $CH_{piperidine}$), 3.28−3.43 (m, 2 H, Me_2CHN), 3.45−3.65 (m, 3 H, H-4', H-5' and H-5"), 3.78 (s, 6 H, CH_3O), 4.12 (br s, 2 H, CH_2OP), 4.62 (br s, 1 H, H-3'), 5.35 (br s, 1 H, H-5), 6.25−6.40 (m, 1 H, H-1'), 6.76−6.90 and 7.16−7.46 (2 m, 13 H, Ar), 7.80 (d, 1 H, *J* = 8.0 Hz, H-6).

^{13}C NMR ($CDCl_3$): δ = 40.87 (C-2'), 55.24 (C-5'), 74.52 (C-3'), 86.03 (C-1'), 86.70 (C-4'), 95.73 (C-5), 144.66 (C-6), 150.49 (C-2), 158.64 (C-4).

^{31}P NMR ($CDCl_3$): δ = 150.34, 150.29.

HRMS: *m*/*z* Calcd for $C_{48}H_{63}N_6O_8P$ 882.444, found 882.435 (M^+), 918.432 (+ $2H_2O$).

5'-*O*-(4,4'-Dimethoxytrityl)-5-methyl-*N*^{4}-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2'-deoxycytidine-3'-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite (2b)

From **11b** (241 mg, 0.34 mmol); yield: 282 mg (91%); mp 126−130 °C; R_f 0.76, 0.83 (EtOAc/*i*-PrOH, 95:5); $[\alpha]_D^{20}$ +17.2 (*c* = 1, MeCN).

UV (MeCN): λ_{max} = 277 (10100), 235 (24200) nm.

IR ($CHCl_3$): ν = 3443, 2992, 1663, 1501, 1464, 1337, 1245, 1177 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.13−1.29 [m, 24 H, $(CH_3)_2C$ and $CH_{3piperidine}$], 1.28 (s, 3 H, C^5-CH_3), 1.41−1.56 (br s, 4 H, $CH_{2piperidine}$), 2.01 (br s, 2 H, H-2' and H-2"), 2.38 (t, 2 H, *J* = 6.0 Hz, CH_2CN), 3.20−3.38 (m, 3 H, Me_2CHN and $CH_{piperidine}$), 3.40−3.65 (m, 3 H, H-4', H-5' and H-5"), 3.76 (s, 6 H, CH_3O), 4.13 (br s, 2 H, CH_2OP), 4.60 (br s, 1 H, H-3'), 6.35−6.44 (m, 1 H, H-1'), 6.79−6.84 and 7.23−7.35 (2 m, 13 H, Ar), 7.68 (s, 1 H, H-6).

^{13}C NMR ($CDCl_3$): δ = 40.72 (C-2'), 55.27 (C-5'), 74.28 (C-3'), 85.87 (C-1'), 86.18 (C-4'), 110.56 (C-5), 144.47 (C-6), 151.27 (C-2), 158.72 (C-4).

^{31}P NMR ($CDCl_3$): δ = 150.50, 150.07.

HRMS: *m*/*z* Calcd for $C_{49}H_{65}N_6O_8P$ 896.460, found 896.459 (M^+), 932.498 (+ $2H_2O$)

Synthesis and Nucleoside Composition Analysis of ODNs

The automatic synthesis of the ODNs was performed at 1 µmol scale on an ABI (Applied Biosystems, Inc., Forster City, CA) 392/8 synthesiser, leaving the terminal 5'-O-DMTr-group on. The labeled phosphoramidites were used for the synthesis of the ODNs without having to change any parameter in the standard DNA synthesis protocol of the machine, in particular the coupling time was kept at 25 s and average coupling yields in the range 96−98% were obtained. Having used Ac-protected dC for the synthesis, the ODNs were cleaved from the solid support by a mixture of ammonia and methylamine (AMA, Beckmann) in 10 min at r.t., then the solution was heated for 10 min at 55 °C in order to deprotect the exocyclic amino groups. After concentration, the samples were loaded onto an HPLC "Pure DNA" Dynamax column module (C4), 300 Å, 5µ, 21.4 × 50 mm eluted at a flow-rate of 6 mL/min. First the failure sequences were removed with a linear gradient of MeCN 3−18% in triethylammonium acetate buffer 0.1 M, pH 7.0, in 15 min. Afterwards the terminal DMTr was removed on column from the full-length ODN by 0.5% TFA. Then the DMTr-free full-length ODN was eluted by a second linear gradient 3−40% MeCN in the same buffer as above, in 20 min. The pooled fractions were lyophilised and checked by PAGE on 20% polyacrylamide, 7 M urea gels (1.5 mm) containing 50 mM Tris-borate buffer pH 8.0, 0.1 mM EDTA. The four labeled (A, B, C, D) and the corresponding unlabeled (A_0, B_0, C_0, D_0) ODNs, each 0.5 O.D., were digested in 100 µL of 10 mM

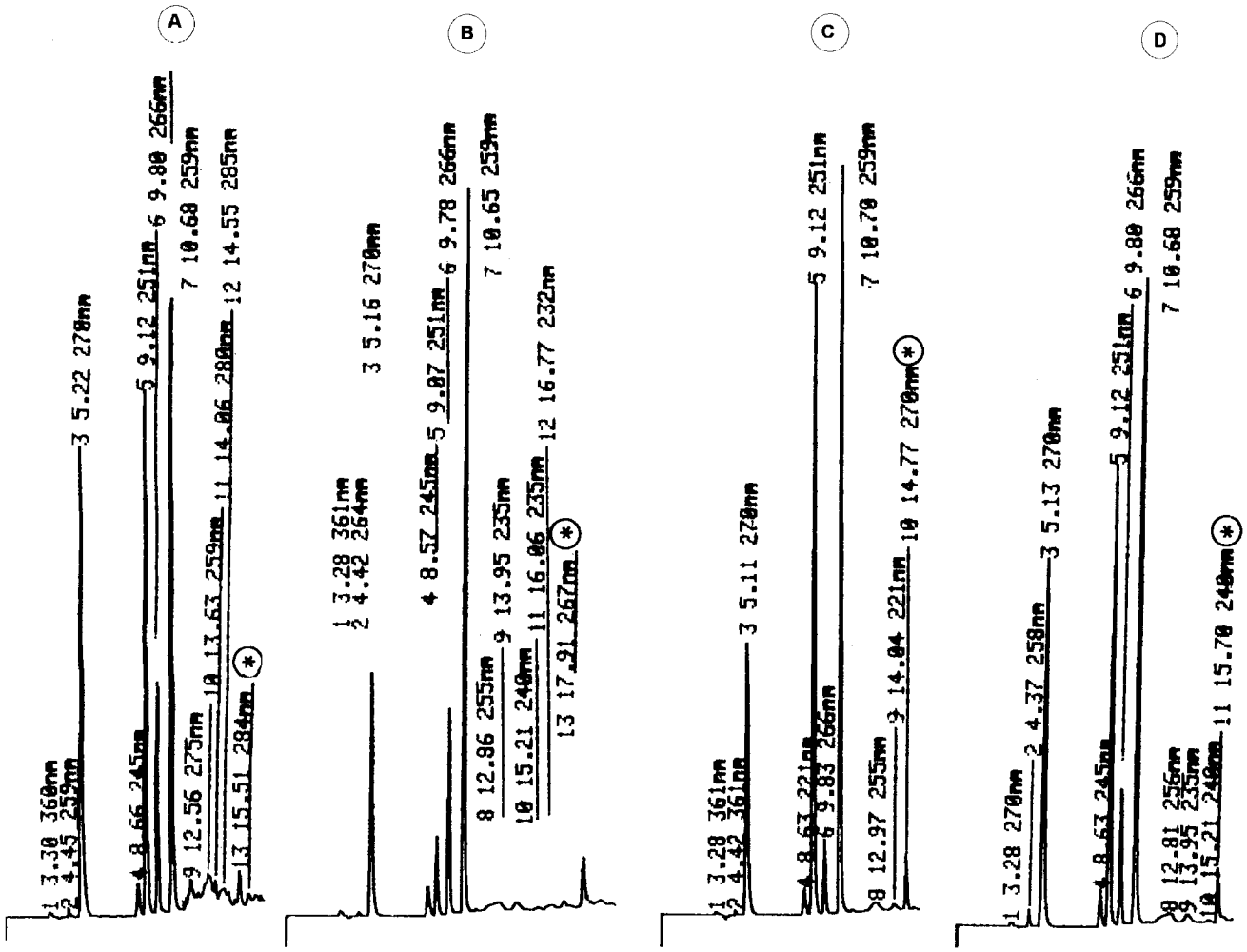


Figure 4 HPLC of enzymatic digestion of the labeled ODNs A ($A_{10}C_{16}G_7T_6$+**6a**), B ($A_7C_7GT_4$+**6b**), C ($A_6C_6G_6T$+**10a**) and D ($A_9C_{11}G_6T_3$+**10b**). The peaks at 5.1−5.2 min correspond to dC, those at ca. 9.1 to dG, those at ca. 9.8 to T, those at 10.6−10.7 to dA. The peaks corresponding to the labeled nucleosides are marked with an asterisk

potassium phosphate buffer, pH 7.0, with a mixture of phosphodiesterase from Crotalus adamanteus, 30 mU (Sigma) and alkaline phosphatase from bovine intestine, 10 U (Sigma), for 6 h at 37 °C, then filtered through a Millex-HV filter unit, 25 mm, 0.45 μm, and analysed by HPLC on a Supelco-C18 column, 250 × 4.6 mm, 5 μm, eluting at 1 mL/min, with a linear gradient of MeCN, 3-40% in 15 min, in triethylammonium acetate buffer, 0.1 M, pH 7.0, monitoring at 255 nm. When the digestion mixtures of the labeled ODNs were analysed, a second linear-gradient step was added, bringing MeCN to 60% in 10 min. One tetramer of composition 5'-d(ACGT)-3' was also synthesised and analysed in the same way, as reference standard mixture. The relative heights of the peaks in the chromatograms, corresponding to the four deoxynucleosides dC, dG, T, dA, which elute in the order, were used for the evaluation of the composition of the eight ODNs above. The peaks of the labeled nucleosides **6a**, **6b**, **10a**, **10b** were matched with the corresponding standards. The digestion mixtures of the labeled ODNs proved to contain also the labeled nucleosides (Figure 4). Nucleoside **6b** resulted only half than expected, although the amount of enzymes and the digestion time were double as usual.[14] The accordance of the theoretical composition of each ODN with the composition evaluated in this way resulted as follows for dC, dG, T and dA, and for the labeled nucleosides, in the order of elution, arbitrarily assigning a value of 1.00 to dC, the one eluting first: (A_0) 1.00, 1.02, 0.92, 0.96; (A) 1.00, 1.06, 0.83, 0.72, 0.73 (**6a**); (B_0) 1.00, 0.95, 0.90, 0.90; (B) 1.00, 0.76, 0.95, 0.80, 0.49 (**6b**); (C_0) 1.00, 1.00, 1.11, 0.89; (C) 1.00, 1.00, 1.00, 1.00, 0.87 (**10a**); (D_0) 1.00, 1.00, 1.00, 0.91; (D) 1.00, 0.90, 0.90, 0.71, 1.10 (**10b**).

References

(1) Lerman, L. S. *DNA Probes*; Lerman, L. S., Ed.; Cold Spring Harbor Laboratory: New York, 1986.
Keller, G. H.; Manak, M. M. *DNA Probes*; Macmillan Publishers Ltd.: Basingstoke, 1990.
(2) Bobst, A. M.; Ozinskas, A. J.; de Clercq, E. *Helv. Chim. Acta* **1983**, *66*, 534.
(3) Kao, S. C.; Bobst, A. M. *J. Magn. Reson.* **1986**, *67*, 125.
Pauly, G. T.; Bobst, E. V.; Bruckman, D.; Bobst, A. M. *Helv. Chim. Acta* **1989**, *72*, 110.
(4) Duh, J. L.; Bobst, A. M. *Helv. Chim. Acta* **1991**, *74*, 739.
Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 1299.
(5) Hoppe, J.; Rieke, E.; Wagner, K.G. *Eur. J. Biochem*. **1978**, *83*, 411.

Hoppe, J.; Wagner, K. G. *Eur. J. Biochem*. **1974**, *48*, 519.
Seiter, M. S.; Bauer, M. P.; Vogel, P. D.; Trommer, W. E. *Synthesis* **1996**, 269.
(6) Kao, S. C.; Bobst, A. M. *Biochemistry* **1985**, *24*, 5465.
Pauly, G. T.; Thomas, I. E.; Bobst, A. M. *Biochemistry* **1987**, *26*, 7304.
Strobel, O. K; Keyes, R. S.; Bobst, A. M. *Biochemistry* **1990**, *29*, 8522.
Keyes, R. S.; Bobst, A. M. *Biochemistry* **1995**, *34*, 9265.
Strobel, O. K.; Keyes, R. S.; Sinden, R. R.; Bobst, A. M. *Arch. Biochem. Biophys.* **1995**, *324*, 357.
Giordano, C.; Pedone, F.; Fattibene, P.; Cellai, L. *Nucleosides, Nucleotides & Nucleic Acids* **2000**, *19*, 1301.
(7) Bannwarth, W.; Schmidt, D. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 977.
(8) Bobst, E. V.; Keyes, R. S.; Cao, Y. Y.; Bobst, A. M. *Biochemistry* **1996**, *35*, 9309.
(9) Mac Millan, A. M.; Verdine, G. L. *J. Org. Chem*. **1990**, *55*, 5931.
(10) Howard, F. B; Frazier, J.; Miles, H. T. *J. Biol. Chem.* **1966**, *241*, 4293.
(11) Seela, F.; Herdering, W.; Kehne, A. *Helv. Chim. Acta* **1987**, *70*, 1649.
Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2253.
Gaffney, B. L.; Marky, L. A.; Jones, R. A. *Tetrahedron* **1984**, *40*, 3.
(12) Markiewicz, W. T. *J. Chem. Res., Synop.* **1979**, 24.
Markiewicz, W. T. *J. Chem. Res., Miniprint* **1979**, 181.
Markiewicz, W. T. *Bull. Pol. Acad. Sci. Chem.* **1984,** *32*, 463; *Chem. Abstr.* **1985**, *103*, 71609.
Saito, I.; Ikehira, H.; Katasani, R.; Watanabe, M.; Matsura, T. *J. Am. Chem. Soc.* **1986**, *108*, 3115.
(13) Lee, T. D.; Keana, F. W. *J. Org. Chem.* **1975**, *40*, 3145.
(14) Coleman, R. S.; Kesicki, E. A. *J. Am. Chem. Soc*. **1994**, *116*, 11636.

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