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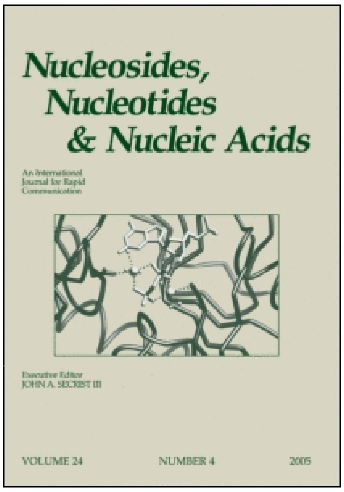

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Synthesis of 2'- and 3'-Ketoadenosines

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NUCLEOSIDES & NUCLEOTIDES, 2(3), 275-289 (1983)

SYNTHESIS OF 2'- AND 3'-KETOADENOSINES

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Abstract: Both 2'- and 3'-ketoadenosines were prepared by the Pfitzner-Moffatt oxidation of the respective N^6-(4-monomethoxytrityl)-3',5'-di-O-(4-monomethoxytrityl)- and N^6-(4-monomethoxytrityl)-2',5'-di-O-(4-monomethoxytrityl)adenosines, followed by removal of the protective groups under anhydrous acidic conditions in chloroform. The products so obtained were stable at least to short-term storage at -20 °C. Solution half lives for 2'-ketoadenosine and 3'-ketoadenosine were found to be ca. 11 h and ca. 84 h, respectively at 25 °C and pH 6.86.

INTRODUCTION

Ketoadenosines have been suggested as intermediates in at least two biological processes. In 1973 Suhadolnik indicated[1] that either 2'- or 3'-ketoadenosine might be the intermediate in the biosynthesis[2,3] of 9-(β-D-arabinofuranosyl)adenine from adenosine by *Streptomyces antibioticus*, as evidenced[3] from tritium-incorporation studies. Palmer and Abeles, while investigating the enzyme-catalyzed reversible hydrolysis of S-adenosyl-L-homocysteine to adenosine and L-homocysteine, concluded that 3'-ketoadenosine was likely involved as an intermediate in that process.[4,5] While in neither study were the ketonucleosides isolated, indirect evidence from tritium-incorporation studies[3] in the first case and from studies on a similar system, 5'-deoxyadenosine, in the second case,[5] point convincingly to the biological significance of these

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compounds. Our goal was to synthesize and purify both 2'- and 3'-ketoadenosine for use in biochemical studies.

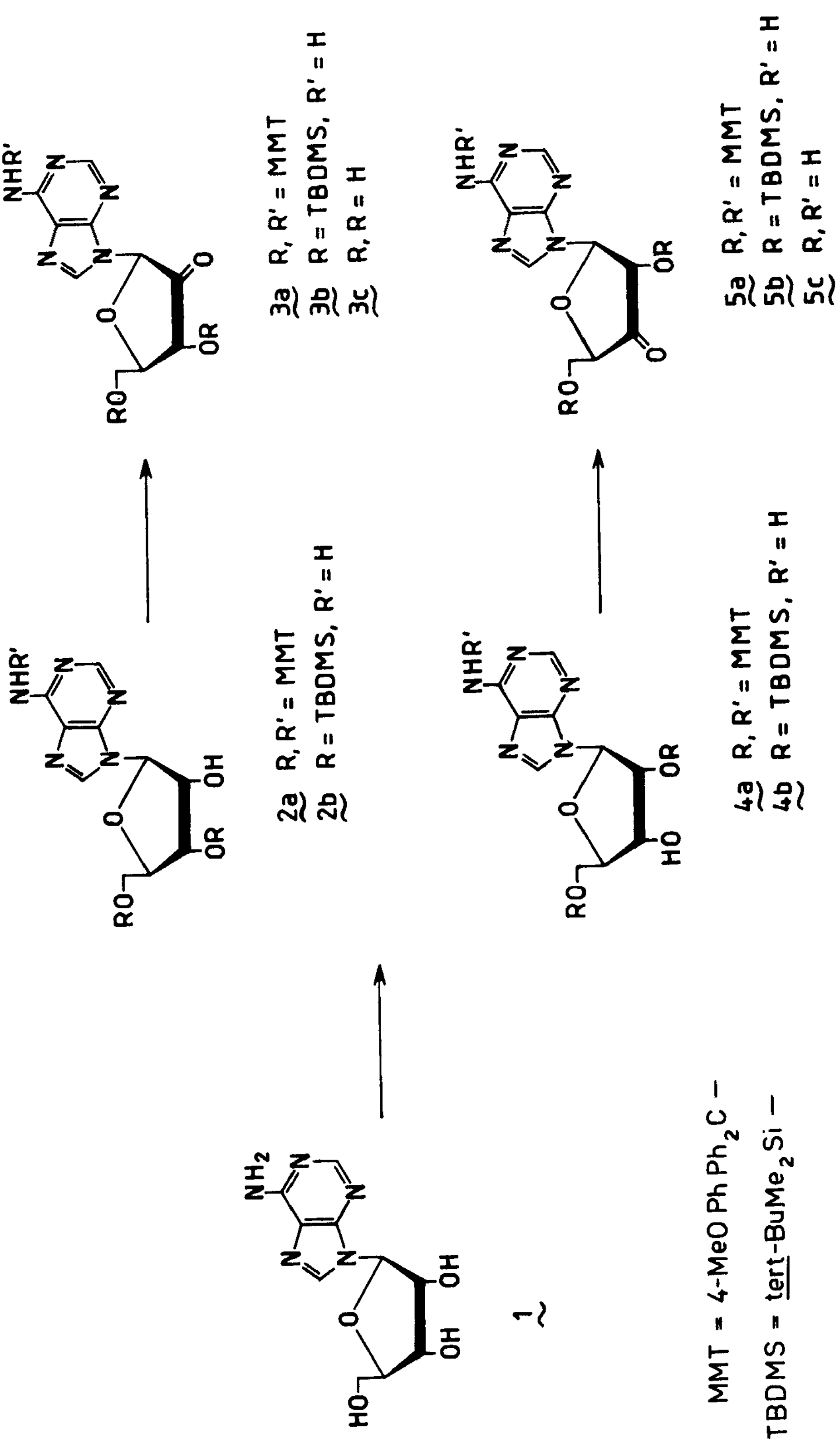
While a number of 2'- and 3'-ketonucleosides have been synthesized, those reported to date have been limited to either protected pyrimidine[6-9] and purine[9-12] nucleosides or the free, underivatized 2'- and 3'-ketouridines.[6] The free-hydroxy ketonucleosides in general, and free-hydroxy adenosines in particular, have decomposed upon attempts at protecting group removal, and consequently the latter have so far eluded the efforts of the synthetic chemist.

RESULTS AND DISCUSSION

In designing a synthesis for both the 2'- and 3'-ketoadenosines, it became evident from trial studies that the most desirable protecting group would be one that would be removable under neutral or mildly acidic conditions, as even slightly basic media were shown to promote decomposition of the keto products, as evidenced by the elimination of adenine from these compounds. Both 2',5'- and 3',5'-di-O-(tert-butyldimethylsilyl)-protected adenosines (**2b** and **4b**), which were synthesized in good yields[13] and were found to be excellent substrates for the Pfitzner-Moffatt oxidation,[14] were found in our hands to be unsuitable for producing the free-hydroxy ketonucleosides, as during deprotection with a number of fluoride reagents, adenine elimination invariably resulted.[15]

In evaluating other potential protecting groups, the 4-monomethoxytrityl group (MMT)[16,17] was considered the best choice when all aspects of stability and ease of removal were evaluated. Reaction of 4-monomethoxytrityl chloride (MMT-Cl) with adenosine essentially as described by Brodbeck and Moffatt[7] for N[4]-acetyl cytidine gave a mixture of N[6]-(4-

SCHEME I



MMT = 4-MeO Ph Ph$_2$C –

TBDMS = *tert*-BuMe$_2$Si –

monomethoxytrityl)-9-[3,5-di-O-(4-monomethoxytrityl)-β-D-ribofuranosyl]adenine (**2a**) and its N^6,2',5'-tri-protected counterpart **4a** that were separated by preparative liquid chromatography (LC) to give **2a** and **4a** in 21% and 12% yields, respectively.[18] Pfitzner-Moffatt oxidation[14] of **2a** yielded the stable, fully protected 2'-ketonucleoside **3a** that could be freed from N,N'-dicyclohexylurea by a precipitation technique, followed by preparative LC to give pure **3a** in 36% yield. ^{1}H NMR analysis of **3a** revealed two distinct sets of resonances, one of which corresponded to the 2'-keto form of the nucleoside, and the other, more predominant set being attributed to the N^6,3',5'-tri-MMT gem diol (i.e., the hydrated ketone). The ratio of keto to gem diol was determined to be highly variable, depending upon the amount of water or deuterium oxide present in the solution. The possibility of a methyl hemiacetal being present cannot be rigorously excluded, although the ^{1}H NMR offers no evidence for such a species. The preponderance of the hydrated species was also evident from the IR spectrum of **3a** which showed only a weak C=O stretch at 1785 cm^{-1}. In an identical manner **4a** gave **5a** in 34% yield. Examination of the ^{1}H NMR spectrum for **5a** showed only one set of resonances that were attributed to the 3'-keto form of the nucleoside, whose presence was also confirmed by the IR spectrum that gave a strong C=O absorption at 1780 cm^{-1}.

Deprotection of **3a** and **5a** was effected using a method similar to that employed for the removal of trityl groups from protected ketocytosines.[7] The protected nucleosides **3a** or **5a**, upon standing for several hours in cold, anhydrous solutions of hydrogen chloride in chloroform, gave the respective ketonucleosides **3c** or **5c** in 43% and 26% optical yields, respectively. Both products were invariably contaminated with 2-4% of adenine, as determined by HPLC; however, these products, when centrifuged, and the solvents decanted at -5 °C under dry nitrogen and stored at -20 °C in the anhydrous state, were stable to further decomposition for periods in excess of 4 weeks.

Studies on the solution stability for both **3c** and **5c** were carried out at 25 °C in pH 6.86 (0.005 M) phosphate buffer, the decomposition being monitored (i.e., increase of adenine, decrease of the ketonucleoside) by HPLC. A 0.8 mmolar solution of 2'-ketoadenosine (**3c**) underwent 50% decomposition in 11 h, while a 0.7 mmolar solution of the 3'-keto counterpart **5c** required 84 h for 50% conversion to adenine. Plots of concentration of either **3c** or **5c** versus time showed excellent linear relationships.

^{1}H NMR analysis of the 2'-ketonucleoside **3c** revealed the keto and gem diol forms present in the product in a ratio of 2:1, respectively. Reduction of **3c** with sodium borodeuteride gave a mixture of 9-(β-D-arabinofuranosyl)adenine-2'-d and adenosine-2'-d in a ratio of ca. 5:1 as determined by HPLC. Integration of the ^{1}H NMR signals confirmed (See Experimental Section.) the above ratio and further indicated that the observed adenosine was indeed adenosine-2'-d. Such criteria have been used to establish the integrity of carbonyl-containing nucleosides[19,9] and are generally more expedient to employ than traditional hydrazone derivatives, etc.

Similar treatment of the N^6,2',5'-tri-MMT-3'-ketoadenosine(**5a**)gave 3'-ketoadenosine (**5c**) in 26% yield. ^{1}H NMR and IR analysis on products which had been equilibrated with water during workup, followed by freeze-drying, curiously revealed a single species, indicated to be the *keto* form of the compound (δ H-1' = 6.16; IR C=O at 1780 cm^{-1}). Reduction of **5c** with sodium borodeuteride and analysis of the products as for **3c** showed a 3.5:1 ratio of 9-(β-D-xylofuranosyl)adenine-3'-*d* to adenosine-3'-*d*. There was no evidence by ^1H NMR for contaminating, non-deuterated D-ribofuranosyl products that would arise had there been incomplete oxidation of **4a**.

In summary, the procedures described in the foregoing are a preparative route to both 2'- and 3'-ketoadenosines that may prove useful as biological probes. Both compounds enjoy considerable stability when prepared in a semi-pure state and kept under anhydrous conditions at -20 °C. Contaminants are limited to ca. 2-4% of adenine, along with some partially deblocked products. The latter can be eliminated by an equilibration between ethyl acetate and water. The purified, non-anhydrous solid products so obtained, however, have stabilities limited to a few hours at room temperature. Solution stabilities at pH 6.86 are considerably longer, indicating that these compounds will probably be useful in biochemical studies.

EXPERIMENTAL

General. All solutions were evaporated at aspirator vacuum at ~40 °C unless otherwise specified. Melting points (mp) were determined using a Thomas-Hoover Unimelt capillary tube device equipped with a Cole-Parmer model 8520-50 Digi-Sense thermocouple thermometer with a 8520-55 probe; as such mp's are considered corrected. IR spectra were determined using a Perkin-Elmer 710B instrument; UV measurements were made using a Cary-14 spectrophotometer; 200-MHz ^1H NMR spectra were determined using a Nicolet NT-200 instrument on ca. 0.1% solutions in the indicated solvent using tetramethylsilane as an internal reference (δTMS = 0.0); optical rotations were measured in a 1-dm cell on a Perkin-Elmer model 241 spectropolarimeter in the indicated solvent. Chromatography products were E. Merck's Silica Gel-60 (TLC: catalog no. 5539; column chromatography: catalog no. 7734, 20-60 μm). Solvent systems include: A, 15:85 ethyl acetate - toluene; B, 20:80 methanol - chloroform, and C, 70:15:15 acetonitrile - 0.1 M ammonium chloride - 0.1 M ammonium acetate. Preparative liquid chromatography (LC) was carried out using a Waters Associates Prep 500-A unit equipped with silica gel cartridges and a refractive index detector. High pressure liquid chromatography (HPLC) was conducted using Altex-Beckman equipment and reverse-phase columns (0.45 x 40-cm) of 10 μm Spherisorb ODS (C-18) packed in house. Conditions include: flowrate, 2 mL/min; solvent, 95:5 0.005 M phosphate buffer, pH 6.86 - acetonitrile; 254-nm UV detection. HPLC peaks were recorded and integrated for the stability studies using a Hewlett-Packard 3390A recorder-integrator. Reagents were reagent grade and were used as supplied. Dimethyl sulfoxide and *N*,*N* -dimethylformamide were distilled *in vacuo* from calcium hydride and stored over 4-A molecular sieves; pyridine was distilled from calcium hydride and stored over 4A molecular sieves; anhydrous chloroform was prepared by passing the reagent (ca. 50 mL) through a 2 x 40-cm column of neutral, activity 1 alumina.

N^6-(4-Monomethoxytrityl)-9-[3,5-di-*O*-(4-monomethoxytrityl)-β-*D*-ribofuranosyl]adenine (2a) and N^6-(4-monomethoxytrityl)-9-[2,5-di-*O*-(4-monomethoxytrityl)-β-*D*-ribofuranosyl]adenine (4a). To a stirred solution of 24.31 g (79 mmol) of 4-monomethoxytrityl chloride (*p*-anisylchlorodiphenylmethane, MMT-Cl, Aldrich) in 170 mL of pyridine at 90 °C was added 10.01 g (37 mmol) of adenosine (1, Aldrich).

The suspension cleared in ca. 15 min, and after 1 h another 13.05 g (42 mmol) of MMT-Cl was added to the reaction. Heating of the mixture was continued for 3 h, at which time 12.53 g (41 mmol) of additional MMT-Cl was added. After 4 h (8 h total reaction time) the reaction was cooled to room temperature and poured into 1.5 L of ice water with stirring. The yellow precipitate was collected by filtration and partitioned between 400 mL of ethyl acetate and 400 mL of 5% aqueous potassium bisulfate. The organic layer was washed with 200 mL of water, dried over magnesium sulfate and evaporated to give 65 g of a dark syrup that was taken up into 150 mL of chloroform. Precipitation of the crude products was effected by the addition of 500 mL of methanol. The mixture was cooled to -16 °C for 2 h, and the precipitate was collected by filtration and washed with methanol to yield 42.5 g of a crude mixture of MMT-substituted adenosine compounds after drying *in vacuo* at 27 °C for 12 h. The gummy precipitate was taken up into a minimum of toluene, split into equal portions and loaded onto two, 200-g (4 x 35-cm) columns of silica gel slurry-packed in toluene. Elution of each column with 300 mL of toluene, followed by a linear gradient of 700 mL of toluene → 700 mL of 1:9 ethyl acetate - toluene, yielded 22 g of a syrupy mixture of the trisubstituted adenosines **2a** and **4a** upon pooling and evaporation of the appropriate fractions as indicated by TLC (solvent A).

The above mixture was taken up into 50 mL of 1:9 ethyl acetate - toluene, divided in two equal volumes and subjected to preparative LC on silica gel equilibrated with the same solvent. Fractions containing the desired products were separately pooled and evaporated, with the first peak yielding 6.30 g of a syrup which, upon precipitation from chloroform - methanol and drying at 100 °C *in vacuo* gave 4.93 g (12%) of (**2a**) as a white solid: mp 150 - 156 °C; $[\alpha]_D^{25} = -12.8^\circ$ (*c* 1, $CHCl_3$); R_f 0.26 (solvent A); UV (THF): 274 nm (ε 26400); IR (KBr) 1600 cm^{-1} (C=N); ^1H NMR (200 MHz, C_6D_6) δ 2.73 [2H, dd, $J_{5',5'a}$ = 10.6, $J_{4',5'a}$ = 3.9, *H*-5'a], 3.23 (3H, s, $OC\underline{H}_3$), 3.25 (3H, s, $OC\underline{H}_3$), 3.27 (3H, s, $OC\underline{H}_3$), ca. 3.30 [1H, m, (partially obscured by OCH_3) *H*-5'], 3.74 [1H, m, (width = 19 Hz), *H*-4'], 4.61 (1H, dd, $J_{2',3'}$ = 5.0, *H*-3'), 4.94 [1H, m, (width = 23 Hz), *H*-2'], 5.75 (1H, d, $J_{2',O\underline{H}}$ = 2.6, 2'-O*H*), 6.06 (1H, d, $J_{1',2'}$ = 5.6, *H*-1'), 6.54 - 7.78 (ca. 42H, m, aryl), 7.94, 8.00 (1, 1, s, s, *H*-2, *H*-8).

Anal. Calcd for $C_{70}H_{61}N_5O_7 \cdot 0.5$ MeOH: C, 76.68; H, 5.75; N, 6.34. Found: C, 76.75; H, 5.78; N, 6.29.

The second peak yielded 10.50 g of a syrup that was precipitated from chloroform - ethanol to give 8.55 g (21%) of **4a** after drying at 100 °C *in vacuo* overnight: mp 130 °C; $[\alpha]_D^{25} = +46.6^\circ$ (*c* 1.2, $CHCl_3$); R_f 0.17 (solvent A); UV (THF): 273 nm (ε 27800); IR (KBr) 1595 cm^{-1} (C=N); ^1H NMR (200 MHz, 9:1 Me_2SO-d_6 - D_2O) δ 2.93 [1H, m, (width = 23 Hz), $J_{5',5'a}$ = 10.3 Hz, *H*-5'a], 3.09 [1H, m, (width = 23 Hz), *H*-5'], 3.27 [1H, m, (width = 19 Hz), *H*-3'], 3.65 (3H, s, $OC\underline{H}_3$), 3.71 (6H, s, $OC\underline{H}_3$), 3.99 [1H, m, (width = 25 Hz), *H*-4'], 5.02 [1H, dd (t), $J_{2',3'}$ = 4.6, *H*-2'], 5.90 (1H, d, $J_{1',2'}$ = 6.0 Hz, *H*-1'), 6.61 - 7.35 (ca. 42H, m, aryl), 7.64, 8.10 (1, 1, s, s, *H*-2, *H*-8).

Anal. Calcd for $C_{70}H_{61}N_5O_7 \cdot 0.5$ EtOH: C, 77.01; H, 5.82; N, 6.32. Found: C, 76.78; H, 5.77; N, 6.42.

$\underline{N}^6$-(4-Monomethoxytrityl)-9-[3,5-di-*O*-(4-monomethoxytrityl)-β-*D*-*erythro*pentofuranos-2-ulos-1-yl]adenine (3a). To a stirred solution of **4.00** g (3.69 mmol) of **2a** and 3.10 g (15.0 mmol) of dicyclohexylcarbodiimide (DCC, Aldrich) in 20 mL of anhydrous dimethyl sulfoxide was added 0.15 mL of dichloroacetic acid. The solution turned the characteristic orange-yellow color, and a precipitate of dicyclohexylurea (DCU) began to form. After the mixture was stirred for 24 h, the reaction was quenched by the addition of 3.0 g of oxalic acid in 20 mL of water and 50 mL of ethyl acetate. The DCU was removed by filtration, the filtrate was transferred to a separatory funnel, and the aqueous layer was extracted with an additional 50 mL of ethyl acetate. The organic layers were combined and extracted with 50 mL of saturated sodium bicarbonate, then with 100 mL of water, dried over magnesium sulfate and evaporated to give a syrup that gave a precipitate from chloroform - methanol to yield 3.1 g of a product free from DCU, but contaminated (TLC) by an unidentified, fast-migrating compound. The precipitate was dissolved in 20 mL of a 8:92 ethyl acetate - toluene and subjected to preparative LC in the same solvent. The fractions containing the product were pooled and evaporated to yield 2.16 g of **3a** as a syrup that was homogeneous by TLC. Precipitation from chloroform - methanol gave 1.44 g (36%) of **3a** as an amorphous solid, shown to be partially hydrated: mp 141 °C dec.; $[\alpha]_{D}^{25} = -12.1^{\circ}$ (*c* 1.0 $CHCl_3$); R_f 0.30 (solvent A); UV (THF): 272 nm (ε26300); IR (KBr) 1600 cm^{-1} (C=N), 1785 cm^{-1} (C=O); ^1H NMR for the 2'-*keto* form: (200 MHz, C_6D_6) δ 2.90 [1H, m, $J_{5',5'a}$ = 8.8, (width = 19 Hz), *H*-5'a], 3.24 (6H, s, OCH_3), 3.29 (3H, s, OCH_3), ca. 3.30 (1H, obscured by OCH_3, *H*-5'), 4.12 [1H, m, (width = 19 Hz), *H*-4'], 4.41 (1H, s, *H*-3'), 6.25 (1H, s, *H*-1'), 6.58 - 7.79 (ca. 42, m, aryl), 8.07, 8.16 (1, 1, s, s, *H*-2, *H*-8); for 2'-*gem*-diol (200 MHz, C_6D_6) δ 3.24 (6H, s, OCH_3), ca. 3.25 (2H, m, obscured by OCH_3, *H*-5', *H*-5'a), 4.31 [1H, m, (width = 20 Hz), *H*-4'], 5.13 (1H, d, $J_{3',4'}$ = 6.5, *H*-3')

5.50 (1H, s, *H*-1'), 6.50 - 7.60 (ca. 42H, m, aryl), 7.96 (1H, s, *H*-2 or *H*-8).[20]

Anal. Calcd for $C_{70}H_{61}N_5O_8 \cdot MeOH$: C, 75.31; H, 5.79; N, 6.19. Found: C, 75.07; H, 5.74; N, 6.27.

***N*^{6}-(4-Monomethoxytrityl)-9-[2,5-di-*O*-(4-monomethoxytrityl)-β-*D*-*erythro*pentofuranos-3-ulos-1-yl]adenine (5a).** By the process described for **3a**, 6.00 g (5.54 mmol) of **4a** was oxidized with 4.56 g (22.1 mmol) of DCC and 0.25 mL of dichloroacetic acid in 30 mL of anhydrous dimethyl sulfoxide to give, after preparative LC, precipitation of the product from chloroform - methanol and drying at 61 °C *in vacuo*, 2.05 g (34%) of pure **5a** as an amorphous solid: mp 145 °C dec.; $[\alpha]_D^{25}$ = +42.0° (*c* 1, $CHCl_3$); R_f 0.35 (solvent A); UV (THF) 272 nm (ε 27500); IR (KBr) 1595 cm^{-1} (C=N), 1780 cm^{-1} (C=O); ^1H NMR (200 MHz, 9:1 Me_2SO-d_6, D_2O) δ 3.02 (1H, m, $J_{5',5'a}$ = 10.6, *H*-5a'), 3.13 (1H, dd, $J_{4',5'}$ = 4.7 Hz, *H*-5'), 3.58 (3H, s, OCH_3), 3.71 (1H, s, OCH_3), 3.72 (3H, s, OCH_3), 4.53 [1H, m, (width = 16 Hz), *H*-4'], 5.77 (1H, d, $J_{1',2'}$ = 7.0, *H*-2'), 6.23 (1H, d, *H*-1'), 6.5 - 7.4 (ca. 42H, m, aryl), 7.56, 8.06 (1, 1, s, s, *H*-2, *H*-8).

Anal. Calcd for $C_{70}H_{59}N_5O_7 \cdot MeOH$: C, 76.53; H, 5.65; N, 6.29. Found: C, 76.49; H, 5.66; N, 6.32.

2'-Ketoadenosine (3c). To a cold solution (-10 °C) of 0.205 g (0.189 mmol) of **3a** and 10 mL of anhydrous chloroform in a septum-stoppered, 200-mL centrifuge bottle was added dropwise 3.2 mL of 0.18 N hydrogen chloride in chloroform. The solution was maintained at -10 °C for 6 h, after which time was added 30 mL of anhydrous ether to precipitate the product. The mixture was centrifuged at -5 °C, and the solvents were removed by transfer tube under a nitrogen atmosphere. The precipitate was washed with 5 x 20 mL of anhydrous ether, with the solvent being removed under nitrogen atmosphere following centrifugation at -5 °C. The crude product was found to be stable for over a month when stored at -20 °C. Final cleanup of the 2'-ketoadenosine, **3c**, was carried out by partitioning the residue between 20 mL of ethyl acetate (previously washed with saturated sodium bicarbonate) and 20 mL of water. The aqueous layer was again extracted with 20 mL of ethyl acetate and lyophilized to yield 21.6 mg (43%) of **3c** (98% pure by HPLC). TLC (solvents B and C) gave as the only UV-active zone one equivalent to that for adenine; HPLC: T_R = 11.36 min for **3c** (T_R = 8.36 min for adenine; T_R = 15.46 min for adenosine); UV (pH 6.86): 258 nm (A_1^1 = 390); ^{1}H NMR for the 2'-keto form: (200 MHz, Me_2SO-d_6) δ ca. 3.67 [2H, m, (width = 33 Hz), (partially obscured by H-4'), H-5', H-5'a], ca. 3.77 [1H, m, (width = 22 Hz), (partially obscured by H-5'), H-4'], 4.07 (1H, d, $J_{3',4'}$ = 7.2, H-3'), 5.95 (1H, s, H-1'), 8.34, 8.39 (1, 1, s, s, H-2 and H-8). ^{1}H NMR for the 2'-gem diol form: (200 MHz, 9:1 Me_2SO-d_6 - D_2O) δ ca. 3.67 [2H, m, (width = 33 Hz), (partially obscured by H-4'), H-5', H-5'a], ca. 3.77 [1H, m, (width = 22 Hz), (partially obscured by H-5'), H-4'], 4.65 (1H, d, $J_{3',4'}$ = 9.0, H-3'), 6.13 (1H, s, H-1'), 8.17, 8.34 (1, 1, s, s, H-2 and H-8).

The 2'-ketoadenosine was further characterized by reduction with sodium borodeuteride.

Reduction of 2'-Ketoadenosine (3c) with Sodium Borodeuteride. To a solution of 4.2 mg of 2'-ketoadenosine (**3c**) in 2 mL of water was added 10 mg (an excess) of sodium borodeuteride. After the evolution of deuterium had ceased, the excess borodeuteride reagent was quenched by adjusting solution to pH 7 with dilute hydrochloric acid. The reduction products were analyzed by HPLC and ^{1}H NMR spectroscopy following lyophilization of the water. HPLC analysis (See methods section.) gave a product ratio of 84% 9-(β-D-arabinofuranosyl)adenine-2'-d (T_R 7.7 min, identical with that of authentic ara-A) to 16% adenosine-2'-d (T_R 15.46 min, identical with that of adenosine). ^{1}H NMR (200 MHz, 9:1 Me_2SO-d_6 - D_2O) integration of the H-1' resonances indicated a ratio of 86% ara-A-2'-d (δ6.26, singlet) to 14% adenosine-2'-d (δ5.88, singlet).

3'-Ketoadenosine (5c). By the same procedure as for 3c, 0.218 g (0.201 mmol) of 5a was deprotected in methanolic hydrogen chloride, washed between ethyl acetate and water and precipitated to give 14.2 mg (26%) of purified 5c (96% pure by HPLC). TLC (solvents B and C) revealed as the only UV-active zone one equivalent to that for adenine; HPLC: T_R = 12.10 min; UV (pH 6.86): 259 nm (A_1^1 = 332); IR (KBr): 1780 cm^{-1} (νC=O, strong); ^{1}H NMR for the keto form: (200 MHz, 9:1, Me_2SO-d_6 - D_2O) δ 3.70 [2H, m, (width = 13 Hz), H-5', H-5'a], 4.38 [1H, m, (width = 11 Hz), H-4'], 4.90 (1H, d, $J_{1',2'}$ = 8.5, H-2'), 6.16 (1H, d, H-1'), 8.20, 8.64 (1, 1, s, s, H-2 and H-8).

The 3'-ketoadenosine was further characterized by reduction with sodium borodeuteride.

Reduction of 3'-Ketoadenosine (5c) with Sodium Borodeuteride. To a solution of 2.9 mg of 3'-ketoadenosine (5c) in 2 mL of water was added 10 mg (an excess) of sodium borodeuteride. Workup and analysis procedures as for 3c gave a product ratio of 78% 9-(β-D-xylofuranosyl)adenine-3'-d (T_R 8.35 min, identical with authentic xyl-A) to 22% adenosine-3'-d (T_R 15.46 min, identical with adenosine). ^{1}H NMR (200 MHz, Me_2SO-d_6) integrations of the H-2' resonances indicated a ratio of 75% xyl-A-2'-d (δ4.32, d, $J_{1',2'}$ = 2.7) to 25% adenosine-2'-d (δ4.61, d, $J_{1',2'}$ = 6.2).

Aqueous Stability Determinations for 2'- and 3'-Ketoadenosines 3c and 5c. Solutions of 2'-ketoadenosine

(3c) (2 mL, 0.8 mmolar) and 3'-ketoadenosine (5c) (2 mL, 0.7 mmolar), respectively, in 0.005 M phosphate buffer (pH 6.86) were maintained at 25 ± 0.1 °C. Aliquots were taken and analyzed immediately by HPLC. The samples at zero time were 97% and 95% pure 2'- and 3'-ketoadenosine, respectively, as determined by HPLC analysis. The only detectable product as determined by HPLC at 254 nm during the experiments was adenine. The solution half-lives for 3c and 5c were determined by extrapolation of the least-squares plot (correlation coefficient for each line was 0.997) drawn from the HPLC data points collected over a period of 24 h and 48 h, respectively. Found: Time for 50% decomposition of 2'-ketoadenosine at pH 6.86, 11 h; for 3'-ketoadenosine at pH 6.86, 84 h.

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20. ^{1}H NMR assignments were based on the observed increase in intensity of one set of signals upon the addition of D$_2$O to the sample. Such hydration could easily be observed for samples of **3a** and **3c**.

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