Preparation of Activated Nucleotides and their Phosphorothioate and Phosphorodithioate Analogues

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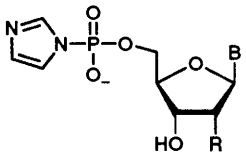
The preparation of nucleoside phosphoro-(1,2,4-triazolides) (*e.g.* **9a**) and their thio- and dithio- analogues (*e.g.* **9b** and **c**, respectively), and a corresponding set of activated nucleotides (*e.g.* **10a, b, c**) derived from 1-hydroxybenzotriazole is described; the reactions between these derivatives and methylamine are also reported.

Ribonucleoside 5'-phosphorimidazolides **1a** were first prepared by Cramer and his coworkers[1] and were shown[2,3] to behave as activated nucleotides in that they reacted with primary amines, alcohols and monoalkyl phosphates to give the corresponding phosphoramidates, phosphodiesters and P^1,P^2-dialkyl pyrophosphates, respectively. Hoard and Ott subsequently demonstrated[4] that the reaction between 2'-deoxyribonucleoside 5'-phosphorimidazolides **1b** and inorganic pyrophosphate constituted a convenient method for the preparation of 2'-deoxyribonucleoside 5'-triphosphates **3a**. This procedure was extended[5] to include the conversion of nucleoside 5'-thiophosphorimidazolides **2a** and **2b** into the corresponding nucleoside 5'-α-thiotriphosphates **3b** (R = OH and H).

Scheit[6] showed that 1,1',1''-phosphinylidynetris-1*H*-imidazole **4a** was an effective reagent for the conversion of suitably-protected nucleoside derivatives into nucleoside 5'-phosphorimidazolides **1**, and Eckstein and Gindl[5] found that the corresponding thio-compound **4b** could be used in the same way in the preparation of 5'-thiophosphorimidazolides **2**. We now report that related phosphorylating agents† derived from 1*H*-1,2,4-triazole (*i.e.* **5a** and **b**) and 1-hydroxybenzotriazole (*i.e.* **6a** and **b**) can readily be used in the preparation of activated nucleotides and their phosphorothioate and phosphorodithioate analogues.

Two substrates (**7** and **8**)[7] were used in our studies. When the thymidine derivative **7** was treated (Table 1, entry 1) with a threefold excess of **5a**[8–10] (Table 1, footnote *a*) in THF



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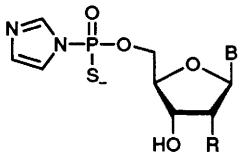
b; R = H



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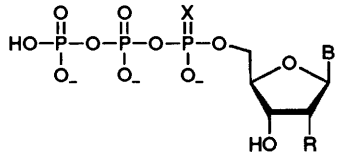
b; R = H



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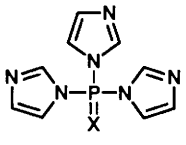
b; X = S



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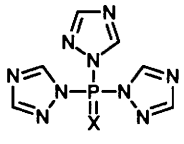

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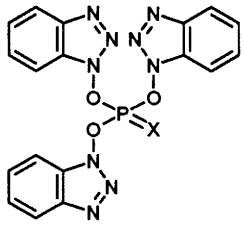
b; X = S

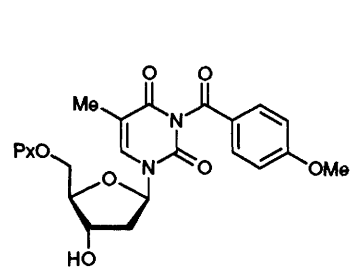


6
a; X = O
b; X = S

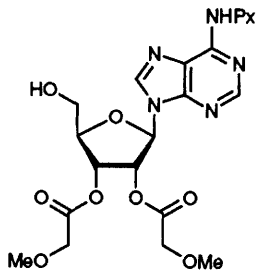
Table 1 Preparation of activated nucleotide derivatives

Entry	Substrate	Phosphorylating agent[a]	Work-up procedure[b]	Product	Eluting solvent (% EtOH)[c]	Yield (%)[d]	δ_P[e]	Purity (%)[f]
1	7	**5a**	A	**9a**	15–20	42	−11.1	94
2	7	**5b**	A	**9b**	8–10	80	43.1	81
3	7	**5b**	B	**9c**	8–10	76	96.3	77
4	7	**6a**	A	**10a**	8–10	81	−0.5	100
5	7	**6a**	B	**10b**	2–4	49	59.1, 59.2	—[g]
6	7	**6b**	A	**10b**	2–4	88	59.1, 59.2	100
7	7	**6b**	B	**10c**	2–4	82	125.0	100
8	8	**6b**	A	**11a**	2–4	70	59.9	99
9	8	**6b**	B	**11b**	2–4	63	125.5	100

[a] Phosphorylation reactions were carried out in the following way: $POCl_3$ (1.5 mmol) or $PSCl_3$ (1.5 mmol) and Et_3N (4.5 mmol) were added to a stirred suspension of 1*H*-1,2,4-triazole (4.5 mmol) or 1-hydroxybenzotriazole (4.5 mmol) in dry THF (20 cm^3) under N_2 at room temp. After 30 min, a solution of substrate (**7** or **8**, 0.5 mmol) in THF (20 cm^3) and 1-methylimidazole (1.0 mmol) were added. Phosphorylation reactions were generally complete within 1 h. [b] Procedure A: Et_3N (18 mmol) and water (1.0 cm^3) were added to the stirred products at room temp. which, after 20 min, were concentrated under reduced pressure and fractionated; procedure B: Et_3N (18 mmol) was added and dry H_2S gas was bubbled through the mixture for 15–30 min, followed by N_2 for 30 min. The products were then filtered, concentrated under reduced pressure and fractionated. [c] Percentage EtOH by volume in $CHCl_3$–EtOH mixtures used for short column chromatography on silica gel. [d] Yields relate to precipitated triethylammonium salts, isolated as colourless solids. [e] NMR spectra were measured in $(CD_3)_2SO$ solution; in all cases satisfactory ^{31}P and ^1H spectra were obtained. [f] Purity was estimated by HPLC on a Jones APEX ODS column eluted with mixtures of 0.1 mol dm^{-3} aqueous triethylammonium acetate (pH 7.0) and acetonitrile. [g] This product was not examined by HPLC but was found to be pure by ^{31}P and ^1H NMR spectroscopy.



7



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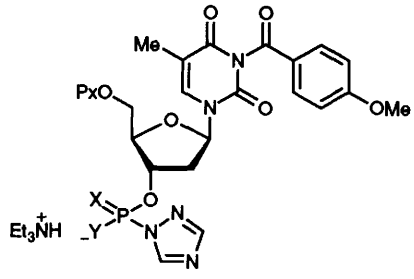
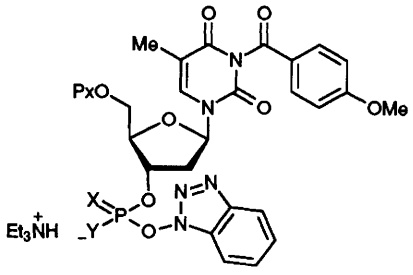


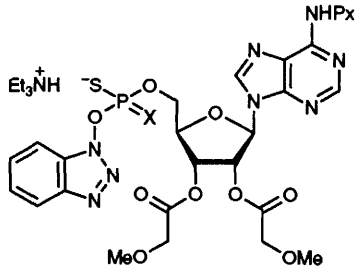
9

a; X = Y = O
b; X = O, Y = S
c; X = Y = S



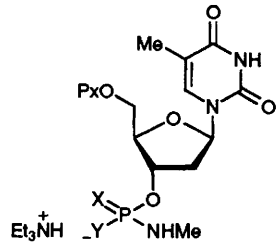
10

a; X = Y = O
b; X = O, Y = S
c; X = Y = S



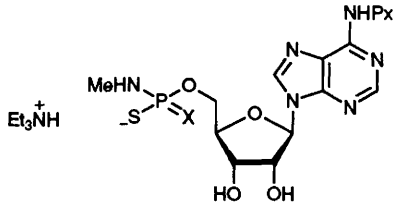
11

a; X = O
b; X = S



12

a; X = Y = O
b; X = O, Y = S
c; X = Y = S



13

a; X = O
b; X = S

Px = 9-phenylxanthen-9-yl

solution and the products worked-up with aqueous triethylamine according to procedure A (footnote *b*), the activated nucleotide **9a** was obtained and isolated as a colourless solid in 42% yield. The corresponding thiophosphoro-(1,2,4-triazolide) **9b** was obtained (entry 2) in 80% isolated yield when **5b** was used as the acylating agent and the products were worked-up in the same way. However, when substrate **7** was treated with the same acylating agent **5b** and the products were worked-up with triethylamine and hydrogen sulfide under anhydrous conditions (entry 3) according to procedure B (footnote *b*), the dithiophosphoro-(1,2,4-triazolide) **9c** was obtained in 76% isolated yield. Although, possibly with the exception of the phosphoro-(1,2,4-triazolide) **9a**, satisfactory yields of 1*H*-1,2,4-triazole derived activated nucleotide derivatives (entries 1–3) were obtained, the estimated purity (by HPLC) of the products was disappointing.

When substrate **7** was allowed to react with a threefold excess of tri(benzotriazol-1-yl) phosphate **6a** (entry 4) and the products then worked-up according to procedure A and fractionated by chromatography on silica gel, the nucleotide derivative **10a** was obtained and isolated as a colourless solid in 81% yield. This material was found to be completely homogeneous by HPLC. When the products of the same phosphorylation reaction were worked-up with triethylamine and hydrogen sulfide (entry 5) according to procedure B, the corresponding phosphorothioate **10b** was obtained. However, the preparation of the latter product **10b** was effected more easily (entry 6) by acylating substrate **7** with tri(benzotriazol-1-yl) phosphorothioate **6b** and then working the products up with aqueous triethylamine according to procedure A. A high yield (88%) of HPLC-homogeneous product was then obtained. When the products of the same acylation reaction were worked-up with triethylamine and hydrogen sulfide under anhydrous conditions (entry 7) according to procedure B, the corresponding phosphorodithioate derivative **10c** was obtained and isolated as an HPLC-homogeneous colourless solid in 82% yield. It can be seen (Table 1) that the 1-hydroxybenzotriazole derived acylating agents **6a** and **b** gave rise to products that were eluted from silica gel with less polar solvent mixtures than were required for the products obtained in reaction involving the 1*H*-1,2,4-triazole derived reagents **5a** and **b**. The fact that the benzotriazole derivatives (**10a**, **b** and **c**) were easier to purify by chromatography on silica gel probably led to generally higher yields of purer products being isolated (Table 1) from reactions involving the 1-hydroxybenzotriazole derived acylating agents **6a** and **b**.

Finally, the adenosine derived building block **8** was allowed to react with **6b**, and the products were worked-up either with

Table 2 Reactions between activated nucleotide derivatives and methylamine in ethanol solution

Entry	Substrate	Reaction time/h[a]	Product	Yield (%)[b]	δ_P[c]	Purity (%)[d]
1	**9a**	2(0.5)	**12a**	83		93
2	**10a**	1(0.2)	**12a**	84	6.6	100
3	**9b**	8(0.7)	**12b**	93		97
4	**10b**	8(0.5)	**12b**	95	60.3, 60.4	97
5	**9c**	18(8)	**12c**	69		97
6	**10c**	18(6)	**12c**	79	108.2	98
7	**11a**	23	**13a**	96	60.1, 60.3	100
8	**11b**	18	**13b**	86	108.1	99

[a] Reactions were carried out by dissolving the substrates (*ca.* 0.2 mmol) in a very large excess (20 cm^3) of 8 mol dm^{-3} alcoholic methylamine at room temp. The numbers in parentheses after the actual reaction times represent the estimated (by HPLC) times required for the reactions to go to completion. [b] Isolated yields as precipitated solids following chromatography on silica gel. [c] NMR spectra were measured in $(CD_3)_2SO$ solution; satisfactory ^1H and ^{31}P NMR spectra were obtained for all products. [d] Purity was estimated by HPLC (see Table 1, footnote *f*).

aqueous triethylamine according to procedure A (entry 8) or with triethylamine and hydrogen sulfide according to procedure B (entry 9). In this way, the corresponding phosphorothioate and phosphorodithioate derivatives **11a** and **b**‡ were obtained and isolated as pure colourless solids in 70 and 63% yield, respectively.

All of the activated nucleotides (**9a–c**, **10a–c**, **11a** and **b**) were found to be effective acylating agents in that they reacted with 8 mol dm^{-3} ethanolic methylamine to give the corresponding *N*-methylphosphoramidates in satisfactory to excellent yields (Table 2). In the case of the thymidine derivatives (**9a–c**, **10a–c**), the more readily isolable (see above) 1-hydroxybenzotriazole derivatives (**10a–c**; entries 2, 4 and 6) were found to be somewhat more reactive and to lead to slightly higher isolated yields of purer products than the corresponding 1*H*-1,2,4-triazole derivatives (**9a–c**; entries 1, 3 and 5). A comprehensive study of the reactions between the above types of activated nucleotide derivatives and various nucleophiles is curently being undertaken in our laboratory.

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Footnotes

† **5a** and **6a** are the putative structures of the products generated *in situ* (Table 1, footnote *a*) from phosphoryl trichloride and 1-*H*-1,2,4-triazole and 1-hydroxybenzotriazole, respectively; **5b** and **6b** are likewise the putative structures of the corresponding products similarly prepared from thiophosphoryl trichloride.

‡ The site of attachment of the 1*H*-1,2,4-triazole and 1-oxybenzotriazole residues to phosphorus has not been firmly established, either in the case of these (**11a** and **b**) or of the other activated nucleotide derivatives (**9a–c**, **10a–c**) described above.

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