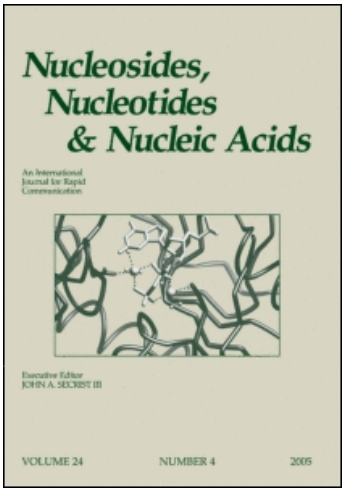

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Synthesis of Oligonucleotide Conjugates with the Aid of *N*-Chloroacetamidohexyl Phosphoramidite Reagent

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SYNTHESIS OF OLIGONUCLEOTIDE CONJUGATES WITH THE AID OF *N*-CHLOROACETAMIDOHEXYL PHOSPHORAMIDITE REAGENT

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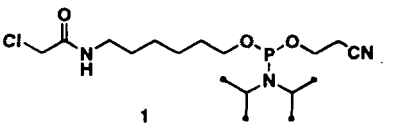
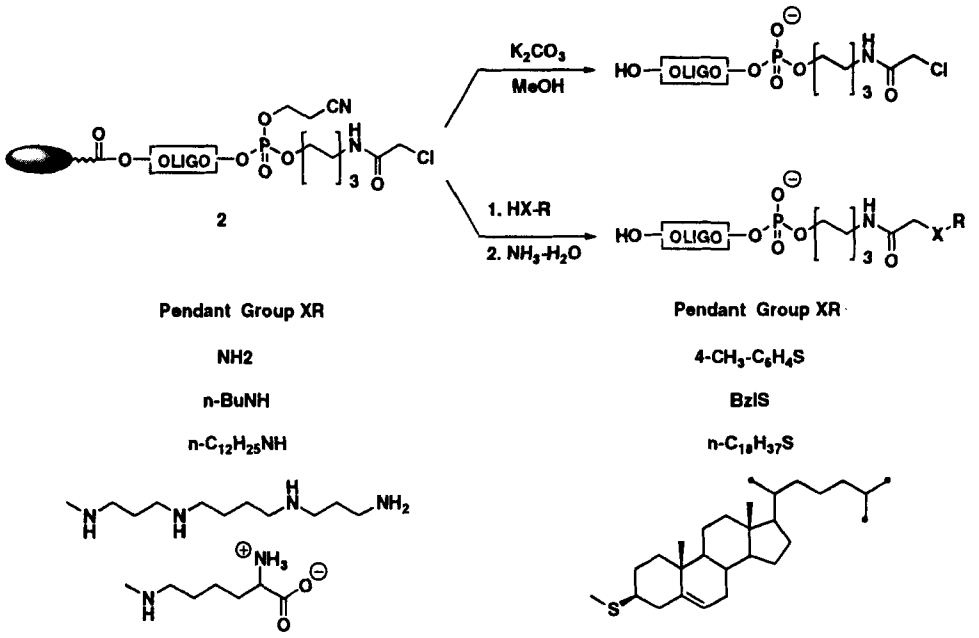
ABSTRACT: A novel phosphoramidite building block derived from *N*-chloroacetyl-6-aminohexanol is attached at the 5'-terminus on the last step of oligonucleotide synthesis. Postsynthetic treatment of support-bound modified oligonucleotides with a variety of amines and mercaptanes affords oligonucleotide conjugates in a high yield.

Synthetic oligonucleotides that bear 5'-terminal functional or reporter group are employed in a number of bioanalytical and antisense applications. Among procedures and reagents that allow their preparation, conjugation of electrophiles to oligonucleotides *via* tethers carrying nucleophilic groups is well known. For the reverse reaction, the available methods to generate electrophilic sites in oligonucleotides are limited. These include periodate oxidation of terminal ribose moiety[1] or the use of an abasic site within the oligonucleotides sequence.[2] Heterobifunctional reagents that bear a phosphoramidite moiety along with an electrophilic functional group that is reactive in orthogonal conditions are of particular interest.[3]



1

Haloacetyl linker that is reactive towards a variety of nucleophiles has been introduced previously into oligonucleotides by postsynthetic reaction in the solution.[4] In this communication we report the synthesis and use in oligonucleotide chemistry of phosphoramidite building block **1**. First, 6-aminohexanol was reacted with succinimido chloroacetate, and the product, 6-(chloroacetamido)hexanol, was successfully converted into **1** by a routine procedure, and characterized by ^{31}P NMR.



In order to examine the usefulness of phosphoramidite **1**, the solid support-bound oligonucleotides **2**, were treated with a variety of either amines or mercaptanes followed by deprotection with concentrated ammonia. The excess reagent may be removed either by treatment with Dowex 50W×8 (PyH^+) or, for mercaptanes and hydrophobic amines, by extraction with an organic solvent. The results suggest that primary alkyl amines, thiophenols in the presence of TEA, and aliphatic mercaptanes in the presence of DBU are highly reactive towards chloroacetamido group in **2**. Practically useful concentration range was estimated as 0.5 to 1.0M for amines and 0.1 to 0.25M for mercaptanes.

REFERENCES

1. Agrawal, S. In *Protocols for Oligonucleotide Conjugates*; Agrawal, S., Ed.; Humana Press: Totowa, **1994**, pp. 93-120.
2. Manoharan, M.; Andrade, L.K.; Mohan, V.; Freier, S.M.; Cook, P.D. *Nucleosides & Nucleotides*, **1997**, *16*, 1741.
3. a) Hovinen, J.; Guzaev, A.; Azhayeva, E.; Azhayev, A.; Lönnberg, H. *J. Org. Chem.* **1995**, *60*, 2205; b) Azhayeva, E.; Azhayev, A.; Guzaev, A.; Hovinen, J.; Lönnberg, H. *Nucleic Acids Res.* **1995**, *23*, 1170; c) Guzaev, A.; Hovinen, J.; Azhayev, A.; Lönnberg, H. *Nucleoides & Nucleotides* **1995**, *14*, 833; d) Hovinen, J.; Guzaev, A.; Azhayev, A.; Lönnberg, H. *J. Chem. Soc. Perkin Trans. I* **1994**, 2745 e) Hovinen, J.; Guzaev, A.; Azhayev, A.; Lönnberg, H. *Tetrahedron*, **1994**, *50*, 7207.
4. Manoharan, M.; Ramasamy, K.S.; Mohan, V.; Cook, P.D. *Tetrahedron Lett.*, **1996**, *37*, 7675.