Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 931–932

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

REGENERATION OF 4-THIO-2′-DEOXYURIDINE RESIDUES IN DNA

Robert S. Coleman* and Richard M. Pires

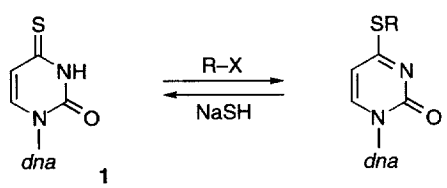
Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210-1185, U.S.A.

Received 13 January

Abstract: We report a facile, quantitative regeneration of the thiocarbonyl group from S-alkylated 4-thio-2′-deoxyuridine residues by treatment with NaSH within oligodeoxynucleotides.

We have described the use of 4-thio-2′-deoxyuridine ($d^{S4}U$) bases for appending chemically reactive functional groups into oligodeoxynucleotides by direct S-alkylation,[1,2,3] and we detailed the use of this methodology for template-directed covalent cross-linking of duplex DNA.[4,5] In the course of our investigations, we developed an extremely simple protocol for the regeneration of the starting $d^{S4}U$ base **1** within the oligonucleotide by displacement of an S-alkyl group of **2** with NaSH under mild conditions. This method, in effect, renders the S-alkylation reaction of $d^{S4}U$ reversible.

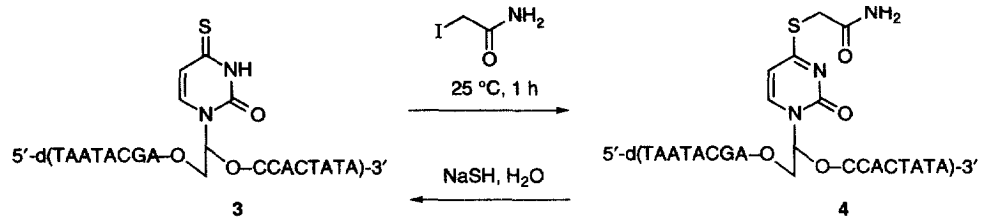
During the incorporation of $d^{S4}U$ residues into synthetic oligonucleotides,[6] we observed that systems such as **2** (R = CH_2CH_2CN)[7] were susceptible to ammonolysis by the ammonium hydroxide used for base deprotection, but that this ammonolysis could be suppressed by addition of 50 mM NaSH to the reagent system. Connolly and Newman had previously made a similar observation with a 6-thioguanosine system,[8] and the susceptibility of thiol groups on pyrimidine and purine systems to act at leaving groups in substitution reactions is well documented.[9] This observation led us to the proposition that the HS^- displacement reaction could be performed on a preparative scale within an oligodeoxynucleotide to regenerate the starting $d^{S4}U$ residue, subsequent to S-alkylation.



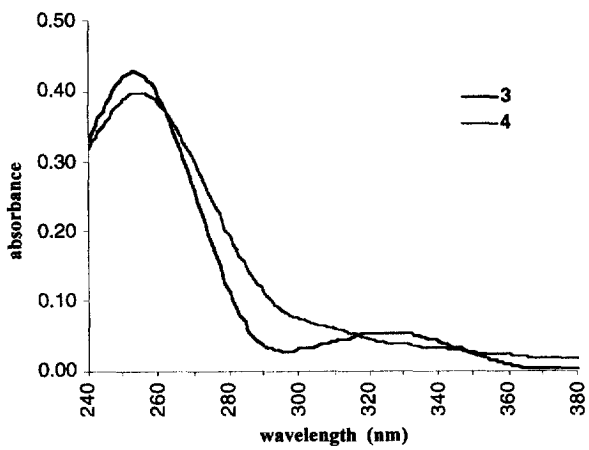
1 **2**

Treatment of $d^{S4}U$-containing oligodeoxynucleotide **3** with iodoacetamide results in quantitative and chemoselective S-alkylation to afford the thioimidate **4**. This compound was characterized by enzymatic digestion to the component nucleosides followed by HPLC analysis of the mixture, as described previously.[2] When **4** was treated with NaSH (0.9 M in dd H_2O) at elevated temperature (95 °C) for 50 min quantitative formation of the parent $d^{S4}U$ oligomer was observed, as evidenced by HPLC analysis.[2] Alternatively, heating at 37 °C for 15 h in the presence of NaSH accomplished the same transformation with equal effectiveness.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00120-1



The conversion of alkylated $d^{S4}U$ back to the unalkylated starting material could also be monitored by UV spectroscopy (Figure 1). Upon treatment of **4** with NaSH, the characteristic 4-thiouracil peak of **3** at 332 nm clearly reappears. This is accompanied by the disappearance of the shoulder at 308 nm, which is characteristic of S-alkylated 4-thiouracil in **4**.



This material was identical in all respects with synthetically prepared starting oligomer **3**, including its ability to undergo a subsequent S-alkylation with α-haloacetamide derivatives. Enzymatic digestion of **3** so obtained provided the expected ratio of nucleosides, including $d^{S4}U$. The methodology described herein allows the "reuse" of thiocarbonyl groups on thionucleosides in alkylation reactions within oligodeoxynucleotides.

Figure 1. UV spectra (H_2O) of **3** obtained from **4** by treatment with NaSH.

Acknowledgments: This work was supported by a grant from the National Institutes of Health (GM-47991). RSC is an Alfred P. Sloan Foundation Research Fellow (1995-98).

References

1. Coleman, R. S., Siedlecki, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 9229.
2. Coleman, R. S., Kesicki, E. A. *J. Am. Chem. Soc.* **1994**, *116*, 11636.
3. For 6-thio-2'-deoxyinosine, see: Coleman, R. S., Arthur, J. C., McCary, J. L. *Tetrahedron* **1997**, *57*, 11191.
4. Coleman, R. S., Kesicki, E. A. *J. Org. Chem.* **1995**, *60*, 6252.
5. Coleman, R. S.; Pires, R. M. *Nucleic Acids Res.* **1997**, *25*, 4771.
6. For the incorporation of thionucleosides into DNA and RNA, see references 2 and 3.
7. Coleman, R. S.; Siedlecki, J. M. *Tetrahedron Lett.* **1992**, *32*, 3033.
8. Connolly, B. A.; Newman, P. C. *Nucleic Acids Res.* **1989**, *17*, 4957.
9. Mayer, R; Morgenstern, J.; Fabian, J. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 2677. Eshaghpour, H.; Söll, D.; Crothers, D. M. *Nucleic Acids Res.* **1979**, *7*, 1485. Kraszewski, A.; Delort, A. M.; Teoule, R. *Tetrahedron Lett.* **1986**, *27*, 861. Roget, A.; Bazin, H.; Teoule, R. *Nucleic Acids Res.* **1989**, *17*, 7643. Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *Nucleosides Nucleotides* **1995**, *14*, 929. Xu, Y.-Z. *Tetrahedron* **1996**, *52*, 10737. Xu, Y.-Z.; Ramesh, V.; Swann, P. F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1179.