N-Alkylation of Cytosine and Its Nucleosides with Trialkyl Phosphates

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Synopsis. Cytosine has been readily alkylated with trialkyl phosphates to give the corresponding *N*-alkyl derivatives, trimethyl phosphate being especially useful for the *N*-methylation of the base moiety of cytosine nucleosides.

The alkylation of nucleic acid components has been the subject of deep interest in organic and biological fields because of the induced physiological effects of alkylated products. The conventional alkylating agents used have been diazomethane,[1] dimethyl sulfate,[2] and alkyl halides.[3]

The alkylation of purines, pyrimidines, imidazole analogs, and pyridones with the alkyl esters of oxoacids of phosphorus,[4] have been reported where it was found that these agents were useful for the alkylation of nitrogen-containing heterocyclic compounds. This paper deals with an application of the method to the alkylation of cytosine and its nucleosides using trialkyl phosphates.

Results and Discussion

The reaction conditions and results are shown in Table 1. Upon heating cytosine (I) with trimethyl phosphate(TMP), I went gradually into solution to give three products, 1-methylcytosine (IIa), 3-methylcytosine (IIIa), and 1,3-dimethylcytosine (IVa, main). Triethyl and triallyl phosphate also converted I into a mixture of the corresponding 1-alkyl-, 3-alkyl-, and 1,3-dialkylcytosines. In general, the reactivity of trialkyl phosphates as alkylating agents appeared in the following order; methyl>allyl>ethyl.

In methylations using *N*,*N*-dimethylformamide (DMF) as the solvent in the presence of triethylamine, the reaction time was considerably reduced and the yield of IIa increased. The employment of protic solvents such as ethanol resulted in decreasing yields of alkylated products, in spite of the addition of triethylamine.

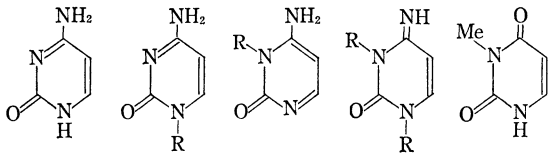
The methylation of I with dimethyl sulfate has been reported to produce only IIIa (25%) and IVa (5%);[2a] the authors have found however this reaction afforded IIa (9%) in addition to IIIa (42%) and IVa (15%) under the same conditions described in the literature[2a] (100 °C, 1 h, sulfate/I=2). The product distribution observed is somewhat different from that in the reaction with TMP (see Table), suggesting that TMP more readily methylates *N*-1 of I than dimethyl sulfate does.

Table 1. Alkylation of cytosine (I) with trialkyl phosphates[a]

R in $(RO)_3PO$	Time h	Temp °C	Solvent	Products yield/% II	III	IV	V
Me	27	100	—	6	10	74	0
	31	80	EtOH	4	20	11	10
	12	90	DMF	18	8	22	0
	12	88	DMF Et_3N	36	4	38	0
	34	100	DMF[b]	16	10	53	0
Et	37	130	—	13	3	71	0
Allyl	56	100	—	16	11	44	0
	17	110	DMF	21	8	36	0

a) Phosphate/I=4, b) Phosphate/I=8.

In the formation of IVa, two pathways (I→IIIa→IVa and I→IIa→IVa) are possible and this was examined briefly. The time-course of the reaction of I with TMP in DMF is depicted in Fig. 1. Figure 2 shows the reactions of IIa or IIIa with TMP under conditions similar to that used for reactions described in Fig. 1, indicating that IIIa is converted to IVa faster than IIa. Since IIa and IIIa are produced in similar yields in the reaction mixture of I and TMP (see Fig. 1), IVa should be produced largely *via* IIIa rather than IIa.



I IIa—c IIIa—c IVa—c V
a: R=Me, b: R=Et, c: R=allyl.

The *N*-1 of IIIa, which exists in the form of a 4-amino-2-oxo structure, is known to be more basic and less sterically hindered than the *N*-3 of IIa,[5] suggesting the former site is more reactive than the latter. The faster rate of formation of IVa from IIIa than from IIa (Fig. 2) may be attributable to these factors.

The methylation of cytidine (VIa) and 2'-deoxycytidine (VIb) was examined using TMP, which appeared to be the most reactive alkylating agent of the trialkyl phosphates studied.

Under neutral conditions in water or anhydrous solvent, alkylating agents such as diazomethane, benzyl

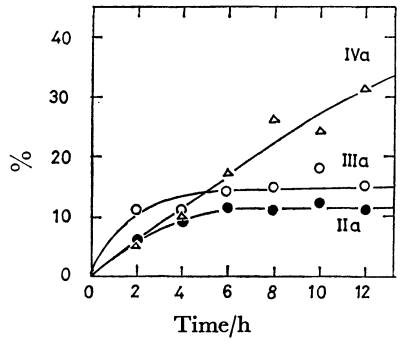


Fig. 1. The reaction of cytosine (I) with trimethyl phosphate (TMP) in DMF at 100 °C. TMP/I=8.

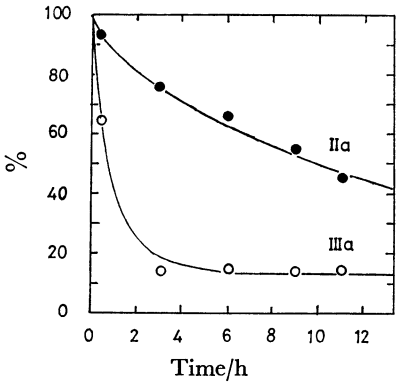
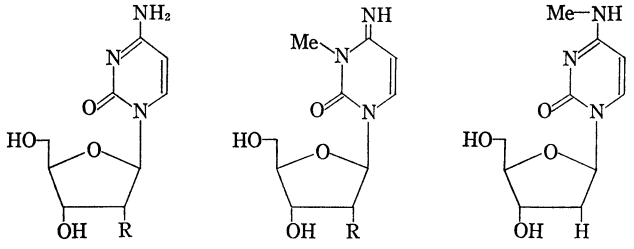


Fig. 2. The reaction of 1-methylcytosine (IIa) and 3-methylcytosine (IIIa) with trimethyl phosphate (TMP) in DMF at 100 °C. TMP:IIa:IIIa=8:1:1.

iodide, dimethyl sulfate, and nitrogen mustard have been shown to give the *N*-3-alkylated derivatives.[1,2a,3a,3b,6] Under alkaline conditions these alkylating agents predominantly alkylate the carbohydrate hydroxyls.[2b,7]

In the reaction with TMP (10 mol-equiv.) in DMF (70 °C; 32 h), compound VIa was smoothly methylated only at the *N*-3 position to give 3-methylcytidine (VIIa) in 81% yield. Under similar conditions, VIb was converted to 3-methyl-2′-deoxycytidine (VIIb; 52 %), but unlike the 4-amino group of VIa, VIb was also methylated to generate N^4-methyl-2′-deoxycytidine (VIII; 11%) as a by-product.[8] Under the present conditions, TLC analysis of the reaction mixture did not show any products which were methylated at the carbohydrate hydroxyls of VIa and VIb.



VIa,b VIIa,b VIII

a: R=OH, b: R=H.

The methylation of VIa with dimethyl sulfate gave VIIa in a yield comparable with that of TMP. Methylations with diazomethane and methyl iodide have been known to be less efficient than dimethyl sulfate or TMP. Thus, the present method would be useful particularly for the *N*-methylation of cytosine and its nucleosides.

Experimental

Commercial reagents were used without further purification. Triallyl phosphate was prepared by the procedure of Toy *et al.*[9] The reaction procedures and product isolations were in a manner similar to that of previous work.[4c] The yields of the reaction products were obtained spectroscopically similarly to that used in the former study.[4d] Results and reaction conditions are given in Table 1.

The majority of the products isolated agreed with the literature values (mp, UV, and NMR).[2a,3b,10,12,13,14]

Physical Constants. IVb; mp 262—264 °C (HCl salt), 237—239 °C (methanol-ether), NMR (D_2O) δ 1.32 (t, J=8 Hz, 3H, CH_3), 1.34 (t, J=8 Hz, 3H, CH_3), 3.96 (q, J=8 Hz, 2H, $-CH_2-$), 4.12 (q, J=8 Hz, 2H, $-CH_2-$), 6.22 (d, J=8 Hz, 1H, C-5), and 7.87 ppm (d, J=8 Hz, 1H, C-6), UV, λ_{max} 277, λ_{min} 242 mμ (pH 7), MS, *m/e* 167 (M^+ for $C_8H_{13}N_3O$): IIc; mp 242—245 °C (ethanol), NMR (D_2O) δ 4.46 (m, 2H, $-CH_2-$), 5.36 (m, 2H, $=CH_2$), 6.10 (d, J=7.5 Hz, 1H, C-5), 6.13 (m, 1H, $-CH=$), and 7.64 ppm (d, J=7.5 Hz, 1-H, C-6), UV, λ_{max} 274, λ_{min} 252 mμ (pH 7), MS, *m/e* 151 (M^+ for $C_7H_9N_3O$): IIIc; UV, λ_{max} 296, λ_{min} 255 mμ (pH 7): IVc; mp 242—244 °C (HCl salt), NMR (D_2O) δ 4.55 (m, 4H, $-CH_2-$), 5.27 (m, 4H, $=CH_2$), 5.95 (m, 2H, $-CH=$), 6.30 (d, J=8 Hz, 1H, C-5), and 7.88 ppm (d, J=8 Hz, 1H, C-6), UV, λ_{max} 277, λ_{min} 243 mμ (pH 7), MS, *m/e* 191 (M^+ for $C_{10}H_{13}N_3O$): VIIb; mp 174—176 °C ($CHCl_3$), NMR (D_2O) δ 2.50 (m, 2H, 2′-H), 3.57 (s, 3H, CH_3), 3.83 (d, J=2 Hz, 1H, 5′-H), 3.93 (s, 1H, 5′-H), 4.18 (m, 1H, 4′-H), 4.53 (m, 1H, 3′H), 6.30 (t, J=7 Hz, 1H, 1′-H), 6.38 (d, J=8 Hz, 1H, C-5), and 8.17 ppm (d, J=8 Hz, 1H, C-6), UV, λ_{max}, λ_{min} mμ (pH), 279, 245 (1), 279, 245 (7), 267, 246 (13), MS, *m/e* 241 (M^+ for $C_{10}H_{15}N_3O_4$).

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