Tetrahedron Letters, Vol.32, No.22, pp 2505-2508, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

5'-O-(4,4'-DIMETHOXYTRITYL)-4-THIOCYANATOTHYMIDINE: A USEFUL INTERMEDIATE FOR THE PREPARATION OF VARIOUS 4-SUBSTITUTED THYMIDINE ANALOGUES

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Abstract: The thiocyanato group in 5'-O-(4,4'-dimethoxytrityl)-4-thiocyanatothymidine 2 is easily displaced by a variety of O-, N- and S-nucleophiles, making this compound a useful intermediate for the preparation of 4-substituted thymidine analogues.

It is believed that the modification of thymidine at 4-O and of deoxyguanosine at 6-O is one of the most important chemical reactions involving N-nitrosoamines and other alkylating agents *in vivo*[1,2]. Therefore, the synthesis of these nucleoside analogues and their incorporation into synthetic oligodeoxynucleotides has recently received considerable interest.

4-O-Alkyl-substituted thymidine analogues have been prepared by one of the following synthetic routes:

a) direct alkylation of thymidine derivatives with diazoalkanes or alkyl halides[3,4]

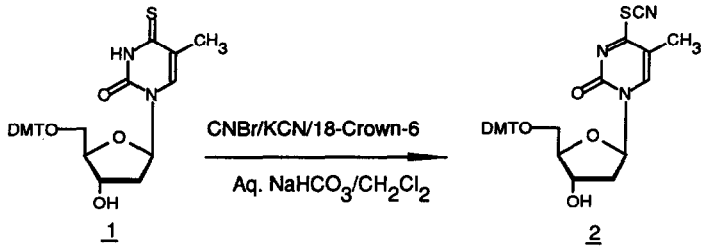
b) base-catalysed substitution of a 3-nitro-1,2,4-triazolo-, 1,2,4-triazolo- or 3-methyl-1-imidazolyl group in the 4-position of thymidine by alkoxy groups[5-8].

The first method generally gives mixtures of 2-O, 3-N and 4-O alkylated products, requiring both careful control of the reaction conditions and separation of the isomers. The second aproach appears to be more generally applicable and has been used for the preparation of oligodeoxynucleotides containing 4-O-methyl- and 4-O-ethylthymidine as well as 5-methyl-4-N,4-N-ethanocytidine. In the last case, aziridine, an N-nucleophile, has been used in the nucleophilic substitution reaction and the product used for DNA-crosslinking experiments. The fact that 4-(1,2,4-triazolyl)-thymidine can be incorporated into synthetic oligodeoxynucleotides using the well-established phosphoramidite chemistry and converted to the desired 4-modified thymidine analogue at the oligonucleotide level makes this approach especially attractive[7,9].

In this article, we wish to report the preparation of 5'-O-(4,4'-dimethoxytrityl)-4-thiocyanatothymidine 2. The extraordinary reactivity of the 4-thiocyanato group of this compound towards displacement by O-, N- and S-nucleophiles makes it an attractive alternative starting material for the preparation of a number of 4-modified thymidine analogues, in a form ready for conversion to the corresponding phosphoramidites and incorporation into synthetic oligodeoxynucleotides.

For the preparation of 2 we first treated 5'-O-(4,4'-dimethoxytrityl)-4-thiothymidine 1[10] with cyanogen bromide in the presence of an organic or inorganic base. The main product of these reactions

always turned out to be the symmetrical disulphide of 1, the yield of the desired 2 being very low. We reasoned that the reaction could be driven to the formation of the desired compound 2 as the main product by adding to the reaction mixture KCN, which should cleave the disulphide to 1 and 2. Indeed, the desired product 2 could be obtained in 85% isolated yield when 1 was treated with CNBr in a two-phase system of aq. $NaHCO_3/CH_2Cl_2$ in the presence of KCN and 18-crown-6 (Scheme 1)[11].

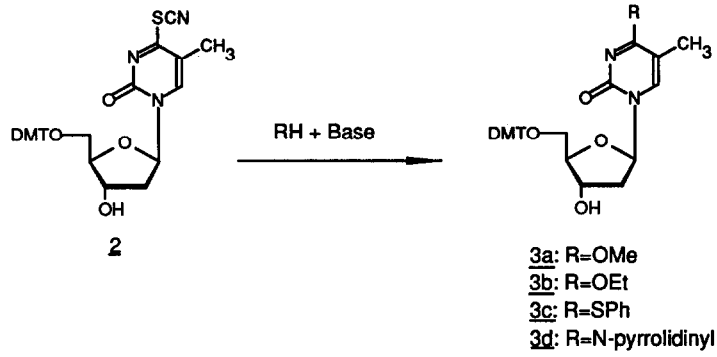


1 2

Scheme 1

As we considered to use the title compound 2 as a protected form of 4-thiothymidine for the incorporation of the latter into synthetic oligodeoxynucleotides we required a method for the removal of the cyano group from the sulphur. During the investigation of several possibilities to achieve this removal we found that the SCN group in 2 is substituted very easily under mild conditions by a number of different nucleophiles (Scheme 2). Thus, treatment of 2 with a methanolic or ethanolic solution of LiOH gave 5′-O-(4,4′-dimethoxytrityl)-4-O-methyl- and 4-O-ethylthymidine, respectively (3a and 3b). Upon treatment of 2 with an ethanolic ammonia solution, 5′-O-(4,4′-dimethoxytrityl)-4-O-ethylthymidine 3b was obtained as the main product (65% isolated yield), the presence of some 5′-O-(4,4′-dimethoxytrityl)-5-methylcytidine could be detected by TLC. Reaction of 2 with $PhS^-Et_3NH^+$ in CH_2Cl_2 led to 5′-O-(4,4′-dimethoxytrityl)-4-(S-phenyl)-thiothymidine 3c, whereas reaction with solid Li_2S in CH_3CN in the presence of 12-crown-4 gave 1. Finally, treatment with an excess of pyrrolidine in CH_2Cl_2 gave 4-pyrrolidinothymidine 3d. All reactions proceeded very rapidly at room temperature. The products were isolated in 65-80% yields after flash chromatography (0.2 mmol reaction scale). All had the expected FAB-MS and ^1H NMR spectra (data not given).

The reaction of 2 with the bulky triphenylmethylmercaptan led to the formation of 1 and the unsymmetrical disulphide 5′-O-(4,4′-dimethoxytrityl)-4-(S-triphenylmethylthio)-thiothymidine in a ratio of about 3:1. Due to steric reasons in this case the nucleophilic attack takes place at the more accessible electrophilic C- and S- atoms of the thiocyanato group.



2

3a: R=OMe
3b: R=OEt
3c: R=SPh
3d: R=N-pyrrolidinyl

Scheme 2

For the use in oligodeoxynucleotide synthesis 2 was converted to the corresponding phosphoramidite derivative and this was incorporated at the X-position of the dodecamer GACGAXATCGTC. Although the coupling of the phosphoramidite of 2 was efficient, HPLC-analysis of the product after standard deprotection (1h at 55°C in conc. NH_3[12]) showed only the presence of an unmodified thymidine at the X-position of the product.

In order to clarify at which stage of the synthesis the thiocyanato group has been lost, a CH_3CN solution of 2 was treated with conc. aq. NH_3 at room temperature. The main products of this reaction were 5-methylcytidine and thymidine, as their 5′-O-(4,4′–dimethoxytrityl) derivatives, in a ratio of about 2:1 as judged by TLC. This result suggests that the highly reactive thiocyanato group of 2 has been displaced by an O-nucleophile already during the synthesis of the modified oligodeoxynucleotide, before the NH_3 treatment. This is most probably caused by acetate during the capping step and clearly means that compound 2 is unsuitable as a protected form of 1 during oligodeoxynucleotide synthesis.

We also prepared a similar thiocyanato derivative of deoxyguanosine, 2′-deoxy-5′-O-(4,4′-dimethoxytrityl)-2-N-benzoyl-6-thiocyanatoguanosine and studied its reactivity under similar conditions. In agreement with previous observations[13], this compound did not show the reactivity pattern of the thymidine derivative 2. Instead, the attack of nucleophiles in this case took place exclusively at the C- and S-atoms of the thiocyanato group. Furthermore, we found that the cyano group represents an alternative protection for the thio-function of 6-thio-2′-deoxyguanosine[14], allowing the successful incorporation of the latter into synthetic oligodeoxynucleotides.

Although our experiments were carried out only with the thymidine-derived 2, it is reasonable to assume that its uridine analogue would behave similarly in these nucleophilic substitution reactions, thus opening a route for the synthesis of the corresponding 4-substituted uridine derivatives.

Acknowledgements: We thank the UK MRC for financial support. BAC is a Fellow of the Lister Institute of Preventive Medicine.

References and Notes

1. Singer, B., Kusmierek, J.T. *Ann. Rev. Biochem.* **1986**, *52*, 655-693.
2. Pegg, A.E. *Adv. Cancer Res.* **1977**, *25*, 195-267.
3. Borowy-Borowski, H., Chambers, R.W. *Biochemistry* **1989**, *28*, 1471-1477.
4. Singer, B., Sagi, J., Kusmierek, J.T. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 4884-4888.
5. Reese, C.B., Skone, P.A. *J. Chem. Soc., Perkin Trans. I* **1984**, 1263-1271.
6. Li, B.F.L., Reese, C.B., Swann, P.F. *Biochemistry* **1987**, *26*, 1086-1093.
7. Fernandez-Forner, D., Palom, Y., Ikuta, S., Pedroso, E., Eritja, R. *Nucl. Acids Res.* **1990**, *18*, 5729-5734.
8. Matsuda, A., Obi, K., Miyasaka, T. *Chem. Pharm. Bull.* **1985**, *33*, 2575-2578.
9. Webb, T.R., Mateucci, M.D. *Nucl. Acids Res.* **1986**, *14*, 7661-7676.
10. Connolly, B.A., Newman, P.C. *Nucl. Acids Res.* **1989**, *17*, 4957-4974.
11. Preparation of 2: 1.68g (3 mmole) 5′-O-(4,4′-dimethoxytrityl)-4-thiothymidine was dissolved in 50 ml CH_2Cl_2. A solution of 960 mg KCN in 20 ml 5% $NaHCO_3$ was added, followed by a few crystals of 18-crown-6. To this stirred two-phase mixture was added slowly at room temperature a solution of 460 mg CNBr in 10 ml CH_2Cl_2. After 10 min the organic phase was washed twice with water, dried and concentrated. The crude product was purified by flash chromatography (eluted with CH_2Cl_2/EtOAc 3:1 to 2:1). The product obtained was dissolved in a minimum amount of CH_2Cl_2 and precipitated into 100 ml of hexane. Yield: 1.48 g (85%).
    ^{1}H NMR: 8.20 (1Hs, H6), 7.40-6.79 (13Hm, dimethoxytrityl aromatic), 6.25 (1Ht, H1′), 4.60 (1Hm, H3′), 4.15 (1Hm, H4′), 3.80 (6Hs, CH_3O), 3.60-3.35 (2Hm, H5′+H″), 2.85-2.70 (1Hm, H2′), 2.45-2.35 (1Hm, H2″), 1.55 (3Hs, CH_3).
    FAB-MS: 303 (100% dmt$^+$), 586 (3%, M+H$^+$).
    IR: 2171 cm^{-1} (weak).
12. In order to minimise the time required for the NH_3-deprotection, the recently introduced fast-deprotecting phosphoramidites were used, see: Vu, H., McCollum, C., Jacobson, K., Theisen, P., Vinayak, R., Spiess, E., Andrus, A. *Tetrahedron Lett.* **1990**, *31*, 7269-7272.
13. Saneyoshi, M., Chihara, G. *Chem. Pharm. Bull.* **1967**, *15*, 909-914.
14. Waters, T.R., Connolly, B.A. *Nucleosides and Nucleotides*, submitted for publication.

(Received in UK 4 March 1991)