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# Synthesis and Properties of α- and β-Oligodeoxynucleotides Containing α- and β-1-(2-*O*-Methyl-D-arabino-furanosyl)thymine

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**Abstract**—Synthesis of the α- and β-anomer of 2′-OMe-araT (α- and β-1-(2-*O*-methyl-D-arabinofuranosyl)thymine) and their incorporation into oligodeoxynucleotide (ODN) analogues is described. Condensation of the key arabinofuranose derivative with silylated thymine afforded the α-anomer and the β-anomer which were converted into the respective phosphoramidite building blocks. Automated synthesis of β-ODNs containing β-2′-OMe-araT (by use of standard β-amidites and phosphoramidite building block **9b**) and α-ODNs containing α-2′-OMe-araT (by use of α-T-amidite and phosphoramidite building block **9a**) allowed evaluation of their properties. With regard to 3′-exonucleolytic degradation, 3′-end incorporation of either β- or α-2′-OMe-araT resulted in considerable stabilization compared to unmodified β-ODNs. Thermal stabilities of duplexes formed between modified ODNs and both unmodified DNA and RNA were evaluated and compared to unmodified controls. In all experiments stable duplexes were formed, but whereas β-ODNs containing β-2′-OMe-araT showed moderately lowered thermal stabilities towards both DNA and RNA, α-ODNs containing α-2′-OMe-araT exhibited significantly increased melting points (compared to β-ODN controls) when complexed with RNA. These results illustrate the potential of using arabino-configurated nucleosides as modified monomers in biologically active ODN-analogues, either as, e.g., 2′-*O*-alkylated or 2′-*O*-functionalized derivatives. 

## Introduction

Oligonucleotides offer potential as inhibitors of gene expression in the antisense strategy. This requires them to show, e.g., enhanced stability towards destructive cellular nucleases and efficient hybridization to target nucleic acids.[1,2] To fulfil these and other criteria, a substantial number of modified oligonucleotide analogues have been synthesized and evaluated recently.[2,3] Carbohydrate modified 2′-*O*-methyloligo-*ribo*nucleotides exhibit increased thermal stability of DNA:DNA and DNA:RNA hybrids,[4,5] and incorporation of 2′-*O*-methylribonucleoside monomers one to five times in oligodeoxynucleotides leads to unchanged or slightly improved hybridization properties.[6] Enzymatic stability tests show that 2′-*O*-methyloligoribonucleotides are resistant towards degradation by RNA- and DNA-specific nucleases although they are degraded by a dual RNA/DNA enzyme such as snake venom phosphodiesterase.[7] The use of 2′-*O*-methyloligoribonucleotides as regulators of gene expression has been reported to be hampered by nonspecific interactions[8] and the fact that the target RNA strand in a 2′-*O*-Me-RNA:RNA duplex is not cleaved by RNase H.[7]

The 2′-*O*-substituent in *ribo*nucleotide monomers is oriented into the minor groove of a duplex, whereas the 2′-*O*-substituent in *arabino*nucleotide monomers points into the major groove. From this background, we have recently synthesized branched ODN-analogues for combined duplex and triplex (in the major groove) high-affinity targeting of complementary strands using 1-(2′-*C*-methyl-β-D-arabinofuranosyl)uracil as a branching monomer.[9,10] To further develop and improve this strategy, we consider arabinonucleosides as promising branching monomers. Oligonucleotides containing either β- or α-arabinonucleosides have been synthesized using 2′-*O*-acetyl-β-,[11,12] 2′-*O*-*p*-nitrophenylethoxycarbonyl-β-[13] or 2′-*O*-benzoyl-α-[14] arabinonucleoside phosphoramidite synthons. Incorporation of one β-arabinonucleoside in the middle of a self-complementary oligonucleotide only slightly weakened the thermal stability of the duplex[11] and all-modified β-oligoarabinonucleotides hybridized with both complementary DNA and RNA with thermal stabilities comparable with those of the corresponding DNA-strands.[12] An all-modified α-ara$T_{15}$ exhibited a large decrease of 26 °C in melting temperature towards a $dA_{15}$-containing complement when compared to $T_{15}$.[14] This decrease is large when compared to what is known for normal α-DNA,[15,16] which forms a more stable duplex with a complementary RNA strand than does a β-DNA strand.[16] This preferred binding to RNA was also observed for α-ara$T_{15}$ (decrease in melting temperature of only 4 °C when compared to $T_{15}$).[14] Keeping in mind these data and the increased thermal stability of duplexes involving 2′-*O*-alkylribonucleotides compared to unmodified DNA,[5] we decided to synthesize and evaluate (enzymatic stability, melting towards DNA and RNA) β- and α-ODN-analogues containing β- and α-2′-OMe-ara-T monomers, respectively.[17] The

Key words: α-Oligodeoxynucleotides; β-oligodeoxynucleotides; α-1-(2-*O*-methyl-D-arabinofuranosyl)thymine; β-1-(2-*O*-methyl-D-arabinofuranosyl)thymine.

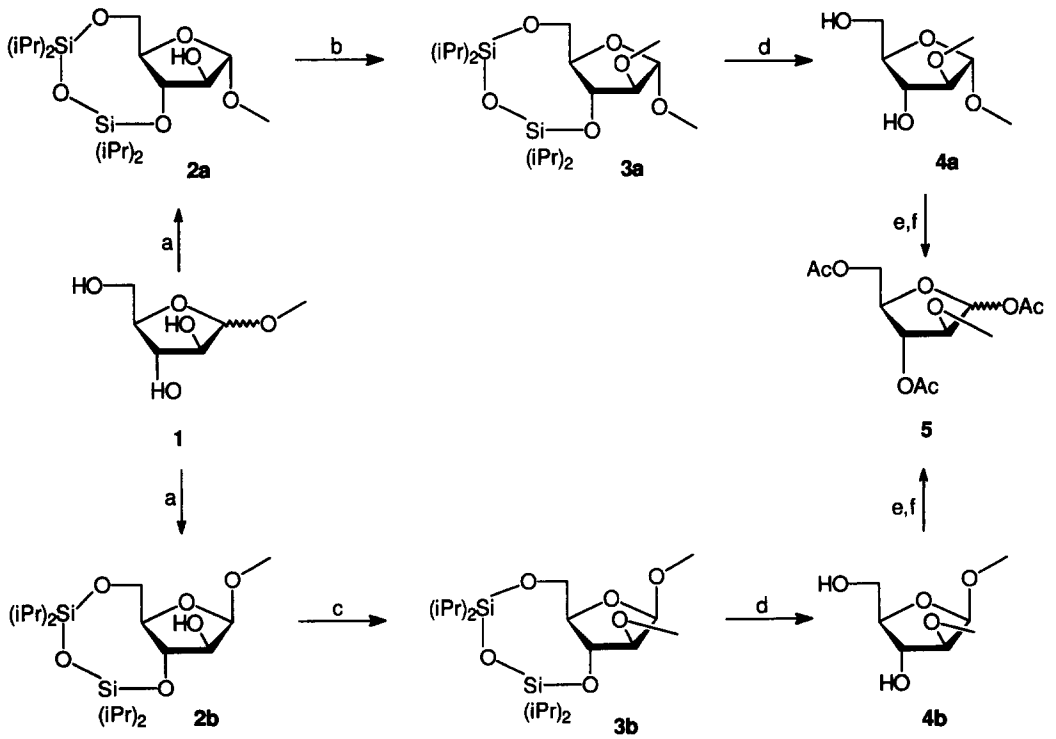
purpose of this work is to approach the answers of the following two questions: (a) are 2′-*O*-alkylated oligoarabinonucleotides promising as effective antisense molecules?; and (b) is it possible to utilize the 2′-*O*-position in arabinonucleotide monomers as attachment point of, e.g., an additional ODN-strand without compromising hybridization properties?

## Results and Discussion

### Synthesis of amidites 9a and 9b

Regioselective 3-*O*- and 5-*O*-protection of methyl D-arabinofuranoside (**1**)[18] was accomplished by use of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) to give an anomeric mixture which after column chromatography afforded the α-anomer **2a** (18% yield) and a mixture of **2a** and the β-anomer **2b** (69% yield, ~1:1 mixture). In addition, the α-anomer **2a** and the β-anomer **2b** were obtained separately in near quantitative yields starting from the individual anomers of methyl D-arabinofuranoside.[18] 2-*O*-Methylation of the α-anomer **2a** was achieved using 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP) and methyl iodide in anhydrous acetonitrile affording **3a** in 42% yield. For the β-anomer **2b**, sodium hydride and methyl iodide in anhydrous DMF was used for methylation affording **3b** in 96% yield. Surprisingly, sodium hydride mediated methylation of **2a** was impossible. Coupling of 2-*O*-methyl derivative **3b** with silylated thymine[19] was unsuccessful due to instability of the disiloxane moiety towards TMS-triflate as reported earlier.[20] Evidence for cleavage of the bridge either at the 3- or at the 5-position was found in the $^{13}C$ NMR spectra in which only three signals originating from the isopropyl groups was observed in pairs. As a consequence, **3a** and **3b** were desilylated by use of tetrabutylammoniumfluoride ($Bu_4NF$) in THF to give **4a** and **4b** in 99% and 81% yields, respectively. Subsequent acetylation and acetolysis (to activate the anomeric position) of a 1:1 mixture of **4a** and **4b** afforded 1,3,5-tri-*O*-acetyl-2-*O*-methyl derivative **5** in 90% yield (Scheme 1).

Coupling between **5** and silylated thymine[19] using the silyl Hilbert–Johnson/Birkofer method as modified by Vorbrüggen et al.[21,22] with TMS-triflate as the Lewis acid catalyst afforded an anomeric mixture of **6a** and **6b** that proved inseparable by standard column chromatography. After separation by HPLC, the α-anomer **6a** was isolated in 40% yield and the β-anomer **6b** in 35% yield. The key mutual NOE contact between H-1′ and H-4′ observed for the β-anomer **6b** [irradiation of H-1′ gave an NOE-effect (1.6%) in H-4′ while irradiation of H-4′ gave an NOE-effect (2.4%) in H-1′], but not for the α-anomer **6a**, confirmed the assigned configuration of the nucleosides. These results were supported by the small coupling constant $J_{1,2}$ for the α-anomer ($J = 1.5$ Hz) compared to the β-anomer ($J = 3.8$ Hz).[23] The convergent strategy for synthesis of 2′-*O*-methyl arabinonucleosides presented here allows straightforward introduction of other nucleobases and other 2′-*O*-substituents. Besides this, it gives access to both anomers which enables evaluation of α- as well as β-ODN-analogues. A similar strategy for synthesis of 2′-*O*-alkylated ribonucleosides was published recently.[24]



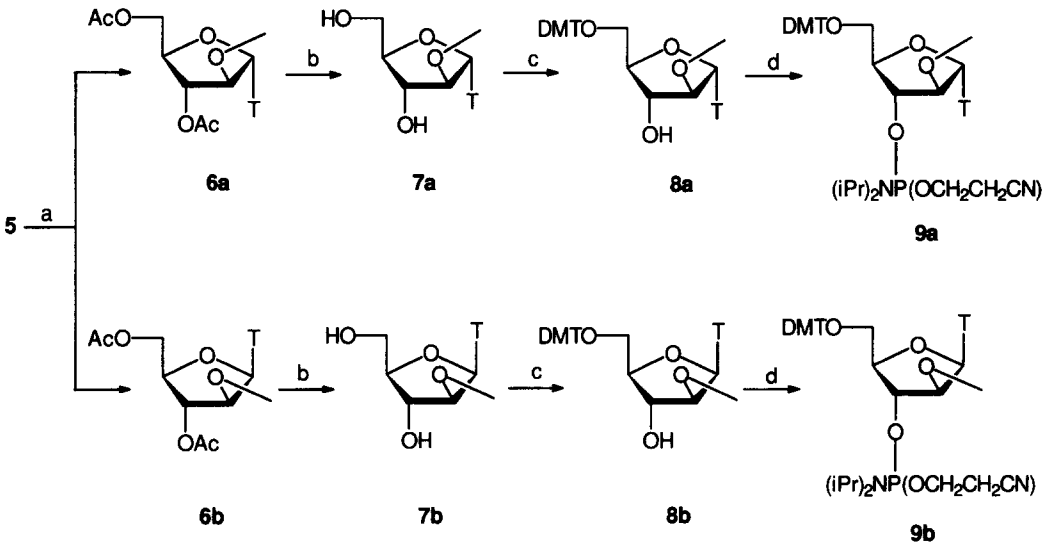

**Scheme 1.** (a) $TIPDSCl_2$, anhydrous pyridine, anhydrous $ClCH_2CH_2Cl$; (b) BDDDP, $CH_3I$, anhydrous $CH_3CN$; (c) NaH, $CH_3I$, anhydrous DMF; (d) $Bu_4NF$ in THF; (e) $Ac_2O$, anhydrous pyridine; (f) glacial AcOH, $Ac_2O$, conc $H_2SO_4$.

Nucleosides **6a** and **6b** were deprotected by use of saturated methanolic ammonia affording **7a** in 89% yield and **7b** in 88% yield. Nucleosides **7a** and **7b** were converted to the phosphoramidite derivatives **9a** and **9b** (Scheme 2) by reaction first with 4,4′-dimethoxytrityl chloride in anhydrous pyridine (yield for **8a**: 88%; yield for **8b**: 70%) and then with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite and *N*,*N*-diisopropylethylamine in anhydrous dichloromethane[25,26] (yield for **9a**: 93%; yield for **9b**: 96%).

## Synthesis and evaluation of modified ODNs

The ODNs **A**–**O** (Tables 1 and 2) were synthesized by the standard phosphoramidite methodology[27] on an automated DNA-synthesizer using the appropriate building blocks [**9a**, **9b**, α-thymidine 3′-*O*-2-(cyanoethyl)phosphoramidite[28] and commercial 2′-deoxy-β-nucleoside 3′-*O*-2-(cyanoethyl)phosphoramidites] as described in the Experimental section. As representative examples, the compositions of ODNs **B** and **L** were verified by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS).[29,30] The observed relative molecular masses (**B**: 5062.1 Da; **L**: 4258.4 Da) correspond within experimental error with those calculated (**B**: 5065.4 Da; **L**: 4256.8 Da). In addition, a 2D NMR structure elucidation of the duplex between ODN **H** and its DNA complement confirmed the composition of **H**.[31]

The hybridization properties of the synthesized ODNs were determined by measuring melting points of DNA–DNA hybrids and DNA–RNA hybrids. In Tables 1 (β-ODNs) and 2 (α-ODNs), the melting temperatures ($T_m$) and the change in $T_m$ between modified and unmodified oligomers per modification ($\Delta T_m$) are shown. As indicated in our preliminary communication,[17] incorporation of **7b** (monomer **X**, Table 1) once or twice in the middle of a sequence (**B**, **C** and **H**) moderately destabilizes the duplex formed with complementary DNA ($\Delta T_m = -5$ and $-6$ °C/modification). Less destabilization is observed for end-modified ODNs **E** and **F** ($\Delta T_m = -0.5$ and $-2$ °C/modification). The situation is similar in melting experiments towards complementary RNA, as melting




**Scheme 2.** (a) Silylated thymine, anhydrous 1,2-dichloroethane, TMS-triflate; (b) saturated methanolic $NH_3$; (c) DMTCl, anhydrous pyridine; (d) $NCCH_2CH_2OP(Cl)N(iPr)_2$, anhydrous $CH_2Cl_2$, *N*,*N*-diisopropylethylamine. T = thymin-1-yl; DMT = 4,4′-dimethoxytrityl.

**Table 1.** Sequences and melting experiments of synthesized β-oligonucleotides

| Sequence | $T_m$ (°C)[a] | $\Delta T_m$ (°C)[a] | $T_m$ (°C)[b] | $\Delta T_m$ (°C)[b] |
|---|---|---|---|---|
| 5′-CACCAACTTCTTCCACA-3′ (**A**) | 64.0 | — | 60.0 | — |
| 5′-CACCAAC**X**TCTTCCACA-3′ (**B**) | 58.0 | −6.0 | 55.5 | −4.5 |
| 5′-CACCAAC**X**TCT**X**CCACA-3′ (**C**) | 54.0 | −5.0 | 52.0 | −4.0 |
| 5′-TTAACTTCTTCACATTC-3′ (**D**) | 54.0 | — | — | — |
| 5′-TTAACTTCTTCACAT**X**C-3′ (**E**) | 53.5 | −0.5 | — | — |
| 5′-TTAACTTCTTCACA**XX**C-3′ (**F**) | 50.0 | −2.0 | — | — |
| 5′-GGCTATATGCG-3′ (**G**) | 45.0 | — | — | — |
| 5′-GGCTA**X**ATGCG-3′ (**H**) | 39.0 | −6.0 | — | — |

A = 2′-deoxyadenosine; C = 2′-deoxycytidine; G = 2′-deoxyguanosine; T = thymidine; **X** = monomer derived from amidite **9b**.
[a]Complexed with DNA.
[b]Complexed with RNA.

point depressions of approximately 4 °C are detected for ODNs **B** and **C** (Table 1).

From the results depicted in Table 1 it is clear, that incorporation of **X** does not prevent stable duplex formation or base pairing. This was confirmed by evaluating the melting of the duplexes between **H** and **G** and their DNA-complement by 1D NMR experiments at various temperatures while observing the imino proton region of the spectra (Fig. 1). The imino resonances appear sharp when they are protected from exchange with the solvent due to hydrogen bonding. Figure 1 show almost identical melting behaviours of the DNA:**H** and DNA:**G** duplexes, the only significant difference being that the imino-resonances are significantly broadened (indicates melting) at 45 and 50 °C, respectively.

**Table 2.** Sequences and melting experiments of synthesized α-oligonucleotides

| Sequence | $T_m$ (°C)[a] | $\Delta T_m$ (°C)[b] |
|---|---|---|
| 5′-TTTTTTTTTTTTTT-3′ (**I**)[c] | 35.0 | 32.0 |
| 5′-α-(TTTTTTTTTTTTTT)-3′ (**J**) | 36.0 | 46.0 |
| 5′-α-(TYTTTTTTTTTTTY)-3′ (**K**) | 34.0 | 43.0 |
| 5′-α-(TTTTYTTTYTTTTT)-3′ (**L**) | 33.5 | 42.0 |
| 5′-α-(TTTYTTTYTTTYTT)-3′ (**M**) | 31.0 | 42.0 |
| 5′-α-(TTTTTYYYYTTTTT)-3′ (**N**) | 31.5 | 38.0 |
| 5′-α-(TTTTYTYTYTYTTT)-3′ (**O**) | 32.5 | 40.0 |

T = thymidine; Y = monomer derived from amidite **9a**; $T_m$ = melting temperature.
[a]Complexed with $dA_{14}$.
[b]Complexed with $rA_{14}$.
[c]Reference β-ODN.

Table 2 shows the results from melting experiments involving α-ODNs **J**–**O**. As expected, β-$T_{14}$ (**I**) and α-$T_{14}$ (**J**) exhibited similar thermal stability towards complementary DNA. Incorporation of the α-arabino anomer **9a** (monomer **Y**, Table 2) two to four times either in the end or in the middle of a 14-mer poly-αT sequence destabilize the duplex only to a small extent (0.5–1 °C per modification) when compared to reference β-ODN **I**. These results are more promising than those reported for α-ara$T_{15}$[14] indicating that alkylation of the free OH-group in the arabino-position might stabilize a duplex with a DNA-complement. However, on the basis of our results it is not possible to extrapolate directly to the melting behaviour of an all-modified 2′-*O*-methyl oligoarabinonucleotide. Results for α-ara$T_{15}$[14] and α-ODNs[16] showed that thermally more stable complexes are formed with RNA than with DNA. The results of melting experiments of RNA-duplexes depicted in Table 2 follows this tendency. Thus, α-$T_{14}$ (**J**) melts 10 °C higher with ribo$A_{14}$ than with $dA_{14}$, and also α-ODNs **K**–**O** containing two to four α-2′-*O*-Me-araT monomers show marked enhance-

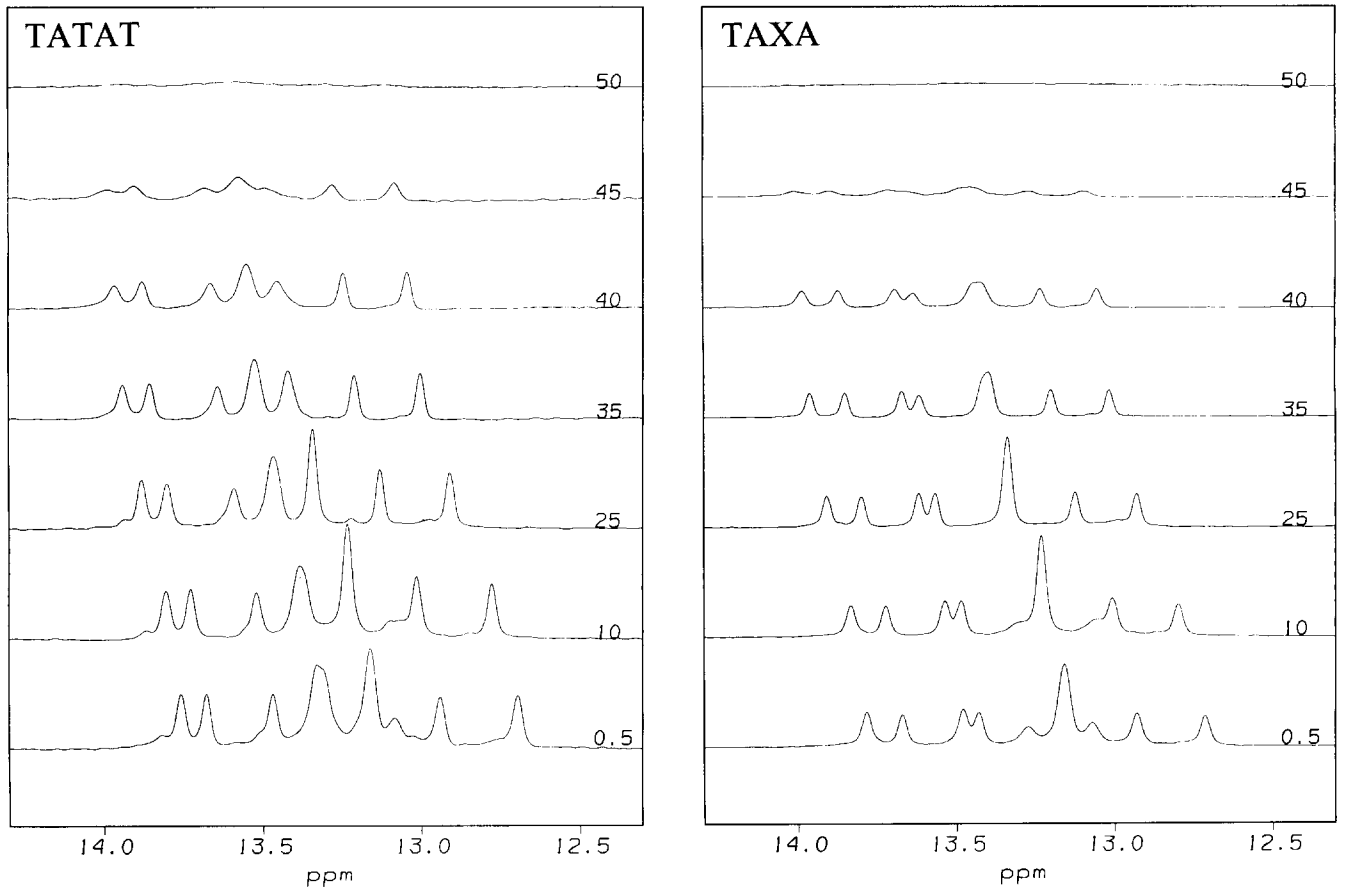



**Figure 1.** 1D NMR spectra of the imino-region for the duplexes DNA:**G** (TATAT) and DNA:**H** (TAXA) at different temperatures. The spectra are referenced to the spectra at 0.5 °C. The duplex concns in the samples were for **G** = 2.0 mM and for **H** = 1.7 mM.

ments of duplex stability when complexed with RNA instead of DNA, and $T_m$ is for all modified α-ODNs significantly higher than for the unmodified control (**I**, β-$T_{14}$).

The enzymatic stability of β-ODNs **A–F** was tested towards snake venom phosphodiesterase (a dual DNA/RNA 3′-exonuclease) as described in the Experimental section. As described earlier,[17] 3′-end modified ODNs **E** and **F** are effectively protected against 3′-exonucleolytic degradation ($t_{1/2} > 30$ min). α-ODNs are known to exhibit increased enzymatic stability towards most nucleases,[32] for which reason we incorporated monomer **Y** twice in the β-ODN 5′-AAAAAAAAAAAYYA-3′ for the enzymatic test. No sign of degradation by snake venom phosphodiesterase was detected after 1 h which indicates that the 2′-*O*-Me-araT monomer **Y** induces excellent resistance towards 3′-exonucleolytic digestion. In fact, it might be more resistant than deoxy-α-ODNs which are slowly degraded by snake venom phosphodiesterase.[32]

## Conclusion

A general synthetic strategy for both the α- and β-anomer of 2′-*O*-alkylated arabinonucleosides has been developed and incorporation of these nucleosides into α- and β-ODNs have been accomplished by use of the phosphoramidite methodology. Incorporation of either α- or β-2′-OMe-araT in the 3′-end of unmodified β-ODNs effectively protects the ODN from 3′-exonucleolytic digestion. Incorporation of β-2′-OMe-araT monomers into β-ODNs moderately lowers the thermal stability of duplexes towards both complementary DNA and RNA, but stable and well defined duplexes are formed. α-ODNs containing α-2′-OMe-araT monomers display excellent hybridisation properties, especially towards complementary RNA.

In conclusion, α-2′-*O*-alkylated oligoarabinonucleotides should be further evaluated as promising antisense molecules. The melting point depressions observed for the β-ODNs containing β-2′-OMe-araT probably exclude their use as single stranded all-modified antisense agents, but as stable duplexes are formed for mono-substituted β-ODNs, attachment of, e.g., major groove binding agents at the 2′-*O*-position is an attractive possibility.

## Experimental

NMR spectra were recorded at 500 MHz for $^1$H NMR, 125 MHz for $^{13}$C NMR and 202 MHz for $^{31}$P NMR on a Varian Unity 500 NMR spectrometer; δ-values are in ppm relative to tetramethylsilane as internal standard ($^1$H and $^{13}$C NMR), relative to 85% $H_3PO_4$ as external standard in $^{31}$P NMR, and relative to HDO in 1D NMR imino-spectra. EI mass spectra were recorded on a Varian Mat 311A spectrometer. Positive FAB mass spectra were recorded on a Kratos MS 50 RF spectrometer. HPLC was performed on Waters Delta Prep 3000 HPLC system. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Snake venom phosphodiesterase (*Crotalus adamanteus*) was obtained from Pharmacia. Matrix assisted laser desorption ionization mass spectra were obtained on a prototype laser desorption mass spectrometer from Applied Biosystem Sweden AB, Uppsala, Sweden. Melting experiments were carried out on a Perkin–Elmer UV/VIS spectrometer fitted with a PTP-6 Peltier temperature programming element. DNA syntheses were performed on a Pharmacia Gene Assembler Special® DNA-synthesizer. Complementary oligoribonucleotides were purchased from DNA Technology, Aarhus, Denmark.

**Methyl 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-D-arabinofuranoside (2a) and methyl 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranoside (2b).** Methyl D-arabinofuranoside[18] (**1**, 3.00 g, 18.28 mmol) was coevaporated with anhydrous pyridine (10 mL) and dissolved in anhydrous pyridine (50 mL) at 0 °C. While stirred under $N_2$, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (6.9 mL, 21.9 mmol) dissolved in anhydrous 1,2-dichloroethane (10 mL) was added dropwise. After 1.5 h, analytical TLC showed complete reaction, and MeOH (20 mL) was added and the solvents subsequently evapd under red. pres. The residue was dissolved in $CH_2Cl_2$ (400 mL) and washed successively with 1 M $NaHCO_3$ (3 × 150 mL) and $H_2O$ (150 mL). The organic phase was dried ($MgSO_4$), filtered, and evapd under red. pres. The residue was column chromatographed on silica gel eluting with a gradient of MeOH (0–1%, v/v) in $CH_2Cl_2$ yielding as clear oils the α-anomer **2a** (yield 1.33 g, 18%) and a mixture of **2a** and the β-anomer **2b** (yield 5.15 g, 69%, ~1:1 mixture). Using analogous reaction conditions, the α-anomer **2a** and the β-anomer **2b** were obtained separately in near quantitative yields starting from the individual anomers of methyl D-arabinofuranoside.[18]

**Compound 2a**. $R_f$ 0.25 ($CH_2Cl_2$:MeOH, 98:2 v/v). $^1$H NMR ($CDCl_3$): δ 1.06 (m, 28H, 4 $(CH_3)_2CH$), 2.26 (br s, 1H, OH), 3.39 (s, 3H, $OCH_3$), 3.86 (m, 1H, H-4), 3.94 (dd, 1H, $J$ = 3.8, 12.7 Hz, H-5a), 3.97 (dd, 1H, $J$ = 3.1, 12.7 Hz, H-5b), 4.16 (m, 2H, H-2, H-3), 4.80 (d, 1H, $J$ = 2.0 Hz, H-1). $^{13}$C NMR ($CDCl_3$): δ 12.51, 12.77, 13.10, 13.46 (4 $(CH_3)_2CH$), 16.93, 16.99, 17.04, 17.09, 17.27, 17.40 (4 $(CH_3)_2CH$), 55.51 ($OCH_3$), 61.41 (C-5), 76.83 (C-3), 80.75, 82.46 (C-2, C-4), 107.94 (C-1). Anal. calcd for $C_{18}H_{38}O_6Si_2$: C, 53.16; H, 9.42. Found: C, 53.02; H, 9.53.

**Compound 2b**. $R_f$ 0.40 ($CH_2Cl_2$:MeOH, 98:2 v/v). $^1$H NMR ($CDCl_3$): δ 1.06 (m, 28H, 4 $(CH_3)_2CH$), 2.28 (d, 1H, $J$ = 10 Hz, OH), 3.39 (s, 3H, $OCH_3$), 3.75 (dd, 1H, $J$ = 8.9, 11.2 Hz, H-5a), 3.84 (ddd, 1H, $J$ = 3.7, 5.7, 8.9 Hz, H-4), 3.96 (dd, 1H, $J$ = 3.7, 11.2 Hz, H-5b), 4.12 (m, 1H, H-2), 4.21 (m, 1H, H-3), 4.74 (d, 1H, $J$ = 4.3 Hz, H-1). $^{13}$C NMR ($CDCl_3$): δ 12.46, 12.79, 13.27, 13.38 (4 $(CH_3)_2CH$), 16.95, 17.02, 17.06, 17.15, 17.22, 17.37, 17.41, 17.50 (4 $(CH_3)_2CH$), 55.12 ($OCH_3$), 66.02 (C-5), 78.70, 79.58 (C-2, C-3), 82.12 (C-4), 101.33 (C-1). Anal.

calcd for $C_{18}H_{38}O_6Si_2$: C, 53.16; H, 9.42. Found: C, 53.07; H, 9.52.

**Methyl 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-methyl-α-D-arabinofuranoside (3a).** Compound **2a** (0.83 g, 2.04 mmol) was dissolved in anhydrous acetonitrile (5.0 mL) under $N_2$ and cooled to 0 °C. BDDDP (0.88 mL, 3.06 mmol) and $CH_3I$ (0.19 mL, 3.06 mmol) were added and the reaction mixture was allowed to warm to room temperature. After 17 h, additional BDDDP (0.3 mL, 1.04 mmol) and $CH_3I$ (0.19 mL, 3.06 mmol) were added and stirring was continued for 5 h. The mixture was evapd to dryness in vacuo, the residue was dissolved in ethyl acetate (15 mL) and petrol (80–100 °C, 40 mL) was added. After filtration, the filtrate was evapd to dryness in vacuo and the residue column chromatographed on silica gel eluting with $CH_2Cl_2$ affording **3a** as an oil (yield 0.37 g, 42%). $R_f$ 0.75 ($CH_2Cl_2$:MeOH, 96:4 v/v). $^1$H NMR ($CDCl_3$): δ 1.07 (m, 28H, 4 $(CH_3)_2CH$), 3.39 (s, 3H, $OCH_3$), 3.43 (s, 3H, $OCH_3$), 3.72 (dd, 1H, $J$=2.3, 5.8 Hz, H-2), 3.88 (m, 1H, H-4), 3.93 (dd, 1H, $J$=4.5, 12.2 Hz, H-5a), 3.99 (dd, 1H, $J$=2.8, 12.2 Hz, H-5b), 4.17 (dd, 1H, $J$=5.8, 8.2 Hz, H-3), 4.78 (d, 1H, $J$=2.3 Hz, H-1). $^{13}$C NMR ($CDCl_3$): δ 12.53, 12.81, 13.14, 13.52 (4 $(CH_3)_2CH$), 16.92, 16.99, 17.03, 17.29, 17.32, 17.43 (4 $(CH_3)_2CH$), 55.10 ($OCH_3$), 58.37 ($OCH_3$), 61.79 (C-5), 76.27 (C-3), 80.77 (C-4), 91.671 (C-2), 106.56 (C-1). FABMS *m/z*: 421 ($M+H^+$, 8%). Anal. calcd for $C_{19}H_{40}O_6Si_2$: C, 54.25; H, 9.58. Found: C, 54.41; H, 9.57.

**Methyl 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-*O*-methyl-β-D-arabinofuranoside (3b).** Compound **2b** (0.71 g, 1.74 mmol) was dissolved in anhydrous DMF (20 mL) and cooled to 0 °C under $N_2$. A 60% dispersion of NaH (0.10 g, 2.61 mmol) was added and the suspension was stirred at 0 °C for 1.5 h. During a period of 1 h a 20% (w/v) solution of $CH_3I$ (2.61 mmol) in dry DMF (1.85 mL) was added, and the temperature was allowed to rise to room temperature. After 1 h the reaction mixture was evapd in vacuo and the residue was column chromatographed on silica gel with a gradient of MeOH (0–1%, v/v) in $CH_2Cl_2$ yielding **3b** as an oil (yield 0.703 g, 96%). $R_f$ 0.5 ($CH_2Cl_2$:MeOH, 98:2 v/v). $^1$H NMR ($CDCl_3$): δ 1.06 (m, 28H, 4 $(CH_3)_2CH$), 3.37 (s, 3H, $OCH_3$), 3.49 (s, 3H, $OCH_3$), 3.79 (m, 2H, H-2 + H-5a), 3.90 (m, 1H, H-4), 3.95 (dd, 1H, $J$=3.5, 9.9 Hz, H-5b), 4.43 (dd, 1H, $J$=5.6, 7.6 Hz, H-3), 4.82 (d, 1H, $J$=4.5 Hz, H-1). $^{13}$C NMR ($CDCl_3$): δ 12.46, 12.77, 13.26, 13.36 (4 $(CH_3)_2CH$), 16.88, 16.90, 16.93, 16.94, 17.34, 17.35, 17.37, 17.50 (4 $(CH_3)_2CH$), 54.86 ($OCH_3$), 58.67 ($OCH_3$), 66.67 (C-5), 78.32 (C-3), 82.47 (C-4), 87.25 (C-2), 100.42 (C-1). Anal. calcd for $C_{19}H_{40}O_6Si_2$: C, 54.25; H, 9.58. Found: C, 54.31; H, 9.75.

**Methyl 2-*O*-methyl-α-D-arabinofuranoside (4a).** A mixture of **3a** (1.43 g, 3.39 mmol) and $Bu_4NF$ (8.5 mL of a 1.0 M solution in THF, 8.5 mmol) in THF (8 mL) was stirred at room temperature. The same procedure as described below for **4b** was used to obtain **4a** as an off-white solid material (yield 601 mg, 99%). $R_f$ 0.25 ($CH_2Cl_2$:MeOH, 95:5, v/v). $^1$H NMR ($CDCl_3$): δ 2.34 (br s, 1H, OH), 2.77 (br s, 1H, OH), 3.43 (s, 3H, $OCH_3$), 3.44 (s, 1H, $OCH_3$), 3.72 (d, 1H, $J$=1.8 Hz, H-2), 3.76 (dd, 1H, $J$=3.7, 11.9 Hz, H-5a), 3.84 (dd, 1H, $J$=3.1, 11.9 Hz, H-5b), 4.09 (br s, 1H, H-3), 4.13 (m, 1H, H-4), 4.96 (s, 1H, H-1). $^{13}$C NMR ($CDCl_3$): δ 54.84 ($OCH_3$), 57.44 ($OCH_3$), 62.41 (C-5), 75.09 (C-3), 86.11 (C-4), 89.47 (C-2), 106.56 (C-1). Anal. calcd for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.01; H, 7.80.

**Methyl 2-*O*-methyl-β-D-arabinofuranoside (4b).** A mixture of **3b** (0.69 g, 1.64 mmol) and $Bu_4NF$ (4.1 mL of a 1.0 M solution in THF, 3.6 mmol) in THF (4 mL) was stirred at room temperature. After 20 min pyridine:MeOH:$H_2O$ (3:1:1, v/v/v, 8 mL) was added together with a suspension of Dowex 50WX2 (8 g) in pyridine:MeOH:$H_2O$ (3:1:1, v/v/v, 16 mL). After stirring for 20 min, the mixture was filtered and washed with pyridine:MeOH:$H_2O$ (3:1:1, v/v/v, 3 × 20 mL) and evapd to dryness in vacuo. The resulting syrup was coevaporated twice with dry toluene (2 × 10 mL) and subsequently column chromatographed on silica gel with a gradient of MeOH (0.5–2%, v/v) in $CH_2Cl_2$ giving **4b** as a white solid (yield 0.24 g, 81%). $R_f$ 0.27 ($CH_2Cl_2$:MeOH, 95:5, v/v). $^1$H NMR ($CDCl_3$): δ 2.72 (br s, 1H, OH), 3.42 (s, 3H, $OCH_3$), 3.46 (s, 3H, $OCH_3$), 3.66 (dd, 1H, $J$=4.8, 12.0 Hz, H-5a), 3.70 (dd, 1H, $J$=4.3, 12.0 Hz, H-5b), 3.76 (dd, 1H, $J$=4.3, 7.3 Hz, H-2), 3.90 (m, 1H, H-4), 4.05 (br s, 1H, OH), 4.26 (t, 1H, $J$=7.3 Hz, H-3), 4.87 (d, 1H, $J$=4.3 Hz, H-1). $^{13}$C NMR ($CDCl_3$): δ 55.45 ($OCH_3$), 58.32 ($OCH_3$), 63.58 (C-5), 74.18 (C-3), 82.65 (C-4), 86.52 (C-2), 101.20 (C-1). Anal. calcd for $C_7H_{14}O_5$(0.15 $H_2O$): C, 46.48; H, 7.97. Found: C, 46.44; H, 8.22.

**1,3,5-Tri-*O*-acetyl-2-*O*-methyl-D-arabinofuranoside (5).** A 1:1 mixture of compounds **4a** and **4b** (0.55 g, 3.09 mmol) was dissolved in anhydrous pyridine (50 mL) and $Ac_2O$ (0.95 g, 9.27 mmol) was added under $N_2$. After 16 h the reaction mixture was evaporated to dryness in vacuo to give a syrup which was used without further purification in the next step. $R_f$ 0.63; ($CH_2Cl_2$:MeOH, 95:5, v/v). A solution of (0.81 g, 3.09 mmol) in glacial AcOH (16 mL) and $Ac_2O$ (3.2 mL) was stirred at 5 °C under dry $N_2$ while conc. $H_2SO_4$ (0.92 mL) was added dropwise. After 24 h the reaction was quenched by pouring the solution into ice-water (60 mL) and the mixture was extracted with $CHCl_3$ (3 × 40 mL). The combined organic phase was washed with satd aq $NaHCO_3$ (2 × 20 mL) and $H_2O$ (20 mL), dried ($MgSO_4$), filtered and evapd under red. pres. The residual oil was column chromatographed on silica gel with a gradient of MeOH (0–1.5%, v/v) in $CH_2Cl_2$ to yield **5** as an oil (α-anomer dominating, yield 805 mg, 90%). $R_f$ 0.63 ($CH_2Cl_2$:MeOH, 97:3, v/v). $^{13}$C NMR ($CDCl_3$): δ (α-anomer) 20.60 ($\underline{C}H_3C{=}O$), 20.66 ($\underline{C}H_3C{=}O$), 20.94 ($\underline{C}H_3C{=}O$), 57.79 ($CH_3O$), 63.46 (C-5), 76.56 (C-3), 82.78 (C-4), 88.02 (C-2), 99.97 (C-1), 169.50 ($CH_3\underline{C}{=}O$), 169.96 ($CH_3\underline{C}{=}O$), 170.54 ($CH_3\underline{C}{=}O$). Anal. calcd for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.48; H, 6.06.

**1-(3,5-Di-*O*-acetyl-2-*O*-methyl-α-D-arabinofuranosyl)-thymine (6a) and 1-(3,5-di-*O*-acetyl-2-*O*-methyl-β-D-arabinofuranosyl)thymine (6b).** Compound **5** (0.75 g, 2.58 mmol) was dissolved in anhydrous 1,2-dichloroethane (60 mL) under $N_2$ and added to silylated thymine[19] (0.78 g, 6.20 mmol) and the mixture was cooled to −30 °C. TMS–triflate (0.75 mL, 3.88 mmol) was added dropwise, and after 5 h at −25 °C and 15 h at 5 °C additional TMS–triflate (0.60 mL, 3.10 mmol) was added. After 9 days at 5 °C, analytical TLC showed complete reaction, and the mixture was diluted with $CH_2Cl_2$ (100 mL) and washed successively with a satd aq soln of $NaHCO_3$ (3 × 50 mL) and $H_2O$ (50 mL). The organic phase was dried ($Na_2SO_4$), evapd under red. pres. and column chromatographed on silica gel eluting with a gradient of MeOH (0–1.5%, v/v) in $CH_2Cl_2$ to give an anomeric mixture of **6a** and **6b**. (α:β ~8:7, yield 0.73 g, 80%). $R_f$ 0.34 ($CH_2Cl_2$:MeOH, 95:5, v/v). The α- and β-anomers were separated by reversed phase HPLC (20% EtOH in $H_2O$, v/v).

**Compound 6a.** $^1$H NMR ($CDCl_3$): δ 1.97 (d, 3H, $J$ = 1.1 Hz, $CH_3$), 2.05 (s, 3H, $CH_3C$=O), 2.14 (s, 3H, $CH_3C$=O), 3.55 (s, 3H, $OCH_3$), 4.01 (m, 1H, H-3′), 4.25 (dd, 1H, $J$ = 5.7, 11.7 Hz, H-5′a), 4.35 (dd, 1H, $J$ = 7.2, 11.7 Hz, H-5′b), 4.61 (m, 1H, H-4′), 5.13 (t, 1H, $J$ = 1.5 Hz, H-2′), 6.03 (d, 1H, $J$ = 1.5 Hz, H-1′), 7.22 (q, 1H, $J$ = 1.1 Hz, H-6), 9.20 (br s, 1H, NH). $^{13}$C NMR ($CDCl_3$): δ 12.51 ($CH_3$), 20.65 ($\underline{C}H_3C$=O), 20.70 ($\underline{C}H_3C$=O), 58.54 ($CH_3O$), 63.22 (C-5′), 76.63 (C-3′), 84.48 (C-4′), 87.98 (C-2′), 91.01 (C-1′), 110.15 (C-5), 135.48 (C-6), 150.06 (C-2), 163.78 (C-4), 169.23 ($CH_3\underline{C}$=O), 170.61 ($CH_3\underline{C}$=O). EIMS *m/z*: 356 ($M^+$, 3%). Anal. calcd for $C_{15}H_{20}N_2O_8(0.5H_2O)$: C, 49.31; H, 5.67; N, 7.67. Found: C, 49.38; H, 5.70; N, 7.78.

**Compound 6b.** $^1$H NMR ($CDCl_3$): δ 1.96 (d, 3H, $J$ = 1.1 Hz, $CH_3$), 2.14 (s, 3H, $CH_3C$=O), 2.16 (s, 3H, $CH_3C$=O), 3.40 (s, 3H, $CH_3O$), 3.91 (dd, 1H, $J$ = 1.0, 3.8 Hz, H-2′), 4.22 (m, 1H, H-4′), 4.35 (dd, 1H, $J$ = 4.4, 11.7 Hz, H-5′a), 4.45 (dd, 1H, $J$ = 6.7, 11.7 Hz, H-5′b), 5.13 (m, 1H, H-3′), 6.20 (d, 1H, $J$ = 3.8 Hz, H-1′), 7.38 (d, 1H, $J$ = 1.1 Hz, H-6), 8.81 (br s, 1H, NH). $^{13}$C NMR ($CDCl_3$): δ 12.45 ($CH_3$), 20.73 ($\underline{C}H_3C$=O), 20.79 ($\underline{C}H_3C$=O), 58.55 ($CH_3O$), 63.05 (C-5′), 75.69 (C-3′), 80.68 (C-4′), 82.37 (C-2′), 85.40 (C-1′), 109.48 (C-5), 137.43 (C-6), 150.32 (C-2), 163.57 (C-4), 169.83 ($CH_3\underline{C}$=O), 170.57 ($CH_3\underline{C}$=O). EIMS *m/z*: 356 ($M^+$, 5%). Anal. calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found: C, 50.52; H, 5.59; N, 8.01.

**1-(2-*O*-Methyl-α-D-arabinofuranosyl)thymine (7a).** Compound **6a** (0.34 g, 0.95 mmol) was dissolved in satd methanolic $NH_3$ (40 mL) under $N_2$. After 24 h at r.t., the solvent was removed under reduced pressure and the product purified following the same procedure as described below for **7b** yielding **7a** as a white foam (yield 230 mg, 89%). $R_f$ 0.21 ($CH_2Cl_2$:MeOH, 9:1, v/v). $^1$H NMR ($CD_3OD$): δ 1.88 (d, 3H, $J$ = 1.2 Hz, $CH_3$), 3.46 (s, 3H, $OCH_3$), 3.63 (m, 2H, H-5′a, H-5′b), 3.93 (t, 1H, $J$ = 2.9 Hz, H-2′), 4.16 (t, 1H, $J$ = 2.9 Hz, H-3′), 4.29 (m, 1H, H-4′), 5.90 (d, 1H, $J$ = 2.9 Hz, H1′), 7.57 (q, 1H, $J$ = 1.2 Hz, H-6). $^{13}$C NMR ($CD_3OD$): δ 12.41 ($CH_3$), 58.51 ($CH_3O$), 62.76 (C-5′), 75.67 (C-3′), 89.65 (C-4′), 91.53 (C-2′), 91.64 (C-1′), 111.10 (C-5), 138,83 (C-6), 152.31 (C-2), 166.53 (C-4). HREIMS *m/z* $C_{11}H_{16}N_2O_6$: calcd 272.1008. Found: 272.0986.

**1-(2-*O*-Methyl-β-D-arabinofuranosyl)thymine (7b).** Compound **6b** (0.20 g, 0.56 mmol) was dissolved in satd methanolic $NH_3$ (35 mL) under $N_2$. After 25 h at room temperature, the solvent was removed under reduced pressure and the product purified by flash chromatography through silica gel eluting with a gradient of MeOH (0–13%, v/v) in $CH_2Cl_2$ yielding **7b** as a white foam (yield 134 mg, 88%). $R_f$ 0.18 ($CH_2Cl_2$:MeOH, 9:1, v/v). $^1$H NMR ($CD_3OD$): δ 1.86 (d, 3H, $J$ = 1.2 Hz, $CH_3$), 3.35 (s, 3H, $OCH_3$), 3.73 (dd, 1H, $J$ = 4.4, 11.5 Hz, H-5′a), 3.80 (m, 2H, H-4′, H-5′b), 3.90 (dd, 1H, $J$ = 4.5, 5.5 Hz, H-2′), 4.16 (dd, 1H, $J$ = 4.5, 5.5 Hz, H-3′), 6.20 (d, 1H, $J$ = 5.5 Hz, H-1′), 7.65 (q, 1H, $J$ = 1.2 Hz, H-6). $^{13}$C NMR ($CD_3OD$): δ 12.34 ($CH_3$), 59.08 ($CH_3O$), 61.65 (C-5′), 74.53 (C-3′), 84.75 (C-4′), 85.26 (C-1′), 87.15 (C-2′), 110.01 (C-5), 139.80 (C-6), 152.44 (C-2), 166.47 (C-4). HREIMS *m/z* $C_{11}H_{16}N_2O_6$: calcd 272.1008. Found: 272.1013. Anal. calcd for $C_{11}H_{16}N_2O_6(0.25H_2O)$: C, 47.74; H, 6.01; N, 10.12. Found: C, 47.88; H, 6.34; N, 10.49.

**1-(5-*O*-(4,4′-Dimethoxytrityl)-2-*O*-methyl-α-D-arabinofuranosyl)thymine (8a).** Compound **7a** (0.11 g, 0.45 mmol) was dissolved in anhydrous pyridine (2 mL) and 4,4′-dimethoxytrityl chloride (0.17 g, 0.50 mmol) was added under $N_2$. After 4.5 h, methanol (2 mL) was added and the solvents were evapd under red. pres. The residue was dissolved in $CHCl_3$ (25 mL) and washed with $H_2O$ (3 × 10 mL). The organic phase was dried ($Na_2SO_4$), filtered and evaporated to dryness in vacuo. The product was purified by column chromatography eluting with a gradient of MeOH (0–8%, v/v) in $CH_2Cl_2$ yielding **8a** as a white foam (yield 215 mg, 88%). $R_f$ 0.37 ($CH_2Cl_2$:MeOH, 9:1, v/v). $^1$H NMR ($CDCl_3$): δ 1.95 (d, 3H, $J$ = 1.2 Hz, $CH_3$), 3.23 (dd, 1H, $J$ = 6, 9.9 Hz, H-5′a), 3.30 (dd, 1H, $J$ = 6, 9.9 Hz, H-5′b), 3.39 (s, 3H, $OCH_3$), 3.57 ( s, 1H, OH), 3.79 (s, 6H, 2 × $CH_3O$), 4.01 (t, 1H, $J$ = 2.3 Hz, H-2′), 4.29 (br s, 1H, H-3′), 4.41 (dt, 1H, $J$ = 3.2, 6 Hz, H-4′), 5.70 (d, 1H, $J$ = 2.3 Hz, H-1′), 6.81–7.46 (m, 14H, DMT, H-6), 8.93 (br s, 1H, NH). $^{13}$C NMR ($CDCl_3$): δ 12.46 ($CH_3$), 55.20 (2 × $CH_3$ O), 57.94 ($CH_3O$), 63.19 (C-5′), 76.33 (C-3′), 86.27 (*C*$Ph_3$), 87.50 (C-4′), 90.57 (C-2′), 92.63 (C-1′), 110.59 (C-5), 113.13, 126.81, 127.84, 128.12 130.05, 136.07, 144.70, 158.50 (DMT), 137.81 (C-6), 150.29 (C-2), 163.83 (C-4).

**1-(5-*O*-(4,4′-Dimethoxytrityl)-2-*O*-methyl-β-D-arabinofuranosyl)thymine (8b).** Compound **7b** (0.13 g, 0.49 mmol) was dissolved in anhydrous pyridine (2.5 mL) and 4,4′-dimethoxytrityl chloride (0.17 g, 0.50 mmol) was added under $N_2$. After 5 h, methanol (1 mL) was added and the solvents were evaporated under red. pres. The residue was dissolved in $CHCl_3$ (20 mL) and washed with $H_2O$ (3 × 10 mL). The organic phase was dried ($Na_2SO_4$), filtered and evapd to dryness in vacuo. The residue was dissolved in hot benzene (10 mL)

and cyclohexane was added until crystallization commenced. After 12 h at 5 °C, the product was filtered off. It was purified further by column chromatography on silica gel eluting with a gradient of MeOH (0–10%, v/v) in $CH_2Cl_2$ yielding **8b** as a white foam (yield 200 mg, 70%). $R_f$ 0.43 ($CH_2Cl_2$:MeOH, 90:10, v/v). $^1$H NMR ($CDCl_3$): δ 1.70 (d, 3H, $J$=1.2 Hz, $CH_3$), 2.35 (d, 1H, $J$=4.2 Hz, OH), 3.33 (3, 3H, $CH_3O$), 3.42 (dd, 1H, $J$=4.2, 10.3 Hz, H-5'a), 3.46 (dd, 1H, $J$=4.2, 10.3 Hz, H-5'b), 3.79 (s, 6H, 2×$CH_3O$), 3.85 (dt, 1H, $J$=6.4, 4.2 Hz, H-4'), 3.92 (dd, 1H, $J$=4.2, 5.6 Hz, H-2'), 4.27 (dt, 1H, $J$=6.4, 4.2 Hz, H-3'), 6.28 (d, 1H, $J$=5.6 Hz, H-1'), 6.82–7.44 (m, 13H, DMT), 7.45 (d, 1H, $J$=1.2 Hz, H-6), 8.53 (br s, 1H, NH). $^{13}$C NMR ($CDCl_3$): δ 12.21 ($CH_3$), 55.24 (2×$CH_3O$), 58.87 ($CH_3O$), 62.39 (C-5'), 75.34 (C-3'), 81.05 (C-4'), 83.47 (C-2'), 85.73 (C-1'), 86.66 ($CPh_3$), 109.65 (C-5), 113.28, 127.07, 127.98, 128.16, 130.06, 130.10, 144.54, 158.73 (DMT), 137.50 (C-6), 150.35 (C-2), 163.62 (C-4). Anal. calcd for $C_{32}H_{34}N_2O_8$(1.0$H_2O$): C, 64.85; H, 6.12; N, 4.73. Found: C, 64.86; H, 6.04; N, 4.66.

**1-(3-*O*-(2-Cyanoethoxy(diisopropylamino)phosphino)-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-methyl-α-D-arabinofuranosyl)thymine (9a).** Compound **8a** (0.36 g, 0.63 mmol) was coevaporated twice with anhydrous $CH_3CN$ (2×5 mL), dried in vacuo overnight and subsequently dissolved in anhydrous $CH_2Cl_2$ (2.3 mL) under $N_2$. *N,N*-Diisopropylethylamine (0.55 mL, 0.79 mmol) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.23 mL, 1.03 mmol). After 1.5 h, anhydrous MeOH (0.1 mL) was added followed by EtOAc (25 mL). The soln was washed successively with satd aq solns of $NaHCO_3$ (2×20 mL) and NaCl (3×20 mL), dried ($Na_2SO_4$), filtered and evapd under red. pres. The residual oil was dissolved in dry toluene (3 mL) and pptd with ice-cold petroleum ether (200 mL) under vigorous stirring. The product was collected by filtration and coevaporated with anhydrous $CH_3CN$ (2×6 mL) yielding **9a** as a white foam (yield 453 mg, 93%). $R_f$ 0.48 (EtOAc:$CH_2Cl_2$:$Et_3N$, 45:45:10, v/v/v). $^1$H NMR ($CDCl_3$): δ 1.08–1.32 (m, 2×6H, 2 $(CH_3)_2C$), 1.92 (d, 3H, $J$=1.2 Hz, $CH_3$), 1.93 (d, 3H, $J$=1.1 Hz, $CH_3$), 2.47, 2.53 (t, 2H, $J$=6.3 Hz; t, 2H, $J$=6.3 Hz, 2×$CH_2CN$), 3.20–3.77 (m, 2×5H, 2×H-5'a, H-5'b, (CH)N, $CH_2O$), 3.40, 3.40 (2×s, 2×3H, 2×$OCH_3$), 3.79, 3.80 (2×s, 2×3H, 2×$OCH_3$), 3.89, 4.00 (2×s, 2×1H, 2×H-2'), 4.42 (d, 1H, $J$=10.4 Hz, H-3'), 4.45 (d, 1H, $J$=10.7 Hz, H-3'), 4.48 (t, 1H, $J$=7 Hz, H-4'), 4.57 (t, 1H, $J$=6.5 Hz, H-4'), 5.92 (d, 1H, $J$=1.2 Hz, H-1'), 5.94 (d, 1H, $J$=1.3 Hz, H-1'), 6.82–7.47 (m, 2×14H, DMT, H-6), 8.63 (br s, 2×1H, NH). $^{31}$P NMR ($CDCl_3$): δ 150.88, 151.69.

**1-(3-*O*-(2-Cyanoethoxy(diisopropylamino)phosphino)-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-methyl-β-D-arabinofuranosyl)thymine (9b).** Compound **8b** (0.10 g, 0.17 mmol) was coevaporated twice with anhydrous $CH_3CN$ (2×3 mL), dried in vacuo overnight and subsequently dissolved in anhydrous $CH_2Cl_2$ (0.9 mL) under $N_2$. *N,N*-Diisopropylethylamine (0.16 mL, 0.23 mmol) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.05 mL, 0.22 mmol). After 1 h anhydrous MeOH (0.04 mL) was added followed by EtOAc (5.5 mL). The solution was washed successively with satd aq solns of $NaHCO_3$ (3×5 mL) and NaCl (3×5 mL), dried ($Na_2SO_4$), filtered and evapd under reduced pressure. The residual oil was dissolved in dry toluene (1 mL) and pptd with ice-cold petroleum ether (200 mL) under vigorous stirring. The product was collected by filtration and coevaporated with anhydrous $CH_3CN$ (2×3 mL) yielding **9b** as a foam (yield 130 mg, 96%). $R_f$ 0.46 (EtOAc:$CH_2Cl_2$:$Et_3N$, 45:45:10, v/v/v). $^1$H NMR ($CDCl_3$): δ 1.01–1.36 (m, 2×$(CH_3)_2C$), 1.64, 1.69 (d, $J$=1 Hz; d, $J$=1 Hz, 2×$CH_3$), 2.36, 2.60 (t, $J$=6.4 Hz; t, $J$=6.4 Hz, 2×$CH_2CN$), 3.30, 3.31 (2×s, 2×$OCH_3$), 3.33–3.65 (m, 2×(H-5'a, H-5'b, (CH)N, $CH_2O$)), 3.79, 3.80 (2×s, 2×$OCH_3$), 3.94, 3.97 (dd, $J$=3.2, 5.0 Hz; dd, $J$=2.4, 4.4 Hz, 2×H-4'), 4.02–4.07 (m, 2×H-2'), 4.41, 4.49 (ddd, $J$=2.5, 4.3, 10.7 Hz; ddd, $J$=3.2, 4.9, 11.2 Hz, 2×H-3'), 6.24, 6.25 (d, $J$=4.6 Hz; d, $J$=5.1 Hz, 2×H-1'), 6.80–7.50 (m, 2×DMT), 7.41, 7.44 (d, $J$=1 Hz; d, $J$=1 Hz, 2×H-6), 8.22 (br s, 2×NH). $^{31}$P NMR: δ 150.43, 151.22. Anal. calcd for $C_{41}H_{51}N_4O_9P$(1.25$H_2O$): C, 61.68; H, 6.88; N, 7.02. Found: C, 61.30; H, 6.69; N, 7.41.

## Oligonucleotide synthesis

Synthesis of oligonucleotides **A–P** was performed on a Pharmacia Gene Assembler Special® DNA-synthesizer in 0.2 μmol scale (ODN **G** and **H** in 10 μmol scale) using Pharmacia primer support™/Pharmacia gene assembler support$^{RF}$ and commercial β-cyanoethylphosphoramidites, αT-cyanoethylphosphoramidite, **9a** and **9b** (5 μmol amidite per cycle). The syntheses followed the regular protocol for the DNA-synthesizer (except for extended coupling times, 12 min instead of 2 min) for amidites **9a** and **9b**. As judged by trityl assay, the stepwise coupling yields for the modified amidites **9a** and **9b** were >90% compared to ~99% for unmodified amidites. The ODNs **A–H** were removed from the solid support by treatment with 25% ammonia for 48 h at room temperature. Purification was done using disposable reversed-phase chromatography cartridges (Oligopurification Cartridges, Cruachem) as described earlier.[32] Synthesis of α-oligonucleotides **I–P** were preformed on a β-support using TOPS-phosphoramidite[33] as a post-synthetically cleavable linker thus allowing synthesis of 3'-end modified oligonucleotides containing a free 3'-OH group. TOPS was cleaved and the oligonucleotides removed from the solid support by treatment with a mixture of 25% ammonia and 40% methylamine (1:1, v/v) at 55 °C for 24 h.[34] The α-ODNs were purified twice on reversed phase purification cartridges.

## Melting experiments

Melting experiment were carried out in medium salt buffer, 1 mM EDTA, 10 mM $Na_2HPO_4$, 140 mM NaCl, pH 7.2, at a concentration of 1.5–2.0 μM for each strand. The increase in absorbance at 260 nm as a

function of time was recorded while the temperature was raised linearly from 10–80 °C at a rate of 1 °C/min.

Temperature-dependent 1D NMR spectra in $H_2O$ of the duplexes **G** and **H** were acquired using a shaped notch pulse sequence to suppress the solvent signal.[35]

### Enzymatic stability of the oligodeoxynucleotides

A solution of the oligodeoxynucleotide (0.2 OD) in 2.0 mL of the following buffer (0.1 M Tris–HCl, pH 8.6, 0.1 M NaCl, 14 mM $MgCl_2$) was degraded with 1.2 U snake venom phosphodiesterase (34 μL of a solution of the enzyme in the following buffer; 5 mM Tris–HCl, pH 7.5, 50% glycerol, v/v) at 25 °C. The hyperchromicity vs time curve of the digestion was plotted and the hyperchromicity and half-life ($t_{1/2}$) of the oligomers were evaluated.[36]

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