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Synthesis of Dinucleoside Phosphates Containing Sulfur Substituted Nucleobases : 4-Thiouracil, 4-Thiothymine and 6-Mercaptopurine

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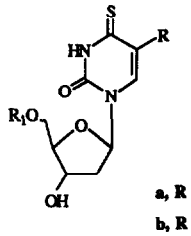
Key words: 4-thiouracil; protection; phosphorylation; nucleoside.

Abstract: S-pivaloyloxymethylation of sulfur substituted nucleobases enables the synthesis in solution of dinucleoside phosphates containing sulfur modified residues such as 4-thiouracil, 4-thiothymine and 6-mercaptopurine.

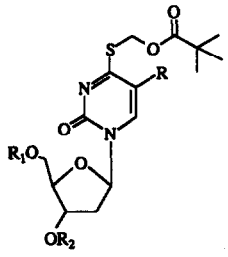
Sulfur-substituted nucleobases manifest interesting chemical and photochemical properties which have been reviewed recently[1]. In this regard several attempts have been made to incorporate 4-thiouridine (**1**) (or its analogues), a naturally occurring nucleoside that is usually found at the 8 position of a number of *E. coli* tRNA[2], in short oligonucleotides[3].

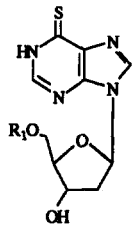
At the present time, a practical preparation method of such compounds, by application of the most recent phosphorylation techniques based on the P(III) chemistry[4,5], is still lacking. One possibility, which we have explored and used with success[6], is based on the 4-triazolylpyrimidinone chemistry as developed by Sung[7]. Such a derivatized pyrimidinone deoxynucleoside can be incorporated in an oligonucleotide[8], then the triazolyl group is displaced by the hydrosulfide ion before the final deprotection step[6]. To simplify this procedure we were prompted to devise another approach which would make use of an appropriately protected derivative of a mercapto substituted nucleoside whose protection has to be compatible with the current phosphorylation conditions.

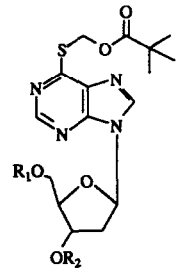
Preliminary experiments with several derivatives of 2'-deoxy-4-thiouridine **2a** having various acyl protecting groups at the sulfur position, such as benzoyl or methoxycarbonyl, suggested that these groups were insufficiently stable to acidic conditions. Finally, the most satisfactory results were obtained with the pivaloyloxymethyl group to mask the thiol fonction of 4-thiouracil.



a, R = H
b, R = CH_3







2 R_1 = H
7 R_1 = Dmt

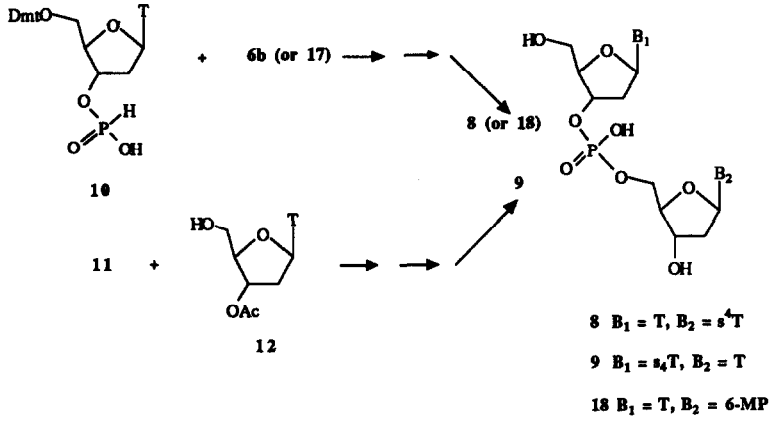
Y = 

3 R_1 = R_2 = H
4 R_1 = Dmt, R_2 = H
5 R_1 = Dmt, R_2 = Ac
6 R_1 = H, R_2 = Ac
11 R_1 = Dmt, R_2 = Y

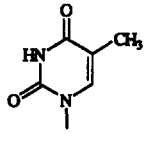
13 R_1 = H
14 R_1 = Dmt

15 R_1 = Dmt, R_2 = H
16 R_1 = Dmt, R_2 = Ac
17 R_1 = H, R_2 = Ac

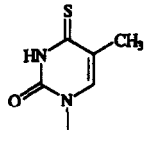
Dmt = dimethoxytrityl



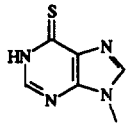
8 B_1 = T, B_2 = s^4T
9 B_1 = s_4T, B_2 = T
18 B_1 = T, B_2 = 6-MP



T



s^4T



6-MP

Treatment of an acetone solution of **2a** with pivaloyloxymethyl chloride in the presence of potassium carbonate at room temperature afforded the 4-(pivaloyloxymethylthio)-pyrimidin-2-one **3a** in 90% yield. Subsequent reaction of **3a** with dimethoxytrityl chloride provided the 5' protected nucleoside **4a** which was acetylated to give **5a**[9].

Compound **5a** served to establish that S-pivaloyloxymethylation of 4-thiouracil was very appropriate to enable a convenient incorporation of 2'-deoxy-4-thiouridine **2a** in oligonucleotides. Indeed, its chemical behaviour was found to be fully compatible with the standard reaction conditions of oligonucleotide synthesis. Thus, detritylation of **5a** (80% acetic acid or 3% DCA in methylene chloride) gave **6a** quantitatively which proved to be stable after a prolonged treatment in methylene chloride containing 3% dichloroacetic acid (DCA). When **5a** was dissolved and kept several hours in a solution of iodine in aqueous pyridine , which is employed to oxidize phosphite di- and triesters into the corresponding phosphates, it was recovered without degradation. Finally, **5a** and **6a** gave 2'-deoxy-5'-O-dimethoxytrityl-4-thiouridine **7a** and 2'-deoxy-4-thiouridine **2a**, respectively after overnight treatment with ammonia:pyridine (1:1) at room temperature.

We observed a similar behaviour with the corresponding derivative **5b** of 4-thiothymidine **2b** which was prepared by following the same procedure.

Encouraged by these promising preliminary observations, we chose to prepare the two dinucleotides **8** and **9** in solution (on a 0.5 mMole scale) by standard application of the H-phosphonate chemistry. Compound **8** was prepared by coupling 5'-O-(dimethoxytrityl)thymidin-3'-yl H-phosphonate (**10**)[5] with derivative **6b** in acetonitrile:pyridine (1:1) using bis(2-oxo-3-oxazolidinyl)phosphinic chloride as condensing agent[10]. The resulting phosphite diester was isolated and oxidized to the corresponding phosphate by treatment with 1.2 equivalent of iodine in pyridine:H_2O (9:1). Deprotection of the dinucleoside phosphate was accomplished in two steps : overnight ammonia treatment (conc. NH_4OH:pyridine;1:1) at room temperature to eliminate the pivaloyloxymethyl- and acetyl groups followed by removal of the dimethoxytrityl group with 80% acetic acid. Final purification of **8** was achieved by reverse phase chromatography over Lichroprep RP 18 (Merck, Art. 13900) using a water:acetonitrile gradient.

The dinucleoside phosphate **9** was obtained in a similar manner. Thus, the H-phosphonate **11b** was prepared using a recently published procedure by treating the protected derivative **4b** in pyridine by phosphorous acid in the presence of pivaloyl chloride[11]. The resulting H-phosphonate was condensed with 3'-O-acetylthymidine **12** to give a derivative which was processed as above in the case of compound **8**. The expected dimers **8** and **9** were obtained in good overall yield (40-50% from the 5' OH component)[9].

Another nucleoside of interest, to be incorporated in oligonucleotides, is 6-mercaptopurine-2'-deoxyriboside **13**. Upon treatment with pivaloyloxymethyl chloride its 5'-O-dimethoxytrityl derivative **14** gave compound **15** which was

acetylated to afford **16**. Detritylation of the latter gave **17** which was combined with **10** to provide in the usual manner the dimer **18**[9].

In conclusion, the pivaloyloxymethyl (POM) group can be used to mask the mercapto function of sulfur containing pyrimidine as well as purine deoxynucleosides to obtain dinucleoside phosphates in solution[12]. Extension of these results to solid phase synthesis to obtain longer sequences will be discussed in the following communication.

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