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The Synthesis of 2-Substituted 1,N^6-Etheno-adenosine-3′,5′-cyclic Phosphate by Ring Reclosure of Alkali-hydrolizate of 1,N^6-Etheno-adenosine-3′,5′-cyclic Phosphate

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An useful procedure for the synthesis of 2-substituted 1,N^6-etheno-adenosine-3′,5′-cyclic phosphates by ring closure of 3-β-D-(3′,5′-cyclic phospho)-ribofuranosyl-4-amino-5-(imidazol-2-yl)-imidazole (II), under relative mild conditions, is reported. Treatment of the compound II with bromine cyanide, triethyl orthoacetate, 1,1′-carbonyldiimidazole and carbon disulfide gave 2-amino-, 2-methyl-, 2-hydroxy-, and 2-mercapto-1,N^6-etheno-adenosine-3′,5′-cyclic phosphate (c-AMP), respectively. Moreover, several 2-alkylmercapto-, 2-benzylmercapto-, and 2-bromo-1,N^6-etheno-c-AMP were synthesized from 2-mercapto-1,N^6-etheno-c-AMP. 2-Methoxy-, 2-azido-, 2-dimethylamino-1,N^6-etheno-c-AMP were synthesized from 2-bromo-1,N^6-etheno-c-AMP. The fluorescent and ultraviolet spectra of 2-substituted 1,N^6-etheno-c-AMP are tabulated.

During the past few years, adenosine-3′,5′-cyclic phosphate (c-AMP) has become recognized as the second messenger of hormones.[2] Numerous derivatives of c-AMP have recently been synthesized in order to obtain substances having specific biological activity and to elucidate the molecular interaction with the receptor.[3] Many 1,N^6-etheno-adenine derivatives were synthesized and their antitumor activity was examined.[4] A number of 8-substituted 1,N^6-etheno-c-AMPs were synthesized and the activity with protein kinase was examined.[5] In the 2-modified 1,N^6-etheno-c-AMP series, only 2-aza-1,N^6-etheno-c-AMP has been reported.[6]

We have previously reported that 2-aza-c-AMP was synthesized by the deblocking reaction of the etheno group of 2-aza-1,N^6-etheno-c-AMP.[7] As one of the series of our studies along this line, the present paper reports the synthesis of new 2-substituted 1,N^6-etheno-c-AMP from 3-β-D-(3′,5′-cyclic phospho)-ribofuranosyl-4-amino-5-(imidazol-2-yl)-imidazole (II). Moreover, several 2-substituted 1,N^6-etheno-c-AMPs were synthesized by the route: 2-SH-1,N^6-etheno-c-AMP⟶2-Br-1,N^6-etheno-c-AMP⟶2-substituted 1,N^6-etheno-c-AMP.

The compound (II) was synthesized by the method of Yip and Tsou.[6a] The reaction of II with bromine cyanide in H_2O in the presence of triethylamine (TEA) at or below room temperature for 1 day afforded a grey precipitate. This precipitate was dissolved in alkaline water and acidified (pH 2) to obtain crystalline 2-amino-1,N^6-etheno-c-AMP (III). The

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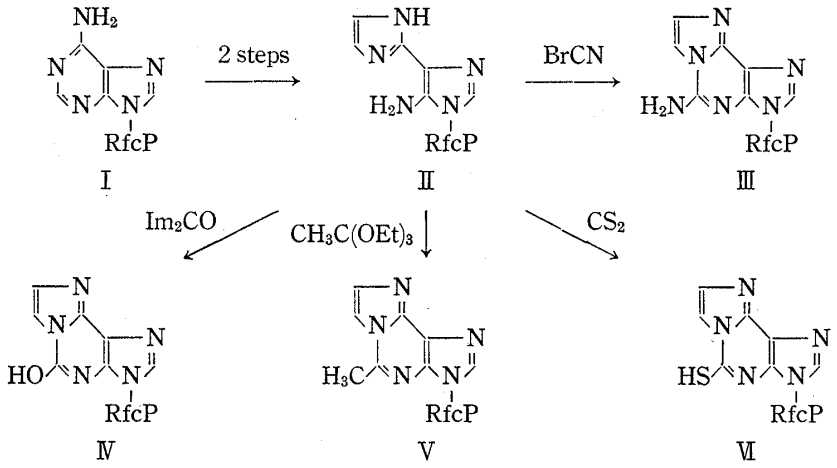
optimal conditions for this reaction are reported in the next section (Discussion). Ring closure of II with 1,1′-carbonyldiimidazole in the presence of TEA and dimethylformamide (DMF) at room temperature for 4 hours gave 2-hydroxy-1,N^6-etheno-c-AMP (IV). Treatment of II (TEA salt) with triethyl orthoacetate in DMF solution at 90° overnight was found to give 2-methyl-1,N^6-etheno-c-AMP (V). Carbon disulfide and II in the presence of anhydrous potassium carbonate and DMF at room temperature afforded 2-mercapto-1,N^6-etheno-c-AMP (VI) in high yield. The ring closure of II with either 1,1′-carbonyldiimidazole or orthoacetate to give 2-hydroxy-1,N^6-etheno-c-AMP (IV) or 2-methyl-1,N^6-etheno-c-AMP (V) was explained by analogy with the same kind of ring closure reaction of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3′,5′-phosphate.[8] But the latter compound and carbon disulfide gave a dark mixture which was difficult to purify and 2-amination of the compound was not reported. Therefore, it seems reasonable to consider that the etheno residue (imidazole ring in II) blocks the multiple reaction and facilitates the synthesis of ring closure products (Chart 1).



RfcP: β-D-(3′,5′-cyclic phospho)-ribofuranosyl

Chart 1

Then, we examined the synthesis of another 2-substituted 1,N^6-etheno-c-AMP which could not be synthesized directly from the ring closure of II. At first, 2-alkylmercapto- and 2-benzylmercapto-1,N^6-etheno-c-AMP (VIIa—VIIg) were synthesized by the alkylation and benzylation of VI with alkyl iodide and benzyl bromide in aqueous alkaline solution. The reaction of VI with bromine in conc. hydrobromic acid yielded 2-bromo-1,N^6-etheno-c-AMP (VIII). The structure of VIII was determined by its conversion to 2-methylmercapto-1,N^6-etheno-c-AMP (VIIa) and 2-hydroxy-1,N^6-etheno-c-AMP (IV) by reaction with methanethiol and sodium hydroxide and by a positive Beilstein test. Therefore, many 2-substituted 1,N^6-etheno-c-AMP derivatives can now be synthesized by substitution of the bromo function in VIII by the same method as reported in the synthesis of 8-substituted c-AMP from 8-bromo-c-AMP.[9] As reported in the previous paper, we synthesized 2-aza-c-AMP from 2-aza-1,N^6-etheno-c-AMP by the deblocking reaction using bromine or N-bromosuccinimide (NBS) in the pH range from 2.5 to 6.0.[7] It seems of interest that the etheno group of compound VI was not eliminated by bromine in highly acidic solution (conc. HBr) but was converted to VIII. The reaction of VIII with dimethylamine in methanol afforded 2-dimethylamino-1,N^6-etheno-c-AMP (IX). 2-Azido-1,N^6-etheno-c-AMP (X) or 2-methoxy-1,N^6-etheno-c-AMP (XI) was synthesized from VIII with sodium azide in DMF or sodium methoxide in methanol, respectively (Chart 2).

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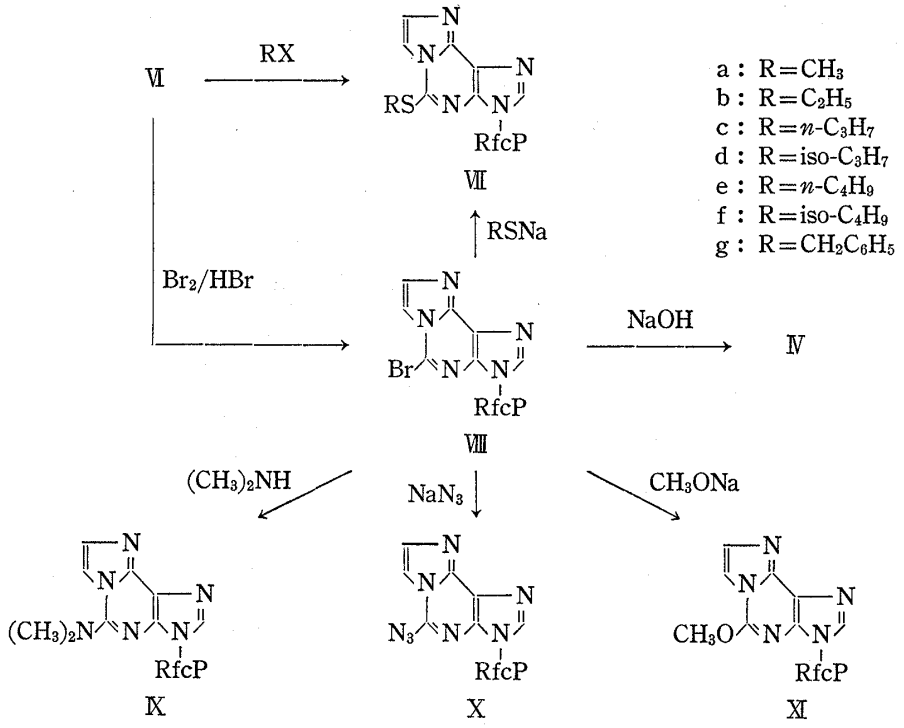

RfcP: β-D-(3′,5′-cyclic phospho)-ribofuranosyl

Chart 2

TABLE I. Physical Constants of the 2-Substituted 1,N⁶- etheno-c-AMP

No.	R	*Rf*[a]		λmax, nm(ε×10⁻³)[b]	
		A	B	0.1 N HCl	0.1 N NaOH
III	NH_2	0.32	0.49	273.5(14.3), 293.5(13.3)	277sh(13.9), 284(14.4), 294(13.6), 306sh(8.43)
IV	OH	0.28	0.34	259.5(7.35), 295.5(15.7), 306sh(12.7)	273sh(14.0), 282(16.4), 292(16.5), 303(10.5)
V	CH_3	0.32	0.55	274(12.0)	255sh(5.15), 265(6.76), 274.5(7.50), 292.5(5.13)
VI	SH	0.34	0.40	240.5(17.2), 310.5(21.5)	249(14.4), 280sh(7.49), 292sh(11.3), 318(15.4)
VIIa	SCH_3	0.42	0.59	237(21.2), 291.5(19.1)	239.5(21.5), 243.5sh(20.7), 273(8.25), 282.5(10.2), 303(9.30)
VIIb	SC_2H_5	0.46	0.68	237.5(19.9), 292.5(18.9)	240(21.1), 244sh(20.5), 273.5(7.93), 283(9.78), 303.5(9.38)
VIIc	S-*n*-C_3H_7	0.53	0.77	238(19.4), 293(18.9)	240.5(20.9), 245sh(20.2), 274(7.96), 283.5(9.82), 304(9.45)
VIId	S-iso-C_3H_7	0.57	0.77	237.5(19.3), 293(19.0)	239.5(20.7), 244sh(20.1), 273.5(7.55), 283(9.27), 306(9.16)
VIIe	S-*n*-C_4H_9	0.61	0.82	238(19.4), 293(19.4)	240.5(20.7), 245sh(20.3), 274(8.08), 283.5(9.93), 305(9.73)
VIIf	S-iso-C_4H_9	0.59	0.84	238(19.2), 293(19.4)	240(20.0), 245sh(19.3), 274(7.69), 283(9.50), 305.5(9.45)
VIIg	$SCH_2C_6H_5$	0.57	0.82	239(19.4), 292(17.9)	238sh(20.4), 273.5(7.63), 283(8.86), 305(8.53)
VIII	Br	0.35	0.55	227.5(30.9), 279.5(12.3)	
IX	$N(CH_3)_2$	0.37	0.63	229.5(19.2), 288(17.7)	288(13.6)
X	N_3	0.34	0.55	233.5(22.3), 287(17.3)	238.5(15.3), 246sh(13.8), 274(10.5), 283.5(10.4), 292sh(8.35), 303sh(6.82)
XI	OCH_3	0.32	0.61	262sh(11.3), 280.5(15.8), 291sh(11.6)	273(13.5)

a) *Rf* on Toyo Filter No. 51A papers in solvent system A (*n*-butanol/acetic acid/water, 5: 2: 3) or B (0.5M ammonium acetate/ethanol, 2: 5). In these systems II has *Rf* (A)=0.35 and *Rf* (B)=0.47.

b) Sh refers to a shoulder.

It is worth pointing out that all derivatives except VI, IX, and XI showed ultraviolet (UV) absorption patterns very similar to 1,N^6-etheno-c-AMP in alkaline solution as shown in Table I.[4b] This spectroscopic feature gave a clue for the structural identification of 2-substituted 1,N^6-etheno-c-AMP products.

Table II. Technical Fluorescence Data[a]

Compound	max	Fluorescence[b] (Emission, nm)		Fluorescence[c] (Excitation, nm)
		+1/2	−1/2	
III	400	455	355	305
IV	410	480	365	305
V	395	440	360	305
VI	410	470	360	305
VIIa-VIIf	430	475	390	315
VIIg	410	460	360	310
VIII	410	470	350	305
IX	440	495	385	310
X	400	470	350	305
XI	400	465	325	295

a) All spectra were recorded in water at the concentrated (5γ/ml).
b) Fluorescence emission spectra were taken by fixing on the fluorescence excitation maximum. Wavelengths representing half heights on each side of the maximum are also given.
c) Fluorescence excitation spectra were taken by fixing on the fluorescence emission maximum.

The close structural similarity between the newly synthesized compounds and 1,N^6-etheno-adenine derivatives suggested that all 2-substituted-1,N^6-etheno-c-AMP derivatives might be fluorescent and this was subsequently confirmed (Table II). All compounds showed emission maxima in the region of 395—430 nm. The fluorescence was strong in the compounds, IV and V, and weak in the compounds, VI, VIIa—VIIg, and VIII. The fluorescent ability of these compounds indicates that they can serve as the means for studying the biological and chemotherapeutic function of c-AMP.[6b]

The study of the deblocking reaction of 2-substituted 1,N^6-etheno-c-AMP is in progress in our laboratory.

Discussion on the Synthesis of 2-Amino-1,N^6-etheno-c-AMP (III)

As judged from the experimental data presented in this paper, 3-β-D-(3′,5′-cyclic phospho)-ribofuranosyl-4-amino-5-(imidazol-2-yl)-imidazole (II) could be effectively converted to 2-amino-1,N^6-etheno-c-AMP (III) by the use of bromine cyanide in the presence of TEA or in buffer solution. As shown in Table III, the use of a limited amount of TEA as an acceptor

Table III. The Synthesis of 2-Amino-1,N^6-etheno-c-AMP (III) in Aqueous Solution[a]

Experiment	2.9M TEA (ml)	H_2O (ml)	BrCN (mg)	Yield (%) of III[b] (day)			
				1	2	3	4
1	0.5	3.5	100	26	25	28	30
2	1.0	3.0	100	39	41	43	43
3	2.0	2.0	100	40	40	37	35
4	4.0	0	100	36	32	28	27

a) Reaction conditions: compound II, 100 mg; and stirred at room temperature
b) The yield of 2-amino-1,N^6-etheno-c-AMP (III) was determined by paper chromatographic separation, followed by the determination of the absorbance in the UV spectrometer. Solvent (saturated ammonium sulfate/1M sodium acetate/2-propanol, 80: 20: 2), Rf=0.09.

of hydrogen bromide during the course of the reaction is necessary. Another base, such as sodium hydroxide or potassium hydroxide was not effective. The use of a large excess of TEA resulted in a decrease of the yield, probably because it affected the intermediate complex and/or bromine cyanide.

From the experiments in buffer solutions of various pH, it could be deduced that pHs lower than 6.0 and higher than 9.0 should be avoided (Table IV). In the former case, the protonation of the amino residue might decrease the nucleophilic susceptibility of II against bromine cyanide. At pHs higher than 9.0, on the other hand, the resulting intermediate and/or bromine cyanide might be decomposed as in the case with an excess of base. Therefore, the most efficient procedure was the use of buffer of pH 8.0.[10]

Table IV. The Synthesis of 2-Amino-1, N^6-etheno-c-AMP (III) in Buffer Solution of Various pH[a]

pH	Buffer	E_{294}/E_{267}[b]		Yield (%)[c]		pH after reaction
		Start	End	III	II	
3	acetate	0.217	0.273	4	39	3.0
4	acetate	0.215	0.229	4	40	4.0
5	acetate	0.215	0.219	3	34	5.0
6	phosphate	0.229	0.541	22	30	5.4
7	phosphate	0.232	0.803	53	17	6.4
8	phosphate	0.267	1.099	66	5	7.0
9	borate	0.353	0.776	40	6	8.8
10	borate	0.302	0.301	6	14	9.5

a) Reaction conditions: compound II, 100 mg; solvent, 5.0 ml; BrCN, 100 mg; reaction time, 1 day; and stirred at room temperature.
b) UV absorption spectra were measured in 0.1N NaOH solution.
c) The yields of 2-amino-1,N^6-etheno-c-AMP (III) and II were determined by paper chromatographic separation, followed by the determination of the absorbance in the UV spectrometer. Solvent (saturated ammonium sulfate/1M sodium acetate/2-propanol, 80: 20: 2), compound III has Rf=0.09 and II has Rf=0.41.

Experimental

All melting points were uncorrected and taken on the Kofler plate. The UV absorption spectra were determined on a Hitachi Model-323 spectrophotometer. The nuclear magnetic resonance (NMR) spectra were obtained using a Hitachi Model-R-24A spectrometer in D_2O–NaOD. The fluorescent spectra were determined on a Hitachi Model-MPF-2 spectrophotometer. Paper chromatograms were run on Toyo Filter No. 51A papers, developing in solvent system A (*n*-butanol/acetic acid/water, 5: 2: 3) or B (0.5M ammonium acetate/ethanol, 2: 5) or C (saturated ammonium sulfate/1M sodium acetate/2-propanol, 80: 20: 2). In all of the NMR spectra, the anomeric proton appeared as an apparent singlet and only C_8 proton (singlet) and etheno protons (a sets of doublets) appeared in aromatic region which were indicative of 2-substituted 1,N^6-etheno-c-AMP.

2-Amino-1,N^6-etheno-c-AMP (III)——i) Using Triethylamine: Two grams (5.83 mmoles) of II was dissolved in 14.5 ml of 0.4M TEA aqueous solution. Into this solution was added 2.4 g of bromine cyanide. This mixture was stirred for 1 day at room temperature and a grey precipitate was obtained from the reaction mixture. This precipitate was filtered and dissolved in 1N NH_4OH. Adjustment of the pH of the solution to 2.0 with 2N HCl caused crystallization of III. The crystals were filtered, washed with water, and dried to give 866 mg (38.4%) of chromatographically pure III, mp 263—266° (decomp.). NMR (in D_2O–NaOD) δ: 5.69 (1H, singlet, $C_1'H$), 6.95 and 7.04 (2H, a sets of doublets, etheno protons), 7.62 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{12}H_{13}O_6N_6P \cdot H_2O$: C, 37.31; H, 3.89; N, 21.76. Found: C, 37.64; H, 3.87; N, 21.65.

ii) Using Phosphate Buffer: Compound II (100 mg. 0.29 mmoles) was dissolved in 1/3M phosphate buffer (pH 8.0, 5.0 ml), followed by the addition of bromine cyanide (100 mg). The reaction mixture was allowed to stand for 1 day at room temperature. The pH of the reaction mixture was adjusted to 2.0 with 2N HCl and applied to a 1.1 × 12.0 cm column of charcoal. The column was washed with water and the nu-

10) The related paper to this topic: see K.F. Yip and K.C. Tsou, *J. Org. Chem.*, **40**, 1066 (1975).

cleotide was eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The appropriate fractions were concentrated and adjustment of the pH of the residue to 2.0 with 2 N HCl caused crystallization of III (50.7 mg, 45.0%). This sample was identical with those obtained in i).

2-Hydroxy-1,N^6-etheno-c-AMP (IV)——i) Two grams (5.83 mmoles) of II was dissolved in the minimum quantity of 0.4 M TEA solution. The solution was evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml of DMF and 4.0 g (24.8 mmoles) of 1,1′-carbonyldiimidazole was added and the mixture was stirred for 4 hr at room temperature. H_2O (100 ml) was added to the reaction solution. The solution was concentrated *in vacuo* and applied to a 2.7×24 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions and recrystallization as in the procedure described in the synthesis of III gave 1.02 g (46.3%) of IV, mp 187—193° (decomp.). NMR (in D_2O–NaOD) δ: 5.75 (1H, singlet, $C_1'H$), 7.21 and 7.35 (2H, a sets of doublets, etheno protons), 7.64 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{12}H_{12}O_7N_5P \cdot 0.5H_2O$: C, 38.10; H, 3.44; N, 18.52. Found: C, 37.88; H, 3.48; N, 18.50.

ii) The compound VIII (20 mg) was dissolved in 0.2 ml of 2 N NaOH and allowed to stand for 5 hr at room temperature. Addition of 0.25 ml of 2 N HCl to the solution caused crystallization to give 6 mg of IV. This material was identical in all respects with those obtained in i).

2-Methyl-1,N^6-etheno-c-AMP (V)——Two grams (5.83 mmoles) of II was synthesized to TEA salt as in the procedure described in the synthesis of IV and dissolved in 40 ml of DMF and added 2.0 ml (11.0 mmoles) of triethyl orthoacetate. The solution was surrounded by a water bath at 90° and stirred overnight. The solution was concentrated *in vacuo* and applied to a 1.5×30 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions and recrystallization with water–acetone gave 1.28 g (54.5%) of V, mp 228—232° (decomp.). NMR (in D_2O–NaOD) δ: 2.51 (3H, singlet, CH_3), 5.88 (1H, singlet, $C_1'H$), 7.18 and 7.32 (2H, a sets of doublets, etheno protons), 8.00 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{13}H_{14}O_6N_5P \cdot 2H_2O$: C, 38.71; H, 4.47; N, 17.37. Found: C, 38.58; H, 4.12; N, 17.15.

2-Mercapto-1,N^6-etheno-c-AMP——A mixture of 5.0 g (14.58 mmoles) of II, 7.5 g of anhydrous potassium carbonate, 50 ml of carbon disulfide, and 450 ml of DMF was stirred overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to 50 ml and adjustment of the pH of the solution to 2.0 with conc. HCl caused precipitation of VI. The precipitate was dissolved in 0.2 N NH_4OH and applied to a 3.5×17 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions and recrystallization as in the procedure described in the synthesis of III gave 5.1 g (83.1%), mp 226—231° (decomp.). NMR (in D_2O–NaOD) δ: 6.10 (1H, singlet, $C_1'H$), 7.34 and 8.03 (2H, a sets of doublets, etheno protons), 7.92 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{12}H_{12}O_6N_5SP \cdot 2H_2O$: C, 34.20; H, 3.80; N, 16.63. Found: C, 33.90; H, 3.78; N, 16.37.

2-Methylmercapto-1,N^6-etheno-c-AMP (VIIa)——i) A mixture of 1.0 g (2.38 mmoles) of VI, 4.0 ml of 2 N NaOH, 1.0 ml (16.06 mmoles) of methyl iodide, 10.0 ml of H_2O, and 4.0 ml of EtOH was stirred for 2 hr at room temperature. The reaction solution was concentrated *in vacuo* and adjustment of the pH of the residue to 2.0 with 2 N HCl caused crystallization of the product. This was recrystallized as in the procedure described in the synthesis of III to give 940 mg (94.9%) of VIIa, mp 240—242° (decomp.). NMR (in D_2O–NaOD) δ: 2.38 (3H, singlet, SCH_3), 5.77 (1H, singlet, $C_1'H$), 6.71 and 6.94 (2H, a sets of doublets, etheno protons), 7.92 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{13}H_{14}O_6N_5SP \cdot H_2O$: C, 37.41; H, 3.84; N, 16.78. Found: C, 37.67; H, 3.68; N, 17.07.

ii) To the solution of 50 mg of VII, 30 mg of sodium methoxide, and 5.0 ml of MeOH was added 0.2 ml of sodium methanethiol. The reaction mixture was stirred for 10 min at room temperature. Adjustment of the pH of the reaction solution to 2.0 with 2 N HCl caused crystallization to give 35 mg (75.5%) of VIIa. This material was identical in all respects with those obtained in i).

2-Ethylmercapto-1,N^6-etheno-c-AMP (VIIb)——Treatment of 1.0 g (2.38 mmoles) of VI with 1.0 ml (12.5 mmoles) of ethyl iodide as in the procedure described in the synthesis of VIIa gave 950 mg (92.8%) of VIIb, mp 233—235° (decomp.). *Anal.* Calcd. for $C_{14}H_{16}O_6N_5SP \cdot H_2O$: C, 38.98; H, 4.18; N, 16.24. Found: C, 39.01; H, 3.94; N, 16.09.

2-Normalpropylmercapto-1,N^6-etheno-c-AMP (VIIc)——A mixture of 1.0 g (2.38 mmoles) of VI, 4.0 ml of 2 N NaOH, 1.0 ml (10.3 mmoles) of *n*-propyl iodide, 3.0 ml of H_2O, and 15.0 ml of DMF was stirred for 2 hr. The reaction mixture was concentrated *in vacuo* and adjustment of the pH of the residue to 2.0 with 2 N HCl caused crystallization of the product. This was recrystallized as in the procedure described in the synthesis of III to give 610 mg (57.7%) of VIIc, mp 214—216° (decomp.). *Anal.* Calcd. for $C_{15}H_{18}O_6N_5SP \cdot H_2O$: C, 40.45; H, 4.49; N, 15.73. Found: C, 40.62; H, 4.55; N, 15.52.

2-Isopropylmercapto-1,N^6-etheno-c-AMP (VIId)——A mixture of 0.5 g (1.19 mmoles) of VI, 2.0 ml of 2 N NaOH, 0.5 ml of isopropyl iodide, and 8.0 ml of DMF was stirred for 5 hr at room temperature. The reaction solution was concentrated *in vacuo*. Acetone (50 ml) was added to the residue and the resulting precipitate was recrystallized as in the procedure described in the synthesis of III to give 260 mg (48.2%) of VIId, mp 224—226° (decomp.). *Anal.* Calcd. for $C_{15}H_{18}O_6N_5SP \cdot 1.5H_2O$: C, 39.65; H, 4.63; N, 15.42. Found: C, 39.53; H, 4.36; N, 15.30.

2-Normalbutylmercapto-1,N^6-etheno-c-AMP (VIIe)——Treatment of 1.0 g (2.38 mmoles) of VI with 1.0 ml (8.79 mmoles) of *n*-butyl iodide as in the procedure described in the synthesis of VIIc gave 900 mg (82.6%)

of VIIe, mp 207—209° (decomp.). *Anal.* Calcd. for $C_{16}H_{20}O_6N_5SP \cdot H_2O$: C, 41.83; H, 4.79; N, 15.25. Found: C, 41.69; H, 4.64; N, 14.92.

2-Isobutylmercapto-1,N^6-etheno-c-AMP (VIIf)——A mixture of 0.5 g (1.19 mmoles) of VI, 20 ml of 2 N NaOH, 0.5 ml (4.64 mmoles) of isobutyl bromide, 2.0 ml of H_2O, and 8.0 ml of DMF was stirred overnight at room temperature. After concentration, adjustment of the pH of the residue to 2.0 with 2 N HCl caused crystallization of the product. This was recrystallized as in the procedure described in the synthesis of III to give 320 mg (57.6%), mp 215—216° (decomp.). *Anal.* Calcd. for $C_{16}H_{20}O_6N_5SP \cdot H_2O$: C, 41.03; H, 4.91; N, 14.96. Found: C, 40.94; H, 4.56; N, 14.88.

2-Benzylmercapto-1,N^6-etheno-c-AMP (VIIg)——A mixture of 0.5 g (1.19 mmoles) of VI, 2.0 ml of 2 N NaOH, 0.5 ml of benzyl bromide, and 8.0 ml of DMF was stirred for 15 min at room temperature. Adjustment of the pH of the reaction mixture to 2.0 with 1 N HCl caused precipitation of the product. The precipitate was dissolved in DMF, silica gel (2.0 g) was added, and the suspension was evaporated to a powder. The powder was added to a silica gel column (20 g, packed with chloroform). The nucleotide was eluted with chloroform–methanol (4: 1 v/v). Evaporation of the appropriate fractions gave 240 mg (41.7%) of VIIg, mp 222—226° (decomp.). *Anal.* Calcd. for $C_{19}H_{18}O_6N_5SP \cdot 0.5H_2O$: C, 47.11; H, 3.93; N, 14.46. Found: C, 47.36; H, 3.78; N, 14.61.

2-Bromo-1,N^6-etheno-c-AMP (VIII)——To an ice-cooled and stirred solution of 47% HBr (140 ml) and VI (3.5 g) was added Br_2 (1.8 ml), dropwise. After 5 hr, the resulting solution was applied to a 3.5×15 cm column of charcoal and the column was washed with water. The nucleotide was eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The appropriate fractions were neutralized with Dowex 50-X8 (H^+) and filtered. The filtrate was concentrated and applied to a 3.5×20 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions gave 2.66 g (71.1%) of VIII, mp 198—205° (decomp.). NMR (in D_2O–NaOD) δ: 5.94 (1H, singlet, $C_1'H$), 7.22 and 7.47 (2H, a sets of doublets, etheno protons), 8.06 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{12}H_{11}O_6N_5PBr \cdot H_2O$: C, 32.00; H, 2.89; N, 15.56, Found: C, 31.90; H, 3.03; N, 15.36.

2-Dimethylamino-1,N^6-etheno-c-AMP (IX)——A mixture of 200 mg (0.44 mmoles) of VIII, 10 ml of MeOH, and 10 ml of 48% dimethylamine solution was refluxed for 3 hr. The reaction mixture was evaporated *in vacuo* and the residue was taken up in 3 ml of water. Adjustment of the pH of the solution to 2.0 with 2 N HCl caused crystallization to give 127 mg (67.6%) of IX, mp 245—250° (decomp.). NMR (in D_2O–NaOD) δ: 2.92 [6H, singlet, $N(CH_3)_2$], 5.87 (1H, singlet, $C_1'H$), 7.23 and 7.46 (2H, a sets of doublets, etheno protons), 7.89 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{14}H_{17}O_6N_6P \cdot 1.5H_2O$: C, 39.72; H, 4.73; N, 19.86. Found: C, 39.78; H, 4.44; N, 19.57.

2-Azido-1,N^6-etheno-c-AMP (X)——A mixture of 200 mg (0.44 mmoles) of VIII, 10 ml of DMF, and 70 mg (1.08 mmoles) of sodium azide was refluxed for 3 hr. Treatment of the reaction mixture as described in the synthesis of IX gave 163 mg (80.2%) of X, mp 157—164° (decomp.). NMR (in D_2O–NaOD) δ: 5.94 (1H, singlet, $C_1'H$), 7.37 and 7.45 (2H, a sets of doublets, etheno protons), 8.11 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{12}H_{11}O_6N_8P \cdot 3.5H_2O$: C, 31.51; H, 3.94; N, 24.51. Found: C, 31.50; H, 3.65; N, 24.43.

2-Methoxy-1,N^6-etheno-c-AMP (XI)——A mixture of 200 mg (0.44 mmoles) of VIII, 10 ml of MeOH, and 200 mg (3.70 mmoles) of sodium methoxide was refluxed for 30 min. The reaction mixture was evaporated *in vacuo* and the residue was taken up in 5 ml of H_2O and applied to a 1.5×28 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions gave 164 mg (83.2%) of XI, mp 226—234° (decomp.). NMR δ: 4.00 (3H, singlet, OCH_3), 5.84 (1H, singlet, $C_1'H$), 7.13 and 7.22 (2H, a sets of doublets, etheno protons), 7.91 (1H, singlet, C_8H). *Anal.* Calcd. for $C_{13}H_{14}O_7N_5P \cdot 2H_2O$: C, 37.23; H, 4.30; N, 16.71. Found: C, 37.07; H, 4.22; N, 16.45.