Reaction Between 2′,3′,5′-Tri-*O*-acetyladenosine and Aryl Chloroformates. 2′,3′,5′-Tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine as an Intermediate in the Synthesis of 6-Ureidopurine Ribosides

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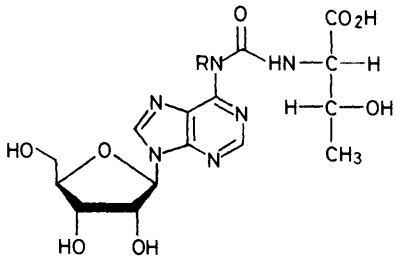
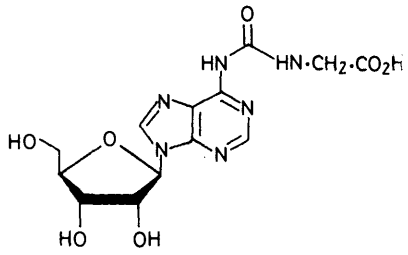
Reaction between 2′.3′.5′-tri-*O*-acetyladenosine (6) and *p*-nitrophenyl chloroformate in pyridine solution at 20 °C or between (6) and phenyl chloroformate in pyridine solution at 70 °C gives the protected symmetrical urea derivative (7b) in moderate or high yield. Deacetylation of (7b) gives the corresponding unprotected urea (7a). Treatment of (6) with an excess of phenyl chloroformate in pyridine solution at 20 °C gives the bisphenoxycarbonyl derivative (10) which, on reaction with morpholine in dioxan solution, is rapidly converted into 2′,3′,5′-tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (3c). Compound (3c) readily reacts with cyclohexylamine, ammonia, and glycine methyl ester at 20 °C to give, after deacetylation, the corresponding 6-ureidopurine riboside derivatives (9b, c, and d; R^1 = H) in high yields.

THE 6-ureidopurine riboside *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-ylcarbamoyl]-L-threonine [1] (1a) occurs as a hypermodified nucleoside at the 3′-ends of the anticodons in yeast lysyl,[2] methionyl,[3] seryl,[4] and other transfer ribonucleic acids (tRNAs). The related *N*-(purin-6-ylcarbamoyl)glycine riboside [5] (2) and (1b), the *N*(6)-methyl derivative [6] of (1a), also occur in tRNA. In connection with our work on the synthesis of oligoribonucleotides,[7] we were interested in developing a convenient general method for the preparation of 6-ureidopurine ribosides such as (1) and (2).



(1) a; R = H
b; R = Me



(2)

The most obvious approach to the synthesis of such unsymmetrical ureas (Scheme 1) involves the reaction between a protected adenosine carbamate (3) and a protected or unprotected amino-acid (4). This approach has been favoured by previous workers, who have used the comparatively unreactive carbamate (3a) as the starting material, thereby making relatively drastic reaction conditions necessary. Thus Chheda and Hong [8] found that the reaction between (3a) and L-threonine in pyridine solution required 5 h at 100 °C; these workers obtained (1a) in *ca.* 45% yield after removal of the protecting groups. Similar results were subsequently obtained in another laboratory.[9] We believed that it would be advantageous to start with a more reactive carbamate than (3a) and therefore investigated the reaction between 2′,3′,5′-tri-*O*-acetyladenosine (6) and aryl chloroformates.

The reaction between 2′,3′,5′-tri-*O*-acetyladenosine (6) and a slight excess of *p*-nitrophenyl chloroformate in pyridine solution proceeded rapidly at 20 °C and gave a product which could not be induced to crystallize. The n.m.r. spectrum of this product, which contained no signals assignable to *p*-nitrophenyl protons, was consistent with its being the symmetrical urea derivative (7b). However, its u.v. absorption spectrum (see below) was quite unlike that of a simple *N*(6)-acyl- or -carbamoyl-adenosine derivative. Treatment of (7b) with an excess of *p*-nitrobenzoyl chloride in pyridine solution gave a crystalline bis-*p*-nitrobenzoyl derivative in 66%

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[1] G. B. Chheda, R. H. Hall, D. I. Magrath, J. Mozejko, M. P. Schweizer, L. Stasiuk, and P. R. Taylor, *Biochemistry*, 1969, **8**, 3278; M. P. Schweizer, G. B. Chheda, L. Baczynskyj, and R. H. Hall, *ibid.*, p. 3283.

[2] J. T. Madison, S. J. Boguslawski, and G. H. Teetor, *Science*, 1972, **176**, 687.

[3] M. Simsek and U. L. RajBhandary, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 508.

[4] H. G. Zachau, D. Dutting, and H. Feldmann, *Angew. Chem. Internat. Edn.*, 1966, **5**, 422; *Z. physiol. Chem.*, 1966, **347**, 212.

[5] M. P. Schweizer, K. McGrath, and L. Baczynskyj, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 1046.

[6] F. Kimura-Harada, D. L. von Minden, J. A. McCloskey, and S. Nishimura, *Biochemistry*, 1972, **11**, 3910.

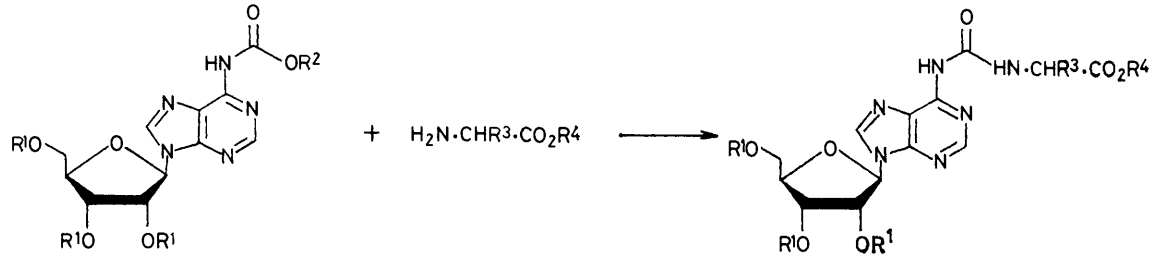
[7] For reviews of this work see C. B. Reese, Colloques Internationaux du C.N.R.S., 1970, No. 182, 319; C. B. Reese, *Phosphorus and Sulfur*, 1976, **1**, 245.

[8] G. B. Chheda and C. I. Hong, *J. Medicin. Chem.*, 1971, **14**, 748.

[9] R. W. Adamiak and M. Wiewiórowski, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1975, **23**, 241.

yield. The u.v. absorption spectrum of the latter was consistent with structure (7c).

When (7b) reacted with methanolic ammonia at 20 °C for 48 h, the unprotected symmetrical urea derivative (7a) was obtained crystalline in high yield. Treatment

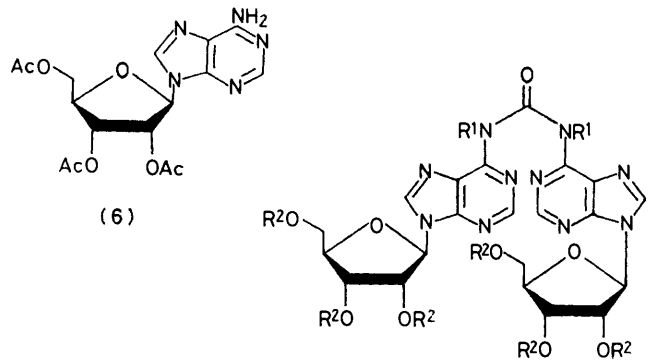


(3) a; R^1 = Ac, R^2 = Et
b; R^1 = Ac, R^2 = 4-$O_2N{\cdot}C_6H_4$
c; R^1 = Ac, R^2 = Ph
d; R^1 = H, R^2 = Me

(5)

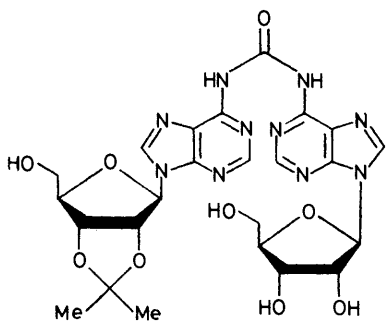
SCHEME 1

of compound (7a) with acetic anhydride in pyridine regenerated (7b). The u.v. absorption spectrum of (7a), which has intense maxima at 283 and 291 nm and



(6)

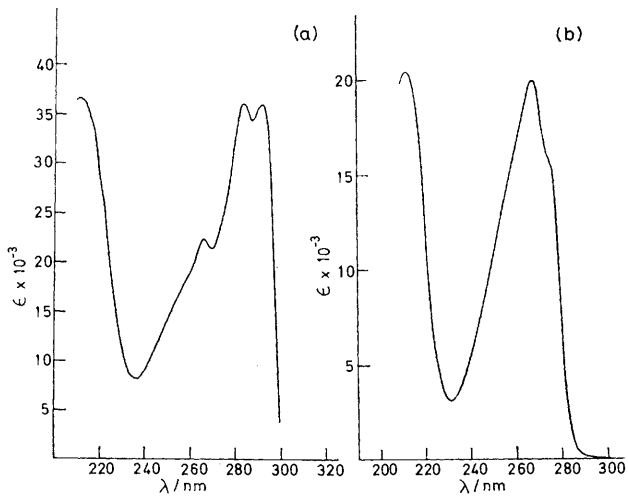
(7) a; R^1 = R^2 = H
b; R^1 = H, R^2 = Ac
c; R^1 = p-$O_2N{\cdot}C_6H_4{\cdot}CO$, R^2 = Ac



(8)

closely resembles that of (7b), is illustrated in Figure 1(a). For comparison, the spectrum of $N(6)$-carbamoyladenosine (9c; R^1 = H) (see below) is also illustrated [see (b)]. The latter spectrum is typical for an $N(6)$-acyl derivative of adenosine. The remarkable u.v. spectrum of (7a), the absence of a molecular ion in its mass spectrum, and several unsuccessful attempts to obtain satisfactory microanalytical data corresponding to anhydrous (7a) led us to seek further confirmation for this structural assignment.

The fact that (7a) contains two identical nucleoside residues was confirmed by converting it, *via* its di-isopropylidene derivative (see Experimental section) into its analytically pure crystalline monoisopropylidene derivative (8). The di-isopropylidene derivative was readily obtained as a crystalline monohydrate in 92% yield by treating (7a) with 2,2-dimethoxypropane [10,11] in the presence of toluene-p-sulphonic acid in dioxan solution.

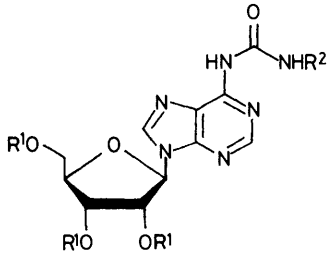


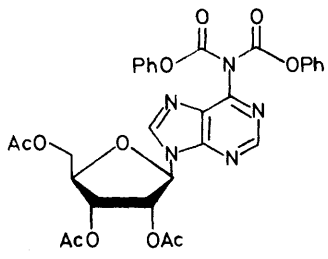
U.v. absorption spectra of (a) *NN'*-bis-[9-(β-D-ribofuranosyl)-purin-6-yl]urea (7a) and (b) $N(6)$-carbamoyladenosine (9c; R^1 = H) in 95% ethanol

The u.v. spectra of both (8) and the di-isopropylidene derivative closely correspond to that of (7a). Further evidence in support of structure (7a) was adduced from

[10] A. Hampton, *J. Amer. Chem. Soc.*, 1961, **83**, 3640.
[11] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2315.

hydrolysis studies. When (7a) was heated in aqueous solution, under reflux, for 4 h, adenosine was obtained as the sole product (isolated in 82% yield). Under the same conditions, a related compound, *N*(6)-phenylcarbamoyladenosine [12] (9a; R^1 = H), prepared by deacetylation of the product from treating 2′,3′,5′-tri-*O*-acetyladenosine (6) with phenyl isocyanate, similarly underwent quantitative hydrolysis to give adenosine. When (7a) was heated in aqueous M-sodium hydroxide at 100 °C for 20 h it was also completely converted into adenosine; however, (7a) was unchanged after 18 h in the same alkaline medium at 20 °C. Additional support for its structural assignment was provided by the observation that when (7a) was heated, under reflux, with an excess of neat cyclohexylamine for 10 min, it was quantitatively converted into adenosine (92% isolated yield) and *N*(6)-cyclohexylcarbamoyladenosine [(9b; R^1 = H); 88% isolated yield]. Compound (9b; R^1 = H) was prepared independently by treating 2′,3′,5′-tri-*O*-acetyladenosine (6) with cyclohexyl isocyanate and then deacetylating the product. The remarkable u.v. spectra of (7a) [Figure 1(a)], (7b), the di-isopropylidene derivative of (7a), and (8) are presumably due to base-stacking or to some other interaction between the two adenine residues in these compounds.[13]





(9) a; R^2 = Ph
b; R^2 = C_6H_{11}
c; R^2 = H
d; R^2 = $CH_2 \cdot CO_2Me$

(10)

After the unsuccessful attempt to prepare 2′,3′,5′-tri-*O*-acetyl-*N*(6)-*p*-nitrophenoxycarbonyladenosine (3b), the preparation of the corresponding phenyl carbamate (3c) was undertaken. Treatment of 2′,3′,5′-tri-*O*-acetyladenosine (6) with 1.2 mol. equiv. of phenyl chloroformate in pyridine solution at 70 °C gave (7b) in high yield; deacetylation of the products with methanolic ammonia gave (7a) as a crystalline solid in 83% overall yield. However, when (6) was treated with a threefold excess of phenyl chloroformate in pyridine solution at 20 °C, 2′,3′,5′-tri-*O*-acetyl-*N*(6),*N*(6)-bisphenoxycarbonyladenosine * (10) was obtained and could be isolated as a crystalline solid in 79% yield. When smaller quantities of phenyl chloroformate were used, t.l.c. did not reveal the presence of the desired phenyl carbamate (3c) but only mixtures of (10) and starting material (6).

The bisphenoxycarbonyl compound (10) proved to be a reactive precursor of 6-ureidopurine riboside derivatives. Thus treatment of (10) with a five-fold excess of cyclohexylamine in dioxan solution at 20 °C for 1 h gave 2′,3′,5′-tri-*O*-acetyl-*N*(6)-cyclohexylcarbamoyladenosine (9b; R^1 = Ac) as the sole nucleoside product, which was isolated as a crystalline solid in 80% yield. An obvious disadvantage which (10) has as a synthetic intermediate is that an excess of what may be a valuable amino-compound must be used in conjunction with it. Fortunately, however, (10) reacted rapidly with a stoicheiometric quantity of morpholine in dioxan solution at 20 °C to give the desired 2′,3′,5′-tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (3c), which was isolated crystalline in 55% yield.

2′,3′,5′-Tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (3c) had precisely the properties required in a precursor of 6-ureidopurine riboside derivatives.† When (3c) was treated with a stoicheiometric quantity of cyclohexylamine in dioxan solution at 20 °C, complete reaction occurred within 1 h. Deacetylation of the products with methanolic ammonia gave *N*(6)-cyclohexylcarbamoyladenosine (9b; R^1 = H), which was isolated crystalline in 92% overall yield. In contrast to the latter experiment, the reaction between *N*(6)-methoxycarbonyladenosine (3d) and 1.5 mol. equiv. of cyclohexylamine in boiling pyridine solution was only *ca.* 75% complete after 4.5 h; after 16 h under these conditions, *N*(6)-cyclohexylcarbamoyladenosine (9b; R^1 = H) was obtained in quantitative yield.

The value of 2′,3′,5′-tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (3c) as a synthetic intermediate was further demonstrated in two other experiments. First, the reaction between (3c) and a saturated solution of ammonia in dioxan was complete within 2 h at 20 °C. Deacetylation of the product with methanolic ammonia gave *N*(6)-carbamoyladenosine (9c; R^1 = H) as a crystalline hemihydrate in 92% overall yield. Secondly, (3c) was treated with slight excesses of glycine methyl ester hydrochloride and triethylamine in dioxan at 20 °C. After 18 h, when the reaction was complete, the products were worked up and treated with sodium methoxide in methanol to give *N*(6)-methoxycarbonylmethylcarbamoyladenosine (9d; R^1 = H) as a crystalline solid in 78% overall yield. Compound (9d; R^1 = H) is the methyl ester of (2), the simplest 6-ureidopurine riboside which occurs naturally [5] in tRNA. Although the reaction between (3c) and glycine methyl ester in dimethylformamide solution was complete within 1 h

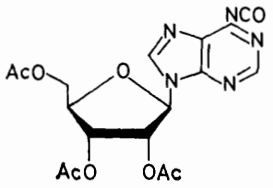
* By comparison with the structures of the corresponding diaroyl derivatives of (6) [P. A. Lyon and C. B. Reese, *J.C.S. Perkin I*, 1974, 2645], this compound is assumed to be an *N*(6),*N*(6)- rather than an *N*(1),*N*(6)-derivative.

† Since the preparation of the manuscript of this paper, R. W. Adamiak and J. Stawiński (personal communication; *Tetrahedron Letters*, 1977, 1935) have reported an alternative synthesis of (3c).

[12] J. J. McDonald, N. J. Leonard, R. Y. Schmitz, and F. Skoog, *Phytochemistry*, 1971, **10**, 1429.

[13] J. Žemlička and J. Owens, *J. Org. Chem.*, 1977, **42**, 517.

at 20 °C, the above reaction conditions in dioxan were found to be more convenient.



(11)

Finally, it seems likely that the mechanism of the reaction between 2′,3′,5′-tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (3c) and amino-compounds involves elimination of phenol [14] to give the 6-isocyanato-derivative (11) as an intermediate which then reacts with the amino-compound.

EXPERIMENTAL

^{1}H N.m.r. spectra were measured at 100 MHz with a Varian HA-100 spectrometer (tetramethylsilane used as internal standard). Mass spectra were recorded with an A.E.I. MS9 spectrometer, i.r. spectra with a Perkin-Elmer 257 G spectrometer, and u.v. spectra with a Pye Unicam SP 1800 recording spectrophotometer.

Thin-layer chromatograms were run on glass plates coated with Merck Kieselgel F_{254} in the following solvent systems: (A) $CHCl_3$–MeOH (80 : 20 v/v), (B) $CHCl_3$–MeOH (95 : 5 v/v), (C) $CHCl_3$–MeOH (97.5 : 2.5 v/v); and on Merck DC–Alufolien Cellulose F_{254} in solvent system (D): butan-1-ol–acetic acid–water (5 : 2 : 3). Reeve Angel Silica Gel/CT was used for short column chromatography. Pyridine was dried by heating under reflux with calcium hydride and then distilled.

Reaction between 2′,3′,5′-Tri-O-acetyladenosine (6) *and* p-*Nitrophenyl Chloroformate.*—*p*-Nitrophenyl chloroformate (0.222 g, 1.1 mmol) was added to a stirred solution of 2′,3′,5′-tri-*O*-acetyladenosine (0.393 g, 1.0 mmol) in anhydrous pyridine (5 ml) at 20 °C. After 2 h, water (0.1 ml) was added and, after a further 1 h the products were concentrated under reduced pressure. The resulting gum was dissolved in chloroform (10 ml), and the solution extracted with saturated aqueous sodium hydrogen carbonate (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was dissolved in dichloromethane and the solution applied to a column of Mallinckrodt SilicAR CC4 (10 g). The column was eluted with (a) $CHCl_3$–CH_2Cl_2 (3 : 1 v/v) and (b) $CHCl_3$–EtOH (99 : 1 v/v). The latter fractions (b) were combined and evaporated under reduced pressure to give NN′-*bis*-[9-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* as a t.l.c. homogeneous [R_F 0.33 (system B)] glass (0.24 g, 59%); τ($CDCl_3$) 1.14 (2 H, s), 1.45 (2 H, s), 3.70 (2 H, d, *J* 5 Hz), 3.94 (2 H, m), 4.28 (2 H, m), 5.57 (6 H, m), 7.86 (6 H, s), 7.90 (6 H, s), and 7.94 (6 H, s); $\lambda_{max.}$ (95% ethanol) 291, 284, and 266; $\lambda_{min.}$ 289, 269, and 235 nm; $\nu_{max.}$($CHCl_3$) 3 360w, 3 020m, 2 990m, 1 745s, 1 610s, and 1 590s cm^{-1}; *m/e* 419 [(*M* − 393)$^+$].

NN′-*Bis*-p-*nitrobenzoyl*-NN′-*bis*-[9-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* (7c).—*p*-Nitrobenzoyl chloride (0.928 g, 5.0 mmol) was added to a stirred solution of NN′-bis-[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]urea (0.406 g, 0.5 mmol) in anhydrous pyridine (10 ml) and the reactants were stirred at 20 °C. After 30 min the products were poured onto ice (60 g) and the mixture was stirred as the ice melted. The precipitate was filtered off and dissolved in chloroform. The solution was extracted with ice-cold saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure. The solid was dissolved in $CHCl_3$–MeOH (99.4 : 0.6 v/v) and fractionated by short column chromatography [85 g of silica gel; $CHCl_3$–MeOH (99.4 : 0.6 v/v)]. The appropriate fractions were combined and concentrated under reduced pressure to give a solid which was carefully recrystallized from hot ethanol (without boiling the solution) to give the crystalline NN′-*bis*-p-*nitrobenzoyl derivative* [Found (material dried *in vacuo* over P_2O_5 at 90°): C, 51.0; H, 3.8; N, 15.1. $C_{47}H_{42}N_{12}O_{12}$ requires C, 50.8; H, 3.8; N, 15.1%], m.p. 125—126°; yield 0.368 g (66%); τ($CDCl_3$) 1.28 (2 H, s), 1.55 (4 H, d, *J* 9 Hz), 1.74 (4 H, d, *J* 9 Hz), 1.91 (2 H, s), 3.92 (2 H, d, *J* 5 Hz), 4.23 (2 H, m), 4.40 (2 H, m), 5.58 (6 H, m), 7.87 (6 H, s), and 7.92 (12 H, s); $\lambda_{max.}$ (95% ethanol) 270 (ε 37 100), $\lambda_{min.}$ 234 nm (ε 17 100).

NN′-*Bis*-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* (7a).—(*a*) 2′,3′,5′-Tri-*O*-acetyladenosine (0.393 g, 1.0 mmol) was treated with *p*-nitrophenyl chloroformate (0.222 g, 1.1 mmol) in pyridine (5 ml) according to the procedure described above and the products were worked-up in the same way but not chromatographed. The crude material was dissolved in methanol (5 ml) and the stirred solution treated with methanolic ammonia (half-saturated at 0 °C; 5 ml) at 20 °C. After 48 h, the products were filtered off and the crystalline residue was washed with ice-cold methanol. Careful crystallization from water gave NN′-*bis*-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea monohydrate* [Found (material dried *in vacuo* over P_2O_5 at 100 °C): C, 43.6; H, 4.3; N, 24.05. $C_{21}H_{24}N_{10}O_9,H_2O$ requires C, 43.6; H, 4.5; N, 24.2%] as crystals, m.p. 165—166°; yield 0.164 g (57%); R_F 0.09 (system A), 0.35 (system D); τ[$(CD_3)_2SO$–D_2O] 1.31 (2 H, s), 1.34 (2 H, s), 3.96 (2 H, d, *J* 5.5 Hz], 5.41 (2 H, m), 5.78 (2 H, m), 5.97 (2 H, m), and 6.32 (4 H, m); $\lambda_{max.}$ (95% ethanol) 298, 284, and 266 (ε 36 200, 36 200, and 22 300), $\lambda_{min.}$ 289, 269, and 235 nm (ε 34 400, 21 300, and 8 000); $\lambda_{max.}$ (0.1M-HCl) 292 and 265 (34 300 and 15 600), $\lambda_{min.}$ 268 and 242 (15 500 and 11 800); $\lambda_{max.}$ (0.1M-NaOH) 322 and 274 (37 000 and 12 600), $\lambda_{infl.}$ 268 (12 100), $\lambda_{min.}$ 286 and 244 (6 000 and 6 700); $\nu_{max.}$ (Nujol) 3 600—3 000br,m and 1 720m cm^{-1}; *m/e* 293 [(*M* − 267)$^+$].

(*b*) Phenyl chloroformate (5.64 g, 36 mmol) was added to a stirred solution of 2′,3′,5′-tri-*O*-acetyladenosine (11.79 g, 30 mmol) in anhydrous pyridine and the reactants were heated to 70 °C. After 1 h, the products were cooled to room temperature and water (0.6 ml) was added; after a further 30 min the products were concentrated under reduced pressure. The resulting gum was dissolved in chloroform (300 ml) and the solution extracted with aqueous sodium hydrogen carbonate (300 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residual gum was dissolved in methanol (150 ml) and the stirred solution treated with methanolic ammonia (half-saturated at 0 °C; 150 ml) at 20 °C. After 48 h the products were filtered and the crystalline residue was washed with ice-cold methanol and then recrystallized from hot water * (600 ml) to give NN′-bis-[9-(β-D-ribofuranosyl)-

* Care should be taken during the recrystallization of this compound as prolonged heating in aqueous solution leads to its conversion into adenosine (see text and below).

[14] M. L. Bender and R. B. Homer, *J. Org. Chem.*, 1965, **30**, 3975.

purin-6-yl]urea monohydrate as crystals, m.p. 165—166°; yield 7.20 g (83%).

Acetylation of NN′-*Bis*-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]-*urea* (7a).—Acetic anhydride (1.63 g, 16 mmol), *NN*′-bis-[9-(β-D-ribofuranosyl)purin-6-yl]urea monohydrate (1.16 g, 2.0 mmol) and anhydrous pyridine (10 ml) were stirred together at 20 °C. After 16 h, methanol (2 ml) was added and, after a further 1 h, the products were concentrated under reduced pressure. The resulting gum was dissolved in chloroform (40 ml) and the solution extracted with saturated aqueous sodium hydrogen carbonate (40 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The glass obtained was identical [R_F(system B); n.m.r., i.r., u.v., and mass spectra] with the *NN*′-bis-[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]urea obtained from the reaction between 2′,3′,5′-tri-*O*-acetyladenosine and *p*-nitrophenyl chloroformate.

NN′-*Bis*-[9-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea*.—2,2-Dimethoxypropane (4.16 g, 40 mmol) was added to a stirred suspension of *NN*′-bis-[9-(β-D-ribofuranosyl)purin-6-yl]urea monohydrate (1.16 g, 2.0 mmol) and toluene-*p*-sulphonic acid monohydrate (0.84 g, 4.4 mmol) in anhydrous dioxan (40 ml) at 20 °C. After 2 h, water (0.6 ml) was added, and after a further 30 min the products were neutralized with methanolic ammonia (half-saturated at 0 °C; *ca.* 1 ml) and then filtered. The filtrate was evaporated under reduced pressure and the residue crystallized from water to give NN′-*bis*-[9-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea monohydrate* as needles (Found: C, 48.9; H, 4.9; N, 21.1. $C_{27}H_{32}N_{10}O_9$,-H_2O requires C, 49.2; H, 5.2; N, 21.3%), m.p. 152—153°; yield 1.21 g (92%); R_F (system A) 0.66; τ[$(CD_3)_2SO$] −1.82br (2 H, s), 1.32 (4 H, s), 3.73 (2 H, d, *J* 2.5 Hz), 4.64 (2 H, dd, *J* 2.5 and 6 Hz), 4.99 (dd, *J* 2.5 and 6 Hz), 5.69 (2 H, m), 6.39 (4 H, m), 8.41 (6 H, s), and 8.64 (6 H, s); $\lambda_{max.}$ (95% ethanol) 292, 285, and 267 (ε 33 800, 35 300, and 20 200), $\lambda_{min.}$ 289, 269, and 235 nm (ε 33 100, 19 600, and 7 000); *m/e* 333 [$(M - 307)^+$].

N-[9-(2,3-O-*Isopropylidene*-β-D-*ribofuranosyl*)*purin*-6-*yl*]-N′-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* (8).—Acetic anhydride (1.0 g, 10 mmol), *NN*′-bis-[9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purin-6-yl]urea monohydrate (1.32 g, 2.0 mmol) and anhydrous pyridine (20 ml) were stirred together at 20 °C. After 16 h, methanol (3 ml) was added, and after a further 1 h the products were concentrated under reduced pressure. The resulting gum was dissolved in ethanol and the solution evaporated. This process was repeated and the residual gum triturated with ether to give a t.l.c. homogeneous [R_F (system B) 0.34] solid (1.30 g).

A portion of the latter was dissolved in 30% aqueous formic acid (30 ml) and the solution stirred at 20 °C. After 22 h, the products were concentrated under reduced pressure, the residual gum was dissolved in ethanol, and the solution was re-evaporated. This process was repeated and the resulting gum triturated with ether to give a solid. This partially dissolved in $CHCl_3$–MeOH (94 : 6 v/v; 10 ml), and the soluble fraction was separated into its pure components by short column chromatography [85 g of silica gel; $CHCl_3$–MeOH (94 : 6 v/v)]. The appropriate fractions were combined and concentrated under reduced pressure to give a t.l.c. homogeneous [R_F (system A) 0.60] glass (0.247 g).

Methanolic ammonia (half-saturated at 0 °C; 5 ml) was added to a solution of the glass in methanol (15 ml) and the resulting solution stirred at 20 °C. After 48 h the products were filtered and the residue was carefully crystallized from hot water to give N-[9-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*purin*-6-*yl*]-N′-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* as crystals [Found (material dried *in vacuo* over P_2O_5 at 85 °C): C, 47.7; H, 4.85; N, 23.5. $C_{24}H_{28}N_{10}O_9$ requires C, 48.0; H, 4.7; N, 23.3%], m.p. 184—185°; yield 0.135 g (22% based on the di-isopropylidene derivative); R_F (system A) 0.37; τ[$(CD_3)_2SO$] −1.85br (1 H, s) and −1.79br (1 H, s); τ[$(CD_3)_2SO$–D_2O] 1.30 (4 H, m), 3.73 (1 H, d, *J* 3 Hz), 3.92 (1 H, d, *J* 5 Hz), 4.63 (1 H, m), 4.98 (1 H, m), 5.38 (1 H, m), 5.71 (2 H, m), 5.95 (1 H, m), 8.41 (3 H, s), and 8.62 (3 H, s); $\lambda_{max.}$ (95% ethanol) 292, 285, and 267 (ε 37 700, 39 200, and 21 100), $\lambda_{min.}$ 289, 269, and 235 nm (ε 37 600, 20 900, and 7 500); *m/e* 307 [$(M - 293)^+$].

Hydrolysis of NN′-*Bis*-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]-*urea* (7a) *under* (a) *Neutral and* (b) *Alkaline Conditions*.—(*a*) The substrate (0.05 g) and water (3 ml) were heated together, under reflux. After 4 h, t.l.c. (system A) revealed that no substrate (R_F 0.09) was present but solely a product with R_F 0.25. The aqueous solution was concentrated under reduced pressure and the resulting gum crystallized from water (1 ml) to give adenosine (0.038 g, 82%), identical with authentic material [m.p. (235 °C), mixed m.p., and u.v. and n.m.r. spectroscopy].

(*b*) The substrate (0.025 g) was dissolved in aqueous M-sodium hydroxide (1 ml) and the solution stirred at 20 °C. After 18 h, t.l.c. (system A) revealed the presence of substrate only. The solution was then heated on a steam-bath. After a further 20 h, t.l.c. (system A) revealed a single product (R_F 0.25) and no substrate. The solution was cooled and treated with an excess of Dowex 50 (pyridinium form) cation-exchange resin, and the mixture was filtered. Concentration of the filtrate under reduced pressure and crystallization of the residual solid from methanol gave a crystalline compound characterized (see above) as adenosine.

N(6)-*Phenylcarbamoyladenosine* (9a; R^1 = H).—Phenyl isocyanate (0.952 g, 8.0 mmol) was added to a stirred solution of 2′,3′,5′-tri-*O*-acetyladenosine (1.572 g, 4.0 mmol) and pyridine (2 ml) in anhydrous dioxan (30 ml). The solution was heated, under reflux, for 1 h, cooled and then treated with water (0.3 ml). After 30 min, the products were concentrated under reduced pressure; the resulting gum was dissolved in methanolic ammonia (half-saturated at 0 °C; 40 ml). After 48 h, the crystalline precipitate was collected, washed with ice-cold methanol, and recrystallized from methanol (400 ml) to give *N*(6)-phenylcarbamoyl-adenosine [12] as crystals [Found (material dried *in vacuo* over P_2O_5 at 90 °C): C, 52.9; H, 4.7; N, 21.7. Calc. for $C_{17}H_{18}N_6O_5$: C, 52.8; H, 4.7; N, 21.75%]; m.p. 190—191°; yield 1.06 g (69%); R_F (system A) 0.48; τ[$(CD_3)_2SO$–D_2O] * 1.31 (1 H, s), 1.33 (1 H, s), 2.3—3.05 (5 H, m), 3.96 (1 H, d, *J* 5.5 Hz), 5.40 (1 H, m), 5.77 (1 H, m), 5.96 (1 H, m), and 6.32 (2 H, m); $\lambda_{max.}$ (95% ethanol) 280 (ε 26 900), $\lambda_{min.}$ 243 nm (ε 8 700); *m/e* 312 [$(M - 74)^+$].

Hydrolysis of N(6)-*Phenylcarbamoyladenosine*.—The substrate (0.03 g) and water (4 ml) were heated together under reflux. After 4 h, t.l.c. (system A) revealed a product with R_F 0.25 and no substrate (R_F 0.48). The mixture was concentrated under reduced pressure to give a gum. Crystallization from methanol gave adenosine, m.p. 235°, identical with an authentic specimen.

* The n.m.r. spectrum of (9a; R^1 = H) in anhydrous $(CD_3)_2SO$ has additional signals at τ −1.82 (1 H, s), −0.04 (1 H, s), 4.4—4.6 (1 H, m), and 4.7—5.0 (2 H, m).

Reaction between NN′-*Bis*-[9-(β-D-*ribofuranosyl*)*purin*-6-*yl*]*urea* (7a) *and Cyclohexylamine*.—*NN*′-Bis-[9-(β-D-ribofuranosyl)purin-6-yl]urea (0.578 g, 1.0 mmol) was dissolved in anhydrous pyridine (20 ml) and the solution evaporated. After this process had been repeated a further four times, anhydrous cyclohexylamine (20 ml) was added to the solid residue and the mixture heated under reflux. After 10 min, when t.l.c. (system A) revealed that no substrate remained, the products were cooled and concentrated under reduced pressure. The residue was triturated with ether (2 × 20 ml) and the resulting powder stirred with water (20 ml). After 1 h, the mixture was filtered and the residue crystallized from water to give colourless crystals of a compound, m.p. 179—180°, which was identical [t.l.c. (system A); mixed m.p.; u.v., i.r., and n.m.r. spectra] with authentic (see below) *N*(6)-cyclohexylcarbamoyladenosine; yield 0.346 g (88%).

The filtrate obtained after the crude products had been stirred with water was concentrated under reduced pressure. Crystallization of the residue from methanol gave adenosine (0.247 g, 92%), m.p. 235°, identical with authentic material.

N(6)-*Cyclohexylcarbamoyladenosine* (9b; R^1 = H).—Cyclohexyl isocyanate (0.75 g, 6.0 mmol) was added to a stirred solution of 2′,3′,5′-tri-*O*-acetyladenosine (1.179 g, 3.0 mmol) and pyridine (2 ml) in anhydrous dioxan (30 ml). The solution was heated, under reflux, for 24 h, cooled, and then treated with water (0.2 ml). After 30 min, the products were concentrated under reduced pressure; the resulting gum was dissolved in methanolic ammonia (half saturated at 0 °C; 30 ml) and the solution was stirred at 20 °C. After 48 h the crystalline precipitate was collected, washed with ice-cold water, and recrystallized from methanol to give N(6)-*cyclohexylcarbamoyladenosine* as crystals [Found (material dried *in vacuo* over P_2O_5 at 90 °C): C, 51.8; H, 6.1; N, 21.2. $C_{17}H_{24}N_6O_5$ requires C, 52.0; H, 6.1; N, 21.4%], m.p. 179—180°; yield 1.10 g (93%); R_F (system A) 0.49; τ[$(CD_3)_2SO$] * 0.45—0.75 (2 H, m), 1.35 (1 H, s), 1.46 (1 H, s), 3.99 (1 H, d, *J* 5 Hz), 4.52 (1 H, d, *J* 6 Hz), 4.75—5.0 (2 H, m), 5.42 (1 H, m), 5.80 (1 H, m), 6.00 (1 H, m), 6.15—6.5 (2 H, m), and 7.9—9.0 (11 H, m); $\lambda_{infl.}$ (95% ethanol) 276 (ε 18 800), $\lambda_{max.}$ 270 (ε 22 200), $\lambda_{min.}$ 234 nm (ε 3 800); *m/e* 392 (M^+).

2′,3′,5′-*Tri*-O-*acetyl*-N(6),N(6)-*bisphenoxycarbonyladenosine* (10).—Phenyl chloroformate (11.8 g, 75 mmol) was added dropwise over 2 min to a stirred solution of 2′,3′,5′-tri-*O*-acetyladenosine (9.825 g, 25 mmol) in anhydrous pyridine (100 ml) at 20 °C. The reactants were stirred for a further 16 h and then concentrated under reduced pressure. The gum obtained was dissolved in chloroform (400 ml); the solution was washed with saturated aqueous sodium hydrogen carbonate (400 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting yellow glass was fractionated by short column chromatography [600 g of silica gel; $CHCl_3$–MeOH (99.25 : 0.75 v/v)]. The appropriate fractions were combined and concentrated under reduced pressure to give a glass which was kept *in vacuo* over P_2O_5 at 20 °C for 16 h. Crystallization from methanol–water (3 l; 1 : 2 v/v) gave 2′,3′,5′-*tri*-O-*acetyl*-N(6),N(6)-*bisphenoxycarbonyladenosine* as crystals (Found: C, 56.8; H, 4.4; N, 11.2. $C_{30}H_{27}N_5O_{11}$ requires C, 56.9; H, 4.3; N, 11.1%), m.p. 66—67°; yield 12.43 g (79%); R_F (system C) 0.54; τ[$(CD_3)_2CO$] 0.96 (1 H, s), 1.18 (1 H, s), 2.25—2.95 (10 H, m), 3.54 (1 H, d, *J* 5.5 Hz), 3.86 (1 H, t, *J* 5.5 Hz), 4.22 (1 H, t, *J* 5.5 Hz), 5.4—5.75 (3 H, m), and 8.0 (s) and 8.04 (s) (9 H); $\lambda_{max.}$ (95% ethanol) 269 (ε 10 100), $\lambda_{infl.}$ 256 (ε 7 500), $\lambda_{min.}$ 236 nm (ε 4 700); *m/e* 496 [$(M - 137)^+$].

Reaction between 2′,3′,5′-*Tri*-O-*acetyl*-N(6),N(6)-*bisphenoxycarbonyladenosine* (10) *and Cyclohexylamine*.—Cyclohexylamine (2.47 g, 25 mmol) was added to a stirred solution of crude, unchromatographed 2′,3′,5′-tri-*O*-acetyl-*N*(6),*N*(6)-bisphenoxycarbonyladenosine [prepared as above from 2′,3′,5′-tri-*O*-acetyladenosine (1.965 g, 5.0 mmol) and phenyl chloroformate (2.35 g, 15 mmol)] in anhydrous dioxan at 20 °C. After 1 h, the products were concentrated under reduced pressure; the gum obtained was dissolved in chloroform (50 ml) and the resulting solution extracted with 3M-hydrochloric acid (50 ml). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue fractionated by short column chromatography [200 g of silica gel; $CHCl_3$–MeOH (98 : 2 v/v)]. The appropriate fractions were concentrated under reduced pressure, the resulting gum was redissolved in ethanol, and the solution was evaporated to give a solid. Recrystallization of this material from ethanol gave 2′,3′,5′-*tri*-O-*acetyl*-N(6)-*cyclohexylcarbamoyladenosine* as needles [Found (material dried *in vacuo* over P_2O_5 at 90 °C): C, 53.0; H, 5.7; N, 16.3. $C_{23}H_{30}N_6O_8$ requires C, 53.3; H, 5.8; N, 16.2%], m.p. 138—139°; yield 2.08 g (80%, based on 2′,3′,5′-tri-*O*-acetyladenosine); R_F (system B) 0.47; τ[$(CD_3)_2SO$] 0.40 (1 H, d, *J* 7 Hz), 0.84 (1 H, s), 1.26 (1 H, s), 1.44 (1 H, s), 3.64 (1 H, d, *J* 5.5 Hz), 3.94 (1 H, t, *J* 5.5 Hz), 4.28 (1 H, dd, *J* 4 and 5.5 Hz), 5.45—5.8 (3 H, m), 7.90 (s), 7.97 (s), and 8.00 (s) (9 H), and *ca.* 8.0—9.0 (11 H, m); $\lambda_{infl.}$ (95% ethanol) 276 (ε 18 600), $\lambda_{max.}$ 270 (ε 21 800), $\lambda_{min.}$ 232 nm (ε 3 000); *m/e* 518 (M^+).

2′,3′,5′-*Tri*-O-*acetyl*-N(6)-*phenoxycarbonyladenosine* (3c).—Morpholine (0.131 g, 1.5 mmol) was added to a stirred solution of 2′,3′,5′-tri-*O*-acetyl-*N*(6),*N*(6)-bisphenoxycarbonyladenosine (0.950 g, 1.5 mmol) in anhydrous dioxan (10 ml) at 20 °C. After 4 min, t.l.c. (system C) revealed no substrate (R_F 0.54) and essentially one product (R_F 0.33). The products were concentrated under reduced pressure and the glass obtained was fractionated by short column chromatography [85 g of silica gel; $CHCl_3$–MeOH (98.5 : 1.5 v/v)]. The appropriate fractions were combined and concentrated under reduced pressure to give a glass which was kept *in vacuo* over P_2O_5 at 20 °C for 16 h. Crystallization from di-isopropyl ether–ethyl acetate (10 : 1 v/v) gave 2′,3′,5′-*tri*-O-*acetyl*-N(6)-*phenoxycarbonyladenosine* as crystals [Found: C, 54.0; H, 4.7; N, 13.4. $C_{23}H_{23}N_5O_9$ requires C, 53.8; H, 4.5; N, 13.65%], m.p. 58—60°; yield 0.423 g (55%); R_F (system C) 0.33; τ[$(CD_3)_2SO$] −1.09br (1 H, s), 1.33 (s) and 1.36 (s) (2 H), 2.4—2.9 (5 H, m), 3.67 (1 H, d, *J* 6 Hz), 3.94 (1 H, m), 4.33 (1 H, m), 5.4—5.8 (3 H, m), and 7.88 (s), 7.96 (s), and 8.00 (s) (9 H); $\lambda_{max.}$ (95% ethanol) 268 (ε 19 000), $\lambda_{min.}$ 231 nm (ε 4 900); *m/e* 419 [$(M - 94)^+$].

Preparation of N(6)-*Cyclohexylcarbamoyladenosine* (9b; R^1 = H) *from* 2′,3′,5′-*Tri*-O-*acetyl*-N(6)-*phenoxycarbonyladenosine* (3c).—A solution of cyclohexylamine (0.049 g, 0.5 mmol) and 2′,3′,5′-tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (0.257 g, 0.5 mmol) in anhydrous dioxan (5 ml) was stirred at 20 °C. After 1 h, t.l.c. (system C) revealed no starting material (R_F 0.33) and a single product (R_F 0.27). The solution was then concentrated under reduced pressure, the gum obtained dissolved in methanolic

* Addition of D_2O causes the signals at τ 0.45—0.75, 4.52, and 4.75—5.0 to disappear.

ammonia (half-saturated at 0 °C; 5 ml), and the resulting solution stirred at 20 °C. After 16 h, the crystalline precipitate was collected, washed with ice-cold water, and recrystallized from methanol–water (1 : 1 v/v) to give *N*(6)-cyclohexylcarbamoyladenosine (0.180 g, 92%), m.p. 179—180°, identical [R_F (system A); mixed m.p.; u.v., n.m.r., and mass spectra] with authentic material.

Reaction between N(6)-*Methoxycarbonyladenosine* (3d) *and Cyclohexylamine*.—Cyclohexylamine (0.025 g, 0.25 mmol) was added to a solution of *N*(6)-methoxycarbonyladenosine [15] (0.054 g, 0.17 mmol) in anhydrous pyridine (2 ml) and the resulting solution heated under reflux. After 4.5 h, t.l.c. (system A) revealed *N*(6)-methoxycarbonyladenosine (R_F 0.34; *ca.* 25%) and a single product (R_F 0.49; *ca.* 75%). After 16 h, when no starting material remained, the products were cooled and concentrated under reduced pressure and the resulting gum was dissolved in chloroform (2 ml). The solution was washed with 3M-hydrochloric acid (2 ml), dried ($MgSO_4$), and evaporated to a gum. This was dissolved in ethanol and the solution re-evaporated. Crystallization of the residue from methanol gave *N*(6)-cyclohexylcarbamoyladenosine, m.p. 179—180°, identical [R_F (system A), mixed m.p., and u.v., n.m.r., and mass spectra] with authentic material.

N(6)-*Carbamoyladenosine* (9c; R^1 = H).—2′,3′,5′-Tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (0.257 g, 0.5 mmol) was dissolved in a stirred, saturated solution of ammonia in dioxan (5 ml) at 20 °C. After 2 h, t.l.c. (system B) revealed no starting material and a single product (R_F 0.25). The solution was concentrated under reduced pressure, the residual gum dissolved in methanolic ammonia (half-saturated at 0 °C; 5 ml), and the resulting solution stirred at 20 °C. After 18 h the crystalline precipitate was collected, washed with ice-cold methanol, and recrystallized from methanol–water (9 : 1 v/v) to give N(6)-*carbamoyladenosine hemihydrate* as crystals [Found (material dried *in vacuo* over P_2O_5 at 90 °C): C, 41.1; H, 4.5; N, 26.1. $C_{11}H_{14}N_6O_5,0.5H_2O$ requires C, 41.4; H, 4.7; N, 26.3%], m.p. 128—129°; yield 0.142 g (92%); R_F (system A) 0.25; τ[$(CD_3)_2SO$–D_2O] 1.24 (1 H, s), 1.33 (1 H, s), 3.88 (1 H, d, *J* 5 Hz), 5.31 (1 H, m), and 5.69 (1 H, m); $\nu_{max.}$ (Nujol) 3 500m, 3 290m, 1 700s, and 1 615s cm^{-1}; $\lambda_{infl.}$ (95% ethanol) 275 (ε 15 800), $\lambda_{max.}$ 268 (ε 19 900), $\lambda_{min.}$ 231 nm (ε 3 100).

N(6)-*Methoxycarbonylmethylcarbamoyladenosine* (9d; R^1 = H).— 2′,3′,5′-Tri-*O*-acetyl-*N*(6)-phenoxycarbonyladenosine (0.256 g, 0.5 mmol) and glycine methyl ester hydrochloride (0.069 g, 0.55 mmol) were stirred together in anhydrous dioxan (5 ml) at 20 °C while triethylamine (0.055 g, 0.55 mmol) was added. After the reactants had been stirred for a further 18 h, t.l.c. (system C) revealed no starting material (R_F 0.33) and a single product * (R_F 0.26). The product was then filtered and the filtrate concentrated under reduced pressure. The gum obtained was fractionated by short column chromatography [50 g of silica gel; $CHCl_3$–MeOH (98 : 2 v/v)]. The appropriate fractions were combined and concentrated under reduced pressure to give a gum which was dissolved in methanolic sodium methoxide (0.5M; 5 ml), and the solution was stirred at 20 °C. After 10 min, the products were neutralized with Amberlite IR 120 cation-exchange resin (H^+ form); the mixture was filtered and the filtrate concentrated under reduced pressure. Crystallization of the solid obtained gave N(6)-*methoxycarbonylmethylcarbamoyladenosine* as crystals [Found (material dried *in vacuo* over P_2O_5 at 90 °C): C, 43.7; H, 4.85; N, 22.0. $C_{14}H_{18}N_6O_7$ requires C, 44.0; H, 4.7; N, 22.0%], m.p. 178—179°; yield 0.149 g (78%); R_F (system A) 0.45; τ[$(CD_3)_2SO$] 0.1—0.4 (2 H, m), 1.36 (1 H, s), 1.47 (1 H, s), 3.96 (1 H, d, *J* 5.5 Hz), 4.4—5.1 (3 H, m), 5.39 (1 H, m), 5.7—6.05 (4 H, m), and 6.1—6.5 (5 H, m); $\lambda_{infl.}$ (95% ethanol) 275 (ε 17 500), $\lambda_{max.}$ 269 (ε 20 800), $\lambda_{min.}$ 233 nm (ε 3 600); *m/e* 293 [$(M - 89)^+$].

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* If the reaction is carried out in dimethylformamide it goes to completion in less than 1 h at 20 °C.

[15] T. Ravindranathan and C. B. Reese, unpublished results.