[Chem. Pharm. Bull. 36(6)2212—2219(1988)]

Studies on the Synthesis of Compounds Related to Adenosine 3′,5′-Cyclic Phosphate. V.[1] Synthesis and Cardiac Effect of N^6-Alkyladenosine 3′,5′-Cyclic Phosphates and Their 8-Benzylthio Derivatives

SHIGEHIRO KATAOKA,*,[a] JUNKO ISONO,[a] NOBUYUKI YAMAJI,[a] MOTOHIKO KATO,[a] TOMIE KAWADA,[b] and SHOICHI IMAI[b]

Research and Development Division, Kikkoman Corporation,[a] 399 Noda, Noda City, Chiba 278, Japan and Department of Pharmacology, Niigata University School of Medicine,[b] 757 Asahimachidori-1-bancho, Niigata City, Niigata 951, Japan

(Received December 1, 1987)

A series of N^6-alkyladenosine 3′,5′-cyclic phosphates (N^6-alkyl cAMPs) (**3**) and N^6-alkyl-8-benzylthio cAMPs (**4**) was synthesized from cAMP (**1**) and 8-benzylthio cAMP (**2**) by means of a one-pot reaction. This reaction proceeded by reductive alkylation in acetic acid with aldehydes and sodium cyanoborohydride. These cAMP derivatives were evaluated for inotropic and chronotropic effects on the isolated guinea pig papillary muscle and right atria. Several N^6-alkyl cAMPs (**3**) were surprisingly more active than compounds **4** in these actions. Among them, N^6-hexyl cAMP (**3f**) and N^6-heptyl cAMP (**3g**) showed strongly positive inotropic effects (PIE) and moderately negative chronotropic effects.

Keywords——N^6-alkyl cAMP; N^6,8-disubstituted cAMP; reductive alkylation; positive inotropic effect; chronotropic effect

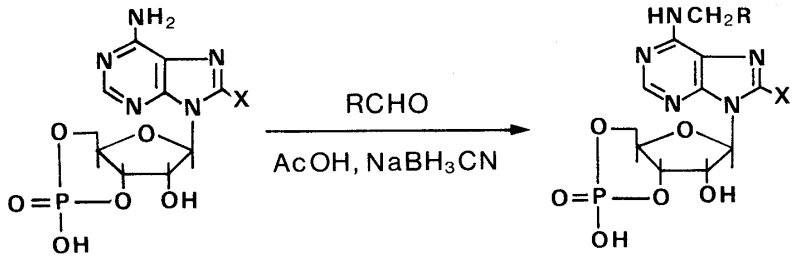
The central role of adenosine 3′,5′-cyclic phosphate (cAMP, **1**) as a second messenger in many diverse biological systems has been established.[2] N^6,2′-*O*-Dibutyryl cAMP and 8-substituted cAMPs such as 8-benzylthio cAMP (**2**) produce a positive inotropic effect (PIE),[3,4] and the regulatory role of endogenous **1** in cardiac contractility has been well established.[5] Therefore, there is now an increased need for research directed toward improving the membrane penetrability of exogenous cAMP derivatives. Miller *et al.* examined the PIE of several N^6,8-disubstituted cAMP derivatives and concluded that 8-benzylthio-N^6-butyl cAMP (BTB cAMP, **4c**) was the most potent cardiotonic agent among them.[6] This fact prompted us to investigate the cardiotonic activity of more N^6-alkyl derivatives and to develop a convenient method for the N^6-alkylation of **1** in order to prepare various derivatives of N^6-alkyl cAMP (**3**). N^6-Alkyl cAMP derivatives have so far been prepared by the following two routes; i) synthesis of N^1-substituted cAMP from **1** with alkyl halide and subsequent Dimroth rearrangement,[6,7] ii) synthesis of 6-chloropurine 3′,5′-cyclic phosphate from inosine 3′,5′-cyclic phosphate with phosphorus oxychloride, followed by substitution with alkylamines.[7]

These methods include tedious steps and are restricted to the introduction of short-chain alkyl groups. As a part of our studies on the synthesis of cAMP derivatives,[8] we examined alkylation at the N^6-position of **1** and found a one-step method of N^6-alkyl cAMP (**3**) synthesis from **1**, which was easily obtained from the culture broth of *Microbacterium* sp. No. 205 (ATCC 21376).[9] In this paper, we report the syntheses of N^6-alkyl cAMPs (**3**) and N^6-alkyl-8-benzylthio cAMPs (**4**) by reductive alkylation and we describe the inotropic

TABLE I. Physical Constants of cAMP Derivatives

Compd. No.	RCH_2	X	Yield (%)	Rf[a]	t_R[b] (min)	Formula[c]	Analysis (%) Calcd (Found)		
							C	H	N
3a	Ethyl	H	48	0.50	4.2	$C_{12}H_{16}N_5O_6P \cdot 1/4\,H_2O$	39.84 (39.70	4.60 4.47	19.36 19.17)
3b	Propyl	H	65	0.57	7.5	$C_{13}H_{18}N_5O_6P \cdot 3/4\,H_2O$	40.58 (40.64	5.11 5.00	18.20 18.09)
3c	Isobutyl	H	43	0.64	13.9	$C_{14}H_{20}N_5O_6P \cdot 2/3\,H_2O$	42.32 (42.15	5.41 5.20	17.63 17.50)
3d	Butyl	H	74	0.60	14.9	$C_{14}H_{20}N_5O_6P \cdot 2/3\,H_2O$	42.32 (42.26	5.41 5.22	17.63 17.61)
3e	Pentyl	H	44	0.70	21.6	$C_{15}H_{22}N_5O_6P \cdot 3/2\,H_2O$	42.26 (42.01	5.91 5.66	16.43 16.23)
3f	Hexyl	H	64	0.72	27.5	$C_{16}H_{24}N_5O_6P \cdot 2/3\,H_2O$	45.18 (45.08	6.00 5.84	16.46 16.31)
3g	Heptyl	H	63	0.74	32.9	$C_{17}H_{26}N_5O_6P \cdot 1/2\,H_2O$	46.76 (46.86	6.24 6.07	16.05 16.02)
3h	Octyl	H	49	0.77	37.3	$C_{18}H_{28}N_5O_6P \cdot 1/2\,H_2O$	48.00 (47.91	6.49 6.37	15.55 15.32)
3i	Nonyl	H	51	0.79	41.7	$C_{19}H_{30}N_5O_6P \cdot 1/2\,H_2O$	49.13 (48.99	6.73 6.53	15.08 15.01)
3j	Decyl	H	46	0.82	45.7	$C_{20}H_{32}N_5O_6P \cdot 1/4\,H_2O$	50.68 (50.90	6.91 6.91	14.77 14.45)
3k	Tetradecyl	H	39	0.87	57.7	$C_{24}H_{40}N_5O_6P \cdot H_2O$	53.03 (53.09	7.79 7.51	12.88 12.76)
3l	Furfuryl	H	57	0.60	8.9	$C_{15}H_{16}N_5NaO_7P \cdot 3/2\,H_2O$	39.31 (39.27	3.95 3.67	15.28 14.96)
3m	Benzyl	H	53	0.62	16.6	$C_{17}H_{18}N_5O_6P \cdot H_2O$	46.72 (46.50	4.58 4.66	16.03 15.84)
4a	Propyl	SBn	56	0.40	4.8	$C_{20}H_{24}N_5O_6PS \cdot H_2O$	46.96 (47.02	5.12 5.15	13.69 13.42)
4b	Isobutyl	SBn	41	0.43	8.7	$C_{21}H_{26}N_5O_6PS \cdot H_2O$	48.00 (47.86	5.37 5.37	13.33 13.22)
4c	Butyl	SBn	83	0.46	9.3	$C_{21}H_{26}N_5O_6PS \cdot H_2O$	48.00 (48.02	5.37 5.43	13.33 13.25)
4d	Pentyl	SBn	78	0.48	15.4	$C_{22}H_{28}N_5O_6PS \cdot H_2O$	48.98 (48.76	5.60 5.58	12.98 12.74)
4e	Hexyl	SBn	75	0.50	19.3	$C_{23}H_{30}N_5O_6PS \cdot H_2O$	49.90 (49.65	5.84 5.75	12.65 12.41)
4f	Heptyl	SBn	66	0.54	22.1	$C_{24}H_{32}N_5O_6PS \cdot H_2O$	50.79 (50.74	6.04 6.00	12.34 12.09)
4g	Octyl	SBn	62	0.58	24.9	$C_{25}H_{34}N_5O_6PS \cdot H_2O$	51.63 (51.63	5.97 6.24	12.04 12.04)
4h	Nonyl	SBn	47	0.59	27.7	$C_{26}H_{36}N_5O_6PS \cdot 4/3\,H_2O$	51.91 (51.96	6.14 6.32	11.64 11.68)
4i	Decyl	SBn	52.5	0.62	30.7	$C_{27}H_{38}N_5O_6PS \cdot 4/3\,H_2O$	52.67 (52.36	6.65 6.37	11.38 11.64)
4j	Tetradecyl	SBn	37	0.71	40.8	$C_{31}H_{46}N_5O_6PS \cdot 5/3\,H_2O$	54.94 (54.69	7.33 7.07	10.33 10.46)
4k	Furfuryl	SBn	58	0.35	6.3	$C_{22}H_{22}N_5O_7PS \cdot H_2O$	48.09 (47.91	4.40 4.37	12.75 12.57)
4l	Benzyl	SBn	87	0.36	11.6	$C_{24}H_{24}N_5O_6PS \cdot 5/3\,H_2O$	50.44 (50.19	4.81 4.70	12.25 11.98)
4m	Phenetyl	SBn	34	0.41	15.0	$C_{25}H_{26}N_5O_6PS \cdot 2/3\,H_2O$	52.91 (52.90	4.86 4.70	12.34 12.05)

SBn = SCH_2Ph, *a*) *Rf* on Toyo No. 51A filter paper; compounds **3**, solvent system (*n*-BuOH : AcOH : H_2O = 5 : 2 : 2); compounds **4**, solvent system (isoamyl alcohol : AcOH : H_2O = 7 : 2 : 2). *b*) Retention time on HPLC; compounds **3** [eluent, i = MeOH, ii = 10 mM acetate buffer (pH 4) containing 1 mM tetra-*n*-butylammonium chloride (TBAC); 40%→100% linear gradient of i over 60 min; detector at 265 nm] and compounds **4** [eluent, i = CH_3CN, ii = 2.5 mM acetate buffer (pH 4) containing 0.25 mM TBAC; 35%→75% linear gradient of i over 40 min; detector at 290 nm]. *c*) Samples were dried over P_2O_5 at 50 °C at 3 mmHg for 3—5 h.



1: X=H
2: X=SCH_2Ph
3: X=H
4: X=SCH_2Ph

Chart 1

and chronotropic actions of these compounds. The synthetic route for the preparation of compounds **3** and **4** is shown in Chart 1.

Treatment of the tri-*n*-butylammonium salt of **1** with aldehydes and sodium cyanoborohydride in acetic acid gave **3**. Compound **4** was also synthesized from 8-benzylthio cAMP (**2**), which was prepared from **1** *via* two steps,[10] by the method described above. These reactions were carried out under acidic conditions, and acetic acid (a good solvent of the starting materials) was used as an acidic solvent. Though they proceeded with sodium borohydride, sodium cyanoborohydride was used owing to its stability under acidic conditions. Physical properties of **3** and **4** are summarized in Table I. Reductive alkylation of **2** proceeded at room temperature, while that of **1** was slow at room temperature and required warming to 50 °C. The long-chain alkyl-substituted derivatives (**3g—k**, **4f—j**) which were not accessible by the previous method were synthesized easily by means of this one-pot reaction. In N^6-alkyl cAMPs **3** and **4**, the butyl derivatives **3d** and **4c** were obtained in the best yields (74% and 83%), and the yields in this reaction decreased with increase of the alkyl chain length. This method is excellent to prepare a variety of N^6-alkyl cAMP derivatives. The method previously used to synthesize BTB cAMP (**4c**), the most potent cardiotonic agent, required the following five steps **1**→N^1-crotyl cAMP→N^6-crotyl cAMP→N^6-butyl cAMP (**3d**)→N^6-butyl-8-bromo cAMP→**4c**, and the preparation of N^6-butyl cAMP (**3d**) required three steps.[6] The butylation process we found involves one step and the synthesis of **4c** from **1** involves three steps (**1**→8-bromo cAMP→**2**→**4c**). The overall yield of **4c** from **1** was increased to 44.5% by the present method, compared with 30% by the previous method. Moreover, as aldehydes are readily available and the alkylation step is a one-pot reaction, the present method is advantageous in comparison with the procedures hitherto reported in view of its wide applicability, suitability for large-scale preparation and simplicity of operation.

Biological Results and Discussion

The PIE and chronotropic effects of most of the compounds **3a—4m** listed in Table I were tested by the use of the papillary muscle of the right ventricle and right atria of guinea pig hearts. The results are shown in Tables II and III. In N^6-alkyl cAMP (**3**), alkyl derivatives in the range of butyl (**3d**) to decyl (**3j**) and aralkyl derivatives such as furfuryl (**3l**) and benzyl (**3m**) were found to exhibit PIE. Among them, the octyl derivative (**3h**) exhibited the maximum PIE. As regards the chronotropic effect, the compounds **3h—j** with longer alkyl groups than octyl and the aralkyl derivatives **3l** and **3m** showed a positive effect, while the compounds **3d—g** with shorter alkyl groups than heptyl showed a negative effect. Such a definite dissociation between the inotropic and chronotropic actions in this series of compounds **3** is very interesting in connection with the further development of superior cardiotonic agents. We previously examined the PIE of 8-substituted cAMPs and reported that 8-benzylthio cAMP (**2**) was a highly active compound.[4] Therefore, N^6-alkyl-8-benzylthio cAMPs (**4**) with compound **2** as a key structure were tested for the above biological activities in the expectation of a superior effect. Miller *et al.*[6] reported that the propyl (**4a**) and butyl

TABLE II. Inotropic and Chronotropic Effects of N^6-Alkyl cAMP Derivatives **3**

Compd.	RCH_2	PIE ED_{30} ($\times 10^{-4}$ M)[a]	Chronotropic effect (%)[b]
3a	Ethyl	$>10^{-3}$	—
3b	Propyl	$>10^{-3}$	—
3c	Isobutyl	$>10^{-3}$	—
3d	Butyl	4.46±0.05	Arrest
3e	Pentyl	2.07±0.52	−63.57±15.39
3f	Hexyl	1.89±0.30	−52.88±10.27
3g	Heptyl	1.60±0.19	−20.10± 7.36
3h	Octyl	0.95±0.21	44.95± 5.07
3i	Nonyl	1.46±0.33	29.92± 6.54
3j	Decyl	6.60±1.60	41.90± 5.49
3k	Tetradecyl	c)	—
3l	Furfuryl	2.48±0.34	48.47± 5.56
3m	Benzyl	3.75±0.49	8.70± 4.62

a) PIE=positive inotropic effect; ED_{30}=concentration required for producing 30% of the maximum response evoked by 10^{-7} M isoproterenol (ISP). Mean±S.E. ($n=5$). *b*) These values were obtained at the dose of PIE ED_{30}, and positive and negative effects were expressed as percent change (plus or minus) from the maximum response evoked by 10^{-7} M ISP. Mean±S.E. ($n=5$). *c*) Caused a negative inotropic effect.

TABLE III. Inotropic and Chronotropic Effects of N^6-Alkyl-8-benzylthio cAMP Derivatives **4**

Compd.	RCH_2	PIE ED_{30} ($\times 10^{-4}$ M)[a]	Chronotropic effect (%)[b]
4a	Propyl	3.23±0.67	−9.90±10.89
4b	Isobutyl	6.73±1.28	7.18± 5.94
4c	Butyl	3.64±0.60	−5.40± 2.26
4d	Pentyl	c)	—
4e	Hexyl	c)	—
4f	Heptyl	c)	—
4g	Octyl	NT	NT
4h	Nonyl	NT	NT
4i	Decyl	NT	NT
4j	Tetradecyl	NT	NT
4k	Furfuryl	6.35±0.64	20.30± 3.22
4l	Benzyl	7.95±0.25	1.18± 4.73
4m	Phenetyl	7.70±0.57	Arrest

a) See footnote *a* in Table II. *b*) See footnote *b* in Table II. *c*) Caused a negative inotropic effect. NT=not tested.

(**4c**) derivatives exhibited PIE, and compound **4c** was the most potent cardiotonic agent among the cAMP derivatives. We confirmed their data, but the enhanced PIE expected from the data for compounds **3** was not seen in long-chain alkyl and aralkyl derivatives of compound **4** (Table III).

Compound **2** showed both the PIE and positive chronotropic effect,[4] while the chronotropic effect of compounds **4a** and **4c** changed to negative. This may be due to the counteracting negative action resulting from the introduction of a short-chain alkyl group at the N^6-position of **1**, as shown in Table II, which overcomes the positive chronotropic effect of compound **2**. The PIEs of the pnetyl (**3e**), furfuryl (**3l**) and benzyl (**3m**) derivatives were equal to or stronger than that of BTB cAMP (**4c**). It is worthy of special mention that the PIEs of the hexyl (**3f**), heptyl (**3g**), octyl (**3h**), and nonyl (**3i**) derivatives were more than twice that of **4c**. Among these compounds, N^6-hexyl (**3f**) and N^6-heptyl cAMP (**3g**) may be the most

promising as positive inotropic agents superior to **4c**, since the positive chronotropic effect is not desirable by reason of an increase of myocardial oxygen consumption. *In vivo* tests of these compounds are in progress. The activities of **3f** and **3g** raise the possibility that superior cardiotonic agents may emerge from further screening of cAMP derivatives in the near future.

Experimental

Proton nuclear magnetic resonance spectra (^{1}H-NMR) were taken at 200 MHz on a JEOL JNM-FX200 NMR spectrometer in dimethyl sulfoxide-d_6. All ^{1}H-NMR data are reported in ppm downfield from tetramethylsilane as an internal standard. High-performance liquid chromatography (HPLC) was performed on a μ-Bondasphere 5 μ C18-100 Å column (3.9 mm × 15 cm) with a flow rate of 1.0 ml/min using a Waters pump (model M600) and a Waters detector (model M490 spectrophotometer), and the eluate was monitored at 265 nm (compounds **3**) or 290 nm (compounds **4**). Ultraviolet (UV) absorption spectra were recorded with a Hitachi 557 spectrophotometer. Infrared (IR) spectra were taken on a JASCO A-202 spectrophotometer. Paper chromatograms were run on Toyo No. 51A filter paper (40 × 40 cm) with the developing solvent system *n*-BuOH–AcOH–H_2O (5 : 2 : 2, v/v) or isoamyl alcohol–AcOH–H_2O (7 : 2 : 2, v/v). Analytical and preparative thin-layer chromatographies (TLC) were performed on Kiesel gel 60F_{254} (Merck) plates.

General Procedure for Preparation of N^6-Alkyl cAMPs (3)——A solution of the tri-*n*-butylammonium salt[11] of **1** (5 g, 9.73 mmol) in 100 ml of AcOH was heated at 50 °C with stirring, and an aldehyde (7.5—10 mol eq) was added to the solution. After 0.5 h, sodium cyanoborohydride (4—5 mol eq) was added. The mixture was stirred at 50 °C for 6—8 h. A small amount of H_2O was added and the reaction solution was evaporated *in vacuo*. The residue was purified by the methods described below.

General Procedure for Preparation of N^6-Alkyl-8-benzylthio cAMPs (4)——An aldehyde (7.5—15 mol eq) was added to a solution of the tri-*n*-butylammonium salt (3.18 g, 5 mmol) of **2**[10] in 100 ml of AcOH with stirring at room temperature. After 0.5 h, sodium cyanoborohydride (4—7 mol eq) was added. The mixture was stirred at room temperature for 4—6 h. A small amount of H_2O was added to the mixture and the reaction solution was evaporated *in vacuo*. The residue was purified by the methods described below.

Method of Purification (A)——The residue was dissolved in a small amount of H_2O, adjusted to pH 2 with 2 N HCl, and applied to a charcoal column (3.2 × 15 cm). After being washed with H_2O, the column was eluted with EtOH–H_2O–28% aqueous NH_3 (10 : 10 : 1, v/v). The eluate was collected and evaporated to dryness *in vacuo*. The resulting residue was dissolved in MeOH, adjusted to pH 2 with 2 N HCl, and subjected to preparative TLC. The plates were developed with MeOH–$CHCl_3$ (1 : 4—2 : 3, v/v), and the appropriate band was extracted with MeOH. The extract was evaporated to dryness *in vacuo*. The residue was dissolved in 2 N NaOH–H_2O, and the solution was adjusted to pH 2 with 2 N HCl to give a product.

Compounds **3a**—**h**, **j**, **l**, **m**, **4k**, and **4l** were purified in the same way (method A).

3a: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3335, 1660. ^{1}H-NMR δ: 1.19 (3H, t, J=7.2 Hz, CH_3), 3.52 (2H, brs, NCH_2), 6.03 (1H, s, H-1′), 8.30 and 8.43 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (15850).

3b: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3330, 2970, 2870, 1665. ^{1}H-NMR δ: 0.90 (3H, t, J=7.2 Hz, CH_3), 1.61 (2H, m, $C\underline{H}_2CH_3$), 3.46 (2H, brs, NCH_2), 6.02 (1H, s, H-1′), 8.29 and 8.43 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17600).

3c: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3245, 2950, 1675. ^{1}H-NMR δ: 0.90 (6H, d, J=6.6 Hz, $(CH_3)_2$), 1.84—2.08 (1H, m, CH), 3.38 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 8.06 (1H, brs, NH), 8.25 and 8.37 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17600).

3d: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3335, 2950, 2860, 1665. ^{1}H-NMR δ: 0.90 (3H, t, J=7.2 Hz, CH_3), 1.20—1.45 (2H, m, $C\underline{H}_2CH_3$), 1.47—1.70 (2H, m, $NCH_2C\underline{H}_2$), 3.53 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 7.95 (1H, brs, NH), 8.24 and 8.35 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17100).

3e: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3240, 2955, 2860, 1675. ^{1}H-NMR δ: 0.70—1.00 (3H, m, CH_3), 1.10—1.44 (4H, m, $(C\underline{H}_2)_2CH_3$), 1.45—1.70 (2H, m, $NCH_2C\underline{H}_2$), 3.47 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 8.15 (1H, brs, NH), 8.27 and 8.40 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (16700).

3f: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3335, 2950, 2920, 1665. ^{1}H-NMR δ: 0.70—0.95 (3H, m, CH_3), 1.12—1.45 (6H, m, $(C\underline{H}_2)_3CH_3$), 1.50—1.72 (2H, m, $NCH_2C\underline{H}_2$), 3.50 (2H, brs, NCH_2), 6.02 (1H, s, H-1′), 8.06 (1H, brs, NH), 8.26 and 8.37 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17700).

3g: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3330, 2950, 2920, 1665. ^{1}H-NMR δ: 0.77—0.92 (3H, m, CH_3), 1.10—1.44 (8H, m, $(C\underline{H}_2)_4CH_3$), 1.50—1.73 (2H, m, $NCH_2C\underline{H}_2$), 3.50 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 8.02 (1H, brs, NH), 8.25 and 8.37 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17800).

3h: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3335, 2920, 1665. ^{1}H-NMR δ: 0.78—0.94 (3H, m, CH_3), 1.12—1.45 (10H, m, $(C\underline{H}_2)_5CH_3$), 1.50—1.70 (2H, m, $NCH_2C\underline{H}_2$), 3.52 (2H, brs, NCH_2), 6.02 (1H, s, H-1′), 8.10 (1H, brs, NH), 8.26 and 8.38 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 268 (17200).

3j: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3340, 2920, 1665. ^{1}H-NMR δ: 0.77—0.92 (3H, m, CH_3), 1.10—1.40 (14H,

m, $(CH_2)_7CH_3$), 1.50—1.70 (2H, m, $NHCH_2CH_2$), 3.50 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 7.96 (1H, brs, NH), 8.24 and 8.35 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17300).

3l: A pale yellow amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 2900, 1620. ^{1}H-NMR δ: 4.76 (2H, brs, NCH_2), 5.81 (1H, brs, OH-2′), 5.93 (1H, s, H-1′), 6.23 (1H, d, J=3.4 Hz, $OCHCHCH$), 6.35 (1H, dd, J=3.4, 2 Hz, $OCHCH$), 7.51 (1H, s like, OCH), 8.18 (1H, brs, NH), 8.26 (2H, s, purine H's overlap). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 266 (16500).

3m: A pale yellow amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 2900, 1620. ^{1}H-NMR δ: 4.78 (2H, brs, NCH_2), 5.83 (1H, d, J=4.4 Hz, OH-2′), 5.93 (1H, s, H-1′), 7.15—7.40 (5H, m, phenyl H's), 8.23 and 8.25 (1H each, s, purine H's), 8.20—8.40 (1H, m, NH). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 268 (18600).

4k: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3350, 2945, 1665. ^{1}H-NMR δ: 4.58 (2H, s, SCH_2), 4.77 (2H, brs, NCH_2), 5.78 (1H, s, H-1′), 6.26 (1H, d, J=3.2 Hz, $OCHCHCH$), 6.37 (1H, dd, J=3.2, 2 Hz, $OCHCH$), 7.20—7.37 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 7.53 (1H, s like, OCH), 8.10—8.25 (1H, m, NH), 8.21 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 289 (17800).

4l: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3240, 3020, 2890, 1620. ^{1}H-NMR δ: 4.57 (2H, s, SCH_2), 4.70—4.90 (2H, brs, NCH_2), 5.78 (1H, s, H-1′), 7.20—7.50 (10H, m, phenyl H's), 8.18 (1H, s, C_2H), 8.20—8.30 (1H, m, NH). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 290 (17400).

Method of Purification (B)——The residue was dissolved in H_2O and adjusted to pH 2 with 2 N HCl. The resulting precipitate was collected by filtration and dissolved in a mixture of CH_2Cl_2 (50 ml) and tri-*n*-butylamine (2 ml). The solution was washed with H_2O (250 ml × 4—8) and the organic layer was dried over Na_2SO_4 and evaporated. The residue was suspended in H_2O–EtOH mixture and dissolved in 2 N NaOH. The solution was adjusted to pH 2 with 2 N HCl to give a product.

Compounds **3i**, **4a**—**i**, and **4m** were purified in the same way (method B).

3i: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3335, 2920, 1665. ^{1}H-NMR δ: 0.77—0.92 (3H, m, CH_3), 1.10—1.42 (12H, m, $(CH_2)_6CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.50 (2H, brs, NCH_2), 6.01 (1H, s, H-1′), 8.01 (1H, brs, NH), 8.25 and 8.36 (1H each, s, purine H's). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 267 (17750).

4a: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3225,2950, 2920, 1670. ^{1}H-NMR δ: 0.91 (3H, t, J=7.2 Hz, CH_3), 1.50—1.73 (2H, m, CH_2CH_3), 3.46 (1H, brs, NCH_2), 4.58 (2H, s, SCH_2), 5.77 (1H, s, H-1′), 7.23—7.38 (3H, m, phenyl H's), 7.39—7.50 (2H, m, phenyl H's), 8.07 (1H, brs, NH), 8.21 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16200).

4b: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3220, 2950, 1665. ^{1}H-NMR δ: 0.91 (6H, d, J=6.6 Hz, $(CH_3)_2$), 1.85—2.10 (1H, m, CH), 3.33 (2H, brs, NCH_2CH_2), 4.59 (2H, s, SCH_2), 5.77 (1H, s, H-1′), 7.20—7.39 (3H, m, phenyl H's), 7.40—7.55 (2H, m, phenyl H's), 8.08 (1H, brs, NH), 8.21 (1H, s, C_2H), UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16600).

4c: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3370, 2950, 2925, 1670. ^{1}H-NMR δ: 0.91 (3H, t, J=7.2 Hz, CH_3), 1.25—1.48 (2H, m, CH_2CH_3), 1.50—1.70 (2H, m, NCH_2CH_2), 3.50 (2H, brs, NCH_2), 4.59 (2H, s, SCH_2), 5.78 (1H, s, H-1′), 7.20—7.40 (3H, m, phenyl H's), 7.43—7.50 (2H, m, phenyl H's), 8.02 (1H, brs, NH), 8.21 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16300).

4d: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3375, 2950, 2920, 1665. ^{1}H-NMR δ: 0.77—0.96 (3H, m, CH_3), 1.20—1.40 (4H, m, $(CH_2)_2CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.48 (2H, brs, NCH_2), 4.58 (2H, s, SCH_2), 5.77 (1H, s, H-1′), 7.20—7.37 (3H, m, phenyl H's), 7.37—7.49 (2H, m, phenyl H's), 8.00 (1H, brs, NH), 8.20 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16500).

4e: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1660. ^{1}H-NMR δ: 0.74—0.92 (3H, m, CH_3), 1.15—1.43 (6H, m, $(CH_2)_3CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.54 (2H, brs, NCH_2), 4.56 (2H, s, SCH_2), 5.78 (1H, s, H-1′), 7.20—7.35 (3H, m, phenyl H's), 7.40—7.49 (2H, m, phenyl H's), 7.78 (1H, brs, NH), 8.17 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16600).

4f: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1665. ^{1}H-NMR δ: 0.72—0.93 (3H, m, CH_3), 1.10—1.40 (8H, m, $(CH_2)_4CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.47 (2H, brs, NCH_2), 4.58 (2H, s, SCH_2), 5.77 (1H, s, H-1′), 7.24—7.38 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 8.02 (1H, brs, NH), 8.20 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16300).

4g: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1665. ^{1}H-NMR δ: 0.78—0.95 (3H, m, CH_3), 1.10—1.47 (10H, m, $(CH_2)_5CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.55 (2H, brs, NCH_2), 4.57 (2H, s, SCH_2), 5.79 (1H, s, H-1′), 7.23—7.37 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 7.73—7.88 (1H, m, NH), 8.17 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16300).

4h: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3200, 2920, 1675. ^{1}H-NMR δ: 0.78—0.95 (3H, m, CH_3), 1.10—1.45 (12H, m, $(CH_2)_6CH_3$), 1.50—1.70 (2H, m, NCH_2CH_2), 3.55 (2H, brs, NCH_2), 4.57 (2H, s, SCH_2), 5.80 (1H, s, H-1′), 7.24—7.38 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 7.87 (1H, brs, NH), 8.18 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16100).

4i: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3200, 2920, 1675. ^{1}H-NMR δ: 0.75—0.98 (3H, m, CH_3), 1.10—1.45 (14H, m, $(CH_2)_7CH_3$), 1.50—1.73 (2H, m, NCH_2CH_2), 3.55 (2H, brs, NCH_2), 4.57 (2H, s, SCH_2), 5.80 (1H, s, H-1′), 7.23—7.39 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 7.84 (1H, brs, NH), 8.18 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 290 (15350).

4m: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3220, 3020, 2890, 1665. ^{1}H-NMR δ: 2.94 (2H, t, J=7.2 Hz, NCH_2CH_2), 3.80 (2H, brs, NCH_2), 4.56 (2H, s, SCH_2), 5.79 (1H, s, H-1′), 7.13—7.38 (8H, m, phenyl H's), 7.40—7.50 (2H, m,

phenyl H's), 7.75—7.88 (1H, m, NH), 8.20 (1H, s, C_2H). UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 292 (16600).

N^6-Tetradecyl cAMP (3k)——A solution of tetradecanal (11 eq, 107 mmol) in $CHCl_3$ (6 ml) was added to a solution of the tri-*n*-butylammonium salt of **1** (5 g, 9.73 mmol) in 100 ml of AcOH and the reaction was performed as in the general procedure for compounds **3**. A small amount of H_2O was added to the mixture and the reaction solution was evaporated *in vacuo*. The residue was suspended in alkaline H_2O (pH 10, 50 ml), and the suspension was extracted with Et_2O (200 ml × 4), then the H_2O layer was adjusted to pH 2 with 2 N HCl and evaporated *in vacuo*. The residue was dissolved in a mixture of CH_2Cl_2 (60 ml) and tri-*n*-butylamine (2 ml) and the solution was washed with H_2O (250 ml × 5). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*, and the residue was purified by silica gel (28 g) chromatography using MeOH–$CHCl_3$ (1 : 9, v/v) as the eluent. The eluate was evaporated *in vacuo*, and the residue was dissolved in 1 N NaOH. Adjustment of the pH of the solution to 2 with 2 N HCl gave **3k** (39%). IR ν_{max}^{KBr} cm^{-1}: 3340, 2920, 1665. ^{1}H-NMR δ: 0.77—0.90 (3H, m, CH_3), 1.10—1.40 (22H, m, $(CH_2)_{11}CH_3$), 1.50—1.68 (2H, m, NCH_2CH_2), 3.48 (2H, br s, NCH_2), 6.01 (1H, s, H-1′), 8.00 (1H, br s, NH), 8.25 and 8.36 (1H each, s, purine H's). UV λ_{max}^{EtOH} nm (ε): 265 (16800).

8-Benzylthio-N^6-tetradecyl cAMP (4j)——A solution of tetradecanal (6 mol eq) in $CHCl_3$ (6 ml) was added to a solution of the tri-*n*-butylammonium salt of **2** (3.18 g, 5 mmol) in 100 ml of AcOH and the reaction was performed as in the general procedure for compounds **4**, but the temperature was maintained at 50 °C. A small amount of H_2O was added to the mixture and the solution was evaporated *in vacuo*. The residue was suspended in H_2O (150 ml), and the suspension was extracted with $CHCl_3$ (150 ml × 2). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in dioxane (50 ml) at 50 °C and the solution was cooled to room temperature to give a precipitate. The precipitate was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in EtOH (50 ml) at 50 °C and treated as described above. The residue was dissolved in H_2O and the solution was adjusted to pH 2 with 2 N HCl. The milky solution was extracted with $CHCl_3$ and the organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by preparative TLC with MeOH–$CHCl_3$ (1 : 4, v/v) as the developing solvent to give **4j** (37%). IR ν_{max}^{KBr} cm^{-1}: 3200, 2915, 1675. ^{1}H-NMR δ: 0.80—0.90 (3H, m, CH_3), 1.10—1.40 (22H, m, $(CH_2)_{11}CH_3$), 1.50—1.70 (2H, m, $NHCH_2CH_2$), 3.56 (2H, br s, NCH_2), 4.56 (2H, s, SCH_2), 5.72 (1H, s, H-1′), 7.23—7.37 (3H, m, phenyl H's), 7.40—7.50 (2H, m, phenyl H's), 8.15 (1H, s, C_2H), 8.26 (1H, s, NH). UV λ_{max}^{EtOH} nm (ε): 289 (16500).

Biological Activity——Male albino guinea pigs weighing 320—680 g were stunned by a blow on the head. The hearts were rapidly removed, and the right atria and the papillary muscle of the right ventricle were dissected out in cold bathing solution and suspended individually in 8 ml organ baths for recording isometric contractions. The bathing solution was the Krebs–Henseleit's solution (32 ± 0.1 °C) containing NaCl 118 (mM); KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25; $MgSO_4$ 1.2; KH_2PO_4 1.2; glucose 11, and was continuously bubbled with 95% O_2 + 5% CO_2. The initial tensions of 0.5 and 0.25 g were applied to the atria preparations and papillary muscle preparations, respectively. After 30 min, the optimal resting tension was determined and maintained thereafter. The right atrium was allowed to beat spontaneously, and the papillary muscle was stimulated by square-wave pulses of 1 msec duration at the frequency of 1 Hz, and at voltages of about 50% above the threshold supplied by a square-wave pulse stimulator (Nihon Kohden MSE-3) *via* a pair of silver-plated electrodes between which the preparations were placed. The isometric contraction was measured by a force-displacement transducer (Toyo Baldwin T7-30-240) connected to a carrier-amplifier (Nihon Kohden RP-5) and the heart rate was counted by a cardiotachometer (Nihon Kohden RT-5). All the measurements were recorded on a thermostylus recorder (Watanabe Sokki Linear Corder Mark V). An equilibration period of 60 min was allowed before starting the experiments. Because of the low solubility of cAMP derivatives, they were dissolved in Krebs–Henseleit's solution and applied to the preparation by replacing less than 1.2 ml of the bathing solution. The PIE of cAMP derivatives was expressed as percent of the maximum response evoked by 10^{-7} M isoproterenol in each preparation. The positive and negative chronotropic effects of them were expressed as percent change (plus or minus) from the maximum response evoked by 10^{-7} M isoproterenol.

Acknowledgments The authors are grateful to Professor T. Hino of Chiba University for his helpful advice and to Associate Professor M. Nakagawa of Chiba University for her encouragement. The authors are also indebted to Dr. S. Nasuno for his interest and help.

References

1) Part IV: N. Yamaji, K. Tahara, and M. Kato, *Chem. Pharm. Bull.*, **28**, 115 (1980).
2) P. Greengard, R. Paoletti, and G. A. Robison, "Advances in Cyclic Nucleotide Research," Vol. 1, Raven Press, New York, 1972.
3) *a*) W. R. Kukovetz and G. Poech, *Arch. Pharmakol. Exp. Pathol.*, **266**, 236 (1970); *b*) S. Imai, T. Otorii, K. Takeda, Y. Katano, and D. Horii, *Jpn. J. Pharmacol.*, **24**, 499 (1974).
4) N. Yamaji, M. Kato, I. Matsubara, N. Shimamoto, K. Miura, and S. Imai, *Chem. Pharm. Bull.*, **28**, 1683 (1980).
5) *a*) P. W. Erhardt, *J. Med. Chem.*, **30**, 231 (1987); *b*) G. I. Drummond and D. L. Severson, *Circ. Res.*, **44**, 145 (1979); *c*) R. W. Tsien, *Adv. Cyclic Nucleotide Res.*, **8**, 363 (1977).

6) J. P. Miller, K. H. Boswell, R. B. Meyer, L. F. Christensen, and R. K. Robins, *J. Med. Chem.*, **23**, 242 (1980).
7) K. H. Boswell, L. F. Christensen, D. A. Shuman, and R. K. Robins, *J. Heterocycl. Chem.*, **12**, 1 (1975).
8) S. Kataoka, J. Isono, N. Yamaji, and M. Kato, *Chem. Lett.*, **1986**, 1221.
9) J. Ishiyama, T. Yokotsuka, and N. Saito, *Agric. Biol. Chem.*, **38**, 507 (1974).
10) K. Muneyama, R. J. Baver, D. A. Shuman, R. K. Robins, and L. N. Simon, *Biochemistry*, **10**, 2390 (1971).
11) J. G. Falbriard, T. Posternak, and E. W. Sutherland, *Biochim. Biophys. Acta*, **65**, 558 (1962).