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REACTION BETWEEN NUCLEOSIDE BASE RESIDUES AND THE PHOSPHORYLATING AGENT DERIVED FROM 1-HYDROXYBENZOTRIAZOLE AND 2-CHLOROPHENYL PHOSPHORODICHLORIDATE

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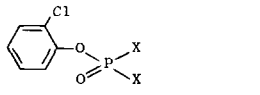
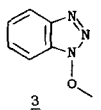
Summary: In the presence of 1-methylimidazole, 2-N-acyl guanine (as in 4a), thymine (as in 5a) and uracil (as in 5b) residues react readily with the phosphorylating agent derived from 2-chlorophenyl phosphorodichloridate (1a) and 1-hydroxybenzotriazole.

For a number of years, we have successfully used 2-chlorophenyl phosphorodi-(1,2,4-triazolide) (1b) as the phosphorylating agent in the first phosphorylation step[1] of the phosphotriester approach[2] to oligonucleotide synthesis. Despite the susceptibility of the 2-N-acylguanine[3], thymine[4] and uracil[3] residues (as in 4, 5a and 5b) to undergo attack at their C-6, C-4 and C-4 positions, respectively, we now confirm (see Table and discussion below) that when phosphorylation is carried out with 1b in tetrahydrofuran solution in the presence of 1-methylimidazole, such side reactions occur only to a small extent.



1 a; X = Cl
b; X = 2
c; X = 3

2



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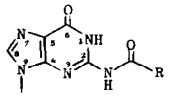
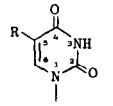


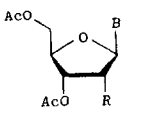
4 a; R = CH_2Ph



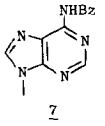
5 a; R = Me
b; R = H

Although 1b is potentially a bifunctional phosphorylating agent and has indeed been used[5] as such, we have found[1] that it can also act as a specifically monofunctional reagent in oligonucleotide synthesis. This is due to the fact that the putative intermediate nucleoside 3'-(2-chlorophenyl) phosphoro-1,2,4-triazolides are relatively much poorer phosphorylating agents than 1b. Recently, van Boom and his coworkers[6] have advocated the use of a more reactive phosphorylating agent (formulated as 1c), obtained by treating 2-chlorophenyl phosphorodichloridate (1a) with *ca.* 2 mol. equiv. each of 1-hydroxybenzotriazole (HOBT) and pyridine in tetrahydrofuran or dioxan solution, in oligonucleotide synthesis. We ourselves have confirmed[7] that the HOBT-activated reagent (1c) is more reactive than 1b, and that it is an effective and useful bifunctional phosphorylating agent. van Boom and his coworkers[6,8] have stressed that, despite its high reactivity in both phosphorylation steps of the phosphotri-

ester approach, the HOBT-activated reagent (1c) is very selective and, unlike 1b [in the first phosphorylation step] and 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT)[9] [in the second phosphorylation step of the phosphotriester approach], it does not attack and thereby modify[3] nucleoside base residues. We now report that, *under the reaction conditions which we normally follow* [i.e. phosphorylation in the presence of 1-methylimidazole in tetrahydrofuran solution[7a]], the HOBT-activated reagent (1c) attacks the base residues with *much greater facility* than 1b.



6 a; R = H
b; R = OAc



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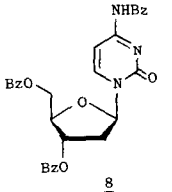

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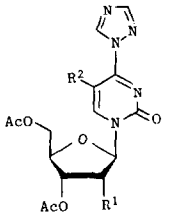
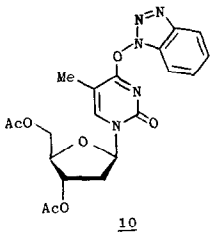


9 a; R^1 = H, R^2 = Me
b; R^1 = OAc, R^2 = H



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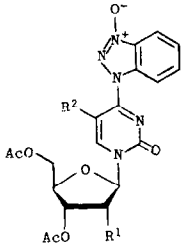
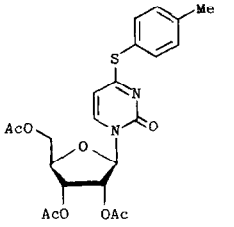


11 a; R^1 = H, R^2 = Me
b; R^1 = OAc, R^2 = H



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Following the approach which we used in connection with our studies[3] on the elucidation of MSNT-promoted side-reactions in oligonucleotide synthesis, we have examined the reactions between the two phosphorylating agents [1b and 1c] and fully esterified nucleoside derivatives in which the amino functions of the base residue are protected by N-acylation [as in 4a, 7 and 8]. Thymine and uracil residues [as in 5a and 5b, respectively] are left unprotected. It can be seen from Table 1 [entries nos. 1 and 2] that 6-N-benzoyl-3',5'-di-O-acetyl-2'-deoxyadenosine (6a; B = 7) and 4-N, 3'-O, 5'-O-tribenzoyl-2'-deoxycytidine (8) are recovered in good yields after they have been treated with *ca.* 3 mol. equiv. of the 1,2,4-triazole derived phosphorylating agent (1b), under the usual phosphorylation conditions[7a], at room temperature for 16 hr. It can also be seen [entries nos. 6 and 7] that similar results are obtained when the latter two substrates [6a; B = 7 and 8] are treated with the HOBT-derived phosphorylating agent (1c)[6,8] under almost the same reaction conditions. However, while 2-N-phenylacetyl-3', 5'-di-O-acetyl-2'-deoxyguanosine (6a; B = 4a) is virtually completely consumed by *ca.* 3 mol. equiv. of 1c after 6 hr [entry no. 8], it may be recovered in 48% yield after it has been allowed to react with 1b under essentially the same conditions [entry no. 3]. As phosphorylation is complete within *ca.* 15 min when an effectively smaller excess[10] of 1b is used in the first step of the phosphotriester approach, it follows that concomitant modification of 2-N-acyl guanine residues (as in 4a) should occur to only a small (≯2%) extent. None of the products of the reactions between the 2'-deoxyguanosine derivative (6a; B = 4a) and the two

phosphorylating agents (1b and 1c) has been isolated or characterized.

TABLE 1. Reactions Between Phosphorylating Agents (1b and 1c) and Protected Nucleoside Derivatives

Entry No.	Parent Nucleoside	Nucleoside Derivative	Reagent[a]	Reaction Time (min)	Nucleoside Derivatives Isolated[b]
1	2'-deoxyadenosine	6a; B = 7	1b	960	starting material (80)
2	2'-deoxycytidine	8	1b	960	starting material (76)
3	2'-deoxyguanosine	6a; B = 4a	1b	360	starting material (48)
4	thymidine	6a; B = 5a	1b	360	starting material (60), 9a (22)[c]
5	uridine	6b; B = 5b	1b	360	starting material (33), 9b (39)[c]
6	2'-deoxyadenosine	6a; B = 7	1c	1,200	starting material (78)
7	2'-deoxycytidine	8	1c	960	starting material (70)
8	2'-deoxyguanosine	6a; B = 4a	1c	360	—[d]
9	thymidine	6a; B = 5a	1c	60	10 (64)
10	uridine	6b; B = 5b	1c	20	12 (77)[e]

[a] The phosphorylating agents (1b and 1c) are prepared by allowing 2-chlorophenyl phosphorodichloridate (1a, *ca.* 1.5 mmol) to react with, respectively, 1,2,4-triazole (*ca.* 3.1 mmol) and 1-hydroxybenzotriazole (*ca.* 3.1 mmol) in the presence of triethylamine (*ca.* 3.2 mmol) in anhydrous tetrahydrofuran (3 ml) at room temperature for 20 min. A solution of nucleoside derivative (*ca.* 0.5 mmol) in tetrahydrofuran (3 ml) and 1-methylimidazole (*ca.* 2.0 mmol) are then added. Reactions are quenched by the addition of triethylamine (*ca.* 2.5 mmol) and water (*ca.* 0.5 ml) after the times indicated.

[b] After quenching, the reaction mixtures are worked up and chromatographed on silica gel: percentage yields are indicated in parentheses.

[c] The percentage yields given are estimates based on the ^{1}H-n.m.r. spectrum of the isolated mixture of products.

[d] A complex mixture of products, containing a small quantity of starting material (6a; B = 4a), was obtained.

[e] Work up and chromatography of the products gave starting material (6b; B = 5b) and its putative 1-hydroxybenzotriazole derivative (11b), both in *ca.* 7% yield. However, when the aqueous triethylamine quench was omitted, and the products were treated with *p*-toluenethiol (8 mol. equiv.) and triethylamine (8 mol. equiv.) in tetrahydrofuran solution at room temperature for 20 min, 12 was obtained and was isolated as a crystalline solid, m.p. 144°C, in 77% yield.

The difference in the reactivities of 1b and 1c towards 3',5'-di-O-acetylthymidine (6a; B = 5a) is even more marked. Thus, while the reaction of the thymidine derivative (6a; B = 5a) with *ca.* 3 mol. equiv. of 1b at room temperature for 6 hr [Table 1, entry no. 4] leads to *ca.* 27% conversion to 9a[4], reaction with 1c under the same conditions for only 1 hr [entry no. 9] leads to the quantitative transformation of the substrate. The reaction with 1b confirms that the latter phosphorylating agent can be used in the first step of the phosphotriester approach without significant (i.e. with ≯1%) concomitant modification of the thymine residues. The product (10) obtained from the reaction between 3',5'-di-O-acetylthymidine (6a; B = 5a) and 1c was isolated as a pure colourless precipitated solid in 85% yield, and was characterized[11] on the basis of its u.v. and ^{1}H-n.m.r. spectra. When 10 (0.22g, 0.5 mmol) is heated in the presence of 1-methylimidazole (0.25 ml) in dry pyridine (0.75 ml) solution at 50°C, it undergoes partial isomerization to give a product to which structure 11a has been provisionally assigned[13]. Work up and chromatography of the products after 24 hr gives 11a (0.125g, 57%) as a crystalline solid (m.p. 158-160°C) and recovered starting material (10, 0.035g, 16%). This would appear to be close to an equilibrium mixture[15] of these isomers as 11a and 10 are obtained in similar proportions (*ca.* 3:1) when pure 11a is treated in the same way. When 10 and 11a are treated with an excess both of 1,2,4-triazole and triethylamine in tetrahydrofuran solution, 9a is obtained and may be isolated as a crystalline solid in yields of 64 and 68%, respectively. Finally, it can be seen from Table 1 [entries nos. 5 and 10] that both phosphorylating agents (1b and 1c) react with 2',3',5'-tri-O-acetyluridine (6b; B = 5b) some 2-3 times more rapidly than with 3',5'-di-O-acetylthymidine (6a; B = 5a). While a stable product (9b)[17] is obtained in the reaction involving 1b, the product obtained with 1c cannot be isolated in satisfactory yield. However, when the latter product [assumed to have structure 11b] is allowed to react with *p*-toluenethiol and triethylamine [see entry no. 10 and footnote[e]], 12 is obtained and may be isolated as a crystalline solid in good yield.

Although the above studies clearly demonstrate that the phosphorylating agent (1c) derived from HOBT and 2-chlorophenyl phosphorodichloridate (1a) reacts readily with 2-N-acyl guanine (e.g. 4a), thymine (5a) and uracil (5b) residues, these side reactions appear, to a large extent, to be promoted by the presence of 1-methylimidazole. When 3',5'-di-O-acetylthymidine (6a; B = 5a, 1.0 mmol) is treated with 1c [3.0 mmol; prepared from 1a, HOBT (2 mol. equiv.) and pyridine (2 mol. equiv.)] in the absence of 1-methylimidazole in tetrahydrofuran solution at room temperature, only *ca.* 65% conversion to 10 occurs in 68 hr. Thus the phosphorylation procedure described by van Boom and his coworkers[6,8] would appear to be quite satisfactory in the absence of 1-methylimidazole. Unfortunately, however, 1-methylimidazole appears to be required[8] in the second phosphorylation step. The results of the present study add weight to a general conclusion that although it is, to some extent, possible to control the side reactions involving base residues in the phosphotriester approach, purer synthetic polynucleotides would most probably be obtained if the 1,6- and 3,4-lactam functions of 2-N-acyl guanine (as in 4), thymine (as in 5a) and uracil (as in 5b) residues were protected[18].

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REFERENCES AND FOOTNOTES

[1] J.B. Chattopadhyaya and C.B. Reese, *Tetrahedron Lett.* 5059 (1979).

[2] C.B. Reese, *Tetrahedron* 34, 3143 (1978).

[3] C.B. Reese and A. Ubasawa, *Tetrahedron Lett.* 21, 2265 (1980); *Nucleic Acids Res. Special Publication No. 7*, pp. 5 *et seq.* (1980).

[4] W.L. Sung, *J. Chem. Soc., Chem. Commun.* 1089 (1981).

[5] J. Stawinski, T. Hozumi, S.A. Narang, C.P. Bahl, and R. Wu, *Nucleic Acids Res.* 4, 353 (1977).

[6] G. van der Marel, C.A.A. van Boeckel, G. Wille, and J.H. van Boom, *Tetrahedron Lett.* 22, 3887 (1981).

[7] (a) C. Christodoulou and C.B. Reese, *Tetrahedron Lett.* 24, 951 (1983); (b) Ö. Kemal, C.B. Reese, and H.T. Serafinowska, *J. Chem. Soc., Chem. Commun.* 591 (1983).

[8] J.E. Marugg, L.W. McLaughlin, N. Piel, M. Tromp, G.A. van der Marel, and J.H. van Boom, *Tetrahedron Lett.* 24, 3989 (1983); C.T.J. Wreesmann, A. Fidder, G.A. van der Marel, and J.H. van Boom, *Nucleic Acids Res.* 11, 8389 (1983); J.E. Marugg, M. Tromp, P. Jhurani, C.F. Hoyng, G.A. van der Marel, and J.H. van Boom, *Tetrahedron* 40, 73 (1984).

[9] C.B. Reese, R.C. Titmas, and L. Yau, *Tetrahedron Lett.* 2727 (1978).

[10] A protected nucleoside or oligonucleotide derivative with a free 3'-hydroxy function is usually phosphorylated with *ca.* 2.5 - 3.0 mol. equiv. of 1b. However, some of the latter reagent is rapidly consumed in the course of the phosphorylation reaction.

[11] The u.v. spectrum (95% EtOH) of 10 (λ_{max} 255, 262 and 292 nm) correlates well with that of 1-methoxybenzotriazole[12]; ^{1}H-n.m.r. spectrum [$(D_3C)_2SO$, 250 MHz]: δ2.06(3H,s), 2.08(3H,s), 2.35 - 2.55(2H,m), 4.31(3H,m), 5.21(1H,m), 6.09(1H, t, J = 6.7 Hz), 7.55(1H,m), 7.68(1H,m), 7.83(1H, d, J = 8.3 Hz), 8.18(1H, d, J = 8.3 Hz), 8.22(1H,m); R_F 0.42[$CHCl_3$-MeOH(19:1 v/v)].

[12] M.P. Servé, P.G. Seybold, W.A. Feld, and M.A. Chao, *J. Heterocyclic Chem.* 13, 509 (1976).

[13] Satisfactory microanalytical data were obtained for 11a; its u.v. spectrum [in 95% ethanol: λ_{max} 269, 349, 359 (ε 9 300, 26 800, 30 600), λ_{infl} 335 nm (ε 15 900)] displays absorption at long wavelengths (>300 nm) like that of 3-methylbenzotriazole-1-oxide[14]. However, the isomeric 2-substituted benzotriazole-1-oxide structure for 11a cannot be excluded on this evidence. ^{1}H-n.m.r. spectrum [$(D_3C)_2SO$, 250 MHz]: δ2.07(3H,s), 2.10(3H,s), 2.41(3H,s), 2.55(2H,m), 4.35(3H,m), 5.26(1H,m), 6.22(1H, t, J = 6.9 Hz), 7.67(1H,m), 7.91(1H,m), 8.05(1H, d, J = 8.3 Hz), 8.21(1H,m), 8.66(1H, d, J = 8.3 Hz); R_F 0.35[$CHCl_3$-MeOH(19:1 v/v)].

[14] F.T. Boyle and R.A.Y. Jones, *J. Chem. Soc., Perkin Trans. II* 160 (1973).

[15] 1-Acetoxybenzotriazole and putative 3-N-acetylbenzotriazole-1-oxide have been shown[16] to equilibrate in acetone-water solution. It is believed[16] that acyl migration in this system is likely to be an intramolecular process.

[16] K. Horiki, *Tetrahedron Lett.* 1897 (1977).

[17] K.J. Divakar and C.B. Reese, *J. Chem. Soc., Perkin Trans. I* 1171 (1982).

[18] S.S. Jones, C.B. Reese, S. Sibanda, and A. Ubasawa, *Tetrahedron Lett.* 22, 4755 (1981).

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