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Studies of Nucleosides and Nucleotides. LVI.[1] A Versatile Method for the Synthesis of 8-Mercaptoadenosine Nucleotides

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We have reported the syntheses of various 8-substituted purine nucleosides[3-5] and nucleotides,[6-9] as well as polynucleotides.[10] The key intermediates 8-bromo derivatives were transformed to 8-mercapto compounds by refluxing with thiourea or by heating with H_2S in pyridine solution. In this paper we describe a versatile method for obtaining 8-mercaptoadenosine derivatives from the corresponding 8-bromo compounds using NaSH in DMF-water solution at room temperature. The yield was generally high and side reactions were limited.

By using this method 8-mercapto AMP, 3′,5′-cyclic 8-mercapto AMP and 8-mercapto ATP were obtained in the yield of 79, 76, and 78%, respectively. 8-Mercapto ATP, thus obtained, was used for the substrate of RNA pyrophosphorylase[11] and 3′,5′-cyclic 8-mercapto-AMP was used for rat liver protein kinase B_2.[12]

Experimental

8-Mercaptoadenosine 5′-Monophosphate——8-Bromo-5′-AMP NH_4 salt[7] (1 mmole) was dissolved in DMF–water (1:1, vol/vol) (7 ml). Into the solution was added 40% NaSH (0.7 ml) and the reaction mixture was kept at room temperature for 21 hr. After the reaction extent was examined by paper electrophoresis (pH 7.5), the mixture was neutralized with 0.1N HCl. Solvent was evaporated *in vacuo*, a small amount of water was added, and filtered. Filtrate was evaporated *in vacuo* and residue was dissolved in water. The water solution was applied to a DEAE–cellulose (bicarbonate form) column (2×50 cm). The column was eluted with triethylammonium bicarbonate buffer (pH 7.5) (0.01—0.2M, 3 l+3 l), using linear gradient technique. Fractions (20 ml each) No. 156—320 were collected and evaporated. After the evaporation with added water several times, the solution was lyophilized. Yield was 79%. UV: $\lambda_{max}^{pH\,2}$ 308 nm (2.74×10^4), $\lambda_{max}^{pH\,7}$ 298 nm (2.74×10^4), $\lambda_{max}^{pH\,11}$ 296 nm (2.59×10^4). Paper chromatography (R_{pA}): in solvent A,[13] 1.34; solvent B, 0.61; solvent C, 0.66. Paper electrophoresis performed in 0.05M ammonium bicarbonate at pH 7.5: R_{pA} 1.25.

8-Mercaptoadenosine 5′-Triphosphate——8-Bromoadenosine 5′-triphosphate[9] (Li salt) (30 mg, 690 A_{265} units, 0.043 mmole) was dissolved in a mixture of DMF (0.35 ml) and water (0.35 ml). Aqueous sodium hydrogensulfide (40%) (90 μl) was added to the mixture and an additional water (0.35 ml) was added to dissolve precipitates. After 16 hr at 25° paper electrophoresis showed no starting material (R_{pA} 1.53). The spot

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13) Solvent A: *n*-Butanol–acetic acid–H_2O (5:2:3, v/v), B: *n*-propanol–conc. NH_3–H_2O (55:10:35, v/v), C: ethanol–1 M ammonium acetate (7:3, v/v), D: sat. ammonium sulfate–isopropanol–conc. ammonia (79:19:2, v/v).

(R_{pA} 1.60) showed ultraviolet (UV) spectrum similar to 8-mercaptoadenosine. The reaction mixture was adjusted to pH 6 with 2N HCl and nitrogen gas was passed through the solution. The solution was again adjusted to pH 7.5 with 2N NaOH and concentrated to *ca.* 5 ml. The concentrated solution was applied to a column (2×60 nm) of Biogel P-2. Fractions of 3 ml were collected and fractions 23—37 were combined. The yield was 890 A_{298} units 0.033 mmole, 78%. UV: $\lambda_{max}^{pH\ 2}$ 261, 236 nm; $\lambda_{max}^{pH\ 7}$ 254 nm; $\lambda_{max}^{pH\ 13}$ 296 nm; $\lambda_{max}^{pH\ 2}$ 261, 236 nm; $\lambda_{max}^{pH\ 7}$ 254 nm; $\lambda_{max}^{pH\ 13}$ 253 nm. Paper chromatography in solvent B: R_{pA}=0.40, in solvent D: R_{pA}=1.74.

8-Mercaptoadensoine 3′,5′-Cyclic Phosphate——8-Bromoadenosine-3′,5′-cyclic phosphate[7] (100 mg) was dissolved in a mixture of DMF (0.3 ml) and water (0.3 ml). Aqueous sodium hydrogen sulfide (407, 100 μl) was added to the mixture. After 15 hr at 25°, paper electrophoresis showed almost complete conversion of the starting material. The reaction mixture was adjusted to pH 7 with 2N HCl and nitrogen gas was passed through the solution, which was adjusted to pH 7.5 with 2N NaOH. The solution was applied to a column (2×50 cm) of DEAE-cellulose (bicarbonate form). The column was eluted with triethylammonium bicarbonate buffer (pH 7.5, 0.01—0.1M, 21+21) using linear gradient technique. Fractions (10 ml each) 58—120 were collected and evaporated. Evaporation was repeated several times with added water and final solution was lyophilized to give a powder. Yield was 76%. UV: $\lambda_{max}^{pH\ 1}$ 220, 244, 308 mμ; $\lambda_{max}^{H_2O}$ 221, 245, 310 mμ; $\lambda_{max}^{pH\ 11}$ 224, 290 nm. PPC: R_{pA} in solvent A 1.65 and in solvent B 0.65. These properties were identical to those reported by Muneyama, *et al.*[14]

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Phosphorylation by Active Ester of Phosphoric Acid. I. Preparation and Reaction of Phenyl 8-Quinolyl Phosphate[1]

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It is well known that active esters of phosphoric acid such as $RO-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-OR'$[3] (diester) and $RO\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-NR'R''$[4] (phosphoramidate) are important intermediates in the synthesis of various kinds of phosphoric acid derivatives.

In the present study, the preparation of phenyl 8-quinolyl phosphate (**4**) and the reactions of the active diester of phosphoric acid (**4**) with alcohols and amines were investigated. It was considered that the phosphate (**4**) would yield a reactive intermediate (**4′**), which in turn would further react with alcohols to give diesters of phosphoric acid as shown in Chart1. The synthesis of diester of phosphoric acid can be effected successfully by this method since it reacts exclusively with nucleophilic reagents such as alcohols and amines under mild conditions.

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