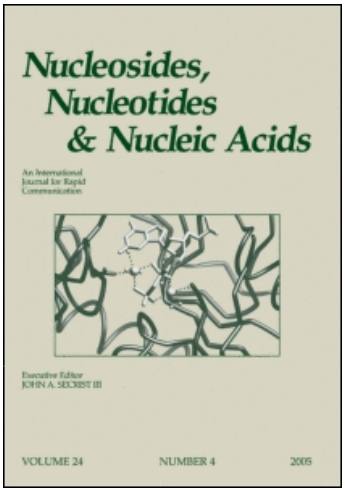

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Qinguo Zheng[a]; Yao-Zhong Xu[a]; Peter F. Swann[a]
[a] Cancer Research Campaign, Nitrosamine-Induced Cancer Research Group, Department of Biochemistry and Molecular Biology, University College London, London

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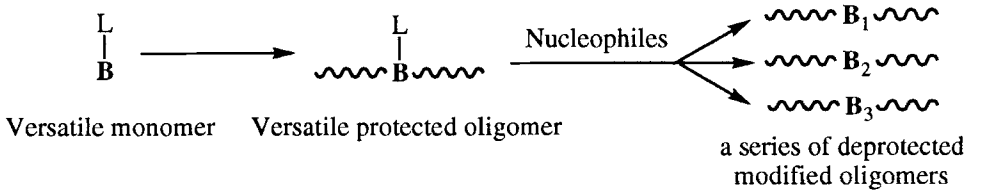
AN IMPROVED POST-SYNTHETIC SUBSTITUTION APPROACH FOR SYNTHESIS OF OLIGODEOXYNUCLEOTIDES CONTAINING LABILE 4-SUBSTITUTED THYMINES

Qinguo Zheng*, Yao-Zhong Xu and Peter F Swann

Cancer Research Campaign, Nitrosamine-Induced Cancer Research Group,
Department of Biochemistry and Molecular Biology,
University College London, Gower Street, London WC1E 6BT

Abstract: A simple procedure is described for the preparation of a versatile oligodeoxynucleotide which contains 4-phenylthiothymidine. This versatile oligomer has been successfully used for synthesis of oligonucleotides containing labile 5-methyl-N^4, N^4-ethanocytosine (**7**) or 4-azido-5-methyl-2-pyrimidinone-1-β-(2'-deoxyriboside) (**8**).

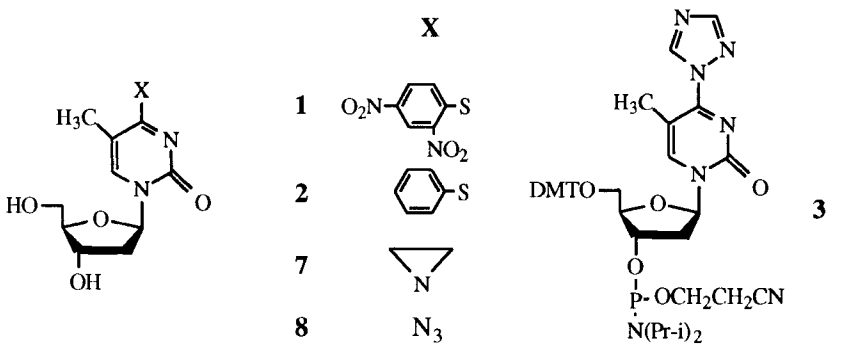
The synthesis of oligodeoxynucleotides containing modified bases at a specific position has been attracting great attention because of their wide applications for the study of DNA repair, DNA-protein or DNA-DNA interactions, and as potential therapeutic agents[1a-b]. Conventionally, these modified oligonucleotides have been synthesized by preparation and incorporation into the oligomer of the protected phosphoramidite of the modified nucleoside. Because of some limitations of this approach, we[2a-c] and others[3a-g] have developed an alternative strategy in which a versatile monomer containing a leaving group (L) is incorporated into the oligomer. This group is sufficiently stable to withstand the conditions used for DNA assembly but can be substituted by a number of nucleophiles at the oligomer level at some point during the deprotection after DNA synthesis (Scheme 1). The main merit of this post-synthetic substitution strategy is that a single DNA synthesis can provide a series of oligodeoxynucleotides each containing a different base including those which would not tolerate the normal conditions of DNA assembly. However, it is still



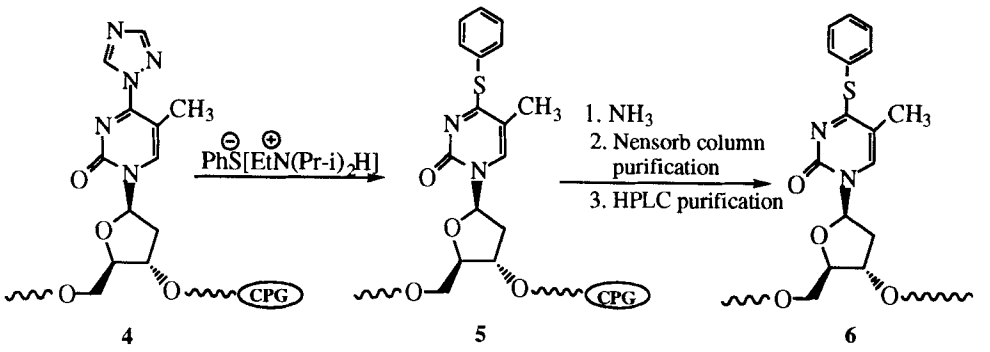
Scheme 1

difficult to apply this strategy to oligomers containing a labile base which can be damaged by a post-synthetic procedure, such as deprotection by conc. aqueous ammonia[3a]. We therefore have been endeavouring to further develop the post-synthetic substitution strategy by preparing a versatile DNA which is fully cleaved from support, deprotected, and purified but with the leaving group (L) intact. This sort of versatile DNA provides the possibility of making DNA containing an extremely reactive base since no post-synthetic treatments are needed after the substitution of "L" by a nucleophile. In this communication, we describe the preparation of such a versatile DNA and its use for making oligomers containing labile 4-modified thymines.

For the strategy to be successful, the thymine analogue must contain at the 4-position a group which is stable during DNA synthesis and to the procedures used to remove protecting groups after synthesis, but still easily displaceable upon treatment with a number of nucleophiles under mild conditions. Leaving groups previously used in post-synthetic substitution approach for the synthesis of oligomers carrying modified pyrimidines are either too reactive to survive during the deprotection procedures[2a, 3a, 3c] or too stable to be substituted by nucleophiles under mild conditions[3b, 3d]. Since previously[2b, 2c] we had successfully used the 2, 4-dinitrophenylthio group at the 6-position of purines in post-substitution strategy, initially we attempted to make 4-(2, 4-dinitrophenyl) thiothymidine (**1**) as the versatile nucleoside. However, dinitrophenylthio group at the 4-position of thymine was found to be too reactive to withstand even a brief treatment (30 min) of conc. aqueous ammonia at room temperature. We therefore decided to use the less reactive phenylthio (PhS-) as the leaving group. Experiments at the nucleoside level indicated that 4-phenylthiothymidine (**2**) was relatively stable toward ammonia treatment at room temperature. No obvious substitution was observed by TLC after 2h, though, as observed before in an attempt of making oligomer containing 4-thiothymine from **2**[4], longer exposure caused the displacement of phenylthio group by ammonia. Most importantly, the 4-phenylthio group underwent clean and quick substitution upon treatment with a number of nucleophiles. To avoid the several chemical synthetic steps needed to make 4-phenylthiothymidine phosphoramidite monomer, we built the versatile oligonucleotides by using 4-triazolothymidine monomer (**3**), which can be prepared in one step from



thymidine phosphoramidite[2a] and is now commercially available (Glen Research, USA). To reduce the possibility of ammonolysis of 4-phenylthiothymine, monomers protected with



4 5 6

Scheme 2

the labile t-butylphenoxyacetyl group on the amino functions of dA, dG, and dC (Expedite monomer, Millipore) were used. These monomers can be deprotected completely with conc. ammonia at room temperature within two hours[5].

4-Triazolothymidine phosphoramidite (**3**) was incorporated into a dodecamer GCTACXGACTGC (X standing for 4-triazolothymidine), and then the protected oligomer **4** was treated at room temperature with 0.1M PhS[EtN(Pr-i)$_2$]H in CH_3CN overnight to substitute the triazolo group. As the oligomer **5** was still attached to CPG-support, the excess PhS[EtN(Pr-i)$_2$]H was washed off with CH_3CN, and then treated with conc. aqueous ammonia to fully cleave and deprotect the oligomer (Scheme 2). The crude oligomer was purified by Nensorb cartridge. Highly pure oligomer **6** was obtained by reverse-phase HPLC because the desired oligomer has longer retention time than those of impurities (mainly oligomer containing 5-methylcytosine) due to the strong hydrophobic effect of phenyl group (Fig.a).

To demonstrate the advantages of this oligonucleotide (**6**), we chose two labile nucleoside analogues 5-methyl-N^4, N^4-ethanocytidine (**7**) and 4-azido-5-methyl-2-pyrimidinone-1-β–(2'-deoxyriboside) (**8**) as the target molecules.

Essentially pure oligomer containing **7** was prepared by treating the versatile oligonucleotide **6** with aqueous aziridine solution at room temperature for 20 min (Fig.b). Although the oligomers containing **7** has been synthesized by incorporation of 4-triazolothymidine into oligomer and subsequently treatment of the still protected oligomer with aziridine, the method did not allow the preparation of oligomer containing **7** with all four natural DNA bases because **7** is not stable to deprotection by conc. aqueous ammonia.

The oligomer containing **8** was prepared simply by treating **6** with NaN_3 in 50% DMF/H_2O at 35°C overnight. Essentially pure oligomer was obtained after removing the excess NaN_3 by NAP-10 column (Fig.c). The desired oligomer could not be obtained by reacting **5** or **6** with NaN_3 before ammonia deprotection because **8** is not stable to NH_3 and oligomers containing 5-methylcytosine, produced by ammonolysis, or containing thymine, formed by hydrolysis, are the main products.

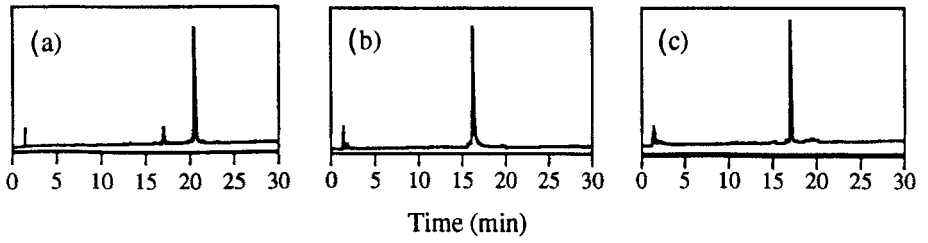


Figure: HPLC profile of oligomers containing **2** (a), **7** (b), **8** (c).

Oligonucleotides containing azidonucleosides have been prepared so far exclusively by the enzymatic incorporation and, to our best knowledge, this is the first report of chemically synthesized oligomer containing an azidonucleoside[6]. Phosphoramidite monomer, the most widely used chemistry for DNA synthesis, are incompatible with azides due to reactivity with the trivalent phosphorus[6].

Above results clearly demonstrated the advantages of using the versatile oligomer **6** for making oligonucleotides containing labile modified bases. Although our experiments were carried out only with the thymidine derivative **2**, it is reasonable to assume that its uridine analogue would behave similarly. We are also developing similar strategy for making oligomers containing labile modified purine bases and the results will be reported elsewhere.

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REFERENCES

1. (a) English, U.; Gauss, D. H. *Angew. Chem. Int. Ed. Eng.* **1991**, 613-629; (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123-6194.
2. (a) Xu, Y.-Z. ; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839-3845; (b) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *Tetrahedron* **1992**, *48*, 1729-1740. (c) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *Tetrahedron Letters* **1992**, *33*, 5837-5840.
3. (a) Webb, T. R.; Matteucci, M. D. *Nucleic Acid Research* **1986**, *14*, 7661-7674; (b) Zhou, X-X.; Chattopadhyaya, J. *Tetrahedron* **1986**, *42*, 5149-5156; (c) Le Brun, S.; Duchange, N.; Namane, A.; Zakin, M. M.; Huynh-Dinh, T.; Igolen, J. *Biochimie* **1989**, *71*, 319-324; (d) MacMillan, A. M.; Verdine, G. L.*Tetrahedron* **1991**, *47*, 2603-2616. (e) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328-4329; (f) Kim, S. J.; Stone, M. P.; Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1992**, *114*, 5480-5481; (g) Gao, H.; Fathi, R.; Gaffney, B. L.; Goswami, B.; Kung, P.-P.; Rhee, Y.; Jin, R.; Jones, R. A. *J. Org. Chem.* **1992**, *57*, 6954-6959.
4. Nikiforov, T. T.; Connolly, B. A. *Tetrahedron Letters* **1991**, *32*, 3851-3854.
5. Sinha, N.; Davis, P.; Usman, N.; Perez, J.; Hodge, R.; Kremsky, J.; Casale, R. *Biochimie* **1993**, *75*, 13-23.
6. Sylvers, L. A.; Wower, J. *Bioconjugate Chemistry* **1993**, *4*, 411-418 and references therein