A CONVENIENT SYNTHESIS OF ADENOSINE 3',5'-CYCLIC PHOSPHATE (cAMP) BENZYL AND METHYL TRIESTERS[1]

Shigehiro Kataoka,* Riichiro Uchida, and Nobuyuki Yamaji
Research and Development Division, Kikkoman Corporation, 399 Noda, Noda-shi, Chiba 278, Japan

Abstract—The benzyl and methyl triesters of cAMP were synthesized from cAMP tri-*n*-butylammonium salt by alkylation with alkyl halides in the presence of Na_2CO_3 in dimethylacetamide (DMA).

Adenosine 3',5'-cyclic phosphate (cAMP, **1**) is an important compound which plays a regulatory role in cells as a second messenger of hormones.[2] Since cAMP (**1**) can not penetrate the cell membrane,[3] we have prepared various alkyl derivatives of **1** to obtain substances having cell membrane permeability and potent biological activity more than **1** itself.[4] Engels and co-workers synthesized the benzyl (**2a**) and methyl (**2b**) triesters by treatment of **1** with diazoalkanes and found that **2a** showed a positive inotropic effect,[5] but their method requires freshly prepared diazoalkanes. In a previous paper, we reported the synthesis of cAMP straight chain alkyl triesters having ethyl, propyl, pentyl, octyl, dodecyl, and cetyl groups by the reaction of tri-*n*-butylammonium salt (TBA salt) of cAMP (**1**·**TBA**) with alkyl bromides (4-10 equiv.) in the presence of excess tri-*n*-butylamine (5-10 equiv.).[4a] However, compounds (**2a,b**) could not be prepared under similar conditions using benzyl bromide (BnBr) and methyl iodide (MeI). Moreover, it was known only that the treatment of alkylammonium salt of **1** with benzyl and methyl halides in DMA or DMSO led to the alkylation at the 1-position of purine to give 1-alkyl cAMP (**3**).[6] We describe here a new and convenient method of synthesis of **2a** and **2b** from **1**·**TBA** by treatment with alkyl halides and the reactivities of the 1-position and phosphate group of **1**·**TBA** toward alkylation.

We examined the formation of **2a** from **1**·**TBA** using inorganic carbonates instead of tri-*n*-butylamine as a proton acceptor and found that Na_2CO_3 gave the best result as shown in

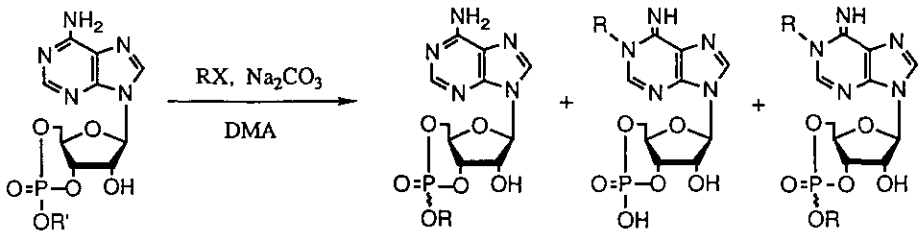
Table I. The compound (**2a**) was formed in about 74% yield at 15 min as analyzed by high-performance liquid chromatography (hplc) of the reaction system using Na_2CO_3, but decreased as the reaction proceeded with increase of 1-alkyl cAMP derivatives (**3a**,**4**) (Entries 1 and 2). The compound **1·TBA** (2 mmol) was treated with Na_2CO_3 (2 equiv.) and BnBr (5 equiv.) in DMA at 80 °C for 15 min to give **2a** (34% yield) and dibenzylated product 1,*P*-*O*-diBn cAMP (**4**, 0.7% yield) as amorphous solids (Scheme 1).

In the synthesis of methyl triester (**2b**) using Na_2CO_3 and MeI **2b** was formed in over 80% yield (on hplc) (Entry 6). The prolonged reaction increased the yield of 1-Me cAMP (**3b**). Work-up of the reaction mixture gave **2b** as an amorphous solid in 68.5% yield (Scheme 1). *The compound* (**2b**) *was found to be less stable than* **2a** *at room temperature and susceptible* to decomposition to **1**, giving two minor compounds which were assumed to be the corresponding 3'- and 5'-diesters of AMP.[5b] The formation of 1,*P*-*O*-diMe cAMP was not found on hplc of the reaction mixture, and this might be due to the lability of the methyl ester moiety.

Table 1. The Effects of Bases on Alkylation of cAMP TBA Salt[a)]

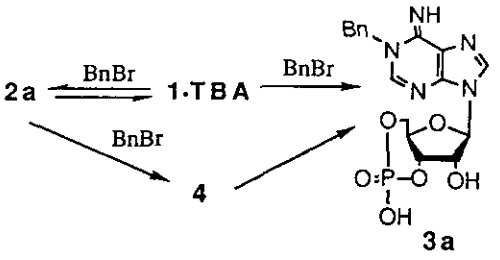
Entry	Base	RX	Products (%)[b)] 1	2	3	4	Time (min)
1	Na_2CO_3	BnBr	17	74	2	5	15
2	Na_2CO_3	BnBr	19	60	8	12	60
3	$NaHCO_3$	BnBr	32	46	13	8	5
4	$CaCO_3$	BnBr	39	46	11	4	5
5	K_2CO_3	BnBr	25	58	8	8	10
6	Na_2CO_3	MeI	10	84	2	—	5

a) Carried out using 1 equiv. of bases, 5 equiv. of RX, and 0.5 mmol of **1·TBA** in DMA at 80 °C.
b) Determined by hplc.

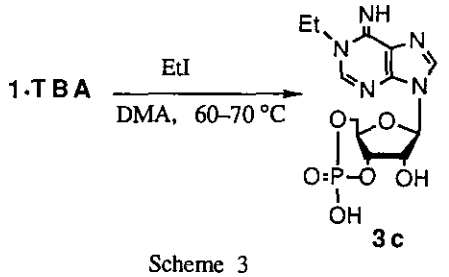


Scheme 1

We next investigated about the mechanism of **3a,b** formation from **1·TBA**, because **2a,b** were obtained under similar conditions to the synthesis of **3a,b**. The synthesis of **3a** from **1·TBA** with BnBr (6 equiv.) was carried out in DMA at 50-60 °C[6c] and **2a** was found to be the major product during 0.2–1 h and then **3a** increased up to 80% (on hplc) along with 1,*P*-*O*-diBn cAMP (**4**) as the reaction proceeded (after 6 h at 50–60 °C and 2 d at room temperature). Compounds (**3a**) and (**4**) were obtained in 60% and 3.5% yields, respectively. When the isolated **2a** was dissolved in DMA at room temperature, its slow decomposition to **1** without the formation of the 1-Bn derivative (**3a**) was observed on hplc. Further, the reactivity of the phosphate group of **1** toward benzylation was examined, and it was found that treatment of **1** (free form, less soluble than alkylammonium salt) with BnBr under the conditions described above, but using a large amount of DMA, did not give **2a** but **3a** as a major product on hplc. These results indicated that phosphate anion formed from **1·TBA** under the reaction conditions was more reactive than nitrogen at the 1-position toward benzylation so that **2a** was predominantly produced in the earlier stage of the reaction. Therefore, we confirmed the mechanism of the 1-Bn cAMP (**3a**) formation: **1·TBA** was benzylated to give mainly **2a** with minor products of **3a** and **4** in the earlier stage of the reaction, and the unstable compound (**2a**) reverted to **1** which was benzylated to give the stable compound (**3a**) as the reaction time was prolonged. Furthermore, **4** was hydrolyzed to **3a** under the reaction conditions (Scheme 2).



Scheme 2



Scheme 3

The same process could occur in the preparation of 1-Me cAMP (**3b**) as we found in the preparation of **2b**. On the other hand, 1–alkyl cAMP (**3**) was not obtained under our previous conditions in the synthesis of ethyl to cetyl triesters.[4a]

There would be two factors for not to give **3** by alkylation of **1·TBA** with alkyl bromides (ethyl to cetyl bromides) and tri-*n*-butylamine. One is that alkyl halides such as ethyl to cetyl bromides are less reactive than BnBr or MeI so that little alkylation of **1** with these

bromides occurs at the 1–position. Even using more reactive EtI than EtBr 1–Et cAMP (**3c**) was synthesized for the first in a very low yield (16%) (Scheme 3). The other factor is very high stability of ethyl to cetyl alkyl triesters in comparison with **2a,b**.[5a]

In conclusion, we developed a convenient synthesis of benzyl (**2a**) and methyl (**2b**) triesters using commercially available alkyl halides and found that the phosphate anion of cAMP (**1**) is more reactive than the nitrogen at the 1–position toward alkylation in the presence of weak base such as Na_2CO_3 or tri-*n*-butylamine.

EXPERIMENTAL

Uv spectra were recorded with a Hitachi 557 spectrophotometer. ^{1}H-Nmr spectra were taken at 200 MHz on a JEOL JNM-FX200 nmr spectrometer. Chemical shifts are reported in ppm downfield from Me_4Si (DMSO-d_6) or $Me_3Si(CD_2)_2CO_2Na$ (D_2O) as an internal standard. Hplc was perfomed on a Finepak SIL C_{18} column (4.6 × 250 mm) with MeOH-10 mM acetate buffer (pH 4.0) containing 1 mM tetra-*n*-butylammmonium chloride as eluent (detection at 260 nm). High-resolution secondary ion mass spectra (hr-sims) were recorded on a Hitachi M-80B spectrometer.

Preparation of cAMP Benzyl and Methyl Triesters (2a,b). General Procedure: Sodium carbonate (424 mg, 4 mmol) was added to a solution of **1a** (1.03 g, 2 mmol) in 100 ml of DMA with stirring at 80 °C and then a solution of alkyl halides (5 –10 mol equiv.) in 5 ml of DMA was added to the mixture. After 15 min (**2a**) and 5 min (**2b**) the solution was evaporated *in vacuo* below 40 °C. The residue was suspended in MeOH and the insoluble material was removed by filtration. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on aluminum oxide (eluent: MeOH/$CHCl_3$) to give a product. The compound (**2a**) was purified by the method described below.

cAMP Benzyl Triester (2a): The resulting residue was purified by preparative tlc (silica gel) with MeCN:EtOH (10:3, v/v) to give **2a** (282 mg, for an axial isomer, 34%) as an amorphous solid and 1,*P*-*O*-diBn cAMP (**4**, 7.2 mg, 0.7%) as an amorphous solid. **2a**: Uv (EtOH) λ_{max} nm (ε): 258 (12100); ^{1}H-nmr (DMSO-d_6) δ: 4.64 (1H, d, *J*=4.6 Hz, H-2'), 5.17 (2H, d, *J*=8.6 Hz, $POCH_2$), 6.05 (1H, s, H-1'), 6.28 (1H, br s, OH-2'), 7.19 (2H, s, NH_2), 7.30–7.60 (5H, m, phenyl H's), 8.02 and 8.26 (1H each, s, purine H's); hr-sims: Calcd for $C_{17}H_{19}N_5O_6P$: 420.1071, Found: 420.1020 $(M+H)^+$. **4**: Identical (ir, uv, ms,

and t_R on hplc) with the authentic sample which was obtained in the synthesis of **3a**.

cAMP Methyl Triester (2b): as an amorphous solid [470 mg, for an isomeric mixture of axial (ax):equatorial (eq) =2.8:1, 68.5%]. Uv (EtOH) λ_{max} nm (ε): 258 (12900); ^{1}H-nmr (DMSO-d_6) (ax:eq=2.8:1) δ: 3.78 and 3.83 (3H, d, *J*=11.4 Hz, $POCH_3$), 6.05 (1H, s, H-1'), 6.28 (1H, d, *J*=4.9 Hz, OH-2'), 7.23 (2H, s, NH_2), 8.12, 8.33, and 8.35 (2H, s, purine H's); hr-sims: Calcd for $C_{11}H_{15}N_5O_6P$:344.0758, Found:344.0623 $(M+H)^+$.

1-Benzylation of 1a: To a solution of **1a** (3.13 g, 6.1 mmol) in 50 ml of DMA was added BnBr (4.3 ml, 36.5 mmol). The mixture was stirred at 50-60 ℃ for 6 h and then at room temperature for 2 d. The solution was evaporated *in vacuo* and the residue was dissolved in MeOH, then added with acetone. The resulting suspension was separated by filtration to solid and mother liquid (A). The solid was dissolved in H_2O by adjusting to pH 7 with dil. aq. NH_4OH and chromatographed on Li-Chroprep RP 18 (Merck, 25–30% MeOH/H_2O, v/v) to afford crude **3a**. The crude product was crystallized from MeOH to give pure 1-Bn cAMP (**3a**, 1.49 g, 60%) as a colorless powder. The mother liquid (A) was evaporated *in vacuo* and the residue was extracted with *n*-BuOH–saturated aq. $NaHCO_3$ (50 ml : 100 ml). The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on aluminum oxide (eluent: 5–10% MeOH/$CHCl_3$, v/v) and the resulting residue was crystallized from MeOH to give 1,*P*–*O*–diBn cAMP (**4**, 109 mg, 3.5%) as a colorless powder. **3a:** Uv (H_2O) λ_{max} nm (ε): 259 (13300); ^{1}H-nmr (DMSO-d_6) δ: 5.61 (2H, s, NCH_2), 5.92 (2H, s, overlapped H-1' and OH-2'), 7.20–7.60 (5H, m, phenyl H's), 8.61 and 8.85 (1H each, s, purine H's), 9.68 (2H, br s, NH_2). Anal. Calcd for $C_{17}H_{18}N_5O_6P \cdot H_2O$: C, 46.69; H, 4.61; N, 16.01. Found: C, 46.41; H, 4.58; N, 15.86. **4:** uv (EtOH) λ_{max} nm (ε): 260 (13000); ^{1}H-nmr (DMSO-d_6) δ: 4.55 (1H, dd, *J*=5.1,4.9 Hz, H-2'), 5.14 (2H, d, *J*=8.6 Hz, $POCH_2$), 5.26 (2H, s, NCH_2), 5.94 (1H, s, H-1'), 6.27 (1H, d, *J*=4.9 Hz, OH-2'), 7.13 (1H, s, NH), 7.20–7.60 (10H, m, phenyl H's) 8.02 and 8.08 (1H each, s, purine H's); hr-sims: Calcd for $C_{24}H_{25}N_5O_6P$: 510.1540, Found : 510.1522 $(M+H)^+$.

1-Ethyl cAMP (3c): To a solution of **1a** (1.55 g, 3 mmol) in 10 ml of DMA was added EtI (2.4 ml, 30 mmol) and the mixture was stirred at 60–70 ℃ for 7 h. The solution was evaporated *in vacuo* and the residue was suspended in $CHCl_3$. The insoluble material was collected by filtration and chromatographed on Li-Chroprep RP 18 (2–3% MeOH/H_2O, v/v) to

afford crude **3c**. The crude product was crystallized from H_2O-acetone to give pure **3c** (176 mg, 16%) as colorless needles. Uv (H_2O) λ_{max} nm (ε): 258 (13100); ^{1}H-nmr (D_2O) δ: 1.52 (3H, t, *J*=7.0 Hz, CH_3), 6.26 (1H, s, H-1'), 8.45 and 8.60 (1H each, s, purine H's). Anal. Calcd for $C_{12}H_{16}N_5O_6P \cdot 1/3H_2O$: C, 39.68; H, 4.62; N, 19.28. Found: C, 39.69; H, 4.75; N, 19.27.

ACKNOWLEDGEMENTS

The authors are grateful to Professor T. Hino (Chiba Univ.) and Associate Professor M. Nakagawa (Chiba Univ.) for their helpful advice. The authors also thank Dr. S. Nasuno and Dr. N. Saito for their encouragement and help, and Mr. T. Matsudo and Miss N. Fukuta for hr-sims measurements.

REFERENCES

1. Part VIII of a series entitled "Studies on the Synthesis of the Compounds Related to Adenosine 3',5'-Cyclic Phosphate." Part VII: S. Kataoka, N. Yamaji, M. Kato, T. Kawada, and S. Imai, *Chem. Pharm. Bull.*, 1990, **38**, 3147 .
2. E. W. Sutherland, I. Oye, and R. W. Bucher, *Recent Prog. Hormone Res.*, 1965, **21**, 623.
3. G. A. Robison, R. W. Bucher, I. Oye, E. W. Sutherland, and H. E. Morgan, *Mol. Pharmacol.*, 1965, **1**, 168.
4. a) S. Kataoka, J. Isono, N. Yamaji, and M. Kato, *Chemistry Lett.*, **1986**, 1221; b) S. Kataoka, J. Isono, N. Yamaji, M. Kato, T, Kawada, and S. Imai, *Chem. Pharm. Bull.*, 1988, **36**, 2212; c) S. Kataoka, J. Imai, N. Yamaji, M. Kato, T. Kawada, and S. Imai, *ibid.*, 1990, **38**, 1596.
5. a) J. Engels and E.-J. Schlaeger, *J. Med. Chem.*, 1977, **20**, 907 ; b) M. Koth and J. Engels, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1979, **310**, 103.
6. a) K. H. Boswell, L. F. Christensen, D. A. Shuman, and R. K. Robins, *J. Heterocycl. Chem.*, 1975, **12**, 1; b) J. P. Miller, K. H. Boswell, R. B. Meyer, Jr., L. F. Christensen, and R. K. Robins, *J. Med. Chem.*, 1980, **23**, 242; c) S. Shibuya, A. Tanaka, and H. Yoshino, Japan. Kokai patent 49-92094 (1974) [*Chem. Abstr.*, 1975, **82**, 140457x].

Received, 8th May, 1991