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A CONVENIENT AND GENERAL APPROACH TO THE SYNTHESIS OF PROPERLY PROTECTED d-NUCLEOSIDE-3'-HYDROGENPHOSPHONATES *VIA* PHOSPHITE INTERMEDIATES

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Abstract: Evidence will be presented to show that the monofunctional phosphitylating reagents *bis*(N,N-di-ethylamino)chlorophosphine and salicylchlorophosphine are very effective for the preparation of 5'-O,N-protected d-nucleoside-3'-hydrogenphosphonates.

The possibility of a H-phosphonate function to serve as a source for the introduction of a 3'-5'-internucleotidic linkage was reported for the first time by Todd et al.[1]. This principle was explored further in more recent studies by several other groups. For instance, Hata et al.[2] introduced a 3'-5'-phosphodiester linkage by coupling the d-nucleoside-3'-phosphonate 4 [B=T; R^1=4,4'-dimethoxytrityl (DMTR)] with a 3'-O-protected d-nucleoside by employing oxidative phosphorylating conditions (i.e. 2,4,6-tri-isopropylbenzenesulphonyl chloride in the presence of 2,2'-pyridinyl disulfide). Garegg et al.[3] and, more recently, Matteucci et al.[4] accomplished the same goal by condensing 4 (B=T; R^1=DMTR) with an incoming d-nucleoside, in the presence of an appropriate condensing reagent [i.e. diphenylchlorophosphate[3] (Todd's reagent[1]) or, more effectively, pivaloyl chloride[4]], to afford an intermediate 3',5'-internucleotidic H-phosphonate bond. The latter can easily be oxidized[5] (iodine/water/pyridine) to give the required phosphodiester linkage. It is also interesting to note that a H-phosphonate function can be used for the preparation of valuable phosphodiesters[6] or modified phosphonates[7] (i.e. phosphorthioates).

The above shortly summarized findings show that H-phosphonates of nucleosides are versatile intermediates for the preparation of nucleic acids derivatives. However, a reliable and economical route to the synthesis of hydrogenphosphonate derivatives of nucleosides has, to our knowledge, not yet been published[8].

We now report a general and simple method for the preparation of this type of compounds using the monofunctional phosphitylating reagents 2b and 5.

In a previous paper[9] we showed that phosphitylation of 1 (B=T; R^1=DMTR) with reagent 2a (R^2=iPr) yielded intermediate 3 [B=T; R^1=DMTR; R^2=isopropyl (iPr)]. The latter could then be converted by acidolysis ($HOAc/H_2O$) into the 3'-phosphonate 4 (B=T; R^1=H). This approach is however not completely satisfactory: removal of the DMTR-group occurs and the rate of hydrolysis of the *bis*(N,N-di-isopropylamino)phosphine function to give 4 (B=T; R^1=H) is rather slow (total reaction time is 8 h at 20°C). In order to eliminate these shortcomings we prepared the sterically less hindered reagent 2b[10] and observed that intermediate 3[11] (B=T; R^2= Et) could be converted, within a reasonable short time and without concomitant release of the DMTR-group, into the 3'-phosphonates 4. For example, the preparation of 4 (B=T; R^1=DMTR) was performed as follows. To a solution of 3 (B=T; R^1=DMTR; R^2=Et, 1 mmol) in dry dioxane (5 ml) was added dry acetic acid (6 mmol). Monitoring of this step by ^{31}P-n.m.r. spectroscopy indicated[12] that the replacement of the diethylamino by acetyl groups was complete within 1 h at

Table: Yields and other relevant data on the synthesis of 4.

Starting product 1[a]	Yield (%) of 4 *via* Method A or B		^{31}P-NMR[b] data of 4	^{1}H-NMR[c] data of 4			R_f-values[d] of 4
				P-H[e]	H1'	OMe	
B=T	87	88	2.08	6.75	6.38	3.96	0.50
B=C^{tol}	86	90	1.96	6.74	6.24	3.90	0.44
B=A^{bz}	85	91	1.99	6.79	6.53	3.72	0.36
B=G^{prop}_{dpc}	91	90	1.93	6.74	6.44	3.81	0.56

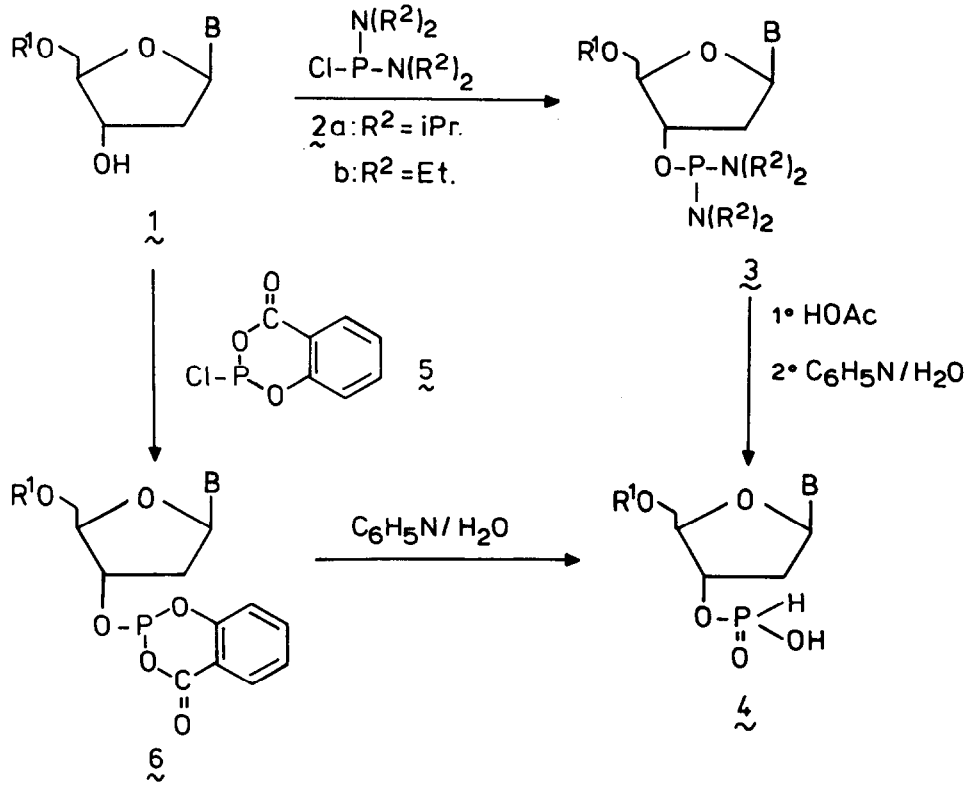
[a] *The exocyclic amino functions of C and A are protected with the 2-methylbenzoyl (tol) and benzoyl (bz) groups, respectively. The 2-amino and O6-amide functions of G are protected with the propionyl (prop) and diphenylcarbamoyl (dpc) groups, respectively* [*see T. Kamimura et al., Tetrahedron Lett., 27, 2775 (1983)*].

[b] *Chemical shifts are given in p.p.m. relative to the external standard H_3PO_4.*

[c] *Solvent: $CDCl_3/CD_3OD$; internal standard TMS; 200 MHz (NMR).*

[d] *Mobile phase: MeOH/EtOAc; 15:85, v/v. Stationary phase: silanized silica gel RP2 (Merck).*

[e] *A PH coupling constant of approximately 636 Hz was observed for all four H-phosphonate derivatives.*



20°C. The intermediate thus obtained was treated with excess pyridine-water and left for 10 min at 20°C. Work-up of the reaction mixture, and purification by column chromatography using silanized silica gel[13] as the stationary phase, gave homogeneous 4 (B=T; R^1=DMTR). Yields and other relevant data on the synthesis of d-nucleoside-3'-phosphonates 4 are recorded in the Table. It can be seen that the three-step route (Method A) described above affords the required compounds in a good yield. Despite these favourable results we felt the need to develop a method in which the H-phosphonate function could be introduced fast and under almost neutral conditions. The accomplishment of such a process would eliminate the acid-hydrolysis step and thus make this method more generally applicable.

A critical survey of the literature revealed that 2-chloro-5,6-benzo-1,3,2-dioxaphosphorin-4-one (salicylchlorophosphite) 5 might fulfil the demands formulated above. The easily accessible and crystalline reagent 5 was synthesized almost one century ago by Anschütz et al.[14], and its structure and fundamental chemistry was established and further corroborated by Young[15]. On the basis of this information we decided to treat a solution of 1 (B=T; R^1= DMTR, 1 mmol) in dioxane (3 ml) and pyridine (1 ml) with a slight excess of 5 (1.0 ml of a 1.25 M stock solution of 5 in dioxane). TLC-Analysis, after 5 min, revealed[16] complete conversion of 1 into a product with zero mobility. Water was now added to the reaction mixture which was further worked-up and purified as mentioned before for the preparation of 4 *via* Method A. Compounds 4 thus obtained were identical (^{31}P- and ^{1}H-n.m.r. spectroscopy) with 4 prepared according to Method A. Further, the good recoveries of 4, prepared *via* Method B (see Table), indicate that this approach may be a convenient and general route to the synthesis of compounds containing a H-phosphonate function. At present we are studying in detail the application of the above described methods towards the preparation of glycophosphates[17] and nucleopeptides[18].

REFERENCES AND NOTES

1. R.H. Hall, A. Todd and R.F. Webb, J. Chem. Soc., 3291 (1957).
2. M. Sekine, H. Mori and T. Hata, Tetrahedron Lett., 1145 (1979).
3. P.J. Garegg, T. Reyberg, J. Stawinsky and R. Strömberg, Chimica Scripta, 25, 280 (1985).
4. B.C. Froehler and M.D. Matteucci, Tetrahedron Lett., 27, 469 (1985).
5. K.K. Ogilvie and M.J. Nemer, Tetrahedron Lett., 21, 4145 (1980).
6. M. Sekine and T. Hata, Tetrahedron Lett., 1711 (1975).
7. T. Hata and M. Sekine, Tetrahedron Lett., 3943 (1974).
8. Thus far, three methods to the synthesis of 5'-O-dimethoxytrityl-3'-thymidine-H-phosphonate (4) have been reported.
   For instance, Hata et al. (see reference 6) condensed 1 (B=T; R^1=DMTR) with phosphorous acid (4 eq) in the presence of 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPSCl; 4 eq) and isolated 4 (B=T; R^1=DMTR), after 25 h at 20°C, in an excellent yield. However, it has to be seen whether this approach using an excess of TPSCl, which may lead to the formation of side-products, (see reference 2), might become a general approach for the preparation of all four properly-protected d-nucleoside-3'-phosphonates 4.
   In another method, Hata et al. (see reference 2) treated 1 (B=T; R^1=DMTR) with excess *tris*(N,N-di-methylamino)phosphine for 24 h at 20°C. Addition of water to the reaction mixture, followed by work-up, gave 4 (B=T; R^1=DMTR) in a good yield. The release, however, of dimethylamine during the phosphitylation step makes this approach not general applicable to the synthesis of 4 containing base-labile protective groups. Finally, Garegg et al. (see reference 3) phosphitylated 1 (B=T. R^1=DMTR) with chloro-2-cyanoethylphosphormorpholidite [Sinha et al., Nucl. Acids Res., 12, 4539 (1984)]. Removal of the morpholine group from the thymidine-3'-phosphite thus obtained with 1-H-tetrazole/water, and subsequently β-elimination of the 2-cyanoethyl group with n-$BuNH_2$ (1 h at 20°C), yielded 4 (B=T; R^1= DMTR) in a good yield. This three-step method requires rather expensive reagents and may not be, due to the extra base-promoted elimination step, of general use for the preparation of 5'-O,N-protected d-nucleoside-3'-phosphonates 4.
9. J.E. Marugg, A. Burik, M. Tromp, G.A. van der Marel and J.H. van Boom, Tetrahedron Lett.,

in press.
10. Houben-Weyl, Methoden der Organische Chemie, Bd. E1, Organische phosphorverbindungen 1, G. Thieme Verlag, Stuttgart 1982, p. 389.
11. This compound was prepared in the same way as previously reported (see reference 9) for the synthesis of 3 (B=T; R^1=DMTR; R^2=iPr).
12. Monitoring of the two-step conversion of 3 into 4 by ^{31}P-NMR spectroscopy showed fast disappearance of 3 (δ-^{31}P: 135 p.p.m.) and the appearance of four phosphorous resonances at 142, 141, 129 and 128 p.p.m. The two resonances at 142 and 141 were slowly converted (within one hour) into the other two, which in turn disappeared, after the addition of pyridine-water, to give one resonance at 2.0 p.p.m. We also observed that the acetic acid mediated hydrolysis of 3 (R^1=DMTR; R^2=iPr) proceeded much slower.
13. This effective chromatography procedure has been used before [see P. Francois et al., Bull. Chem. Soc. Belg., 94, 821 (1985)] for the purification of 5'-O-protected (DMTR) d-nucleoside-3'-phosphodiesters.
14. R. Anschütz and W.O. Emery, Ann., 239, 301 (1887).
15. R.W. Young, J. Am. Chem. Soc., 74, 1672 (1952).
16. Monitoring of the phosphitylation of 1 with 5 (δ-^{31}P: 148.5 p.p.m.) showed rapid formation of 6 (δ-^{31}P: 124.5 and 124.3 p.p.m.), which was also readily converted by the addition of pyridine/water into 4.
17. P. Westerduin, G.H. Veeneman, J.E. Marugg, G.A. van der Marel and J.H. van Boom, Tetrahedron Lett., 27, 1211 (1986).
18. E. Kuyl-Yeheskiely, P.A.M. van der Klein, G.M. Visser, G.A. van der Marel and J.H. van Boom, Recl. Trav. Chim. (Pays-Bas), 105, 69 (1986).

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