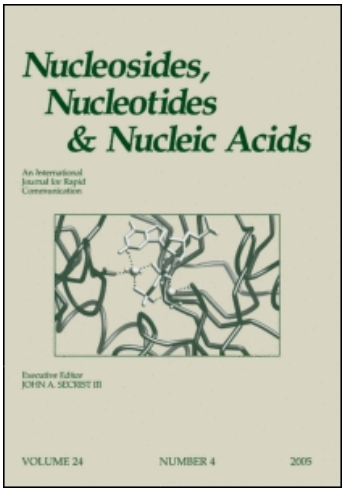

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3'-*C*-HYDROXYMETHYLTHYMIDINE: SYNTHESIS AND INCORPORATION INTO OLIGODEOXYNUCLEOTIDE ANALOGUES

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Abstract: The stereoselective synthesis of 3'-*C*-hydroxymethylthymidine (**5**) in five steps from thymidine has been accomplished and this nucleoside has been incorporated into oligodeoxynucleotides (ODNs) in different ways.

Compared with thymidine, 3'-*C*-hydroxymethylthymidine (**5**) contains an extra primary hydroxy functionality which enables its incorporation into oligodeoxynucleotides (ODNs) in several ways. Incorporation using the phosphoramidite **7a** afforded ODNs containing an unaltered backbone and a 3'-*C*-hydroxymethyl group. This group orients into the major groove of a DNA:DNA duplex without influencing the hybridization properties.[1] This group may therefore prove useful as an attachment site, e.g. for covalently linked intercalating agents or lipophilic carriers. Attempts to synthesize ODNs containing compressed phosphodiester backbones (3'-*C*-hydroxymethyl to 3'-hydroxyl) were done using the phosphoramidite **7b**. The third possibility, incorporation of **5** with an extended backbone (5'-hydroxyl to 3'-*C*-hydroxymethyl), was accomplished using the phosphoramidite **9a**. To verify the hybridization properties of **9a**, the N^3-analogue **9b** was synthesized. Introduction of a N^3-methyl group reduces the ability of the thymine nucleobase to form hydrogen-bonds with a complementary adenine.

The synthesis of 3'-*C*-hydroxymethylthymidine was performed as follows (Figure 1): Oxidation of 5'-*C*-(4,4'-dimethoxytrityl)thymidine (**1a**) using pyridinium dichromate (PDC) afforded 5'-*O*-(4,4'-dimethoxytrithyl)-3'-ketothymidine **2a** in 81% yield. Lombardo methylenation of **2a** afforded 2',3'-dideoxy-3'-*C*-methylene nucleoside **3a** in 79% yield.

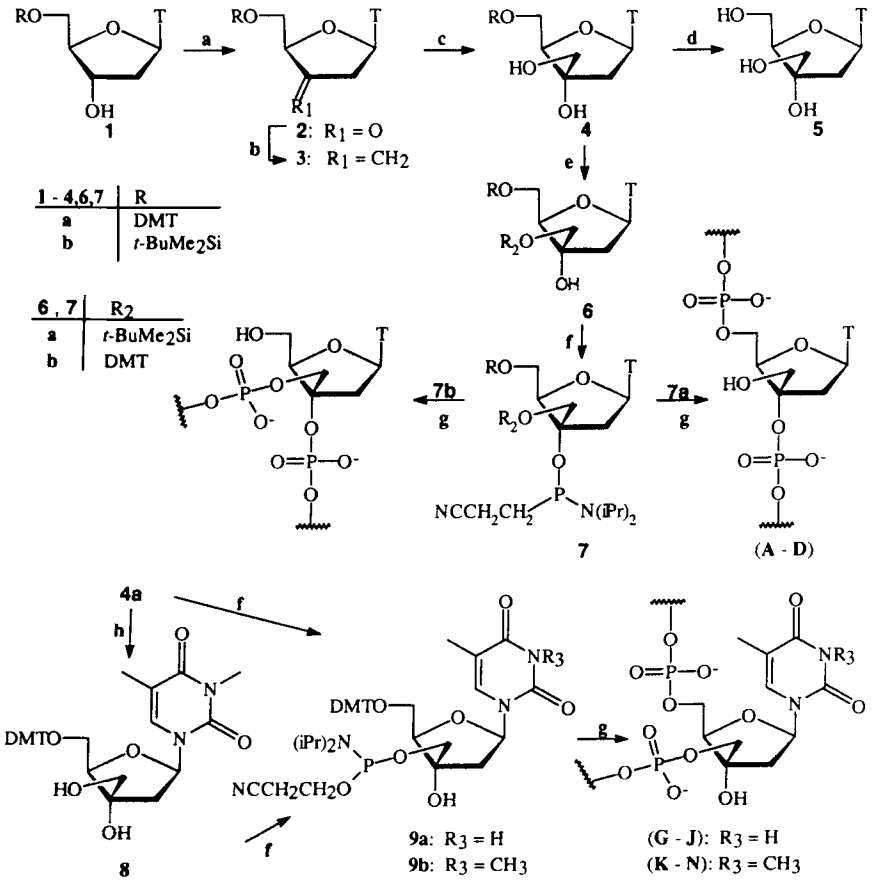

FIGURE 1: **a**) PDC/3Å molecular sieve powder/CH_2Cl_2, **b**) Zn/CH_2Br_2/$TiCl_4$/THF/CH_2Cl_2, **c**) OsO_4/*N*-methylmorpholine *N*-oxide/*t*-butanol/pyridine/H_2O, **d**) 3% dichloroacetic acid, **e**) *tert*-butyldimethylsilyl-chloride/imidazol/DMF (**6a**) or DMTCl/pyridine (**6b**), **f**) 2-cyanoethyl-*N,N*-diisopropylchlorophosphor-amidite/*N,N*-diisopropylethylamine/CH_2Cl_2, **g**) DNA-synthesizer, **h**) CH_3I/BDDDP/CH_3CN.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-hydroxymethylthymidine (**4a**) was subsequently obtained in 70% yield by stereoselective catalytic osmium tetroxide oxidation of **3a** using *N*-methylmorpholine *N*-oxide as co-oxidant. Deprotection of **4a** using dichloroacetic acid gave in 90% yield 3'-*C*-hydroxymethylthymidine (**5**), which was found inactive against HSV-1 and HIV-1. Reaction of **4a** with *tert*-butyldimethylsilyl chloride using imidazole as catalyst afforded in 81% yield 3'-*C*-(*tert*-butyldimethylsilyl)oxymethyl nucleoside **6a** which was phosphitylated[2] using 2-cyanoethyl-*N,N*-diisopropylaminophosphoramidochlori-dite to obtain the nucleoside phosphoramidite **7a** in 90% yield. Using the same synthetic

TABLE 1: Sequences synthesized, hybridization data and enzymatic stability

Sequence[a]		T_m(°C)[b]	ΔT_m(°C)[c]	$t_{1/2}$(sec)[d]
5'-(CACCAAC**X**TCTTCCACA)-3'	**(A)**	60.0	0.0	50
5'-(CACCAAC**X**TCT**X**CCACA)-3'	**(B)**	59.5	0.5	100
5'-(TTAACTTCTTCACAT**X**C)-3'	**(C)**	50.0	2.0	200
5'-(TTAACTTCTTCACA**XX**C)-3'	**(D)**	48.0	2.0	400
5'-(CACCAAC**Y**TCTTCCACA)-3'	**(E)**	56.5	3.5	30
5'-(CACCAAC**YY**C**Y**TCCACA)-3'	**(F)**	45.0	5.0	60
5'-(TTAACTTCTTCACAT**Y**C)-3'	**(G)**	49.5	2.5	200
5'-(TTAACTTCTTCACA**YY**C)-3'	**(H)**	46.5	2.8	300
5'-(CACCAAC**Z**TCTTCCACA)-3'	**(I)**	43.5	16.5	-
5'-(CACCAAC**Z**TCT**Z**CCACA)-3'	**(J)**	33.5	13.3	-
5'-(TTAACTTCTTCACAT**Z**C)-3'	**(K)**	46.0	6.0	300
5'-(TTAACTTCTTCACA**ZZ**C)-3'	**(L)**	41.5	5.3	>500
3'-(CACCAACTTCTTCCACA)-5'	**(M)**	60.0	-	40
3'-(TTAACTTCTTCACATTC)-5'	**(N)**	52.0	-	80

[a] A = 2'-deoxyadenosine, C = 2'-deoxycytidine, G = 2'-deoxyguanosine, T = thymidine, **X = 7a**, **Y = 9a**, **Z = 9b**. [b] T_m = melting temperature. [c] ΔT_m = decrease in T_m per modification compared to unmodified ODNs. [d] $t_{1/2}$ = half-life

route as described for **1a**, 5'-*O*-(*tert*-butyldimethylsilyl)thymidine (**1b**) was transformed into 5'-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-hydroxymethylthymidine (**4b**) in an overall yield of 25%. Reaction of **4b** with 4,4'-dimethoxytrityl chloride in dry pyridine afforded **6b** in 47% yield. Subsequent phosphitylation afforded the phosphoramidite building block **7b** in 69% yield. Regioselective phosphitylation of **4a** afforded the primary phosphoramidite **9a** in 74% yield. **9a** was successfully applied on a DNA-synthesizer without protection of the tertiary hydroxy group. Methylation of **4a** was accomplished with CH_3I in the presence of the organic base 2-*t*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP) to give **8** in 81% yield. The N^3-methyl phosphoramidite **9b** was obtained in 98% yield.

ODNs **A** - **N** (table 1) were synthesized by standard phosphoramidite methodology on an automated solid phase DNA-synthesizer using the appropriate building blocks (**7a**, **9a**, **9b** and commercial 2'-deoxynucleoside-β-cyanoethylphosphoramidites). Deprotection and purification of the ODNs was performed as described.[1]

The composition of the ODNs was verified by matrix assisted laser desorption mass spectrometry. The melting points and the enzymatic stability of the modified ODNs

towards snake venom phosphordiesterase (3'-exonuclease) was evaluated as previously described.[3] The results are depicted in Table 1.

The following observations were made: incorporation of this nucleoside into ODNs causes, in the case of **7a**, no (middle-modification) or only minor (3'-end modification) destabilization of the resulting DNA:DNA duplex. Incorporation of the building block **9a** results in a decrease in T_m of 2.5 - 5.0 °C per modification. As expected, a large destabilization is observed after incorporation of **9b** in a 17-mer. Comparison of the results from incorporation of **9a** and **9b** shows that oligomers containing **9a** retain the ability to hybridize with a complementary DNA sequence. It is evident, that the most promising way of incorporation **5** into ODNs is through 5'-hydroxyl to 3'-hydroxyl, as it exhibits very good melting points and is interesting because of the possibility of the extra 3'-*C*-hydroxymethyl group to serve as the attachment site for other molecules.

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