Direct Sulfhydrolysis of Cyclic AMP: A One-Step Synthesis of the Cyclic Ribonucleotide of 6-Mercaptopurine (1).

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Treatment of adenosine cyclic 3′,5′-phosphate with liquid H_2S in aqueous pyridine provided a convenient, one-step synthesis of 9-β-D-ribofuranosylpurine-6(1*H*)thione cyclic 3′,5′-phosphate.

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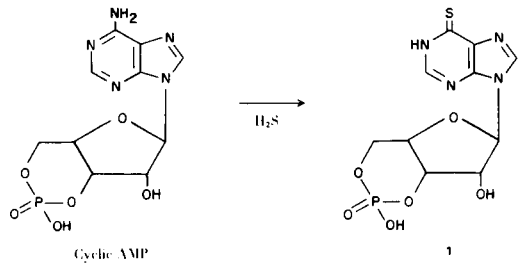
A major difficulty in the clinical treatment of leukemia by chemotherapy is acquired resistance to many of the antimetabolites used in the therapeutic regimens. The purine antimetabolite 6-mercaptopurine is converted to 9-β-D-ribofuranosylpurine-6(1*H*)thione 5′-phosphate (6-HS-RMP), the first of a series of active metabolites, by hypoxanthine-guanine phosphoribosyl transferase (HGPRT), and deficiency or lack of this enzyme is a major cause of mercaptopurine resistance (2,3). The potential importance of purine analogs which can bypass HGPRT has been pointed out (2).

5′-Nucleotides have met with little success as chemotherapeutic agents due to lack of cell membrane pentration. Cyclic 3′,5′-nucleotides, however, appear to penetrate cell membranes intact, as judged by widespread use of *N*,2′-*O*-dibutyryladenosine cyclic 3′,5′-phosphate as an exogenous cyclic AMP analog which stimulates cyclic AMP-dependent processes in living cells. Once inside the cell cyclic nucleotides are cleaved to the corresponding 5′-phosphates by phosphodiesterase. Montgomery (4) used this concept in the design of 9-β-D-ribofuranosylpurine-6-(1*H*)thione cyclic 3′,5′ phosphate (**1**). This agent was designed to be able to enter cancer cells and be hydrolyzed to 6-HS-RMP, the active metabolite of mercaptopurine, by the ubiquitous phosphodiesterase. Lepage and Hersh (5) later showed that **1** was active in animals bearing Ehrlich carcinomas which lacked HPGRT.

Recently we have prepared a series of 2′-*O*-acyl derivatives of **1** and found excellent activity against murine lymphoma cells which lack HGPRT. This study necessitated large quantities of **1**, and the three previously described routes (4,6,7) were fairly lengthy. One of these routes (7) is based on the sulfhydrolysis of N^6-methoxyadenosine cyclic 3′,5′-phosphate in analogy to the sulfhydrolysis of N^6-methoxyadenosine described by Ueda (8,9). They found that adenosine and 5′-AMP gave extensive decomposition under the conditions used, although 5′-AMP gave a very low yield of sulfhydrolysis product. Apparently the methoxylamine is a much better leaving group than ammonia under these conditions.

The presence of the cyclic 3′,5′-phosphate ring imparts substantial stability to nucleosides (10), and we have now found that cyclic AMP undergoes reaction with hydrogen sulfide to give **1** under somewhat more vigorous conditions than those used previously. The reaction is performed



Cyclic AMP 1

conveniently on a large scale, with the yield of this important nucleotide being 60% based on recovered cyclic AMP.

Many variations in the reaction conditions were investigated in order to improve the yield. The best yields were obtained when the water/pyridine/hydrogen sulfide solution of cyclic AMP was maintained at 90° for several days. Higher temperatures lead to extensive decomposition. This was found to be a limiting factor in the reaction of hydrogen sulfide with 5′-AMP at 100° (8). Lower temperatures gave much less product.

It is essential that the hydrogen sulfide be in a molar excess over the amount of pyridine used; otherwise the ratios of water, pyridine and hydrogen sulfide have little effect. Use of a catalytic amount of 4-dimethylaminopyridine, an agent known to hasten acylation reactions (11), had no effect, nor did addition of acetic acid as an acid catalyst. In the absence of pyridine no product was obtained.

Separation of unreacted cyclic AMP from **1** is readily accomplished by either anion or cation exchange column chromatography, but is complicated on a large scale by crystallization in the column of unreacted cyclic AMP and the product. The preparation given below reflects this difficulty.

EXPERIMENTAL (12)

9-β-D-Ribofuranosylpurine-6(1*H*)thione Cyclic 3′,5′-Phosphate (**1**).

A 500 ml. high pressure reaction vessel was charged with a solution of 32.9 g. (0.1 mole) of adenosine cyclic 3′,5′-phosphate, 150 ml. of water, and 10 ml. of pyridine. The vessel was cooled to -78° and 80 ml. of pyridine/liquid hydrogen sulfide (1/1, v/v) was added. The vessel was sealed and heated at 85°-90° for 7 days. After venting excess hydrogen sulfide (through aqueous sodium hypochlorite), the reaction mixture was filtered and evaporated *in vacuo*. Water (100 ml.) was twice added and evaporated. The residue was dissolved in 200 ml. of hot water and 2 volumes of hot

methanol was added. Seeding with cyclic AMP and cooling gave 19 g. of the pyridinium salt of cyclic AMP, removed by filtration, containing a trace of **1**. The filtrate was concentrated to 150 ml. and 40 ml. of Dowex 50 x 8 (H^+, 100-200 mesh) was added to this solution. Some precipitation resulted. This mixture was warmed and percolated through a funnel containing an additional 40 ml. of Dowex 50 x 8. The resin was washed with 300 ml. of boiling water, and the eluate was evaporated giving 6.86 g. pure **1**. An additional 300 ml. of boiling water was passed through the resin and concentrated to the minimum volume necessary to maintain solution (150 ml.). This solution was passed through a 100 ml. column of Dowex 50 x 8 and the appropriate fractions were evaporated to give 2.82 g. of **1** and 1.66 g. of cyclic AMP. Elution of the original resin bed with an additional 700 ml. of boiling water gave 2.28 g. of pure cyclic AMP. Thus 60% of the original cyclic AMP was recovered (as the pyridinium salt and free acid) and 9.68 g. of **1** (26.6% overall, 64.7% based on unrecovered cyclic AMP) was obtained as the monohydrate, identical by tlc (silica gel, 0.1 *N* ammonium chloride/acetonitrile, 3/7, v/v), nmr, and uv to authentic material (7).

REFERENCES AND NOTES

(1) This work was supported by grants from the University of California Cancer Research Coordinating Committee and from the Academic Senate, San Francisco Division, University of California.

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(12) Evaporations were conducted on a rotary evaporator under high vacuum, with a bath temperature $\leqslant 50^\circ$. Uv spectra were determined on a Beckman Acta C III.