

[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

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(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 cardiogenic agent among them.⁶⁾ This fact prompted us to investigate the cardiogenic 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 (%)	<i>R_f</i> ^{a)}	<i>t_R</i> ^{b)} (min)	Formula ^{c)}	Analysis (%) Calcd (Found)		
							C	H	N
3a	Ethyl	H	48	0.50	4.2	C ₁₂ H ₁₆ N ₅ O ₆ P · 1/4 H ₂ O	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 ₅ O ₆ P · 3/4 H ₂ O	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 ₅ O ₆ P · 2/3 H ₂ O	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 ₅ O ₆ P · 2/3 H ₂ O	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 ₅ O ₆ P · 3/2 H ₂ O	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 ₅ O ₆ P · 2/3 H ₂ O	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 ₅ O ₆ P · 1/2 H ₂ O	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 ₅ O ₆ P · 1/2 H ₂ O	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 ₅ O ₆ P · 1/2 H ₂ O	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 ₅ O ₆ P · 1/4 H ₂ O	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 ₅ O ₆ P · H ₂ O	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 ₅ NaO ₇ P · 3/2 H ₂ O	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 ₅ O ₆ P · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · H ₂ O	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 ₅ O ₆ PS · 4/3 H ₂ O	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 ₅ O ₆ PS · 4/3 H ₂ O	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 ₅ O ₆ PS · 5/3 H ₂ O	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 ₅ O ₇ PS · H ₂ O	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 ₅ O ₆ PS · 5/3 H ₂ O	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 ₅ O ₆ PS · 2/3 H ₂ O	52.91 (52.90)	4.86 (4.70)	12.34 (12.05)

SBn = SCH₂Ph, a) *R_f* on Toyo No. 51A filter paper; compounds 3, solvent system (*n*-BuOH : AcOH : H₂O = 5 : 2 : 2); compounds 4, solvent system (isoamyl alcohol : AcOH : H₂O = 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 = CH₃CN, 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 P₂O₅ at 50 °C at 3 mmHg for 3–5 h.

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

Compd.	RCH ₂	PIE ED ₃₀ (× 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; ED₃₀=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 ED₃₀, 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 (× 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 pntetyl (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 (^1H -NMR) were taken at 200 MHz on a JEOL JNM-FX200 NMR spectrometer in dimethyl sulfoxide- d_6 . All ^1H -NMR data are reported in ppm downfield from tetramethylsilane as an internal standard. High-performance liquid chromatography (HPLC) was performed on a μ -Bondasphere $5\ \mu$ C18-100 Å column (3.9 mm $\times$ 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 $\times$ 40 cm) with the developing solvent system *n*-BuOH-AcOH-H₂O (5:2:2, v/v) or isoamyl alcohol-AcOH-H₂O (7:2:2, v/v). Analytical and preparative thin-layer chromatographies (TLC) were performed on Kiesel gel 60F₂₅₄ (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 H₂O 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 H₂O 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 H₂O, adjusted to pH 2 with 2 *N* HCl, and applied to a charcoal column (3.2 $\times$ 15 cm). After being washed with H₂O, the column was eluted with EtOH-H₂O-28% aqueous NH₃ (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-CHCl₃ (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-H₂O, 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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3335, 1660. ^1H -NMR δ : 1.19 (3H, t, $J=7.2$ Hz, CH₃), 3.52 (2H, brs, NCH₂), 6.03 (1H, s, H-1'), 8.30 and 8.43 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (15850).

3b: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3330, 2970, 2870, 1665. ^1H -NMR δ : 0.90 (3H, t, $J=7.2$ Hz, CH₃), 1.61 (2H, m, CH₂CH₃), 3.46 (2H, brs, NCH₂), 6.02 (1H, s, H-1'), 8.29 and 8.43 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (17600).

3c: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3245, 2950, 1675. ^1H -NMR δ : 0.90 (6H, d, $J=6.6$ Hz, (CH₃)₂), 1.84–2.08 (1H, m, CH), 3.38 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 8.06 (1H, brs, NH), 8.25 and 8.37 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (17600).

3d: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3335, 2950, 2860, 1665. ^1H -NMR δ : 0.90 (3H, t, $J=7.2$ Hz, CH₃), 1.20–1.45 (2H, m, CH₂CH₃), 1.47–1.70 (2H, m, NCH₂CH₂), 3.53 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 7.95 (1H, brs, NH), 8.24 and 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (17100).

3e: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3240, 2955, 2860, 1675. ^1H -NMR δ : 0.70–1.00 (3H, m, CH₃), 1.10–1.44 (4H, m, (CH₂)₂CH₃), 1.45–1.70 (2H, m, NCH₂CH₂), 3.47 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 8.15 (1H, brs, NH), 8.27 and 8.40 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (16700).

3f: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3335, 2950, 2920, 1665. ^1H -NMR δ : 0.70–0.95 (3H, m, CH₃), 1.12–1.45 (6H, m, (CH₂)₃CH₃), 1.50–1.72 (2H, m, NCH₂CH₂), 3.50 (2H, brs, NCH₂), 6.02 (1H, s, H-1'), 8.06 (1H, brs, NH), 8.26 and 8.37 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (17700).

3g: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3330, 2950, 2920, 1665. ^1H -NMR δ : 0.77–0.92 (3H, m, CH₃), 1.10–1.44 (8H, m, (CH₂)₄CH₃), 1.50–1.73 (2H, m, NCH₂CH₂), 3.50 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 8.02 (1H, brs, NH), 8.25 and 8.37 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 267 (17800).

3h: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3335, 2920, 1665. ^1H -NMR δ : 0.78–0.94 (3H, m, CH₃), 1.12–1.45 (10H, m, (CH₂)₅CH₃), 1.50–1.70 (2H, m, NCH₂CH₂), 3.52 (2H, brs, NCH₂), 6.02 (1H, s, H-1'), 8.10 (1H, brs, NH), 8.26 and 8.38 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{0.1\ \text{N NaOH}}$ nm (ϵ): 268 (17200).

3j: A white powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3340, 2920, 1665. ^1H -NMR δ : 0.77–0.92 (3H, m, CH₃), 1.10–1.40 (14H,

m, (CH₂)₇CH₃), 1.50—1.70 (2H, m, NHCH₂CH₂), 3.50 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 7.96 (1H, brs, NH), 8.24 and 8.35 (1H each, s, purine H's). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 267 (17300).

3l: A pale yellow amorphous solid. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 2900, 1620. ¹H-NMR δ : 4.76 (2H, brs, NCH₂), 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 $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 266 (16500).

3m: A pale yellow amorphous solid. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 2900, 1620. ¹H-NMR δ : 4.78 (2H, brs, NCH₂), 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 $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 268 (18600).

4k: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3350, 2945, 1665. ¹H-NMR δ : 4.58 (2H, s, SCH₂), 4.77 (2H, brs, NCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 289 (17800).

4l: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3240, 3020, 2890, 1620. ¹H-NMR δ : 4.57 (2H, s, SCH₂), 4.70—4.90 (2H, brs, NCH₂), 5.78 (1H, s, H-1'), 7.20—7.50 (10H, m, phenyl H's), 8.18 (1H, s, C₂H), 8.20—8.30 (1H, m, NH). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 290 (17400).

Method of Purification (B)—The residue was dissolved in H₂O and adjusted to pH 2 with 2N HCl. The resulting precipitate was collected by filtration and dissolved in a mixture of CH₂Cl₂ (50 ml) and tri-*n*-butylamine (2 ml). The solution was washed with H₂O (250 ml $\times$ 4—8) and the organic layer was dried over Na₂SO₄ and evaporated. The residue was suspended in H₂O—EtOH mixture and dissolved in 2N NaOH. The solution was adjusted to pH 2 with 2N HCl to give a product.

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

3i: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3335, 2920, 1665. ¹H-NMR δ : 0.77—0.92 (3H, m, CH₃), 1.10—1.42 (12H, m, (CH₂)₆CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.50 (2H, brs, NCH₂), 6.01 (1H, s, H-1'), 8.01 (1H, brs, NH), 8.25 and 8.36 (1H each, s, purine H's). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 267 (17750).

4a: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3225, 2950, 2920, 1670. ¹H-NMR δ : 0.91 (3H, t, $J=7.2$ Hz, CH₃), 1.50—1.73 (2H, m, CH₂CH₃), 3.46 (1H, brs, NCH₂), 4.58 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16200).

4b: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3220, 2950, 1665. ¹H-NMR δ : 0.91 (6H, d, $J=6.6$ Hz, (CH₃)₂), 1.85—2.10 (1H, m, CH), 3.33 (2H, brs, NCH₂CH₂), 4.59 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16600).

4c: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3370, 2950, 2925, 1670. ¹H-NMR δ : 0.91 (3H, t, $J=7.2$ Hz, CH₃), 1.25—1.48 (2H, m, CH₂CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.50 (2H, brs, NCH₂), 4.59 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16300).

4d: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3375, 2950, 2920, 1665. ¹H-NMR δ : 0.77—0.96 (3H, m, CH₃), 1.20—1.40 (4H, m, (CH₂)₂CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.48 (2H, brs, NCH₂), 4.58 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16500).

4e: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400, 2920, 1660. ¹H-NMR δ : 0.74—0.92 (3H, m, CH₃), 1.15—1.43 (6H, m, (CH₂)₃CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.54 (2H, brs, NCH₂), 4.56 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16600).

4f: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400, 2920, 1665. ¹H-NMR δ : 0.72—0.93 (3H, m, CH₃), 1.10—1.40 (8H, m, (CH₂)₄CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.47 (2H, brs, NCH₂), 4.58 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16300).

4g: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400, 2920, 1665. ¹H-NMR δ : 0.78—0.95 (3H, m, CH₃), 1.10—1.47 (10H, m, (CH₂)₅CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.55 (2H, brs, NCH₂), 4.57 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16300).

4h: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3200, 2920, 1675. ¹H-NMR δ : 0.78—0.95 (3H, m, CH₃), 1.10—1.45 (12H, m, (CH₂)₆CH₃), 1.50—1.70 (2H, m, NCH₂CH₂), 3.55 (2H, brs, NCH₂), 4.57 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 292 (16100).

4i: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3200, 2920, 1675. ¹H-NMR δ : 0.75—0.98 (3H, m, CH₃), 1.10—1.45 (14H, m, (CH₂)₇CH₃), 1.50—1.73 (2H, m, NCH₂CH₂), 3.55 (2H, brs, NCH₂), 4.57 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\max}^{0.1\text{ NNaOH}}$ nm (ϵ): 290 (15350).

4m: A white powder. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3220, 3020, 2890, 1665. ¹H-NMR δ : 2.94 (2H, t, $J=7.2$ Hz, NCH₂CH₂), 3.80 (2H, brs, NCH₂), 4.56 (2H, s, SCH₂), 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, C₂H). UV $\lambda_{\text{max}}^{0.1\text{N NaOH}}$ nm (ϵ): 292 (16600).

N⁶-Tetradecyl cAMP (3k)—A solution of tetradecanal (11 eq, 107 mmol) in CHCl₃ (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 H₂O was added to the mixture and the reaction solution was evaporated *in vacuo*. The residue was suspended in alkaline H₂O (pH 10, 50 ml), and the suspension was extracted with Et₂O (200 ml $\times$ 4), then the H₂O layer was adjusted to pH 2 with 2N HCl and evaporated *in vacuo*. The residue was dissolved in a mixture of CH₂Cl₂ (60 ml) and tri-*n*-butylamine (2 ml) and the solution was washed with H₂O (250 ml $\times$ 5). The organic layer was dried over Na₂SO₄ and evaporated *in vacuo*, and the residue was purified by silica gel (28 g) chromatography using MeOH–CHCl₃ (1:9, v/v) as the eluent. The eluate was evaporated *in vacuo*, and the residue was dissolved in 1N NaOH. Adjustment of the pH of the solution to 2 with 2N HCl gave **3k** (39%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3340, 2920, 1665. ¹H-NMR δ : 0.77–0.90 (3H, m, CH₃), 1.10–1.40 (22H, m, (CH₂)₁₁CH₃), 1.50–1.68 (2H, m, NCH₂CH₂), 3.48 (2H, br s, NCH₂), 6.01 (1H, s, H-1'), 8.00 (1H, br s, NH), 8.25 and 8.36 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 265 (16800).

8-Benzylthio-N⁶-tetradecyl cAMP (4j)—A solution of tetradecanal (6 mol eq) in CHCl₃ (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 H₂O was added to the mixture and the solution was evaporated *in vacuo*. The residue was suspended in H₂O (150 ml), and the suspension was extracted with CHCl₃ (150 ml $\times$ 2). The organic layer was dried over Na₂SO₄ 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 H₂O and the solution was adjusted to pH 2 with 2N HCl. The milky solution was extracted with CHCl₃ and the organic layer was washed with H₂O, dried over Na₂SO₄, and evaporated *in vacuo*. The residue was purified by preparative TLC with MeOH–CHCl₃ (1:4, v/v) as the developing solvent to give **4j** (37%). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3200, 2915, 1675. ¹H-NMR δ : 0.80–0.90 (3H, m, CH₃), 1.10–1.40 (22H, m, (CH₂)₁₁CH₃), 1.50–1.70 (2H, m, NHCH₂CH₂), 3.56 (2H, br s, NCH₂), 4.56 (2H, s, SCH₂), 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, C₂H), 8.26 (1H, s, NH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ 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 $\pm$ 0.1 °C) containing NaCl 118 (mM); KCl 4.7; CaCl₂ 2.5; NaHCO₃ 25; MgSO₄ 1.2; KH₂PO₄ 1.2; glucose 11, and was continuously bubbled with 95% O₂ + 5% CO₂. 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 cardiometer (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.

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