Synthesis of the 3',5'-Cyclic Phosphates from Unprotected Nucleosides

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Unprotected nucleosides were phosphorylated with phosphoryl chloride in trialkyl phosphates and subsequently cyclized with base to give 3',5'-cyclic nucleotides in good yields.

Cyclic nucleotides play an important role in numerous biochemical processes. The binding region of the involved target receptor proteins can be mapped by deliberately selected analogues of the natural ligands.[1] Thus, cyclic nucleotide derivatives are valuable tools for characterization of the molecular interactions responsible for the binding process.

A common synthetic route to nucleoside 3',5'-cyclic phosphates is the phosphorylation of an unprotected riboside to its 5'-monophosphate[2] which is cyclized using a carbodiimide[3] or other condensing[4] agents. Another method is the cyclization with base of 5'-nitrophenyl phosphates of 2',3'-protected nucleosides.[5]

Several other procedures or variants have also been published.[6-10]

However, none of these methods appeared to be appropriate for the synthesis of more lipophilic cyclic nucleotide analogues including those with the nucleobase benzimidazole.

Recently, we found that thiophosphorylation of unprotected nucleosides with thiophosphoryl chloride and subsequent cyclization in a refluxing mixture of aqueous potassium hydroxide and acetonitrile, resulted in 3',5'-cyclic phosphorothioates in high yields.[11] We now report the successful application of this procedure to the synthesis of 3',5'-cyclic phosphates.

Nucleosides **1** were dissolved in trialkyl phosphates and treated with phosphoryl chloride to form presumably the dichloridate[12] **2**. However, instead of hydrolysis to the corresponding 5'-monophosphates the solution was added dropwise to a large volume of 0.08 M potassium hydroxide in a mixture of water/acetonitrile (4:6).

While cyclization to phosphorothioates required high temperatures the formation of cyclic phosphates **3** had to be performed with ice cooling. The procedure gave high yields for all nucleosides tested (Table). A main side product was the corresponding 5'-monophosphate.

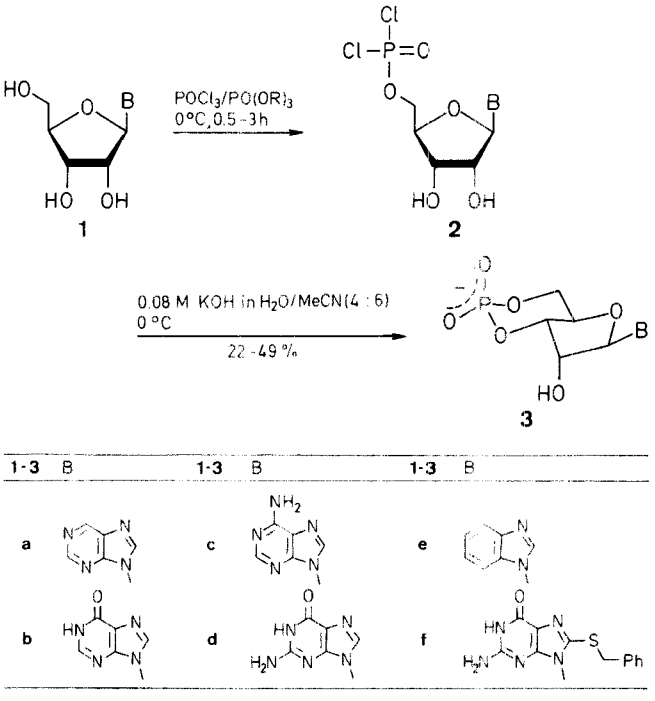

Table. 3',5'-Cyclic Phosphates **3** of Nucleotides Prepared[a]

Product	Reaction Time (h)	Yield (%)	Molecular Formula	FAB-MS $[M-H]^-/[M+H]^+$ *m/z*	^{1}H-NMR $\delta_{H-1'}$	^{31}P-NMR δ
3a	1	28	$C_{10}H_{11}N_4O_6P$ (314.2)	313/315	6.30[b]	−0.95[b]
3b	3	35	$C_{10}H_{11}N_4O_7P$ (330.2)	329/331	6.11[b]	−0.90[b]
3c	0.5	49	$C_{10}H_{12}N_5O_6P$ (329.2)	328/330	6.08[c]	−2.88[c]
3d	1	49	$C_{10}H_{11}N_5O_7P$ (345.2)	344/346	5.69[c]	−2.95[c]
3e	3	45	$C_{12}H_{13}N_2O_6P$ (312.2)	311/313	6.57[c]	−2.70[c]
3f	1.5	22	$C_{17}H_{19}N_5O_7PS$ (467.7)	466/468	5.68[c]	−2.55[c]

[a] All compounds were characterized by Fast Atom Bombardment mass spectrometry (FAB-MS) and by ^{31}P- and ^{1}H-NMR spectrometry focusing on the ribose H-1' proton.
[b] In D_2O.
[c] In DMSO-d_6.

The use of reactive dichloridates **2** for subsequent substitution reactions[13] and even their direct cyclization to 3′,5′-cyclic phosphates[14] has already been reported. However, the yields reported for the cyclization of 2′-alkylated nucleosides[14] (49%) could not be achieved by us with 2′-unprotected ribosides (12%) without the use of acetonitrile as cosolvent. It seems possible that cyclization in the presence of acetonitrile might further increase the yields of cyclic phosphates of 2′-alkylated nucleosides considerably.

All starting materials were purchased from Pharma Waldhof, Düsseldorf. LiChroprep RP-8 and LiChrosorb RP-18 were purchased from Merck, Darmstadt. 8-Benzylthioguanosine (**1f**) was synthesized according to Ref. 15.

FAB – MS spectra were recorded on a Finnigan MAT (Modell 8222). NMR spectra were performed on a Bruker WH 360 spectrometer (360 MHz).

3′,5′-Cyclic Phosphates 3 of Nucleosides; General Procedure:
The nucleoside (1 mmol) is dried over P_2O_5 and dissolved in freshly distilled trimethyl or triethyl phosphate (5 mL) by heating. The solution is cooled to room temperature, freshly distilled $POCl_3$ (186 μL, 2 mmol) is added, and the mixture is stirred at 0 °C. The reaction is monitored by HPLC on octadecyl-modified silica gel with MeOH/100 mM triethylammonium formate buffer. When the nucleoside has reacted completely the mixture is added to a stirred mixture of 0.08 M KOH in H_2O/MeCN (4:6, 120 mL) at 0 °C. Immediately afterwards, the mixture is neutralized with aq. HCl and then evaporated to a volume of 5 mL. This residue is extracted with dry Et_2O (2 × 100 mL). The residue is suspended in MeOH (100 mL) to precipitate insoluble salts which are filtered off. The filtrate is fractionated by isocratic column chromatography (25 cm × 3 cm) on octyl-modified silica with 0.1 M triethylammonium formate buffer in MeOH. Lyophilization of the product-containing fraction yields the cyclic nucleotide. The spectral data from the nucleotides synthesized were identical to those obtained from authentic samples.

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