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Studies on the Syntheses of Compounds related to Adenosine 3′,5′-cyclic Phosphate. II.[1] Removal of Etheno Group of 2-Substituted 1,N^6-Etheno-adenosine 3′,5′-cyclic Phosphates[2]

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Treatment of 2-substituted 1,N^6-etheno-c-AMP (c-AMP: adenosine 3′,5′-cyclic phosphate) derivatives with N-bromosuccinimide (NBS) under mild conditions gave the corresponding 2-substituted-c-AMP. Therefore, the route (c-AMP→1,N^6-etheno-c-AMP→2-substituted 1,N^6-etheno-c-AMP→2-substituted c-AMP) offers an excellent way to synthesize the last compound from c-AMP. The yields of this deblocking reaction of the etheno group were increased by the adjustment of the solution to alkaline after bromination. The probable reaction mechanism involving the bromination and the subsequent hydrolysis has been proposed.

Keywords——cyclic AMP derivative; etheno group; N-bromosuccinimide; bromination; Warburg manometer; UV; mass

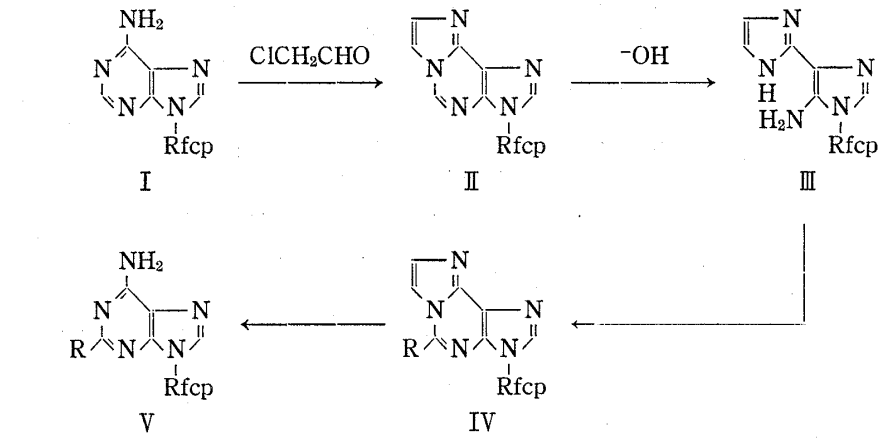
During the past few years, adenosine 3′,5′-cyclic phosphate (c-AMP) has become to be recognized as the most important low molecular weight compound having physiological activity.[4] Numerous derivatives of c-AMP have been synthesized to obtain substances that might have a better biological action than the original nucleotide (c-AMP) and to find substances that might act as antagonists to the original nucleotide.[5] But the synthesis of 2-substituted c-AMP from c-AMP is very difficult, because it requires the fission of purine ring and the subsequent recyclization.

So, a convenient synthesis of 2-substituted c-AMP is very important to obtain further information concerning the unique properties of 2-substituted c-AMP analogues in the biological system. 2-Substituted c-AMP derivatives have been prepared by the following two routes, i) synthesis of 2-substituted adenosine 5′-monophosphates from 2-substituted adenosines and the subsequent cyclization reaction;[6] ii) synthesis of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3′,5′-phosphate from c-AMP and the subsequent cyclization reaction.[7] These methods include tedious steps and unstable intermediates, and are not feasible for a large scale preparation of 2-substituted c-AMPs. Our method utilizes c-AMP as starting material like route ii, which is easily accessible from the culture broth of

1) Paper I in this series, N. Yamaji, Y. Yuasa, and M. Kato, *Chem. Pharm. Bull.* (Tokyo), **24**, 1561 (1976).
2) Presented in part at the 4th Symposium on Nucleic Acids Chemistry of Japan, Kyoto, November 27, 1976.
3) Location: *a) 399, Noda, Noda-shi, Chiba, 278, Japan*; *b) 9–500–1, Nagareyama-shi, Chiba, 270–01, Japan.*
4) *a)* B. Jastorff and W. Freist, *Angew. Chem. Intern. Ed. Engl.*, **11**, 713 (1972); *b)* P. Greengard, R. Paoletti, and G.A. Robison (ed.), "Advances in Cyclic Nucleotide Research," Vol. 1, Raven Press, New York, 1972.
5) *a)* R.B. Meyer, Jr. and J.P. Miller, *Life Sci.*, **14**, 1019 (1974); *b)* L.N. Simon, D.A. Shuman, and R.K. Robins, "Advances in Cyclic Nucleotide Research," Vol, 3, ed. by P. Greengard and G.A. Robison, Raven Press, New York, 1973, pp. 225—353.
6) *a)* R. Marumoto, T. Nishimura, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **23**, 2295 (1975); *b)* T. Posternak and G. Cehovie, *Ann. N.Y. Acad. Sci.*, **185**, 42 (1971).
7) R.B. Meyer, Jr., D.A. Shuman, and R.K. Robins, *J. Am. Chem. Soc.*, **96**, 4962 (1974).

Corynebacterium murisepticam or *Microbacterium sp.*[8] We have previously reported the synthesis of 2-aza-c-AMP by the reaction of 2-aza-1,N^6-etheno-c-AMP with N-bromosuccinimide (NBS) or bromine.[9] As one of the series of our studies along this line, 2-substituted 1,N^6-etheno-c-AMP derivatives were synthesized easily from 3-β-D-(3′,5′-cyclic phospho)-ribofuranosyl-4-amino-5-(imidazol-2-yl)-imidazole.[1]

In the present paper we report the synthesis of the corresponding 2-substituted c-AMPs from the 2-substituted 1,N^6-etheno-c-AMPs with NBS under mild condition. So, we now established a new synthetic route to 2-substituted c-AMPs from c-AMP (Chart 1).



a : R=CH_3 e : R=OCH_3
b : R=OH f : R=$N(CH_3)_2$
c : R=NH_2 g : R=Br
d : R=SCH_3 h : R=N_3

a : CH_3 f : R=$N(CH_3)_2$
b : OH g : R=Br
c : NH_2 h : R=N_3
d : SCH_3 i : R=SH
e : OCH_3

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

Chart 1

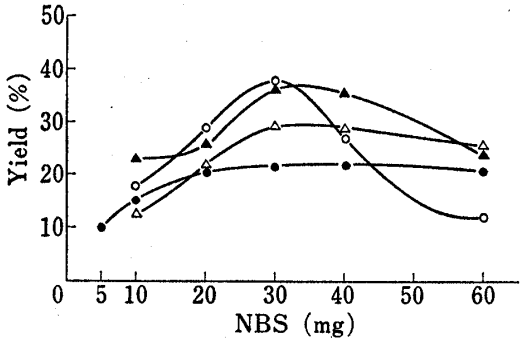


Fig. 1. Synthesis of c-AMP (I) from 1,N^6-Etheno-c-AMP (II)

a) Reaction conditions: 1,N^6-etheno-c-AMP, 20 mg; 0.5 M acetate buffer solution, 5.0 ml; reaction time, 18 hr (under stirring) and reaction temp., 30°.
b) The yield of c-AMP was determined by high pressure liquid chromatography.
—●—: pH 3, —○—: pH 4,
—▲—: pH 5, —△—: pH 6.

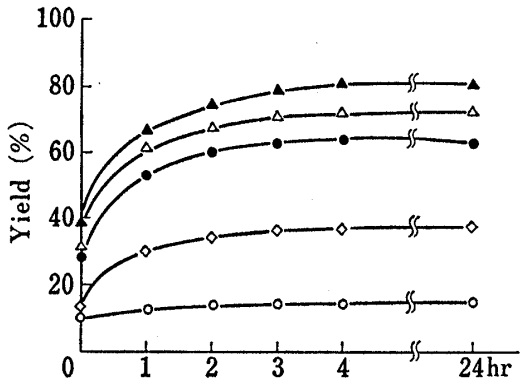


Fig. 2. Yield of I from II by Alkalization aften Bromination

Reaction conditions: 1,N^6-etheno-c-AMP, 20 mg; 0.5 M acetate buffer solution (pH 4.5), 5.0 ml. After stirring at 30° for 18 hr, added the same volume of 1 N NaOH.
—○—: NBS 10 mg, —●—: NBS 20 mg,
—▲—: NBS 30 mg, —△—: NBS 40 mg,
—◇—: NBS 60 mg.

8) *a*) J. Ishiyama, T. Yokotsuka, and N. Saito, *Agric. Biol. Chem.* (Tokyo), **38**, 507 (1974); *b*) J. Ishiyama, *Nippon Nogeikagaku Kaishi*, **50**, 295 (1976).
9) N. Yamaji and M. Kato, *Chemi. Lett.*, **1975**, 311.

At first, in order to investigate the deblocking reaction of the etheno group, we studied the synthesis of c-AMP from 1,N^6-etheno-c-AMP in detail. This reaction proceeded in acetate buffer solution with NBS, but the yield was not high (40%). When a large amount of NBS was used, the yield of c-AMP was decreased, because of the formation of 8-bromo-c-AMP (Fig. 1).

In order to increase the yield of this reaction, we examined the DMF–H_2O solvent instead of acetate buffer solution but it was not so effective as the synthesis of 2-aza-c-AMP from 2-aza-1,N^6-etheno-c-AMP.[9] This reaction was then perfomed in acetate buffer solution at pH 4.5 and the reaction mixture was made alkaline with NaOH above pH 13.0. The yield was increased up to 80% by standing for 4 hr (Fig. 2).

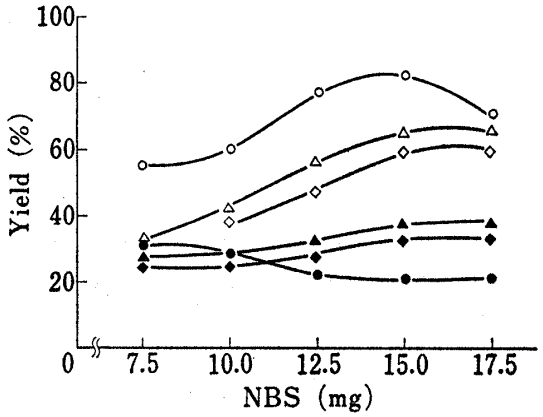


Fig. 3. Synthesis of 2-CH_3-c-AMP (Va) from 2-CH_3-1,N^6-Etheno-c-AMP (IVa)

Reaction conditions: 2-CH_3-1,N^6-etheno-c-AMP, 20 mg; 0.5 M acetate buffer solution, 5.0 ml. After stirring at 30° for 18 hr, added the same volume of 1 N NaOH.
—●—: pH 4, —○—: alkali,
—▲—: pH 5, —△—: alkali,
—◆—: pH 6, —◇—: alkali.

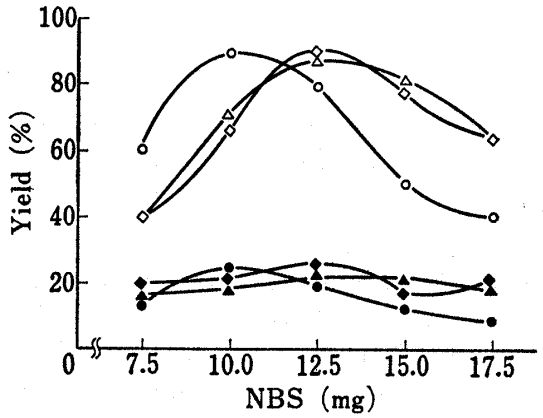


Fig. 4. Synthesis of 2-OH-c-AMP (Vb) from 2-OH-1,N^6-Etheno-c-AMP (IVb)

Reaction conditions: 2-OH-1,N^6-etheno-c-AMP, 20 mg; 0.5 M acetate buffer solution, 5.0 ml. After stirring at 30° for 18 hr, added the same volume of 1 N NaOH.
—●—: pH 4, —○—: alkali,
—▲—: pH 5, —△—: alkali,
—◆—: pH 6, —◇—: alkali.

The yields of 2-methyl-c-AMP (Va) and 2-hydroxy-c-AMP (Vb) from 2-methyl-1,N^6-etheno-c-AMP (IVa) and 2-hydroxy-1,N^6-etheno-c-AMP (IVb) respect to the amount of NBS were illustrated by typical representative patterns shown in Figs. 3 and 4. The yield of (Vb) increased about 4 times by alkalization of the reaction mixture to that in acidic solution.

In the syntheses of 2-bromo-c-AMP (Vg) and 2-azido-c-AMP (Vh), the yields were not high in acetate buffer solution, but the yields of the both compounds were increased in the reaction using aq. hydrogen chloride solution and the faster alkalization (Fig. 5).

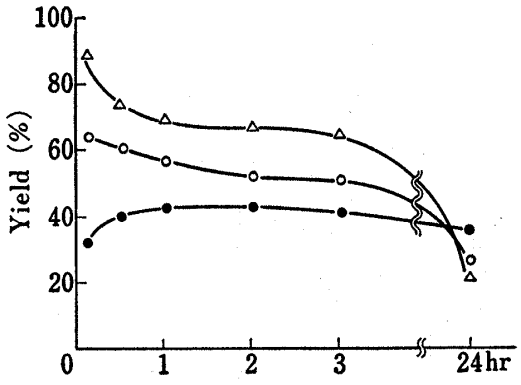


Fig. 5. Synthesis of 2-Br-c-AMP (Vg) from 2-Br-1,N^6-Etheno-c-AMP (IVg)

Reaction conditions: 2-Br-1,N^6-etheno-c-AMP, 20 mg; NBS, 30 mg; 0.5 M acetate buffer solution or 2 N HCl, 5.0 ml. After stirring at 30°, added the same volume of 1 N NaOH in the case of former and 3 N NaOH in the case of latter.
—△—: 2 N HCl, —○—: pH 3, —●—: pH 5.

These results suggest that strongly acidic condition is preferred for bromination of these etheno compounds and that the brominated intermediates must be hydrolyzed quickly by alkalization since they are unstable in this condition.

We examined the detailed conditions of this reaction of all compounds by changing the amounts of NBS, pH and reaction time. The optimum conditions thus obtained are shown in Table I.

Table I. Yield of 2-Substituted-c-AMP from the Corresponding Etheno Compound[a]

Compound No.	R	Reaction pH	Yield (%)[b] Standing	Addition (NaOH)	NBS (mole equivalent)
II	H	4.5	38	80	2.58
Va	CH_3	3.0	26	81	1.43
Vb	OH	4.5	31	93	1.44
Vc	NH_2	3.0	24	73	1.44
Vd	SCH_3	3.0	33	67	1.56
Ve	OCH_3	4.5	50	63	1.94
Vf	$N(CH_3)_2$	4.5	45	78	1.00
Vg	Br	0.2	8	88	3.45
Vh	N_3	1.0	Trace	69	1.54

a) Reaction conditions: 2-substituted-1,N^6-etheno-c-AMP, 10 mg; solvent, 5.0 ml; temperature, 30°; and reaction time, 2-N_3-c-AMP and 2-Br-c-AMP (10 min), others (18 hr).
b) The yield of 2-substituted-c-AMP was determined by high pressure liquid chromatography.

The reaction of the synthesized 2-amino-c-AMP (Vc) with sodium nitrite in dilute acetic acid afforded 2-hydroxy-c-AMP (Vb) and the latter was shown to be identical in all respects with the compound which was synthesized by the deblocking reaction of 2-hydroxy-1,N^6-etheno-c-AMP (IVb). The reaction of 2-bromo-c-AMP (Vg) which was synthesized by the deblocking reaction of 2-bromo-1,N^6-etheno-c-AMP (IVg) with dimethylamine under reflux for 3 hr afforded 2-dimethylamino-c-AMP (Vf). This was identical with the compound synthesized by the deblocking reaction of 2-dimethylamino-1,N^6-etheno-c-AMP (IVf).

The reaction of (III) with carbon disulfide afforded 2-mercapto-1,N^6-etheno-c-AMP (IVi) in high yield.[1] But the reaction of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3′,5′-phosphate (AICARcP) with carbon disulfide resulted in the formation of a dark mixture which was difficult to be purified[7] and the synthesis of 2-amino-c-AMP (Vc) from

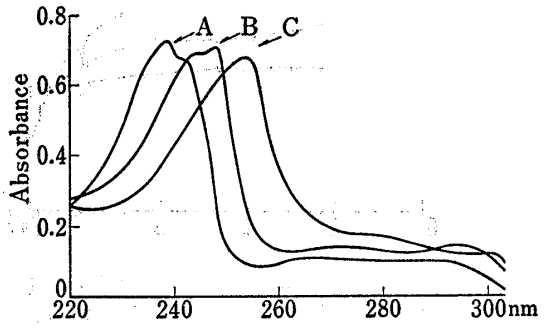


Fig. 6. The Ultraviolet Spectra of Fluorescent Spots (A, B, C) on Paper Chromatogram of 2-Aza-c-AMP Synthesis

Paper chromatography: solvent, *n*-BuOH/acetic acid/H_2O, 5:2:3: spot A, 2-aza-1,N^6-etheno-c-AMP (*Rf*=0.35); spot B, compound X (*Rf*=0.43); spot C, compound Y (*Rf*=0.52).

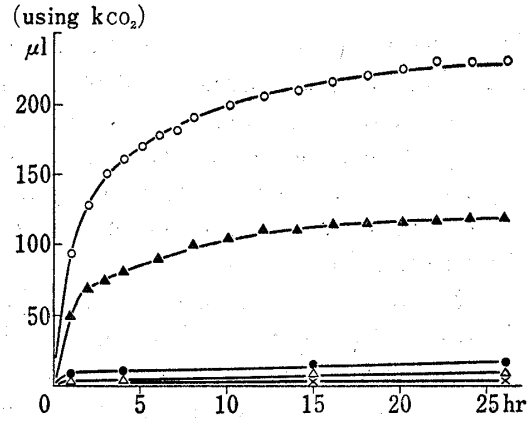


Fig. 7. Gas Evolution from the Reaction Mixtures of 2-Aza-c-AMP Synthesis

Reaction conditions

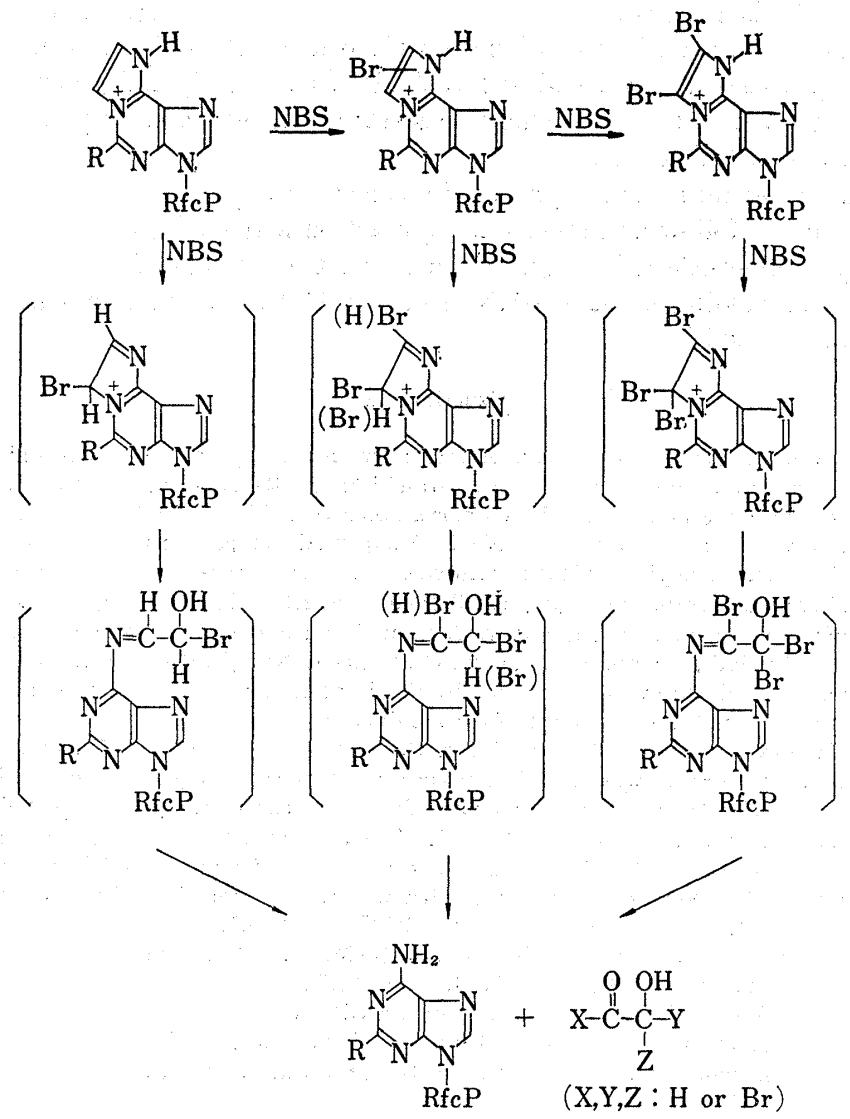
	main chamber 2-aza-1,N^6-etheno-c-AMP (1.5 ml)	side chamber NBS (0.5 ml)	center well 1 N KOH (0.2 ml)
—○—	5.0×10^{-6} M	3.0×10^{-5} M	–
—▲—	2.5×10^{-6} M	1.5×10^{-5} M	–
—×—	5.0×10^{-6} M	3.0×10^{-5} M	+
—●—	—	3.0×10^{-5} M	–
—△—	5.0×10^{-6} M	—	–

solvent, 1 M acetate buffer solution (pH 4.0), temperature, 30±0.5°; apparatus; Warburg manometer.

AICARcP was not reported. Therefore, it seems reasonable to consider that the etheno group (imidazole ring in (III)) blocks the complex reaction and facilitates the synthesis of ring closure products.

Moreover, 2-mercapto-1,N^6-etheno-c-AMP (IVi) was not only methylated easily (yield 94.9%) but also afforded 2-bromo-1,N^6-etheno-c-AMP (IVg) by the reaction with bromine in 48% hydrogen bromide solution (yield 71.1%).[1] The yield of 2-methylmercapto-c-AMP (Vd) from 2-mercapto-c-AMP was only 38%[7] and the synthesis of 2-bromo-adenosine derivative from 2-mercapto-adenosine derivative was not reported. The reaction of 2-bromo-c-AMP (Vg) with sodium azide in DMF solution at refluxing temperature did not afford 2-azido-c-AMP (Vh), but 2-azido-1,N^6-etheno-c-AMP (IVh) was easily synthesized from 2-bromo-1,N^6-etheno-c-AMP (Vg).[1] From the above reasons, it is very useful to use etheno group for the synthesis of many 2-substituted c-AMP derivatives.

The present method provides not only a facile general synthesis of V from I, but also provides a new simple route to prepare various 2-substituted c-AMPs whose syntheses have hitherto been found to be difficult or complicated.



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

Chart 2

Reaction Mechanism

In the synthesis of 2-aza-c-AMP from 2-aza-1,N^6-etheno-c-AMP,[9] there were two fluorescent spots of intermediates (compound X and Y) on paper chromatography. Fluorescent compound Y was synthesized using two moles of NBS and was isolated by charcoal treatment and the subsequent Dowex 50-X8 (H^+, 100—200 mesh) column chromatography. This compound was assigned to be α,β-dibromo-2-aza-1,N^6-etheno-c-AMP on the basis of spectral data, elemental analytical data, and the positive Beilstein test. Though this compound was stable in aqueous solution, it changed to 2-aza-c-AMP by the use of NBS. Fluorescent compound X may be α- or β-monobromo-2-aza-1,N^6-etheno-c-AMP on the basis of the similarity to the ultraviolet curves of the two compounds (compound Y and 2-aza-1,N^6-etheno-c-AMP) and the fact that the maximum absorption of compound X (244.0 nm and 248.0 nm) exists between that of 2-aza-1,N^6-etheno-c-AMP (239.0 nm) and compound Y (253.5 nm) (Fig. 6). The maximum amount of compound X was observed using 0.5 moles of NBS.

Then the released compound from the etheno group was examined using the Warburg manometer and the evolved gas which could be absorbed in KOH solution was found to be two moles by using the flask constant of carbon dioxide (Fig. 7).

We attempted to isolate the released compound from the reaction mixture of 2-dimethylamino-c-AMP synthesis using equimolar NBS. Phenylhydrazone derivative could be isolated from the reaction mixture and was assigned to glyoxal bis-phenylhydrazone on the basis of spectra and the synthesis from glyoxal or glycolaldehyde. As glyoxal was not recognized by the colorimetric method,[10] the compound released from the etheno group may be glycolaldehyde or its analogues (*cf.* Chart 2).

From the above examination, a proposed mechanism is given in Chart 2. This reaction seems to proceed in the following three routes, i) reaction with equimolar NBS like the synthesis of 2-dimethylamino-c-AMP, ii) reaction with over three moles of NBS like the synthesis of 2-aza-c-AMP *via* α,β-dibromo-2-aza-1,N^6-etheno-c-AMP, iii) reaction with two moles of NBS between route i) and ii).

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The UV absorption spectra were determined on a Hitachi Model-R-323 spectrophotometer. Mass spectra (MS) were measured by the method of A.M. Lawson, *et al.* on a Hitachi Model RMU-7M mass spectrometer.[11] Paper chromatograms were run on Tokyo Filter No. 51 A papers, developing in solvent system, isopropanol/28 % NH_4OH/water (7 : 1 : 2). The yields of 2-substituted c-AMP derivatives were determined on a Hitachi 634 high pressure liquid chromatography; packing, #2632; column, 2.1 m ID × 500 mmL, flow rate, 1.1 ml/min; pressure, 50 kg/cm²; temperature, 70°; detector, 254 nm filter; and eluent, except compounds (Vg) and (Vh); 0.07 M NaCl/0.008 M HCl, compounds (Vg) and (Vh); 1.00 M NaCl/0.016 M HCl. The quantity of gas evolved from the reaction mixture was determined by Warburg manometer by the use of flask with one side arm, at 30±0.5° and the use of flask constant of carbon dioxide.

2-Methyl-c-AMP (Va)——To a solution of 2-methyl-1,N^6-etheno-c-AMP[1] (500 mg) was added NBS (375 mg) and the reaction mixture was stirred at room temperature overnight. The solution was adjusted to above pH 13 with 1 N NaOH and stirred for 4 hr. The pH of the solution was adjusted to 2.0 with HCl and passed through a column of charcoal (1.4 × 20 cm). The column was washed with water and eluted with EtOH–H_2O–28% NH_4OH (10 : 10 : 1). The eluate was concentrated and applied to a 1.4 × 20 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions gave 250 mg (53.5%) of (Va), mp 223—225° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm: 256.5, 265.5 (shoulder), $\lambda_{max}^{0.1N\text{-}NaOH}$ nm: 262.5. Paper chromatography; Rf=0.59. MS m/e: 560 (M^+, (Va)(TMS)$_3$). *Anal.* Calcd. for $C_{11}H_{14}N_5O_6P \cdot 1.5H_2O$: C, 35.68; H, 4.59; N, 18.92. Found: C, 35.42; H, 4.36; N, 18.72.

2-Hydroxy-c-AMP (Vb)——i) To a solution of 2-hydroxy-1,N^6-etheno-c-AMP[1] (500 mg) in 0.5 M acetate buffer (pH 4.5, 125 ml) was added NBS (325 mg) and the reaction mixture was stirred at room temperature overnight. Treatment of the solution as in the procedure in the synthesis of (Va) gave 305 mg

10) C.S. Wise, C.L. Methltretter, and J.W. Van Cleve, *Anal. Chem.*, 31, 1241 (1959).
11) A.M. Lawson, R.N. Stollwell, M.M. Tacker, K. Tsuboyama, and J.A. McClosky, *J. Am. Chem. Soc.*, 93, 1014 (1971).

(65.2%) of (Vb), mp 198—200° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$nm; 236, 281.5, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 254.5, 284.5. Paper chromatography; Rf=0.45. MS m/e: 633 (M^+, (Vb) $(TMS)_4$). *Anal.* Calcd. for $C_{10}H_{12}N_5O_7P$: C, 34.78; H, 3.48; N, 20.29. Found: C, 34.62; H, 3.54; N, 20.46.

ii) To an ice cooled suspension of 2-amino-c-AMP (10 mg) in 0.5 ml of water and 1.0 ml of AcOH was added 12.5 mg of $NaNO_2$. The flask was loosely covered and stirred for 2 hr in the ice bath, then an additional 50.0 mg of $NaNO_2$ was added. After standing for 20 hr at room temperature, the solution was concentrated. The residue was passed through a 0.9×10 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and the product was eluted with water. Evaporation of the appropriate fractions gave 2 mg of (Vb). This material was identical in all respects with that obtained in i).

2-Amino-c-AMP (Vc)——To a solution of 2-amino-1,N^6-etheno-c-AMP[1] (500 mg) in 0.5 M acetate buffer (pH 3.0, 125 ml) was added NBS (350 mg) and the reaction mixture was stirred at room temperature overnight. Treatment of the solution as in the procedure in the synthesis of (Va) gave 187 mg (40.0%) of (Vc), mp 233—235° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$nm; 253, 293.5, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 257.5, 280. Paper chromatography; Rf=0.30. MS m/e; 632 (M^+, (Vc)$(TMS)_4$). *Anal.* Calcd. for $C_{10}H_{13}N_6O_6P\cdot H_2O$: C, 33.15; H, 4.14; N, 23.20. Found: C, 33.26; H, 3.89; N, 23.03.

2-Methylmercapto-c-AMP (Vd)——To a solution of 2-methylmercapto-1,N^6-etheno-c-AMP[1] (500 mg) in 0.5 M acetate buffer (pH 3.0, 125 ml) was added NBS (350 mg) and the reaction mixture was stirred at room temperature overnight. Treatment of the solution as in the procedure in the synthesis of (Va) gave 200 mg (42.6%) of (Vd), mp 206—208° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 268.5, 290 (shoulder), $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 235, 274.5. Paper chromatography; Rf=0.55. MS m/e; 591 (M^+, (V)d$(TMS)_3$). *Anal.* Calcd. for $C_{11}H_{14}N_5O_6PS\cdot 2.5H_2O$: C, 31.43; H, 4.52; N, 16.67. Found: C, 31.18; H, 4.31; N, 16.52.

2-Methoxy-c-AMP (Ve)——To a solution of 2-methoxy-1,N^6-etheno-c-AMP[1] (500 mg) in 0.5 M acetate buffer (pH 4.5, 50 ml) was added NBS (450 mg) and the reaction mixture was stirred at room temperature overnight. Treatment of the solution as in the procedure in the synthesis of (Va) gave 227 mg (48.4%) of (Ve), mp 187—191° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 245.5, 275, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 252 (shoulder), 266.5. Paper chromatography; Rf=0.45. MS m/e; 575 (M^+, (Ve) $(TMS)_3$). *Anal.* Calcd. for $C_{11}H_{14}N_5O_7P\cdot 1.5H_2O$: C, 34.20; H, 4.40; N, 18.13. Found: C, 34.23; H, 4.07; N, 18.39.

2-Dimethylamino-c-AMP (Vf)——i) To a solution of 2-dimethylamino-1,N^6-etheno-c-AMP[1] (500 mg) in 0.5 M acetate buffer (pH 4.5, 125 ml) was added NBS (225 mg) and the reaction mixture was stirred at room temperature overnight. Treatment of the solution as in the procedure in the synthesis of (Va) gave 290 mg (61.7%) of (Vf), mp 212—215° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 214.5, 269, 305.5, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 226, 263, 295. Paper chromatography; Rf=0.53. MS m/e; 588 (M^+, (Vf)$(TMS)_3$). *Anal.* Calcd. for $C_{12}H_{17}N_6O_6P\cdot 1.5H_2O$: C, 36.09; H, 5.01; N, 21.05. Found: C, 35.93; H, 4.75; N, 21.29.

ii) A solution of 2-bromo-c-AMP (20 mg) in 10 ml of methanol and 0.5 ml of dimethylamine was refluxed for 3 hr. The reaction mixture was evaporated to dryness and the residue was dissolved in water. Adjustment of the pH of the solution to 2.0 caused crystallization of (Vf). The crystals were filtered, washed with water and dried to give 12 mg of (Vf). This material was identical in all respects with that obtained in i).

2-Bromo-c-AMP (Vg)——To a suspension of 2-bromo-1,N^6-etheno-c-AMP[1] (1.0 g) in 2 N HCl (250 ml) was added NBS (1.25 g) and the reaction mixture was stirred at room temperature for 2 min. Treatment of the solution as in the procedure in the synthesis of (Va) gave 520 mg (55.2%) of (Vg), mp 195—197° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 265, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 265.5. Paper chromatography; Rf=0.59. MS m/e; 624 (M^+, (V)g$(TMS)_3$). *Anal.* Calcd. for $C_{10}H_{11}BrN_5O_6P\cdot 0.5H_2O$: C, 28.78; H, 2.88; N, 16.79. Found: C, 28.78; H, 3.01; N, 17.00.

2-Azido-c-AMP (Vh)——To a suspension of 2-azido-1,N^6-etheno-c-AMP[1] (100 mg) in 0.1 N HCl (25 ml) was added NBS (60 mg) and the reaction mixture was stirred at room temperature for 10 min. Treatment of the solution as in the procedure in the synthesis of (V)a gave 45 mg (47.9%) of (V)h, mp 178—180° (dec.). UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 274, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 270. Standing overnight, this absorption curve changed to $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 279, 314.5. This shows the presence of the azidoazomethine-tetrazole equilibrium.[12] Paper chromatography; Rf=0.61. MS m/e; 586 (M^+, (Vh)$(TMS)_3$). *Anal.* Calcd. for $C_{10}H_{11}N_8O_6P\cdot 0.5H_2O$: C, 31.66; H, 3.17; N, 29.55. Found: C, 31.58; H, 3.26; N, 29.42.

α,β-Dibromo-2-aza-1,N^6-etheno-c-AMP——2-Aza-1,N^6-etheno-c-AMP[9] (250 mg) was stirred overnight in DMF–H_2O (1:1, 50 ml) in the presence of NBS (315 mg). The reaction mixture was passed through a column of alumina, washed with water and eluted with EtOH–H_2O–28% NH_4OH (5:5:1, 200 ml). The eluate was concentrated and applied to a Dowex 50-X8 (H^+, 100—200 mesh) column and eluates (eluting solvent, H_2O) were collected. The appropriate fractions were concentrated and again applied to a Dowex 50-X8 (H^+, 100—200 mesh) column and eluted with water. Evaporation of the appropriate fractions gave the product (47.5 mg), UV $\lambda_{max}^{0.1N\text{-}HCl}$ nm; 253.5, $\lambda_{max}^{0.1N\text{-}NaOH}$ nm; 252.0. *Anal.* Calcd. for $C_{11}H_9BrO_6P\cdot H_2O$: C, 24.92; H, 2.07; N, 15.85. Found: C, 24.98; H, 2.00; N, 15.52.

Glyoxal Bis-phenylhydrazone——To the solution of 2-dimethylamino-1,N^6-etheno-c-AMP (20.0 mg) in 0.5 M acetate buffer (pH 4.0, 5.0 ml) was added NBS (8.99 mg) and stirred at room temperature overnight. The reaction mixture was applied to a 1.1×12 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and washed

12) C. Temple, Jr., C.L. Kussner, and J.A. Montgomery, *J. Org. Chem.*, 31, 2210 (1966).

with water (30 ml). The passing and washing were concentrated to dryness, then water (2.0 ml) and 10% phenylhydrazine–EtOH solution (0.4 ml) were added to the residue. The solution was kept standing at room temperature for 5 hr. The filtration of the precipitate gave the bis-phenylhydrazone (4.50 mg). Spectral data of this compound were identical with those obtained from phenylhydrazone derivative of glycolaldehyde or glyoxal.

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