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A Simple Synthesis of Adenosine 3',5'-Cyclic Phosphate Alkyl Triesters

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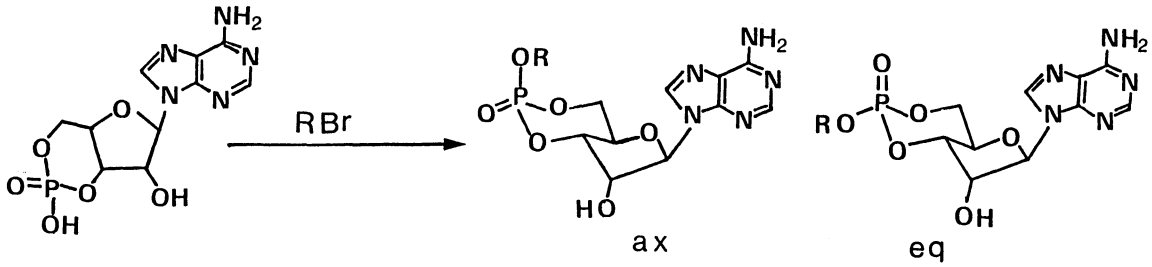
A series of alkyl triesters of c-AMP has been prepared from c-AMP tri-n-butylammonium salt by treating with readily available alkyl halides in the presence of tri-n-butylamine in N,N-dimethylacetamide. By this method the axial isomers were obtained predominantly.

Adenosine 3',5'-cyclic phosphate (c-AMP) plays an important role in the regulation of cellular processes in eukaryotic organisms,[1] and its many derivatives have been synthesized to obtain substances that might have better biological action than the original nucleotide.[2] We have reported synthesis of 2-substituted c-AMP.[3] Triesters of c-AMP, which possess an enhanced lipophilicity to penetrate the cell membrane,[4] have been reported to show the inhibition of tumor growth[4d,5] and the positive inotropic effects.[6] Recently, synthesis of benzyl triesters of 8-substituted c-AMP derivatives has been reported.[7] Reported alkyl triesters of c-AMP have been prepared by the following two routes: i) phosphate activation with a sulfonyl chloride, followed by esterification with dry alcohols;[4a] ii) direct alkylation of phosphate with diazocompounds.[4b,c] The former method includes tedious processes and gives low yield. The latter method requires freshly prepared diazocompounds and long reaction time, and examples were limited to methyl, ethyl, and benzyl triesters. Furthermore, both methods are not feasible for a large scale preparation. As to long chain alkyl triester of c-AMP, the cetyl triester was once synthesized in only 5% yield by the method i.

Utilization of cheap and readily available alkyl halide as the alkylating agent is obviously attractive for the preparation of the alkyl triester, but the reaction of c-AMP with alkyl halide in DMSO and 1,8-diazabicyclo[5.4.0]-undec-7-ene or in aqueous NaOH has been reported to give N^1-alkyl c-AMP[8] or 2'-O-alkyl c-AMP.[9] We have found, however, that tri-n-butylammonium salt of c-AMP is easily converted to the alkyl triesters by treatment with alkyl halides in the presence of tri-n-butylamine in N,N-dimethylacetamide.



R= C_2H_5, C_3H_7, C_5H_{11}, C_8H_{17}, $C_{12}H_{25}$, $C_{16}H_{33}$,

The present method is advantageous in comparison with the procedures hitherto reported in view of its wide applicability, operation simplicity and the low cost of the reagent. In particular, long chain alkyl triesters of c-AMP could be synthesized easily. Representative results are summarized in Table 1. In the case of long chain alkyl halides, higher reaction temperature was required than that for short chain alkyl halides.

In the synthesis of methyl, ethyl, benzyl triesters of c-AMP, Engels reported that the axial and equatorial isomers of these triesters were formed approximately 1 : 1 by the method ii.[4c] In our conditions, the amounts of the axial isomers of c-AMP triesters were 2.2 — 6.5 times those of the equatorial isomers. We observed that in the earlier stage of the reaction the axial and equatorial isomers were produced in the similar amounts and the axial isomers increased with the progress of reaction. This is presumably due to the thermodynamical stability of the axial isomers reported by Engels.[4c]

A typical procedure is illustrated as follows (entry 5 in Table 1): Tri-n-butylammonium salt of c-AMP (3.13 g, 6.08 mmol) was dissolved in N,N-dimethylacetamide (70 ml) and tri-n-butylamine (10 ml). Into this solution, a solution of n-dodecyl bromide (7.53 ml, 30.2 mmol) in N,N-dimethylacetamide (20 ml) was added dropwise at 100 - 110 °C with stirring. After 1.5 h, the

Table 1. Preparation of c-AMP alkyltriesters[a)]

	RBr		Conditions		Alkyl product	
Entry	R	m equiv.	Temp/°C	Time/h	Yield/%[b)]	ax : eq ratio[c)]
1	C_2H_5	10	75-90	1.7	78.9	2.8 : 1
2	C_3H_7	10	85-95	2.0	62.1	2.2 : 1
3	C_5H_{11}	7.5	85-95	2.9	40.6	6.5 : 1
4	C_8H_{17}	5.2	90-100	1.5	58.1	6.5 : 1
5	$C_{12}H_{25}$	5.0	100-110	1.0	39.1	6.0 : 1
6	$C_{16}H_{33}$	3.9	100-110	1.5	26.9	3.5 : 1

a) The reaction was carried out in the presence of tri-n-butylamine in N,N-dimethylacetamide.
b) Isolated yield.
c) Ratio was determined on the basis of HPLC (Finepak SIL C18 ; eluent, MeOH : 10 mmol acetate buffer (pH 4.0) containing 1 mmol tetra-n-butylammonium chloride).

solution was evaporated under reduced pressure. The residue was washed with n-hexane (30 ml x 3), and dissolved in a small amount of chloroform and chromatographed over aluminumoxide (40 g) with methanol-chloroform (1:9) as eluent. The appropriate fractions were evaporated under reduced pressure and the residue was purified by preparative TLC (silica gel) with methanol-chloroform (1:9) to give n-dodecyl triester of c-AMP (1.18 g) in 39.1% yield. ^{1}H-NMR (DMSO-d_6) δ 8.35 (s,1H,C_8- H), 8.20 (s,1H,C_2-H), 6.06 (s,1H,C_1'-H), 1.65-1.80 (m,2H,O-C-CH_2), 1.25-1.50 (m,18H,$(CH_2)_9$), 0.86 (t,J=6.5 Hz,3H, CH_3). UV λ_{max}^{EtOH} 260 nm. Anal. Found: C,52.18; H,7.24; N,13.61%. Calcd for $C_{12}H_{36}N_5O_6P_1.0.5H_2O$: C,52.17; H,7.36; N,13.83%.

Antitumor activity of alkyl triesters thus prepared will be published elsewhere.

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