Bis[2,2,2-tribromoethyl] Phosphorochloridate: A Suitable Phosphorylating Agent for Nucleosides

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In spite of the great variety of monofunctional phosphorylating agents[1] known, there still seems to be a need for a mild procedure that will result in a stable triester. Our aim is to introduce the 5'-phosphomonoester functionality to deoxy- and ribonucleotides containing phosphotriester linkages via phosphotriester intermediates. The 5'-phosphates are necessary for a variety of enzymatic reactions. The widely used procedure[2] which predominantly gives 5'-phosphorylated ribonucleosides has been shown in later work to yield also some mixtures of phosphorylated nucleosides[3] as well as cyclic phosphates[4] and phosphoramidates[5].

Since 2,2,2-trichloroethyl phosphates[6] can be split off by a variety of reductive procedures, we tried to substitute the trichloroethyl by the tribromoethyl group. The difference in the reduction potential between trichloro- and tribromoethyl groups amounts to almost 1 eV[7] and makes the 2,2,2-tribromoethyl group attractive for a mild reducing procedure.

Efforts to synthesize bis[2,2,2-tribromoethyl] phosphorochloridate in an analogous fashion to the preparation of bis[2,2,2-trichloroethyl] phosphorochloridate[6] were not successful. In addition, the reaction of tribromoethyl phosphorodichloridate[8] with 2,2,2-tribromoethanol in the presence of various bases such as triethylamine, 1,2,4-triazole, 1-methylimidazole, or 5-chloro-1-ethyl-2-methylimidazole[9] furnished the desired product, though in variable amounts and it was impossible to isolate the analytically pure compound. We therefore developed a new route to the target compound by constructing the phosphorochloridate functionality in the last step via a Michaelis-Arbuzov type reaction. Starting from the easily accessible ethyl phosphorodichloridite[10] (1) and 2,2,2-tribromoethanol (2), the ester 3 was synthesized and directly transformed by treatment with phosgene[11] to the reagent 4 in 60% overall yield.
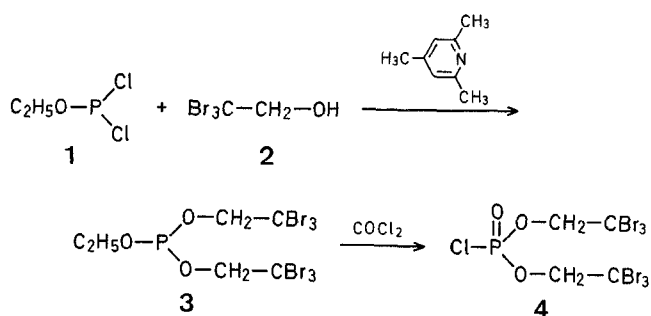
This phosphorylating agent is fairly stable and can be handled in the open air. Phosphorylation of ribonucleosides in pyridine was very slow. Activation of the chloridate 4 by 1,2,4-triazole and/or 1-methylimidazole or 4-dimethylaminopyridine enhanced the reactivity but lowered the selectivity for the 5'-position. Thus, we decided to protect the 2',3'-hydroxy functions before phosphorylation, with the goal to cleave off the protecting groups in one step during electrodeblocking.

We started from the commercially available 2',3'-isopropylidene nucleosides 5a-e. Phosphorylation with 4 was accomplished in good to excellent yields giving the fully protected 5'-phosphorylated nucleosides 6a-e. These stable and crystalline compounds can be stored at room temperature without detectable decompo-

sition for a long time. Although it was possible to deprotect these triesters with various zinc-type procedures[1] and the zinc-copper couple[6], the results were poorly reproducible and the yields quite unsatisfactory. Our final deprotection to the 5'-phosphates was nicely accomplished by electrochemical deblocking[7], a method which had also worked very satisfactorily in the case of diphenyl 2-haloethyl phosphates[12] and oligodeoxynucleotides[13]. In the presence of water the reaction, as depicted, should directly furnish the unprotected 5'-phosphates 7a-e.
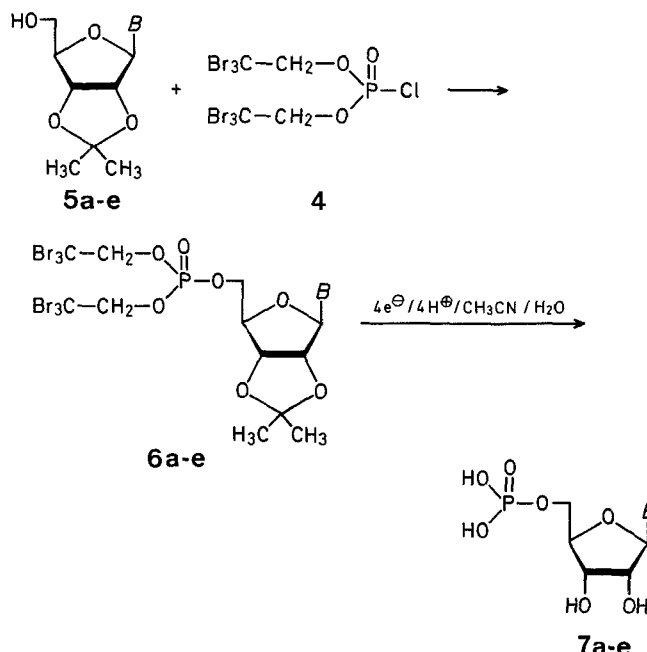
Due to the different stabilities of the isopropylidene protecting groups, the reaction times varied.

We were thus able to employ the anodically generated protons for the removal of the isopropylidene group. If necessary, for acid-sensitive functions such as the monomethoxytrityl group, we consequently resolved this problem through simply buffering the anolyte with e.g. sodium hydrogen carbonate. Our reagent 4 thus can introduce a phosphomonoester functionality to nucleosides and nucleotides such as 2',5'-ApApA[14].

Bis[tribromoethyl] Phosphorochloridate (4):

C₄H₄Br₆ClO₃P   calc.   C 7.43   H 0.69   Hal 38.40

(645.9)	found	7.46	0.49	38.35

^{1}H-N.M.R. (CDCl₃): δ = 4.93 ppm (d, J = 7 Hz).

^{31}P-N.M.R. (dioxan/85% H₃PO₄, ext.): δ = −4.65 ppm.

Table. Phosphorylation of Nucleosides 5a-e with Bis[2,2,2-tribromoethyl]Phosphorochloridate (4)

Nucleo-side	Nucleo-tide	Yield [%]	m.p. [°C]	Molecular formula[a]	^{1}H-N.M.R. (CDCl$_3$/DMSO-d_6/TMS) δ [ppm]	U.V. (CH$_3$OH) λ_{max} [nm] (log ε)
5a	6a	80	190–191°	C$_{16}$H$_{18}$Br$_7$N$_2$O$_9$P (972.6)	1.33, 1.55 (2s, 6H); 4.83 (d, 4H, $J=6$ Hz); 5.80 (d, 1H, $J=2$ Hz); 8.07 (s, 1H)	272 (3.89)
5b	6b	73	156–160°	C$_{17}$H$_{19}$Br$_6$N$_4$O$_8$P (917.7)	1.51, 1.73 (2s, 6H); 4.85, 4.90 (2d, 4H, $J=6$ Hz); 6.22 (d, 1H, $J=2$ Hz); 8.06, 8.26 (2s, 2H)	242 (4.04)
5c	6c	86	171–173°	C$_{17}$H$_{19}$Br$_6$N$_4$O$_7$PS (933.8)	1.39, 1.61 (2s, 6H); 4.76, 4.79 (2d, 4H, $J=6$ Hz); 6.23 (d, 1H, $J=3$ Hz); 8.06, 8.26 (2s, 2H)	320 (4.36)
5d	6d	94	——[b]	C$_{17}$H$_{20}$Br$_6$N$_5$O$_7$P (960.9)	1.39, 1.64 (2s, 6H); 4.75, 4.79 (2d, 4H, $J=6$ Hz); 6.11 (d, 1H, $J=3$ Hz); 7.94, 8.04 (2s, 2H)	258 (4.15)
5e	6e	90	210° (dec.)	C$_{17}$H$_{20}$Br$_6$N$_5$O$_8$P (932.7)	1.41, 1.62 (2s, 6H); 4.83, 4.89 (2d, 4H, $J=6$ Hz); 6.11 (d, 1H, $J=1–2$ Hz); 7.72 (s, 1H)	251 (4.17) [268 (4.01)][c]

[a] Satisfactory microanalyses obtained: C ±0.45, H ±0.19, N ±0.26.

[b] Freeze dried from dioxan, analysis calculated with 0.5 mol dioxan according to ^{1}H-N.M.R. spectrum.

[c] Shoulder.

Phosphorylation of Nucleosides 5a–e with Reagent 4:

The protected nucleosides 5a–e (2 mmol) are dried by coevaporation with anhydrous pyridine and dissolved or suspended in dry pyridine (5 ml). At 4°C, a solution of 4 (2 g, 3 mmol) in dry pyridine (5 ml) containing 1,2,4-triazole (275 mg, 4 mmol) and 1-methylimidazole (660 mg, 6 mmol) is added over 2 h and stirring is continued overnight at 4°C. The organic phase is evaporated, partitioned between chloroform (50 ml) and water (25 ml), the organic layer dried and evaporated, and the residue crystallized from methanol (see Table).

Electrochemical Deprotection of Nucleotides 6a–e:

The triesters 6a–e (0.25 mmol), dissolved in acetonitrile[15]/dimethylformamide (27/3 ml) and lithium perchlorate trihydrate (20 mmol) are filled as catholyte in a divided (Nafion 125 membrane) electrolysis cell[12] (auxiliary electrode: platinum wire, working electrode: mercury pool, quasi reference electrode: silver wire) and 0.4 molar lithium perchlorate trihydrate solution in acetonitrile (10 ml) as anolyte. The cathode potential is set at -0.55 V (Ag-wire) and electrolysis is monitored coulometrically. After the current has reached its residual value the reaction is followed chromatographically (T.L.C. on cellulose with 7:1:4 isopropanol/ammonia/water) to monitor the isopropylidene cleavage which, for the case of guanosine requires warming to 50°C for completion. The phosphates 7a–e are either purified by DEAE-Sephadex chromatography or more simply by RP 8 column filtration by first eluting the lithium perchlorate salt with acetonitrile followed by water giving the following chromatographically and U.V. pure 5'-phosphates; yields: 7a (82%); 7b (89%); 7c (55%); 7d (80%); 7e (86%); identical with authentic samples (Böhringer Mannheim, Sigma).

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