Studies on the Synthesis of Compounds Related to Adenosine-3′,5′-cyclic Phosphate. VI.[1] Synthesis and Cardiac Effects of N^6,N^6,2′-*O*-Trialkyl-, N^6,2′-*O*-Dialkyl-, and 2′-*O*-Alkyladenosine-3′,5′-cyclic Phosphates

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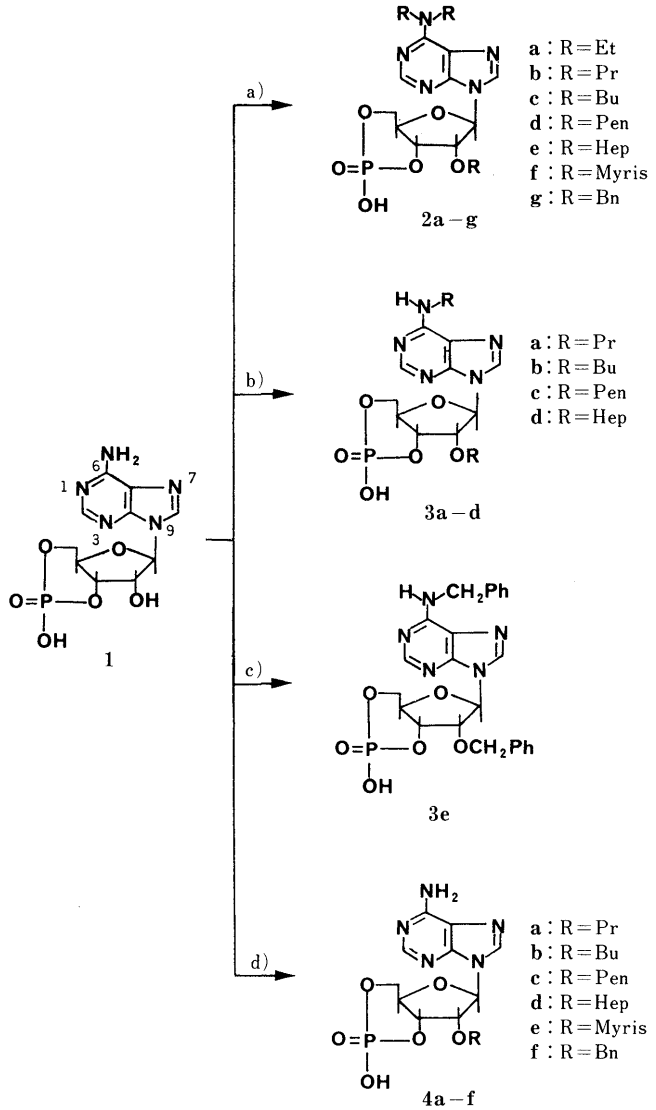
The alkylation of adenosine-3′,5′-cyclic phosphate (cAMP, 1) with alkyl bromides was investigated and various new alkylated cAMP derivatives, N^6,N^6,2′-*O*-trialkyl cAMPs (2), N^6,2′-*O*-dialkyl cAMPs (3) and 2′-*O*-alkyl cAMPs (4), were prepared by a one step reaction without the introduction of a protecting group into 1. Compounds (2) were synthesized from 1 by treatment with alkyl bromides in the presence of NaH or potassium *tert*-butoxide in dimethyl sulfoxide. Compounds (3) were also synthesized from 1 under conditions similar to those of the synthesis of 2 except for the use of MeONa as a base. Compounds (4) were prepared from 1 by treatment with alkyl bromides in the presence of 18-crown-6 in dioxane–aqueous KOH solution. N^6,2′-*O*-Dibenzyl cAMP (3e) was obtained from 1 by the same method as the preparation of 4. These new alkylated derivatives were evaluated for cardiotonic activity *in vitro*. Some of them showed weak positive inotropic effects and strong negative chronotropic effects. Thus, the presence of the 2′-hydroxyl group seemed to be essential for the appearance of potent positive inotropic activity caused by cAMP derivatives.

Keywords alkylation; N^6,N^6,2′-*O*-trialkyl cAMP; N^6,2′-*O*-dialkyl cAMP; 2′-*O*-alkyl cAMP; inotropic effect; chronotropic effect

Adenosine-3′,5′-cyclic phosphate (cAMP, **1**) is involved in many biological reactions as a second messenger of various hormones.[2] A great many cAMP derivatives have been synthesized to obtain more biological effects than that of cAMP itself in whole cell systems and some of them were found to have pharmacological activities.[3] In addition to the regulatory role of **1** in cardiac contractility,[4] N^6,2′-*O*-dibutyryl cAMP (DB cAMP),[3e] 8-benzylthio cAMP,[3f] N^6-alkyl cAMP,[1,3g] and N^6-alkyl-8-benzylthio cAMP[3h] have been found to produce a positive inotropic effect (PIE). We have studied the alkylation of **1** to obtain more lipophilic derivatives of **1** which might be expected to have enhanced biological activities due to their higher permeability to cell membranes.[1] There are three reactive protons capable of being transformed in **1**: (a) the 6-amino proton, (b) the 2′-hydroxyl proton and (c) the phosphoryl proton. In previous papers we reported the conversion of the phosphoryl group with alkyl halides to alkyl triesters[5] and the reductive alkylation of the 6-amino group with aldehydes to N^6-monoalkyl cAMPs.[1] Among the latter compounds, the hexyl to nonyl derivatives exhibited potent PIE.[1] Therefore, we designed the syntheses of various alkylated cAMPs at the 2′-hydroxyl or both the 6-amino and 2′-hydroxyl groups to obtain a more potent cardiotonic agent and to find the structure–activity relationships. Although the preparation of 2′-*O*-methyl and 2′-*O*-ethyl cAMPs[6] has been reported, the *O*,*N*-tri- and *O*,*N*-dialkylated cAMPs have not been synthesized. We report now the alkylation of **1** to synthesize N^6,N^6,2′-*O*-trialkyl cAMPs (**2**), N^6,2′-*O*-dialkyl cAMPs (**3**), and 2′-*O*-alkyl cAMPs (**4**) by appropriate combinations of bases, solvents and reaction conditions.

Synthesis of Alkylated cAMPs When tri-*n*-butylammonium salt of **1** was treated with alkyl bromides in the presence of a strong base such as NaH in dimethyl sulfoxide (DMSO) at room temperature, trialkylated derivatives (**2**) were obtained as major products **2a** (58% yield), **2b** (46%), **2c** (47%), and **2d** (44%) (Chart 1). Potassium *tert*-butoxide (*tert*-BuOK), instead of NaH, gave better results for the preparation of longer alkyl derivatives (**2e**, 52%), (**2f**, 43%) and the benzyl derivative (**2g**, 38%). The ultraviolet absorption maximum of **2** occurred at 277 nm (pH 13) and 268 nm (pH 1), which was characteristic of N^6,N^6-dialkyl cAMP[7] and differed from 1, N^6-dimethyl adenosine.[8] The structures of **2** were further supported by elemental analyses (Table III) and proton nuclear magnetic resonance (^{1}H-NMR).

Thus far, the syntheses of N^6,2′-*O*-dialkyl cAMPs (**3**) have not been reported. Various combinations of bases, solvents and reaction times were examined for butylation of **1** with butyl bromide (Table I). The dibutyl derivative (**3b**) was obtained in good yields (69.8% and 68.0% on high performance liquid chromatography (HPLC)) by using MeONa–DMSO and *n*-butylLi (*n*-BuLi)–DMSO as base-solvent systems, but the trialkyl derivative (**2c**) was also detected as a by-product (10.7% and 27.5% on HPLC). Therefore, we selected the former system (MeONa–DMSO) for the preparation of **3** (Chart 1). Thus, tri-*n*-butylammonium salt of **1** was treated with excess MeONa and alkyl bromides in DMSO at room temperature, or 50 °C, to afford **3a** (17% yield), **3b** (20%), **3c** (32%), and **3d** (31%). The trial of **1** with benzyl bromide (BnBr) under similar conditions, however, gave tribenzyl derivative (**2g**, 62% on HPLC) as the major product, and the desired compound (**3e**, 22% on HPLC) as the minor product. The *O*,*N*-dibenzyl derivative (**3e**) was obtained in 41% yield by the reaction of **1** with excess BnBr at 50 °C in aqueous dioxane solvent containing KOH and 18-crown-6 which was the best condition for the 2′-*O*-monoalkylation of **1** (see below). Among the compounds (**3a**—**e**), the dibutyl derivative (**3b**) was expected to have some biological action because of its structural resemblance to DB cAMP which has cardiotonic activity. Compounds (**3**) had almost the same absorption maximum (267 nm) as N^6-monoalkyl cAMP.[1] The ^{1}H-NMR spectra and elemental analyses (Table III) further supported the structures of **3**.



a) RBr, NaH or *tert*-BuOK, DMSO, r.t.; b) RBr, MeONa, DMSO, r.t. or 50 °C; c) $PhCH_2Br$, KOH, 18-crown-6, H_2O–dioxane, 50 °C; d) RBr, KOH, 18-crown-6, H_2O–dioxane, r.t. or 50 °C

Chart 1

For the preparation of 2′-*O*-alkyl cAMP (**4**), Tazawa *et al.*[6] have reported the synthesis of 2′-*O*-methyl and 2′-*O*-ethyl cAMPs from **1** in aqueous NaOH solution, but this method was not successful in obtaining longer chain alkylated cAMP derivatives. Miller *et al.* reported an inhibitory effect on phosphodiesterase of 2′-*O*-methyl cAMP prepared from adenosine.[9] When we followed up on the dialkylation of **1** to **3** by HPLC, we found an intermediate different from N^6-monoalkyl cAMP and identified it as 2′-*O*-alkyl cAMP (**4**). Based on these results, it was assumed that the 2′-hydroxyl group was alkylated faster than the 6-amino group. But compounds (**4**) could not be obtained selectively by the treatment of **1** with a limited amount of a base such as NaH, *tert*-BuOK, or MeONa under the milder conditions. After many trials of reaction conditions, **4** was obtained by the treatment of **1** with KOH and alkyl bromides in the presence of 18-crown-6 in aqueous dioxane solution (Chart 1). Aqueous KOH was used for dissolving **1** and acting as a moderate base. The decomposition of **1** to 3′- and 5′-AMPs was observed and **4b** could not be

Table I. Dibutylation of cAMP (**1**) with Various Bases[a]

Base	Solvent	Products (%)[b] 3b	2c	Time (d)
NaH	DMF	38.7	46.5	3
	DMA	48.0	20.0	3
	Pyridine	8.0	—	2
tert-BuOK	DMF	N.R		
	DMA	28.0	33.0	3
	Pyridine	8.0	—	2
n-BuLi	DMF	N.R		
	DMA	N.R		
	DMSO	68.0	27.5	3
	Pyridine	17.9	—	2
MeONa	DMA	37.4	—	4
	DMF	33.0	—	4
	DMSO	69.8	10.7	3
	Pyridine	19.4	—	2

a) Carried out with 6—12 mol eq of bases and of butyl bromide, 100 mg of tri-*n*-butylammonium salt of cAMP in 5 ml of solvent at room temperature for 2—4 d. *b*) Determined by HPLC.

Table II. Solvent Effect on 2′-*O*-Monoalkylation of cAMP (**1**)[a]

Entry	Solvent[b] Dioxane : H_2O	R	Time (d)	Yield[c] (%) 3b or 3d	4b or 4d	1
1	5 : 1	C_4H_9	2	29.3	52.3	7.1
2	1 : 1	C_4H_9	2	7.5	66.0	18.0
3	1 : 5	C_4H_9	3	2.4	54.5	24.8
4	5 : 1	C_7H_{15}	2	17.9	44.2	16.4
5	1 : 1	C_7H_{15}	3	3.5	39.1	29.9
6	1 : 5	C_7H_{15}	3	—	7.4	58.3

a) Carried out with 8 mol eq of KOH, 3 mol eq of 18-crown-6, 6 mol eq of alkyl bromide and 1 mmol of **1** at 50—60 °C for 2—3 d. *b*) Total 18 ml (v/v). *c*) Determined by HPLC.

obtained when 18-crown-6 was absent in the case of entry 1 of Table II. The compositions of the solvents were explored to find the best condition for this alkylation (Table II). In butylation, the reaction in even parts of dioxane and water (entry 2) predominantly gave the desired monoalkylated product (**4b**, 66.0% on HPLC) and a more dioxane-containing solvent (entry 1) gave a more dialkylated derivative (**3b**, 29.3% on HPLC) as a by-product. The reaction proceeded slowly in more water-containing systems (entry 3). The heptylation in analogous solvent systems was similar to butylation, but the best result was obtained in the more dioxane-containing solvent (entry 4). The differences in the reaction conditions between the synthesis of **4b** and **4d** may be due to the weaker reactivity of heptyl bromide compared to that of butyl bromide. Compounds (**4a** and **4b**) were prepared in dioxane–H_2O (1 : 1, v/v) and compounds (**4c—e**) were in dioxane–H_2O (5 : 1, v/v). The general procedure for the synthesis of **4** was as follows. The treatment of the aqueous KOH solution of **1** with a solution of 18-crown-6 in dioxane, followed by the addition of excess alkyl bromides at 50 °C (**4a—e**), or room temperature (**4f**), gave desired products **4a** (19% yield), **4b** (25%), **4c** (14%), **4d** (18%), **4e** (11%), and **4f** (51%), respectively. The production of corresponding dialkyl compound (**3**) and alkylated adenine derivatives[10] were observed as by-products. The benzylation of **1** under these conditions, however, gave the dibenzyl derivative (**3e**) as described above. On the other hand, the monobenzyl derivative (**4f**)

TABLE III. Yields and Physical Constants of Alkylated cAMPs

Compd. No.	R	Yield (%)	Rf[a]	UV (nm)[b] (ε)	Formula[c]	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
2a	Et	58	0.30	277 (18700)	$C_{16}H_{24}N_5O_6P\cdot 1/2H_2O$	45.50	5.96	16.58	45.48	5.86	16.51
2b	Pr	46	0.37	277 (18800)	$C_{19}H_{30}N_5O_6P\cdot 3/4H_2O$	48.66	6.47	14.93	48.37	6.47	14.70
2c	Bu	47	0.42	277 (18400)	$C_{22}H_{36}N_5O_6P\cdot 2/3H_2O$	51.86	7.38	13.74	52.21	7.19	13.42
2d	Pen	44	0.46	277 (18800)	$C_{25}H_{42}N_5O_6P\cdot 1/4H_2O$	55.18	7.87	12.87	55.36	7.86	12.78
2e	Hep	52	0.58	277 (17400)	$C_{31}H_{54}N_5O_6P\cdot 5/4H_2O$	57.61	8.81	10.84	57.63	8.48	10.76
2f	Myris	43	0.69	277 (19200)	$C_{52}H_{96}N_5O_6P\cdot 3/2H_2O$	66.06	10.55	7.41	65.86	10.28	7.41
2g	Bn	38	0.42	277 (21000)	$C_{31}H_{30}N_5O_6P\cdot 1/3H_2O$	61.48	5.10	11.56	61.70	5.12	11.51
3a	Pr	17	0.47	266 (17200)	$C_{16}H_{24}N_5O_6P\cdot 2/3H_2O$	45.18	6.00	16.46	45.32	5.85	16.24
3b	Bu	20	0.53	267 (16400)	$C_{18}H_{28}N_5O_6P\cdot H_2O$	47.06	6.58	15.24	46.85	6.24	14.94
3c	Pen	32	0.58	267 (15900)	$C_{20}H_{32}N_5O_6P\cdot H_2O$	49.28	7.03	14.37	49.26	6.89	14.10
3d	Hep	31	0.64	267 (16500)	$C_{24}H_{40}N_5O_6P\cdot 3/5H_2O$	53.73	7.74	13.05	54.02	7.71	12.72
3e	Bn	41	0.56	267 (18400)	$C_{24}H_{24}N_5O_6P\cdot 3/4H_2O$	55.12	4.91	13.39	55.31	4.74	13.06
4a	Pr	19	0.58	258 (13200)	$C_{13}H_{18}N_5O_6P\cdot 5/4H_2O$	39.65	5.25	17.78	39.92	5.16	17.53
4b	Bu	25	0.60	258 (14100)	$C_{14}H_{20}N_5O_6P\cdot 5/4H_2O$	41.23	5.56	17.17	41.29	5.29	16.90
4c	Pen	14	0.63	258 (14500)	$C_{15}H_{22}N_5O_6P\cdot 1/2H_2O$	44.12	5.68	17.15	44.07	5.69	17.26
4d	Hep	18	0.66	258 (13600)	$C_{17}H_{26}N_5O_6P\cdot H_2O$	45.84	6.34	15.72	46.02	6.35	15.37
4e	Myris	11	0.71	258 (12400)	$C_{24}H_{40}N_5O_6P\cdot 3/4H_2O$	53.47	7.76	12.99	53.76	7.70	12.76
4f	Bn	51	0.64	258 (14700)	$C_{17}H_{18}N_5O_6P\cdot 1/2H_2O$	47.67	4.47	16.34	47.61	4.23	16.20

a) Rf on Kiesel gel $60F_{254}$ (Merck) plate; compounds **2**, solvent system (MeOH–$CHCl_3$, 3 : 7, v/v); compounds **3**, solvent system (MeOH–$CHCl_3$, 4 : 6, v/v); compounds **4**, solvent system (0.1 N NH_4Cl–MeOH–CH_3CN, 1 : 1 : 4, v/v). *b*) Solvent; compounds **2a**—**d** and **2g**, **3** and **4** in 0.1 N NaOH; compounds **2e** and **2f** in EtOH. *c*) Samples were dried over P_2O_5 at 50 °C at 3 mmHg for 4—5 h.

was prepared with an equimolar of benzyl bromide at room temperature. This reaction gave compounds (**4**) predominantly with small amounts of the dialkyl derivatives (**3**), but no detectable amounts of N^6-monoalkyl cAMPs. The compounds (**4**) had practically the same absorption maximum (258 nm) as **1**. The ^{1}H-NMR spectra and elemental analyses (Table III) also supported the structures of **4**.

The results of the synthesis of alkylated cAMPs (**2**, **3**, and **4**) showed that the alkylation of **1** occurred at 2′-hydroxyl group first and then at 6-amino group. This difference in the speeds of alkylation was similar to that of acylation of **1** to synthesize N^6,2′-O-diacyl cAMP.[11] The well known reaction of **1** with alkyl halides was the alkylation at 1-position in the presence of a weak base in dimethyl acetamide or DMSO and gave only 1-methyl, 1-crotyl, and 1-benzyl cAMPs.[3h,12] Reactive alkyl halides such as methyl iodide, crotyl bromide and BnBr were used in this reaction, but the introduction of a longer alkyl group was difficult in this reaction.[3h] The preparation of many 1-alkyl cAMPs was not possible, so the synthesis of N^6-alkyl cAMP derivatives by using the Dimroth rearrangement was restricted to a few compounds. On the other hand, our methods of alkylation of **1** with such a strong base as NaH, *tert*-BuOK, MeONa, or KOH gave various derivatives of N^6,N^6,2′-O-trialkyl cAMP (**2**), N^6,2′-O-dialkyl cAMP (**3**), and 2′-O-alkyl cAMP (**4**) without the introduction of a protecting group by one step alkylation due to the difference of reactivity between the 6-amino and 2′-hydroxyl groups.

Biological Results and Discussion The inotropic and chronotropic effects of several new alkylated cAMPs were tested by the use of papillary muscle of the right ventricle and right atria, respectively, of guinea pig hearts. The results are shown in Table IV. In the previous report, we have shown that N^6-monoalkyl cAMPs showed increasing PIE and changes in their chronotropic effects from negative to positive for a longer alkyl group (increasing lipophilicity) among ethyl and octyl side chains.[1] New alkylated cAMPs (**2**, **3**, and **4**) were interesting in the influence of the alkylated positions and the length of the alkyl group on cardiac action. Though compounds (**2**) have high lipophilicity, their inotropic potency at a high concentration of 1 mM reached only 10—36% of the maximum PIE evoked by 10^{-7} M isoproterenol. Compounds (**2**) are one order of magnitude less potent than N^6-octyl cAMP which had the most positive inotropic potency among N^6-monoalkyl cAMPs.[1,13] Compounds (**2c** (butyl) and **2g** (benzyl)) exhibited strong negative chronotropic effects while other compounds of **2** showed weak negative ones. Compounds (**3**) exhibited weak negative actions in both effects except **3e** (benzyl) which had a strong negative chronotropic effect. Compounds (**4**) were almost inactive in cardiac effect. These negative chronotropic effects of **2** and **3** might be caused by adenosine- or AMP-like action on the surface membrane.[3e,g]

TABLE IV. Inotropic and Chronotropic Effects of Alkylated cAMPs

Compd. No.	R	Inotropic effect (%)[a] (1 mM)	Chronotropic effect (%)[a] (1 mM)
2a	Et	13.1±9.3	−1.3± 1.4
2b	Pr	15.4±5.4	−10.4±12.8
2c	Bu	36.4±9.3	−112.4±35.2
2d	Pen	10.5±5.9	−16.4± 7.5
2g	Bn	24.9±5.3	−52.9± 6.5
3b	Bu	−0.1±1.6	−22.8± 4.5
3d	Hep	−8.4±1.8	−12.7±10.4
3e	Bn	−12.6±7.7	−138.5±13.0
4b	Bu	6.3±2.4	9.8± 4.6
4d	Hep	2.2±2.7	18.4±14.3
4f	Bn	2.8±1.9	−8.4± 3.8

a) These values were expressed as percent changes (plus or minus) form the maximum response evoked by 10^{-7} M isoproterenol. Each value represents the mean±S.E. (n=4—5).

Factors which influence the inotropic potency of a cAMP

derivative are the abilities of the compound to cross the cell membrane, to activate cAMP dependent protein kinase and to inhibit or resist the action of phosphodiesterase. It is considered that the less potent cardiac effects of **2**, **3**, and **4** are due to the substitution at 2′-hydroxyl group of **1**, since in the report of Miller *et al.* the potencies of 2′-*O*-substituted derivatives such as 2′-*O*-acetyl, 2′-*O*-butyryl, and 2′-*O*-methyl cAMPs to activate protein kinase were less than 1/100 as active as cAMP.[9] This view is supported by the results that N^6,2′-*O*-diheptyl cAMP (**3d**) did not exhibit the PIE and that N^6-heptyl cAMP possessing no substituent at 2′-hydroxyl position showed potent PIE and its ED_{30} value on the PIE was 1.60 ± 0.19 ($\times 10^{-4}$ M).[1] The weak PIE of **2** may be due to their high lipophilicity, which causes penetration of **2** through cell membrane and the resultant increase in the intracellular level of cAMP by the action of **2** as inhibitors of phosphodiesterase in the cells. 2′-*O*-Substituted cAMPs are inhibitors of phosphodiesterase[9] and N^6,N^6-diethyl cAMP is an inhibitor of the bovine heart phosphodiesterase.[14] The length of alkyl group of **2**, **3**, and **4** did not show marked differences on the inotropic effect. The appearance of PIE by cAMP derivatives was found to require a 2′-hydroxyl group. These results suggest that the biologically active form of DB cAMP is N^6-butyryl cAMP.[15] We are preparing a more potent cardiotonic agent on the basis of this view.

Experimental

^{1}H-NMR spectra were taken at 200 MHz on a JEOL JNM-FX200 NMR spectrometer in DMSO-d_6 or chloroform-d_1. All ^{1}H-NMR data are reported in ppm downfield from tetramethylsilane as an internal standard. Ultraviolet (UV) absorption spectra were recorded with a Hitachi 557 spectrophotometer. Infrared (IR) spectra were taken on a JASCO A-202 spectrophotometer. HPLC was performed on a Finepak SIL C_{18} column (4.6 mm × 25 cm) with MeOH–10 mM acetate buffer (pH 4) containing 1 mM tetra-*n*-butylammonium chloride (5 : 5—8 : 2, v/v) as eluants. Detection was at 275 nm (compounds **2**), 265 nm (compounds **3**) and 260 nm (compounds **4**). Analytical and preparative thin-layer chromatographies (TLC) were performed on Kiesel gel $60F_{254}$ (Merck) plates. Chromatographic separation was done on Merck Silica gel 60 or Dowex 50w-×8 (H^+) with the indicated eluant.

General Procedure for the Preparation of N^6,N^6,2′-*O*-Trialkyl cAMP (2) Method A: Sodium hydride (4—8 mol eq) was added to a solution of tri-*n*-butylammonium salt[16] of **1** (2.06 g, 4 mmol) in 20 ml of DMSO with stirring and then an alkyl bromide (4—8 mol eq) was added to the mixture. The mixture was stirred at room temperature for 5 h—3 d. The reaction solution was adjusted to pH 2 with 2 N HCl and evaporated *in vacuo*. The resulting residue was dissolved in benzene and washed with H_2O (150 ml × 4). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in MeOH and purified by preparative TLC with MeOH–$CHCl_3$ or column chromatography (silica gel, eluant: MeOH–$CHCl_3$) to give a product.

Method B: This procedure was the same as method A except for the use of potassium *tert*- butoxide (4—8 mol eq). Compounds **2a—d** and **2e—g** were respectively prepared by method A and by method B.

2a: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2975, 2930, 1640, 1585. ^{1}H-NMR δ: 1.16 (3H, t, J=7.1 Hz, CH_3), 1.19 (3H, t, J=7.1 Hz, 2 × CH_3), 3.60—3.80 (2H, m, OCH_2), 3.80—4.35 (7H, m, CH_2NCH_2, H-4′ and H_2-5′), 4.42 (1H, d, J=5.4 Hz, H-2′), 4.74—4.90 (1H, m, H-3′), 6.06 (1H, s, H-1′), 8.24 and 8.28 (1H each, s, purine H's).

2b: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2955, 2925, 2870, 1620, 1580. ^{1}H-NMR δ: 0.88 (3H, t, J=7.3 Hz, CH_3), 0.89 (6H, J=7.2 Hz, 2 × CH_3), 1.40—1.80 (6H, m, 3 × $C\underline{H}_2CH_3$), 3.50—3.70 (2H, m, OCH_2) 3.75—4.20 (7H, m, CH_2NCH_2, H-4′ and H_2-5′), 4.35 (1H, d, J=5.4 Hz, H-2′), 4.60—4.78 (1H, m, H-3′), 6.01 (1H, s, H-1′), 8.23 (2H, s, purine H's).

2c: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2955, 2925, 2860, 1590, 1565. ^{1}H-NMR δ: 0.80—1.00 (9H, m, 3 × CH_3), 1.20—1.45 (6H, m, 3 × $C\underline{H}_2CH_3$), 1.45—1.75 (6H, m, 3 × CH_2), 3.50—3.80 (2H, m, OCH_2), 3.80—4.20 (7H, m, CH_2NCH_2, H-4′ and H_2-5′), 4.34 (1H, d, J=4.9 Hz, H-2′), 4.60—4.73 (1H, m, H-3′), 6.00 (1H, s, H-1′), 8.23 (2H, s, purine H's).

2d: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 2925, 2855, 1585, 1565. ^{1}H-NMR δ: 0.75—1.00 (9H, m, 3 × CH_3), 1.15—1.45 (12H, m, 3 × $(C\underline{H}_2)_2CH_3$), 1.45—1.80 (6H, m, 3 × CH_2), 3.50—3.70 (2H, m, OCH_2), 3.75—4.20 (7H, m, CH_2NCH_2, H-4′ and H_2-5′), 4.35 (1H, d, J=5.1 Hz, H-2′), 4.60—4.75 (1H, m, H-3′), 6.00 (1H, s, H-1′), 8.22 (2H, s, purine H's).

2e: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 2950, 2925, 2850, 1585, 1565. ^{1}H-NMR δ: 0.70—0.95 (9H, m, 3 × CH_3), 1.10—1.45 (24H, m, 3 × $(C\underline{H}_2)_4CH_3$), 1.45—1.80 (6H, m, 3 × CH_2), 3.50—3.70 (2H, m, OCH_2), 3.75—4.25 (7H, m, CH_2NCH_2, H-4′ and H_2-5′), 4.35 (1H, d, J=5.3 Hz, H-2′), 4.65—4.80 (1H, m, H-3′), 6.05 (1H, s, H-1′), 8.21 and 8.23 (1H each, s, purine H's).

2f: An oil. IR ν_{max}^{KBr} cm^{-1}: 2925, 2850, 1585, 1560. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (9H, s like, 3 × CH_3), 1.24 (72H, s like, 3 × $(C\underline{H}_2)_{12}CH_3$), 4.80—5.00 (1H, m, H-3′), 6.04 (1H, s, H-1′), 8.25 (2H, s, purine H's).

2g: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 3015, 2900, 1590, 1580. ^{1}H-NMR δ: 3.90—4.20 (3H, m, H-4′ and H_2-5′), 4.56 (1H, d, J=5.4 Hz, H-2′), 4.70 (1H, d, J=12.1 Hz, OCH_a), 4.85 (1H, d, J=12.1 Hz, OCH_b), 5.20 (4H, br s, CH_2NCH_2), 6.16 (1H, s, H-1′), 7.15—7.50 (15H, m, phenyl H's), 8.29 and 8.34 (1H each, s, purine H's).

General Procedure for the Preparation of N^6,2′-*O*-Dialkyl cAMP (3) Sodium methoxide (7—20 mol eq) was added to a solution of tri-*n*-butylammonium salt of **1** (3.1 g, 6 mmol) in 50 ml of DMSO with stirring and then an alkyl bromide (7—16 mol eq) was added to the mixture. The mixture was stirred at room temperature (**3a**, **c**, and **d**), or 50 °C (**3b**), for 20—35 h, followed by an adjustment of the pH to 7 with 2 N HCl and evaporated *in vacuo*. The resulting residue was dissolved in a small amount of H_2O, adjusted to pH 2 with 2 N HCl, and applied to a charcoal column (2.1 × 34 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 with MeOH–$CHCl_3$ (1 : 3—2 : 3, v/v) to give a product which was recrystallized from H_2O adjusted to pH 2 with 2 N HCl or was applied to column chromatography (Dowex 50w-×8, eluant: EtOH–H_2O).

N^6,2′-*O*-Dibenzyl cAMP (3e) This procedure was the same as the synthesis of **4** (method D).

3a: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2955, 2925, 1610, 1585^{sh}. ^{1}H-NMR δ: 0.89 (3H, t, J=7.3 Hz, CH_3), 0.90 (3H, t, J=7.3 Hz, CH_3), 1.45—1.80 (4H, m, 2 × $C\underline{H}_2CH_3$), 3.40—3.80 (4H, m, NCH_2 and OCH_2), 4.36 (1H, d, J=5.1 Hz, H-2′), 4.60—4.80 (1H, m, H-3′), 6.01 (1H, s, H-1′), 7.73 (1H, br s, NH), 8.21 and 8.22 (1H each, s, purine H's).

3b: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3360, 2950, 2860, 1620, 1580. ^{1}H-NMR δ: 0.89 (3H, t, J=7.2 Hz, CH_3), 0.90 (3H, t, J=7.2 Hz, CH_3), 1.25—1.45 (4H, m, 2 × $C\underline{H}_2CH_3$), 3.40—3.80 (4H, m, NCH_2 and OCH_2), 4.35 (1H, d, J=4.9 Hz, H-2′), 4.65—4.80 (1H, m, H-3′), 6.00 (1H, s, H-1′), 7.70 (1H, br s, NH), 8.20 and 8.22 (1H each, s, purine H's).

3c: A colorless amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 2925, 2850, 1620, 1580^{sh}. ^{1}H-NMR δ: 0.80—0.95 (6H, m, 2 × CH_3), 1.20—1.45 (8H, m, 2 × $(C\underline{H}_2)_2CH_3$), 1.45—1.70 (4H, m, 2 × CH_2), 3.45—3.80 (4H, m, NCH_2 and OCH_2), 4.34 (1H, d, J=5.4 Hz, H-2′), 4.65—4.78 (1H, m, H-3′), 6.00 (1H, s, H-1′), 7.68 (1H, br s, NH), 8.18 and 8.21 (1H each, s, purine H's).

3d: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3300, 2925, 2850, 1615, 1580^{sh}. ^{1}H-NMR δ: 0.75—1.00 (6H, m, 2 × CH_3), 1.10—1.45 (16H, m, 2 × $(C\underline{H}_2)_4CH_3$), 1.45—1.70 (4H, m, 2 × CH_2), 3.40—3.80 (4H, m, NCH_2 and OCH_2), 4.35 (1H, d, J=5.6 Hz, H-2′), 4.62—4.80 (1H, m, H-3′), 5.99 (1H, s, H-1′), 7.72 (1H, br s, NH), 8.21 (2H, s, purine H's).

3e: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3250, 3010, 1618, 1580. ^{1}H-NMR δ: 4.50 (1H, d, J=4.2 Hz, H-2′), 4.60—4.90 (5H, m, NCH_2, OCH_2 and H-3′), 6.11 (1H, s, H-1′), 7.15—7.45 (10H, m, phenyl H's), 8.24 (2H, s, purine H's), 8.29 (1H, br s, NH).

General Procedure for the Preparation of 2′-*O*-Alkyl cAMP (4) Method C (**4a**,**b**): To a stirred solution of KOH (5—8 mol eq) in 90 ml of H_2O was added 3.3 g (10 mmol) of **1** and a solution of 18-crown-6 (2.5—3 mol eq) in 90 ml of dioxane. An alkyl bromide (4—8 mol eq) was added to the solution and stirred at 50 °C for 6 h—5 d. The mixture was adjusted to pH 7 with 2 N HCl and evaporated *in vacuo*. The resulting residue was dissolved in a small amount of H_2O and adjusted to pH 2 with 2 N HCl to give a crude product. This was dissolved in MeOH–2 N NaOH and subjected to preparative TLC (MeOH–$CHCl_3$). The resulting residue was dissolved in H_2O and adjusted to pH 2 with 2 N HCl to give a product.

Method D (**4c—e**): This procedure was the same as method C except

for the use of 30 ml of H_2O and 150 ml of dioxane as solvents.

4a: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 2955, 1700, 1610. ^{1}H-NMR δ: 0.89 (3H, t, J=7.4 Hz, CH_3), 1.45—1.68 (2H, qt, J=7.4, 7.0 Hz, CH_2), 3.61 (2H, t, J=7.0 Hz, OCH_2), 4.51 (1H, d, J=4.6 Hz, H-2′), 4.90—5.10 (1H, m, H-3′), 6.13 (1H, s, H-1′), 7.73 (2H, br s, NH_2), 8.25 and 8.41 (1H each, s, purine H's).

4b: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3350, 2950, 2855, 1690, 1605. ^{1}H-NMR δ: 0.88 (3H, t, J=7.3 Hz, CH_3), 1.23—1.45 (2H, m, CH_2CH_3), 1.45—1.65 (2H, m, CH_2), 3.64 (2H, t, J=6.2 Hz, OCH_2), 4.50 (1H, d, J=5.2 Hz, H-2′), 4.95—5.10 (1H, m, H-3′), 6.12 (1H, s, H-1′), 7.68 (2H, br s, NH_2), 8.24 and 8.41 (1H each, s, purine H's).

4c: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3320, 3075, 2925, 2855, 1690, 1605. ^{1}H-NMR δ: 0.86 (3H, t, J=6.9 Hz, CH_3), 1.20—1.43 (4H, m, $(CH_2)_2CH_3$), 1.45—1.65 (2H, m, CH_2), 3.55—3.70 (2H, m, OCH_2), 4.51 (1H, d, J=4.6 Hz, H-2′), 4.96—5.10 (1H, m, H-3′), 6.12 (1H, s, H-1′), 7.69 (2H, br s, NH_2), 8.24 and 8.41 (1H each, s, purine H's).

4d: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3310, 3080, 2925, 2850, 1685, 1610. ^{1}H-NMR δ: 0.78—0.95 (3H, m, CH_3), 1.10—1.40 (8H, m, $(CH_2)_4CH_3$), 1.45—1.63 (2H, m, CH_2), 3.58—3.75 (2H, m, OCH_2), 4.50 (1H, d, J=5.1 Hz, H-2′), 4.95—5.10 (1H, m, H-3′), 6.11 (1H, s, H-1′), 7.62 (2H, br s, NH_2), 8.22 and 8.39 (1H each, s, purine H's).

4e: A white powder. IR ν_{max}^{KBr} cm^{-1}: 3090, 2925, 2850, 1685, 1610. ^{1}H-NMR δ: 0.75—0.93 (3H, m, CH_3), 1.05—1.43 (22H, m, $(CH_2)_{11}CH_3$), 1.43—1.67 (2H, m, CH_2), 3.50—3.75 (2H, m, OCH_2), 4.47 (1H, d, J= 4.9 Hz, H-2′), 4.95—5.10 (1H, m, H-3′), 6.12 (1H, s, H-1′), 7.56 (2H, br s, NH_2), 8.23 and 8.40 (1H each, s, purine H's).

2′-*O*-Benzyl cAMP (4f) To a stirred solution of 1.5 g (20 mmol) of KOH in 60 ml of H_2O was added 3.3 g (10 mmol) of **1** and a solution of 2.6 g (10 mmol) of 18-crown-6 in 150 ml of dioxane. Benzyl bromide (1.7 g, 10 mmol) was added to the solution and stirred at room temperature for 20 h. The mixture was adjusted to pH 7 with 2 N HCl and evaporated *in vacuo*. The residue was purified by the same procedure as method C described above to give 2.1 g of **4f** (51%) as a white powder. IR ν_{max}^{KBr} cm^{-1}: 3430, 3100, 3050, 1685, 1608. ^{1}H-NMR δ: 4.66 (1H, d, J=5.1 Hz, H-2′), 4.72 (1H, d, J=11.9 Hz, OCH_a), 4.80 (1H, d, J=11.9 Hz, OCH_b), 5.05—5.20 (1H, m, H-3′), 6.24 (1H, s, H-1′), 7.20—7.50 (5H, m, phenyl H's), 7.86 (2H, br s, NH_2), 8.28 and 8.41 (1H each, s, purine H's).

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 dissected out in cold bathing solution, and were suspended individually in 8 ml organ baths for recording isometric contractions. The bathing solution was 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 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 ms duration at a 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 inotropic and chronotropic effects of cAMP derivatives were expressed as percent change (plus or minus) from the maximum response evoked by 10^{-7} M isoproterenol in each preparation.

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References and Notes

1) Part V: S. Kataoka, J. Isono, N. Yamaji, M. Kato, T. Kawada, and S. Imai, *Chem. Pharm. Bull.*, **36**, 2212 (1988).
2) G. I. Drummond, P. Greengard, and G. A. Robison, "Advances in Cyclic Nucleotide Research," Vol. 5, Raven Press, New York, 1975, pp. 265—279.
3) *a*) I. Adachi, I. Kusunoki, T. Ishizuka, A. Yasuta, T. Nakagawa, and I. Horikoshi, *Chem. Pharm. Bull.*, **34**, 3861 (1986); *b*) J. Nagyvary, R. N. Gohil, C. R. Kirchner, and J. D. Stevens, *Biochem. Biophys. Res. Commun.*, **55**, 1072 (1973); *c*) Y. S. Cho-Chung, *Cancer Res.*, **34**, 3492 (1974); *d*) R. M. Niles, K. W. Ludwig, and J. S. Makarshi, *J. Natl. Cancer Inst.*, **63**, 909 (1979); *e*) S. Imai, T. Otorii, K. Takeda, Y. Katano, and D. Horii, *Jpn. J. Pharmacol.*, **24**, 499 (1974); *f*) N. Yamaji, M. Kato, I. Matsubara, N. Shimamoto, K. Miura, and S. Imai, *Chem. Pharm. Bull.*, **28**, 1683 (1980); *g*) T. Kawada, Y. Yoshida, and S. Imai, *Br. J. Pharmacol.*, **97**, 371 (1989); *h*) J. P. Miller, K. H. Boswell, R. B. Meyer, L. F. Christensen, and R. K. Robins, *J. Med. Chem.*, **23**, 242 (1980).
4) *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, "Advances in Cyclic Nucleotide Research," Vol. 8, Raven Press, New York, 1977, pp. 363—420.
5) S. Kataoka, J. Isono, N. Yamaji, and M. Kato, *Chem. Lett.*, **1986**, 1221.
6) I. Tazawa, S. Tazawa, J. L. Alderfer, and P. O. P. Ts'O, *Biochemistry*, **11**, 4931 (1973).
7) R. B. Meyer, D. A. Shuman, R. K. Robins, R. J. Baver, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, **11**, 2704 (1972).
8) Absorption maxima of 1,N^6-dimethyl adenosine was 263, 305^{sh} nm (pH 13) and 261 nm (pH 1). B. Singer, L. Sun, and H. Fraenkel-Conrat, *Biochemistry*, **13**, 1913 (1974).
9) J. P. Miller, D. A. Shuman, M. B. Scholten, M. K. Dimmitt, C. M. Stewart, T. A. Khwaja, R. K. Robins, and L. N. Simon, *Biochemistry*, **12**, 1010 (1973).
10) These structures were indicated by ^{1}H-NMR spectrum since the signals of ribofuranose protons disappeared and the signals of alkyl and purine (C_2 and C_8) protons were present in the ^{1}H-NMR spectrum. E. R. Garrett reported that the formation of adenine was found in the alkaline hydrolysis of adenosine nucleosides. E. R. Garrett and P. J. Metha, *J. Am. Chem. Soc.*, **94**, 8542 (1972).
11) J. G. Falbriard, T. Posternak, and E. W. Sutherland, *Biochim. Biophys. Acta*, **148**, 99 (1967).
12) K. H. Boswell, L. H. Christensen, D. A. Shuman, and R. K. Robins, *J. Heterocycl. Chem.*, **12**, 1 (1975).
13) The ED_{30} value of N^6-octyl cAMP on the PIE was 0.95±0.21 ($\times 10^{-4}$ M).
14) J. P. Miller, C. C. Sigman, H. L. Johnson, T. Novinson, R. N. Springen, K. Senga, D. E. O'Brien, and R. K. Robins, "Advances in Cyclic Nucleotide and Protein Phosphorylation Research," Vol. 16, Raven Press, New York, 1984, pp. 277—290.
15) G. I. Drummond, S. J. Hemmings, and R. B. Warneboldt, *Life Sci.*, **15**, 319 (1974).
16) J. G. Falbriard, T. Posternak, and E. W. Sutherland, *Biochim. Biophys. Acta*, **65**, 558, (1962).