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Novel Oligodeoxynucleotide Analogues Containing A 2'-*O*-Methylarabinonucleoside

Charlotte H. Gotfredsen, Jens Peter Jacobsen, and Jesper Wengel*

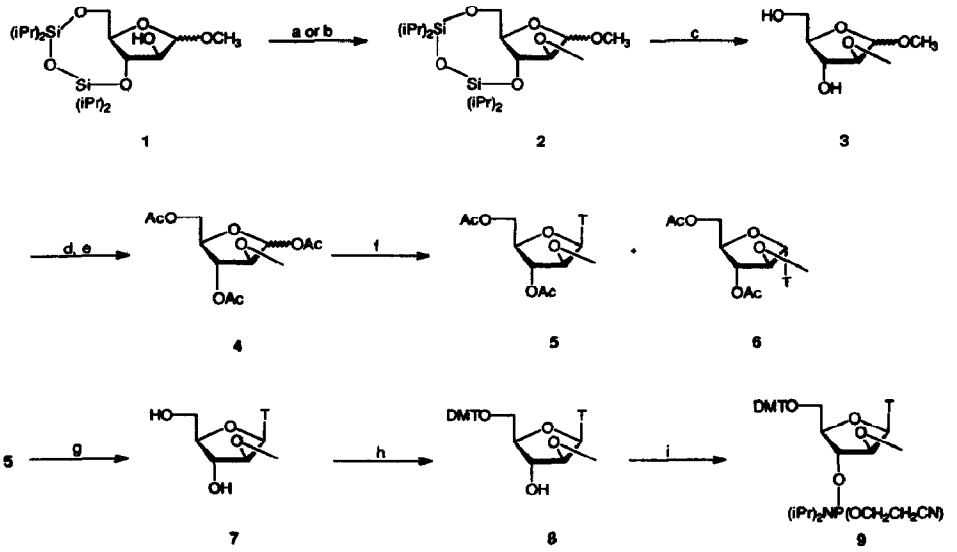
Department of Chemistry, Odense University, DK-5230 Odense M, Denmark.

Abstract: Synthesis of 1-(2-*O*-methyl-β-D-arabinofuranosyl)thymine (7) has been accomplished starting from methyl D-arabinofuranoside. Subsequent incorporation of the phosphoramidite monomer 9 into oligodeoxynucleotides afforded the first oligonucleotide analogues containing 2'-*O*-methylarabinonucleoside monomers.

Basic requirements for antisense oligonucleotides as potential inhibitors of gene expression include, for example, enhanced stability towards cellular nucleases and efficient hybridization to target nucleic acids.[1,2] Chemically modified oligonucleotide analogues[3] may fulfil these criteria, and 2'-*O*-methyloligo*ribo*nucleotides have been used as chemically and enzymatically stable RNA-substitutes for various biological experiments.[4,5] Thus, uniform 2'-*O*-methyl-*ribo* derivatization of a DNA-strand increases the thermal stability of DNA:DNA and DNA:RNA hybrids,[4] and incorporation of a 2'-*O*-methylribonucleoside one to five times in oligodeoxynucleotides conserves or slightly improves the hybridization properties.[6] In addition, 2'-*O*-methyloligoribonucleotides are resistant towards degradation by RNA- and DNA-specific nucleases although they are degraded by a dual RNA/DNA active enzyme.[5] The gene regulatory potential of 2'-*O*-methyloligoribonucleotides is hampered by nonspecific interactions[7] and inability to stimulate RNase H activity.[5] Oligonucleotides containing arabinonucleosides, have been synthesized using 2'-*O*-acylated[8] as well as 2'-*O*-unprotected[9] arabinonucleoside phosphoramidite synthons. Incorporation of one arabinonucleoside in the middle of a self-complementary oligodeoxynucleotide only slightly weakens the thermal stability of the duplex.[8] The above lead us to develop a versatile synthetic strategy of the novel 2'-*O*-methyl*arabino*nucleoside **7**, which was subsequently incorporated into novel oligodeoxynucleotides using the phosphoramidite synthon **9**. These oligomers are the first examples of oligonucleotide analogues containing 2'-*O*-alkyl arabinonucleoside monomers.

Selective 3'-*O*- and 5'-*O*-protection of methyl D-arabinofuranoside[10] was accomplished in 87% yield using the bidentate reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to give **1** (scheme 1). 2'-*O*-Methylation of **1** was achieved using either sodium hydride/methyl iodide in anhydrous DMF (for the β-anomer, affording **2β** in 96% yield) or 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP)/methyl iodide in anhydrous acetonitrile (for the α-anomer, affording **2α** in 42% yield). Use of the sterically hindered organic base BDDDP and methyl iodide for methylation is well described for ribo-

nucleosides.[11] Coupling of 2'-*O*-methyl derivative **2** with silylated thymine[12] was unsuccessful due to instability of the disiloxane moiety towards TMS-triflate as reported earlier.[13] Therefore, the anomeric mixture **2** was deprotected using tetra-*n*-butylammoniumfluoride in THF to give **3** in 81% yield. Subsequent acetylation and acetolysis afforded 1,3,5-tri-*O*-acetyl-2'-*O*-methyl derivative **4** in 90% yield. Coupling between **4** and silylated thymine[12] was achieved using the silyl Hilbert-Johnson/Birkofer method as modified by Vorbrüggen *et al.*[14,15] with TMS-triflate as the Friedel-Craft catalyst to give, after 9 days at 5 °C, an anomeric mixture of **5** and **6** (inseparable using conventional column chromatography). After HPLC-separation (eluting with 20% ethanol in H_2O, v/v) the β-anomer **5** was isolated in 35% yield and the α-anomer **6** in 40% yield. The configuration of the anomers was confirmed by ^1H NOE-difference experiments. The key NOE contact between H-1' and H-4' was especially useful: it was not observed for the α-anomer **6** but for the β-anomer **5** (irradiation of H-1' gives a NOE-effect (1.6%) to H-4' while irradiation of H-4' gives a NOE-effect (2.4%) to H-1'). These results were supported from evaluation of the coupling-constant $J_{1,2}$ which is smaller for the α-anomer (J = 1.5 Hz) compared to the β-anomer (J = 3.8 Hz).[16] Nucleoside **5** was deprotected using saturated methanolic ammonia affording 1-(2-*O*-methyl-β-D-arabinofuranosyl)thymine (**7**) in 88% yield.[17] Synthesis of the corresponding cytosine and uracil derivatives has been reported earlier using a troublesome and low yield strategy.[18-20] Reaction of **7** with 4,4'-dimetoxytritylchloride in anhydrous pyridine gave the 5'-*O*-protected nucleoside **8** in 70% yield. Phosphitylation[21] of **8** by reaction with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite and *N,N*-diisopropylethylamine in anhydrous dichlormethane afforded the nucleoside phosphoramidite **9** in 96% yield after precipitation from petroleum ether.[22,23]



a) NaH, CH_3I, anhydrous DMF; b) BDDDP, CH_3I, anhydrous CH_3CN; c) *n*-Bu_4NF in THF; d) Ac_2O, anhydrous pyridine; e) glacial AcOH, Ac_2O, conc. H_2SO_4; f) silylated thymine, anhydrous 1,2-dichlorethane, TMS-triflate; g) saturated methanolic NH_3; h) DMTCl, anhydrous pyridine; i) *N,N*-diisopropylethylamine, $NCCH_2CH_2OP(Cl)N(iPr)_2$, anhydrous CH_2Cl_2. T = thymin-1-yl; DMT = 4,4'-dimethoxytrityl

Scheme 1

Oligomers A-H were synthesized on an automated DNA synthesizer using **9** and commercial 2'-deoxynucleoside-β-cyanoethylphosphoramidites. The coupling efficiency of the modified phosphoramidite **9** was approximately 93% (12-min coupling) compared to approximately 99% for unmodified monomers (2-min coupling) as monitored by the release of the dimethoxytrityl cation. The DMT-protected oligonucleotides were removed from the solid support by treatment with concentrated ammonia for 2 days at room temperature, and disposable reverse-phase chromatography cartridges were used for purification. As a confirmation of the syntheses of the novel oligodeoxynucleotide analogues the composition of oligomer **B** (containing one modified monomer) was verified by matrix assisted laser desorption mass spectrometry: The observed relative molecular mass (5062.1 Da) corresponds within experimental error with the calculated (5065.4 Da). Besides, we are currently performing a 2D-NMR-structure analysis of the duplex of **H** with its complementary DNA-strand. Preliminary data from this analysis indicate that a stable duplex exists at room temperature (figure 1) as the expected intra- and interstrand connectivities are present.

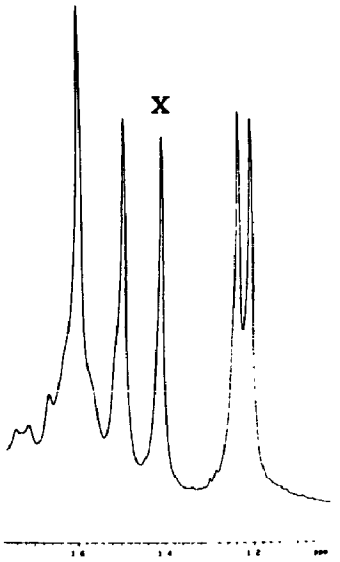


Figure 1. ^{1}H NMR spectrum of T-*Me* region of duplex between **H** and complementary DNA

Table 1. Sequences synthesized, hybridization properties, and enzymatic stability

Sequence	T_m/°C	$t_{1/2}$/min	H_e
5'-CACCAACTTCTTCCACA-3' (**A**)	64.0	~1	1.17
5'-CACCAACXTCTTCCACA-3' (**B**)	58.0	~1	1.05
5'-CACCAACXTCTXCCACA-3' (**C**)	54.0	~1	1.04
5'-TTAACTTCTTCACATTC-3' (**D**)	54.0	~1	1.15
5'-TTAACTTCTTCACATXC-3' (**E**)	53.5	>30	1.07
5'-TTAACTTCTTCACAXXC-3' (**F**)	50.0	>30	1.06
5'-GGCTATATGCG-3' (**G**)	45.0		1.21
5'-GGCTAXATGCG-3' (**H**)	39.0		1.10

A = 2'-deoxyadenosine; C = 2'-deoxycytidine; G = 2'-deoxyguanosine; T = thymidine; X = 1-(2-*O*-methyl-β-D-arabinofuranosyl)thymine (**7**); T_m = melting temperature; $t_{1/2}$ = hyperchromicity half-life; H_e = hyperchromicity (enzymatic)

As depicted in Table 1, incorporation of 2'-*O*-methyl arabinonucleoside **7** one or two times in the middle of a sequence (**B**, **C** and **H**) destabilizes (but not prevents) the duplex with complementary DNA (ΔT_m = 4-6 °C/modification) while one or two end-modifications (**E** and **F**) weaken the duplex stability to only a small extent (ΔT_m = 0.5-2 °C/modification). The enzymatic stability of oligomers **A**-**F** was tested towards snake venom phosphodiesterase (3'-exonuclease). The increase in absorbance (260 nm) during digestion was fol-

lowed and the enzymatic hyperchromicities calculated. 3'-End modified oligomers (**E** and **F**) are effectively protected against 3'-exonucleolytic degradation ($t_{1/2}$ > 30 min). Results from similar experiments on **B** and **C** indicate a rapid degradation from the 3'-end ($t_{1/2}$ ~ 1 min, H_e = 1.04, 1.05) affording a 3'-end protected 12- and 8-mer, respectively.

In conclusion, 1-(2-*O*-methyl-β-D-arabinofuranosyl)thymine (**7**) has been obtained from methyl-D-arabinofuranoside using a generally practicable synthetic strategy. Incorporation of this novel nucleoside into oligodeoxynucleotides induces a significant increase in the stability towards 3'-exonucleolytic degradation while conserving the duplex-forming capacity. Futher evaluation of α- and β-2'-*O*-methylarabino oligonucleotides are in progress and will be reported in due course.

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17. ^{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 = 11.5, 4.4 Hz, H-5'a), 3.80 (m, 2H, H-4', H-5'b), 3.90 (dd, 1H, J = 5.5, 4.5 Hz, H-2'), 4.16 (dd, 1H, J= 5.5, 4,5 Hz, H-3'), 6.20 (d, 1H, J = 5.5 Hz, H-1'), 7.65 (q, 1H, J = 1.2 Hz, H-6).
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22. ^{31}P NMR ($CDCl_3$) δ = 150.4, 151.2.
23. All new compounds **1-9** exhibited satisfactory spectral and analytical data.

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