Tetrahedron Letters,Vol.25,No.14,pp 1437-1440,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

SYNTHESIS OF DEOXYNUCLEOSIDE METHYLPHOSPHONATES VIA A PHOSPHONAMIDITE APPROACH

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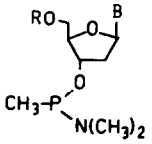
Deoxynucleoside methylphosphonamidites 1 have been synthesized as building monomers for the stepwise synthesis of methylphosphonate analogs of oligonucleotides.

Recently we introduced CH_3PCl_2 as a reagent for the synthesis of phosphonate analogs of oligodeoxyribonucleotides [1]. Following this, a similar approach has been applied by Köster et al. for solid phase synthesis [2]. A serious drawback of this method, however, poses the instability of the intermediate reactive deoxyribonucleoside methylphosphonochloridites towards hydrolysis as well as the unrepressible formation of symmetrical 3',3'-dinucleoside methylphosphonates.
We therefore envisaged the use of a bifunctional phosphonylation reagent with improved selectivity yielding also more stable intermediates. Nucleoside methylphosphonamidites of type 1 were found to be suitable intermediates for this purpose. As phosphonylation agent for their synthesis we finally selected $CH_3P(NMe_2)_2$, the amino groups of which can be exchanged under quite different conditions.

The synthetic procedure for 1a is as follows: 5'-O-tritylthymidine (1.00 mmol) is dissolved in 6 ml of dry $CHCl_3$ under an atmosphere of nitrogen and $CH_3P(N(CH_3)_2)_2$ (2.00 mmol) is added. Reaction is complete after 12 h, but the reaction time can be reduced to 2 h by the addition of catalytic (0.1 mmol) amounts of collidine hydrochloride. The purification procedure consists of twofold extraction with an aqueous saturated solution of NaCl (50 ml, 0.1 ml NEt_3 added), drying over anhydr. Na_2SO_4 and evaporation to a foam. The remainder is stirred with 50 ml of pentane for 2 h, filtered off, dissolved in 2 ml of diethyl ether and added dropwise to 40 ml of pentane. After filtering off the suspension and drying 82-89 % of 1a are obtained as a white powder. Similarly 1b can be isolated in 85-93 % yield. Compounds 1 give satisfactory elementary analyses; they were also characterized by their oxidation products 2a and 2b which can be obtained in good yields by treatment of 1a or 1b with t-butylhydroperoxide.
Characteristic ^{31}P-NMR show contamination of 1a with up to 3 % of an unidentified compound at -207 ppm, up to 3 % of hydrolyzed product (nucleoside methylphosphinate) and 1 % of

phosphonylation agent, but no traces of 3',3'-dinucleoside phosphonite could be detected. Stored as a powder at -20°C 1a is stable for at least a month without decomposition by moisture or air oxidation.



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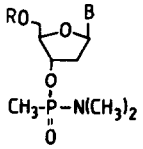
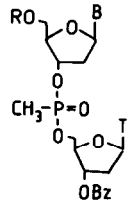
	R =	B =	Yield	^{31}P-NMR[a] (ppm)
1a	Tr	T	82-89 %	-139.6/-140.7
1b	MMTr	A^{Bz}	85-93 %	-145.3
1c	DMTr	C^{Bz}	94 %[b]	-146.2/-145.8
1d	DMTr	G^{ibu}	95 %[b]	-144.8
2a	Tr	T	75 %	- 40.1
2b	MMTr	A^{Bz}	64 %	- 40.7



2

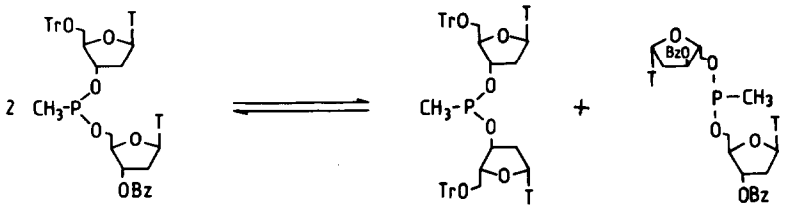
a) in $ClCH_2CH_2Cl$, relative to 85 % H_3PO_4;
b) estimated yield from P-NMR.

A variety of phosphonylation reagents of type $CH_3PCl(NR_2)$ (NR_2 = NMe_2, NEt_2, $Npr^i{}_2$, N(Me)Ph, $N Ph_2$) has also been tested, but $CH_3P(N(CH_3)_2)_2$ proved to be the ideal reagent of choice due to its simple preparation and purification [3], its stability against hydrolysis or air oxidation and its easy handling. Furthermore, reaction of some of the phosphonochloridites give irreproducible yields of phosphonylated products with a lower purity.
Compounds 1 are stable towards catalytic amounts of acids such as collidine hydrochloride or trialkylammoniumchlorides, but like nucleoside phosphoramidites [4] they can be activated by the additions of more than equimolar amounts of acid [5]. We have found that a threefold excess of 1H-benzotriazole is suitable for this purpose. For the condensation reaction 0.20 mmol of 3'-O-benzoylthymidine and 0.80 mmol of 1H-benzotriazole are separately dried by evaporation with CH_3CN, dissolved in 1.00 ml of CH_3CN and added to 0.20 mmol of solid 1a. After 1 min. at RT reaction is complete and the resulting very labile (both to air and moisture) phosphonite can be oxidized with 0.25 mmol of t-but-OOH. 81 % of dinucleoside methylphosphonate 3a are obtained. Analysis via high pressure liquid chromatography and comparison with pure reference materials showed 1 % of 3',3'-phosphonate as contamination, but no trace of the corresponding 5',5'-phosphonate was present. Similar reaction of 1b with 3'-benzoylthymidine gave 3b in 80 % yield.



	R =	B =	Yield
3a	Tr	T	81 %
3b	MMTr	A^{Bz}	80 %

For the condensation reaction chloroform or acetonitrile can be used as solvents while THF or pyridine require longer reaction times and give rise to side reactions. Even in $CHCl_3$ or in CH_3CN a short reaction time is desirable if pure products are to be obtained. The side reaction, a slow acid-catalyzed ligand exchange reaction of the unsymmetrical phosphonous diester I, occurs to give a statistical mixture of all possible diesters I, II and III after 1 day. Even after 15 min. at RT 6 % of III are formed.

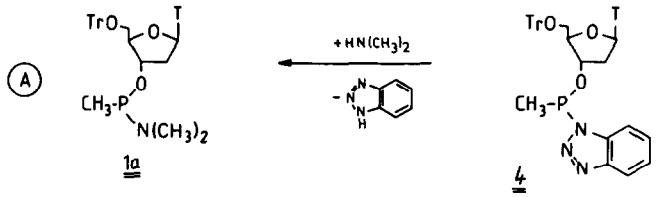


I II III

This reaction is even more rapid with tetrazole as activating agent. Similar disproportionation reactions have been observed by Hoffmann et al. for dialkyl alkylphosphonites [6], and for the reorganization of trialkylphosphites Moedritzer et al. established a strong catalytic effect of acids [7].

Tetrazole-catalyzed activation of nucleoside phosphoramidites seems to be based on the formation of a reactive tetrazolide intermediate [4]; similar intermediates have been inferred for the reaction of phosphoramidites with alcohols catalyzed by acetic acid [8a] or aniline hydrochloride [8b]. We could not detect such an intermediate during the action of 1H-benzotriazole on 1a in the absence or presence of other nucleophiles. When this reaction was monitored in the absence of an alcohol no immediate change in its ^{31}P-NMR-spectrum could be found. Longer reaction times (4 h) led, instead, to a 50 % disproportionation of 1a into 3',3'-phosphonite II and $CH_3P(N(CH)_3)_2)_2$.

Spectroscopic evidence shows that 4 does exist and is a stable compound which can be prepared by reaction of CH_3PCl_2 with 2 equiv. of 1H-benzotriazole (in the presence of an acid scavenger) followed by the addition of 1 equiv. of 5'-O-tritylthymidine (^{31}P NMR for 4: -135/-137 ppm). For 4 a qualitatively similar rate of substitution with 3'-O-benzoylthymidine was found as for 1a in the presence of 1.5 equiv. of activating agent. This rules out the possibility of 4 as being a highly reactive intermediate in the condensation reaction. If a solution of 4 is treated, however, with dimethylamine, 4 disappears and phosphonamidite 1a is formed quantitatively. Reaction A (see below) therefore seems to be a rapid acid catalyzed reaction where the equilibrium is shifted completely to the left. The catalytic function of 1H-benzotriazole therefore is essentially based on its acidic properties (pK_a = 8.6 [9]), i.e. its ability to facilitate the release of amine from phosphonamidite 1a but not through formation of an intermediate as 4.



The experiments described above indicate that dimethylaminophosphanes of type 1 can be used under appropriate conditions for the synthesis of deoxyribonucleoside methylphosphonates. Furthermore, this method should be compatible with Caruther's solid-phase synthesis of oligonucleotides [4] to produce compounds where a phosphodiester is selectively replaced by a methylphosphonate group.

LITERATURE

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(Received in Germany 30 December 1983)