Studies on the Synthesis of Compounds Related to Adenosine 3',5'-Cyclic Phosphate. IX.¹⁾ Synthesis and Cytotoxic Effect of Adenosine 3',5'-Cyclic Alkylphosphoramidates

Makoto SAITO,*^a Shigehiro KATAOKA,^b Ayako NASU,^b Nobuyuki YAMAJI^b and Atsushi ICHIKAWA^c

Noda Institute for Scientific Research,^a 399 Noda, Noda-shi, Chiba 278, Japan, Research and Development Division, Kikkoman Corp.,^b 399 Noda, Noda-shi, Chiba 278, Japan and Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University,^c Yoshida, Sakyo-ku, Kyoto 606, Japan. Received May 28, 1991

A series of adenosine 3',5'-cyclic monophosphoramidates (3, cAMP amidates), including long-chain alkyl amidates, were synthesized from adenosine 3',5'-cyclic monophosphate (1, cAMP) by means of a one-pot reaction. This reaction proceeded by the treatment of cAMP tributylammonium salt (2) with phosphorus pentachloride (PCl₅) and alkylamine in *N,N*-dimethylformamide (DMF). Compounds 3 synthesized were investigated to determine their cytotoxic activities on the growth of mouse mastocytoma P-815 cells, mouse mammary tumor FM3A cells, and human mammary tumor ZR-75 cells in culture. It was found that compounds 3h–m showed significant cytotoxic activities against these cell lines, and that cAMP decylamidate (3j) was the most cytotoxic compound (the concentration required for 50% inhibition of cell growth, ID₅₀ = 6.0, 15.0, 2.2 μM, respectively); the antitumor effect on P-815 cells by a total packed cell volume method showed 81.8% inhibition. The cytotoxic activity of 3 increased with the increase in alkyl chain length up to 10 carbon atoms and decreased in compounds having longer alkyl chain.

Keywords cAMP phosphoramidate; cytotoxicity; phosphorus pentachloride; alkylamine; one-pot reaction; mastocytoma P-815 cell

Introduction

Adenosine 3',5'-cyclic monophosphate (1, cAMP) is an important intracellular "second messenger" mediating various hormonal actions. In addition to its well-known mediating activity, 1 is assumed to have a role in cell proliferation.^{2,3)} The cellular levels of 1 vary as the cell division cycle proceeds, and it generally accumulates at the G₁ or G₂ phases of growing cells, which result in the inhibition of deoxyribonucleic acid (DNA) synthesis^{4,5)} or cell division,⁶⁾ respectively. Furthermore, *N*⁶,2'-*O*-dibutyryl cAMP^{2,7)} has been shown to have a weak cytostatic activity against the growth of several tumor cell lines in culture. On the basis of these observations, various derivatives of 1 have been synthesized and examined for cytotoxic activity against the growth of cultured cancerous cells, and many effective compounds such as 8-amino cAMP and *N*⁶-benzyl cAMP were reported.^{2,8)} We previously synthesized many cAMP derivatives^{9–11)} to examine their biological activities^{10,11)} and found that cAMP alkyltriester strongly inhibited the growth of mouse mastocytoma P-815 cells and mammary FM3A cells.¹¹⁾

Basically, the chemical modification of 1 is focused on the improvement of its poor cellular permeability, which is mostly derived from a single negative charge on the phosphate moiety of 1. Along with triesterification,^{9d,12)} the introduction of alkylamines to the phosphate is another appropriate way to increase the cell permeability of 1. A few cAMP amidates such as cAMP dimethylamidate and cAMP methylamidate have already been synthesized^{13–15)} but nothing is known about their cytotoxic effect on the growth of tumor cells. We found that long-alkyl chain derivatives of cAMP alkyltriester were more potent in cell cytotoxicity than the short-alkyl chain derivatives.¹¹⁾ Such circumstances prompted us to investigate the effect of a series of cAMP amidates with long-alkyl chain on cell growth. In this paper, we report the synthesis of a series of cAMP amidates using a newly developed one-pot reaction from 1, and the examination of their cytotoxic activities against the growth of cultured mastocytoma P-815 cells.

Results and Discussion

Chemistry The synthetic route for the preparation of cAMP amidates (3) is shown in Chart 1. Treatment of the tri-*n*-butylammonium salt (2) of 1 with phosphorus pentachloride and alkylamine in absolute *N,N*-dimethylformamide (DMF) gave 3. Physical properties of 3 are summarized in Table I. The *R_f* values of 3 by paper chromatography and those by thin-layer chromatography (TLC) became larger according to the increase of alkyl chain length. All these compounds showed the maximal ultraviolet (UV) absorption at 259 nm in EtOH and the similar infrared (IR) spectral pattern, except for the intensity of the absorption by methylene in the vicinity of 2950 cm⁻¹. Though the yields of compound 3 were in the range from 18 to 48% (Table I), the formation of the by-product was almost negligible on TLC (CHCl₃:MeOH = 75:25) and the starting material was recovered.

We noticed in our preliminary experiment that the long-chain alkyl-substituted derivatives (3b–o) were hard to synthesize by other methods¹³⁾ as described in various published papers which indicated the need for the protection for 2'-hydroxyl and 6-amino groups. The present method is advantageous in its wide applicability for large-scale preparation and simplicity of operation in the

Chart 1

TABLE I. Physical Constants of cAMP Amidates

Compd. No.	R	Yield (%)	Rf_1^a	Rf_2^b	Formula ^c	Analysis (%)					
						Calcd			Found		
						C	H	N	C	H	N
3a	Methyl	26.2	0.44	0.51	$C_{11}H_{15}N_6O_5P \cdot 3/2H_2O$	35.78	4.91	22.76	35.68	4.75	22.62
3b	Ethyl	48.3	0.53	0.57	$C_{12}H_{17}N_6O_5P \cdot 2/3H_2O$	39.14	5.02	22.82	38.91	4.72	22.64
3c	Propyl	41.3	0.58	0.66	$C_{13}H_{19}N_6O_5P \cdot 7/9H_2O$	40.63	5.39	21.87	40.40	5.11	21.54
3d	Butyl	28.8	0.63	0.68	$C_{14}H_{21}N_6O_5P \cdot 1/4H_2O$	43.25	5.57	21.61	43.20	5.64	21.37
3e	Pentyl	24.9	0.66	0.70	$C_{15}H_{23}N_6O_5P \cdot 1/2H_2O$	44.23	5.94	20.63	44.44	5.82	20.30
3f	Hexyl	26.2	0.69	0.71	$C_{16}H_{25}N_6O_5P \cdot 1/4H_2O$	46.10	6.17	20.16	46.34	6.29	19.88
3g	Heptyl	25.4	0.73	0.74	$C_{17}H_{27}N_6O_5P \cdot 1/2H_2O$	46.89	6.48	19.30	46.73	6.40	19.03
3h	Octyl	27.4	0.75	0.75	$C_{18}H_{29}N_6O_5P \cdot 3/4H_2O$	47.63	6.77	18.51	47.90	6.61	18.22
3i	Nonyl	29.7	0.77	0.76	$C_{19}H_{31}N_6O_5P \cdot H_2O$	48.30	7.04	17.79	48.43	6.75	17.83
3j	Decyl	32.0	0.78	0.77	$C_{20}H_{33}N_6O_5P \cdot 1/2H_2O$	50.31	7.18	17.60	50.58	7.26	17.25
3k	Undecyl	24.6	0.78	0.80	$C_{21}H_{35}N_6O_5P \cdot 1/3H_2O$	51.63	7.36	17.20	51.79	7.20	17.24
3l	Dodecyl	29.5	0.78	0.84	$C_{22}H_{37}N_6O_5P \cdot 1/5H_2O$	52.83	7.54	16.80	53.02	7.58	16.93
3m	Tetradecyl	36.2	0.78	0.87	$C_{24}H_{41}N_6O_5P \cdot 1/4H_2O$	54.48	7.91	15.88	54.68	7.88	15.68
3n	Cetyl	33.3	0.78	0.87	$C_{26}H_{45}N_6O_5P \cdot 1/4H_2O$	56.05	8.23	15.08	56.20	8.36	14.73
3o	Stearyl	18.5	0.77	0.87	$C_{28}H_{47}N_6O_5P \cdot 3/4H_2O$	56.60	8.57	14.14	56.43	8.36	14.13

a) Rf by paper chromatography(0.5M ammonium acetate/EtOH=2/5). b) Rf by TLC(25% methanol in chloroform). c) Samples were dried over P_2O_5 at 50 °C at 3 mmHg for 3–5 h.

TABLE II. Effect of cAMP Amidates on the Growth of Mastocytoma P-815 Cells

Compd. No.	R	Cytotoxic activity (%) ^a				
		P815 ^b			FM3A ^c	ZR-75 ^d
		1 μ M	10 μ M	20 μ M	20 μ M	20 μ M
1	—	0	0	0	0	0
3a	Methyl	1.7	0			
3b	Ethyl	1.7	0.8	5.2	3.0	18.6
3c	Propyl	0	0			
3d	Butyl	0	0	22.4	2.2	35.6
3e	Pentyl	0	0			
3f	Hexyl	0	0			
3g	Heptyl	0	9.3	45.2	4.8	62.0
3h	Octyl	0	43.2			
3i	Nonyl	4.7	50.9			
3j	Decyl	7.6	54.2	90.0	45.0	85.2
3k	Undecyl	0	34.0			
3l	Dodecyl	0	33.0	42.3	36.5	62.4
3m	Tetradecyl		22.0		26.4	43.4
3n	Cetyl		17.9			
3o	Stearyl		1.4	15.9	31.2	27.5

a) Percent inhibition of the growth of tumor cells treated with compounds to untreated controls. Values are the mean of duplicate assays. b) Mastocytoma P-815 cell. c) Mouse mammary tumor FM3A cells. d) Human mammary tumor ZR-75 cells.

synthesis of a series of cAMP amidates, especially those with long-alkyl chain. Similar reaction was recently reported by Bottka and Tomasz,¹⁵⁾ who synthesized only methyl and dimethyl cAMP amidate with $POCl_3$ and alkylamines in trimethyl phosphate.

Biological Studies The compounds (3a–o) listed in Table I were tested for growth inhibition against mouse mastocytoma P-815 cells, mouse mammary tumor FM3A cells, and human mammary tumor ZR-75 cells in culture. As shown in Table II, 3j was the most active compound in inhibiting cell proliferation of these cell lines. ID_{50} values (the concentration required for 50% inhibition of cell growth) of 3j were 6, 15.0, and 2.2 μ M, respectively. The ID_{50} of 6-mercaptopurine (6-MP) and 5-fluorouracil

Fig. 1. Inhibition of $[^3H]$ TdR Incorporation by 3 in Mastocytoma P-815 Cells

Details for this experiment are described in Experimental. The final concentration of 3 was 20 μ M. The percent inhibition of the $[^3H]$ TdR incorporation into DNA was the ratio of the radioactivity in a treated group to that in the control (924800 ± 33640 dpm).

(5-FU) against FM3A cells was 13 and 1.9 μ M in our experiment. Comparing these results, the cytotoxicity of compound 3j was similar to 6-MP. Then, the compound (3j) was tested for antitumor effect on P-815 cells *in vivo* by the total packed cell volume method and showed potent antitumor activity of 81.8% growth inhibition on day 6. Body weight of the animal did not decrease during this experiment. In a preliminary experiment, compound 3j did not show toxicity after a single i.p. administration of 1 g/kg into ICR female mice.

Effects of 3 on the biosynthesis of DNA in P-815 cells were determined by measuring the incorporation of radioactivity into an acid-insoluble fraction. Among various cAMP amidates tested at 20 μ M, the derivatives 3i and 3j showed the most potent inhibition of $[^3H]$ thymidine (TdR) incorporation into macromolecules (Fig. 1). It was found that the cytotoxic activity of a series of cAMP amidates increased with the increasing length of the side chain from 6 to 10 carbon atoms and decreased with

compounds having longer alkyl residues. This inhibition related to the number of carbon atoms was comparable to the growth inhibition of P-815 cells.

Further studies are needed to clarify the detailed mechanism of the inhibition of growth of mastocytoma P-815 cells by **3j**.

Experimental

UV absorption spectra were recorded with a Hitachi 557 spectrophotometer. IR spectra were taken on a JASCO A-202 spectrophotometer. Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were taken at 200 MHz on a JEOL JNM-FX200 NMR spectrometer in dimethylsulfoxide ($\text{DMSO}-d_6$). All $^1\text{H-NMR}$ data are reported in ppm downfield from tetramethylsilane as an internal standard. All melting points were determined on a micro-hot stage (Yanagimoto) and are uncorrected. Paper chromatograms were run on Toyo No. 51A filter paper (40×40 cm) with the developing solvent system of 0.5M ammonium acetate-EtOH (2/5, v/v). Analytical TLC were performed on Kiesel gel 60F₂₅₄ (Merck) plates. Column chromatography was carried out on YMC octadecyl silica (ODS) AQ-120 gel (YMC Co., Japan). Mass spectra (MS) were measured by the method of Lawson *et al.*¹⁶ on a Hitachi RMU-7M spectrometer.

General Procedure for Preparation of cAMP Amidates¹⁷⁾ A solution of the tributylammonium salt (**2**)¹⁸⁾ of **1** (2 g, 3.89 mmol) in 50 ml of absolute DMF was cooled at 0°C, and phosphorus pentachloride (1 g, 1.2 moleq) was added to the solution with stirring. After 20 min, alkylamine·HCl or alkylamine (10 moleq) and 10 ml of triethylamine were added to the solution and stirred for 20 min. Then, the mixture was evaporated *in vacuo*.

Method of Purification The residue was dissolved in an appropriate amount of water. The solution was directly poured onto an ODS column (3.5×30 cm). After washing with 500 ml of water, compound **3** was eluted from the column with a solution of acetonitrile (stepwise increase from 0 to 80%) in water. Each fraction was checked on TLC (CHCl_3 :MeOH=75:25) and *R_f* values are shown in Table I. Fractions containing **3** were collected and evaporated to dryness *in vacuo*. The residue was recrystallized from MeOH or EtOH-acetone to give a product.

3a: A white powder, mp 151–155°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 2.45 (3H, d, CH_3), 5.10–5.35 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.18, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (13400). MS *m/z*: 558 ($\text{M}(\text{TMS})_3$)⁺.

3b: A white powder, mp 140–147°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 1.06 (3H, t, CH_3), 2.81–2.94 (2H, m, P-N- CH_2), 5.35–5.44 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.17, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14400). MS *m/z*: 572 ($\text{M}(\text{TMS})_3$)⁺.

3c: A white powder, mp 144–147°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.80 (3H, t, CH_3), 1.22–1.58 (2H, m, CH_2), 2.60–2.85 (2H, m, P-N- CH_2), 5.30–5.64 (1H, m, P-NH), 6.06 (1H, s, H-1'), 7.32 (2H, s, NH_2), 8.20, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (15900). MS *m/z*: 586 ($\text{M}(\text{TMS})_3$)⁺.

3d: A white powder, mp 134–138°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.84 (3H, t, CH_3), 1.16–1.60 (4H, m, $(\text{CH}_2)_2$), 2.73–2.85 (2H, m, P-N- CH_2), 5.24–5.43 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.17, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (13900). MS *m/z*: 600 ($\text{M}(\text{TMS})_3$)⁺.

3e: A white powder, mp 132–135°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.87 (3H, t, CH_3), 1.15–1.55 (6H, m, $(\text{CH}_2)_3$), 2.72–2.90 (2H, m, P-N- CH_2), 5.28–5.44 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.18, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14500). MS *m/z*: 614 ($\text{M}(\text{TMS})_3$)⁺.

3f: A white powder, mp 119–124°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.87 (3H, t, CH_3), 1.16–1.57 (8H, m, $(\text{CH}_2)_4$), 2.72–2.85 (2H, m, P-N- CH_2), 5.26–5.44 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.18, 8.34 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14200). MS *m/z*: 628 ($\text{M}(\text{TMS})_3$)⁺.

3g: A white powder, mp 168–173°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.87 (3H, t, CH_3), 1.25–1.56 (10H, m, $(\text{CH}_2)_5$), 2.76–2.89 (2H, m, P-N- CH_2), 5.28–5.42 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.18, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14100). MS *m/z*: 642 ($\text{M}(\text{TMS})_3$)⁺.

3h: A white powder, mp 160–162°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.85 (3H, t, CH_3), 1.15–1.60 (12H, m, $(\text{CH}_2)_6$),

2.70–2.90 (2H, m, P-N- CH_2), 5.24–5.43 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.17, 8.34 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14500). MS *m/z*: 656 ($\text{M}(\text{TMS})_3$)⁺.

3i: A white powder, mp 143–149°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.83 (3H, t, CH_3), 1.20–1.60 (14H, m, $(\text{CH}_2)_7$), 2.70–2.90 (2H, m, P-N- CH_2), 5.24–5.43 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.18, 8.34 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14500). MS *m/z*: 670 ($\text{M}(\text{TMS})_3$)⁺.

3j: A white powder, mp 160–165°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.80 (3H, t, CH_3), 1.20–1.58 (16H, m, $(\text{CH}_2)_8$), 2.60–2.85 (2H, m, P-N- CH_2), 5.30–5.64 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.32 (2H, s, NH_2), 8.22, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14200). MS *m/z*: 684 ($\text{M}(\text{TMS})_3$)⁺.

3k: A white powder, mp 162–165°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.83 (3H, t, CH_3), 1.15–1.55 (18H, m, $(\text{CH}_2)_9$), 2.73–2.88 (2H, m, P-N- CH_2), 5.26–5.44 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.17, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14800). MS *m/z*: 698 ($\text{M}(\text{TMS})_3$)⁺.

3l: A white powder, mp 162–167°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.84 (3H, t, CH_3), 1.16–1.60 (20H, m, $(\text{CH}_2)_{10}$), 2.70–2.90 (2H, m, P-N- CH_2), 5.24–5.43 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.17, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (13600). MS *m/z*: 712 ($\text{M}(\text{TMS})_3$)⁺.

3m: A white powder, mp 157–162°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.85 (3H, t, CH_3), 1.15–1.60 (24H, m, $(\text{CH}_2)_{12}$), 2.70–2.90 (2H, m, P-N- CH_2), 5.26–5.42 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.17, 8.34 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14900). MS *m/z*: 740 ($\text{M}(\text{TMS})_3$)⁺.

3n: A white powder, mp 161–164°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.85 (3H, t, CH_3), 1.15–1.60 (28H, m, $(\text{CH}_2)_{14}$), 2.72–2.88 (2H, m, P-N- CH_2), 5.26–5.44 (1H, m, P-NH), 6.01 (1H, s, H-1'), 7.24 (2H, s, NH_2), 8.17, 8.34 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (13900). MS *m/z*: 768 ($\text{M}(\text{TMS})_3$)⁺.

3o: A white powder, mp 176–179°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3120, 2950, 1660. $^1\text{H-NMR}$ δ : 0.87 (3H, t, CH_3), 1.16–1.58 (32H, m, $(\text{CH}_2)_{16}$), 2.73–2.90 (2H, m, P-N- CH_2), 5.24–5.44 (1H, m, P-NH), 6.00 (1H, s, H-1'), 7.25 (2H, s, NH_2), 8.17, 8.35 (1H each, s, purine H's). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 259 (14700). MS *m/z*: 796 ($\text{M}(\text{TMS})_3$)⁺.

Measurement of Cytotoxicity Mastocytoma P-815 cells were grown as suspension culture in Fischer's medium supplemented with 10% fetal bovine serum (FBS). Mouse mammary tumor FM3A cells were maintained as suspension culture in RPMI 1640 medium supplemented with 10% FBS and human mammary tumor ZR-75 cells were maintained as monolayer culture in the same medium. All tumor cell lines were cultured in a 5% carbon dioxide humidified incubator at 37°C. For determination of cell growth inhibition, cells (2×10^5) were seeded in a Falcon 6 well plate (3 ml/well). Cells were grown in the presence of compounds (**3**) at concentration of 1, 10 or 20 μM . After incubation for 48 h at 37°C, number of P-815 cells and FM3A cells was determined using a Coulter cell counter. ZR-75 cells which were cultured for 7 d at 37°C were counted using a Coulter counter after treatment with 0.25% trypsin.

Stock solutions of the drugs were made in DMSO and added to duplicate wells of the above cell lines. DMSO without drugs added to duplicate wells served as the control.

Antitumor Effect of cAMP Decyl Amidate (3j**) on P-815 Cells** Male BDF₁ mice of 20–30 g were used to test the antitumor activity of **3j** against mastocytoma P-815 cells. Groups of six mice were inoculated with P-815 cells (2×10^6) intraperitoneally. From the following day, **3j** at a dose of 0.2 mg/mouse was administered intraperitoneally once daily for 5 d; the animals were then killed by exsanguination under deep Nembutal anesthesia and the packed volume of P-815 cells in ascitic fluid was measured to determine antitumor activity.

Measurement of [^3H]TdR Incorporation into Macromolecules After the preincubation of P-815 cells (2×10^6) in phosphate-buffered saline (PBS) solution for 10 min with test compound (**3**), the cells were incubated for 1 h at 37°C with radiolabeled precursor (0.2 $\mu\text{Ci/ml}$). They were then collected on Whatman GF/C filter paper and washed three times with 3 ml each of ice-cold PBS and 10% HClO_4 . The radioactivity was counted using a toluene based scintillation solution.

Acknowledgment The authors wish to express their hearty thanks to Dr. S. Ishii for his help. They are also indebted to Mr. T. Tomikura for his help.

References

- 1) Part VIII: S. Kataoka, R. Uchida and N. Yamaji, *Heterocycles*, **32**, 1351 (1991).
- 2) R. R. Burk, *Nature* (London), **219**, 1272 (1968).
- 3) W. L. Ryan and M. L. Heidrick, *Science*, **162**, 1484 (1968).
- 4) W. D. Wicks, "The Role of Cyclic Nucleotides in Carcinogenesis," ed. by J. Schultz and H. G. Gratzner, Academic Press, New York, 1972, pp. 103—124.
- 5) M. C. Willingham, G. S. Johnson and I. Pastan, *Biochem. Biophys. Res. Commun.*, **48**, 743 (1972).
- 6) M. Negishi, A. Ichikawa, N. Oshio, K. Yatsunami and K. Tomita, *Biochem. Pharmacol.*, **31**, 173 (1982).
- 7) J. Francis, R. Thompson, S. D. Bernal, G. D. Luk and S. B. Baylin, *Cancer Res.*, **43**, 639 (1983).
- 8) C. I. Hong, G. L. Tritsch, A. Mittelman, P. Hebborn and G. B. Chheda, *J. Med. Chem.*, **18**, 465 (1975); J. W. Koontz and W. D. Wicks, *Mol. Pharmacol.*, **18**, 65 (1980); M. Saito, A. Nasu, S. Kataoka, K. Ohshita and N. Yamaji, *J. Pharmacobio-Dyn.*, **12**, 357 (1989).
- 9) a) N. Yamaji, Y. Yuasa and M. Kato, *Chem. Pharm. Bull.*, **24**, 1516 (1976); b) N. Yamaji, K. Suda, Y. Onoue and M. Kato, *ibid.*, **25**, 3239 (1977); c) N. Yamaji, K. Tahara and M. Kato, *ibid.*, **26**, 2391 (1978); d) S. Kataoka, J. Isono, N. Yamaji and M. Kato, *Chem. Lett.*, **1986**, 1221.
- 10) S. Kataoka, J. Isono, N. Yamaji, M. Kato, T. Kawada and S. Imai, *Chem. Pharm. Bull.*, **36**, 2212 (1988); S. Kataoka, J. Imai, N. Yamaji, M. Kato, T. Kawada and S. Imai, *ibid.*, **38**, 1596 (1990).
- 11) S. Kataoka, J. Imai, N. Yamaji, M. Kato, M. Saito, T. Kawada and S. Imai, *Nucleic Acids Res. Symp. Ser.*, **21**, 1 (1989).
- 12) J. Nagyvary, R. N. Gohil, C. R. Kirchner and J. D. Stevens, *Biochem. Biophys. Res. Commun.*, **55**, 1072 (1973); J. Engels and E. J. Schlaeger, *J. Med. Chem.*, **20**, 907 (1977); M. Korth and J. Engels, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **335**, 77 (1987).
- 13) R. B. Meyer, Jr., D. A. Shuman and R. K. Robins, *Tetrahedron Lett.*, **1973**, 269; J. Baraniak and W. J. Stec, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 1645; J. Tomasz, S. Bottka, J. Ludwig and I. Pelczer, *Phosphorus Sulfur*, **30**, 597 (1987); J. Beres, W. G. Bentrude, L. Parkanji, A. Kalman and A. E. Sopchik, *J. Org. Chem.*, **50**, 1271 (1985).
- 14) S. Bottka and J. Tomasz, *Tetrahedron Lett.*, **26**, 2909 (1985).
- 15) S. Bottka and J. Tomasz, *Nucleosides and Nucleotides*, **7**, 307 (1988).
- 16) A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsuboyama and J. A. McCloskey, *J. Am. Chem. Soc.*, **93**, 1014 (1971).
- 17) N. Yamaji, T. Tomikura and S. Nasuno, Japan. Patent 82175200 (1982) [*Chem. Abstr.*, **98**, 143796 (1983)].
- 18) J. G. Falbriard, T. Posternak and E. W. Sutherland, *Biochim. Biophys. Acta*, **65**, 558 (1962).