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SYNTHESIS OF NUCLEOSIDE PHOSPHOTRIESTERS CONTAINING AMIDATE OR THIOATE FUNCTIONS

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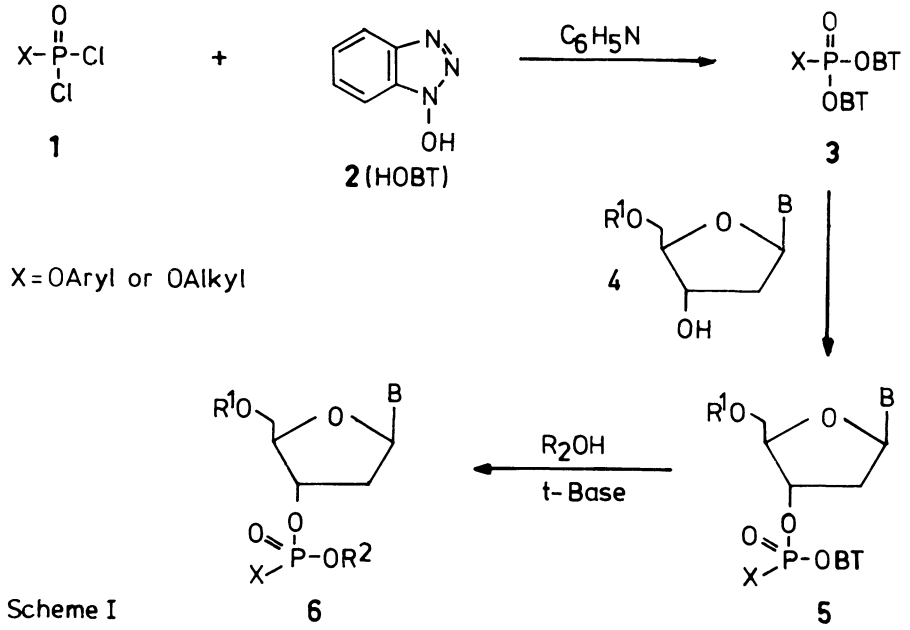
Phosphorylation of properly protected nucleosides with aryl or alkyl phosphorodichloridates, in the presence of 1-hydroxybenzotriazole, gives phosphorylated intermediates which react smoothly with different amine or thiol functions to afford the corresponding amidate or thioate phosphotriester derivatives.

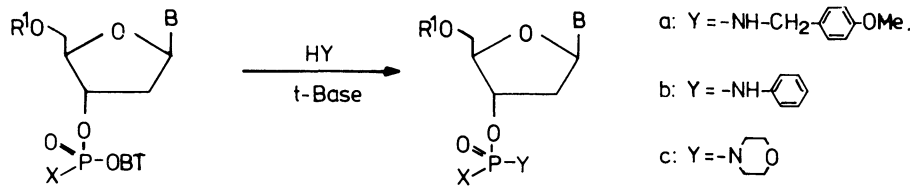
In a previous paper we demonstrated[1] that aryl or alkyl phosphoryl derivatives 3, which are easily accessible by the reaction (see Scheme I) of aryl or alkyl phosphorodichloridates 1 with 1-hydroxybenzotriazole (HOBT) 2, are convenient phosphorylating agents. Thus, phosphorylation of d-nucleoside 4 with 3 afforded intermedites 5 (X=OAryl or OAlkyl). The latter derivatives reacted smoothly with alcoholic functions (R^2OH) to give valuable phosphotriesters 6.



Scheme I

In this paper we wish to report that intermediates 5 are equally well suitable for the synthesis of phosphotriesters having amidate or thioate functions (e.g. compounds 7a-e in Scheme II). Up to now the most common route to the synthesis of mixed phosphotriesters 7 (X=-OR; Y=-NHR or -SR) consisted of treating a properly protected nucleoside with a monofunctional phosphorylating agent 11 (X=OR; Y=NHR or SR). For instance, reagent 11 (X=2,4-$Cl_2C_6H_3O$- ; Y=SMe) has been applied[2a,b] to the synthesis of phosphorothioate triester 7 (X=2,4-$Cl_2C_6H_3O$- ; Y=SMe). Further, several reagents 11 having amidate functions [e.g., X=C_6H_5O- ; Y=-$NHCH_2CH_2OMe$ or -NHC_6H_{11}[3] or X=CBr_3CH_2O- ; Y=-$N(CH_2CH_2)_2O$[4]] have been designed to introduce ami-

date phosphotriester functions. However, we found an attractive alternative to the above described method for the preparation of mixed phosphotriesters 7 in the reaction of intermediates 5 with different amines and thiols. For example, treatment of 5 (R^1=t-butyldimethylsilyl ; X=2,4-$Cl_2C_6H_3O$-; 1 mmol) in THF (6 ml) with 4-methoxybenzylamine (2.5 mmol) in the presence of Et_3N (4 mmol) for 1 h at 20°C gave, after work-up and purification by short-column chromatography, 7a (X=2,4-$Cl_2C_6H_3O$- ;Y=-$NHCH_2C_6H_4OMe$) in a high yield. In an analogous way, we prepared other phosphotriesters having different amidate functions (i.e., 7b,c in Scheme II). The results we obtained and the conditions we applied are summarized in the Table.



5 7

Scheme II

a: Y=-NH-CH_2-⟨⟩-OMe.
b: Y=-NH-⟨⟩
c: Y=-N⟨O⟩
d: Y=-SC_3H_7
e: Y=-S-⟨⟩-Me.

TABLE: Experimental conditions and physical data of compounds 7a-e.

Intermediates[a] 5 (B=T;R^1=TBDMS)	Nucleophiles HY	mmol	t-Base mmol	Time h	Yield 7a-e %	^{31}P-NMR[b] data
X=2,4-$Cl_2C_6H_3O$-	$NH_2CH_2C_6H_4OMe$	2.5	Et_3N 4	1	a 85[c]	3.79 3.56
X=Br_3CCH_2O-	$NH_2C_6H_5$	3.0	N-MeIm 6	3	b 80[d]	1.97 1.63
X=2,4-$Cl_2C_6H_3O$-	$NH(C_2H_4)_2O$	5.0	-	0.5	c 90[c]	2.40 2.27
X=2,4-$Cl_2C_6H_3O$-	HSC_3H_7	2.0	Et_3N 4 N-MeIm 4	4	d 54	26.55 26.04 20.59
X=2,4-$Cl_2C_6H_3O$-	HSC_6H_4Me	2.0	N-MeIm 4	2	e 60	20.30

a) Intermediates 5 were prepared by adding 4 (B=T;R^1=TBDMS:1 mmol) to a stirred and filtered soln. (THF, 6 ml) of 3 (1.2 mmol), which in turn was obtained by adding 1 (1.2 mmol) in THF (0.5 ml) to a cooled (0°C) soln. of HOBT (2.4 mmol) in THF (5.5 ml) containing pyridine (2.4 mmol).

b) Solvent: $CDCl_3$: δ-values in ppm relative to the external standard H_3PO_4.

c) Basic hydrolysis of X followed by acidic hydrolysis of Y afforded thymidine-3'-phosphate[7].

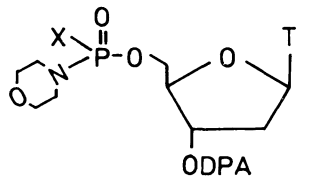
d) Reductive removal of X followed by deblocking of Y by isopentylnitrite treatment gave thymidine-3'-phosphate[7].

Intermediate 5 (X=2,4-$Cl_2C_6H_3O$-) reacted also, and in a satisfactory way, with different thiols. Thus, treatment of 5 (X=2,4-$Cl_2C_6H_3O$-) with n-propanethiol in the presence of N-methylimidazole (N-MeIm) and Et_3N afforded, after work-up and purification by short-column chromatography, the nucleoside phosphorothioate 7d (X=2,4-$Cl_2C_6H_3O$-;Y=-SC_3H_7) in an acceptable yield. In the same way, we prepared the S-p-methylphenyl phosphorothioate triester 7e (X=2,4-$Cl_2C_6H_3O$-;Y=-SC_6H_4Me). Yields and analytical data on the preparation of 7d,e are summarized in the Table. Both thioate triesters 7d,e could be converted[5] into the diesters 7 (X=OH;Y=-SC_3H_7 or -SC_6H_4Me) by treatment with N^1,N^1,N^2,N^2-tetramethylguanidinium syn-4-nitrobenzaldoximate[6].

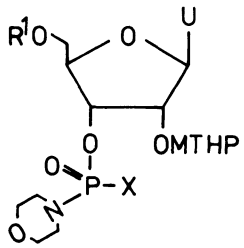
^{31}P-NMR spectroscopy of the diesters showed the presence of only one resonance for each compound. Further, oxidation of 7 (X=OH;H=-SC_3H_7 or -SC_6H_4Me) with iodine in pyridine/water, followed by the removal of the R^1 (TBDMS) group with acid, gave exclusively[7] thymidine-3'-phosphate.

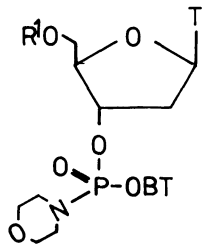
The above described method to the synthesis of thioate derivatives presents an alternative route to the methods developed to introduce thioate functions by a phosphotriester approach, which is based on the use of monofunctional phosphorylating agents 11. Thus compound 7d (X=2,4-$Cl_2C_6H_3O$-;Y=-SCH_3) is in principle accessible by phosphorylation of 4 with 11[2a] (X=2,4-$Cl_2C_6H_3O$-;Y=-SCH_3). Compound 7d (X=2,4-$Cl_2C_6H_3O$-;Y=-SCH_3) thus obtained can be converted[2b] by oximate treatment into 7d (X=OH;Y=-SCH_3). Further, the preparation of 7e (X=OH;Y=-SC_6H_5) could also be accomplished by the phosphorylation of 4 with S,S-diphenyl phosphorodithioate[8], in the presence of an activating agent (e.g., 2,4,6-triisopropylbenzenesulfonyl chloride) to give 7e (X=Y=-SC_6H_5). Selective removal of one thiophenol function from 7e thus obtained by pyridinium phosphonate[8] would afford 7e (X=OH;Y=-SC_6H_5).

The HOBT approach is also applicable to the phosphorylation of hydroxyl groups which are less or more hindered than the secondary hydroxyl groups of d-nucleosides 4. For instance, phosphorylation of 3'-O-diphenylacetyl thymidine[9] (1 mmol) with 3 (X=4-$NO_2C_6H_4O$-;1.2 mmol) in acetonitrile (6 ml) folowed, after 1.5 h, by the addition of morpholine (5 mmol) gave, after work-up and purification by short column chromatography, pure 8[10] in 86% yield. In this particular case, we observed the formation of a small quantity (5%) of symmetrical product (i.e., the product formed when 3'-O-diphenylacetyl thymidine reacts two times with 3). Deblocking of 8 by base (sodium hydroxide) or tetrabutylammonium acetate[11] followed by acid (0.01 N HCl, pH 2) afforded pure thymidine-5'-phosphate[7].



8 X=-O- 





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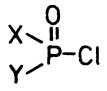

11

9 a: X=-OCH_2CBr_3.

b: X=-O- 

Phosphorylation of the sterically hindered secondary hydroxyl group of 2'-O-methoxytetrahydropyranyl-5'-O-levulinoyl uridine[12] with 3 (X=-OCH_2CBr_3 or 4-$NO_2C_6H_4O$-) followed by the addition of morpholine gave 9a[10] or 9b in a yield of 70% and 78%, respectively. In this respect, it is interesting to note that the monofunctional phosphorylating agents 11 [X=-OCH_2CBr_3[4] or 4-$NO_2C_6H_4O$-[13] and Y=-$N(CH_2CH_2)O$] failed to react[13] with the 3'-OH group of the before mentioned uridine derivative.

Finally, we made the following interesting observation by phosphorylation of 4 (R^1 =t-butyldimethylsilyl) with 3[14] [X=-$N(CH_2CH_2)O$]. In this particular case, we isolated - short-column chromatography - the relatively stable triester 10 [yield 80%;

^{31}P-NMR ($CDCl_3$) data: δ-values in ppm 9.48 and 8.88]. Derivative 10 could quantitatively be converted - 35 h at 20°C - in pyridine/water into 7c [X=OH;Y=-N(CH_2CH_2)$_2$O; ^{31}P-NMR ($CDCl_3$/CD_3OD) data: δ-value in ppm 1.82] which, after acid (0.01 N HCl, pH 2) treatment, afforded solely thymidine-3'-phosphate[7]. The latter phosphorylation procedure proved to be very effective for the synthesis of 5'-phosphorylated DNA fragments[15].

In conclusion, the HOBT approach described in this paper promised to be an attractive and economic route to the introduction of a wide variety of functionalized phosphotriester functions in organic molecules.

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