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Note

Synthesis, stereochemistry, intramolecular cyclization, and rates of hydrolysis of adenosine 2′,3′-acetals

VENKATESALU BAKTHAVACHALAM, LEE-GIN LIN, XAVIER M. CHERIAN, AND ANTHONY W. CZARNIK*
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)
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As part of our work on the design of artificial RNA glycosylases, we have been examining ways of positioning functional groups near to the carbohydrate ring-oxygen atom of nucleosides. One such method could involve an anchoring to ribonucleosides *via* their 2′,3′-hydroxyl groups, accomplished by using the well-known condensation reaction of adenosine with suitable carbonyl compounds in the presence of an acid catalyst[1–9]. The products of these reactions with an aldehyde (RCHO) are the corresponding bicyclic acetals that, if R is not a hydrogen atom, may be diastereomeric mixtures due to the introduction of a new stereocenter (see formulas **1** and **2**).

Prior to the initiation of our enzyme-modelling efforts, we had to answer several fundamental questions concerning nucleoside acetals that had not been addressed in the literature to date, namely, (*1*) can acetals between adenosine and *hindered* aldehydes be prepared, (*2*) can the stereochemistry of nucleoside acetals be established unequivocally, and (*3*) is the glycosidic bond in nucleoside acetals stable relative to acetal hydrolysis under mildly acidic conditions? We now communicate our synthetic, spectroscopic, and kinetic results that specifically answer these questions.

Synthesis. — Adenosine acetals constitute an extremely well-known class of compounds, and many of the derivatives (**4, 5, 9a, 9b, 10a, 10b, 11,** and **12**) used in this study had previously been prepared. Treatment of adenosine with various aldehydes, ketones, or orthoesters in the presence of anhydrous *p*-toluenesulfonic acid in dry *N,N*-dimethylformamide gave the corresponding 2′,3′-*O*-substituted adenosine derivatives, whose ^{1}H-n.m.r. data are summarized in Table I. Reactions leading to the ethylidene (**4**; ref. 1), propylidene (**5**; ref. 3), isobutylidene (**6**), *tert*-butylmethylene (**7**), decylidene (**8**), and ethoxymethylene (**11**; ref. 2) derivatives all gave only one isomer, based on both t.l.c. and n.m.r. spectroscopy of the crude reaction-products.

*To whom correspondence should be addressed.

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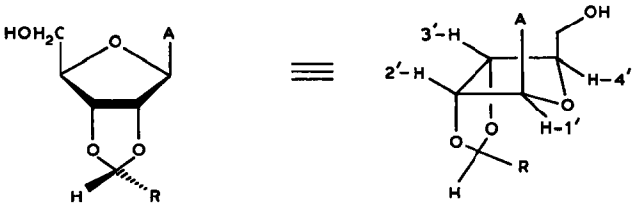
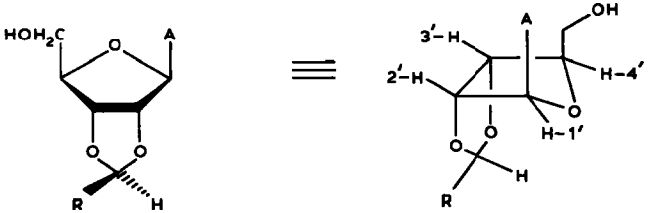


endo 1

A = adenin-9-yl



exo 2

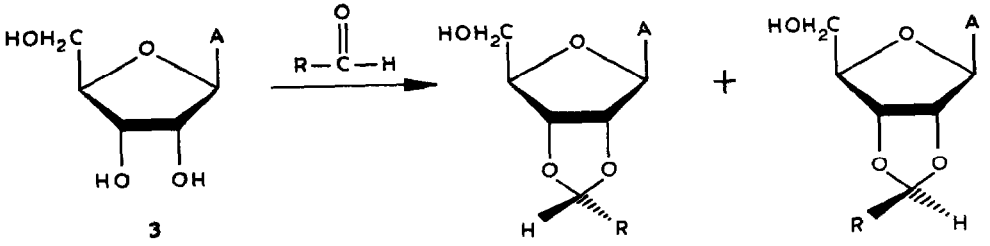
A second isomer was isolated in only two cases. In the reaction of benzaldehyde with adenosine at room temperature, two isomers (**9a** and **9b**) were obtained[4] and were isolated by medium-pressure liquid chromatography; in the other case, reaction with trimethyl orthoformate gave a second isomer (**10b**) as the major product when the reaction was conducted under reflux[5]. The *exo* form of (methoxy-

TABLE I

THE CHEMICAL SHIFTS OF THE PROTONS IN THE SPECTRA OF ACETALS AND ORTHOESTERS OF ADENOSINE

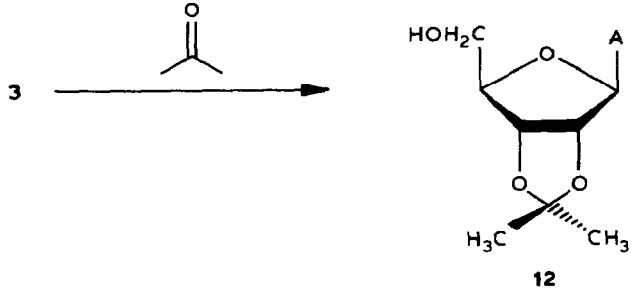
Compound	*Chemical shift (p.p.m.)*[a]							
	2 and 8	*1'*	*2'*	*3'*	*4'*	*5'*	*H*[b]	*R*[c]
4	8.37, 8.14	6.26	5.42	4.98	4.27	3.56	6.07	3.36
5	8.34, 8.15	6.12	5.30	4.89	4.24	3.54	5.06	1.72, 0.94
6	8.36, 8.17	6.14	5.33	4.91	4.25	3.55	4.89	1.91, 0.97
7	8.38, 8.16	6.14	5.34	4.92	4.26	3.54	4.78	0.97
8	8.33, 8.14	6.11	5.30	4.88	4.23	3.53	5.08	0.70–1.70
9a	8.38, 8.16	6.30	5.51	5.09	4.37	3.56	6.02	7.57, 7.45
9b	8.36, 8.16	6.28	5.48	5.08	4.29	3.61	6.24	7.51, 7.42
10a	8.36, 8.14	6.25	5.42	4.98	4.27	3.56	6.06	3.36
10b	8.33, 8.17	6.15	5.49	5.07	4.20	3.54	6.18	3.32
11	8.39, 8.17	6.28	5.41	4.99	4.31	3.59	6.14	3.67, 1.22

[a]Spectra of solutions in Me_2SO-d_6 with internal standard of Me_4Si. [b]Acetal or orthoester hydrogen atom. [c]Acetal or orthoester substituent.

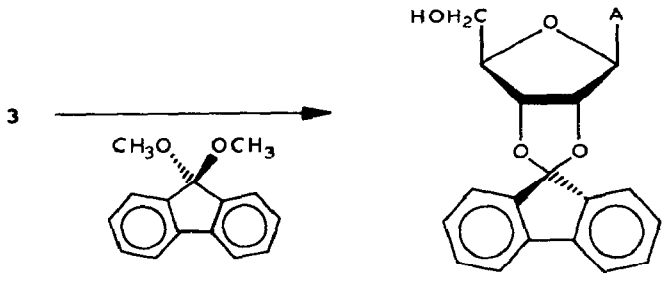


3

R	endo	exo
CH_3	4	
C_2H_5	5	
$CH(CH_3)_2$	6	
$C(CH_3)_3$	7	
C_9H_{19}	8	
C_6H_5	9a	9b
OCH_3	10a	10b
OC_2H_5	11	



12



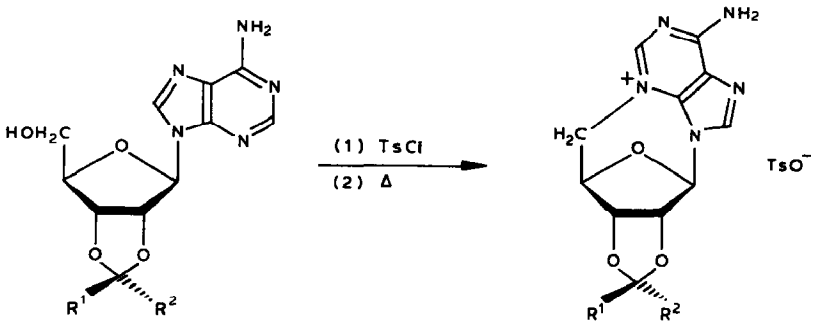
13 14

methylene)adenosine (**10b**) had not previously been separated from the *endo* form[5]; we found that a homogeneous sample of the *exo* isomer may be obtained by precipitation from water.

To the best of our knowledge, acetals **6** and **7** have not hitherto been reported, and the possibility existed that acetal formation in these cases would be so slow (as compared to the competing depurination reaction under the conditions employed) that it could not be achieved. However, we were able to prepare compounds **6, 7,** and **8,** albeit in varied yields. Fluorenone acetal **14** could not be prepared by condensation in DMF; the only product obtained was adenine. We therefore employed a variation of the conditions reported by Heath *et al.*[10], and the desired product **14** was isolated as a light-yellow solid. It is worth noting that all our efforts to condense guanosine with aliphatic aldehydes or dimethyl acetals have been unsuccessful.

Stereochemical assignments based on homonuclear n.O.e. effects. — In the reaction of ribonucleosides with aldehydes, it is generally presumed that the *endo* isomer is the kinetic product, and the *exo* isomer, the thermodynamic product[11]; however, no unequivocal method for the determination of this stereochemistry has been reported to date. Although n.m.r.-spectral methods have been employed to establish product ratios in the syntheses of, *e.g.*, nucleoside acetals[12], dimethylaminomethylene acetals[13], and orthoesters[14], such methods are inherently equivocal as tools for structure assignment; a critical assumption must be made regarding the degree to which a certain proton is deshielded in one isomer relative to that in the other. Our solution was to use a through-space n.O.e. in order to assign relative distances, and therefore relative configurations. In the aldehyde case, in an *exo* isomer, the aldehyde hydrogen atom is underneath the ribosyl ring whereas in the *endo* isomer, the aldehyde substituent (R) is underneath it (see **1** and **2**); an examination of molecular models revealed that H-1 and H-4 of ribose are closer to the *endo* substituent, independent of the conformation. The *endo* isomers, which were prepared at room temperature, showed no enhancement of the acetal-hydrogen signals upon irradiation of the H-4′ atom of the D-ribosyl group, whereas the *exo* isomers **9b** and **10b** respectively showed 4.2 and 2.4% enhancement of the acetal-hydrogen signals. Although the number of *exo* isomer examples used is less than would be ideal (**9b** and **10b** are the only examples known to date), all of the available compounds gave results that were consistent with assignments made previously. It seems probable that this n.O.e. method could also be used to assign the stereochemistry of other 2′,3′-*O*-alkylidenenucleoside derivatives.

Intramolecular cyclization. — During the course of our n.O.e. studies, we considered the possibility that intramolecular cyclization between N-3 of the adenine and C-5 of the ribose would afford a more rigid 2′,3′-*O*-alkylidenenucleoside, which might give larger signal enhancements. This type of cyclization is well-precedented[6] for 2′,3′-*O*-isopropylideneadenosine (**12**→**15**), as well as for other nucleosides. We carried out this conversion on alkylidene derivatives **5, 7,** and **8.** Tosylation of the unprotected 5′-hydroxyl group gave the covalent tosylate



	R^1	R^2	
12	CH_3	CH_3	15
7	H	$C(CH_3)_3$	16
8	H	$(CH_2)_8CH_3$	17
5	H	CH_2CH_3	18

that was heated in acetone to effect cyclization, yielding compounds **16–18** as colorless, crystalline solids. Studies of solutions of these compounds in Me_2SO-d_6 by n.O.e. gave results that were not substantially different from those reported in Table II, and therefore this approach was not examined further.

Rate of hydrolysis in aqueous buffer–1,4-dioxane. — The relative ease with which the acetal groups of nucleoside acetals can be removed under strongly acidic conditions has been studied by Hampton *et al.*[15] and by Chládek and Smrt[9]. Acetal hydrolysis under less acidic conditions had not been examined previously, but might be expected to compete with glycosidic hydrolysis at near neutral pH values, owing

TABLE II

NUCLEAR OVERHAUSER EFFECTS IN THE ^{1}H-N.M.R. SPECTRA OF ACETALS AND ORTHOESTERS OF ADENOSINE

Compound	*% Enhancement on irradiation of the 4'-hydrogen atom*[a]					
	1'	*2'*	*3'*	*5'*	*Acetal-H*	*R*
4	1.88	—	2.37	6.82	—	—
5	1.66	—	2.18	6.72	—	—
6	0.78	—	—	—	—	—
7	1.46	—	2.15	4.50	—	—
8	—	—	1.39	4.57	—	—
9a	1.92	0.34	2.01	5.72	—	2.01
10a	2.69	—	2.98	5.56	—	3.00
11	2.49	—	2.00	5.69	—	1.79
9b	1.62	—	—	3.71	4.17	—
10b	2.11	—	2.60	2.19	2.40	—

[a]In this Table, enhancements >0.3% are reported. In fact, we were able to detect enhancements as small as 0.05%; none of the blank entries in the "Acetal-H" column were ≥0.05%.

TABLE III

KINETICS OF HYDROLYSIS OF ACETALS AND ORTHOESTERS OF ADENOSINE[a]

Compound	*Half-life (h)*	*Compound*	*Half-life (h)*
4	170	**10a**	0.8
5	160	**10b**	0.5
6	240	**11**	0.4
7	250	**12**	14
8	Insoluble	**14**	190
9a	13		

[a]Estimated half-lives (±20%) for the hydrolysis of adenosine acetals to adenosine at 100 ±5° in 4:1 0.15M acetate buffer (pH 5)–1,4-dioxane.

to the greater basicity of the N-1 atom of adenosine as compared to the acetal ring oxygen atoms. For our enzyme-model work, it was essential to determine the rate of acetal hydrolysis as compared to that of glycosidic cleavage. Because some of the adenosine derivatives were insoluble in water, we measured the rates of hydrolysis in 4:1 buffer (pH 5)–1,4-dioxane, and the results are reported in Table III. The ordering of hydrolysis rates is comparable to that observed under more acidic conditions, and closely follows the stability of the intermediate oxonium ion. Monosubstituted acetals are more resistant to hydrolysis than are disubstituted acetals, followed by the very reactive orthoester derivatives **10a, 10b,** and **11**. However, benzylidene acetal **9a** is approximately as reactive as isopropylidene derivative **12**, as anticipated by the benzylic-type carbenium ion stabilization possible in **9a**. Of particular interest is the very slow hydrolysis of the novel fluorenone acetal **14**. As anticipated, the hydrolysis of this acetal is dramatically slowed as compared to benzylidene acetal **9a**, owing to the antiaromatic oxonium ion through which this reaction must occur. This rigid acetal derivative is almost ideal for our enzyme-modeling work.

EXPERIMENTAL

Adenosine was purchased from Sigma Chemical Company and was dried in a vacuum oven for 48 h at 70°. Anhydrous *N,N*-dimethylformamide (DMF) was distilled from BaO after being stored over KOH pellets for 24 h. Anhydrous *p*-TsOH was obtained by heating *p*-TsOH · H_2O at 100° under vacuum, and the resulting material was immediately dissolved in dry DMF, to make a solution of 4 g of *p*-TsOH per 10 mL of solution. Zinc chloride was fused before use. Aldehydes and orthoesters were used as received from the Aldrich Chemical Company, Milwaukee, Wisconsin. Compounds **4** (ref. 4), **5** (ref. 3), **12** (ref. 7), and **15** (ref. 6) were prepared as described previously. A mixture of **9a** *(endo)* and **9b** *(exo)* was afforded by the literature procedure[4]; the two isomers had R_F values of 0.25 and 0.30, respectively, in 19:1 $CHCl_3$–MeOH (silica), and were isolated by medium-pressure liquid chromatography.

Melting points were determined with an Electrothermal melting-point

apparatus and are uncorrected. ^{1}H-Nuclear magnetic resonance spectra were recorded with a 90-MHz Varian EM-390 spectrometer and a 500-MHz Bruker AM-500 F.t.-n.m.r. spectrometer. Chemical shifts (δ) are reported downfield from Me_4Si, in parts per million (p.p.m.) of the applied field. Mass spectra were recorded with a Kratos MS-30 mass spectrometer at The Ohio State University Campus Chemical Instrument Center.

Column chromatography was performed with E. Merck silica gel 60 (230–400 ASTM mesh), and plastic sheets precoated with silica gel 60 F_{254} (0.2 mm thickness), from E. Merck were used for t.l.c. Medium-pressure liquid chromatography was performed in a Labar column, size C (440-37), pre-packed with Lichroprep™ Si 60 (63–125 μm) supplied by E. M. Reagents. Elemental analyses were performed by Canadian Microanalytical Service, Ltd., Vancouver, B.C.

endo-2′,3′-O-Isobutyleneadenosine (**6**). — A mixture of isobutyraldehyde (3.80 g, 53 mmol), *p*-toluenesulfonic acid (4.22 g, 28 mmol), trimethyl orthoformate (5 mL, 46 mmol), and DMF (35 mL) was stirred for 6 h at room temperature. A slurry of adenosine (2.81 g, 10.5 mmol) was then added, the mixture was stirred for 144 h at room temperature and ethanolic 2M NH_3 (16 mL) was added. The mixture was cooled in an ice bath, the ammonium tosylate was removed by vacuum filtration, and the filtrate was subjected to rotary evaporation with a vacuum pump, to remove DMF, affording a light-yellow resin-like residue, which was treated with water, and the organic product extracted with $CHCl_3$. The extract was separated, dried ($MgSO_4$), and evaporated to an oil which crystallized after several hours. This crude product was collected, and recrystallized from MeOH, to give 0.26 g (8%) of colorless solid **6**; m.p. 213–216°; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 0.97 [s, 6 H, $C(CH_3)_2$], 1.91 (m, 1 H, $CHMe_2$), 3.55 (m, 2 H, H-5′), 4.25 (m, 1 H, H-4′), 4.89 [s, 1 H, $(RO)_2CH$], 4.91 (dd, 1 H, H-3′), 5.22 (t, 1 H, OH-5′), 5.33 (dd, 1 H, H-2′), 6.14 (dd, 1 H, H-1′), 7.32 (br s, 2 H, NH_2), 8.17 (s, 1 H, H-2), and 8.36 (s, 1 H, H-8).

Anal. Calc. for $C_{14}H_{19}N_5O_4$: C, 52.3; H, 6.0; N, 21.8. Found: C, 52.1; H, 6.0; N, 21.8.

*endo-2′,3′-O-(*tert*-Butylmethylene)adenosine* (**7**). — A mixture of trimethylacetaldehyde (17.2 g, 0.2 mol), *p*-toluenesulfonic acid (9.51 g, 55 mmol), trimethyl orthoformate (25 mL, 0.23 mol), and DMF (150 mL) was stirred for 24 h at room temperature. A slurry of adenosine (13.5 g, 0.05 mol) and *p*-toluenesulfonic acid (19.02 g, 0.11 mol) was then added, and the mixture was stirred for 168 h at room temperature, heated for 3 h at 65°, cooled, and 2M NH_3 in ethanol (125 mL) added. The mixture was processed as for compound **6**, giving an oil which was treated with ether. After several hours, a colorless solid (~6.4 g) precipitated; this was recrystallized from MeOH, to give colorless needles (2.80 g, 17%) of **7**; m.p. 215–218°; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 0.97 [s, 9 H, $C(CH_3)_3$], 3.54 (m, 2 H, H-5′), 4.26 (m, 1 H, H-4′), 4.78 [s, 1 H, $(RO)_2CH$], 4.92 (dd, 1 H, H-3′), 5.12 (t, 1 H, OH-5′), 5.34 (dd, 1 H, H-2′), 6.14 (dd, 1 H, H-1′), 7.32 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-2), and 8.38 (s, 1 H, H-8); *m/z* (% intensity) 335 (M^+, 1), 320 (1), 305 (3), 278 (24), 248 (5), 218 (29), 202 (8), 190 (6), 164 (36), and 135 (100).

Anal. Calc. for $C_{15}H_{21}N_4O_5$: C, 53.7; H, 6.3; N, 20.9. Found: C, 53.6; H, 6.3; N, 20.8.

endo-2',3'-O-Decylideneadenosine (**8**). — To a stirred suspension of adenosine (**3**; 12.0 g, 45 mmol) in dry DMF (100 mL) and decanal (42.0 g, 270 mmol) at 0° was added a solution of anhydrous *p*-TsOH (40 g, 230 mmol) in DMF (100 mL), in small portions. The clear mixture was stirred for two weeks at 25°, and then treated with 2M ethanolic NH_3 (150 mL) and concentrated to a small volume. A solution of the viscous residue in chloroform (400 mL) was washed with water (2 × 200 mL), dried ($MgSO_4$), and concentrated, and the concentrate was loaded onto a column of silica gel, and successively eluted with $CHCl_3$ (1000 mL) and 19:1 $CHCl_3$–MeOH (1000 mL). Evaporation of pooled fractions provided a colorless solid which, on recrystallization from methanol, gave **8** as a granular solid (6.7 g, 36%); m.p. 176–178°; t.l.c. R_F 0.3 in 10% MeOH in $CHCl_3$ on silica gel; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 0.7–0.9 (br t, 3 H, C*H*$_3$), 1.20–1.75 (m, 16 H, 8 C*H*$_2$), 3.53 (m, 2 H, H-5'), 4.23 (dt, 1 H, H-4'), 4.88 (dd, 1 H, H-3'), 5.08 (t, 1 H, acetal H), 5.19–5.23 (br t, 1 H, O*H*), 5.30 (dd, 1 H, H-2'), 6.11 (d, 1 H, H-1'), 7.32 (br s, 2 H, N*H*$_2$), 8.14 (s, 1 H, H-2), and 8.33 (s, 1 H, H-8); *m/z* (% intensity) 405 (M^+, 3), 375 (5), 316 (3), 278 (10), 248 (11), 218 (50), 164 (51), and 135 (100).

Anal. Calc. for $C_{20}H_{31}N_5O_4$: C, 59.2; H, 7.7; N, 17.3. Found: C, 59.3; H, 7.7; N, 17.2.

endo-2',3'-O-(Methoxymethylene)adenosine (**10a**). — A mixture of adenosine (5.0 g), *p*-TsOH (4.4 g), trimethyl orthoformate (6 mL), and DMF (50 mL) was stirred at room temperature. Trimethyl orthoformate (1 mL) was added after 24 h and again after 48 h. After 66 h, 2M NH_3 in ethanol (13 mL) was added, and the solution was evaporated to dryness *in vacuo*. The residue was mixed with hot water (30 mL), the suspension filtered, and the filtrate chilled in a refrigerator, to give a white precipitate which was recrystallized from ethanol to give 1.12 g of the known *endo* isomer **10a**; m.p. 208–216° (dec.) [lit.[5] m.p. 215° (dec.)]; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 3.36 (s, 3 H, OC*H*$_3$), 3.56 (m, 2 H, H-5'), 4.27 (m, 1 H, H-4'), 4.98 (dd, 1 H, H-3'), 5.11 (t, 1 H, O*H*-5'), 5.42 (dd, 1 H, H-2'), 6.06 [s, 1 H, (RO)$_3$C*H*], 6.25 (dd, 1 H, H-1'), 7.30 (br s, 2 H, N*H*$_2$), 8.14 (s, 1 H, H-2), and 8.36 (s, 1 H, H-8).

exo-2',3'-O-(Methoxymethylene)adenosine (**10b**). — Using the procedure of Reese *et al.*[5] for the synthesis of an *endo–exo* mixture, a mixture of adenosine (5.0 g), *p*-TsOH (4.1 g), and trimethyl orthoformate (25 mL) was refluxed in a hot oil bath for 75 min, cooled, kept overnight at room temperature, and 2M NH_3 in ethanol (15 mL) added. The solid material was removed by suction filtration and the filtrate was evaporated to give a gel-like residue that contained a mixture of the *endo* and *exo* isomers. On treating this with water (30 mL) the solid gradually dissolved and white crystals formed after several hours at 5°. The crude product was recrystallized from ethanol to give 1.14 g (19%) of the pure *exo* isomer; m.p. 199–201°; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 3.32 (s, 3 H, OC*H*$_3$), 3.54 (m, 2 H, H-5'), 4.20 (m, 1 H, H-4'), 5.07 (dd, 1 H, H-3'), 5.18 (t, 1 H, O*H*-5'), 5.49

(dd, 1 H, H-2'), 6.15 (d, 1 H, H-1'), 6.18 [s, 1 H $(RO)_3CH$], 7.33 (br s, 2 H, NH_2), 8.17 (s, 1 H, H-2), and 8.33 (s, 1 H, H-8).

Anal. Calc. for $C_{12}H_{15}N_5O_5$: C, 46.6; H, 4.9; N, 22.6. Found: C, 46.5; H, 4.9; N, 22.5.

endo-2',3'-O-(Ethoxymethylene)adenosine (**11**). — *Method 1.* The compound was prepared according to the literature procedure[2]: m.p. 232–236°; ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 1.22 (t, 3 H, CCH_3), 3.59 (m, 2 H, H-5'), 3.67 (m, 2 H, OCH_2C), 4.31 (m, 1 H, H-4'), 4.99 (dd, 1 H, H-3'), 5.13 (t, 1 H, O*H*-5'), 5.41 (dd, 1 H, H-2'), 6.14 [s, 1 H, $(RO)_3CH$], 6.28 (dd, 1 H, H-1'), 7.33 (br s, 2 H, NH_2), 8.17 (s, 1 H, H-2), and 8.39 (s, 1 H, H-8).

Method 2. On following the procedure described for preparing compound **10b**, adenosine (5.0 g) gave a colorless product (0.16 g) that was identical in all respects to that obtained by method 1.

9,9-Dimethoxyfluorene (**13**). — A mixture of 9-fluorenone (9.0 g), *p*-TsOH (0.38 g), trimethyl orthoformate (12 mL), and CH_3OH (60 mL) was stirred for 40 h at room temperature. The clear yellow solution was made neutral with an excess of 2M NH_3 in ethanol, and evaporated to a yellow residue which was mixed with $CHCl_3$, and the suspension filtered to remove the tosylate salt. The filtrate was evaporated to dryness, and the residue crystallized and then recrystallized from CH_3OH to give 7.43 g (66%) of colorless needles; m.p. 86–88°; ^{1}H-n.m.r. data ($CDCl_3$): δ 3.3 (s, 6 H, 2 OCH_3), and 7.2–7.7 (m, 8 H, ArH).

2',3'-O-(o,o'-Biphenylmethylene)adenosine (**14**). — A suspension of adenosine (2.40 g) 9,9-dimethoxyfluorene (3.05 g), and pyridinium tosylate (50 mg) in dichloroethane (60 mL) was refluxed for 48 h. The resulting yellow suspension was cooled, and the solvent evaporated to give a yellow residue which was treated with $CHCl_3$ to precipitate 1.52 g (63% recovery) of pure adenosine, removed by filtration. The filtrate was concentrated, and the concentrate was loaded onto a column of silica gel. 9-Fluorenone and 9,9-dimethoxyfluorene were eluted off first, with $CHCl_3$, and the product was then eluted with 1:19 CH_3OH–$CHCl_3$. The fractions containing the product were pooled, and evaporated, to give a yellow residue which crystallized from ethanol to afford 77 mg (2%; 5.5% based on unrecovered adenosine) of a yellow solid; m.p. 243–245°; ^{1}H-n.m.r. data (Me_2SO-d_6): δ 3.6–3.8 (m, 2 H, H-5'), 4.5–4.7 (m, 1 H, H-4'), 5.3 (t, 1H, O*H*-5', exchanges with D_2O), 5.5–5.7 (dd, 1 H, H-3'), 5.9–6.1 (dd, 1 H, H-2'), 6.6 (d, 1 H, H-1'), 7.2–7.9 (m, 8 H, ArH), 8.3 (s, 1 H, H-8), and 8.6 (s, 1 H, H-2); *m/z* 431 ($[M + 2]^+$) and 429 (M^+). High-resolution mass spectrum: Calc. for $C_{23}H_{19}N_5O_4$: 429.150; Found: 429.147.

Anal. Calc. for $C_{23}H_{19}N_5O_4$: C, 64.3; H, 4.5; N, 16.3. Found: C, 64.0; H, 4.6; N, 16.3.

3,5'-Anhydro-2',3'-O-(tert-butylmethylene)adenosine p-toluenesulfonate (**16**). — A slurry of **7** (0.5 g) in warm pyridine (5 mL) was cooled in an ice bath. Tosyl chloride (0.50 g) was added, and the yellow mixture was cooled for 45 h at −10° with occasional stirring, poured into ice–water (75 mL), and extracted with $CHCl_3$

(3 × 25 mL). The extracts were combined, successively washed with dilute sulfuric acid (until the aqueous phase was acidic) and H_2O (25 mL), dried ($MgSO_4$), and evaporated, to give 0.73 g (100%) of a colorless solid: m.p. 308–310° (dec.). Without purification, this compound (300 mg) was suspended in acetone (50 mL), and the mixture was heated at reflux for 20 h under a nitrogen atmosphere. The starting material dissolved after several minutes, and, within 1 h, colorless crystals had formed. After 20 h, the suspension was cooled, and the solid was recrystallized from MeOH–acetone, to give 205 mg (68%) of colorless crystals; m.p. 322–323° (dec.); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 0.93 [s, 9 H, C(CH_3)$_3$], 2.29 (s, 3 H, tosyl-CH_3), 4.49–5.05 (m, 5 H, H-2′,3′,4′,5′), 4.61 [s, 1 H, (RO)$_2$C*H*], 6.67 (s, 1 H, H-1′), 7.10–7.48 (dd, 4 H, tosyl-ArH), 8.50 (s, 1 H, ArH), 8.65 (s, 1 H, ArH), and 9.40 (two broad singlets, 2 H, NH_2).

Anal. Calc. for $C_{22}H_{27}N_5O_6S$: C, 54.0; H, 5.6; N, 14.3; S, 6.6. Found: C, 53.8; H, 5.5; N, 14.3; S, 6.6.

3,5′-Anhydro-2′,3′-O-decylideneadenosine p-*toluenesulfonate* (**17**). — Decylidene acetal **8** (1.0 g, 2.5 mmol) was dissolved in anhydrous pyridine (15 mL) by warming. To the cooled solution was added *p*-toluenesulfonyl chloride (1.0 g, 5.2 mmol), and the mixture was kept for 18 h at 0°, washed with aq. $NaHCO_3$ (2 g in 20 mL), and evaporated to dryness *in vacuo* below 30°. The colorless residue was extracted into chloroform (200 mL), the extract evaporated to dryness, the residue dissolved in acetone (150 mL), and the solution heated at reflux for 6 h. The colorless solid that separated was filtered off, washed with acetone, and dried; yield, 790 mg (57%); m.p. 260–262° (dec.). An analytical sample was prepared by recrystallization from ethanol–acetone; m.p. 265–266°; λ_{max}^{MeOH} 220 (ε_{mM} 20.86) and 276 (14.57); ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 0.86 (t, 3 H, CH_3), 1.25 (m, 14 H, 7 CH_2), 1.65 (m, 2 H, CH_2), 2.28 (s, 3 H, tosyl CH_3), 4.4–4.8 (m, 2 H, H-3′,4′), 4.88–4.90 (m, 2 H, H-2′ and acetal H), 5.0–5.03 (m, 2 H, H-5′), 6.74 (s, 1 H, H-1′), 7.09–7.10 (d, 2 H, tosyl-ArH), 7.47–7.49 (d, 2 H, tosyl-ArH), 8.51 (s, 1 H, ArH), 8.65 (s, 1 H, ArH), and 9.37–9.45 (two broad singlets, 2 H, NH_2); ^{13}C-n.m.r. data (125 MHz) (Me_2SO-d_6): δ 13.8, 20.71, 22.03, 23.09, 28.62, 28.78, 28.83, 28.90, 31.23, 32.48, 57.52, 79.86, 84.01, 85.46, 90.53, 106.41, 119.56, 125.44, 127.96, 137.51, 139.16, 139.97, 145.71, 149.23, and 156.67; f.a.b.-mass spectrum *m/z* (% intensity) 388 (100).

Anal. Calc. for $C_{27}H_{37}N_5O_6S$: C, 57.9; H, 6.7; N, 12.5; S, 5.7. Found: C, 58.0; H, 6.6; N, 12.6; S, 5.8.

3,5′-Anhydro-2′,3′-O-propylideneadenosine p-*toluenesulfonate* (**18**). — Compound **5** (1.6 mmol) was dissolved in pyridine (10 mL) by gentle warming. To the cooled solution was added *p*-TsCl (3.5 mmol) and the mixture was kept for 48 h at −15°. The yellow mixture was poured into cold water (50 mL), and extracted with chloroform (2 × 50 mL). The extract was successively washed with 2M H_2SO_4 (2 × 30 mL) and cold water, dried ($MgSO_4$), and evaporated, to give the covalent tosylate, which was converted into the ionic tosylate in refluxing acetone (150 mL) during 24 h. The precipitated salt was filtered off, washed with acetone, and dried,

to afford a colorless solid in 48% yield: m.p. 282–283° (dec.). An analytical sample was obtained by recrystallization from ethanol–acetone: ^{1}H-n.m.r. data (500 MHz) (Me_2SO-d_6): δ 0.91–0.94 (t, 3 H, CH_3), 1.65–1.70 (m, 2 H, CH_2), 2.28 (s, 3 H, tosyl-CH_3), 4.47–4.53 (m, 2 H, H-3′,4′), 4.86–4.88 (t, 1 H, acetal H), 4.91–4.92 (d, 1 H, H-2′), 5.01–5.04 (m, 2 H, H-5′), 6.75 (s, 1 H, H-1′), 7.10–7.11 (d, 2 H, tosyl-ArH), 7.47–7.49 (d, 2 H, tosyl-ArH), 8.51 (s, 1 H, ArH), 8.65 (s, 1 H, ArH), and 9.36–9.45 (two broad singlets, 2 H, NH_2); ^{13}C-n.m.r. data (125 MHz) (Me_2SO-d_6): δ 7.36, 20.64, 25.54, 57.25, 79.83, 83.93, 85.41, 90.47, 109.02, 119.49, 125.35, 127.91, 137.5, 139.1, 139.9, 145.6, 149.2, and 156.6; f.a.b.-mass spectrum *m/z* (% intensity) 290 (100).

Anal. Calc. for $C_{20}H_{23}N_5O_6S$: C, 52.0; H, 5.0; N, 15.2; S, 7.0. Found: C, 51.9; H, 5.0; N, 15.1; S, 6.9.

Nuclear Overhauser enhancement experiments. — The n.O.e. experiments were conducted with a Bruker AM-500 500-MHz F.t.-n.m.r. instrument and were performed by Dr. C. E. Cottrell at The Ohio State University Campus Chemical Instrument Center. The irradiation pulse-width varied from 1.0 to 8.0 Hz, depending on the proximity of neighboring signals. The error range is estimated to be ±10% of the reported enhancement.

Determination of rates of hydrolysis of acetals. — A solution of the adenosine acetal (10 mg) in hot 1,4-dioxane (2 mL) was added to 0.15M acetate buffer, pH 5 (8 mL) preheated to 100°, and the resulting solution was heated to gentle reflux. At appropriate time-intervals, an aliquot was withdrawn and analyzed by t.l.c. using 4:1:1 1-butanol–glacial acetic acid–water on cellulose plates. In all cases, the acetal had an R_F value of 0.7–0.9, whereas adenosine had R_F 0.5. By spotting samples at regular time-intervals from 0% to 95% reaction, it was possible to choose the time at which the spots for the starting material and the product were of equal intensity. This method relies on the reasonable assumption that adenosine and its acetals have approximately equal extinction coefficients, and is accurate to ~±20%. This level of accuracy is sufficient for the qualitative comparisons sought in this work. It is of interest that adenosine is only very slowly depurinated under these conditions, only becoming observable by our t.l.c. methods after ~100 h.

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