NOVEL PREPARATION OF CYTIDINE 5′-PHOSPHATE AND CYTIDINE 3′,5′-CYCLIC PHOSPHATE*

W.S.Zieliński**, J.Smrt and J.Beránek

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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N,O$^{2'}$,O$^{3'}$-Tribenzoylcytidine (*III*) reacts with dianilidophosphochloridate to afford N,O$^{2'}$,O$^{3'}$--tribenzoylcytidine 5′-phosphodianilidate (*IV*). On successive treatment with isoamyl nitrite and aqueous ammonia, compound *IV* is converted to cytidine 5′-phosphate (*V*). The N,N′-dicyclohexyl-4-morpholinecarboxamidinium salt of the phosphate *V* reacts with N,N′-dicyclohexylcarbodiimide in a mixture of pyridine and dimethylformamide with the formation of cytidine 3′,5′-cyclic phosphate (*VI*).

The general synthesis of ribonucleoside 3′,5′-cyclic phosphates consists in reaction of 5′-phosphates with N,N′-dicyclohexylcarbodiimide[1]. In view of the insolubility of the unprotected nucleotide, the N-benzoyl derivative has been used in the case of cytidine 5′-phosphate[1]. An unprotected cytidine amino group has been used in the cyclisation of cytosine arabinoside 3′-phosphate[2]. In the present paper, we wish to report the cyclisation of cytidine 5′-phosphate bearing an unprotected amino group to cytidine 3′,5′-cyclic phosphate along with a novel preparation of the starting cytidine 5′-phosphate consisting in phosphorylation with dianilidophosphochloridate which has been recently shown to represent an efficient phosphorylating agent[3,4].

The starting cytidine was converted by the action of dimethylformamide dimethylacetal to N^4-dimethylaminomethylenecytidine[5] (*I*) which was not isolated. On treatment with dimethoxytrityl chloride, compound *I* was transformed to 5′-O-dimethoxytrityl-N^4-dimethylaminomethylenecytidine (R_F 0·45 in S_1) which afforded by the action of aqueous ammonia 5′-O-dimethoxytritylcytidine (*II*; R_F 0·35 in S_1). On the successive treatment with benzoyl chloride and 90% aqueous acetic acid, there was obtained N,O$^{2'}$,O$^{3'}$-tribenzoylcytidine (*III*) which was reacted with a small excess of dianilidophosphochloridate in pyridine. After two days, the excess reagent was hydrolysed with aqueous potassium acetate and the required N,O$^{2'}$,O$^{3'}$-tri-

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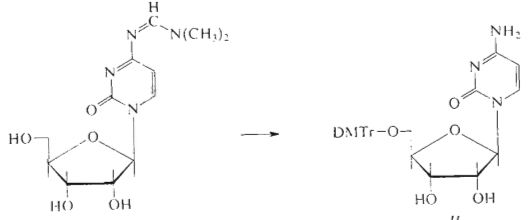
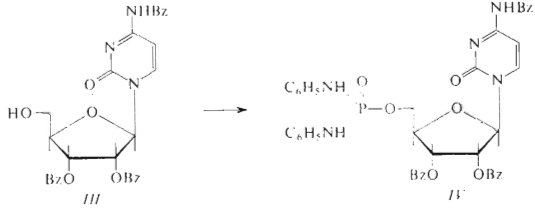
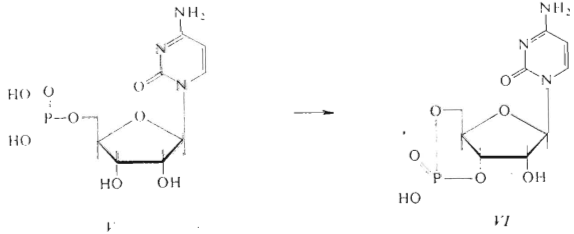
** Present address: Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Lodz, Poland.

benzoylcytidine 3'-phosphodianilidate (*IV*) isolated by extraction with chloroform in 95% yield. The aromatic phosphoamidic groups were removed by the action of isoamyl nitrite in the pyridine–acetic acid mixture[6] and the debenzoylation was performed with aqueous ammonia. The thus-obtained cytidine 5'-phosphate (*V*) was isolated in the form of the calcium salt. The phosphate *V* was cyclised by refluxing its N,N'-dicyclohexyl-4-morpholinecarboxamidinium salt with N,N'-dicyclohexylcarbodiimide in the pyridine–dimethylformamide mixture. The final cytidine 3',5'--cyclic phosphate (*VI*) was isolated by chromatography on DEAE-cellulose and the







contaminating trimethylammonium chloride was removed by means of Dowex 50 ion exchange resin. The yield of the final *VI* was 16%.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, chloroform–methanol (9 : 1), and S_2, chloroform–ethyl acetate (8 : 2). Descending paper chromatography was performed on paper Whatman No 1 in the solvent system S_3, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Electrophoresis was performed on the same paper dipped in tetrachloromethane with the use of 0·05M triethylammonium hydrogen carbonate (pH 7·5).

N,$O^{2'}$,$O^{3'}$-Tribenzoylcytidine (*III*)

A suspension of cytidine (10 g), dimethylformamide (50 ml), and dimethylformamide dimethyl acetal (24 ml) is stirred at 40°C until a solution is obtained. After 20 h at 20°C, the solution is evaporated at 25°C/1 Torr, the residue dissolved in 50% aqueous dioxane (50 ml), the stirred solution neutralised with solid carbon dioxide, and after elapse of 30 min evaporated. The residue is coevaporated with three portions of pyridine and finally dissolved in pyridine (100 ml). The solution is shaken with dimethoxytrityl chloride (14 g) until homogeneous. After 20 h, there is added conc. aqueous ammonia (100 ml), the mixture kept for additional 20 h at room temperature, diluted with water until turbid, and extracted with three 100 ml portions of chloroform. The extracts are dried over anhydrous magnesium sulfate, evaporated, the residue dissolved in ethyl acetate (100 ml), the solution filtered through Cellite, and the filtrate evaporated. The residue is dissolved in chloroform (30 ml), the solution added dropwise into stirred hot cyclohexane (300 ml), the precipitate dissolved in pyridine (200 ml), the solution concentrated to half of the original volume, the concentrate treated at −30°C with benzoyl chloride (15·2 ml), the whole stirred for 3 h, and poured onto ice (500 g). The mixture is extracted with chloroform (two 200 ml portions), the extract dried over anhydrous magnesium sulfate, and evaporated. The residue is coevaporated with two portions of toluene and finally dissolved in 90% aqueous acetic acid. After 3 h at 20°C, the solution is evaporated at 20°C/1 Torr, the residue is dissolved in chloroform (200 ml), the solution washed with four 100 ml portions of saturated aqueous sodium hydrogen carbonate, dried, and evaporated. The residue is dissolved in a mixture (200 ml) of chloroform and benzene (1 : 1) and applied to a 500 ml column of silica gel. The column is washed with chloroform–benzene (1 : 1; 1 500 ml) and the product is eluted with chloroform (800 ml). The eluaet is evaporated, the residue dissolved in chloroform (100 ml), and the solution poured into stirred cyclohexane (800 ml). The precipitate is collected with suction, washed with cyclohexane, and dried to afford 6·8 g (33%) of compound *III*, m.p. 178−180°C (reported[7], m.p. 185°C); R_F value 0·15 in the solvent system S_2.

N,$O^{2'}$,$O^{3'}$-Tribenzoylcytidine $O^{5'}$-Phosphodianilidate (*IV*)

To a solution of compound *III* (6·8 g) in pyridine (100 ml), there is added dianilidophosphochloridate (3·45 g), the whole is shaken until homogeneous and then kept at room temperature for 30 h. A solution of potassium acetate (5 g) in water (200 ml) is added, the whole stirred for 30 min, and extracted with chloroform. The extract is dried over anhydrous magnesium sulfate, evaporated, the residue coevaporated with toluene, and dried under diminished pressure. Yield, 8·6 g (95%) of compound *IV* in the form of a solid foam, R_F value 0·07 in S_2. For $C_{42}H_{36}N_5O_9P$ (785·6) calculated: 64·12% C, 4·58% H, 8·91% N, 3·95% P; found: 63·74% C, 4·72% H, 8·63% N, 4·07% P.

Cytidine 5′-Phosphate (*V*)

To a solution of compound *IV* (6·3 g) in a mixture (200 ml) of pyridine and acetic acid (1 : 1) there is added isoamyl nitrite (21·5 ml), the whole stirred for 20 h, and evaporated at 20°C/1 Torr. The residue is coevaporated with two 50 ml portions of pyridine, the final residue dissolved in 50% aqueous pyridine, and the solution washed with three 100 ml portions of ether. The aqueous layer is concentrated to half of its original volume, and the concentrate kept with an equal volume of conc. aqueous ammonia overnight. The solution is evaporated, the residue dissolved in water (100 ml), and the solution washed with three 50 ml portions of chloroform. In the aqueous phase, there is dissolved calcium chloride (8 g), the solution diluted with ethanol (600 ml), and kept for 20 h to deposit a solid which is collected with suction, washed with three portions of ethanol and one portion of ether, and dried. Yield, 2·3 g (62%) of the calcium salt of compound *V*.

Cytidine 3′,5′-Cyclic Phosphate (*VI*)

Cytidine 5′-phosphate (*V*) pyridinium salt (2 mmol) is coevaporated with five portions of pyridine and to the final residue there is added N,N′-dicyclohexyl-4-morpholinecarboxamidine (558 mg) and dimethylformamide (200 ml). The mixture is briefly refluxed to obtain a solution which is evaporated at 25°C/1 Torr. The residue is dissolved in dimethylformamide (50 ml), the solution is added dropwise over 70 min into a refluxing solution of N,N′-dicyclohexylcarbodiimide (2·31 g) in pyridine (500 ml), the refluxing is continued for 2 h, the mixture kept at room temperature for 20 h, and concentrated to the volume of about 50 ml under diminished pressure. The concentrate is diluted with water (150 ml), washed with ether (200 ml), and filtered. The aqueous filtrate is evaporated, the residue adjusted to pH 1 with conc. hydrochloric acid, kept at room temperature for 5 min, adjusted to pH 7 with 10% aqueous sodium hydroxide, and applied to a column (2000 ml) of DEAE-cellulose. The elution is performed with the use of a linear gradient (4 l of water in the mixing chamber and 4 l of 0·15M triethylammonium hydrogen carbonate in the reservoir). The UV-absorbing fractions eluted with 0·08M buffer solution (at the top of the column) are evaporated, the residue coevaporated with three portions of ethanol, and dried. The crystals are dissolved in water (20 ml) and the solution is applied to a column (100 ml) of Dowex 50 (H^+) ion exchange resin. The column is eluted with water until the pH value of the eluate is 5. The column is then eluted with 10% triethylamine in 30% aqueous ethanol, the eluate concentrated to a small volume, and the concentrate freeze-dried to afford 130 mg (16%) of the triethylammonium salt of the cyclic phosphate *VI*, R_{Up} 2·5 in the solvent system S_3 (paper). Electrophoretical mobility, $0{\cdot}45_{Up}$ (pH 7·5).

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