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Studies on the Synthesis of Compounds related to Adenosine 3′,5′-Cyclic Phosphate. III.[1] The Synthesis of 2-Halogenoadenosine 3′,5′-Cyclic Phosphate

Nobuyuki Yamaji,[2a] Kyoko Tahara,[2b] and Motohiko Kato[2a]

Central Research Laboratories of Kikkoman Shoyu Co., Ltd.[2a] *and Research Laboratory of Seishin Pharmaceutical Co., Ltd.*[2b]

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Because of the presence of etheno group, the reactivity of mercapto residue at 2 position in purine skeleton is so high that 2-chloro-1,N^6-etheno adenosine 3′,5′-cyclic phosphate (2-chloro-1,N^6-etheno c-AMP) and 2-iodo-1,N^6-etheno c-AMP were easily synthesized from 2-mercapto-1,N^6-etheno c-AMP. The deblocking reaction of the etheno group from these two halogenated products with N-bromosuccinimide (NBS) gave 2-chloro-c-AMP and 2-iodo-c-AMP. When 2-amino-c-AMP, which was synthesized from 2-amino-1,N^6-etheno c-AMP by the same deblocking reaction, was diazotized with sodium nitrite in 42% fluoroboric acid (HBF_4) at −10——20°, 2-fluoro-c-AMP was obtained in a yield of 17.8%. Since 2-bromo-c-AMP has been similarly obtained in previous work, the deblocking reaction of etheno group offers an excellent new route to synthesis of 2-halogeno-c-AMPs from c-AMP.

Keywords——cyclic AMP derivative; etheno group; N-bromosuccinimide; halogenation; Schiemann reaction; mass spectrum; UV

Numerous halogenopurine and halogenopyrimidine nucleosides have been synthesized in order to obtain substances having a specific biological activity. Pyrimidine nucleosides halogenated at various positions are interesting, because the 5-fluoro- and 5-trifluoromethyl derivatives were reported to be active against cancer cells and viruses.[3] In the purine nucleoside series, 2-halogeno-[4] and 8-halogenoadenosines[5] were synthesized. In the 2-halogenoadenosine 3′,5′-cyclic phosphate (2-halogeno-c-AMP) series, only 2-chloro- and 2-bromo-c-AMP were synthesized by the cyclization reaction of the corresponding 2-substituted adenosine 5′-phosphate[6] and 2-chloro c-AMP was also synthesized from guanosine 3′,5′-cyclic phosphate.[7] However, the direct synthesis of 2-halogeno-c-AMPs from c-AMP has not been reported as yet.

During the studies on the synthesis of 2-substituted 1,N^6-etheno c-AMPs, we have found the high reactivity of the mercapto residue of 2-mercapto-1,N^6-etheno c-AMP. By the

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2) Location: *a) 399, Noda, Noda-shi, Chiba, 278, Japan*; *b) 9–500–1, Nagareyama-shi, Chiba, 270–01, Japan.*
3) L. Bosch, E. Harbers, and C. Heidelberger, *Cancer Res.*, **18**, 335 (1958).
4) *a)* J.A. Montgomery and K. Hewson, *J. Org. Chem.*, **33**, 432 (1968); *b)* J.A. Montgomery, A.T. Shortnacy, and H.J. Thomas, *J. Med. Chem.*, **17**, 1197 (1974); *c)* P.C. Srivastava and R.K. Robins, *J. Carbhydr. Nucleosides Nucleotides*, **4**, 93 (1977); *d)* M.H. Maguire and M.K. Sim, *Eur. J. Biochem.*, **23**, 229 (1971); *e)* R. Marumoto, Y. Yoshioka, S. Shima, K. Imai, K. Kawazoe, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **23**, 759 (1975).
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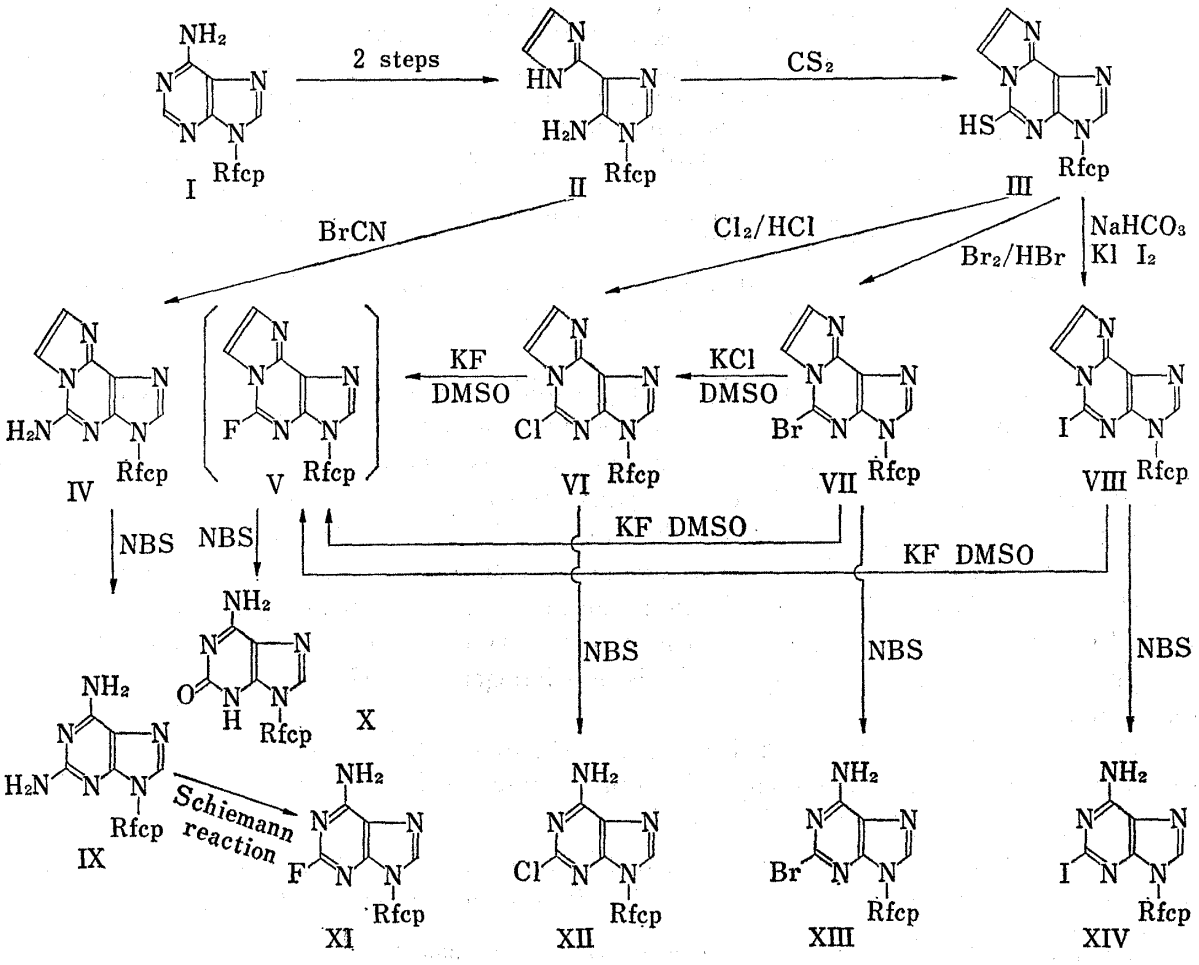
utilization of this reactivity of the mercapto function, we now found a new and general preparative method of 2-chloro-, 2-bromo-, and 2-iodo-c-AMP from 2-mercapto-1,N^6-etheno c-AMP (readily prepared from c-AMP produced from the culture broth of *Corynebacterium murisepticum* or *Microbacteriun sp.*[8]), by replacement of 2-mercapto group with halogens followed by the deblocking reaction of the etheno function with N-bromosuccinimide (NBS). Though 2-fluoro-c-AMP could not be prepared by this method owing to too high reactivity of the fluoro group to be retained for the deblocking reaction, this compound has been synthesized successfully from 2-amino-c-AMP, obtained from the deblocking reaction of the etheno group from 2-amino-1,N^6-etheno c-AMP,[1] by the application of the Schiemann reaction.

Thus, we now established convenient synthetic routes to all kinds of 2-halogeno-c-AMPs from c-AMP, which seem to be important compounds in obtaining further informations concerning the unique properties of c-AMP analogues in the biological system. The details of these procedures, as shown schematically in Chart 1, will be reported in this paper.



Rfcp: β-D-ribofuranosyl cyclic 3',5'-phosphate

Chart 1

5-Amino-1-β-D-(3',5'-cyclic phospho)-ribofuranosyl-4-(imidazol-2-yl)-imidazole (II) was synthesized from c-AMP by the method of Yip and Tsou.[9] This compound was converted to 2-mercapto-1,N^6-etheno c-AMP (III) by the method described in a previous paper.[10] The

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) K.F. Yip and K.C. Tsou, *Tetrahedron Lett.*, **1973**, 3087.
10) N. Yamaji, Y. Yuasa, and M. Kato, *Chem. Pharm. Bull.* (Tokyo), **24**, 1561 (1976).

reaction of III with chlorine in an ice-cooled conc. hydrochloric acid gave 2-chloro-1,N^6-etheno c-AMP (VI) in a high yield. The bubbling of chlorine gas was terminated at the time when the ultraviolet (UV) spectrum of the reaction mixture completely changed to that of 2-chloro-1,N^6-etheno c-AMP. The same compound (VI) was also obtained by treatment of VII with potassium chloride in dimethyl sulfoxide (DMSO) at 50° for 5 days. The removal of the etheno group from VI by treatment with NBS in 2N hydrochloric acid afforded 2-chloro-c-AMP (XII). Although the reaction of III with iodine in an ice-cooled 57% hydriodic acid gave 2-iodo-1,N^6-etheno c-AMP (VIII) in a poor yield, a more satisfactory synthesis of VIII was realized by an application of the method previously employed in the synthesis of 2'-deoxy-8-iodo-adenosine.[5c] Thus, by treatment of III with iodine in potassium iodide solution in the presence of sodium bicarbonate, VIII was obtained in 67% yield. 2-Iodo-c-AMP (XIV) was obtained from VIII by the deblocking reaction of the etheno group in the usual manner. Since 2-bromo-c-AMP (XIII) was synthesized previously by us essentially in the same manner as above *via* the corresponding etheno compound (VII), we now complete syntheses of c-AMPs having chlorine (XII), bromine (XIII), and iodine (XIV) at the 2-position *via* the corresponding etheno compounds (VI, VII, and VIII).

The structures of 2-halogeno-c-AMPs (XII—XIV) and the etheno compounds (VI—VIII) were determined by elemental analyses and mass spectra. The UV spectra (Table I) are also indicative of the 2-halogenopurine structure.

Table I. Physical Constants of the 2-Halogeno-1,N^6-Etheno c-AMP and 2-Halogeno-c-AMP

Compound No.	Residue	*Rf*[a]		Retention time (min)	λ_{max} (nm), ($\varepsilon \times 10^{-3}$)[b]	
		A	B		0.1N HCl	0.1N NaOH
VI	Cl-1,N^6-etheno	0.44	0.50	14.5[c]	225.0(30.5), 279.0(11.9)	
VIII	I-1, N^6-etheno	0.42	0.52	43.0[c]	223.5(23.3), 233.0 sh (22.3) 241.0 sh (21.8), 284.5(12.5),	
XI	F	0.53	0.45	9.5[d]	261.5(13.4), 269.0 sh (11.7)	261.5(15.0), 269.0 sh (11.8)
XII	Cl	0.54	0.47	16.8[d]	264.5(14.9)	264.5(15.9)
XIII	Br	0.55	0.45	18.4[d]	265.0(14.3)	265.5(14.8)
XIV	I	0.46	0.46	33.0[d]	265.5(14.3)	267.0(14.8)

a) *Rf* on Toyo Filter No. 51A paper in solvent system A (*n*-butanol/acetic acid/water, 5: 2: 3) or B (0.5M ammonium acetate/ethanol, 2: 5).
b) sh refers to a shoulder.
c) Retention time on high pressure liquid chromatograms using the eluent (0.07M NaCl/0.008M HCl).
d) Retention time on high pressure liquid chromatograms using the eluent (1.00M NaCl/0.016M HCl).

These three etheno compounds (VI—VIII) with potassium fluoride in DMSO solution at 30° for several days afforded the common product showing retention time (9.5 min) on high pressure liquid chromatogram. Although the crude reaction product was obtained by precipitation by the addition of ethanol and ether to the reaction mixture, the attempted purification of it failed owing to its high reactivity. Thus, for example, this changed to 2-hydroxy-1,N^6-etheno c-AMP either in 1N sodium hydroxide solution or 1N hydrochloric acid within 10—30 min at room temperature, and to 2-methoxy-1,N^6-etheno c-AMP in 50% aq. methanol within a few hours at room temperature. This extraordinary reactivity of the product may be due to the strong inductive effect of fluorine reminiscent of that of 8-fluoro-adenosine reported by Kobayashi *et al.*[11] In addition to these facts, the UV spectrum ($\lambda_{max}^{0.1N\ HCl}$: 278.0

11) Y. Kobayashi, I. Kumadaki, A. Ohsawa, S. Murakami, K. Yamamoto, and T. Nakano, The Third Symposium on Nucleic Acids Chemistry of Japan, Sapporo, 1975, p. 17.

nm: the maximum appeared in a comparatively shorter wavelength region than those of VI, VII, and VIII) and the behavior on the high pressure liquid chromatography (relative retention times of VI, VII, and VIII are 14.5, 22.0, and 43.0 min, respectively) indicated clearly that the product was 2-fluoro-1,N^6-etheno c-AMP (V). Attempted removal of the etheno group from the crude product (V) resulted in the formation of 2-hydroxy-c-AMP (X), and the desired 2-fluoro-c-AMP (XI) was detected only on high pressure liquid chromatogram in a trace amount.

In order to obtain XI, we investigated the application of the Schieman reaction to 2-amino-c-AMP (IX) which was easily synthesized through 4 steps from c-AMP by our deblocking reaction of the etheno group.[1] When we used about 2 moles of sodium nitrite to IX as in the case of the synthesis of 2-fluoropurine ribosides,[4a-c] the desired product (XI) was obtained in a poor yield. The use of a smaller amount of sodium nitrite, however, was found to give a better result (Table II).

Table II. The Synthesis of 2-Fluoro-c-AMP (XI) from 2-Amino-c-AMP (IX)[a]

$NaNO_2$ (equivalent mol)	Compound IX (mg)	Yield (%) of XI[b] (hr)			
		0.5	1.0	2.0	4.0
0.75	100.0	32.1	41.2	42.6	43.0
1.00	100.0	48.6	52.1	53.9	52.4
1.25	100.0	38.6	44.9	46.5	40.1
1.50	100.0	36.4	36.3	33.1	33.0

a) Reaction conditions: $NaNO_2$ in 0.5 ml of H_2O was added to a solution of IX in 10.0 ml of 42% HBF_4 for 5 min at −10—−20° and stirring of the reaction mixture was continued at 0—−5°.
b) The yield of 2-fluoro-c-AMP (XI) was determined by high pressure liquid chromatography.

After the Schiemann reaction had been completed, the reaction mixture was passed through a column of charcoal using 28% NH_4OH–H_2O–EtOH (1: 10: 10 v/v) as eluent. The eluate was then applied to a Dowex-50 (H^+, 100—200 mesh) column (eluting solvent: H_2O) and the eluates were collected. Finally the eluate was applied to a silica gel column and evaporation of the appropriate fractions using MeOH–$CHCl_3$ (15: 85 v/v) gave XI in an actual isolation yield of 17.8% (This reaction condition corresponds to the optimal conditions shown in Table II). The structure of XI was determined by elemental analysis, UV (Table I) and mass spectrum. The direct applicability of the Schiemann reaction to 2-amino-c-AMP without acetylation as verified in the present experiment indicates the stability of both the nucleoside linkage as well as the cyclic phosphate linkage in these compounds (IX and XI).

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV absorption spectra were determined on a Hitachi Model-R-323 spectrophotometer. Mass spectra were measured by the method of Lawson *et al.*[12] on a Hitachi Model RMU-7M mass spectrometer. 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 (0.5 M ammonium acetate/ethanol, 2: 5). The yields of 2-substituted c-AMPs and their retention times were determined on a Hitachi 634 high pressure liquid chromatogram; packing, #2632; column, 2.1 mm ID × 500 mmL; flow rate, 1.1 ml/min; pressure, 50 kg/cm²; temperature, 70°; detector, 254 nm filter; and eluent, 2-halogeno-1,N^6-etheno c-AMPs: 0.07 M NaCl/0.008 M HCl, 2-halogeno-c-AMPs: 1.00 M NaCl/0.016 M HCl.

Crude 2-Fluoro-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (V)——A mixture of 250 mg (0.55 mmol) of 2-bromo-1,N^6-etheno c-AMP (VII),[10] 250 mg (4.30 mmol) of potassium fluoride, and 75 ml of dimethyl sulfoxide was stirred for 24 hr at 30°. The precipitated compound was filtered off. Ethanol (50 ml) and

12) A.M. Lawson, R.N. Stollwell, M.M. Tacker, K. Tsuboyama, and J.A. McClosky, *J. Amer. Chem. Soc.*, 93, 1014 (1971).

ether (400 ml) were added to the filtrate and the filtration of the precipitate gave 222.6 mg of crude V. UV $\lambda_{max}^{0.1N\ HCl}$ nm: 278.0.

2-Chloro-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (VI)——To an ice-cooled and stirred solution of III (2.0 g) in conc. HCl (50 ml) was bubbled Cl_2 gas. The bubbling of Cl_2 gas was terminated at the time when the UV spectrum of the reaction mixture changed to $\lambda_{max}^{H_2O}$; 278.5 nm from $\lambda_{max}^{H_2O}$; 310.0 nm. Water (50 ml) was added to the solution and the solution was applied to a 2.2×14 cm column of charcoal. The column was washed with water and was eluted with water–ethanol–28% NH_4OH (20: 20: 1 v/v). The appropriate fractions were neutralized with Dowex 50-X8 (H^+) and filtered. The filtrate was concentrated and applied to a 1.4×15 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and the column was eluted with water. Evaporation of the appropriate fractions gave 1.345 g (67.0%) of VI, mp 181—183° (dec.). MS *m/e*: 531.5 (M^+, VI-(trimethylsilyl (TMS))$_2$). *Anal.* Calcd. for $C_{12}H_{11}N_5O_6PCl\cdot H_2O$: C, 35.51; H, 3.21; N, 17.26. Found: C, 35.37; H, 3.20; N, 17.08.

2-Iodo-1,N^6-etheno Adenosine 3′,5′-Cyclic Phosphate (VIII)——A mixture of 1.0 g (2.38 mmol) of III, 50 ml of H_2O, 3.0 g (35.7 mmol) of $NaHCO_3$, 2.0 g (12.1 mmol) of KI, and 3.0 g (11.8 mmol) of I_2 was stirred overnight at room temperature. The reaction mixture was applied to a 1.2×10 cm column of charcoal. The column was washed with water and the nucleotide (VIII) 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 VIII. This was washed with ethanol and dissolved in 1 N NaOH. The solution was applied to a 1.5×10 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and eluted with water. Evaporation of the appropriate fractions gave 833 mg (67.2%) of VIII, mp 183—185° (dec.). MS *m/e*: 623 (M^+, VIII-(TMS)$_2$). *Anal.* Calcd. for $C_{12}H_{11}N_5O_6PI\cdot 2H_2O$: C, 27.96; H, 2.91; N, 13.59. Found: C, 27.96; H, 2.61; N, 13.31.

2-Fluoro-adenosine 3′,5′-Cyclic Phosphate (XI)——2-Amino-c-AMP (500 mg, 1.38 mmol) was dissolved with stirring in cold (−10 to −20°) 42% aqueous fluoroboric acid (20 ml). The resulting solution was maintained below −10° and a solution of sodium nitrite (100 mg, 1.45 mmol) in water (1 ml) was added dropwise. After the addition was complete (5 min), the solution was stirred for an additional 4 hr below 0°. The reaction mixture was applied to a 1.2×15 cm column of charcoal. The column was washed with water and the nucleotide (XI) was eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The appropriate fractions were concentrated and applied to a 1.4×30 cm column of Dowex 50-X8 (H^+, 100—200 mesh). The nucleotide was eluted with water and the appropriate fractions were concentrated. To the residue was added silica gel (200 mg) and the suspension was evaporated to a powder. The powder was applied to a silica gel column (5.0 g, packing in chloroform) and the nucleotide was eluted with chloroform–methanol (85: 15 v/v). Evaporation of the appropriate fractions gave 92 mg (17.8%) of XI, mp 195—198° (dec.). MS *m/e*: 563 (M^+, XI-(TMS)$_3$). *Anal.* Calcd. for $C_{10}H_{11}N_5O_6PF\cdot 1.5H_2O$: C, 32.09; H, 3.74; N, 18.72. Found: C, 32.39; H, 3.56; N, 18.48.

2-Chloro-adenosine 3′,5′-Cyclic Phosphate (XII)——To a suspension of VI (1.0 g, 2.47 mmol) in 2 N HCl (100 ml) was added NBS (500 mg, 2.81 mmol) and the reaction mixture was stirred at room temperature for 5 min. The solution was made alkaline (pH>13) with 6 N NaOH and stirred for 4 hr. Finally, the pH of the solution was adjusted to 2.0 with conc. HCl and the whole was passed through a column of charcoal (1.4×30 cm). The column was washed with water and eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The eluate was concentrated and applied to a 1.4×30 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and the column was eluted with water. Evaporation of the appropriate fractions gave 423 mg (45.7%) of XII, mp 197—199° (dec.). MS *m/e*: 579.5 (M^+, XII-(TMS)$_3$). *Anal.* Calcd. for $C_{10}H_{11}N_5O_6PCl\cdot 1.5H_2O$: C, 31.45; H, 3.41; N, 18.35. Found: C, 31.72; H, 3.33; N, 18.38.

2-Iodo-adenosine 3′,5′-Cyclic Phosphate (XIV)——To a suspension of VIII (500 mg, 0.97 mmol) in 2 N HCl (50 ml) was added NBS (250 mg, 1.41 mmol) and the reaction mixture was stirred at room temperature for 5 min. The solution was made alkaline (pH>13) with 6 N NaOH and stirred for 4 hr. Finally, the pH of the solution was adjusted to 2.0 with conc. HCl and the whole was passed through a column of charcoal (1.4×30 cm). The column was washed with water and eluted with water–ethanol–28% NH_4OH (10: 10: 1 v/v). The eluate was concentrated and the residue was applied to a 1.4×30 cm column of Dowex 50-X8 (H^+, 100—200 mesh) and the column was eluted with water. Evaporation of the appropriate fractions gave 207 mg (44.2%) of XIV, mp 192—195° (dec.). MS *m/e*: 671 (M^+, XIV-(TMS)$_3$). *Anal.* Calcd. for $C_{10}H_{11}N_5O_6PI\cdot 1.5H_2O$: C, 24.90; H, 2.90; N, 14.52. Found: C, 24.76; H, 2.62; N, 14.29.