Synthesis of 3′-*C*-(Hydroxymethyl)thymidine: Introduction of a Novel Class of Deoxynucleosides and Oligodeoxynucleotides

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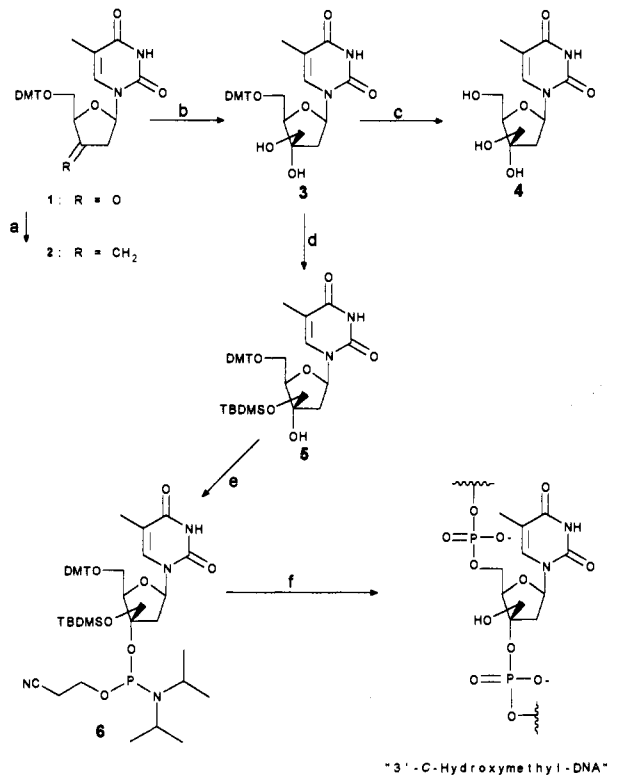
Important requirements for antisense or antigene oligonucleotides are enhanced stability against destructive nucleases and efficient hybridization to target nucleic acids.[1] As a consequence, such oligonucleotides need to be chemically modified in the nucleobase, the carbohydrate, or the internucleoside linkage.[2] We consider the development of oligonucleotide analogues containing carbohydrate-modified nucleoside monomers as the most promising strategy, as modifications in the phosphate moiety (e.g., phosphorothioates,[3] phosphoramidates,[4] and methylphosphonates[5]) often result in highly heterogeneous oligomers because of introduction of uncontrolled chirality at phosphorus. Besides, routine preparation of oligonucleotide sequences containing neutral achiral dephospho linkages[6] requires often laborious syntheses of up to 16 different dimeric building blocks compared to only four when using monomeric building blocks. This communication describes the synthesis of the first 3′-*C*-hydroxymethyl deoxyribonucleoside **4** and its incorporation using the phosphoramidite synthon **6** into novel oligodeoxynucleotide analogues showing promising properties.

The synthesis of the monomeric phosphoramidite building block **6** was performed as follows (Scheme 1). Oxidation of 5′-*O*-(4,4′-dimethoxytrityl)thymidine[7] using pyridinium dichromate (PDC) in dry dichloromethane in the presence of 3-Å molecular sieve powder afforded 5′-*O*-(4,4′-dimethoxytrityl)-3′-ketothymidine (**1**) in 81% yield. Wittig reaction on **1** was unsuccesful because of base-induced β-elimination of the nucleobase.[8] However, methylenation of **1** was accomplished with the electrophilic reagent $Zn/CH_2Br_2/TiCl_4$ in THF[9,10] to give the 2′,3′-dideoxy-3′-*C*-methylene nucleoside **2** in 79% yield.[11] 5′-*O*-(4,4′-Dimethoxytrityl)-3′-*C*-(hydroxymethyl)thymidine (**3**) was obtained in 70% yield[12] by stereoselective catalytic osmium tetraoxide oxidation[13,14] of **2** in basic aqueous *tert*-butyl alcohol using *N*-methylmorpholine *N*-oxide as cooxidant. *N*-Methylmorpholine *N*-oxide is reported to be preferable compared to a variety of other known cooxidants (e.g., hydrogen peroxide,[13,14] *tert*-butyl hydroperoxide,[14,15] sodium hypochlorite[14,16]) because it avoids overoxidation, thus preventing the formation of keto or acid byproducts. The configuration of **3** was unambigously established by a ^1H NOE difference experiment. Especially the key NOE contact between 3′-*C*-CH_2 and H-5′ confirmed the positioning of the 3′-*C*-substituent at the β-face of the pentofuranose ring. Deprotection of **3** using dichloroacetic acid gave in 90% yield 3′-*C*-(hydroxymethyl)-thymidine (**4**), the first example of this novel class of nucleosides. Phosphorylation of **4** is possible at the 3′-*C*- as well as the 4′-*C*-hydroxymethyl group, thus rendering the 2′-deoxy-3′-*C*-hydroxymethyl nucleosides interesting, e.g., as potentially antiviral compounds structurally related to known biologically active D-apio-,[17] oxetanosin-,[18] 2′,3′-dideoxy-3′-*C*-*erythro*-hydroxymethyl-,[19] and isonucleoside[20] analogues. Next we decided to incorporate **4** into oligodeoxynucleotides, and **3** was reacted with *tert*-butyldimethylsilyl chloride in dry DMF using imidazole as catalyst to afford the 3′-*C*-((*tert*-butyldimethylsilyl)oxy)methyl

Scheme 1



[a] (a) $Zn/CH_2Br_2/TiCl_4/THF/CH_2Cl_2$ (79%), (b) OsO_4/*N*-methylmorpholine *N*-oxide/*tert*-butyl alcohol/pyridine/H_2O (70%), (c) 3% dichloroacetic acid in CH_2Cl_2 (v/v) (90%), (d) *tert*-butyldimethylsilyl chloride/imidazole/DMF (81%), (e) $NCCH_2CH_2OP(Cl)N(iPr)_2$/*N*,*N*-diisopropylethylamine/CH_2Cl_2 (90%), (f) DNA synthesizer.

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Table 1. Sequences Synthesized, Hybridization Data, and Enzymatic Stability

sequence[a]	T_m/°C[b]	$t_{1/2}$/s[c]	H[d]
5′-(CACCAACTTCTTCCACA)-3′ (**A**)	60.0	50	1.45
5′-(CACCAACXTCTTCCACA)-3′ (**B**)	60.0	50	1.13
5′-(CACCAACXTCTXCCACA)-3′ (**C**)	59.5	100	1.16
5′-(TTAACTTCTTCACATTC)-3′ (**D**)	52.0	100	1.19
5′-(TTAACTTCTTCACATXC)-3′ (**E**)	50.0	200	1.12
5′-(TTAACTTCTTCACAXXC)-3′ (**F**)	48.0	400	1.11
3′-(AATTGAAGAAGTGTAAG)-5′ (**G**)			
3′-(GTGGTTGAAGAAGGTGT)-5′ (**H**)			

[a] A = 2′-deoxyadenosine, C = 2′-deoxycytidine, G = 2′-deoxyguanosine, T = thymidine, X = 3′-*C*-(hydroxymethyl)thymidine (**4**). [b] T_m = melting temperature. [c] $t_{1/2}$ = hyperchromicity half-life. [d] H = enzymatic hyperchromicity.

nucleoside **5** in 81% yield. Phosphitylation[21] of **5** by reaction with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite ($NCCH_2CH_2OP(Cl)N(iPr)_2$) in the presence of *N*,*N*-diisopropylethylamine in anhydrous dichloromethane afforded the nucleoside phosphoramidite **6** in 90% yield after column chromatographic purification and precipitation from petroleum ether.

Synthesis of oligomers **A–H** (Table 1) was performed using standard phosphoramidite methodology on a Pharmacia Gene Assembler Special DNA synthesizer using **6** and commercial 2′-deoxynucleoside-β-cyanoethylphosphoramidites. The coupling efficiency of the modified phosphoramidite **6** was approximately 60% (12-min coupling), compared to approximately 99% for standard phosphoramidites (2-min coupling). The low coupling yield of **6** is probably due to steric hindrance caused by the bulky silyl protecting group. The dimethoxytrityl-protected oligodeoxynucleotides **B**, **C**, **E** and **F**, were removed from the solid support by treatment with concentrated ammonia at 20 °C for 48 h, which also removes the phosphate and nucleobase protecting groups. Subsequent purification using disposable reverse-phase chromatography cartridges, detritylation, desilylation, and desalting afforded the pure oligomers.[22]

The composition of the oligodeoxynucleotide **E** was verified by matrix-assisted laser desorption mass spectrometry, which has become an important analytical tool for mass analysis of oligonucleotides.[23] Oligomer **E** contains one modification, resulting in an enhancement of the mass of 30 Da compared to the unmodified 17-mer **D**. The measured mass (5102.4 Da) corresponds excellently with the calculated (5101.4 Da), and we therefore conclude that the modified nucleoside building block **6** is incorporated once in **E** as contemplated. Because of the homogeneous results from the syntheses of all the modified oligonucleotides **B**, **C**, **E**, and **F**, we consider their composition verified.

Hybridization studies[24] (Table 1) indicate that incorporation of one or two 3′-*C*-hydroxymethyl nucleoside(s) in the middle of a 17-mer (**B** and **C**) induces no destabilization of the duplexes formed between the oligodeoxynucleotides and their complementary sequence **H**. Substitution in the 3′-end with one or two modified nucleosides (**E** and **F**) causes a minor decrease in T_m (ΔT_m/modification = 2 °C).

It was reported[25] that 3′-phosphodiesterase activity is the major cause of degradation of unmodified oligonucleotides *in vivo*. We therefore tested the enzymatic stability of the modified oligodeoxynucleotides **B**, **C**, **E**, and **F** toward snake venom phosphodiesterase (SV PDE, 3′-exonuclease).[26] The increase in absorbance (hyperchromicity) at 260 mn was followed[27] during digestion and the half-life ($t_{1/2}$) estimated (Table 1). Incorporation of the modified 3′-*C*-hydroxymethyl nucleoside one or two times in the middle of a 17-mer (**B** and **C**) has no apparent effect on the enzymatic stability of the full-length oligomers, while two 3′-end substitutions result in a 4-fold increase in half-life.

In summary, the stereoselective synthesis of the novel 3′-*C*-(hydroxymethyl)thymidine (**4**) in four steps from 5′-*O*-(4,4′-dimethoxytrityl)thymidine has been accomplished. Incorporation of this nucleoside into oligodeoxynucleotides causes no (middle modifications) or only minor (3′-end modifications) destabilization of the resulting DNA:DNA duplex. 3′-End-capped sequences exhibit enhanced stability toward SV PDE. The "3′-*C*-(hydroxymethyl)DNA" introduced here incorporates extra primary hydroxy functionalities into oligonucleotides which, e.g., may prove useful as attachment sites for covalently linked intercalating agents or lipophilic carriers. We are currently further investigating oligonucleotide analogues containing 3′-*C*-hydroxymethyl nucleosides and derivatives thereof as a new class of interesting oligonucleotide analogues.

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Supplementary Material Available: Experimental details and NMR data for compounds **1–6** and laser desorption mass spectrum of oligodeoxynucleotide **E** (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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