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A NEW APPROACH TO THE SYNTHESIS OF PHOSPHOTRIESTER INTERMEDIATES OF NUCLEOSIDES AND NUCLEIC ACIDS

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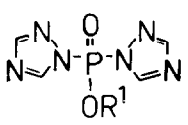
Summary: Aryl phosphorodichloridates can be converted by means of 1-hydroxybenzotriazole into an effective phosphorylating agent, which can be applied to the synthesis of phosphotriester intermediates of nucleic acids.

Some years ago, Katagiri et al.[1] introduced a procedure to convert a bifunctional phosphorylating agent (e.g., 6; R^1= 4-ClC_6H_4) into an arylphosphorodi-(1,2,4-triazolide) derivative 1 (R^1= 4-ClC_6H_4). The latter reagent had properties comparable with those of a monofunctional phosphorylating agent (e.g., 2 ; R^1=4-ClC_6H_4; R^2= $-CH_2CH_2CN$). For instance, preparation of the 3'-phosphotriester derivative 5 (R^1=4-ClC_6H_4; R^2= $-CH_2CH_2CN$) could be performed according to Eq. A. Thus cyanoethylation of monotriazolide 4 with an excess of 3-hydroxypropionitrile gave[1] phosphotriester 5 (R^1=4-ClC_6H_4; R^2= $-CH_2CH_2CN$). Intermediate 4 (R^1=2-ClC_6H_4) can also be hydrolyzed[2] to yield valuable nucleoside 3'-phosphodiester derivatives 5 (R^1=2-ClC_6H_4; R^2= H). An-



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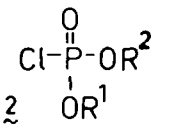

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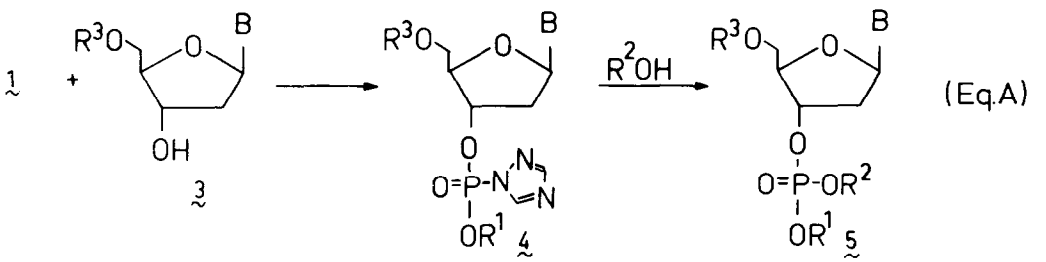


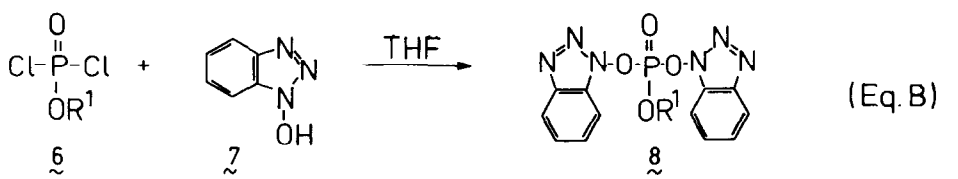
(Eq.A)

other important finding was that condensation of monotriazolide 4 with an alcohol could be catalysed by the addition of N-methylimidazole [3,4a],4-(dimethylamino)pyridine[4] or the triethylammonium salt of p-toluenesulphonic acid[5]. In this way intermediate 4 could now be applied directly to the formation of 3'-5'-internucleotide phosphotriester linkages (e.g., compounds 5 ; R^1=4-ClC_6H_4; R^2=3'-protected d-nucleoside).

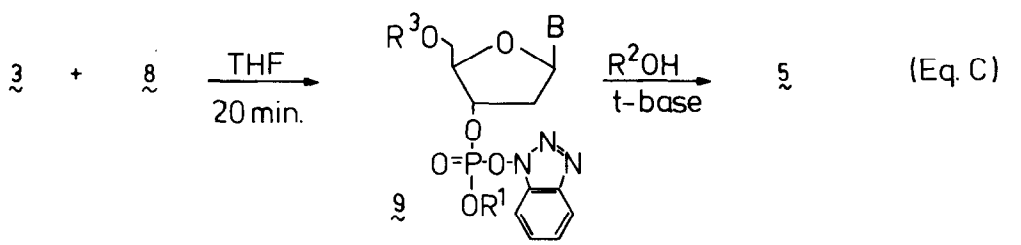
We now wish to report that 1-hydroxybenzotriazole can be used to convert a bifunctional phosphorylating agent (i.e., 6 ; R^1=2-ClC_6H_4) into an effective and efficient phosphorylating reagent (i.e., compound 8).

In analogy with the application of 1-hydroxybenzotriazole (HOBT), in the presence of DCC, to the formation of peptide bonds[6] , we reasoned that HOBT, instead of 1,2,4-triazole, could be used for the modification of a bifunctional phosphorylating agent. Thus, aryl phosphorodi-

chloridate 6 (1 mmol; R^1=2-ClC_6H_4) was added to a stirred solution of HOBT (2 mmol) in dry THF (5 ml) in the presence of pyridine (2 mmol), and left for 1 h at 20°C. The amount of pyridinium HCl salt recovered, after filtration of the reaction mixture, indicated that complete formation of the di-benzotriazole derivative 8 (Eq. B) had occurred[7]. In order to evaluate the synthetic applicability of reagent 8, we investigated its phosphorylating properties (Eq. C) towards 5'-protected d-nucleosides 3 (B=T, C^{An}, G^{DPA} and A^{Bz}; R^3=t-butyldimethylsilyl). Thus, d-nucleoside 3 (1 mmol; B=T; R^3=t-butyldimethylsilyl) was added at once to a stirred solution of 8 (1.1 mmol) in dry THF (5 ml). After 30 min at 20°C, TLC-analysis ($CHCl_3$:MeOH,92:8,v/v) showed complete conversion of starting product 3 into base-line material. In this respect it is interesting to note that no symmetrical product (i.e., 8 reacts two times with 3) was observed. Hydrolysis of intermediate 9 with aqueous triethylamine, followed by concentration, gave, after precipitation with pet-ether, a solid compound. Analysis of this compound by ^{31}P NMR($CDCl_3$) spectroscopy revealed the presence of one resonace at δ=-7.11 ppm (relative to H_3PO_4), Furthermore, the compound was in every aspect - ^1H and ^{31}P NMR spectroscopy - identical with the compound prepared starting from 3 (B=T; R^3=t-butyldimethylsilyl) and using 1 (Eq.A; R^1=2-ClC_6H_4) as the phosphorylating agent. Having established the favorable phosphorylating properties of 8 (Eq. B), we were anxious to find out if intermediate 9 (R^3=t-butyldimethylsilyl; R^1=2-CLC_6H_4) could react with alcoholic functions (R^2OH in Eq.C), to afford phosphotriester derivatives 5.



6 7 8 (Eq. B)



9 (Eq. C)

The conditions we used and the results we obtained are summarized in the Table. Close inspection of the data in the Table reveals that most alcoholic functions, which are currently in use for the preparation of valuable d-nucleoside 3'-phosphotriester intermediates 5, can easily be phosphorylated in the presence of the tertiary base pyridine or N-methylimidazole. Thus the β-cyanoethyl[1] or the 2,2,2-tribromoethyl[8] group (Exp. no. 1-4 in the Table) can be introduced using pyridine, and the recently introduced 4-nitrophenylethyl[9] and the 5-chloro-8-quinolyl[10] groups with the tertiary base N-methylimidazole. The latter base increases the rate of phosphorylation four times in comparison with pyridine. An important observation we made in the preparation of the 3'-phosphotriester derivatives 5 was that no side-reactions[11] on guanine or cytosine were detected in the phosphorylation step. Intermediate 9 (Eq.C) proved also to be very effective for the introduction of 3'-5'-internucleotide phosphodiester linkages. To a stirred solution of 9(B=T;R^3=t-butyldimethylsilyl;R^1=2-ClC_6H_4), obtained by treating 3

(1.0 mmol) with 8 (1.1 mmol; R^1=2-ClC_6H_4) under the conditions as previously described, was added 3'-O-diphenylacetylthymidine[12] (1.0 mmol). After 15 min, N-methylimidazole (4 mmol)

TABLE Conditions used for the preparation of 3'-phosphotriesters 5 (Eq.C).

Exp. No.	Compound 9[a] (1.1 mmol)	Alcohol (R^2OH) (2.0 mmol)	Tertiary base	Time (h)	Yield[b] of 5[f] (%)
1.	B=T	R^2=-CH_2CH_2CN	pyridine[c]	3.5	95
2.	B=C^{An}	R^2=-CH_2CBr_3	pyridine[c]	3.5	90
3.	B=G^{DPA}[d]	R^2=-CH_2CBr_3	pyridine[c]	3.5	90
4.	B=A^{Bz}	R^2=-CH_2CBr_3	pyridine[c]	3.5	88
5.	B=T	R^2=4-nitro-phenylethyl	N-methyl-imidazole[e]	1.0	88
6.	B=T	R^2=5-chloro-8-quinolyl	N-methyl-imidazole[e]	1.0	64

a) R^3=t-butyldimethylsilyl; R^1=2-ClC_6H_4. b) Based on nucleoside 3. The yield of 5 has not been optimized. c) To a solution of 9 in THF (5 ml) was added dry pyridine (2 ml). d) DPA=diphenylacetyl[12]. e) To a solution of 9 in THF (5 ml) was added dry N-methylimidazole (4 mmol). f) For ^{31}P NMR data see references and notes no. 18.

was added and the reaction was left at 20°C. TLC-analysis, after 45 min, showed the reaction to be complete. Work-up and purification of the crude product by short column chromatography[13], afforded fully protected dimer TpT (0.93 mmol). In the same way, we prepared the fully protected dimers ApT, CpT and GpT in an overall yield of 80%. The four fully protected dimers thus obtained were in every aspect - TLC-analysis, ^{31}P NMR spectroscopy - identical with the dimers prepared via another phosphotriester approach[11a]. Further, removal (oximate[14] followed by fluoride-ion and finally aq. ammonia treatment) of all protective groups afforded the corresponding deprotected dimers, which contained solely 3'-5'-phosphodiester linkages as evidenced by ^{31}P NMR spectroscopy and HPLC-analysis in combination with enzymatic digestion using the enzymes venom and spleen phosphodiesterase. In connection with the preparation of the dimers, we made the remarkable observation that the synthesis of the fully protected dimer GpT proceeded without any side products or coloration of the reaction mixture. In our long experience in the synthesis of DNA fragments we always observed, depending on the phosphotriester approach we followed, less or more formation of side-products[11] and coloration of the reaction mixture in the synthesis of G-containing DNA fragments.

The methodology was also applicable to the synthesis of RNA fragments. Thus reacting together 5'-O-levulinoyl-2'-O-(4-methoxytetrahydropyranyl)-uridine[15] (1 mmol) with 8 (1.1 mmol) and 2',3'-O-methoxymethylene-uridine[16] (1.0 mmol), under the conditions used for the synthesis of d-nucleoside dimers, afforded fully protected UpU (0.82 mmol)[17]. Also in this case, no side reaction on the uracyl moiety[11d] could be detected.

In conclusion, the methodology described in this paper presents an economic and efficient route to the introduction of phosphotriester functions in organic molecules. We are presently

engaged in ascertaining the scope of this new phosphorylation procedure. Preliminary experiments indicate that the new approach can be applied successfully to the synthesis of DNA fragments on a solid support. Further, intermediate 9 (Eq. C) reacts very smoothly, in the presence of N-methylimidazole, with R^2XH (X=S or N) to afford phosphotriesters which are up to now only accessible by using the appropriate monofunctional phosphorylating agents [19].

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11. Several side reactions may occur during the synthesis of phosphotriester intermediates of nucleic acids. For instance, phosphorylation of d-cytidine derivative 3 ($B=C^{An}$; R^3=p-chlorophenoxyacetyl) with 2 (R^1=2-ClC_6H_4; R^2=-CH_2CCl_3) afforded a side product, which we tentatively assigned (a) as a phosphorylated 4-N-anisoylcytosine derivative lacking the deoxyribose sugar moiety. Further, synthesis of triester derivatives 5, starting from a 3'-phosphodiester 5 (R^1=Aryl; R^2=H) an appropriate alcohol (R^2OH) and in the presence of a condensing agent (i.e., 2,4,6-triisopropylbenzenesulfonyl tetrazole (b) / 3-nitro-1,2,4-triazole (a) or mesitylenesulfonyl 3-nitro-1,2,4-triazole (c)), may lead to modification (d) of guanine and uracyl moieties.
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