# SYNTHESIS OF 6-AMINOHEXYL ESTERS OF URIDINE NUCLEOTIDES*

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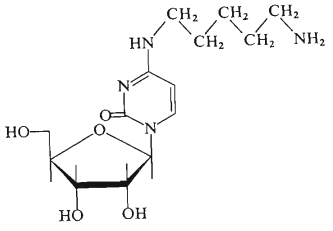
6-Aminohexyl esters of uridine 3'-phosphate (*V*) and uridine 5'-phosphate (*VI*) were prepared by condensation of 6-trifluoroacetamidohexanol (*IV*) with pyridinium salts of the protected corresponding phosphates by means of N,N'-dicyclohexylcarbodiimide followed by removal of protecting groups.

Covalently bound nucleosides and nucleotides[1] to insoluble matrices provide useful media for affinity chromatography. A nucleoside ligand, $N^4$-(5-aminopentyl)cytidine[2] (*I*), was recently synthetized from 4-thiouridine and 1,5-diaminopentane and used for chromatography of uridine kinase. For the same purpose, nucleotide compounds derived from uridine 3'-phosphate (*V*) and uridine 5'-phosphate (*VI*), whose phosphoryl groups were esterified by 6-aminohexanol, were prepared.



*I*

The key intermediate for the synthesis of both phosphodiesters was 6-trifluoroacetamidohexanol (*IV*). This compound was prepared by the action of trifluoroacetic anhydride on 6-aminohexanol in the presence of triethylamine. The O-trifluoroacetyl derivative simultaneously formed was hydrolysed by aqueous pyridine[3]. Analogous

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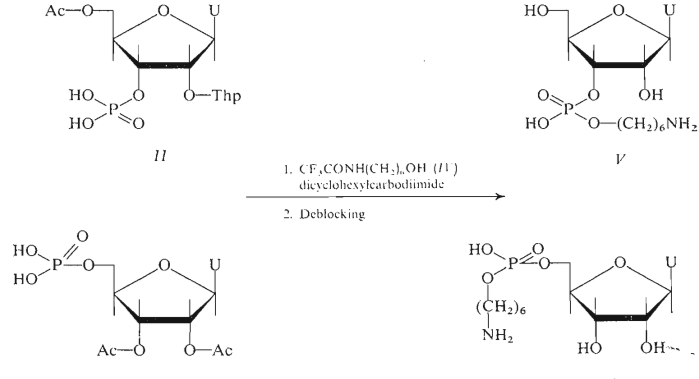
procedure was recently used in the preparation of a trifluoroacetamido nucleoside[4]. For the preparation of phosphodiester *V*, the intermediate *IV* was condensed with pyridinium salt of 2'-O-tetrahydropyranyl-5'-O-acetyluridine 3'-phosphate[5] (*II*). To remove the protecting groups, the product was treated with dilute aqueous ammonia and then with aqueous acetic acid, and isolated by linear gradient of triethylammonium hydrogen carbonate on DEAE cellulose. 6-Aminohexyl ester of uridine-5'-phosphate (*VI*) was prepared similarly starting from pyridinium salt of 2',3'-di-O-acetyl-uridine 5'-phosphate (*III*).




*II* *V* *III* *VI*

The products were characterised by their electrophoretic mobility, ninhydrin test and by enzymic degradation.

## EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol $UV_{254}$ silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system $S_1$, 2-propanol–conc. ammonia–water (7 : 1 : 2). Electrophoresis was performed on paper Whatman No 1, dipped in tetrachloromethane in 0·1M triethylammonium hydrogen carbonate (pH 7·5) at 20 V/cm. Unless stated otherwise, solutions were taken down on a rotatory evaporator equipped with dry ice condenser at 20°C, 1 Torr. Pyridine dried over calcium hydride and stored over molecular sieves was used.

### 6-Trifluoroacetamidohexanol (*IV*)

6-Aminohexanol (50 mmol) is evaporated with two 50 ml portions of pyridine and dissolved in a mixture of pyridine (100 ml) and triethylamine (7·7 ml). While stirring and colling in an

ice-water bath, trifluoroacetic anhydride (7·25 ml) is added dropwise. The mixture is allowed to stir for 1 h at room temperature and evaporated (40°C, 15 Torr). The residue is dissolved in 10% aqueous pyridine (100 ml) and, after 10 min, extracted with chloroform (100 ml). The chloroform layer is washed with water (50 ml), dried over anhydrous magnesium sulphate and evaporated (40°C, 15 Torr). The residue is evaporated with two 20 ml portions of toluene and dried under diminished pressure. Yield 6·1 g (57%) of *IV*, m.p. 47–48°C. For $C_8H_{14}F_3NO_2$ (213·2) calculated: 45·40% C, 7·56% H, 6·62% N; found: 45·71% C, 6·77% H, 6·88% N.

Uridine 3′-Phosphate 6-Aminohexyl Ester (*V*)

A suspension of 2′-O-tetrahydropyranyl-5′-O-acetyluridine 3′-phosphate[5] (calcium salt; 5 mmol), Dowex-50 (pyridinium) ion exchange resin (10 ml) and 50% aqueous pyridine (30 ml) immersed in an ice-water bath is stirred till the salt dissolves (2 h). The mixture is then poured on the top of a column of Dowex-50 (pyridinium; 20 ml) and eluted with precooled (0°C) 50% aqueous pyridine (60 ml). The eluate is concentrated to one fifth of the original volume and the concentrate is evaporated with six 50 ml portions of pyridine to sirupy consistence. To the residue, 6-trifluoroacetamidohexanol (*IV*; 10 mmol) and pyridine (30 ml) is added and the solution evaporated. Pyridine (40 ml) and N,N′-dicyclohexylcarbodiimide (5 g) are added and the mixture kept at room temperature for 2 days. Water (20 ml), conc. ammonia (30 ml) and cyclohexane (30 ml) are added, the mixture shaken and allowed to stand overnight. The insoluble material is filtered off, washed with 50% aqueous pyridine (10 ml), the lower layer of the filtrate evaporated (40°C, 15 Torr) and the residue evaporated with two 50 ml portions of toluene. 50% Aqueous acetic acid (40 ml) is added, the mixture is heated to 50°C for 1 h and evaporated. The residue is dissolved in 50% aqueous ethanol and applied to a column (2 l) of DEAE cellulose ($HCO_3^-$) equilibrated with 50% aqueous ethanol. The column is washed with 50% ethanol (2 l) and then eluted with the use of a linear gradient (2 l 50% aqueous ethanol in the mixing chamber and 2 l 0·15M triethylammonium hydrogen carbonate in 50% ethanol in the reservoir). The peak eluted at 0·12M buffer concentration on the top of the column is evaporated, the residue evaporated with three 50 ml portions of ethanol and dried at 40°C, 0·1 Torr. Yield 1·6 g (60%) of triethylammonium salt of *V*, $R_F$ ($S_1$) 0·62. $E_{Up}$ 0·34. Pancreatic ribonuclease digests the product to uridine 3′-phosphate.

Uridine 5′-Phosphate 6-Aminohexyl Ester (*VI*)

The solution of sodium salt of uridine 5′-phosphate (1 mmol) in water is passed through a column (5 ml) of Dowex-50 ($H^+$) and the column is washed with water (10 ml). Pyridine (5 ml) is added and the solution is evaporated (30°C, 15 Torr). The residue is evaporated with two 10 ml portions of pyridine and dissolved in a mixture of pyridine (5 ml) and acetic anhydride (3 ml). After 20 h, the mixture is evaporated (40°C, 15 Torr), the residue evaporated with two 10 ml portions of pyridine and dissolved in 50% aqueous pyridine (10 ml). After 2 h, the solution is evaporated, the residue dissolved in pyridine (5 ml) and added dropwise to stirred ether (100 ml). The pyridinium salt of 2′,3′-di-O-acetyluridine 5′-phosphate separated is collected by filtration, washed with ether and dried under diminished pressure (450 mg). The mixture of this salt and 6-trifluoroacetamidohexanol (2 mmol) is dissolved in pyridine (10 ml), the solution evaporated, and the residue dissolved in pyridine (10 ml). N,N′-Dicyclohexylcarbodiimide (1 g) is added and the mixture kept at room temperature for 2 days. 50% Aqueous pyridine (20 ml), conc. ammonia (20 ml) and cyclohexane (10 ml) are added, the mixture shaken and allowed to stand for 20 h. The insoluble material is filtered off, washed with 50% aqueous pyridine (5 ml) and the lower layer of the filtrate evaporated. The residue is dissolved in 50% aqueous ethanol and applied

to a column (500 ml) of DEAE cellulose ($HCO_3^-$) equilibrated with 50% aqueous ethanol. The isolation of the product is performed by the same procedure used for compound *V*. Yield 190 mg (36%) of triethylammonium salt of *VI*, $R_F$ ($S_1$) 0·60. $E_{Up}$ 0·35. Snake venom diesterase digests the product relatively slowly to uridine 5'-phosphate.

*Analyses were performed in the Analytical Department (Dr J. Horáček, Head) of this Institute.*

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