Arch. Pharm. Res. Vol.20, No.2, pp. 176-179, 1997

The Facile and Efficient Synthesis of 8-Chloroadenosine 3',5'-cyclic monophosphate by Phosphorylative Cyclization of 8-Chloroadenosine and its Characterization by ^{1}H and ^{13}C NMR Spectroscopy

Nam-Tae Woo[1]*, Sun Yong Jin[1], Dae Jin Cho[1], Nam Sun Kim[1], Eun Hyung Bae[1], Jee-Hyung Jung[2], Won Hun Ham[3] and Young Hoon Jung[3]

[1]Il-Yang Central Research Institute, Hagal 182-4, Kiheung, Yongin, Kyungki, 449-900, Korea, [2]College of Pharmacy, Pusan National University, Pusan 609-735, Korea, [3]College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

(Received December 28, 1996)

Purine nucleosides were chlorinated by the reaction of acyl chloride in DMF with MCPBA under mild conditions with moderate yields. And, satisfactory method for the synthesis of ribonucleoside-3',5'-cyclic phosphates and its characterization by ^{1}H and ^{13}C nmr spectroscopy is described.

Key words : 8-Chloroadenosine-3',5'-cyclic monophosphate, antitumor agent, antineoplastic drug, c-AMP binding site, protein kinase isozyme

INTRODUCTION

The halo substituted purine nucleosides have exhibited chemotherapeutic, biomedical and biophysical properties. The target compound, 8-chloroadenosine-3',5'-cyclic phosphate, is known as an antitumor agent. Consideration of the cancer process as a problem of blocked oncogeny makes the approach to the control of cancer through differentiation theraphy using non-toxic biological agents an attractive one. cAMP, discovered by Sutherland in 1957 to be a mediator of hormonal signals, has long been considered to have a role in the regulation of cell growth and differentiation in a variety of cell type.

However, the potential for the clinical application of cAMP was only recently appreciated, when 8-Cl cAMP, a site-selective cAMP analog, was selected by the National Cancer Institute (NCI) as clinical phase I antineoplastic drug which is currently under phase II clinical trial.

It was discovered that 8-Cl cAMP, the most potent site-selective cAMP analog, exhibits growth inhibition *in vitro* and *in vivo* to various human carcinomas, such as fibrosarcomas and leukemias, without causing cytotoxicity. The use of these cAMP analogs, in fact, greatly advanced our understanding of the mechanism of cAMP action in growth control. Unlike cAMP, site-selective cAMP analogs demonstrate selective binding toward either one of the two known cAMP binding sites, Site A (site 2) and Site B (site 1) in the R subunit, resulting in preferential binding and activation of either protein kinase isozyme. With the use of site-selective cAMP analogs that demonstrate, with high affinity, selectivity toward protein kinase isozyme, it becomes possible to correlate the specific effect of cellular protein kinase isozymes with cAMP mediated responses in intact cells. That is, 8-Cl cAMP binds to R II of protein kinase type II with a high affinity at site B, but with a low affinity at site A, while binding with moderately high affinity for both Site A and Site B to RI, the dissociation of the RI Subunit and the down-regulation of protein kinase type I. Therefore, it was important to produce 8-Cl cAMP, the most potent site-selective cAMP analog.

The chlorination of purine derivatives have been less extensively studied than bromination. Previous methods for chlorination of purine derivatives at C-5 have included the use of Cl_2-H_2O in the presence of UV irradiation (Fukuhara *et al.*, 1955) and of N-chlorosuccinimide-acetic acid (Kikugawa *et al.*, 1975). But, unlike the easy method of bromination at C-8 of purine derivatives, (Long *et al.*, 1967) greater difficulty have been noted with regard to chlorination.

Recently, direct chlorination at C-8 of adenosine

Correspondence to: Nam-Tae Woo, Il-Yang Central Research Institute, Hagal 182-4, Kiheung, Yongin, Kyungki, 449-900, Korea

and its nucleotides has been performed using tetrabutylammonium iodotetrachloride (Brentnall *et al.*, 1972), or t-butyl hypochlorite (Ikehara *et al.*, 1977), although the yields are relatively poor.

MATERIALS AND METHODS

All reactions were conducted under an atmosphere of dry N_2. As necessary, most of solvents and reagents were dried over 3Å or 4Å molecular sieves and distilled. Melting points were uncorrected. ^1H and ^{13}C nmr spectra were collected at 200 MHz (^1H) or 500 MHz (^1H) from samples in the specified deuterated solvent. Assay yields of product were determined by HPLC analysis using the corresponding pure products as standards.

Preparation of 8-chloroadenosine (2)

To a solution of adenosine (0.0187 mole) in DMF (100 ml) was added a slight excess of acetyl chloride (7.0 ml, 0.0748 mole) followed by a solution of MCPBA (12.7 g, 0.0748 mole) in DMF (50 ml) over a period of 10 min at room temperature. The reaction mixture was stirred for 20 min, and then poured into cold water (100 ml). The resulting precipitate was filtered and washed with water.

The combined filtrate was washed with ether (500× 3 ml), basified and concentrated under reduced pressure to a syrup. The syrup was applied to a silica gel column (Merck silica gel 70~230 mesh, prepacked by a slurry of silica gel in 5% MeOH-$CHCl_3$), and eluted with 5% MeOH in $CHCl_3$. Fractions were combined according to tlc pattern. Evaparation of solvent gave the desired product. (2.2 g, 40.0%) ^1H NMR (200 MHz, DMSO-d_6): 3.66 (m. H5', H5"), 3.97 (m. H4'), 4.18 (m. H3'), 5.03 (t, H2"), 5.84 (d, H1'), 7.01 (br, NH2), 8.16 (s, H2): mp 188~190°C.

Preparation of 8-chloroadenosine 3',5'-cyclic monophosphate (4) from Adenosine 3',5'-cyclic monophosphate (Method A)

Adenosine 3',5'-cyclic monophosphate (5.0 g, 15.2 mmole) and N,N'-dicyclohexyl-4-morpholine carboxamidine (4.46 g, 15.2 mole) were dissolved in pyridine (300 ml) and the solution was concentrated to dryness. After removal of water by co-evaporation with pyridine (250 ml), the nucleotide salt was dissolved in DMF (140 ml).

The solution was added dropwise to tetra *n*-butyl ammonium iodotetrachloroide (11.66 g, 22.8 mmole) in DMF (140 ml). The reaction mixture was stirred for 12h. The completion of the reaction was judged by hydrolysis with H_2O (200 ml). The reaction mixture was washed with $CHCl_3$ (500 ml × 3). After evaporation of $CHCl_3$, a crude yellow compound was obtained and basified with 0.5 N NaOH. The yellow crude compound was purfied by C-18 column chromatography eluting with 5% MeOH in H_2O. Freeze-dry of the solution gave the desired product (1.32 g, 29.4%).

Preparation of 8-chloroadenosine 3',5'-cyclic monophosphate (4) from 8-chloroadenosine (2) (Method B)

To a solution of 8-chloroadenosine (**2**) (10 g, 0.0331 mole) in DMF (100 ml) was added a slight excess of freshly distilled phosporous oxychloride (3.7 ml, 0.0398 mole) over a period of 10 min at room temperature and the reaction mixture was stirred for 30 min at room temperature. After addition of excess H_2O, the reaction mixture was stirred for 2hr at room temperature. After usual workup, 8-chloroadenosine 5'-monophosphate (**3**) was obtained, which was directly used in following reaction without purification. Crude 8-chloroadenosine 5'-monophosphate (**3**) (11.0 g, 26.2 mmole) and N,N'-dicyclohexyl-4-morpholine carboxamidine (7.69 g, 26.2 mmole) were dissolved in pyridine (300 ml) and the solution was concentrated to dryness. After removal of water by co-evaporation with pyridine, the nucleotide salt was dissolved in pyridine (200 ml). The solution was added dropwise to boiling solution of DCC (10.8 g, 52.4 mmole), in pyridine (200 ml). After completion of addition of the 8-chloroadenosine 5-phosphate solution, the reaction mixture was heated under reflux for 6hr.

After the reaction was completed, the resulting precipitate was filtered and the combined filtrates was concentrated under reduced pressure to a syrup. The resulting yellow syrup was adjusted to pH 9~10 using 40% NH_4OH (20 ml). The solution was washed with CH_2Cl_2 (300 ml). Evaporation of aqueous solution gave crude syrup. The crude syrup was purified by C-18 column chromatography eluting with 1% AcOH and 10% MeOH in H_2O. Freeze-dry of eluent gave the desired product (2.47 g, 25.9%).

Preparation of 8-chloroadenosine 3',5'-cyclic monophosphate (4) from 8-chloro adenosine (2) (Method C)

A solution of 300 ml of triethyl phosphate and 10.0 g (33.1 mmole) of 8-chloroadenosine (**2**) were heated at 60°C for 1hr. A solution of 5.98 ml (66.3 mmole) of freshly distilled $POCl_3$ was added and the resulting reaction mixture was stirred at 0°C for 6hr. The reaction mixture was adjusted to pH 11.5 using 0.08 M NaOH solution. After additional 2hr of stirring, the solution was extracted with CH_2Cl_2 (500 ml × 3) and concentrated under reduced pressure to a white solid. The crude compound was isolated by C-18 column chromatography eluting with 1% AcOH and 10% MeOH in H_2O. The desired product was monitored under ultraviolet light. Evaporation of solvent gave the desired product (2.7 g, 23.1%).

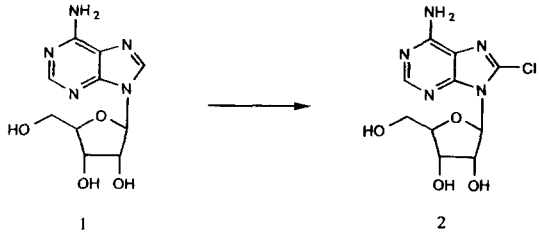
^{1}H NMR (200 MHz, D_2O): 4.25 (ddd, H4'), 4.34 (ddd, Hax5'), 4.51 (ddd, Heq5'), 5.00 (d, H2'), 5.22 (ddd, H3'), 6.11 (s, H1'), 8.16 (s, H2): ^{13}C NMR (50 MHz, DMSO-d_6): δ 69.82 (C5'), 73.79 (C2'), 74.58 (C 4'), 79.51 (C3'), 94.62 (C1'), 119.93 (C5), 141.0 (C8), 152.15 (C4), 155.73 (C2), 156.75 (C6). mp: 237°C.

RESULTS AND DISCUSSION

A facile method for the direct chlorination of purine nucleoside has been developed using MCPBA and HCl in an aprotic solvent such as DMF, DMA or HMPA (Ryu *et al.*, 1981). Herein, we describe the first example of oxidative chlorination of the nucleosides and bases using an acyl and aroyl chloride, such as benzoyl or acetyl chloride, as chlorinating agent. The other chloride ion sources such as p-toluenesulfonyl chloride, phosphorous oxychloride, phosphorous pentachloride were investigated. However, the use of acyl chloride, especially benzoyl chloride has several advantages over the other reagents described above in term of the yields, the mild reaction conditions and the ease of handling as well as facile workups.

Thus, to solutions of various ribonucleosides or their corresponding bases in DMF was added a slight excess of acetyl chloride followed by a solution of MCPBA in DMF over a period of 10 min. at room temperature. After 20~30 min, tlc examination of the reaction mixture on silica gel 60F-254 (Merck) using $CHCl_3$-MeOH (1:3) revealed that the starting material had disappeared. After appropriate workup procedure, the pure chlorinated analog was separated with column chromatography on silica gel using 5% MeOH in $CHCl_3$. The 8-chloro substituted adenosine was prepared in moderated yields. The use of other solvents such as dimethylacetamide (DMA) or hexamethylphosphoramide (HMPA) gave nearly similar results in the limited experiments and no advantage over DMF (Scheme 1).

The HPLC examination of the reaction mixture showed several byproducts which were not investigated in detail. The possible byproducts such as N-oxides resulting from this reaction conditions could not be detected. It is of interest to note that the reaction of a nucleoside (e.g. adenosine) with a complex of benzoyl or acetyl chloride in DMF in the absence of MCPBA on gentle warming (40~50°C) for 2 days, afforded 8-chloroadenosine (2).

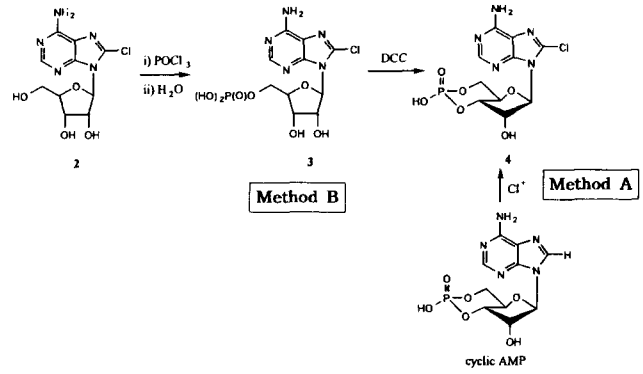


Reaction condition : CH_3COCl/DMF/MCPBA, RT, <20min
or CH_3COCl/DMF, 40-50°C, 2days

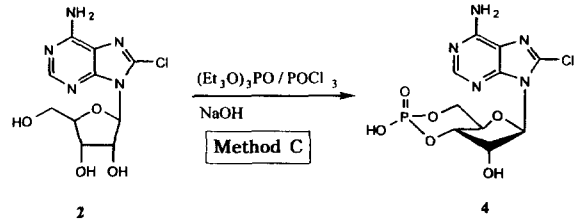
Scheme 1.

However by addition of MCPBA to this reaction mixture at room temperature, 8-chloro adenosine (2) was obtained within 30 min.

And then, we performed an efficient and economic phosphorylative cyclization. Direct conversion of 8-chloroadenosine (2) to its 5-monophosphate (3) proceeds cleanly in high yields using phosphoryl chloride with technical improvements in the general method of Yoshikawa *et al.* (Yoshikawa *et al.*, 1967) Cyclizations to the 3',5'-cAMP (4) by the dicyclohexyl carbodiimide (DCC) procedure of Khorana and co-workers (Smith *et al.*, 1961) proceed in more than 70%



Scheme 2.



Scheme 3.

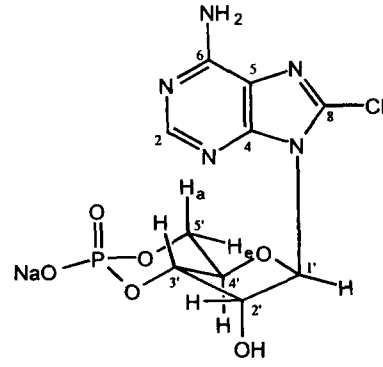


Fig.

Table I. ^{1}H (500 MHz) and ^{13}C (125 MHz) nmr spectral data of 4 (D_2O)

position	$^{13}C\delta$	$^{1}H\delta$ (m,J)#
6	156.75	
2	155.73	8.16 (s)
4	152.15	
8	141.00	
5	119.93	
1'	94.62	6.11 (s)
3'	79.51	5.22 (ddd, 11.0, 5.5, 2.1)
4'	74.58	4.25 (ddd, 11.0, 11.0, 4.8)
2'	73.79	5.00 (d, 5.5)
5'a	69.82	4.34 (ddd, 11.0, 11.0, 1.7)
5'e	69.82	4.51 (ddd, 21.3, 11.0, 4.8)

#m: multiplicity, J: coupling constant

yields.

A new method employing base-catalyzed cyclization of 5-trichloromethyl phosphonates makes these 3',5'-cAMP derivatives readily accessible (Marumoto *et al.*, 1975). Especially, this procedure has advantage over the chlorination method employing expensive cyclic AMP (Scheme 2).

During the course of an investigation on accelerators for the phosphorylation, it was found that the use of trialkyl phosphate highly facilitated the phosphorylation with phosphoryl chloride. In addition, nucleosides are moderately soluble in anhydrous trialkyl phosphates, such as trimethyl and triethyl phosphates.

These facts suggest the effectiveness of these esters as useful solvents for the phosporylation. Herein, the conversion could proceed as a base-catalyzed intramolecular phosphorylative cyclization to give the cyclic phosphate (Scheme 3).

8-Chloroadenosine 3',5'-cyclic monophosphate (4) was characterized based on ^{1}H, ^{13}C, COSY and HMQC nmr data (Table 1). ^{1}H nmr spectrum of 8-chloroadenosine 3',5'-cyclic monophosphate (4) displayed the characteristic three-bond proton-phosphorus coupling ($^{3}J_{H\text{-}3',P}$, $^{3}J_{H\text{-}5'e,P}$, and $^{3}J_{H\text{-}5'a,P}$) (Fig).

REFERENCES CITED

Brentnall, H. J. and Hutchinson, D. W., Preparation of 8-chloroadenosine and its phosphate esters. *Tetrahedron Lett.*, 2595 (1972).

Fukuhara, T. K. and Visser, D. W., Cytidine derivatives, *J. Am. Chem. Soc.*, 77, 2393 (1955).

Ikehara, M., Ogiso, Y. and Maruyama, T., Studies of Nucleosides and Nucleotides. LXXIII. Chlorination of Adenosine and its N^6-methyl Derivatives with t-Butyl Hypochlorites. *Chem. Pharm. Bull.*, 25, 575 (1977).

Kikugawa, K., Kawada, I. and Ichino, M., Alteration of 5-chloropyrimidine nucleosides in alkaline. *Chem. Pharm. Bull.*, 23, 35 (1975).

Long, R. A., Robins, R. K. and Townsend, L. B., Purine Nucleosides. XV. The synthesis of 8-Amino-and 8-substituted aminopurine nucleosides. *J. Org. Chem.*, 32, 2751 (1967).

Marumoto, R., Nishimura, T. and Honjo, M., New method for synthesis of nucleoside cyclic 3',5'-phosphates. Cyclization of nucleoside 5'-trichloromethylphosphonates. *Chem. Pharm. Bull.*, 23, 2295 (1975).

Ryu, E. K. and MacCoss, Malcoll, New procedures for the chlorination of pyrimidine and purine nucleosides. *J. Org. Chem.*, 46, 2819 (1981).

Smith, M., Drummond, G. I. and Khorana, H. G., Cyclic phosphates. IV. Ribonucleotide-3',5'-cyclic phosphates. A general method of synthesis and some properties. *J. Am. Chem. Soc.*, 83, 698 (1961).

Yoshikawa, M., Kato, T., and Takenishi, T., A Novel method for phosphorylation of nucleosides to 5'-nucleotides. *Tetrahedron Lett.*, 5065 (1967).