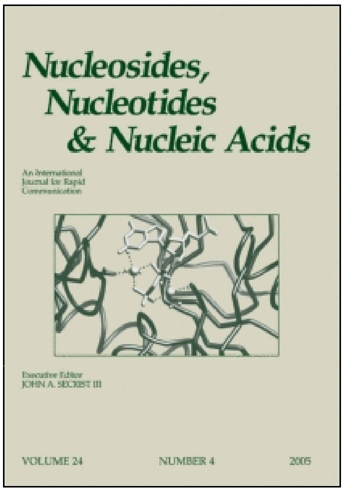

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Synthesis of 2-Azido-1,N^6-Etheno and 2-Azido Analogs of Deoxyadenosine as Nucleotide Photoaffinity Probes

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SYNTHESIS OF 2-AZIDO-1,N^6-ETHENO AND 2-AZIDO ANALOGS OF DEOXYADENOSINE AS NUCLEOTIDE PHOTOAFFINITY PROBES

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We prepared 2-azido-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (**7b**) by activation of the 2-thio analog **4b** by two methods. In the preferred method, **4b** was treated with 2,4-dinitrofluorobenzene in 50% aqueous acetonitrile, and the resultant thioether was cleaved with hydrazoic acid, yielding the 2-azido-1,N^6-etheno analog **7b** in 70% isolated yield. N-bromoacetamide oxidized the etheno group of **7b**, giving the 2-azido analog of dAMP **9b** in 78% yield.

The 2-azido and 8-azido analogs of adenosine and their phosphates and the corresponding fluorescent probes based on 1,N^6-ethenoadenosine are nucleotide photoaffinity labels used extensively to characterize ribonucleotide binding sites in purified enzymes and *in vitro* systems. Schaefer and Thomas[1] first synthesized 2-azidoadenosine by treatment of 2-chloroadenosine with hydrazine, followed by nitration of the resulting 2-hydrazino with sodium nitrite. The 2-thio analog of 1,N^6-ethenoadenosine and its phosphates are important precursors to the 2-azido-1,N^6-etheno analogs. For example, bromination of 2-thio-1,N^6-etheno-cAMP in HBr yields the 2-bromo, and treatment of the 2-bromo with azide yields the 2-azido.[2] In contrast, Yip and Tsou[3] synthesized 2-azido-1,N^6-ethenoadenosine by hydrogen peroxide oxidation of the corresponding 2-thio followed by displacement of the resultant 2-sulfo with hydrazine and nitration of the 2-hydrazino with sodium nitrite.

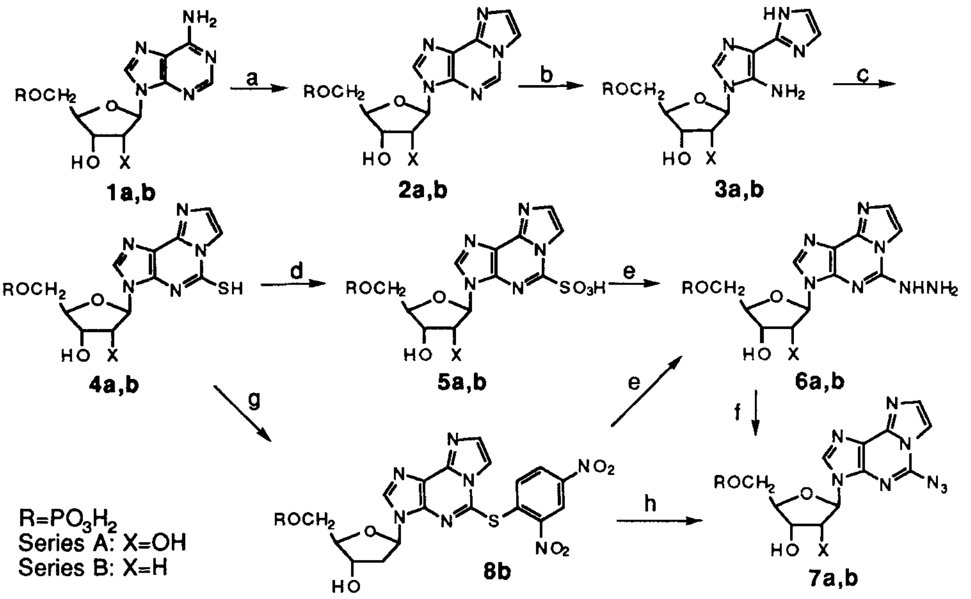
In the 2'-deoxyribose series, the 5'-triphosphate analog of 8-azido-2'-deoxyadenosine, synthesized by bromination of the corresponding deoxyadenosine with Br_2 followed by displacement of the 8-bromo with azide,[4] is a photoaffinity label of the deoxynucleotide binding sites of the *E. coli* DNA polymerase I Klenow fragment[5] and terminal deoxynucleotidyl transferase,[6,7] and when incorporated into DNA is a protein-DNA crosslinking reagent.[8,9] However, the corresponding 2-azido analogs of deoxyadenosine and the corresponding 1,N^6-etheno analogs have apparently not been reported, although the 2-azido analogs are often preferred as photoaffinity labels since the 8-azido substituent shifts the equilibrium between the syn and anti conformers of adenosine 5'-phosphates.[10] In the present study we report convenient synthetic routes to 2-azido-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (2-N_3-ε-dAMP)[11] and 2-azido-2'-deoxyadenosine 5'-monophosphate (2-N_3-dAMP) and the corresponding ribonucleotides, and reverse phase HPLC and ion-exchange chromatography methods for their purification.

RESULTS AND DISCUSSION

Synthesis of 2-N_3-1,N^6-ε-dAMP

We based the initial synthetic route (Scheme 1) to the target compound on the reported synthesis of 2-substituted 1,N^6-ethenoadenosine analogs by Yip and Tsou[3] and Yamaji *et al.*[2] The first step in the synthetic scheme was addition of chloroacetaldehyde to adenosine monophosphate as described by Secrist and coworkers.[12] We obtained quantitative yields for 1,N^6-ethenoadenosine 5'-monophosphate (**2a**) and slightly lower yields (87%) for the 2'-deoxyribose analog **2b**. Treatment of **2a** and **2b** with three equivalents of sodium hydroxide led to hydrolytic ring opening and loss of the 2-carbon to yield the 4-amino-5-(imidazol-2-yl)-imidazole derivatives **3a** and **3b**, respectively. Subsequent treatment of the tetrabutylammonium salts of crude **3a** and **3b** with carbon disulfide and potassium carbonate in dimethylformamide gave the 2-thio-1,N^6-etheno analogs **4a** and **4b**, respectively. Compounds **4a** and **4b** were purified from potassium salts by column chromatography; the products elute from a DEAE column with a low pH ammonium acetate buffer, but they do not elute with ammonium bicarbonate (pH 8.5) up to 1.0 M. Because the 2-thio analogs slowly decomposed with exposure to oxygen and light, we protected the reaction mixture and purification solvents from both and stored the products at -20°C.

The next step in the synthesis required oxidation of **4a** and **4b** to **5a** and **5b**, respectively. Literature procedures[13,14] for the oxidation of 2-thioadenosine called for the addition of H_2O_2 at pH 9. However, these conditions yielded a mixture of **5** and 2-hydroxy-1,N^6-ethenoadenosine 5'-monophosphate for the etheno series. When treated with base, **5a** rapidly formed the 2-hydroxy, suggesting that the 2-hydroxy formed under basic conditions after the oxidation. However, when the pH of the reaction solution was buffered between 5.5-6.5, the amount of this hydrolysis product diminished. Subsequent addition of **5a** and **5b**



a) ClCH$_2$CHO, 37°C; b) NaOH; c) CS$_2$, K$_2$CO$_3$; d) H$_2$O$_2$, pH 5.5-6.5; e) NH$_2$NH$_2$; f) CH$_3$CO$_2$H, NaNO$_2$; g) DNFB, pH 8.7; h) NaN$_3$, 50% acetic acid

Scheme 1

to hydrazine gave the 2-hydrazino analogs **6a** and **6b**, respectively. The 2-hydrazino products were easily identified by TLC since they decomposed on the silica plate to give a blue streak at or near the origin. The 2-hydrazino analogs were not stable under basic conditions as they decomposed rapidly to unknown products, but were more stable under acidic or neutral conditions. Although the initial ethanol precipitate contained a mixture of product, starting material, the 2-amino and the 2-hydroxy analogs, the product could be purified by elution through a short DEAE column using an ammonium acetate gradient at pH 4.5.

The 2-hydrazino analogs **6a** and **6b** yielded the corresponding 2-azido analogs **7a** and **7b**, respectively, after treatment with sodium nitrite in acetic acid at 0°C according to the method of Yip and Tsou[3] in a foil-covered or amber-colored reaction vessel. The reactions were followed by TLC based on the disappearance of the blue spot characteristic of **6a** and **6b** and the appearance of products with Rf of 0.36 and 0.35 (Solvent A) for **7a** and **7b**, respectively, which darkened instantly on the TLC plate upon exposure to UV light. Besides product, the 2-amino analog was observed as a by-product in this reaction for both 2-N$_3$-ε-

AMP and 2-N_3-ε-dAMP. Product yields were significantly lower for the 2'-deoxy analog, apparently because of the instability of the 2-hydrazino-1,N^6-etheno deoxyribose nucleotide (the 2-azido analogs were stable to these acidic conditions).

Because of the instability of the 2-sulfo analogs to hydrolysis and the low yields for the conversion of the 2-sulfo **5b** to the 2-azido **7b**, we activated **4b** by a second method. Treatment of the 2-thio analog **4b** with one equivalent of 2,4-dinitrofluorobenzene (DNFB) in 50% aqueous acetonitrile at pH 8.7 yields the 2-[S-(2,4-dinitrophenyl)thio]-1,N^6-etheno analog **8b** (Scheme 1). Formation of the dinitrophenylthioether activates the nucleotide C-2 position for nucleophilic substitution, and the 2,4-dinitrothiobenzene moiety serves as a good leaving group. When treated with a 10 fold excess of KOH, an analytical sample of **8b** hydrolyzed to the 2-hydroxy analog in quantitative yield based on comparison of the ^{1}H-NMR and uv spectra of the product with a standard. The thioether **8b** was treated with excess hydrazine at 0°C for 4 hours at room temperature, and the 2-hydrazino was subsequently converted to the 2-azido by treatment of the crude reaction mixture with $NaNO_2$ in 10% acetic acid. The 2-amino analog was the major product observed; yields of the 2-azido **7b** were variable, with the highest yield (about 25%) observed with fresh hydrazine.

Because the 2-hydrazino-1,N^6-etheno deoxynucleotide analog was unstable and decomposed to the 2-amino analog, we also attempted to obtain the 2-azido by direct treatment of **8b** with sodium azide. However, because the side-products of this reaction are potentially explosive, we investigated the reactivity of **8b** on a 2 mg scale using HPLC (solvent system A given in experimental section) to analyze for product distributions. Sodium azide cleaved **8b** to form the 2-thio analog **4b** and presumably 2,4-dinitroazidobenzene (the products were treated with 2-mercaptoethanol to reduce the azides before disposal) in DMSO, DMF, and neutral, aqueous solution; the 2-azido was not observed. This chemistry was the first evidence that the thioether **8b** cleaves at either the C-2 position of the nucleotide or the C-1 position of the dinitrophenyl ring. However, under acidic conditions cleavage occurred preferentially at the C-2 position of the nucleotide; azide cleaved **8b** in 50% acetic acid to yield the 2-azido and 2-thio analogs in a ratio of 85:15 (the nucleophilicity of hydrazoic acid prepared by the addition of azide to acetic acid was reported by Fieser and Hartwell[15]). We attribute this change in **8b**'s reactivity to nucleotide base protonation in acidic solution, an electronic effect that activates the C-2 position for nucleophilic substitution. Neither the 2-amino analog nor nucleotide decomposition was observed in the reaction of **8b** with azide in acetic acid even with extended reaction times. The 2-N_3-ε-dAMP obtained from 125 mg of crude **8b** was easily purified to homogeneity in 70% yield by ion-exchange or HPLC chromatography, and thus this procedure appears to be the preferred route to the target photoaffinity probes.

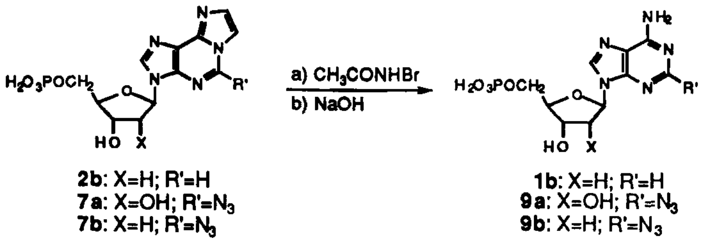
Additional attempts to synthesize 2-N_3-ε-dAMP were unsuccessful. For example, attempted conversion of **4a** and **4b** to the 2-halogeno analogs with Cl_2/HCl or Br_2/HBr were unsuccessful.[16] In the ribose series this reaction lead to oxidation of the etheno ring, and in the deoxyribose series this chemistry also led to nucleotide cleavage. Although **7a** was obtained from the 2-amino ribose analog by diazotization in the presence of $CuCl_2$[17] followed by nucleophilic displacement of the 2-chloro with azide, the yield was only 5%, and the only product isolated after similar diazotization of the 2-amino-2'-deoxyribose analog was 2-chloro-1,N^6-ethenoadenine.

Deprotection of 1,N^6-ethenoadenosine nucleotides

Yamaji *et al.*[16,18] reported the synthesis of 2-substituted cAMP analogs from the 1,N^6-etheno derivatives by removal of the etheno bridge (deprotection) using N-bromosuccinimide (NBS). Because these conditions did not yield the requisite deoxynucleotides, two alternative methods were attempted. In the first, the etheno bridge was oxidized with sodium hypobromite (NaOBr) in HBr for 30 minutes at 0°C, followed by hydrolysis of the oxidized etheno ring with excess sodium hydroxide. Although highest yields (52%) were obtained with 0.5M HBr, these conditions were too acidic for the deoxyribose analogs. Therefore, we used N-bromoacetamide as the source of bromine (Scheme 2). The reactions were performed with three equivalents of N-bromoacetamide in 0.5 M acetate buffer, pH 4.5, followed by the addition of NaOH to facilitate cleavage of the oxidized etheno group. This procedure gave yields of 70-99% for deprotection of **2a**, **2b**, **7a**, and **7b**. Compounds **9a** and **9b** both showed characteristic ir absorption bands for the azido functionality around 2120 Å, but the ^{1}H-NMR spectra indicated the presence of the azido and N^1- and N^3- tetrazole isomers.[19,20]

Experimental Methods

CAUTION: Sodium azide and aryl azides are explosive and hydrazoic acid is explosive and poisonous. Reactions using or generating these reagents should be performed in a well-ventilated hood behind an explosion-proof shield. We have worked with these reagents and procedures on mg scales and to date have



Scheme 2

observed no explosions. Nevertheless, use extreme care when repeating or scaling-up these procedures.

Materials and Methods: Ultraviolet spectra were acquired on a Hewlett-Packard 8450A diode array spectrophotometer, and NMR were obtained on a Varian XL-300 spectrometer. Proton shifts are reported relative to HOD as measured in D_2O. Infrared spectra were obtained on a Perkin-Elmer 1450 ratio recording spectrometer. Mass spectra were obtained on a ZAB-HS system. Adenosine 5'-monophosphate monohydrate and 2'-deoxyadenosine 5'-monophosphate monohydrate were purchased from Aldrich (Milwaukee, WI). All other reagents were of analytical grade and obtained commercially. TLC silica plates were purchased from Sigma (St. Louis, MO). TLC analysis was performed in the following solvent systems: Solvent A: isopropanol: ammonium hydroxide: water (20:12:3); Solvent B: butanol: acetic acid: water (4:1:1). DEAE Sephadex C-25, SP Sephadex A-25, and QAE Sephadex C-25 were purchased from Pharmacia (Piscataway, NJ). HPLC analysis and purification were performed on a Beckman dual pump gradient HPLC system, with System Gold software, on a Vydac C-18 analytical (1 ml/min) or semi-preparative (2 ml/min) reverse phase column using the following solvent systems: System A, 30 min gradient from 0-50% acetonitrile in 0.1% trifluoroacetic acid; System B, 30 min gradient from 0-50% acetonitrile in 10mM ammonium acetate. In general, the nucleotide salts **7** and **9** were very hygroscopic and decomposed with in vacuo drying at elevated temperatures. Therefore, the purity of these final products was determined by analytical HPLC with detection at 275 nm and from ^{1}H-NMR spectra.

1,N^6-Ethenoadenosine 5'-monophosphate (2a) was prepared by the addition of adenosine 5'-monophosphate monohydrate **1a** (5.00 g, 13.7 mmol) and sodium acetate (68.5 mmol, 5 equivalents) to 50 ml of chloroacetaldehyde (50% wt in H_2O); the solution was heated at 37°C for 12 hours. The solution was extracted with three 50 ml volumes of ethyl acetate, and the product was precipitated by slow addition of the aqueous layer into ethanol. The precipitate was collected by centrifugation and converted to the free acid by the addition of one equivalent (16.83 ml) of 1.0M HCl. The solution was dropped into ethanol (1:10 dilution), and the resulting precipitate was collected by vacuum filtration and washed with ethanol and ethyl ether. Drying under vacuum yielded 5.08 g of **2a** in 99% yield as an off-white, amorphous powder: mp 194-198°C (dec.) [lit.[12]

mp190-192 (dec.)]; ^1H NMR (300 MHz, D_2O) δ 4.15 (m, 2H, C5'H/C4'), 4.44 (m, 1H, C3'H), 4.57 (m, 1H, C2'H), 6.19 (d, J = 5.5 Hz, 1H, C1'H), 7.48 (d, 1H, C10H), 7.86 (d, 1H, C11H), 8.55 (s, 1H, C8H), 8.94 (s, 1H, C2H); MS (-FAB, glycerol) m/e 370 ($M-H^+$).

1,N^6-Etheno-2'-deoxyadenosine 5'-monophosphate (2b) was prepared from 2'-deoxyadenosine 5'-monophosphoric acid monohydrate **1b** (1.00 g, 2.86 mmol) as described above for the preparation of **2a** yielding 0.89 g (87%) of **2b** (>99% pure by ^{1}H-NMR): mp 188-190°C (dec.); UV λ_{max} ($\varepsilon x10^{-4}$): 0.1N HCl, 221(1.78), 274(0.67); 0.1N NaOH, 228(2.50), 258sh(0.48), 266(0.56), 275(0.56), 293(0.29); IR (KBr) 3365, 1640, 1482, 1380, 1330, 1295, 1220, 1180, 1090, 1055, 930, 695; ^1H NMR (300 MHz, D_2O) 2.65 (m, 1H, C2'H1), 2.89 (m, 1H, C2'H2), 4.11 (m, 2H, C5'H), 4.34 (m, 1H, C4'H), 4.79 (C3'H partially obscured by HOD), 6.58 (t, J = 6.8 Hz, 1H, C1'H), 7.53 (d, J = 1.3 Hz, 1H, C10H), 7.90 (d, J = 1.3 Hz, 1H, C11H), 8.47 (s, 1H, C8H), 9.01 (s, 1H, C2H); high-resolution MS (-FAB, glycerol) m/e 354.0592 (354.0603 calculated for $C_{12}H_{13}N_5O_6P$)

3-(β-D-5'-phosphoribofuranosyl)-4-amino-5-(imidazol-2-yl)-imidazole (3a) was prepared by addition of **2a** (1.15 g, 3.09 mmol) to 10.8 ml (3.2 equivalents) of 1.0 N NaOH. After stirring for 5 hours, 1 N HCl (3.5 equivalents) was added to the solution, and the product was precipitated in 200 ml of ethanol. The precipitate was isolated by centrifugation, washed with 20 ml of ethanol and again isolated by centrifugation. The solid was dried in a vacuum desiccator to yield 0.87 g (78%) of crude **3a** (about 88% of total nucleotide is product by ^{1}H-NMR) as an amorphous powder: UV λ_{max}: 0.1N NaOH,268; NMR(300 Hz, D_2O) δ 4.09 (s, 2H, C5'H), 4.35 (m, 1H, C4'H), 4.46 (m, 1H, C3'H), 4.67 (m, 1H, C2'H), 5.75 (d, J = 7Hz, 1H, C1'H), 7.36 (s, 2H, imidazolyl C4,5H), 7.72 (s, 1H, C2H); MS (-FAB, glycerol) m/e 394 ($M + (NH_4)_2 - H$).

3-(β-D-5'-phospho-2'-deoxyribofuranosyl)-4-amino-5-(imidazol-2-yl) imidazole (3b) was prepared from **2b** (400 mg, 1.06 mmole) as described above for the synthesis of **3a** to give 0.31 g (86%) of crude **3b** (>99% of total nucleotide is product by ^{1}H-NMR): UV λ_{max} (0.1N NaOH) 267; ^1H NMR (300 MHz, D_2O) δ 2.37 (m, 1H, C2'H1), 2.64 (m, 1H, C2'H2), 3.89 (m, 2H, C5'H), 4.13 (m, 1H, C4'H), 4.61 (m, 1H, C3'H), 6.03 (t, J = 6.6 Hz, 1H, C1'H), 7.14 (s, 2H, imidazolyl C4,5H), 7.60 (s, 1H, C2H).

Ammonium 2-thio-1,N^6-ethenoadenosine 5'-monophosphate (4a) was prepared from the tetrabutylammonium salt of **3a**. Compound **3a** (750 mg, 2.08 mmol) was dissolved in a minimum volume of water containing 1.36 ml (1 equivalent) of 1.54 M tetrabutylammonium hydroxide, and the solution was stirred and lyophilized to dryness. The resulting solid was dissolved in 20 ml of DMF and treated with K_2CO_3 (1.25 g, 13 mmol) and CS_2 (5.3 ml, 88 mmol). After stirring for 50 hours, the solution was evaporated in vacuo and the suspension was filtered. The filtrate was dissolved in water (50 ml) and extracted with four volumes of methylene chloride. The aqueous portion was then evaporated on a rotary evaporator, and the residue was washed with ether. The resulting precipitate was dissolved in water and eluted on a silica gel column with isopropanol/NH_4OH/H_2O(20:12:4). The appropriate fractions were pooled and roto-evaporated to dryness. The resulting solid was dissolved in water and loaded onto a 30 ml DEAE Sephadex column. The column was first eluted with a 7-2.5 pH gradient of 50 mM ammonium acetate, and the elution was continued with a 50 mM - 250 mM concentration gradient of ammonium acetate, pH 2.5. The major peak was collected and lyophilized to give 544 mg of **4a** (>99% pure by ^{1}H-NMR) in 65% yield as an amorphous powder: mp 220-225°C (dec.);^{1}H NMR (300 MHz, D_2O) δ 4.16 (m, 2H, C5'H1,2), 4.41 (m, 1H, C4'H), 4.56 (m, 1H, C3'H), 4.77 (m, 1H, C2'H), 6.18 (d, J = 5.0 Hz, 1H, C1'H), 7.61 (d, 1H, C10H), 8.22 (d, 1H, C11H), 8.42 (s, 1H, C8H); MS (-FAB, glycerol) m/e 402 ($M-H^+$).

Ammonium 2-thio-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (4b) was prepared from the tetrabutylammonium salt of **3b** (2.74 g, 7.9 mmol) by the method described above for the synthesis of **4a** with the following exceptions. The residue obtained from roto-evaporation of the reaction mixture was dissolved in water (50 ml) and extracted with three volumes of methylene chloride. CM-Sephadex A-25 ammonium form (20 ml) was added to the aqueous solution with gentle stirring, and the mixture was acidified to pH 5 with acetic acid. After 15 minutes the resin was removed by filtration through a sintered glass funnel, and the filtrate was lyophilized. The residue was dissolved in 10 mM acetic acid (60 ml) and filtered though celite. The filtrate was then chromatographed by FPLC on a fast-flow Q-Sephorose column (2.5 x 15 cm^2) eluting with a 0.01 to 2 M ammonium acetate gradient, pH 4, to give 2.52 g (82%) of **4b** (>99% pure by ^{1}H-NMR): mp 210-212°C (dec.); UV λ_{max} (ϵx10^{-4}): 0.1N HCl, 240 (1.46) , 312 (1.78); 0.1N NaOH, 249 (1.27), 295sh (1.05), 320 (1.37) ; IR (KBr)3140, 1670, 1550, 1505, 1424, 1368, 1210, 1075, 1050, 935, 733; ^{1}H NMR (300 MHz, D_2O) δ 2.55 (m, 1H, C2'H1), 2.75 (m, 1H, C2'H2), 3.95 (m, 2H, C5'H), 4.32 (m, 1H, C4'H), 4.65 (m, 1H, C3'H), 6.42 (t, J = 6.8 Hz, 1H, C1'H), 7.53 (d, J = 1.6 Hz, 1H, C10H), 8.16 (d, J = 1.6 Hz, 1H, C11H), 8.30 (s, 1H, C8H); MS (-FAB, glycerol) m/e 386 ($M-H^+$).

2-Sulfo-1,N^6-ethenoadenosine 5'-monophosphate (5a) was prepared by oxidation of an aqueous solution (2 ml) of **4a** (350 mg, 0.90 mmol) at 0°C at pH 9 by the addition of hydrogen peroxide (1.0 ml of 30% in water). After stirring for one hour, the solution was dropped into ethanol, and the resulting precipitate was collected by filtration and dried. The crude solid was dissolved in H_2O, loaded onto a 30 ml DEAE column (formate form) and eluted with a gradient of 150 mM ammonium formate, pH 3.5 - 1.5 (400 ml total volume). The appropriate fractions were pooled and lyophilized to give 200 mg of crude **5a** (>98% pure by ^{1}H-NMR) in 51% yield: ^{1}H NMR (300 MHz, D_2O) δ 4.22 (m, 2H, C5'H1,2), 4.47 (m, 1H, C4'H), 4.59 (m, 1H, C3'H), C2'H obscured by HOD, 6.42 (d, J = 4.4 Hz, 1H, C1'H), 7.75 (d, 1H, C10H), 8.40 (d, 1H, C11H), 8.76 (s, 1H, C8H); MS (-FAB, glycerol) m/e 450 ($M-H^+$).

2-Sulfo-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (5b) was prepared by oxidation of an aqueous solution of **4b** (500 mg, 1.22 mmol) at 0°C by the addition of hydrogen peroxide. The pH of the reaction mixture was monitored and maintained at pH 5.5-6.5 by the addition of 0.1 N NaOH. After two hours, the solution was dropped into ethanol. The resulting precipitate was isolated by filtration and dried under vacuum to yield 397 mg (75%) of crude **5b**

(about 88% pure by ^{1}H-NMR): UV λ_{max} : 0.1N HCl, 272sh, 280 ;^{1}H NMR (300 MHz, D_2O) δ 2.73 (m, 1H, C2'H1), 2.93 (m, 1H, C2'H2), 4.03 (m, 2H, C5'H), 4.32 (m,1H, C4'H), C3'H obscured by HOD, 6.77 (t, J = 6.4 Hz, 1H, C1'H), 7.73 (d, J = 1.9 Hz, 1H, C10H), 8.37 (d, J = 1.9 Hz, 1H, C11H), 8.75 (s, 1H, C8H); MS (-FAB, glycerol) m/e 433 (M-H$^+$).

2-(S-[2,4-dinitrophenyl]thio)-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (8b) was prepared by the addition of 2,4-dinitrofluorobenzene (44 mg, 0.23 mmol) in 1.5 ml acetonitrile to an aqueous solution (1.5 ml) of the sodium salt of **4b** (100 mg, 0.23 mmol; prepared by elution of **4b** through a column of the sodium form of SP Sephadex with water). The solution was stirred at room temperature for 2 hours during which time the pH of the solution was maintained at 8.7 with 1N KOH. The reaction was then diluted with 5 ml of water, the solution was acidified to pH 4.5 with 0.1N HCl, and the solution was extracted with 3 volumes of ethyl ether. The crude product (128 mg; >95% pure by ^{1}H-NMR and HPLC eluting with solvent System A) was obtained by lyophilization of the aqueous layer. If necessary, the product can be purified by reverse phase HPLC eluting with System A (retention time=25 min): mp 116-118°C; UV λ_{max} (εx10^{-4}): 0.1N HCl, 278 (4.09), 339 (2.25); IR(KBr) 1344, 1530 (NO_2); ^{1}H-NMR(D_2O) 2.56 (m, 1H, C2'H1), 2.83 (m, 1H, C2'H2), 3.92 (m, 2H, C5'H), 4.18 (m, 1H, C4'H), 4.56 (m, 1H, C3'H), 6.51 (t, J = 6.6 Hz, 1H, C1'H), 7.6 (d, J = 9.0 Hz, 1H, PhH), 7.95 (br s, 1H, C10H), 8.24 (br s, 1H, C11H), 8.40 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H, PhH), 8.74 (s, 1H, C8H), 9.18 (d, J = 2.4 Hz, 1H, PhH); MS (-FAB, glycerol) m/e 552 (M-H+).

Ammonium 2-hydrazino-1,N^6-ethenoadenosine 5'-monophosphate (6a) was prepared by the slow addition of **5a** (200 mg, 0.44 mmol), as a solid, to 2 ml of 98% hydrazine. After stirring the solution for an additional 15-20 minutes, the solution was concentrated in vacuo by rotary evaporation before and after the addition of ethanol (1 volume), and the solution was roto-evaporated several times with ethanol and water. The resulting residue was dissolved in water and precipitated from ethanol. The precipitate was filtered and dried under vacuum to give 132 mg of crude **6a** (about 75% pure by ^{1}H-NMR) in 75% yield: ^{1}H NMR (300 MHz, D_2O) δ 4.20 (m, 2H, C5'H), 4.41 (m, 1H, C4'H), 4.58 (m, 1H ,C3'H), C2'H obscured by HOD, 6.08 (d, J = 5.0 Hz, 1H, C1'H), 7.37 (d, 1H, C10H), 7.46 (d, 1H, C11H), 8.17 (s, 1H, C8H); MS (-FAB, glycerol) m/e 400 (M-H+), 385 (M-NH).

Ammonium 2-hydrazino-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (6b) was prepared by the slow addition of **5b** (300 mg, 0.63 mmole) to 2.5 ml of 98% hydrazine. The solution was stirred for 15 minutes and concentrated on a rotary evaporator. The remaining residue was dissolved in 100 ml of 50 mM acetate buffer, and the pH was adjusted to 4.5. The solution was loaded onto a 1.5x30cm DEAE Sephadex column, and product was eluted with a gradient of 50 -150 mM ammonium acetate, pH 4.5 (400 ml total volume). The fractions corresponding to the major peak were pooled and lyophilized, yielding 200 mg (82%) of crude **6b** (about 85% pure by ^{1}H-NMR) as a dark colored solid: UV λ_{max} : 0.1N HCl, 272sh, 285; 0.1N NaOH, 292; ^{1}H NMR (300 MHz, D_2O) δ 2.55 (m, 1H, C2'H1), 2.90 (m, 1H, C2'H2), 4.01 (m, 2H, C5'H), 4.21 (m,1H, C4'H), 4.71 (C3'H partially obscured by HOD), 6.12 (t, J = 6.3 Hz, 1H, C1'H), 7.32 (d, 1H, C10H), 7.47 (d, 1H, C11H), 8.11 (s, 1H, C8H); MS (-FAB, glycerol) m/e 384 (M-H+).

Ammonium 2-azido-1,N^6-ethenoadenosine 5'-monophosphate (7a) was prepared by the dropwise addition of sodium nitrite (19.5 mg, 0.28 mmole) in 0.4 ml of water to 2-hydrazino-1,N^6-etheno-AMP **6a** (100 mg, 0.25 mmol) in ice cold 85% acetic acid in a fume hood behind an explosion-proof shield. The solution was stirred at 0°C for 30 minutes. The solution was diluted with water (5 ml), roto-evaporated in vacuo to dryness, and the resulting residue was dissolved in 30 ml of water and loaded onto a 1.5 cm x 20 cm DEAE Sephadex column. The column was washed with 100 mM ammonium acetate, pH 7, and the product was eluted with a gradient of 150 mM ammonium acetate, pH 7 to pH 3.5 (300 ml total volume). The fractions containing product were pooled and lyophilized to yield 47.1 mg (46%) of **7a** (>95% pure by ^{1}H-NMR): mp 160-163°C (dec.); IR (KBr), 2150 (N_3); ^{1}H NMR (300 MHz, D_2O) δ 4.14 (m, 2H, C5'H), 4.40 (m, 1H, C4'H), 4.62 (m, 1H, C3'H), C2'H obscured by HOD, 6.22 (d, J = 4.4 Hz, 1H, C1'H), 7.47 (d, 1H, C10H), 7.64 (d, 1H, C11H), 8.47 (s, 1H, C8H); MS (-FAB, glycerol) m/e 411 (M-H+), 385 (M-N_2+H).

Ammonium 2-azido-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate (7b): Method A. Compound **7b** was prepared from **6b** (200 mg, 0.5 mmol) by the procedure described above for the synthesis of **7a** from **6a**, yielding 57.6 mg (29%). The product was purified to >99% homogeneity by HPLC reverse phase chromatography on a C-18 column eluting with System B (retention time=17 min): mp 160-161°C (dec.); UVλ_{max} (εx10^{-4}): 0.1N HCl, 233 (1.92), 287 (1.51) ; 0.1N NaOH, 238 (2.21), 275 (0.78), 284 (0.86), 315 (0.65); IR (KBr) 2145 (N_3); ^{1}H NMR (300 MHz, D_2O) δ 2.54 (m, 1H, C2'H1), 2.90 (m, 1H, C2'H2), 4.00 (m, 2H, C5'H), 4.18 (m,1H, C4'H), C3'H obscured by HOD, 6.50 (t, J = 6.8 Hz, 1H, C1'H), 7.40 (d, J = 1.6, 1H, C10H), 7.60 (d, J = 1.61Hz, 1H, C11H), 8.29 (s, 1H, C8H); high-resolution MS (-FAB, glycerol) m/e 395.0597 (395.0617 calculated for $C_{12}H_{12}N_8O_6P$).

Method B: Crude thioether **8b** was suspended in 3.9 ml of glacial acetic acid and added to 0.139 g (10 equivalents) of sodium azide dissolved in 3.9 ml of water in a fume hood behind an explosion-proof shield, and the solution was stirred at room temperature for 30 hours. The mixture was diluted with water to 1.0M in acetic acid, and the solution was adjusted to pH 4.5 with 6N NH_4OH and extracted three times with ethyl ether (the ether layer was treated with 2-mercaptoethanol before disposal). The aqueous layer was separated and lyophilized, and the crude product was purified on a DEAE column (1.5x30 cm) by gradient elution with 0.01M to 1.0M NH_4OAc (150 ml each side). Lyophilization of the major peak yielded 70 mg (70% based on 2-SH-ε-dAMP starting material) of **7b**. The product was >99% pure as evidenced by ^{1}H-NMR and its elution as a single, symmetrical peak from a C-18 reverse phase column eluting with solvent System B.

Ammonium 2-azido-adenosine 5'-monophosphate (9a) was prepared by stirring a solution of N-bromoacetamide (4 mg, 0.03 mmol) and **7a** (5.0 mg, 0.01

mmol) in 1 ml of 0.5 M acetate buffer, pH 4.5 overnight. The pH of the solution was adjusted to 13 with 1.0 N NaOH and stirred for an additional 4 hours. The solution was neutralized to pH 7 with acetic acid, diluted to 20 ml, and loaded onto a 0.75 cm x 6 cm DEAE Sephadex column, and product was eluted with a gradient of 100 mM ammonium acetate, pH 7 to pH 4.5. The UV active fractions were pooled and lyophilized, yielding 3.0 mg (77%) of **9a** (>95% pure by ^{1}H-NMR) as a mixture of the azido and N1 and N3 tetrazoles: mp 125-130°C (dec.); UV λ_{max}: 0.1 N HCl, 277, 308sh; 0.1N NaOH, 274, 315; IR(KBr) 2120 (N_3); ^{1}H NMR (D_2O) δ 4.03 (m, C5'H), 4.40 (m, C3'/C4'H), 4.52 (m,1H,C2'H), 6.19, 6.04, 5.91 (d, 1H, J=5.5, C'1H), 8.69, 8.43 (1H, singlet, C8H); MS (-FAB, glycerol) m/e 387 (M-H$^+$).

Ammonium 2-azido-2'-deoxyadenosine 5'-monophosphate (9b) was prepared from **7b** (50 mg, 0.12 mmol) by the procedure described above for the synthesis of **9a** to give 36.4 mg (78%) of **9b** (>95% pure by ^{1}H-NMR) as a mixture of the azido and N1 and N3 tetrazoles: mp 131-135°C (dec.); UV: λ max (εx10^{-4}): 0.1 N HCl, 279; 1N NaOH, 275, 316; IR (KBr) 2125 (N_3); ^{1}H NMR (D_2O) δ 2.51-2.77 (m, C2'H), 3.97 (m, 2H, C5'H), 4.20 (m, 1H, C4'H), C3'H obscured by HOD, 6.45, 6.28, 6.18 (1H, triplet, C'1H), 8.51, 8.22, 8.07 (1H, singlet,C8H); high-resolution MS (-FAB, glycerol) m/e 371.0611 (371.0617 calculated for $C_{10}H_{12}N_8O_6P$).

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(11) Abbreviations used: 2-N_3-ε-dAMP, 2-azido-1,N^6-etheno-2'-deoxyadenosine 5'-monophosphate; 2-N_3-dAMP, 2-azido-2'-deoxyadenosine 5'-mono-

phosphate; 2-SH-ε-dAMP, 2-thio-1,N^6-etheno-2'deoxyadenosine-5'-monophosphate.

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