Pergamon

*Tetrahedron Letters*, Vol. 38, No. 14, pp. 2393-2396, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00386-9

# Synthesis of 5'-*C*-Hydroxyalkylthymidines and Their Incorporation into Oligodeoxynucleotides

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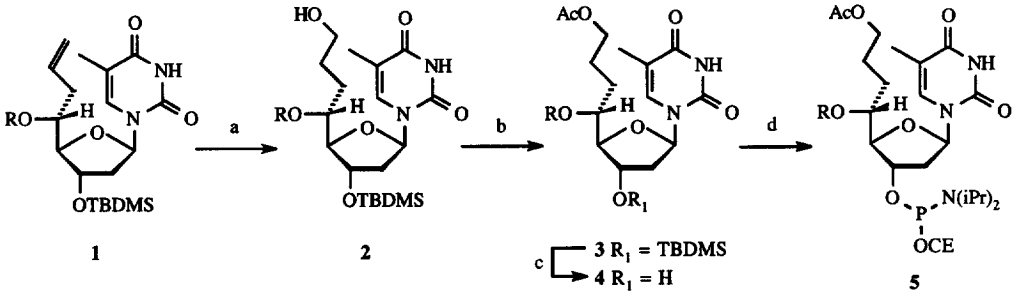
*Abstract*: 5'-*C*-Hydroxymethyl-, 5'-*C*-hydroxyethyl, and 5'-*C*-hydroxypropylthymidine derivatives were prepared through four synthetic routes, which constitute an approach to introduce hydroxyalkyl of different lengths at C5' of thymidine. 5'-*C*-hydroxymethyl- and 5'-*C*-hydroxypropylthymidines were converted to the corresponding phosphoramidites and incorporated into oligodeoxynucleotides.


In search for oligonucleotide analogs that can meet the criteria for human therapeutics, a variety of chemical modifications have been explored.[1-3] Recently, oligonucleotides containing *C*-branched nucleosides have received considerable attention.[4-8] Oligonucleotides containing 5'-*C*-branched nucleosides have shown good hybridization to both DNA and RNA and exhibited improved stability to cellular nucleases as compared to unmodified oligonucleotides.[4-5] Recently, we have independently explored a variety of sugar modifications aimed at increasing enzyme stability of antisense oligonucleotides. In a previous communication,[5] we reported the synthesis and properties of oligodeoxynucleotides containing 5'-*C*-branched thymidines. With the extension of the research program, we have recently prepared a few hydroxyalkylthymidines and incorporated them into oligodeoxynucleotides. Although 5'-*C*-hydroxyalkylthymidines can be prepared in many ways,[9-10] preparation of the corresponding building blocks for synthesis of oligonucleotides requires a special consideration in hydroxyl protection strategy. As we described earlier,[5] introduction of a DMT group at the secondary 5'-hydroxyl of 5'-*C*-branched nucleosides required heating for days and use of silver triflate, but even so, compounds containing a bulky substituent at C5' may not be tritylated. Therefore, a protecting group for the hydroxyalkyl must be selected from those that are relatively small and be stable to potential attack by the secondary 5'-hydroxyl under the tritylation conditions. It may require a serious effort to develop such a protection strategy. Instead, we took a different approach. First, a linker having a properly selected functionality at the end was attached to C5' of thymidine; second, 5'-hydroxyl of 5'-*C*-branched thymidines was protected with DMT; and finally, the functionality at the end of the linker was converted to hydroxyalkyl. By this approach, the building blocks for introducing 5'-*C*-hydroxyalkylthymidines into oligonucleotides were readily prepared. In this communication, we will describe synthesis of 5'-*C*-hydroxymethyl-, 5'-*C*-hydroxyethyl-, and 5'-*C*-hydroxypropylthymidine and their incorporation into oligodeoxynucleotides.

Synthesis of (5*R*)'-*C*-hydroxypropylthymidine derivatives is shown in Scheme 1. **1** was prepared from thymidine according to a published procedure.[5] **2** was prepared by a procedure similar to that used for synthesis of 4'-hydroxyalkylthymidine.[11] **1** was reacted with borane-methyl sulfide and the resulting product treated with

sodium perborate to give **2**, which was converted to **3** by acetylation. Removal of TBDMS from **3** with TBAF yielded **4**, which was converted to **5** by treatment with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite. The racemic (5*R*,*S*)'-*C*-hydroxypropylthymidine phosphoramidites were also prepared. This synthetic route is useful for preparation of 5'-*C*-hydroxyalkylthymidine derivatives having a trimethylene or longer linker.

**Scheme 1.**

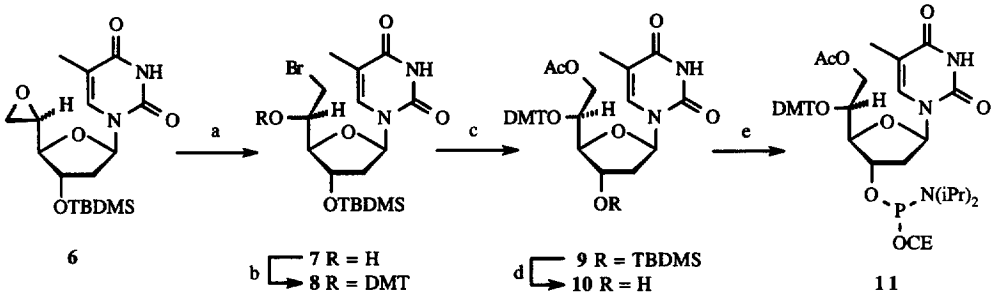



1 2 5

R = 4,4'-dimethoxytrityl (DMT). a) 1. $BH_3SMe_2$, THF, 0 °C, 2.5 h; 2. $NaBO_3$, EtOH, $H_2O$, r.t., 2 h, 64%; b) $Ac_2O$, pyridine, r.t., 2 h; c) 1.0 M TBAF, THF, r.t., 1 h, 81% overall yield from b and c; d) $NCCH_2CH_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, $CH_2Cl_2$, r.t., 0.5 h, 79%.

For preparation of 5'-*C*-hydroxymethyl derivatives, a different synthetic route was used as shown in Scheme 2. **6** was prepared according to a published procedure.[5] Reaction of **6** with methylmagnesium bromide did not yield the normal Grignard reaction product, the 5'-*C*-ethylthymidine derivative, instead, the bromide **7** was obtained in good yield. It seems that the real reagent of the reaction was magnesium bromide since **7** was also prepared quantitatively from the reaction of **6** with magnesium bromide. Treatment of **7** with DMT-Cl in the presence of silver triflate[12] yielded **8**, which was heated with anhydrous NaOAc in DMF to give **9**. After removal of TBDMS, **10** was converted to **11**.

**Scheme 2.**



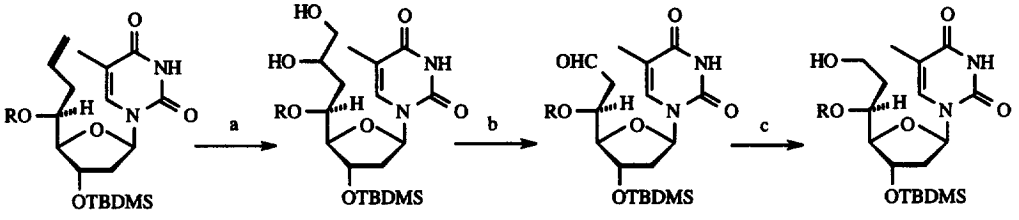

6 11

a) $CH_3MgBr$, THF, 0 °C, 1.5 h, 73%; b) DMT-Cl, AgOTf, Pyridine, 45 °C, 24 h, 70%; c) NaOAc, DMF, 95 °C, 4 days, 66%; d) 1.0 M TBAF, THF, pH 7.5, r.t., 16 h, 81%; e) $NCCH_2CH_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, $CH_2Cl_2$, r.t., 0.5 h, 88%.

5'-*C*-Hydroxyethylthymidine derivatives can, in principle, be prepared according to the synthetic route in Scheme 1. However, the synthesis of 5'-*C*-vinyl derivative of 3'-*O*-t-butyldimethylsilylthymidine from reaction of the aldehyde **16** with vinylmagnesium bromide was not successful owing to the rapid formation of polymerized products. A synthetic route based on alkene cleavage, as shown in Scheme 3, was successfully

used for synthesis of the 5'-*C*-hydroxyethyl derivatives. The allyl derivative **12**[5] was converted to the diol **13** by treatment with $OsO_4$. Cleavage of **13** with lead tetraacetate yielded the aldehyde **14**, which was readily reduced to **15a** with $LiAlH_4$. **15a** should be readily converted to the corresponding phosphoramidite by the same procedures as shown in Scheme 1.

## Scheme 3.

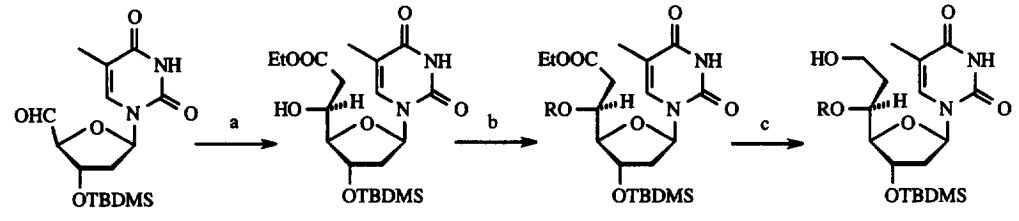



**12** **13** **14** **15a**

R = DMT. a) $OsO_4$, 4-methylmorpholine N-oxide, t-BuOH, 70 °C, 5 h, 55-60%; b) $Pb(OAc)_4$, benzene, r.t. 1.5 h, 88%; c) $LiAlH_4$, THF, -10 °C, 2 h, 70%.

Alternatively, **15a** was also prepared from the aldehyde **16**[13] as shown in Scheme 4. The Reformasky reaction of **16** with ethyl bromoacetate and freshly prepared zinc powder in dry benzene yielded **17** in good yield (two isomers, about 1:1 ratio). Heated with DMT-Cl in the presence of silver triflate in pyridine, **17a** (higher $R_f$ isomer, silica, EtOAc-hexanes 1:1) was converted to **18a** (about 80% conversion), which was separated from the intact **17a** by chromatography. Reduction of **18a** with $LiAlH_4$ yielded **15a**, which has the identical $R_f$ and $^1H$ NMR spectrum as does the product from the synthetic route in Scheme 3. Likewise, the lower $R_f$ Reformasky reaction product **17b** (not shown) was converted to **15 b** (not shown). Through **15a**, the stereochemistry of the Reformasky reaction products is also assigned since all the reactions in Scheme 3 and 4 except Reformasky reaction do not alter the chirality at C5' of thymidine.

## Scheme 4.




**16** **17a** **18a** **15a**

R = DMT. a) Zn, $BrCH_2COOEt$, Benzene, 60 °C, 5 h, 70-75% (total yield of both isomers); b) DMT-Cl, AgOTf, 65 °C, 2 days, 89%; c) $LiAlH_4$, THF, -5 °C, 2 h, 65%.

**17a** was treated with $LiAlH_4$ to give 3'-*O*-TBDMS-5'-*C*-hydroxyethylthymidine (a diol, not shown). During the preparation of this manuscript, there appeared a communication letter that describes the preparation of 5'-*C*-hydroxyethylthymidine derivatives by a different synthetic route,[10] but does not address how to add DMT

protecting group. As mentioned above, for the diol to be useful in oligonucleotide synthesis, a new protection strategy must be developed to attach DMT to 5'-hydroxyl without affecting the hydroxyl of C5'-hydroxyalkyls, or a new protecting group that can replace DMT and be readily introduced must be available. We did not make effort in this direction, but we believe the approach we described above is useful and convenient.

By using the phosphoramidites **11** and **5**, (5*S*)'-*C*-hydroxymethyl- and (5*R*)'-*C*-hydroxypropylthymidine were incorporated into oligodeoxynucleotides by phosphoramidite chemistry.[14] The coupling time for the modified phosphoramidites and the unmodified phosphoramidites adjacent to the modified was raised up to five minutes and good coupling yields (96-98%) were achieved. The synthesized sequences were deprotected according to the standard procedure,[15] purified by a C8 reverse phase column with 0.1 M TEAA buffer (containing 5% acetonitrile, pH 7.5) on a preparative HPLC. The purified oligonucleotides were obtained in more than 70 ODs on 1.0 μmol scale. After desalting on OPC (oligonucleotide purification cartridge, ABI), the purified oligonucleotides were characterized by electrospray mass spectrometry.

In summary, 5'-*C*-hydroxymethyl-, 5'-*C*-hydroxyethyl-, and 5'-*C*-hydroxypropylthymidines have been synthesized and two of them incorporated into oligodeoxynucleotides. The approach described in this letter is not only useful for synthesis of 5'-*C*-hydroxyalkylthymidines that have an alkyl linker of any reasonable size, but also suitable for incorporation of 5'-*C*-hydroxyalkylthymidines into oligodeoxynucleotides at any position of a sequence. The reactions used in the synthetic routes of this letter are expected to be applicable to other nucleosides.

**Acknowledgment.** We are indebted to Vesna Stoisavljevic for synthesis of oligonucleotides.

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(*Received in USA* 26 *November* 1996; *revised* 14 *February* 1997; *accepted* 17 *February* 1997)