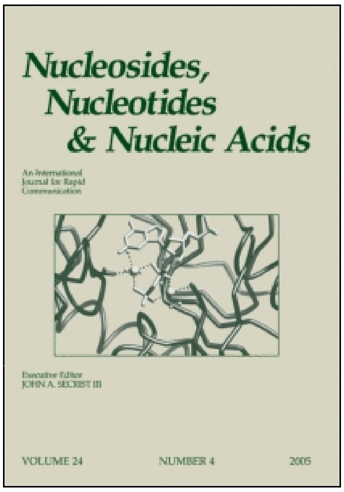

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Partial Protection of Carbohydrate Derivatives. Part 9[1]. Equilibration between 2', 5'- and 3', 5'-DI-O-Benzoyladenosine Derivatives Substituted at the N^6-Position, on Silica Gel

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PARTIAL PROTECTION OF CARBOHYDRATE DERIVATIVES. PART 9[1]. EQUILIBRATION BETWEEN 2',5'- AND 3',5'-DI-O-BENZOYLADENOSINE DERIVATIVES SUBSTITUTED AT THE N^6-POSITION, ON SILICA GEL

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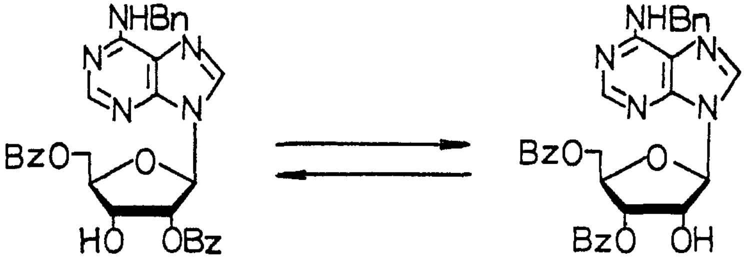
Abstract: Equilibration between 2',5'- and 3',5'-di-O-benzoyladenosine derivatives on Wakogel C-300 and Merck 60 silica gel was effectively induced to give a mixture predominantly containing the latter; further potential factors involved were also investigated.

Acyl migration reactions have been reported to occur very easily in a vicinal diol system[2], especially in the 2',3'-*cis*-diol system of the ribonucleosides[3]. Moreover, the reaction on silica gel has also been observed by Chacon-Fuertes and Martin-Lomas[4] on separation of a 1:5 mixture of 1,6-anhydro-β-D-galactopyranoside 2,3- and 2,4-diacetate, whose proportion was converted into 1:1. On the other hand, regioselective 2'-O-deacylation of fully acylated purine and pyrimidine ribonucleosides through hydrazine hydrate in 1:4 acetic acid – pyridine[5] and through hydroxylaminium acetate in pyridine[6] have been successful due to the chromatographic isolation of the resulting dibenzoate mixtures on Wakogel C-300 (silica gel); this gave an equilibrated mixture containing the corresponding 3',5'-dibenzoates predominantly or preferentially; *e.g.*, a 3:7 mixture of 2',5'- and 3',5'-di-

O-benzoyladenosine afforded a mixture containing no 2',5'-dibenzoate. In contrast, the equilibration was slowed down considerably on a column of Mallinckrodt silica gel (100-mesh)[6]; this silica gel has been used by Johnston[7] for the separation of mixtures involving adenosine or uridine diacetates, in which the equilibration through acyl migration reaction has never been noticed. The results prompted us to investigate potential factors concerned with silica gel by the use of series of 2',5'- and 3',5'-di-*O*-benzoyladenosine derivatives substituted at the *N*^{6}-position; the results thus obtained will be described in detail herein.

In the first place, the effects of five species of silica gel on the equilibration between 2',5'- and 3',5'-dibenzoate were compared by the use of 2',5'- (**1**) and 3',5'-di-*O*-benzoyl-*N*^{6}-benzyladenosine (**2**) as the model compounds, since they are detected as clearly separated peaks in l.l.c., making a quantitative analysis possible. A 95:5 mixture of **1** and **2** was adsorbed on a column of one of the five species for 7 h and 18 h, respectively, and the resulting mixture



1 **2**

was eluted out with chloroform and then with 99:1 chloroform - methanol, in turn. Their resultant proportions in each of the isolated mixtures were determined by l.l.c. and the results thus obtained are summarized in TABLE 1 together with *p*H values *etc.* given to each commercial silica gel. Merck 60 (Entry 3) and Wakogel C-300 (Entry 10) were much more effective for the equilibration towards **2** than Merck 40 (Entry 4), Merck 100 (Entry 8), and Mallinckrodt (Entry 16) silica gel. It has been reported, on the other hand, that catalysis in the reactions in the presence of silica gel was due to acidic impurities contained in silica gel, and that purified silica gel showed little catalytic activity[8]. In contrast to this, Merck 40 (Entry 1) and Mallinckrodt (Entry 16) were much less effective for the equilibration towards **2**, although they have *p*H values of 5.5 and 4.7, respectively. Moreover, Merck 100 and Wakogel C-300 have the same *p*H value, but the latter was far more effective for the equilibration (Entries 8 and 10). Wakogel C-300, which was previously treated with a solution of methanolic ammonia in chloroform and washed with chloroform, showed almost the same activity for the equilibration (Entry 11), compared with the original one (Entry 10). Accordingly, we could not find any linear relationship between the *p*H values of each silica gel used and the effect on the equilibration. In addition, it was difficult to correlate these data with the surface-area, cavity-volume, or cavity-diameter of the silica gels, which are shown in the table.

In the second place, the effect of the water adsorbed on silica gel as well as of the acidic hydroxyl groups of silicic acid on the equilibration towards **2** were investigated; these might actually be concerned with the formation of hydrogen-bonds with heterocyclic moieties or the carbonyl oxygens of the acyl groups of the ribonucleoside diacylates. The temperature range for removing the adsorbed water without loss of any hydroxyl groups of silicic acid has been reported as 150 – 200°C[9]. Removal of the water was thus conducted at 200°C for 3 h, resulting in a decrease of the effect on the equilibration towards **2** for some silica gels (Entries 2, 4, 9, and 12), but no significant change in the case of Mallinckrodt (Entry 17). On the other hand, conversion of silicic acid into siloxane has been reported to begin at a temperature about 200°C[10]. Consequently, the effect of Merck 60 and Wakogel C-300 on acyl migration was similarly examined after heating at 400°C (Entries 5 and 13), at 620°C (Entries 6 and 14), and at 700°C (Entries 7 and 15),

TABLE 1. Proportions of 2',5'- (**1**) and 3',5'-Di-*O*-benzoyl-*N*^{6}-benzyladenosine (**2**) after Equilibration on a Silica Gel[a]

Entry	Silica gel	Cavity[b] volume (mL/g)	Surface-area (m^2/g)	*p*H[b]	Temp. for heating (°C)	Proportions of **1** and **2** obtained by	
						7 h adsorption	18 h adsorption
1	Merck 40	0.65	650	5.5	——	75 : 25	40 : 60
2	Merck 40				200		93 : 7
3	Merck 60	0.75	500	7.0	——	21 : 79	19 : 81
4	Merck 60				200		33 : 67
5	Merck 60				400		44 : 56
6	Merck 60				620		45 : 55
7	Merck 60				700		69 : 31
8	Merck 100	1.00	400	7.5	——	73 : 27	41 : 59
9	Merck 100				200		78 : 22

10	Wakogel C-300	0.70	337	7.5	——	30 : 70	14 : 86
11	Wakogel C-300				——	20 : 80[d]	20 : 80[d]
12	Wakogel C-300				200		44 : 56
13	Wakogel C-300				400		80 : 20
14	Wakogel C-300				620		80 : 20
15	Wakogel C-300				700		78 : 22
16	Mallinckrodt	0.36	322	4.7	——		82 : 18
17	Mallinckrodt				200		90 : 10

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a) All the columns for equilibration were prepared in the same way as has been reported[6]; a 95:5 mixture of 1 and 2 (100 mg) and each silica gel (33 g) were used and their resulting proportions were determined by l.l.c. (see ref. 6).

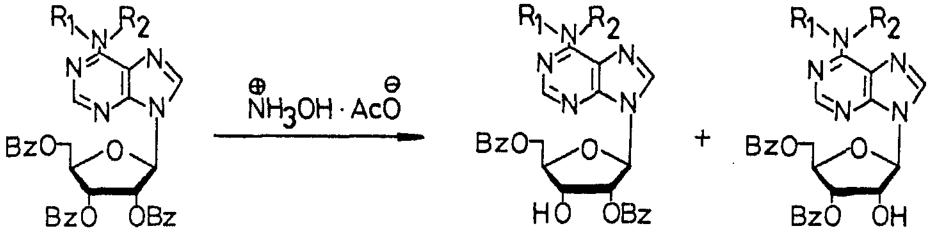
b) This specification is given by the company supplying the silica gel.

c) Heated for 3 h.

d) Before adsorption of the material on the column, this silica gel was washed with chloroform containing methanolic ammonia and then with chloroform.

respectively, for 3 h. Clearly acyl migration was decreased due to the heating of the silica gel. Therefore, adsorbed water and the hydroxyl functions of silicic acid were demonstrated to be important for the equilibration from 1 towards 2 through acyl migration from the 2' and 3' position.

In the last place, the effect of substituents at the *N*^{6}-position of adenosine on the equilibration was investigated in terms of the proportions of the corresponding 2',5'- and 3',5'-dibenzoates, which were obtained by the hydroxylaminolysis of 2',3',5'-tri-*O*-benzoyl-^{6} (3), -*N*^{6}-benzyl-^{6} (4), -*N*^{6},*N*^{6}-dibenzyl- (5), and -*N*^{6}-trityladenosine (6), followed by chromatography on a column of Wakogel C-300. The results thus obtained are summarized in TABLE 2. Comparing the proportions of the dibenzoates before and after chromatography, those of adenosine (Entry 1) and *N*^{6}-benzyladenosine (Entry 2) were remarkably equilibrated towards the corresponding 3',5'-dibenzoates, but those of *N*^{6},*N*^{6}-dibenzyl- (Entry 3) and *N*^{6}-trityladenosine (Entry 4) were substantially the same as seen from the data. Incidentally, the proportions of 2',5'- and 3',5'-dibenzoates of a series of ribonucleosides have been reported to vary after chromatography from 0:100 to 35:65[6], depending upon the structure of their heterocyclic moieties. This suggests that their hetero-



3 $R_1=R_2=H$
4 $R_1=Bn, R_2=H$
5 $R_1=R_2=Bn$
6 $R_1=Tr, R_2=H$

TABLE 2. Equilibration of a Mixture of 2',5'- and 3',5'-Di-*O*-benzoyladenosine Derivatives Substituted at the *N*^{6}-Position Obtained from Hydroxylaminolysis, on Wakogel C-300[a]

Entry	R^1	R^2	Proportions of 2',5'- and 3',5'-Bz$_2$ prior to equilibration[b]	Proportions of 2',5'- and 3',5'-Bz$_2$ after equilibration
1	H	H (3)	40 : 60[c]	0 : 100[c](84% yield)
2	Bn	H (4)	36 : 64[d]	10 : 90[d](82% yield)
3	Bn	Bn (5)	33 : 67[e]	26% yield : 51% yield
4	Tr	H (6)	33 : 67[e]	24% yield : 42% yield

a) All reactions were performed by the use of hydroxylaminium acetate (4 mol. Equiv.) in pyridine at room temperature similar to the reported method (see *ref.* 6), and the products were isolated by column chromatography on Wakogel C-300.

b) These are of the resulting mixtures from hydroxylaminolysis.

c) These were estimated by ^{1}H-n.m.r. spectroscopy; see also *ref.* 6.

d) These were estimated by l.l.c. technique; see also *ref.* 6.

e) These were estimated by ^{1}H-n.m.r. spectroscopy in terms of area-ratios of anomeric proton signals of the corresponding dibenzoates.

cyclic moieties probably interact with the surface of silica gel and the difference in the strength of the interaction brings about significant differences in their electron-withdrawing effect onto the *D*-ribofuranosyl ring; such differences may affect the correlative activity of the ester function at 2' and 3' positions. We may assume that the equilibration towards 2 on silica gel through acyl migration should be facilitated if the difference in the correlative activity of the ester groups was enhanced by a stronger interaction between a nucleoside derivative and the surface of silica gel. The results thus obtained here might be accepted on the same basis as above if it is possible to ascribe the decrease of acyl migration in Entries 3 and 4 to the steric hindrance by substituents at the *N*^{6}-position to the surface on silica gel.

EXPERIMENTAL SECTION

Melting points were determined with a Yanagimoto Micro Melting Point Apparatus and are uncorrected. Specific rotations were measured with a JASCO DIP-4 apparatus. ^{1}H-n.m.r. spectra were recorded with a Varian T-60 instrument for solutions in chloroform-d and tetramethylsilane as the internal standard. U.v. spectra were recorded with a Hitachi EPS-3T spectrophotometer for solutions in ethanol. T.l.c. was performed on Merck 60 F$_{254}$ silica gel precoated plates (thickness 0.25 mm) employing 9:1 benzene – methanol or 9:1 chloroform – methanol as eluents. L.l.c. was performed with a Varian LC-8520 apparatus [column of MicroPak Si-5 (30 cm x 4 mm); mobile phase hexane (solvent A) and 40% 2-propanol in dichloromethane (solvent B); solvent composition 2 – 15% B with a slope of 1%/min; detection by u. v. at 270 nm (Variscan appratus)]. Elementary analysis was performed with a Perkin-Elmer 240-002 instrument.

Equilibration Test of 2',5'- (1) and 3',5'-Di-O-benzoyl-N^6-benzyladenosine (2) on Silica Gel.

A 95:5 mixture of 1 and 2[6] (100 mg) was adsorbed on a column of a silica gel (33 g), prepared with commercial chloroform, and left for a time as shown in TABLE 1. After the time, the resulting mixture was eluted out with commercial chloroform and, then, with 99:1 chloroform – methanol; material balances throughout the chromatographic operation were all excellent(greater than 95% recovery). The mixtures obtained in the manner described above were subjected to l.l.c. determination, and the results thus obtained are shown in TABLE 1.

2',3',5'-Tri-O-benzoyl-N^6,N^6-dibenzyladenosine (5).

To a solution of 6-chloro-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-purine[6] (2 g, 3.3 mmol) in benzene (100 mL), was added dibenzylamine (2.6 mL, 13.2 mmol), and the solution was refluxed for 12 h, after which 1 M hydrochloric acid (100 mL) was added and the mixture was stirred for several hours. The precipitated salt was removed by filtration, and the filtrate was washed successively with aqueous sodium bicarbonate solution and saturated aqueous sodium chloride.

After drying over anhydrous sodium sulfate, the organic solution was evaporated to dryness, and the residue was chromatographed on a column of silica gel. The fractions containing 5 were gathered and the residue obtained by evaporation was crystallizedd to give 5 (2.3 g, 92% yield); m.p. 139 – 140.5°C (from methanol), $[\alpha]_D^{25}$ -101.2° (c 0.84, chloroform), λ_{max} (EtOH) 275 nm (ε 23900).

Anal. Calcd for $C_{45}H_{37}N_5O_7$: C, 71.14; H, 4.91; N, 9.21. Found: C, 71.12; H, 4.96; N, 9.15.

2',5'- (7) and 3',5'-Di-O-benzoyl-N^6,N^6-dibenzyladenosine (8).

Compound 5 (0.76 g, 1 mmol) was treated with hydroxylaminium acetate (0.37 g, 4 mmol) in pyridine (10 mL) with stirring at room temperature for 1 day. After quenching the resulting solution with acetone (*ca*. 10 mL), the mixture was evaporated to a syrup, whose ^{1}H-n.m.r. spectrum gave the ratio of 33:67 for 7 and 8 (TABLE 2) in terms of the area-ratio of their anomeric proton signals. Chromatographic separation on a column of silica gel was successful to give 7 (0.17 g, 26% yield) and 8 (0.33 g, 51% yield) in addition to recovered 5 (0.08 g, 11%).

Compound 7 was amorphous; $[\alpha]_D^{25}$ -73.9° (c 0.93, chloroform), λ_{max} (EtOH) 277 nm (ε 15400), ^{1}H-n.m.r. ($CDCl_3$) δ 4.3 – 4.7 (3-proton multiplet, H-4', 5', and 5"), 5.0 – 5.3 (1-proton multiplet, H-3'), 5.92 (1-proton multiplet, $J_{2',3'}$ 3.5 Hz, H-2'), 6.10 (1-proton doublet, $J_{1',2'}$ 3.5 Hz, H-1').

Anal. Calcd for $C_{38}H_{33}N_5O_6$: C, 69.60; H, 5.07; N, 10.68. Found: C, 69.95; H, 5.33; N, 10.37.

Compound 8 had m.p. 180 – 182°C (from methanol); $[\alpha]_d^{25}$ -88.3° (c 0.80, chloroform), λ_{max} (EtOH) 278 nm (ε 18000), ^{1}H-n.m.r. ($CDCl_3$) δ 4.5 – 4.8 (3-proton multiplet, H-4', 5', and 5"), 5.5 – 5.8 (1-proton multiplet, H-3'), 5.0 (1-proton triplet, $J_{2',3'}$ 5.5 Hz, H-2'), 5.94 (1-proton doublet, $J_{1',2'}$ 5.5 Hz, H-1').

Anal. Calcd for $C_{38}H_{33}N_5O_6$: C, 69.60; H, 5.07; N, 10.68. Found: 69.56; H, 4.94; N, 10.71.

2',3',5'-Tri-O-benzoyl-N^6-trityladenosine (6).

To a solution of 2',3',5'-tri-O-benzoyladenosine[11] (3) (2.90 g, 5 mmol) in pyridine (30 mL), was added trityl chloride (2.90 g, 10 mmol) and the resulting solution was stirred at 70 – 75°C for 2 days, after which the solution was quenched with water and diluted with chloroform. The organic layer was separated and washed successively with 1 M hydrochloric acid, aqueous saturated solution of sodium bicarbonate, and water. The organic solution was, after drying over anhydrous sodium sulfate, evaporated toa syrup, which was then chromatographed on a column of silica gel to give glassy 6 (6.66 g, 81% yield); $[\alpha]_D^{21}$ -75° (c 1.0, chloroform), λ_{max} (EtOH) 274 nm (ε 26200), λ_{min} (EtOH) 250 nm (ε 15400).

Anal. Calcd for $C_{50}H_{39}N_5O_7$: C, 73.70; H, 4.78; N, 8.52. Found: C, 73.29; H, 4.78; N, 8.52.

2',5'- (9) and 3',5'-Di-O-benzoyl-N^6-trityladenosine (10).

To a solution of 6 (1.64 g, 2 mmol) in pyridine (15 mL), was added hydroxylaminium acetate (0.74 g, 8 mmol), and the resulting solution was stirred at room temperature for 24 h. After quenching the solution with acetone (ca. 10 mL) and diluting with chloroform, the organic layer was separated and washed successively with 1 M hydrochloric acid, aqueous sodium bicarbonate solution, and water. The organic solution was dried over anhydrous sodium sulfate, and evaporated to a syrup, whose ^{1}H-n.m.r. spectrum gave the ratio of 33:67 for 9 and 10 (TABLE 2) in terms of their anomeric proton signals. Chromatographic separation of the mixture on a column of silica gel gave 9 (0.36 g, 24% yield) and 10 (0.63 g, 42% yield).

Compound 9 was a glass; $[\alpha]_D^{21}$ -61° (c 0.5, chloroform), λ_{max} (EtOH) 276 nm (ε 25400), λ_{min} (EtOH) 249 nm (ε 10700), ^{1}H-n.m.r. ($CDCl_3$) δ 4.3 – 4.8 (3-proton multiplet, H-4', 5', and 5"), 5.92 (1-proton triplet, H-2'), 6.10 (1-proton doublet, $J_{1',2'}$ 5.0 Hz, H-1').

Anal. Calcd for $C_{43}H_{35}N_5O_6 \cdot 1/2\ H_2O$: C, 71.06; H, 4.99; N, 9.63. Found: C, 70.96; H, 4.98; N, 9.48.

Compound **10** was a glass; $[\alpha]_D^{21}$ -48° (c 0.7, chloroform), λ_{max} (EtOH) 275 nm (ε 31100), λ_{min} (EtOH) 248 nm (ε 13700), ^{1}H-n.m.r. ($CDCl_3$) δ 4.5 – 4.8 (3-proton multiplet, H-4', 5', and 5"), 5.06 (1-proton triplet, H-2'), 5.60 – 5.85 (1-proton multiplet, H-3'), 5.93 (1-proton doublet, $J_{1',2'}$ 5.0 Hz, H-1').

Anal. Calcd for $C_{43}H_{35}N_5O_6 \cdot 1/2\ H_2O$: C, 71.06; H, 4.99, N, 9.63. Found: C, 70.69; H, 4.91; N, 9.73.

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REFERENCES

1. For Part 8, see I. Hirao, K. Itoh, N. Sakairi, Y. Araki, and Y. Ishido, accepted for publication in Carbohydr. Res. [Manuscript No. T(U)-1333].

2. A. Haines, Adv. Carbohydr. Chem. Biochem., **33**, 11 (1976).

3. C. B. Reese and O. R. Trentham, Tetrahedron Lett., 2459 and 2467 (1965); C. S. Mclaughlin and V. M. Ingram, Biochem., **4**, 1448 (1965).

4. M. E. Chacon-Fuertes and E. Martin-Lomas, Carbohydr. Res., **42**, C4 (1975).

5. Y. Ishido, N. Nakazaki, and N. Sakairi, J. Chem. Soc., Perkin Trans. 1, 2088 (1979).

6. Y. Ishido, N. Sakairi, K. Okazaki, and N. Nakazaki, J. Chem. Soc., Perkin Trans. 1, 563 (1980).

7. G. A. R. Johnston, Tetrahedron, **24**, 6987 (1968).

8. E. Heftmann, " Chromatography," 2nd ed., Reihold Publishing Corp., 1967, p. 52.

9. R. Hernandez, R. Hernandez, Jr., and L. R. Axelrod, Anal. Chem., **33**, 370 (1961).

10. A. V. Kiselev," The Structure and Properties of Porous Materials," Academic Press, Inc., New York, 1958, p. 195.

11. Y. Ishido, N. Nakazaki, and N. Sakairi, J. Chem. Soc., Perkin Trans. 1, 657 (1977).

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