Partial Protection of Carbohydrate Derivatives. Part 4.[1] Regioselective 2'-*O*-Deacylation of Fully Acylated Purine and Pyrimidine Ribonucleosides with Hydroxylaminium Acetate

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Like hydrazine hydrate, hydroxylamine was found to be useful for the regioselective 2'-*O*-deacylation of fully acylated purine and pyrimidine ribonucleosides as its salt with acetic acid; the partial *O*-deacylation reactions (which were not accompanied by undesirable discolouration as happens with hydrazine hydrate) gave the corresponding di-*O*-acylribonucleosides in superior yields; *e.g.* 2',3'-di-*O*-benzoyladenosine (74% yield), 3',5'- (64% yield) and 2',5'-di-*O*-benzoyl-N^6-benzyladenosine (63% yield on performing the reaction in ethanol), N^2,3',5'-tri-*O*-benzoylguanosine (66% yield), N^2,2'.5'-tri-isobutyrylguanosine (48% yield), and 3',5'-di-*O*-benzoyluridine (61% yield) were obtained using hydroxylaminium acetate in pyridine. Treatment of fully acetylated ribonucleosides with an excess of hydroxylaminium acetate gave the corresponding 5'-*O*-acetylribonucleosides in quantitative yields.

The excellent regioselectivity observed in the present partial *O*-decyclation was confirmed on the basis of chromatographic separation; the mixtures of di-*O*-acylribonucleosides, which had already been equilibrated in pyridine. were re-equilibrated on the silica gel during separation, *e.g.* a 70 : 30 mixture of 3',5'- and 2',5'-di-*O*-benzoyladenosine was completely converted into the former based on ^{1}H n.m.r. spectroscopy. The acetates of 9-β-D-*xylo*- and -*arabino*-furanosyladenine were also found to give predominantly the corresponding 3',5'-diacetates on hydroxylaminolysis.

CHEMICAL discrimination between the hydroxy-groups at the 2'- and 3'-positions of the β-D-ribofuranosyl moiety of ribonucleosides has been a long-standing problem in the field of nucleic acid chemistry, from the standpoint of both ribonucleotide oligomer or polymer synthesis and nucleoside chemistry, although there have been a number of attempts at partial protection. The assumption that 2'-*O*-acyl groups of fully acylated ribonucleosides should be the most labile toward an appropriate nucleophile has recently brought about a novel procedure for regioselective 2'-*O*-deacylation *via* hydrazinolysis, *i.e.* treatment of the ribonucleoside acyl derivatives with hydrazine hydrate in acetic acid–pyridine (1 : 4 v/v).[1] Since then we have searched for other reagents more effective than hydrazine hydrate, and found that hydroxylamine is superior to hydrazine hydrate, by using it as hydroxylaminium acetate for the partial *O*-deacylation. We now report the results in detail, as

TABLE 1

Attempted partial *O*-deacylation of 2',3',5'-tri-*O*-benzoyladenosine (1a) by means of aminolyses [a]

Amine	pK_b	[Amine]/[(1a)]	Solvent [b]	Time/d	Products (yield %) 3',5'- and 2',5'-Dibenzoates	5'-Benzoate (4a)	Recovery of (1a)
Guanidine	0.41	1.2	A	2	*c*		
		1.2	B	2	*c*		
Pyrrolidine	2.73	1.2	A	2	5	38	57
		4	B	4	28	8	64
Diethylamine	3.02	1.2	A	2	4	90	5
		4	B	4	<1		>99
Cyclohexylamine	3.53	1.2	A	2	8	62	30
		4	B	4	<1		>99
1,2-Ethylenediamine	4.03	0.6	A	2	11	81	8
	(6.35)	2	B	4	26	10	64
Benzylamine	4.64	1.2	A	2	11	34	55
		4	B	4	<1		>99
Ammonia	4.75	1.2	A	4	<1	<1	>98
		4	B	4	<1		>99
Hydrazine hydrate	5.90	1.2	A	2	10	69	21
		4	B	4	26	65	9
Hydroxylamine	5.95	1.2	A	2	15	64	21
		4	B	4	20	65	15
Phenylhydrazine	6.79	4	A	2	19	28	53
		4	B	6	<1		>99
NN-Dimethylhydrazine	8.79	4	A	2	10	25	65
		4	B	6	<1		>99
Methoxyamine	9.4	4	B	7	18 [d]		78
		4	A	2	<1		>99
Hydrazinoformamide	10.54	4	B	6	<1		>99

[a] All the reactions were performed with (1a) (0.1 mmol) at room temperature. [b] A, $CHCl_3$–MeOH (1 : 1 v/v); B, pyridine. [c] Free adenosine was produced. [d] This is of the isolated products.

well as an interesting finding on the crucial point of obtaining excellent regioselectivity in 3′-5′-di-*O*-acylribonucleoside formation, as a result of re-equilibration of the mixtures, which have already been equilibrated during column chromatography of the aminolysis fraction on silica gel.

RESULTS AND DISCUSSION

The remarkable utility of hydrazine hydrate in the partial *O*-deacylation of fully acylated ribonucleosides [1] prompted us to search for other amine species to bring about similar selectivity in di-*O*-acylribonucleoside formation. In the light of the effectiveness of hydrazine hydrate, we chose the series of amines shown in Table 1 as potential deacylating agents, and 2′,3′,5′-tri-*O*-benzoyladenosine (1a) as a model substrate. The reaction conditions used and the results thus obtained are summarized in Table 1. Surveying all the results in comparison with those obtained with hydrazine hydrate, hydroxylamine was found to give a selectivity comparable with hydrazine hydrate. The results obtained with benzylamine were also comparable, but the reaction was too slow for practical use. Other agents (methoxyamine, *NN*-dimethylhydrazine, and hydrazinoformamide) were unexpectedly unreactive; the stereochemical bulkiness of the substituents on the oxygen or nitrogen atoms is presumably the reason for these unexpected results.

Both hydroxylamine and hydrazine hydrate are of comparable nucleophilicity judging from their chemical properties in connection with the ' α-effect '.[2] Accordingly, we compared their utility (as their carboxylate salts) in the partial *O*-debenzoylation of (1a) under the conditions summarized in Table 2. The results clearly demonstrate the superiority of hydroxylamine over hydrazine hydrate in giving di-*O*-benzoyladenosines, when used in combination with acetic or benzoic acids. In addition, little discolouration was observed in the reaction with hydroxylamine, in contrast to the reaction with hydrazine hydrate.

On the basis of these results, we made a detailed investigation on the conditions appropriate for the potentially regiospecific 2′-*O*-deacylation of fully acylated ribonucleosides, using 2′,3′,5′-tri-*O*-benzoyl-N^6-benzyladenosine (1b) as the substrate with hydroxylamine. The 3′,5′- (2b) and 2′,5′-dibenzoates (3b) resulting from the hydroxylaminolysis of (1b) were clearly detectable separate peaks in the high performance l.l.c., unlike 3′,5′- (2a) and 2′,5′-di-*O*-benzoyladenosine (3a) from (1a) which were detected as overlapping peaks with almost the same retention times. The material balance of each reaction was monitored by means of l.l.c. under conditions *B* and *C* (see Experimental section). Compound (1b) was prepared from 2′,3′,5′-tri-*O*-benzoylinosine (1d) in 67% overall yield by chlorination with thionyl chloride–*NN*-dimethylformamide (DMF), followed by benzylamination.

The conditions used and the results obtained from reaction with hydroxylaminium acetate are summarized in Table 3. The unreacted (1b) in the reaction could not be determined accurately because the l.l.c. peak of (1b) appeared as a shoulder on the l.l.c. peak of pyridine, and is therefore not shown in the Table. As can be seen from the Table, the partial *O*-debenzoylation was best performed by use of 4 mol equiv. of hydroxylaminium acetate for 3—4 d. It is interesting to note that the proportions of (2b) and (3b) shown in the fourth column were, by and large, the same; this was confirmed to have arisen from their equilibration in pyridine containing hydroxylaminium acetate, as will be described later.

It was shown that the proportion of acetic acid to hydrazine hydrate was crucial in obtaining good selectivity in the formation of (2a) and (3a) in the partial hydrazinolytic *O*-debenzoylation.[1] On the other hand, it has been reported that acyl migration in the 2′,3′-*cis*-diol system could be suppressed in an acidic medium.[3]

TABLE 2

A comparative study on utility of hydroxylaminium and hydrazinium acylates for the partial *O*-debenzoylation of 2′,3′,5′-tri-*O*-benzoyladenosine (1a) *

		Product yields (%)		
Agent–acid (1 : 1)	[Agent]/[(1a)]	Mixture of 3′,5′- and 2′,5′-dibenzoates	5′-benzoate	Recovery of (1a)
H_2NOH–AcOH	4	36		64
H_2NOH–AcOH	6	48	<1	51
H_2NOH–BzOH	4	28		72
H_2NOH–BzOH	6	41		59
H_2NNH_2–AcOH	4	12	62	26
H_2NNH_2–BzOH	2	37		63
H_2NNH_2–BzOH	4	63	31	6
H_2NNH_2–BzOH	8	35	65	

* All the reactions were performed with (1a) (0.1 mmol) in pyridine at room temperature for 4 d.

TABLE 3

Effect of molar ratios of hydroxylaminium acetate, and reaction times, on the partial *O*-debenzoylation of 2′,3′,5′-tri-*O*-benzoyl-N^6-benzyladenosine (1b) in pyridine *

		Product yield (%)		
$[NH_3OH^+AcO^-]$/[(1b)]	Reaction time/d	Di-benzoates	3′,5′- (2b) : 2′,5′-isomer (3b)	5′-Benzoate (4b)
2	1	35	66 : 34	
2	2	43	74 : 26	
2	3	56	71 : 29	
2	4	60	65 : 35	
2	5	61	69 : 31	7
4	1	49	70 : 30	
4	2	64	70 : 30	
4	3	77	68 : 32	10
4	4	73	67 : 33	16
4	5	72	67 : 33	24
6	1	62	71 : 29	
6	2	66	71 : 29	15
6	3	73	63 : 37	22
6	4	61	69 : 31	43
6	5	51	66 : 34	44

* All the reactions were performed with (1b) (0.1 mmol) in pyridine (3 ml) at room temperature. The yields and proportions of products were determined by l.l.c.

Therefore, we next examined the effects of the proportion of acetic acid to hydroxylamine; the conditions used and the results obtained are summarized in Table 4. It was confirmed that the use of 1—3 mol equiv. of acetic acid to hydroxylamine is the most advantageous for obtaining good selectivity in the formation of (2b) and (3b). As seen from the Table, the proportions of (2b) and (3b) were substantially the same regardless of the proportions of acetic acid and hydroxylamine, *ca.* 2 : 1.

TABLE 4

Effect of molar ratio of acetic acid : hydroxylamine on partial *O*-debenzoylation of (1b) in pyridine *

[AcOH]/[NH_2OH]	Product yield (%)		
	Dibenzoates	(2b) : (3b)	5′-Benzoate (4b)
0	65	62 : 38	25
1	78	62 : 38	19
3	76	64 : 36	11
7	57	64 : 36	
16	48	64 : 36	

* All the reactions were performed with (1b) (0.1 mmol) and hydroxylamine (0.4 mmol) in pyridine (3 ml) at room temperature for 3 d. The yields and proportions of products were determined by l.l.c.

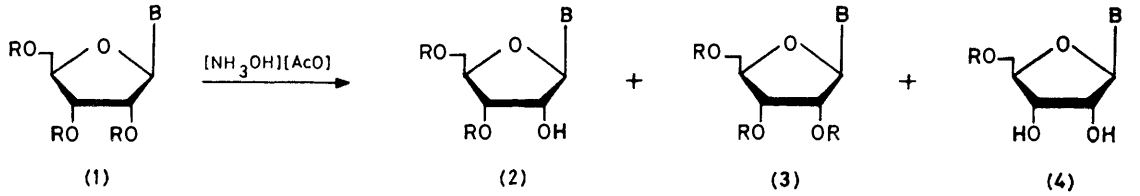
As in the hydrazinolytic reaction,[1] we examined the solvent effect on the hydroxylaminolysis by using (1b) in a series of solvents; the conditions used and the results thus obtained are summarized in Table 5. As seen from the Table, pyridine was found to be the best solvent for obtaining (2b) and (3b) in good yields, and, moreover, it is of great interest that the reactions in methanol and ethanol gave more (3b) than (2b), and (3b) was found to crystallize from the alcohols more easily than (2b); during both reactions, (3b) began to precipitate as pure white crystals. Thus from (1b), we can prepare either (2b) or (3b) by selecting the appropriate solvent for partial *O*-debenzoylation.

TABLE 5

Effect of solvent on the partial *O*-debenzoylation of (1b) with hydroxylaminium acetate [a]

Solvent	Reaction period/d	Product yield (%)			
		Di-benzoates	(2b) : (3b)	5′-Benzoate (4b)	Recovery of (1b)
Pyridine	3	78	66 : 34	19	*b*
DMF	7	62	63 : 37	32	6
DMSO	7	64	68 : 32	0	36
1,4-Dioxan	7	33	54 : 46	0	67
Chloroform	7	6		0	94
Benzene	7	5		0	95
Methanol	7	59	32 : 68	14	27
Ethanol	7	71	19 : 81	13	16

[a] All the reactions were performed with (1b) (0.1 mmol) and hydroxylaminium acetate (0.4 mmol) in the appropriate solvent (3 ml) at room temperature. The yield of products and their proportions were determined by l.l.c. [b] The yields could not be determined because the l.l.c. peak of (1b) appeared as a shoulder on that of pyridine.



	B	R
a;	Adenin-9-yl	Bz
b;	N^6-Benzyladenin-9-yl	Bz
c;	N^2-Benzoylguanin-9-yl	Bz
d;	Hypoxanthin-9-yl	Bz
e;	Uridin-1-yl	Bz
f;	Adenin-9-yl	Ac
g;	N_2-Acetylguanin-9-yl	Ac
h;	N_2-Benzoylguanoin-9-yl	Ac
i;	Hypoxanthin-9-yl	Ac
j;	Uridin-1-yl	Ac
k;	N^4-Acetylcytin-1-yl	Ac
l;	N^2-Isobutyrylguanin-9-yl	Bu

Based on the results obtained so far, we performed partial *O*-deacylation reactions on (1a), (1b), N^2,2′,3′,5′-

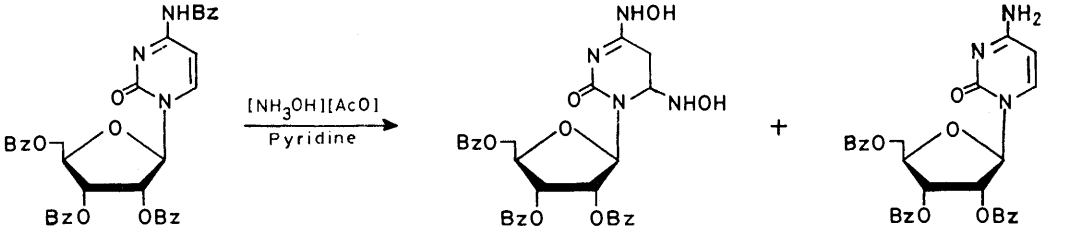
TABLE 6

Partial *O*-deacylation of fully acylated purine and pyrimidine ribonucleosides by hydroxylaminolysis [a]

Substrate	Solvent	Reaction conditions [NH_3OH][AcO]/[Substrate]	Reaction time/d	Product yield (%)			
				(2)	(3)	[(2) + (3)]	(4)
(1a)	Pyridine	4	1	74		[84]	10
(1b)	Pyridine	4	2	64		[82]	
(1b)	Ethanol	8	6		63 [b]		
(1c)	Pyridine	4	1	66		*c*	
(1d)	Pyridine	4	1	72		*c*	10
(1e)	Pyridine	4	1	61		[76]	6
(1f)	Pyridine	1.2	2	54		[83]	6
(1g)	Pyridine	1.2	1	57		[78]	7
(1h)	Methanol	4	3	58 [b]			
(1i)	Pyridine	1.2	1		56	*c*	18
(1j)	Pyridine	1.2	1	53		[72]	15
(1k)	Pyridine	2.2	1			[68]	
(1l)	Pyridine	1.5	3		43	[63]	

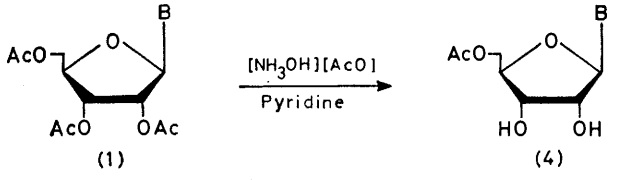
[a] All the reactions were performed at room temperature. [b] These were obtained in pure crystalline form from the resulting mixture by simple suctional filtration. [c] These were obtained as mixtures with benzohydroxamic or acetohydroxamic acids, and the yields could not be calculated.

tetrabenzoylguanosine (1c), (1d), 2′,3′,5′-tri-*O*-benzoyluridine (1e), 2′,3′,5′-tri-*O*-acetyladenosine (1f), N^2,2′,3′,5′-tetra-acetylguanosine (1g), 2′,3′,5′-tri-*O*-acetyl-N^2-benzoylguanosine (1h), 2′,3′,5′-tri-*O*-acetylinosine (1i), 2′,3′,5′-tri-*O*-acetyluridine (1j), N^4,2′,3′,5′-tetra-acetylcytidine (1k), and N^2,2′,3′,5′-tetraisobutyrylguanosine (1l); the conditions used and the results obtained are summarized in Table 6. In contrast with the hydrazinolysis,[1] the hydroxylaminolysis gave the corresponding di-*O*-acylates in good yields (pyridine as solvent). The reactions of (1b) in ethanol and of (1h) in methanol gave (3b) and (2h), respectively, both of which precipitated out during the reactions. In the case of (1k), the corresponding 3′,5′- and 2′,5′-diacetates were obtained as an inseparable syrupy mixture. Hydroxylaminolysis of N^4,2′,3′,5′-tetrabenzoylcytidine (1m) was impossible since the 4-amino-group and 5,6-double bond



(1m)

were susceptible to substitution and addition, respectively, by the hydroxylamine; similar reactions have been reported by Brown and Shell [4] for cytidine derivatives. These results further prompted us to prepare 5′-*O*-acetylribonucleosides using (1f), (1g), (1i), and (1j) as starting materials; the conditions used and the results obtained are summarized in Table 7. The use of 4 mol equiv. of hydroxylaminium acetate gave the corresponding 5′-acetates (4f), (4g), (4i), and (4j) in quantitative yields.



(1) (4)

B = adenin-9-yl (f)
B = N^2-acetylguanin-9-yl (g)
B = hypoxanthin-9-yl (i)
B = uracil-1-yl (j)

Subsequently, we made an investigation on the potential acyl migration involved in this procedure, to resolve the inconsistency between the proportions of (2b) and (3b) (*ca.* 2 : 1) given in Table 3 and the unexpectedly good yield of isolated (2b) (64% yield) given in Table 6. The proportion might be within the limits proposed for the proportions of 3′- and 2′-*O*-acylribonucleosides, *i.e.* 1.7 : 1—3 : 1, which have been already reported with respect to 3′- and 2′-*O*-acyl- and -aminoacylribonucleosides, respectively, by Reese and Trentham [5] and McLaughlin and Ingram.[6] Also, Griffin *et al.*[3] reported that acyl migration in such systems is much faster than hydrolysis of the acyl groups. Therefore, we first examined the proportions of the corresponding 3′,5′- and 2′,5′-diacylates resulting from the hydroxylaminolyses of the fully acylated ribonucleosides; the conditions used and the results obtained are summarized in Table 8. It was shown that all the proportions obtained were in the range 2 : 1—3 : 1. Consequently, we next examined potential acyl migration during the hydroxylaminolysis by use of (2b) [containing 5% of

TABLE 7

Preparation of some 5′-*O*-acetylribonucleosides by hydroxylaminolysis of the corresponding fully *O*-acetylated ribonucleosides *

Ribonucleoside acetate	Yield (%) of 5′-*O*-acetylribonucleosides
2′,3′,5′-Tri-*O*-acetyladenosine (1f)	92
N^2,2′,3′,5′-Tetra-acetylguanosine (1g)	97
2′,3′,5′-Tri-*O*-acetylinosine (1i)	91
2′,3′,5′-Tri-*O*-acetyluridine (1j)	95

* All the reactions were performed with hydroxylaminium acetate (4 mol equiv. to each substrate) in pyridine at room temperature for 1d.

TABLE 8

Proportions of 3′,5′- and 2′,5′-di-*O*-acylribonucleosides in the mixtures resulting from the hydroxylaminolysis and those after the chromatographic separation on Wakogel C-300 [a]

Entry	Starting material	Proportions of 3′,5′- and 2′,5′-diacylates: Hydroxylaminolytic mixture	Proportions of 3′,5′- and 2′,5′-diacylates: After chromatography
1	(1a)	70 : 30	100 : 0
2	(1b)	65 : 35 [b]	90 : 10 [b]
3	(1c)	65 : 35	90 : 10 [c]
4	(1d)	65 : 35	80 : 20 [c]
5	(1e)	*d*	80 : 20
6	(1f)	75 : 25	90 : 10
7	(1g)	*d*	70 : 30
8	(1h)	65 : 35	70 : 30 [c]
9	(1i)	70 : 30	70 : 30
10	(1j)	*d*	60 : 40

[a] All the reactions were performed with the benzoates (1a)—(1e) (0.2 mmol) and hydroxylaminium acetate (0.8 mmol), or the acetates (1f)—(1j) (0.3 mmol), and hydroxylaminium acetate (0.36 mmol) in pyridine at room temperature. The proportions were calculated from the ^{1}H n.m.r. spectra of the corresponding products. [b] These proportions were calculated by 1.1.c. [c] Obtained as a mixture with the corresponding hydroxamic acids. [d] Proportions could not be calculated from the ^{1}H n.m.r. spectra because of interference from the signals of the starting acylated ribonucleosides.

TABLE 9

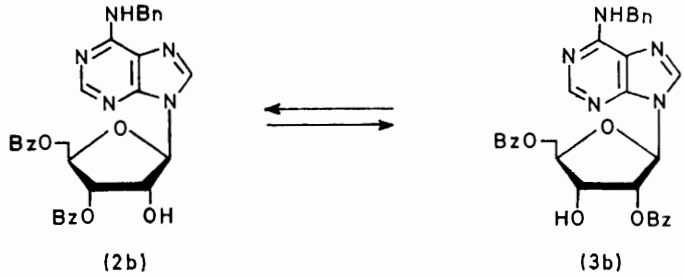
Acyl migration reactions on silica gel (Wakogel C-300) 3′,5′- and 2′,5′-di-*O*-acylribonucleosides [a]

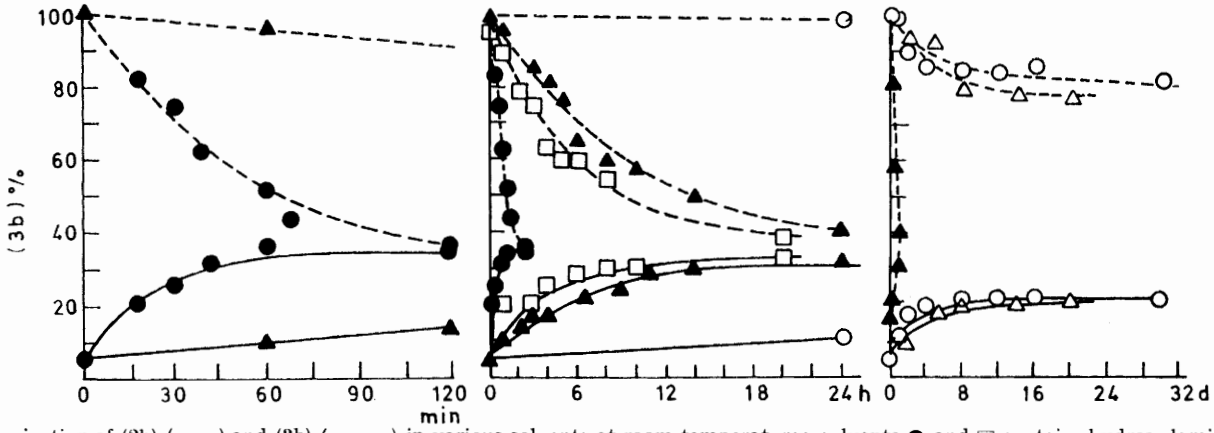
	Ratio of 3′,5′- : 2′,5′-isomers	Resulting ratios of 3′,5′-2′,5′-isomers
(2a) + (3a)	60 : 40	100 : 0
(2b) + (3b)	95 : 5 [b]	96 : 4 [b]
(2b) + (3b)	0 : 100 [b]	94 : 6 [b]
(2c) + (3c)	0 : 100	90 : 10
(2e) + (3e)	60 : 40	65 : 35
(2f) + (3f)	60 : 40	80 : 20
(2i) + (3i)	0 : 100	80 : 20

[a] Ratios calculated from ^{1}H n.m.r. spectroscopy. [b] Ratios calculated by means of l.l.c.

(3b) as contaminant] and (3b) as the model substrates of the monodeacylated ribonucleoside acylates, since they are detectable as separate peaks in l.l.c. as described earlier. The results thus obtained are shown in the Figure, from which it was demonstrated that (*i*) the isomerization of (2b) to (3b) and *vice versa* in anhydrous pyridine, and in pyridine containing 2 mol equiv. of acetic acid was so slow that only 15—20% of the substrates was isomerized even after 20—30 d; a similar trend was observed in the reaction in 1 : 1 v/v chloroform–methanol: and (*ii*) the solvent systems containing 2 mol equiv. of hydroxylaminium acetate, in contrast, induced rapid acyl migration to give the equilibrated mixture composed of *ca.* 2 : 1 of (2b) : (3b). It was thus confirmed that the reaction system for partial *O*-deacylation facilitates acyl migration between the 2′- and 3′-positions on the D-ribofuranosyl moiety. The acyl migration was also effectively induced in aqueous pyridine.

On the other hand, it is of great interest to note that the di-*O*-benzoyladenosine resulting from the chromatographic separation on a column of Wakogel C-300 silica gel contains no 2′,5′-dibenzoate, but the mixture from the hydroxylaminolytic reaction prior to the chromatographic separation contains 30% of the 2′,5′-dibenzoate on the basis of ^{1}H n.m.r. spectroscopy (Table 8, column 4). Accordingly, we compared the proportions of 3′,5′- and 2′,5′-di-*O*-acylribonucleosides before and after chromatographic separation. The results in the third and fourth columns of Table 8 showed that significant changes in the proportions had occurred in some cases (entries 1–4, and 6). In order to confirm the importance of the chromatographic separation process, we then used various arbitrary proportions of the diacylates for the treatment, and the results are summarized in Table 9. We then concluded that the crucial step of the present procedure for obtaining excellent regioselectivity was actually the chromatographic separation process. In marked contrast to Wakogel C-300, considerably slower acyl migration was observed on Mallinckrodt silica gel (100 mesh), which was used by Johnston [7] for the separation of adenosine and uridine diacetates; such acyl migration proved here was not mentioned at all.* Treatment of (3b) on Mallinckrodt silica gel, followed by elution after adsorption on it for





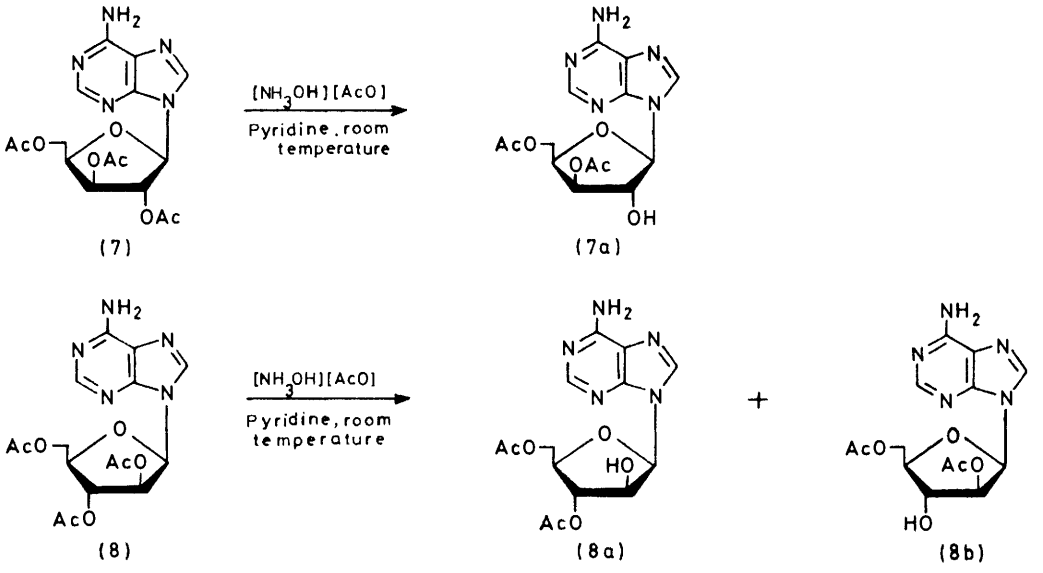
Isomerization of (2b) (——) and (3b) (– – – –) in various solvents at room temperature; solvents ● and □ contains hydroxylaminium acetate (2 mol equiv. to each nucleoside): pyridine (●); water–pyridine (2 : 98 v/v) (▲); acetic acid–pyridine (1 : 4 v/v) (□); anhydrous pyridine (○); pyridine containing acetic acid (2 mol equiv. to each nucleoside) (△)

* The difference observed between these silica gels may reflect their surface structure, *e.g.* if they involve plenty of free acidic hydroxy-functions and/or cavity-volume on their surface.

8 d, gave an 83 : 17 mixture of (2b) : (3b) (16 : 84 after 7 h; 42 : 58 after 1 d; and 74 : 26 after 3 d). The period needed for adsorption on Wakogel C-300, on the other hand, was only a few hours. On Wakogel C-300, the delicate difference between 2′- and 3′-*O*-acyl groups in the *activity* as esters was well discriminated to re-equilibrate the mixtures of the diacylates, which had already been equilibrated in the hydroxylaminolytic system, and bringing about the excellent regioselectivity observed. This may also be the case in the hydrazinolytic partial *O*-deacylation procedure.

Subsequently, we attempted partial *O*-deacylation using ribonucleosides with different acyl groups at the 2′- and 3′-positions, *i.e.* 3′,5′-di-*O*-benzoyl-N^6-benzyl-2′-*O*-*m*-toluoyl- (5) and 2′,5′-di-*O*-benzoyl-N^6-benzyl-3′-*O*-*m*-toluoyladenosine (6), in order to acquire more direct evidence than that we had obtained previously on partial hydrazinolysis of 2′,5′-di-*O*-acetyl-3′-*O*-methyl- and 3′,5′-di-*O*-acetyl-2′-*O*-methyladenosine; [1] *viz.*, that the 2′-*O*-acetyl group was far more labile than the 3′-*O*-acetyl group. Compounds (5) and (6) were respectively prepared by *m*-toluoylation of (2b) [containing 5% of (3b)] and (3b) with *m*-toluoyl chloride in anhydrous pyridine.* They were then treated with 4 mol equiv. of hydroxylaminium acetate under similar conditions. Compound (5) gave a 4 : 1 mixture of di-*O*-benzoyl, and *O*-benzoyl-*O*-*m*-toluoyl derivatives in 71% yield, and (6) gave a 1 : 9 mixture of the derivatives in 68% yield; such a large difference might indeed confirm the assumption that 2′-*O*-acyl functions are the most active in the *O*-acetyl groups on the D-ribofuranosyl ring. These results led us to the conclusion that 2′-*O*-acyl groups were removed with 80—90% regioselectivity in the hydroxylaminolysis; both (5) and (6) might possibly contain the corresponding isomer as a contaminant, based on the acyl migration results, and the actual regioselectivity could thus possibly exceed that which was found.

Based on these results, we were further interested in the behaviour of nucleoside acylates with D-xylo- and D-arabino-furanosyl moieties in place of the D-ribofuranosyl one. As model substrates for this purpose we chose 9-(2,3,5-tri-*O*-acetyl-β-D-xylo- and -arabinofuranosyl)adenine (7) and (8), respectively. Compound (7) was prepared according to the method of Lee *et al.*,[8] followed by acetylation as usual. Compound (8) was prepared from 3′,5′-di-*O*-benzoyladenosine (2a) by DMSO oxidation, followed by $NaBH_4$ reduction and acetylation, in the light of the method reported by Brodbeck and Moffatt.[9] Reist *et al.*,[10] Glaudemans and Fletcher,[11] Ikehara and Ogiso,[12] and Sowa and Tsunoda [13] have reported the synthesis of 9-β-D-arabinofuranosyladenine. However, the present method was found to be the best for preparing the nucleoside (63% overall yield), although it involves a purification procedure involving column chromatography on a strongly basic ion-exchange resin.[14] On hydroxylaminolysis under similar conditions, (7) gave 9-(3,5-di-*O*-acetyl-β-D-xylofuranosyl)adenine (7a) (64% yield) with 100% selectivity, and (8) gave an 85 : 15 mixture of 9-(3,5- and -2,5-di-*O*-acetyl-β-D-arabinofuranosyl)adenine (8a,b) (59% yield in addition to a further 13% yield as a 1 : 2 mixture with acetohydroxamic acid). On the basis of these ratios, we can assume that the 2′-*O*-acyl groups are also the most active ester function in (7) and (8), due to the electronic effect of the aglycone moieties, as we have discussed in the hydrazinolytic partial *O*-deacylation of fully acylated ribonucleosides [1] in comparison with the results obtained with methyl β-D-ribofuranoside acylates, whose 2-*O*-acyl groups were found to be apparently less active than those of ribonucleoside acylates.[15] pK_a Values of *ca.* 12 have been obtained for naturally



(7) (7a) (8) (8a) (8b)

* *m*-Toluoylation reactions of (2b) and (3b) are probably accompanied by little benzoyl migration, although we could not discriminate (5) from (6) and *vice versa* by means of t.l.c., l.l.c., and ^1H n.m.r. spectroscopy.

occurring nucleosides [16] and 9-β-D-xylofuranosyladenosine,[17] and were assumed to be due to the 2′- and 3′-hydroxy-groups, respectively, the anions of which are stabilized by intramolecular hydrogen bonding [as O(2′) · · · H–O(3′) [18] and O(3) · · · H–O(5′),[19] respectively]. The unusual acidity of β-D-arabinofuranosyl nucleosides has similarly been explained through intramolecular hydrogen bonding, *i.e.* O(2′) · · · H–O(5′).[20] However, a recent investigation on chromatographic behaviour of 2′-, 3′-, and 5′-*O*-methyl derivatives of 9-β-D-xylofuranosyladenine on a column of Dowex 1 × 2 (OH^-) ion-exchange resin led to the conclusion that the 2′-hydroxy-group is the most acidic.[21] Therefore, the electronic effect of the heterocyclic aglycone moieties on the 2′-hydroxy-groups seems to be important for explaining these aspects. Moreover, the proportions obtained in the hydroxylaminolysis of (7) and (8) may reflect steric hindrance, by the adenyl moiety, on the attack of hydroxylaminium acetate on the 2′-*O*-acetyl groups. Furthermore, the problem of how the remaining *O*-acyl groups at 2′- or 3′-position of ribonucleoside diacylates can survive under the conditions used may be explained by taking into consideration the results of a kinetic study by Griffin *et al.*[3] on rates of hydrolysis and equilibration of 2′- and 3′-*O*-acylribonucleosides, *i.e.* by assuming that acyl migration in such a system is much faster than the aminolysis.

EXPERIMENTAL

U.v. spectra were recorded with a Hitachi EPS-3T spectrometer for solutions in ethanol. Specific rotational values were determined with a Hitachi PO-B or Carl-Zeiss LEP A-1 polarimeter. ^1H N.m.r. spectra were recorded with Varian EM-390 or T-60 instruments for solution in [$^2H_6$]dimethyl sulphoxide or [$^2H_1$]chloroform (tetramethylsilane as internal standard); two or three drops of deuterium oxide were added, if necessary. T.l.c. was performed on Merck silica gel $60F_{254}$ pre-coated plates (thickness 0.25 mm) employing benzene–methanol (9 : 1 v/v) or chloroform–methanol (9 : 1 v/v) as eluant. Column chromatography was performed on Wakogel C-300 employing chloroform–methanol. Liquid–liquid chromatography (l.l.c.) was performed with a Varian LC-8520 apparatus [Condition *A*: column of MicroPak CN-10 (25 cm × 2 mm); mobile phase hexane (solvent A) and 20% propan-2-ol in dichloromethane (solvent B); solvent composition 25—60% B, composition changing by 4% min^{-1}; flow rate 100 ml h^{-1}; detected by u.v. at 260 nm (Variscan apparatus). Condition *B*: column of MicroPak Si-10 (25 cm × 2 mm); solvent composition 10—40% B, composition changing by 3% min^{-1}; flow rate 100 ml h^{-1}; detected by u.v. at 280 nm. Condition *C*: column of MicroPak Si-10 (25 cm × 2 mm); solvent composition 3—15% B, changing by 1% min^{-1}; flow rate 100 ml h^{-1}; detected by u.v. at 280 nm]. Elemental analyses were performed by the members of Laboratory of Organic Analysis, Tokyo Institute of Technology.

Pyridine used here was pre-treated with 5% aqueous potassium permanganate solution at 50—60 °C before distillation, and redistilled from barium oxide, as usual.

General Procedure for Reactions.—The procedure for the reactions of (1a) was the same as has previously been reported.[1] Reactions of (1b) were similarly performed by use of 0.1 mmol of hydroxylaminium acetate in a solvent (3 ml), under the conditions described in the footnotes of the corresponding Tables. Each of the resulting solutions was quenched with acetone and then diluted with chloroform to a volume of 10 ml. The diluted solutions were subjected to l.l.c. analysis; under condition *B*, (1b), the mixture of (2b) and (3b), and 5′-*O*-benzoyl-*N*^{6}-benzyladenosine (4b) were detected as peaks with retention times of *ca.* 2, 4, and 14 min, respectively; under condition *C*, (1b), (2b), and (3b) were detected as separate peaks with retention times of *ca.* 7, 10, and 12 min, respectively.

2′,3′,5′-*Tri*-O-*benzoyl*-N^6-*benzyladenosine* (1b).—Thionyl chloride (10 ml, 145 mmol) and DMF (1 ml, 16 mmol) were dissolved in chloroform (200 ml), and the resulting solution was set aside at room temperature for 30 min. To this solution was added 2′,3′,5′-tri-*O*-benzoylinosine [22] (1d) (28 g, 48.5 mmol), and the resulting solution was diluted with chloroform (400 ml) and dichloromethane (400 ml). The solution was heated under reflux for 10 h, poured into ice–water, and the organic layer was separated; the aqueous layer was further extracted with chloroform. The organic layers were combined and successively washed with an aqueous saturated solution of sodium hydrogencarbonate and water, and then dried over anhydrous sodium sulphate. Evaporation yielded a syrup which on crystallization gave 6-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine [23] (24.5 g, 84% yield), m.p. 114—115 °C (from methanol) (Found: C, 62.25; H, 3.86; N, 9.35. $C_{31}H_{23}ClN_4O_7$ requires C, 62.16; H, 3.85; N, 9.35%). This product (16 g, 26.7 mmol) was then dissolved in benzene (500 ml) containing benzylamine (11.4 g, 107 mmol), and the solution was refluxed for 4 h. The resulting solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to a syrup, which was chromatographed on a column of silica gel to give (1b) as a glass (12.3 g, 90% yield) [24] (Found: C, 68.03; H, 4.75; N, 10.42. $C_{38}H_{31}N_5O_7$ requires C, 68.15; H, 4.67; N, 10.46%).

3′,5′-*Di*-O-*benzoyladenosine* (2a).—To a solution of (1a) (1 160 mg, 2 mmol) in pyridine (30 ml) was added hydroxylaminium acetate (740 mg, 8 mmol), and the resulting solution was stirred at room temperature for 1 d. After quenching the resulting solution with acetone (*ca.* 10 ml) with stirring, it was evaporated to a syrup, which was then chromatographed on a column of silica gel [chloroform–methanol (96 : 4 v/v)] to give syrupy di-*O*-benzoyladenosine (808 mg, 84% yield); crystallization of the product gave (2a) (730 mg, 74% yield), m.p. 193—194 °C (from methanol) (lit.,[1] m.p. 193—194 °C). The 2′,5′-dibenzoate had δ_H ([$^2H_6$]DMSO) 6.40 (d, $J_{1',2'}$ 4.0 Hz, H-1′). In addition to this, was obtained 5′-*O*-benzoyladenosine (4a) (74 mg, 10% yield), m.p. 128—129 °C (from methanol) (lit.,[1] m.p. 126—129 °C).

3′,5′-*Di*-O-*benzoyl*-N^6-*benzyladenosine* (2b).—A solution of (1b) (1 340 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) in pyridine (30 ml) was stirred at room temperature for 2 d, and the resulting mixture was worked up in the same way as above. The mixture of dibenzoates (syrup, 932 mg, 82% yield) thus obtained, on crystallization, gave (2b) as a white powder (728 mg, 64% yield), m.p. 168—169 °C (from methanol); $[\alpha]_D^{18}$ −73° (*c* 1.5 in DMF); $\lambda_{max.}$ (EtOH) 269 (ε 18 300) and 230 nm (ε 25 700); $\lambda_{min.}$ (EtOH) 248 nm (ε 10 000); δ_H ($CDCl_3$–D_2O) 4.5—4.9 (5 H, m, H-4′, -5′, and -5″, and CH_2Ph), 5.10 (1 H, t, $J_{1',2'}$ 5.5 Hz, H-2′), 5.63—5.83 (1 H, m, H-3′), 6.03 (1 H, d,

H-1'), 7.08 (1 H, s, H-8), and 8.20 (1 H, s, H-2) (Found: C, 65.55; H, 4.75; N, 12.35. $C_{31}H_{27}N_5O_6$ requires C, 65.85; H, 4.8; N, 12.4%). L.l.c. of this sample showed that it contained 5% of the corresponding isomer (3b). The m.p. and l.l.c. were reproducible; isomerization of (2b) into (3b) is hard to postulate during l.l.c. on MicroPak Si-10, since (3b) (see below) gave a single peak on l.l.c.

2',5'-Di-O-benzoyl-N⁶-benzyladenosine (3b).—Compound (1b) (1 340 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in ethanol (50 ml) at room temperature for 4 d; further hydroxylaminium acetate (740 mg, 8 mmol) was then added and stirring continued for 2 d. The resulting crystalline precipitate was filtered off and dried to give (3b) (710 mg, 63% yield), m.p. 101—102.5 °C; $[\alpha]_D^{18}$ −64° (*c* 1.5 in DMF); $\lambda_{max.}$ (EtOH) 267—272 (ε 19 000) and 230 nm (ε 25 800); $\lambda_{min.}$ (EtOH) 248 nm (ε 10 800); δ_H ($CDCl_3$–D_2O) 4.4—5.0 (5 H, m, H-4', -5', -5'', and CH_2Ph), 5.06 (1 H, t, $J_{2',3'}$ 6.0 Hz and $J_{3',4'}$ 6.0 Hz, H-3'), 6.13 (1 H, dd, $J_{1',2'}$ 4.5 Hz, H-2'), 6.36 (1 H, d, H-1'), 8.13 (1 H, s, H-8), and 8.20 (1 H, s, H-2) (Found: C, 65.65; H, 4.85; N, 12.3. $C_{31}H_{31}N_5O_7$ requires C, 65.85; H, 4.3; N, 12.4%).

N²,3',5'-Tribenzoylguanosine (2c).—*N²,2',3',5'*-Tetrabenzoylguanosine [25] (1c) (1 400 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d and the resulting mixture was worked up in the same way as described in the preparation of (2a). Chromatographic separation gave the dibenzoates as a mixture with benzohydroxamic acid; however, crystallization gave (2c) (790 mg, 66% yield), m.p. 230—232 °C (from methanol) (lit.,[1] 230—231 °C); (1c) (126 mg, 9% yield) was recovered unchanged.

3',5'-Di-O-benzoylinosine (2d).—2',3',5'-Tri-*O*-benzoylinosine (1d) (1 130 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d, and the resulting mixture was worked up in the same way as above to give a mixture of di-*O*-benzoylinosine and benzohydroxamic acid, crystallization of which gave (2d) (701 mg, 72% yield), m.p. 167—168 °C (from methanol) (lit.,[1] m.p. 167—168 °C). The corresponding 2',5'-dibenzoate had δ_H ([$^2H_6$]DMSO) 6.46 (d, $J_{1',2'}$ 4.5 Hz, H-1'). 5'-*O*-Benzoylinosine (4d) (174 mg, 18% yield), m.p. 169 °C (from methanol) (lit.,[1] m.p. 169 °C) was also obtained.

3',5'-Di-O-benzoyluridine (2e).—2',3',5'-Tri-*O*-benzoyluridine [26] (1e) (1 110 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d; the resulting mixture was then worked up in the same way as above to give a mixture of di-*O*-benzoyluridine (688 mg, 76% yield), crystallization of which gave (2e) (553 mg, 61% yield), m.p. 199—200 °C (from methanol) (lit.,[1] m.p. 199.5—200.5 °C). The 2',5'-dibenzoate had δ_H ([$^2H_6$]DMSO) 6.08 (d, $J_{1',2'}$ 3.0 Hz, H-1'). 5'-*O*-Benzoyluridine (4e) (42 mg, 6% yield), m.p. 163—164 °C (from methanol) (lit.,[1] 163—164 °C) was also obtained.

3',5'-Di-O-acetyladenosine (2f).—2',3',5'-Tri-*O*-acetyladenosine [27] (1f) (1 182 mg, 3 mmol) and hydroxylaminium acetate (340 mg, 3.6 mmol) were stirred in pyridine (30 ml) at room temperature for 2 d, after which acetone (*ca.* 10 ml) was added to quench the excess of reagent; the reaction was further stirred for several hours, and then evaporated to a syrup. Chromatography on a column of silica gel gave a mixture of di-*O*-acetyladenosine (876 mg, 83% yield), which, on crystallization yielded (2f) (565 mg, 54% yield), m.p. 172—173 °C (from methanol) (lit.,[1] m.p. 172—173 °C). The 2',5'-diacetate had δ_H ([$^2H_6$]DMSO) 6.20 (1 H, d, $J_{1',2'}$ 4.5 Hz, H-1'). Also obtained was 5'-*O*-acetyladenosine (4f) (60 mg, 6% yield), m.p. 131—134 °C (from methanol) (lit.,[28] m.p. 143 °C); $[\alpha]_D^{18}$ −55° (*c* 1.5 in DMF); $\lambda_{max.}$ (EtOH) 259 nm (ε 11 800); δ_H ([$^2H_6$]DMSO) 4.0—4.4 (4 H, m, H-3', -4', -5', and -5''), 4.67 (1 H, m, H-2'), 5.92 (1 H, d, $J_{1',2'}$ 4.5 Hz, H-1'), 5.33 (1 H, d, $J_{3',3'}$ 6.0 Hz, 3'-OH), 5.53 (1 H, d, $J_{2',2'\text{-OH}}$ 6.0 Hz, 2'-OH), 7.25 (2 H, br s, NH_2), 8.17 (1 H, s, H-8), and 8.32 (1 H, s, H-2) (Found: C, 42.0; H, 5.2; N, 20.6. $C_{12}H_{15}N_5O_5{\cdot}2H_2O$ requires C, 41.75; H, 5.55; N, 20.3%).

N²,3',5'-Triacetylguanosine (2g).—*N²,2',3',5'*-Tetra-acetylguanosine [27] (1g) (1 410 mg, 3.1 mmol) and hydroxylaminium acetate (340 mg, 3.6 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d; the resulting mixture was then worked up as above to give a mixture of diacetates (987 mg, 78% yield), which, on crystallization, gave (2g) (781 mg, 57% yield), m.p. 131—132 °C (from methanol) (lit.,[1] m.p. 131—132 °C). *N²,5'*-Diacetylguanosine (4g) (85 mg, 7% yield) was also obtained, m.p. 207—208.5 °C (from methanol) (lit.,[29] m.p. 210—211 °C); $[\alpha]_D^{18}$ +6.4° (*c* 0.5 in DMF), $\lambda_{max.}$ (EtOH) 279 (ε 8 500) and 260—253 nm (ε 12 100); $\lambda_{max.}$ (EtOH) 271 nm (ε 8 200); δ_H ([$^2H_6$]DMSO–D_2O) 4.0—4.35 (4 H, m, H-3', -4', -5', and -5''), 4.50 (1 H, t, $J_{1',2'}$ 5.0 Hz, H-2'), 5.83 (1 H, d, H-1'), and 8.15 (1 H, s, H-8) (Found: C, 43.9; H, 4.95; N, 17.95. $C_{14}H_{17}N_5O_5{\cdot}H_2O$ requires C, 43.65; H, 4.95; N, 18.2%).

3',5'-Di-O-acetyl-N²-benzoylguanosine (2h).—2',3',5'-Tri-*O*-acetyl-*N²*-benzoylguanosine [30] (1h) (16.6 g, 31.8 mmol) was dissolved in methanol (300 ml), and the solution was evaporated to a volume of *ca.* 100 ml; hydroxylaminium acetate (11.5 g, 127 mmol) was then added, and the reaction stirred at room temperature for 1 d. The resulting crystalline precipitate was filtered off, and the filtrate was stirred for a further 2 d, when the second crop of crystals, was filtered off, to give (2h) (total 8.5 g, 56% yield), m.p. 126—127 °C (lit.,[1] m.p. 126—127 °C).

2',5'-Di-O-acetylinosine (3i).—2',3',5'-Tri-*O*-acetylinosine [27] (1i) (1 180 mg, 3 mmol) and hydroxylaminium acetate (340 mg, 3.6 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d. After the usual work-up, the resulting mixture was chromatographed on a column of silica gel to give the diacetates as a mixture with acetohydroxamic acid. Crystallization of the mixture gave (3i) (600 mg, 56% yield), m.p. 210—211 °C (from methanol) (lit.,[1] 210—211 °C). 5'-*O*-Acetylinosine (4i) (170 mg, 18% yield), m.p. 117—118 °C (re-melt at 171 °C) (from methanol) [lit.,[1] m.p. 117—118 °C (re-melt at 171 °C)] was also obtained).

3',5'-Di-O-acetyluridine (2j).—2',3',5'-Tri-*O*-acetyluridine [31] (1j) (1 080 mg, 3 mmol) and hydroxylaminium acetate (340 mg, 3.6 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d. The mixture of diacetates (660 mg, 72% yield) obtained by work-up was chromatographed to give (2j) (510 mg, 53% yield), m.p. 150—152 °C (from methanol) (lit.,[1] 150—152 °C), and 5'-*O*-acetyluridine (4j) (130 mg, 15% yield), m.p. 162—163 °C (lit.,[1] m.p. 162—163 °C).

2',5'- (2k) *and 3',5'-Di-O-acetylcytidine* (3k).—A solution of *N⁴,2',3',5'*-tetra-acetylcytidine [32] (1k) (1 235 mg, 3 mmol) and hydroxylaminium acetate (340 mg, 3.6 mmol) were treated in pyridine as above (30 ml), and the resulting mixture was worked up as above to give a syrupy mixture (2k) and (3k) (670 mg, 68% yield), the ^1H n.m.r. spectrum

of which was identical with that reported previously.[1] In addition to this, syrupy 5′-*O*-acetylcytidine (4k) (165 mg, 15% yield) was also obtained; the ^{1}H n.m.r. spectrum was also identical to that reported previously.[1]

N^2,2′,3′,5′-*Tetraisobutyrylguanosine* (1l).—Guanosine (5 g) was treated with isobutyric anhydride (30 ml) in pyridine (50 ml) under reflux for 10 h. The resulting mixture was, after cooling, poured into ice–water and stirred for several hours. The mixture was extracted with chloroform (3 × 30 ml); the organic layers were combined, washed successively with 1M hydrochloric acid, aqueous saturated sodium hydrogencarbonate, and water, and dried over anhydrous magnesium sulphate. The organic solution was then evaporated to give (1l) as a glass (89% yield, 8.9 g) (Found: C, 55.75; H, 6.8; N, 12.2. $C_{26}H_{37}N_5O_9$ requires C, 55.4; H, 6.6; N, 12.45%).

N^2,2′,5′-*Tri-isobutyrylguanosine* (3l).—Compound (1l) (1 690 mg, 3 mmol) and hydroxylaminium acetate (430 mg, 4.5 mmol) were stirred in pyridine (30 ml) at room temperature for 3 d, and the resulting solution was worked up in the same way as above to give (1l) (430 mg, 25% recovery) and a syrupy mixture of di-isobutyrates, crystallization of which gave (3l) (607 mg, 48% yield), m.p. 121—122 °C (from acetone–hexane); $[\alpha]_D^{19}$ −2.7° (*c* 1.0 in DMF); $\lambda_{max.}$ (EtOH) 280 nm (ε 11 900); δ_H ([$^2H_6$]DMSO–D_2O) 1.00—1.35 (18 H, m, 6 × Me), 2.5—3.0 (3 H, m, 3 × Me_2CHCO_2), 4.2—4.5 (3 H, m, H-4′, -5′, and -5″), 4.7—4.88 (1 H, m, H-3′), 5.55 (1 H, t, $J_{1',2'}$ 4.5 Hz and $J_{2',3'}$ 4.5 Hz, H-2′), 6.10 (1 H, d, H-1′), and 8.03 (1 H, s, H-8) (Found: C, 53.45; H, 6.3; N, 14.15. $C_{22}H_{31}N_5O_8$ requires C, 53.55; H, 6.35; N, 14.2%).

Attempted Partial Debenzoylation of N^4,2′,2′,5′-*Tetrabenzoylcytidine* (1m) *with Hydroxylaminium Acetate*.—Compound (1m) [33] (1 290 mg, 2 mmol) and hydroxyaminium acetate (740 mg, 8 mmol) were stirred in pyridine (30 ml) at room temperature for 2 d. The usual work-up and chromatography gave 5,6-dihydro-*N*^{4}-hydroxy-6-hydroxyamino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)cytosine (413 mg, 68% yield), m.p. 176—178 °C (from ethanol); $[\alpha]_D^{18}$ +33° (*c* 1.5 in DMF); $\lambda_{max.}$ (EtOH) 230 nm (ε 47 800) (Found: C, 59.6; H, 4.8; N, 9.2. $C_{30}H_{25}N_3O_9$ requires C, 59.6; H, 4.65; N, 9.25%). 2′,2′,5′-Tri-*O*-benzoylcytidine (110 mg, 10% yield), m.p. 183—184 °C (from ethanol) (lit.,[34] m.p. 183—184 °C), was also obtained.

Preparation of (4f).—Compound (1f) (787 mg, 2 mmol) and hydroxylaminium acetate (744 mg, 8 mmol) were stirred in pyridine (10 ml) at room temperature for 1 d. The resulting mixture was treated with acetone (*ca.* 10 ml), stirred for 2—3 h, and evaporated to a syrup, crystallization of which gave (4f) (604 mg, 92% yield), m.p. 130—131 °C (from ethanol).

Preparation of (4g).—Compound (1g) (440 mg, 1 mmol) and hydroxylaminium acetate (370 mg, 4 mmol) were stirred in pyridine (10 ml) at room temperature for 1 d, and similar treatment to that above gave (4g) (357 mg, 97% yield), m.p. 207—208.5 °C (from methanol).

Preparation of (4i).—Compound (1i) (780 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in pyridine (10 ml) at room temperature for 1 d, and the syrup resulting from evaporation gave (4i) (581 mg, 91% yield), m.p. 117—118 °C (re-melted at 178 °C) (from methanol).

Preparation of 5′-O-*Acetyluridine* (4j).—Compound (1j) (720 mg, 2 mmol) and hydroxylaminium acetate (740 mg, 8 mmol) were stirred in pyridine (10 ml) at room temperature for 1 d, and the syrup obtained by evaporation gave (4j) (545 mg, 95% yield), m.p. 162—163 °C (from ethanol).

Isomerization of (2b) *and* (3b).—Compounds (2b) and (3b) (28 mg, 0.05 mmol) were separately dissolved in a solvent (1 ml), and set aside at room temperature to monitor the ratio of (2b) : (3b) by means of l.l.c. under condition *C*. The results thus obtained are shown in the Figure. No products other than (2b) and (3b) were observed.

Examination of Acyl Migration Reactions on a Silica Gel Column.—Compounds (2a), (2e), and (2f) (each *ca.* 100 mg) were dissolved in [$^2H_6$]DMSO (*ca.* 0.5 ml), to which was added a small amount of D_2O, and the solutions were heated at 70—80 °C for 5—6 h in order to make mixtures of the corresponding 2′,5′- and 3′,5′-diacylates. The relative proportions of the diacylates were then determined by means of ^{1}H n.m.r. spectroscopy. Subsequently, the resulting mixtures and pure samples of (2b), (3b), (3c), and (3i) were adsorbed on the column of silica gel, and allowed to stand for several hours, after which the resulting mixtures were eluted out with chloroform–methanol (96 : 4 v/v). The proportions of the di-*O*-acyl derivatives in each mixture were calculated from the areas of their anomeric proton signals in the ^{1}H n.m.r. spectra, except for the ratio (2b) : (3b), which was determined by means of l.l.c. All the results thus obtained are summarized in Table 9.

m-*Toluoylation of* (2b).—To a solution of (2b) (1 130 mg, 2 mmol) in pyridine (30 ml), was added dropwise *m*-toluoyl chloride (1.1 ml, 8 mmol) with stirring and cooling with ice–water, and the mixture was stirred overnight. It was then poured into ice–water, and the solution was extracted with chloroform (3 × 30 ml). The organic layers were combined and washed successively with 1M hydrochloric acid, aqueous saturated sodium hydrogencarbonate, and water, and then dried over anhydrous sodium sulphate. Evaporation of the organic solution gave a syrup which was then purified by chromatography on a column of silica gel to afford 3′,5′-di-*O*-benzoyl-*N*^{6}-benzyl-2′-*O*-*m*-toluoyladenosine (5) as a glass (1 140 mg, 83% yield) (Found: C, 68.25; H, 5.0; N, 9.9. $C_{39}H_{33}N_5O_7$ requires C, 68.5; H, 4.85; N, 10.25%).

m-*Toluoylation of* (3b).—The same performance of *m*-toluoylation reaction on (3b) (1 130 mg, 2 mmol) as above gave 2′,5′-di-*O*-benzoyl-*N*^{6}-benzyl-3′-*O*-*m*-toluoyladenosine (6) as a glass (1 210 mg, 88% yield) (Found: C, 68.95; H, 4.8; N, 9.95. $C_{39}H_{33}N_5O_7$ requires C, 68.5; H, 4.85; N, 10.25%).

Partial O-*Deacylation* of (5) *with Hydroxylaminium Acetate*.—Compound (5) (684 mg, 1 mmol) and hydroxylaminium acetate (370 mg, 4 mmol) were stirred in pyridine (20 ml) at room temperature for 2 d. The resulting mixture was quenched with acetone, evaporated, and then chromatographed on silica gel to give a mixture of diacylates (406 mg, 71% yield). The proportion of the nucleosides bearing *m*-toluoyl group was calculated from the ^{1}H n.m.r. spectra, and it was found that of the resulting mixture of nucleoside diacylates, *ca.* 20% carried an *m*-toluoyl group.

Partial O-*Deacylation of* (6) *with Hydroxylaminium Acetate*.—Compound (6) and hydroxyaminium acetate (370 mg, 4 mmol) were stirred in pyridine (20 ml) at room temperature for 2 d, and the resulting mixture was worked up in the same way as above to give a mixture of diacylates (392 mg, 68% yield). From the ^{1}H n.m.r. spectrum it was found that *ca.* 90% of the *m*-toluoyl group survived in the resulting mixture of the nucleoside diacylates.

9-(2,3,5-*Tri*-O-*acetyl*-β-D-*xylofuranosyl*)*adenine* (7).—9-β-D-Xylofuranosyladenine prepared by the procedure reported by Lee *et al.*[10] was acetylated as usual [35] to give (7), δ_H ($CDCl_3$) 2.06 (3 H, s, OCOMe), 2.09 (3 H, s, OCOMe), 2.16 (3 H, s, OCOMe), *ca.* 4.5 (3 H, m, H-4′, -5′, and -5″), 5.23—5.66 (2 H, m, H-2′ and -3′), 6.15 (1 H, d, $J_{1',2'}$ 2.5 Hz, H-1′), 8.39 (1 H, s, H-8), and 8.61 (1 H, s, H-2).

9-β-D-*Arabinofuranosyladenine* (9).—A solution of (2a) (1 430 mg, 3 mmol) in anhydrous dimethyl sulphoxide (20 ml)–acetic anhydride (10 ml) was set aside at room temperature overnight. The resulting mixture was poured onto ice, and the mixture was stirred for several hours. The mixture was then extracted with ethyl acetate (3 × 100 ml), and the combined extracts were successively washed with aqueous saturated sodium hydrogencarbonate and water, dried over anhydrous sodium sulphate, and evaporated to give a syrup, which was then dissolved in benzene–ethanol (50 ml) (1 : 1 v/v) and the resulting solution treated with sodium borohydride (150 mg) and allowed to stand at 0 °C with stirring for 2 h. The solution was then evaporated to dryness, and the residue was dissolved in anhydrous methanol (20 ml) and treated with 2M methanolic sodium methoxide solution (several drops); the solution was then stirred overnight at room temperature. The syrup obtained by evaporation of the resulting solution was subjected to chromatographic purification on a column (25 × 5 cm) of Dowex 1 × 2 (OH^-) ion-exchange resin, eluting successively with 60% aqueous methanol (500 ml) and 0.1M aqueous ammonium hydrogencarbonate (3 000 ml). The eluate obtained by use of the latter was evaporated to dryness and the residue recrystallized from water to give compound (9) (540 mg, 63% yield), m.p. 255—257 °C (lit.,[12] 257—257.5 °C); $[\alpha]_D^{18}$ −10° (*c* 1.0 in DMF); $\lambda_{max.}$ (EtOH) 258.5 nm (ε 13 800) (Found: C, 45.0; H, 4.9; N, 19.85. $C_{10}H_{13}N_5O_5$ requires C, 44.95; H, 4.9; N, 20.2%).

9-(2,3,5-*Tri*-O-*acetyl*-β-D-*arabinofuranosyl*)*adenine* (8).—Compound (9) was acetylated according to the procedure of Reist *et al.*[35] to give (8) as a syrup; δ_H ($CDCl_3$) 1.94 (3 H, s, OCOMe), 2.15 (3 H, s, OCOMe), 2.20 (3 H, s, OCOMe), *ca.* 4.5 (3 H, m, H-4′, -5′, and -5″), 5.40—5.66 (2 H, m, H-2′ and -3′), 6.62 (1 H, d, $J_{1',2'}$ 4.0 Hz, H-1′), 8.15 (1 H, s, H-8), and 8.27 (1 H, s, H-2).

Partial O-*Deacetylation of* (7) *with Hydroxylaminium Acetate*.—Compound (7) (395 mg, 1 mmol) and hydroxylaminium acetate (110 mg, 1.2 mmol) were stirred in pyridine (20 ml) at room temperature for 1 d, and the resulting mixture was quenched with acetone and evaporated to a syrup; chromatography on a column of silica gel then gave (7) (81 mg, 20% recovery) and 9-(3,5-di-*O*-acetyl-β-D-xylofuranosyl)adenine (7a) (253 mg, 72% yield). Recrystallization of the sample gave pure (7a) (225 mg, 64% yield), m.p. 211—212.5 °C (from methanol); $[\alpha]_D^{18}$ −18° (*c* 1.0 in DMF), $\lambda_{max.}$ (EtOH) 260 nm (ε 14 300); δ_H ([$^2H_6$]-DMSO–D_2O) 2.00 (3 H, s, OCOMe), 2.06 (3 H, s, OCOMe), *ca.* 4.4 (3 H, m, H-4′, -5′, and -5″), 4.60 (1 H, t, $J_{1',2'}$ 2.5 Hz, H-2′), 5.25 (1 H, dd, $J_{2',3'}$ 2.5 and $J_{3',4'}$ 4.0 Hz, H-3′), 6.05 (1 H, d, H-1′), 8.17 (1 H, s, H-8), and 8.22 (1 H, s, H-2) (Found: C, 47.7; H, 4.9; N, 19.85. $C_{14}H_{17}N_5O_6$ requires C, 47.85; H, 4.9; N, 19.95%).

Partial O-*Deacetylation of* (8) *with Hydroxylaminium Acetate*.—Compound (8) (790 mg, 2 mmol) and hydroxylaminium acetate (210 mg, 2.2 mmol) were stirred in pyridine (30 ml) at room temperature for 1 d, and the resulting mixture was worked up and chromatographed as above to give (8) (71 mg, 9% recovery yield) and a syrupy mixture of 9-(3,5- (8a) and 9-(2,5-di-*O*-acetyl-β-D-arabinofuranosyl)adenine (8b) (411 mg, 59% yield), whose ^1H n.m.r. spectrum showed the ratio of (8a) : (8b) in the mixture to be 85 : 15; δ_H for (8a) ([$^2H_6$]DMSO–D_2O) 2.06 (3 H, s, OCOMe), 2.13 (3 H, s, OCOMe), *ca.* 4.4 (4 H, m, H-2′, -4′, -5′, and -5″), 5.7—5.30 (1 H, m, H-3′), 6.40 (1 H, d, $J_{1',2'}$ 3.0 Hz, H-1′), 8.22 (2 H, s, H-2 and -8); (for (8b) 5.36 (1 H, t, $J_{1',2'}$ 6.0 Hz, H-2′) and 6.57 (1 H, d, H-1′) (Found: C, 48.0; H, 4.8; N, 19.5. $C_{14}H_{17}N_5O_6$ requires C, 47.85; H, 4.9; N, 19.95%). In addition, a 1 : 2 mixture of (8a,b) and acetohydroxamic acid was obtained [120 mg, yield of (8a,b) 13%].

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