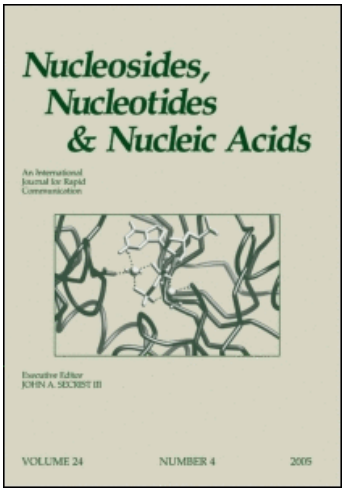

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Synthesis of Terminal Nucleoside Phosphates and Thiophosphates via Phosphoramidite Chemistry

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SYNTHESIS OF TERMINAL NUCLEOSIDE PHOSPHATES AND THIOPHOSPHATES VIA PHOSPHORAMIDITE CHEMISTRY

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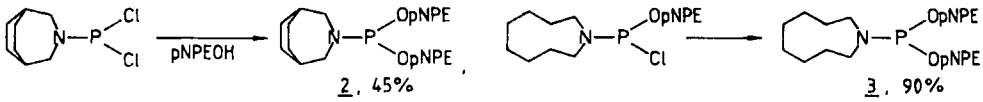
Summary: Synthetic oligonucleotides for use in biological processes often require a 5'-terminal phosphate function. To study enzymatic reactions this group can be replaced by a thiophosphate function. Most chemical methods for the synthesis of these termini base on phosphotriester chemistry.

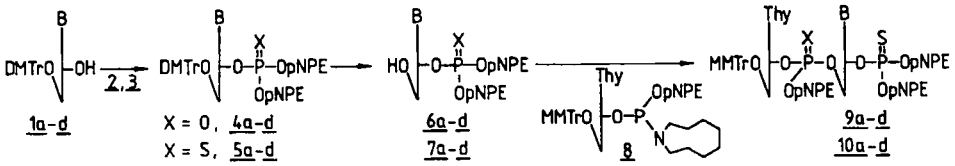
Synthesis of nucleotides with terminal phospho- and thiophosphotriester functions via the phosphoramidite approach demands the preparation of a monovalent phosphitylating agent. Reaction of a dichlorophosphoramidite (1) with two eq. of p-nitrophenylethanol or of a p-nitrophenylethoxy-chlorophosphoramidite (2) with one eq of alcohol yields the desired phosphites 2 and 3, respectively.

Upon activation with tetrazole the bis-(p-nitrophenylethoxy)-phosphoramidites 2 or 3 are condensed with the free 3'-OH of the N-protected 5'-dimethoxytritylated nucleosides 1a - d in acetonitrile. Subsequent oxidation with sulphur or iodine/water yields the fully protected 3'-terminal nucleoside thiophosphates (1) 5a - d and phosphates 4a - d, respectively, in good yields. Detritylation using 2 % toluenesulfonic acid in CH_2Cl_2/CH_3OH (4:1) leads to the protected nucleotides 6a - d and 7a - d. From these the 3'-terminal thiophosphates 7a - d were condensed in acetonitrile with tetrazole activated thymidine phosphoramidite 8 (2).



2, 45% 3, 90%

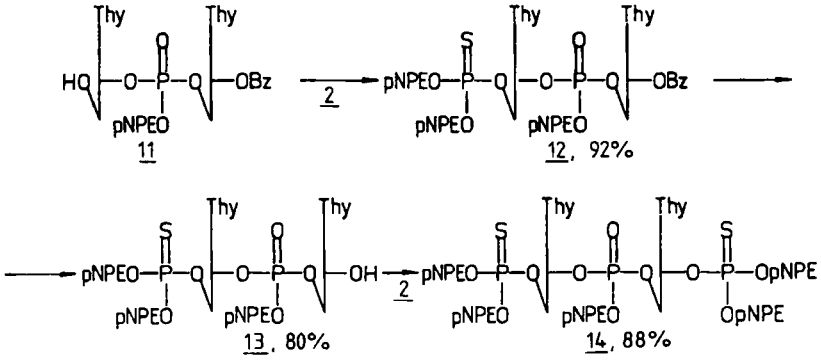
Scheme 1



a = Ade^{pNPEoc}, b = Cyt^{pNPEoc}, c = Gua$^{pNPE}_{iBu}$, d = Thy

4a 86%	5a 82%	6a 90%	7a 88%	9a 77%	10a 79%
4b 87%	5b 86%	6b 88%	7b 88%	9b 78%	10b 77%
4c 88%	5c 88%	6c 92%	7c 94%	9c 81%	10c 86%
4d 92%	5d 86%	6d 92%	7d 92%	9d 81%	10d 80%

Scheme 2



Scheme 3

Following the oxidation with iodine water or sulphur dinucleotides with internucleotidic phosphate and terminal thiophosphate function 9a - d or internucleotidic and terminal thiophosphate function 10a - d were isolated in good yields demonstrating the stability of the terminal thiophosphotriester function to the reaction and work-up conditions.

Additionally a dithymidine phosphotriester 11 protected at the 3'-end with the benzoyl group was condensed with tetrazole activated bis-(p-nitrophenylethoxy)-phosphoramidite 2 in acetonitrile. The intermediate phosphite triester was oxidized with elemental sulphur and the 5'-thiophosphate 12 isolated in good yield. After debenzoylation using methanolic ammonia the resulting dimer 13, which was obtained in 80% yield, was again reacted

with the phosphoramidite 2 in the presence of tetrazole. Again the resulting phosphite triester was oxidized with sulphur. The dithymidine triphosphate analogue 14, in which as well the internucleotidic phosphate as also the terminal thiophosphates are solely protected by the p-nitrophenylethyl group, was obtained in quite good yield. Phosphate derivatives of this type were already synthesized earlier using a chlorophosphodiester for the introduction of the terminal phosphotriester functions (3).

This method is not limited to synthesis in solution but can be applied for the chemical 5'-phosphorylation of oligonucleotides in automated DNA synthesis (4).

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