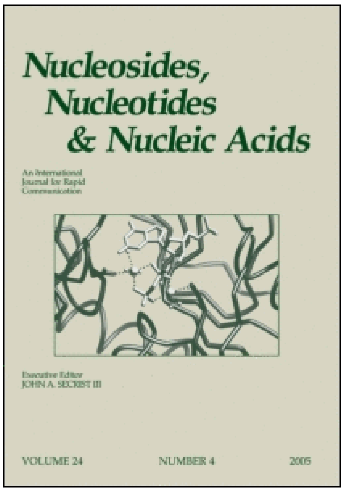

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An Improved Preparation of Rp and Sp N^6, N^6, O$^{2'}$ - Tri-Benzoyl-Adenosine-3', 5'-Cyclic Phosphoranilidates

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AN IMPROVED PREPARATION OF Rp and Sp $N^6,N^6,O^{2'}$-TRI-BENZOYL-ADENOSINE-3',5'-CYCLIC PHOSPHORANILIDATES

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Abstract: The conversion of the benzoylated cAMP (2) to the diastereomeric mixture of the anilidates (3) was improved. Replacing the triphenylphosphine/carbon tetrachloride mixture by oxalyl chloride in the presence of a catalytic amount of DMF followed by the addition of aniline not only increased the yield from 27 to 96% but rendered much easier the separation of the diastereoisomers (3), which were formed in approximately equal amounts. This greatly improved the accessability of Rp and Sp-cAMPS (1).

The discovery[1] that one of the diasteroisomers of adenosine cyclic 3',5'-phosphorothioates (Rp-cAMPS) (1) acts as an antagonist of cyclic adenosine mono phosphate (cAMP) prompted us to search for an improved synthesis of Rp-cAMPS.

The published procedures[2] for the preparation of Rp-cAMPS and Sp-cAMPS call for the conversion of the $N^6,N^6,O^{2'}$-tribenzoyl adenosine 3',5'-cyclic phosphate pyridinium or trialkyl ammonium salt (2) to a mixture of diastereomeric phosphoranilidates (3) via treatment of (2) with triphenylphosphine and carbon tetrachloride in pyridine in the presence of aniline (Appel reaction[3]) to give a 27% yield of the desired anilidates (3) as a 2:1 mixture of Rp and Sp diastereoisomers.[4] These were separated by chromatography on Silica gel.

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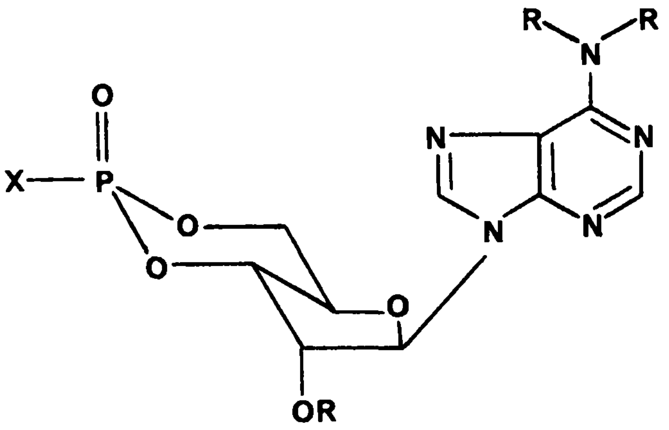
When we repeated these procedures, our attempts to separate the diastereoisomers were frustrated by the presence of the triphenylphosphine oxide produced during the reaction. On flash chromatography columns, this polar side product would overlap with considerable amounts of the desired products while on HPLC its retention time was too long because of tailing. This rendered a preparative separation on HPLC uneconomical due to considerable time lost for column regeneration.



1: X = SH; R = H (Rp-cAMPS)
2: X = OH; R = benzoyl
3: X = NH-Phenyl; R = benzoyl (Sp shown only)

We therefore searched for procedures avoiding the generation of triphenylphosphine oxide. Oxalyl chloride is extensively used to convert acids to acid chlorides under mild conditions. Since the molecule at hand was fully protected, side reactions were expected to be limited to a minimum for the conversion of cyclic phosphoric acid to the corresponding acid chloride. The cyclic phosphate (2) was thus treated with oxalyl chloride in methylene chloride in the presence of a catalytic amount of DMF (Vilsmeier reagent[5]). The formation of the corresponding cyclic phosphoric acid chloride was indicated by the presence of the molecular ion in the mass spectrum of the crude product. As expected the chloride consisted of a mixture of diastereoisomers as indicated by the ^{31}P NMR spectrum of the crude material.[6] The crude acid chloride was converted in 96% yield (over two steps) to a mixture of the diastereomeric anilidates (3) via the addition of aniline.

The crude mixture was separated by a fully integrated and automated HPLC system (RAININ) on a DYNAMAX™ preparative Silica gel column. This gave the pure diastereomeric Rp and Sp anilidates (3) in 50% and 46% yield, respectively.

Experimental Section

All solvents and reagents used were of the highest purity available.

Preparation of Rp- and Sp-Anilidates (3)

A solution of 7.0 g (2.5 ml, 0.029 mol) of oxalyl chloride in 200 ml of HPLC grade of methylene chloride containing 5 drops of abs. DMF was cooled in an ice bath. A solution of 5.0 g (0.007 mol) of the protected cAMP (2) in 150 ml of methylene chloride was added dropwise. After the addition was complete the ice bath was removed and the mixture was stirred at room temperature for 30 min. The solvent was evaporated and the residue was dried under high vacuum. The residue was redissolved in 100 ml of methylene chloride and cooled to 5-10°. A solution of 4.0 g (0.043 mol) of distilled aniline in 40 ml of methylene chloride was added. The mixture was stirred at room temperature overnight. The mixture was diluted with additional solvent, washed with water, dried over $MgSO4$. The solvent was evaporated to give 7.3 g of crude anilidates (3). The mixture was separated on a preparative HPLC column using a combination of methylene chloride/acetonitrile as mobile phase. This gave 2.5 g (50%) of Rp-anilidate (m.p. 175-177°; m/e 717 [MH+]) and 2.3 g (46%) of Sp-anilidate (m.p. 182-184°; m/e 717 [MH+]). The spectral data (H, ^{13}C and ^{31}P NMR) for both compounds were in agreement with those published in the literature.

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6. The chemical shifts observed for the crude were 8.8 and -1.0 ppm, respectively (external H_3PO_4) with a ratio of 2:1. When aniline was added to the probe the peak at -1.0 disappeared faster.

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