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BIOACTIVATABLE DERIVATIVES OF 8-SUBSTITUTED cAMP-ANALOGUES

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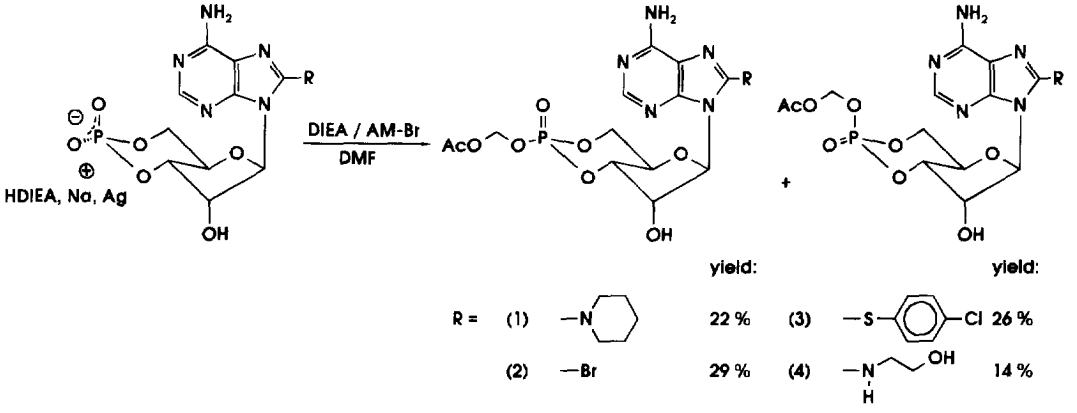
Abstract: Four 8-substituted derivatives of cAMP were converted into their membrane-permeant acetoxymethyl esters (AM-esters). The activity of these bioactivatable compounds to induce chloride secretion of T_{84} cells was up to 100-fold increased compared to the non-modified cAMP derivatives.

In most eucaryotic tissue adenosine 3',5'-cyclic monophosphate (cAMP) regulates numerous cellular processes through the activation of the cAMP dependent protein kinases type I and type II (PKAs).[1] Membrane-permeant cAMP-derivatives, i.e. 8-substituted analogues with increased lipophilicity, activate the PKAs and therefore mimick the effect of an increased intracellular cAMP level. These derivatives are usually metabolized more slowly and therefore show higher and more longlasting biological effects. However, a major problem of most known cAMP-analogues including the 8-substituted derivatives continues to be the relative membrane impermeability, leaving most of the compound wasted in extracellular space, when applied to living cells. Because of the ionic structure of cAMP and its analogues the extracellular doses nessecary were often in the millimolar range. Recently this problem was tackled for nucleotides,[2,3] cyclic nucleotides,[4,5] and even inositol polyphosphates[6] by masking the phosphates with bioactivatable acetoxymethyl ester (AM-esters), hence leading to derivatives with greatly improved membrane-permeability. Extending this method, we here describe the convertion of a number of 8-substituted cAMP-analogues to the corresponding AM-esters with some crucial modifications in the procedure.



Scheme 1: Alkylation of 8-substituted cAMP derivatives with acetoxymethyl bromide yielded the uncharged diastereomeric *Sp*- and *Rp*-acetoxymethyl esters 1 - **4**.

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Some of the new membrane-permeant cAMP/AM derivatives were applied extracellularly to cells of the human colonic epithelial cell line T_{84} and their potency to increase the chloride secretion (Cl^--secretion) of these cells was compared to the non-modified nucleotides.

Results and Discussion

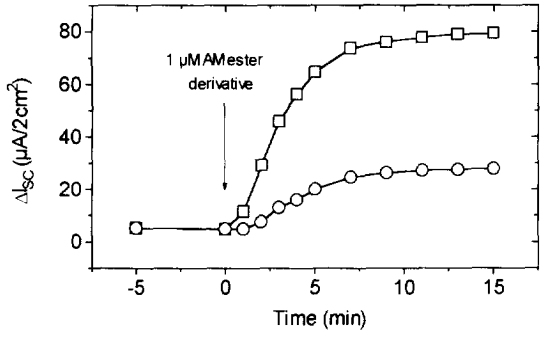
8-Bromo-cAMP (8-Br-cAMP) and 8-p-chlorophenylthio-cAMP (8-pCPT-cAMP) belong to the most widely used cAMP derivatives, although they have often to be applied to living cells in concentrations around 1 mM and 100 µM, respectively. The alkylation of the cAMP-derivatives to the *Rp*- and *Sp*-isomers of 8-piperidino-cAMP/AM (8-PIP-cAMP/AM) **1**, 8-bromo-cAMP/AM (8-Br-cAMP/AM) **2**, 8-p-chlorophenylthio-cAMP/AM (8-pCPT-cAMP-/AM) **3** and 8-hydroxyethylamino-cAMP/AM (8-HEA-cAMP/AM) **4**, respectively, is shown in Scheme 1. Reaction to the AM-ester of cAMP analogues in their commercially available form as sodium- or triethylammonium salt failed because of their insufficient solubility in acetonitrile. Experiments to convert the cAMP-derivatives into the corresponding free acids on an ion exchange column were mostly unsatisfactory. For this reason we chose a solvent with aprotic dipolar behaviour and an acceptable boiling-point: *N,N*-dimethylformamide (DMF). Now even the sodium salts were alkylated successfully in about one hour. Acetoxymethyl bromide (AM-Br) and diisopropylethylamine (DIEA) were added in an excess of five equivalents each. In all cases both diastereomers of the cAMP/AM-esters were produced. Table 1 shows selected physicochemical data of the new cAMP/AM-esters.

compound	FAB-MS [m/z] ($M+H^+$) (pos. mode)	^{31}P-NMR [ppm]	relative lipophilicity[d] [logK_w]
8-PIP-cAMP/AM **1**	485	- 4.2; -5.8[a]	> 3.5
8-Br-cAMP/AM **2**	482	- 4.9; - 6.9[c]	> 2.0
8-pCPT-cAMP/AM **3**	544	- 4.9; - 6.8[b]	> 4.0
8-HEA-cAMP/AM **4**	461	- 4.8; - 6.8[b]	> 2.0

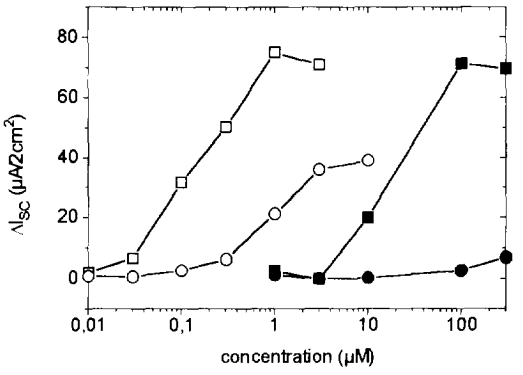
Table 1: AM-esters of 8-substituted cAMP-derivatives: abbreviations, corresponding numbers and some physicochemical data (measured in: [a]CD_3OH; [b]d_6-DMSO; [c]D_2O). [d] lipophilicity relative to cAMP (logK_w = 1.0).

The cAMP-mediated regulation of electrolytes through epithelial cells represents one of the best characterized signalling pathways in intestine.[7,8] To test the potency of the most lipophilic cAMP derivatives (Table 1) the Cl^--secretion of the human intestinal epithelial cell line T_{84} was investigated. The effect was continuously monitored after treatment of confluent monolayers previously mounted into modified Ussing chambers.[9] Figures 1*A* and 1*B* illustrate the Cl^--secretion measured as short circuit current (ΔI_{SC}) across the monolayer. 1 µM solutions of the acetoxymethyl ester analogues (8-pCPT-cAMP/AM **3** and 8-PIP-cAMP/AM **1**, respectively) was added to the basolateral reservoir 5 minutes after mounting the cells (Figure 1*A*). Dose-response relations for the cAMP/AM

derivatives and their unmodified mother compounds are shown in Figure 1*B*. The acetoxymethyl ester of 8-pCPT-cAMP had the highest activity with a half maximal effective concentration (EC_{50}) of 0.15 µM, while 8-PIP-cAMP/AM **1** showed an EC_{50} of 10 µM. Surprisingly, the Cl^--secretion induced by **1** did not exceed 50 % of the maximal response. This interesting phenomena may result from half-maximal activation of PKAs and should by checked in future experiments regarding the activation of isolated kinases. The dose-response curve of 8-pCPT-cAMP showed an EC_{50} of 20 µM. The results indicated that the AM-ester group resulted in a 100-fold increase of the biological activity compared to the unmodified derivatives. Neither the hydroxy ethyl amino analogue nor its AM-ester had an effect on the Cl^--secretion in this concentration range (data not shown).



A



B

Figure. 1: Time course of the Cl^--secretion induced in T_{84} cells (**A**) and concentration-response relationships (**B**) measured 12 minutes after the addition of the cAMP analogs 8-pCPT-cAMP [-■-] and 8-PIP-cAMP [-●-] as well as their uncharged acetoxymethyl esters 8-pCPT-cAMP/AM **3** [-□-] and 8-PIP-cAMP/AM 1 [-○-].

Materials and Methods

N,N-Dimethylformamide (DMF) was stored over activated molecular sieves (3 Å) for at least 2 weeks. CH_3CN was destilled three times from P_4O_{10} and stored over molecular sieves (4 Å). *N,N*-Diisopropylethylamine (DIEA) was destilled from CaH_2. Acetoxymethyl bromide (AM-Br) was prepared according to a known procedure (ref. 10). 8-pCPT-cAMP was purchased from Biolog Life Science Inst., Bremen. 8-Br-cAMP, 8-PIP-cAMP and 8-HEA-cAMP were synthesized as described previously.[11,12] All NMR spectra were recorded on a Bruker WH-360 spectrometer (360 MHz). Proton and ^{31}P NMR spectra were obtained in DMSO, $CDCl_3$ or D_2O, respectively. Fast atom bombardment mass spectroscopy was performed with a Finnigan MAT, Modell 8222. To estimate the lipophilicity $\log K_W$ values were determined by a recently developed gradient method, which was shown to be fully compatible to classical procedures.[13]

General method for the synthesis. 100 µmol of each cyclic nucleotide was dissolved in 1 ml dry DMF and treated with 500 µmol DIEA and 500 µmol AM-Br. After stirring the solution for one hour at 25 °C all volatile components were removed in high vacuum. Purification was performed by preparative HPLC (10 x 250 mm, LiChrospher 100, RP-18, 10 µm), except for 8-PIP-cAMP/AM **1** which was purified on a semi-preparative HPLC (4 x 250 mm, Si 60, 10 µm). All products gave satisfactory NMR and mass spectroscopy data.

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References and notes

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