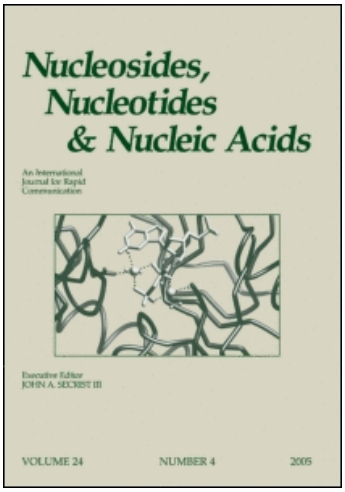

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Efficient Synthesis of Deoxyribonucleoside Phosphoramidites by Eliminating the Use of Additional Activator

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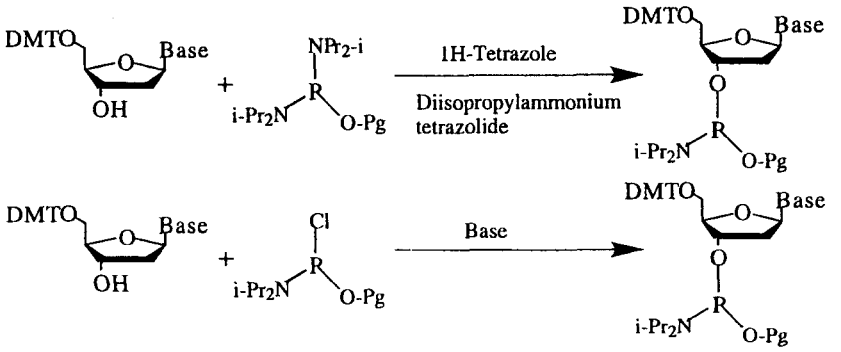
Abstract: An efficient, safe and cost effective synthesis of deoxyribonucleoside phosphoramidites is described. The amine hydrochloride produced as a by-product during the synthesis of nucleoside phosphorbisamidite is used as an internal activator in the synthesis of phosphoramidites.

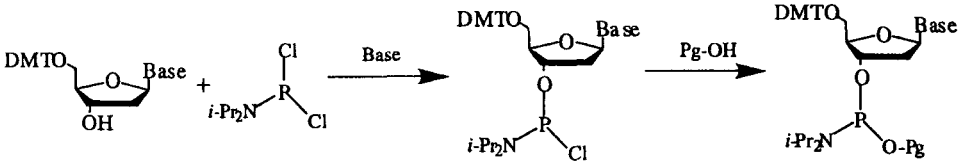
The antisense therapeutic strategy has aroused much interest in the past two decades as a tool to interfere with disease processes at the gene level and as the most basic approach to rational drug design.[1-6] Among the many DNA analogs that have been designed and synthesized as potential antisense drugs, oligodeoxyribonucleotide phosphorothioates have stood out and reached advanced clinical studies against multiple diseases.[7-9] Recent advances in phosphoramidite coupling chemistry[10-14] and solid phase synthesis methodology, together with current state of the art large-scale synthesizers, allow complete assembly of a 20-mer deoxyribonucleotide phosphorothioate at 150 mmole scale (1 kilogram) in just 8 hours. Due to its high efficiency, phosphoramidite coupling followed by stepwise sulfurization of the trialkyl phosphite linkage is the current preferred method, providing >98.5% average coupling yields at 1.75-fold molar amidite excess.[15] Hence there is strong demand for safe and economical ways of preparing deoxyribonucleoside phosphoramidite synthons.

Traditionally, deoxyribonucleoside phosphoramidites have been prepared according to one of the following schemes (Pg = protecting group):

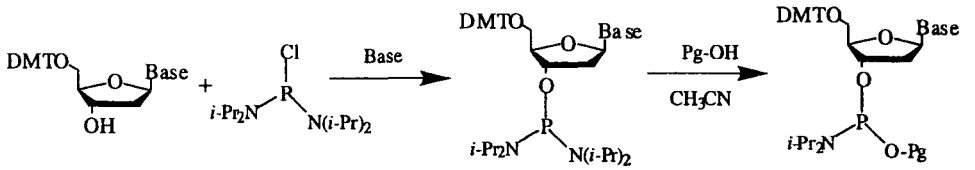


The first scheme[16-19] involves the use of 1*H*-tetrazole or tetrazole salts like diisopropylammonium tetrazolide as activators. In addition to being health hazardous, the use of tetrazole also evokes disposal problems due to its explosive nature.[20] The second synthesis[21-22] involves the preparation of the chloro phosphitylating reagent which is also explosive in nature. Recently trimethylsilyl[23] and 4,5-dicyanoimidazole[24] have been reported as activators for the synthesis of phosphoramidites.

An alternate dichloridite pathway to the synthesis of amidites was proposed[25] by Tanaka *et al.* as shown below:



However, for the very large scale synthesis of deoxyribonucleoside phosphoramidites, atom economy and efficient use of by-products are very crucial. Herein we report a method where potentially explosive activators like tetrazole and separate synthesis of explosive phosphitylating reagents have been eliminated. In addition, the amine hydrochloride by-product formed in the



Pg = *p*-NC-$CH_2C_6H_4CH_2OH$; $HOCH_2CH_2CN$

first step has been used as an activator in the second step, for reaction with the alcohol. Finally, the procedure is carried out in one-pot, making it very attractive for large scale phosphoramidite syntheses. Thus, reaction of 5'-*O*-DMT protected nucleoside with bis(diisopropylamino)chlorophosphine in the presence of triethylamine gave the nucleoside phosphorobisamidite[26-29] in less than 1 h. Without isolation, addition of the protecting group alcohol at room temperature afforded the phosphoramidites as colorless solids in 60-75% isolated yields. Other bases like triethylamine and pyridine were investigated for use in the reaction. Activation by triethylamine hydrochloride was found to be slow (16-20 h, pKa=10-11) but much cleaner. On the other hand, activation by pyridinium hydrochloride (pka = 5.6) was fast but led to the formation of dinucleoside phosphite (5-10%). In either case, purification by column chromatography afforded the products as colorless solids. In the case of 4-cyanomethylbenzyl alcohol,[30] both conditions led rapidly to the formation of phosphoramidites. It was also observed that acetonitrile was a better solvent for the reaction than dichloromethane and gave higher yields of isolated products.

General Procedure for Synthesis of Phosphoramidites: To a stirred solution of bis(diisopropylamino)chlorophosphine (1.18 g; 4.44 mmol; 1.2 eq) and anhydrous pyridine (0.44 g; 5.55 mmol; 1.5 eq.) in anhydrous acetonitrile (50 ml) in a 250 ml round bottomed flask under argon was added 5'-*O*-dimethoxytrityl-N^2-isobutyryl-2'-deoxyguanosine as a solid. The reaction mixture was stirred at room temperature for 30 min. and then a solution of 4-cyanomethylbenzyl alcohol (0.63 g; 4.28 mmole; 1.15 eq.) in anhydrous acetonitrile (dried over 4A sieves) was added. After stirring the reaction mixture for 30 min., all the volatiles were evaporated under vacuum and the residue partitioned between dichloromethane (100 ml) and saturated sodium bicarbonate solution (100 ml). The aqueous layer was back extracted with dichloromethane (100 ml). The combined organic extracts was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography using silica gel and hexanes/ethyl acetate as eluants. Yield: 2.52 g (2.75 mmol; 74%).[31]

References and Notes:

1. Crooke, S.T. In *Therapeutic Applications of Oligonucleotides*, (Ed.: Crooke, S.T.), R.G. Landes Co., **1995**, and references cited therein.
2. Crooke, S. T. In *Burger's Medicinal Chemistry and Drug Discovery*; M. E. Wolff, Ed.; John Wiley & Sons: New York, **1995**; Vol. 1; pp 863.
3. Crooke, S.T. In *Progress in Oligonucleotide Therapeutics*, American Society for Pharmacology and Experimental Therapeutics, Spring Meeting, April 24-28, **1994**, Anaheim, CA, USA.
4. Sanghvi, Y.S. In *Comprehensive Natural Products Chemistry*, (Eds.: Barton, D.H.R.; Nakanishi, K.) in Volume 7: *DNA and Aspects of Molecular Biology*, (Ed. Kool, E.T.) Pergamon Press, in press.
5. Knorre, D. G.; Vlassov, V. V.; Zarytova, V. F.; Lebedev, A. V.; Federova, O. S. *Design and targeted reactions of Oligonucleotide derivatives*; CRC Press: Boca Raton, **1994**.
6. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
7. Kisner, D. L. *12th International Roundtable on Nucleosides and Nucleotides*, September 19, **1996**, La Jolla, CA, USA.
8. Agrawal, S.; Zhang, X.; Lu, Z.; Zhao, H.; Tamburin, J. M.; Yan, J.; Cai, H.; Diasio, R. B.; Habus, I.; Jiang, Z.; Iyer, R. P.; Yu, D.; Zhang, R. *Biochem. Pharmacol.*, **1995**, *50*, 571.
9. Crooke, S. T.; Grillone, L. R.; Tendolkar, A.; Garrett, A.; Fraktin, M. J.; Leeds, J.; Barr, W. H. *Biochem. Pharmacol.*, **1994**, *56*, 641.
10. McBridge, L.C., Caruthers, M.H. *Tetrahedron Lett.* **1983**, *24*, 245.
11. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223.
12. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123.
13. Sinha, N.D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843.
14. Lyttle, M.H.; Wright, P.B.; Sinha, N.D.; Bain, J.D.; Chamberlin, A.R. *J. Org. Chem.* **1991**, *56*, 4608.
15. Scozzari, A.N. *Large Scale Oligonucleotide Synthesis : Production, Purification and Analysis*, IBC Conference, October 28-29, **1997**, San Diego, CA, USA.
16. Nielsen, J.; Taagaard, M.; Marugg, M.; van Boom, J.H.; Dahl, O. *Nucleic Acids Res.* **1986**, *14*, 7391.

17. Nielsen, J.; Marugg, M.; van Boom, J.H.; Honnens, J.; Taagaard, M.; Dahl, O. *J. Chem. Res. (S)* **1986**, 26.
18. Hamamoto, S.; Takaku, H. *Chem. Lett.*, **1986**, 1401.
19. Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 3626.
20.. Sigma-Aldrich Library of Chemical Safety 2; Sigma-Aldrich: Milwaukee, **1990**.
21. Hering, G.; Stöcklein-Schneiderwind, R.; Ugi, I.; Pathak, T.; Balgobin, N.; Chattopadhyaya, J. *Nucleosides Nucleotides* **1985**, *4*, 169.
22. Ugi, I.; Bauer, J.; Fontain, E.; Götz, J.; Hering, G.; Jakob, P.; Landgraf, B.; Karl, R.; Lemmen, P.; Stöcklein-Schneiderwind, R.; Schwarz, R.; Sluka, P.; Balgobin, N.; Chattopadhyaya, J.; Pathak, T.; Zhou, X.X. *Chem. Scr.* **1986**, *26*, 205.
23. Dabkowski, W.; Tworowska, I.; Michalski, J.; Cramer, F. *J. Chem. Soc., Chem. Commun.*, **1997**, 877.
24. Vargeese, C.; Carter, J.; Yegge, J.; Krivjansky, S.; Settle, A.; Kropp, E.; Peterson, K.; Pieken, W. *Nucleic Acids Res.*, **1998**, *26*, 1046.
25. Tanaka, T.; Tamatsukuri, S.; Ikehara, M. *Tetrahedron Lett.* **1986**, *27*, 199.
26. Marugg, J.E.; Burik, A.; Tromp, M.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.* **1986**, *27*, 2271.
27. Uznanski, B.; Wilk, A.; Stec, W.S. *Tetrahedron Lett.* **1987**, *28*, 3401.
28. Yamana, K.; Nishijima, Y.; Negishi, K.; Yashiki, T.; Nishio, K.; Nakano, H.; Sangen, O. *Tetrahedron Lett.* **1991**, *32*, 4721.
29. Yamana, K.; Nishijima, Y.; Oka, A.; Nakano, H.; Sangen, O.; Ozaki, H.; Shimidzu, T. *Tetrahedron* **1989**, *45*, 4135.
30. 4-Cyanomethylbenzyl group has been found to be a useful group for the synthesis of oligodeoxyribonucleotide phosphorothioates.
31. Capaldi, D.C.; Eleuteri, A.; Cole, D.L.; Ravikumar, V.T. *Nucleic Acids Res.* **1998** (submitted).

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