Tetrahedron Letters,Vol.27,No.20,pp 2271-2274,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

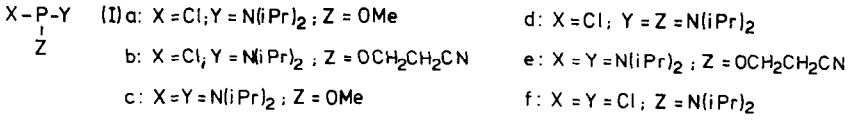
A NEW AND VERSATILE APPROACH TO THE PREPARATION OF VALUABLE DEOXYNUCLEOSIDE 3'-PHOSPHITE INTERMEDIATES

J.E. Marugg, A. Burik, M. Tromp, G.A. van der Marel and J.H. van Boom

Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Abstract: The easily accessible and crystalline monofunctional phosphitylating reagent bis-(diisopropylamino)chlorophosphine has been used for the synthesis of phosphoramidites and H-phosphonates of d-nucleosides and, also, the formation of 3'-5'-internucleotidic phosphonate bonds.

The introduction of more reliable and versatile phosphitylating reagents has made the phosphite-triester approach, as originally devised by Letsinger et al.[1], a powerful method for the preparation of deoxyoligonucleotides on a solid-support. For instance, the development and application[2] of various methyl monochlorophosphoramidites of secondary amines such as N,N-diisopropylamino (i.e. reagent Ia) or N-morpholino was a major breakthrough in the phosphite-triester methodology. Further, the advent of a new class of phosphitylating reagents[3] (e.g. Ib), in which the protective groups at P(V) could be deblocked selectively by mild basic hydrolysis, increased the effectiveness of the unmasking of the protected intermediate DNA fragments obtained by solid-support synthesis (i.e. P(V) as well as O- and N-acyl protective groups are removed in one step by ammonolysis). Finally, the use[4] of reagent Ic enabled the *in-situ* preparation of properly-protected d-nucleoside 3'-phosphoramidites (i.e. compounds IV; R=Me), which play a pivot role in the solid-phase synthesis of DNA. Recently, we showed[5] that the easily accessible and crystalline reagent bis(N,N-diisopropylamino)chlorophosphine (Id) could be used for the preparation of Ie, which showed to be an improvement[6] over the earlier mentioned phosphitylating reagent Ic.



We now report that reagent Id can be used successfully towards the synthesis of 3'-phosphoramidites IV carrying different protective groups at P(III), H-phosphonates VII (R=H) and the introduction of 3'-5'-internucleotidic phosphonate linkages (see compound X).

The preparation of the d-nucleoside 3'-phosphoramidites IV was performed as follows. To a stirred solution of a 5'-O,N-acyl-protected[7] d-nucleoside II (1.0 mmol) under a blanketing atmosphere (N_2) in dry dioxane (5 ml) containing triethylamine (1.5 mmol) was added crystalline Id (1.2 mmol). TLC-analysis, after 20 min, indicated complete conversion of II into a product with a higher R_f-value. Removal of the $(Et_3)NHCl$-salts by filtration and concentration of the filtrate *under vacuo* afforded crude III. The latter was dissolved in acetonitrile (5 ml) and the appropriate alcohol (1.5 mmol) together with 1H-tetrazole (0.5 mmol) was added. TLC-

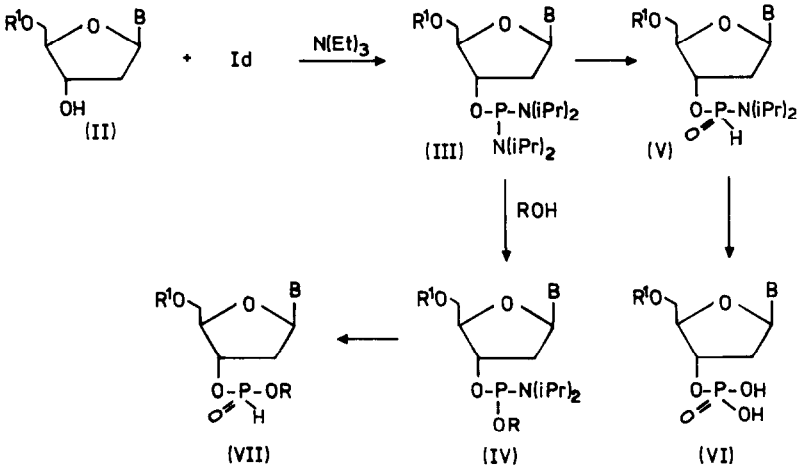
analysis, after 30 min, showed the reaction to be complete. Further work-up and purification by short-column chromatography afforded homogeneous IV (see Table for further information).

Table: Yields and other relevant data on compounds IV.

Starting product II	Product IV yield (%)[a]	^{31}P-NMR data of IV[b]	R_f-values of IV[c]
B=T	75 ($R=CH_2CH_2CN$)	149.0; 148.9	0.53; 0.49
$B=A^{bz}$	80 ($R=CH_2CH_2CN$)	149.0	0.61; 0.58
$B=C^{tol}$	85 ($R=CH_2CH_2CN$)	149.3; 149.0	0.55; 0.49
$B=G^{dpa}$	70 ($R=CH_2CH_2CN$)	148.9	0.25; 0.14
B=T	73 ($R=CH_2CH=CH_2$)	148.5; 148.2	0.60
B=T	85 [$R=CH_2C_6H_4(2-NO_2)$]	149.7; 149.3	0.46

[a] Based on II. [b] Solvent $CDCl_3$; δ-^{31}P values expressed in p.p.m. relative to the external reference 85% H_3PO_4. [c] Eluens: EtOAc/CH_2Cl_2/$(Et_3)N$, 50/45/5, v/v.

It can be seen in the Table that the yield of IV ($B=G^{dpa}$) is not as high as the yields of the other derivatives of IV. Analytical (^{31}P-NMR and TLC) data indicated that the relatively low yield of IV ($B=G^{dpa}$) is due to concomitant phosphitylation by Id of the lactam function in the guaninine moiety of II ($B=G^{dpa}$).



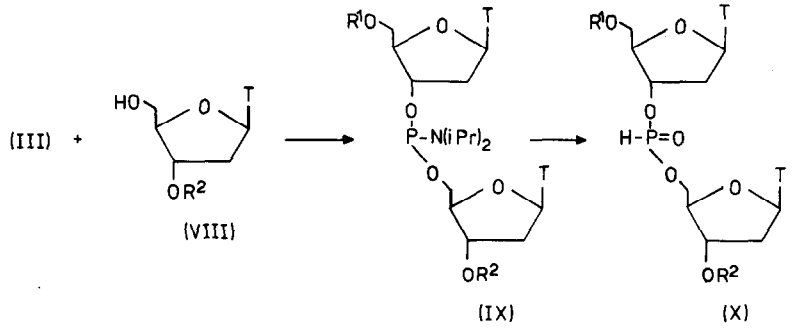
R^1=4,4'-dimethoxytrityl

Attempts to isolate key intermediate III (B=T) were, due to the extreme acid lability of the bis-amidite function, not successful: homogeneous III (B=T, δ-^{31}P 116.1 p.p.m.) could be isolated after silica gel chromatography in a yield of 30%. Treatment of *in-situ* prepared III (B=T; 1 mmol) in acetonitrile (5 ml) with 1H-tetrazole (0.5 mmol) and water (0.1 ml) for 30 min at 20°C gave, after work-up and purification by column chromatography, homogeneous[8] V (B=T) in high yield. Acidolysis of V (B=T) with $HOAc/H_2O$ (8/1, v/v) for 8 h at 20°C afforded 3'-

phosphonate VII[8] (B=T; R=R^1=H) in an excellent yield.

The 3'-phosphoramidites IV are, apart from suitable building units for the solid-phase synthesis of DNA, valuable starting compounds for the preparation of compounds VII (R=H). For example, conversion of IV (B=T; R=CH_2CH_2CN or $CH_2CH=CH_2$), using the same conditions as mentioned for the preparation of V (B=T), afforded VII (B=T; R=CH_2CH_2CN or $CH_2CH=CH_2$) in a good yield. Removal of the β-cyanoethyl group with n-butylamine[9] or the allyl group with $Pd[P(C_6H_5)_3]_4/P(C_6H_5)_3/n\text{-}BuNH_2$[10] afforded, after work-up, the phosphonate derivative VII (B=T; R=H) in an excellent yield.

The scope of intermediate III was further illustrated by the synthesis of the DNA dimer X containing a 3'-5'-internucleotidic phosphonate linkage. The preparation of precursor IX was easily accomplished as mentioned above for the conversion of III into IV, to give, after work-up and purification, pure IX in an acceptable yield. Thus coupling of III (B=T; 1 mmol) with VIII (R^2=benzoyl; 1.1 mmol) under similar conditions as used for the conversion of III (B=T) into IV (B=T) afforded, after work-up and column chromatography, pure[8] IX in a yield of 70%. The latter was quantitatively converted into X[8] by the same conditions as applied for the preparation of VII (B=T; R=CH_2CH_2CN) starting from IV (B=T; R=CH_2CH_2CN). Dimer X thus obtained was oxidized ($I_2/H_2O/C_6H_5N$) followed by acidolysis (removal of R^1) and finally ammonolysis (removal of R^2), to yield, after DEAE-Sephadex A25 chromatography, the fully-deprotected dimer d-TpT, which was in every aspect - ^{31}P- and ^1H-NMR spectroscopy - identical with an authentic sample of d-TpT.



In conclusion, the easily accessible reagent Id is an alternative for the recently introduced[11] bifunctional reagent If for the preparation of compounds IV. Further, the pathway we followed for the formation of X, starting from *in-situ* prepared III, may open the way to a convenient approach towards the synthesis of DNA on a solid-support[12]. Finally, it is interesting to note that the conversion of III into V or VII (R=H) may be regarded as a new and mild procedure for the introduction[13] of phosphate functions in organic molecules.

REFERENCES AND NOTES

1. R.L. Letsinger, J.L. Finnan, G.A. Heavner and W.B. Lunsford, J. Am. Chem. Soc. 97, 3278 (1975).
2. M.A. Dorman, S.A. Noble, L.J. McBride and M.H. Caruthers, Tetrahedron 40, 95 (1984), and references cited therein.
3. So far the following protective groups at P(V), which can be removed by ammonolysis in a phosphite-triester approach, have been published. a) The -CH_2CH_2CN group; N.D. Sinha et al., Nucl. Acids Res. 12, 4539 (1984). c) The -CH(Me)CH_2CN or -$C(Me)_2CH_2CN$ groups; J.E.

Marugg et al., Recl. Trav. Chim. (Pays-Bas) 103, 97 (1984). c) The $-CH_2CH_2SO_2Me$ group; C.A.A. Claesen et al., Tetrahedron Lett. 25, 1307 (1984). d) The $-CH_2CH_2SO_2C_6H_5$ group; N. Balgobin et al., Acta Chem. Scand. B39, 883 (1985).

4. a) S.L. Beaucage, Tetrahedron Lett. 25, 375 (1984). b) A.D. Barone, J.-Y. Tang and M.H. Caruthers, Nucl. Acids Res. 12, 4051 (1984). c) M.F. Moore and S.L. Beaucage, J. Org. Chem. 50, 2019 (1985).
5. J. Nielsen, J.E. Marugg, J.H. van Boom, J. Honnens, M. Taagaard and O. Dahl, J. Chem. Res. (S), 26 (1986).
6. J. Nielsen, J.E. Marugg, M. Taagaard, J.H. van Boom and O. Dahl, Recl. Trav. Chim. (Pays-Bas 105, 33 (1986).
7. The exocyclic amino groups of d-A, d-G and d-C are protected with the benzoyl (bz), diphenylacetyl (dpa) and 2-methylbenzoyl (tol) groups, respectively.
8. ^1H- and ^{31}P-NMR data of compounds V, VII, IX and X.
V (B=T): δ-^{31}P ($CDCl_3$) 13.75; 13.41 (JP-H 638 Hz). δ-^1H ($CDCl_3$) 8.5 (d); 5.3 (d), (2xP-H, JP-H 638 Hz); 7.60 (s), 7.59 (s), (2xH6); 6.5 (m, 2xH1'); 1.42 (s), 1.38 (s), (2x5Me).
VII (B=T; R=H): δ-^{31}P (D_2O; P-H decoupled) 2.57. (P-H coupled) 6.43, 6.31, -1.21, -1.33 (JP-H 617 Hz). δ-^1H (CD_3OD) 8.3 (s), 5.1 (s). (2xP-H, JP-H 632 Hz); 7.7 (s, H6); 6.3 (s, H1'); 1.3 (s, 5Me).
IX (R^1=DMTR; R^2=Bz): δ-^{31}P (D_2O) 149.68, 149.28.
X (R^1=DMTR; R^2=Bz): δ-^{31}P (D_2O) 10.21, 9.42 (JP-H 725 Hz).
9. P.J. Garegg, T. Regberg, J. Stawiński and R. Strömberg, Chimica Scripta 25, 280 (1985).
10. Y. Havakawa, M. Uchivama, H. Kato and R. Noyori, Tetrahedron Lett. 26, 6505 (1985).
11. T. Tanaka, S. Tamatsukuri and M. Ikehara, Tetrahedron Lett. 27, 199 (1986).
12. In a recent publication [B.C. Froehler and M.D. Matteucci, Tetrahedron Lett. 27, 649 (1986)] the preparation of immobilized X was accomplished by coupling VII (B=T; R^1=DMTR; R=H) in the presence of pivaloyl chloride with VIII (R^2=succinyl silica).
13. A preliminary experiment showed (^{31}P-NMR data) that V (B=T; R^1=DMTR) could be oxidized, although slowly, with $I_2/H_2O/C_6H_5N$ to afford a 3'-diisopropylamino phosphate intermediate, which was rapidly hydrolyzed with acid (pyridinium-HCl salt), to give VI (B=T; R^1=DMTR) identical with authentic VI [δ-^{31}P (D_2O) -2.0 p.p.m.] prepared by a phosphotriester approach [C.T.J. Wreesmann et al., Tetrahedron Lett. 26, 933 (1985)].

(Received in UK 17 March 1986)