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5'-*C*-Branched Thymidines: Synthesis, Stereochemistry, and Incorporation into Oligodeoxynucleotides

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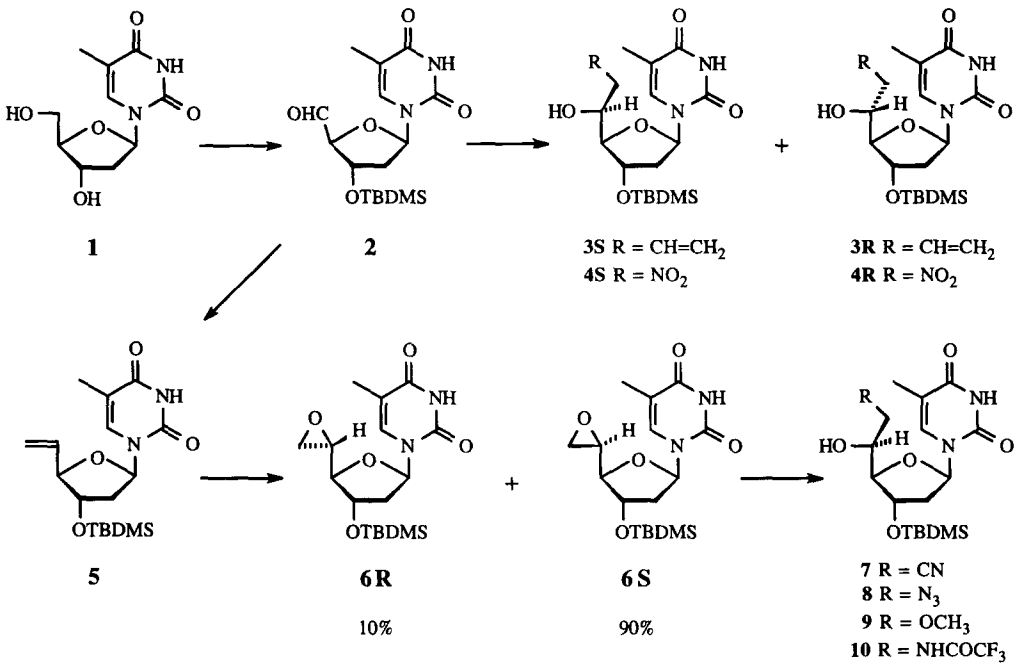
Abstract: Thymidine was converted to its 5'(*S*)-epoxy derivative, which reacted with nucleophiles to give 5'(*S*)-*C*-aminomethyl-, 5'(*S*)-*C*-azidomethyl-, 5'(*S*)-*C*-cyanomethyl-, and 5'(*S*)-*C*-methoxymethyl-thymidine with defined stereochemistry. 5'-*C*-Allyl- and 5'-*C*-nitromethylthymidines were prepared from an aldehyde derivative. Stereochemistry of 5'-*C*-branched thymidines was assigned with the help of NOE experiments. Four 5'-*C*-branched thymidines were incorporated into oligodeoxynucleotides.

Antisense oligonucleotides have shown the potential for treatment of viral and cancerous diseases.[1-3] To have therapeutic importance, however, antisense oligonucleotides must be stable to cellular nucleases and hybridize efficiently and specifically to target RNA. In efforts to meet these criteria, a variety of oligonucleotide analogs have recently been explored.[1,4] Oligonucleotide analogs having modifications at C2' positions of nucleosides have shown certain superior properties.[5-7] Very recently, oligonucleotide analogs containing 3'-*C*-hydroxymethylthymidine,[8] 4'-*C*-hydroxymethylthymidine,[9,10] and 5'-*C*-methyl nucleosides[11,12] have been reported. These sugar-modified oligonucleotides have significantly increased enzyme stability while they retain good hybridization properties. It seems that oligonuceotides containing branched nucleosides have the potential for therapeutic and diagnostic purposes and deserve a further evaluation. Recently, we have independently explored a variety of sugar modifications in search of oligonucleotide analogs that will have sufficient stability to cellular nucleases and binding affinity to complementary DNA and RNA as well as the ability to activate RNase H. Among sugar-modified nucleosides, 5'-*C*-branched nucleosides have not attracted much attention and their synthesis and stereochemistry have not been well established. In this communication we will describe synthesis and configurational assignment of 5'(*S*)-*C*-aminomethyl-, 5'(*S*)-*C*-azidomethyl-, 5'(*S*)-*C*-cyanomethyl-, 5'(*S*)-*C*-methoxymethyl-, 5'(*S*)-*C*-allyl-, 5'(*R*)-*C*-allyl-, and 5'(*R*,*S*)-*C*-nitromethylthymidine. Synthesis and properties of 5'-*C*-branched oligonucleotides will be briefly described.

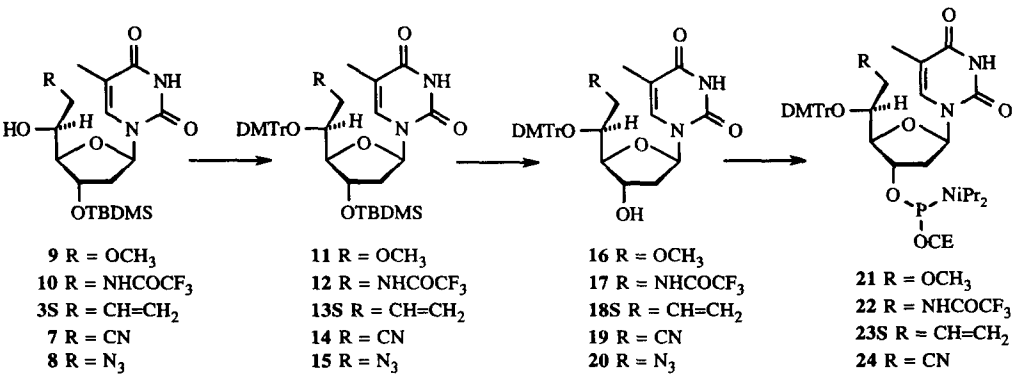
Synthesis of 5'-*C*-branched thymidines is shown in Scheme 1. Thymidine was converted, in four steps,[13] to an aldehyde derivative **2**. Grignard reaction of **2** with vinylmagnesium chloride and cuprous cyanide in THF gave **3S** and **3R** (about 1:1 ratio) in 63% yield. Nucleophilic addition of nitromethane to **2** in the presence of triethylamine afforded **4S** and **4R** (2:1 ratio) in 86% yield. Both these reactions gave a mixture of 5'(*R*)- and 5'(*S*)-branched thymidines, and therefore, the configurations at C5' of these products need to be assigned individually. For introducing substituents at C5' of thymidine in a defined configuration, we took advantage of epoxy group which can readily react with a variety of nucleophiles and is selectively attacked at its less hindered

Scheme 1.



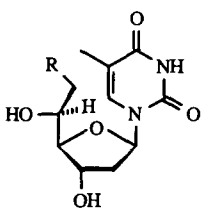
carbon by neutral or alkaline nucleophiles. Apparently, if stereochemistry of a 5'-epoxy derivative of thymidine is determined, stereochemistry of its ring-opening products is simultaneously assigned. 5'-Epoxy derivatives of thymidine **6S** and **6R** (10:1 ratio) were prepared from epoxidation of the alkene **5** with MCPBA in 85% yield (the reaction was not complete, and the yield was based on **5** consumed). **5** was prepared from **2** by Wittig reaction in 80% yield. Reaction of **6S** with potassium cyanide gave **7** in 70% yield and reaction of **6S** with sodium azide gave **8** in 67% yield. Treatment of **6S** with methanol over alumina (ICN super I neutral) at room temperature for 6 weeks (or at 55 °C for 2 weeks) afforded **9** in 70-73% yield. Reaction of **6S** with ammonia in methanol, followed by treatment with *S*-ethyl thiotrifluoroacetate, afforded **10** in 82% yield.

Scheme 2.

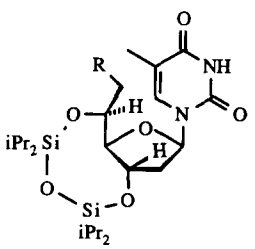


3S, **3R**,[14] **7**, **8**, **9**, and **10** were heated with excess dimethoxytrityl chloride in the presence of silver triflate in pyridine at 55 °C for 1-3 days to yield 5'-*O*-DMT derivatives **11-15** in 75-85% yields (Scheme 2). Silver triflate is indispensable in these reactions since it converts dimethoxytrityl chloride to dimethoxytrityl triflate, a more reactive alkylating reagent. After removal of TBDMS with TBAF, the resulting products **16-19** reacted with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite to yield the phosphoramidites **21-24** in 70-85% yields, respectively.

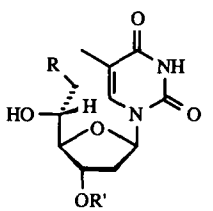
For determining configurations at C5' of 5'-*C*-branched thymidines we took advantage of the NOE of spatially adjacent protons in ^{1}H NMR. Since rigid orientations of the substituents at C5' are essential for NOE experiments, a tetraisopropyldisilyl (TIPDS) bridge was introduced to connect O3' and O5' of the thymidine derivatives to form a rigid ring, where the 5'-proton orients either toward the 3'-proton or away from the 3'-proton. For introducing the TIPDS bridge, TBDMS groups of **3S**, **3R**, **9** and **29** (the (5'*R*)-isomer of **9**, prepared from **6R**) were removed with TBAF, and the resulting products **25**, **26**, **30**, and **31** treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and imidazole in DMF to yield **27**, **28**, **32**, and **33**, respectively. For determining the configurations at C5', the 3'-protons of **27**, **28**, **32**, and **33** were saturated and NOE enhancements of the 5'-protons recorded. For 5'-*C*-allylthymidines, 4.8 % NOE enhancement was observed in the isomer (**27** or **32**) prepared from the lower R_f Grignard reaction product (**3S** or **3R**, silica, EtOAc in $CHCl_3$). Apparently, this isomer is (5'*R*)-isomer **32** and the lower R_f Grignard reaction product is **3R**. No NOE was observed in the isomer prepared from the higher R_f Grignard reaction product. Clearly, this isomer is (5'*S*)-isomer **27** and the higher R_f Grignard reaction product is **3S**. Without X-ray crystallography direct determination of configurations at C5' of **6S** and **6R** is a challenge. However, their configurations at C5' can be indirectly assigned by determining a pair of their ring-opening products such as **9** and **29** since the ring-opening reaction used does not alter chirality at C5'. Similarly to **27** and **32**, for 5'-*C*-methoxymethylthymidines, 5.1 % NOE enhancement was observed in the isomer (**28** or **33**) prepared from the lower R_f epoxide (**6S** or **6R**, silica, EtOAc-Hexane). Apparently, this isomer is **33** and the lower R_f epoxide is **6R**. No NOE was observed in the isomer prepared from the higher R_f epoxide. Clearly, this isomer is **28** and the higher R_f epoxide is **6S**.



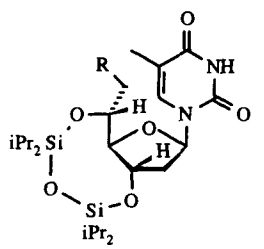
25 R = CH=CH$_2$
26 R = OCH$_3$



27 R = CH=CH$_2$
28 R = OCH$_3$



29 R = OCH$_3$, R'= TBDMS
30 R = CH=CH$_2$, R' = H
31 R = OCH$_3$, R'= H



32 R = CH=CH$_2$
33 R = OCH$_3$

By using the phosphoramidites **21**, **22**, **23S**, and **23R** (the (5'*R*)-isomer of **23S**, prepared from **3R**), four 5'-*C*-branched thymidines were incorporated into oligodeoxynucleotide (ATCTCTCCGCTTCCTTTC) at 3'-end, 5'-end, and internal regions. For the modified phosphoramidites coupling time of 2-5 minutes was used and the coupling yields (97-99%) were comparable to those for the unmodified. The purified oligonucleotides were obtained in 60-90 ODs on 1.0 μmol scale and characterized by electrospray mass spectrometry. Hybridization and enzyme stability data of a few selected sequences containing 5'-*C*-branched thymidines are

Table 1. Hybridization and enzyme stability data of selected sequences

	Sequence	Tm °C DNA	ΔTm °C/Mod.	Tm °C RNA	ΔTm °C/Mod.	$t_{1/2}$ min.
1.	5'-ATCTCTCCGCTTCCTTTC-3'	58.3		64.4		4.4
2.	5'-ATCTCTCCGCTTCCTXXC-3'	57.1	-0.6	63.8	-0.3	144
3.	5'-ATCTCTCCGCTTCCYTYC-3'	57.7	-0.3	63.6	-0.4	177
4.	5'-ATCTCTCCGCTTCCTZ_SZ_SC-3'	55.6	-1.4	63.3	-0.6	114
5.	5'-ATCTCTCCGCTTCCTZ_rZ_rC-3'	56.0	-1.2	63.3	-0.6	64

X = 5'(*S*)-*C*-methoxymethylthymidine, Y = 5'(*S*)-*C*-aminomethylthymidine, Z_S = 5'(*S*)-*C*-allylthymidine, and Z_r = 5'(*R*)-*C*-allylthymidine. The samples for Tm experiments contain 2 μM of modified oligos and 2.0 μM of either complementary DNA or RNA in a buffer (10 mM sodium phosphate, 0.1 mM EDTA, and 100 mM of sodium chloride, pH = 7.0). The half-lives were calculated from UV absorbance curves of oligonucleotide samples during degradation by SV phosphodiesterase at 37 °C.

listed in Table 1. Thermodynamic melting (Tm) experiments have shown that these modified sequences have comparable hybridization to the unmodified with both complementary DNA and RNA. For preliminary enzyme stability studies a similar procedure to that described by Wengel and coworkers[9] was employed. As can be seen in Table 1, replacement of two thymidines at 3'-region with two 5'-*C*-branched thymidines has significantly increased stability of these sequences to SV phosphodiesterase. Detailed biophysical and biological evaluation will be published elsewhere.

In summary, we have demonstrated synthesis and configurational assignment of 5'(*S*)- and 5'(*R*)-*C*-branched thymidines as well as their incorporation into oligodeoxynucelotides. The synthetic procedures are expected to apply to other nucleosides as well. Preliminary hybridization and enzyme stability studies have revealed that these sugar modifications seem promising for oligonucleotide therapeutics and diagnostics.

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REFERENCES AND NOTES.

1. Crooke, S.T.; Lebleu, B. *Antisense Research and Applications*, CRC Press (1993).
2. Lisziewicz, J.; Sun, D.; Metelev, V.; Zamecnik, P.; Gallo, R.C.; Agrawal, S. *Proc. Natl. Acad.Sci. USA* **1993**, *90*, 3860.
3. Stein, C.A.; Cheng, Y.-C. *Science* **1993**, *261*, 1004.
4. Uhlmann, E.; Peyman, A. *Chemical Reviews* **1990**, *90*, 543-584.
5. Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miuta, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131.
6. Lesnik, E.A.; Guinosso, C.J.; Kawasaki, A.M.; Sasmor, H.; Zounes, M.; Cummins, L.L.; Ecker D.J.; Cook, P.D.; Freier, S.M. *Biochemistry* **1993**, *32*, 7832.
7. Sproat, B.S.; Lamond, A.I.; Beijer, B.; Neuner, P.; Ryder, U. *Nucleic Acids Res.* **1989**, *17*, 3373.
8. Jorgensen, P.N.; Stein, P.C.; Wengel, J. *J. Am. Chem. Soc.* **1994**, *116*, 2231;
9. Fensholdt, J.; Thrane, H.; Wengel, J. *Tetrahedron Letts.* **1995**, *36*, 2535;
10. Thrane, H.; Fensholdt, J.; Regner, M.; Wengel, J. *Tetrahedron* **1995**, *51*, 10389.
11. Saha, A.K.; Caulfield, T.J.; Hobbs, C.; Upson, D.A.; Waychunas, C.; Yawman, A.M. *J. Org. Chem.* **1995**, *60*, 788.
12. Beigelman, L.; Karpeisky, A.; Usman, N. *Nucleosides & Nucleotides* **1995**, *14*, 901.
13. O-Yang C.; Wu, H.Y.; Faser-Smith, B.F.; Walker, K.A.M. *Tetrahedron Letts.* **1992**, *33*, 37.
14. **3R** is the (5'R)-isomer of **3S** (not shown in Scheme 2), which was subject to the same transformations as **3S** to yield the phosphoramidite **23R**.

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