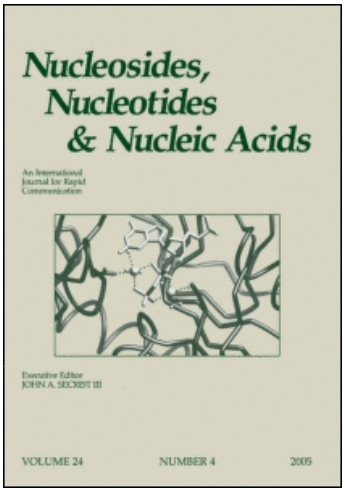

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Hering, G. , Stöcklein-schneiderwind, R. , Ugi, I. , Pathak, T. , Balgobin, N. and Chattopadhyaya, J.(1985) 'Preparation and Properties of Chloro-N, N-dialkylamino-2,2,2-trichloroethoxy- and Chloro-N, N-dialkylamino-2,2,2-trichloro-1,1-dimethylethoxyphosphines and their Deoxynucleoside Phosphiteamidates', Nucleosides, Nucleotides and Nucleic Acids, 4: 1, 169 — 171
To link to this Article: DOI: 10.1080/07328318508077846
URL: http://dx.doi.org/10.1080/07328318508077846

NUCLEOSIDES & NUCLEOTIDES, 4(1&2), 169-171 (1985)

Preparation and Properties of Chloro-N,N-dialkylamino-2,2,2-trichloroethoxy- and Chloro-N,N-dialkylamino-2,2,2-trichloro-1,1-dimethylethoxyphosphines and their Deoxynucleoside Phosphiteamidates.

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Summary: Trichloroethyl- and trichloro-t-butyl phosphiteamidates building blocks,(2) to (11), have been prepared for DNA synthesis and their relative rates of activation with several acids have been estimated along with two model oligothymidylic acid syntheses on a solid support.

Significant developments have been made in the chemical synthesis of DNA fragments using solid phase methodologies in the phosphotriester[1] and the phosphite-triester [2,3] approaches. In the latter approach, the internucleotide linkage is usually protected by a methyl group[4,5] ; however, there are also literature reports of 2,2,2-trichloroethyl-[2] and 2,2,2-trichloro-1,1-dimethylethyl-[6] groups, using the corresponding dichloridites as phosphitylating agents. It has been recently shown that the 5'-protected-2'-deoxynucleoside phosphoramidates of the general formula (1) are useful intermediates for DNA synthesis[3]. We describe here synthetic procedures for the preparation of building blocks, (2) to (9), using either Fourrey's procedure[7], or through the use of monofunctional phosphitylating agents,(12) to (14), and subsequently show some of their properties.

General procedure for the preparation of Chloro-N,N-dialkylamino-(2,2,2-trichloro-1,1-dimethylethoxy)phosphine: (12) to (14)

Dimethylamine (56.8g, 1.26 mol) in dry diethyl ether (100 ml) was added dropwise at -10°C to a dry diethyl ether (300 ml) solution of 2,2,2-trichloro-1,1-dimethylethoxydichlorophosphine (176g, 0.63 mol), and stirred overnight at 21°C. The precipitated dimethylamine hydrochloride was removed by filtration. The residue was washed with dry diethyl ether (500 ml). After evaporation of the combined filtrate and the residue was distilled in vacuo to give 163g (90.4%) of (12); bp: 74-78°/0.5 torr; mp: 34-38°; ^{31}P-NMR: 167.7 ppm.

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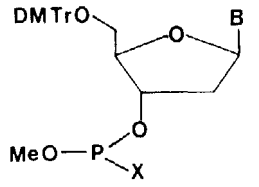
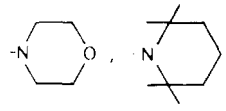
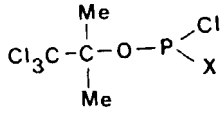


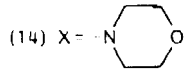
X= NMe_2, $N(i\text{-}Pr)_2$, -N

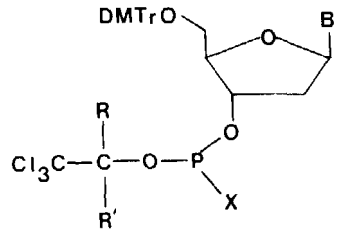


General formula (1)



(12) X = $-NMe_2$ (13) X = $-N(i\text{-}Pr)_2$





(2) R=R'=H ; X= $-NMe_2$; B= A^{mClBz}

(3) R=R'=H ; X= $-NMe_2$; B= C^{Bz}

(4) R=R'=H ; X= $-NMe_2$; B= G^{TBB}

(5) R=R'=H ; X= $-NMe_2$; B= T

(6) R=R'=Me; X= $-NMe_2$; B= A^{mClBz}

(7) R=R'=Me; X= $-NMe_2$; B= C^{Bz}

(8) R=R'=Me; X= $-NMe_2$; B= G^{TBB}

(9) R=R'=Me; X= $-NMe_2$; B= T

(10) R=R'=Me; X= $-N(i\text{-}Pr)_2$; B= T

(11) R=R'=Me; X= -N O ; B= T

A^{mClBz} = 6-N-(m-chlorobenzoyl)-9-adeninyl-

C^{Bz} = 4-N-benzoyl-1-cytosinyl

G^{TBB} = 2-N-(t-butylbenzoyl)-9-guaninyl-

^{1}H-NMR ($CDCl_3$): δ 1.9 (6H; Cl_3C-CMe_2-); 2.6 & 2.8 (two s, 6H, NMe_2).

Compounds (13) and (14) were similarly obtained, using the above general procedure, in 68 and 87% yields respectively as crystalline solids.

Compound (13): bp 104-109°C / 0.5 torr; mp 46-51°C; ^{31}P-NMR 169.2

Compound (14): bp 98-100°C / 0.06 torr; mp 52-53°C; ^{31}P-NMR 158.4

General procedures for the preparation of 5'-O-dimethoxytrityl-3'-O-(N,N-dialkylamino) phosphoramidates: (2) to (11)

Method A: To a well stirred solution of the 5'-DMTr-2'-deoxyribonucleoside block (1mmol) and diisopropylethylamine (7 mmol) in dichloromethane (4 ml) was added the solid monochloridite (2 mmol) and the reaction mixture stirred for 40 min. The reaction mixture was then poured into ethylacetate (60 ml) and washed with saturated sodium chloride (4X40 ml). The ethylacetate layer was dried ($MgSO_4$) and coevaporated with toluene. The residue was dissolved in a mixture of dichloromethane-ethylacetate-triethylamine (2:2:1; v/v/v) and a short silica gel column was run, using the same mixture as an eluant[8]. The desired compound was rapidly eluted from the column, coevaporated with toluene and then precipitated into hexane (at -78°C) and dried. Compounds (6), (7), (8) and (9) were thus obtained in 80, 91, 20 and 90% yields respectively with the following ^{31}P-NMR absorptions ($CDCl_3$): 140.2 & 140.2 for (6); 140 & 141 for (7);

140.4 & 140.9 for (8); 139.8 & 140.7 for (9).

Method B: This corresponds to a one-pot procedure that is described by Fourrey and his coworkers[7]. The mononucleotide blocks (6) to (9), were obtained using this procedure in 80, 94, 86 and 85 % yields, respectively. Building blocks, (2) to (5), were also obtained using method B in 94, 93, 90 and 86% yield, respectively. It should be noted that the trichloroethyl amidate blocks were not stable to the column chromatographic conditions that are described in method A. Crude blocks, (2) to (5), however had following ^{31}P-NMR ($CDCl_3$) absorptions: 148.8 for (2); 148.1 & 148.7 for (3); 148.3 & 149.2 for (4); 148.3 & 148.9 for (5).

In order to evaluate the usefulness of these building blocks,(2) to (11), we have subsequently attempted to estimate relative rates of their activation by several acidic catalyst using ^{31}P-NMR. Thus a reaction of (5) with 1-H-tetrazole, N-methylimidazole.HCl, 5-trifluoromethyltetrazole (3 eq. in CH_2Cl_2) showed activation times of 5, 2, and 2 min., respectively; similarly, a reaction of (11) with the above acidic catalysts, under an identical condition, showed activation periods of 5,2 and 2 min., respectively. These activation periods were also independently evaluated by their ability to react with 3'-acetyl thymidine to give fully protected TpT. It was found during these experiments that 5-trifluoromethyltetrazole, followed by N-methylimidazole.HCl, gave the best relative overall results among all activating agents, including N,N-dimethylaniline.HCl and N,N-dimethylaminopyridine.HCl. Details of this work will be reported in a full paper.

Finally we have employed (5) and (9) in the synthesis of dodeca- and octa-thymidylic acids using solid phase methodology reported in the literature[3]. It emerged during these model solid phase studies that compound (5) gave an average yield of 90% for each condensation reaction during the dodecathymidylic acid synthesis while compound (9) gave an average yield of 70%; The desired compound was subsequently deprotected and purified by HPLC[9] and the compounds that were eluted under the last peak were characterized to be the titled compounds by ^{32}P-labelling and electrophorsis with authentic materials.

Acknowledgements:
I.U. and his coworkers acknowledge gratefully the support by Deutsche Forschungsgemeinschaft (SFB 145) and Fonds der Chemischen Industrie; J.C. and his coworkers thank the Swedish board for Technical Development for generous financial assistance.

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