SYNTHESIS OF DEOXYURIDYLYL-(3′ → 5′)-RIBONUCLEOSIDE [P-(2-HYDROXYETHYL) ESTERS]*

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Deoxyuridylyl-(3′→5′)-uridine [P-(2-hydroxyethyl) ester] (*Va*), deoxyuridylyl-(3′→5′)-adenosine [P-(2-hydroxyethyl) ester] (*Vb*), and deoxyuridylyl-(3′→5′)-guanosine [P-(2-hydroxyethyl) ester] (*Vc*) were prepared by condensation of 5′-O-dimethoxytrityldeoxyuridine (*I*) with pyridinium salts of the peracetyl derivatives *IIa*—*IIc* of uridine 5′-phosphate, adenosine 5′-phosphate, and guanosine 5′-phosphate in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride, followed by treatment with O-monomethoxytrityl ethylene glycol and removal of protecting groups by the action of methanolic ammonia and then 90% aqueous acetic acid.

It is hardly possible to affect a biological system by oligonucleotidic sequences bearing a base-coded information, since the acidic phosphodiesters do not penetrate the cell wall[1] and the oligonucleotides are degraded by phospholytic enzymes. The transport barrier of the cell wall might be overcome by the use of electroneutral phosphotriesters which may be expected to be stable towards phosphorolytic enzymes. The absence of an acidic function is of course accompanied by the loss of solubility in water. The solubility in water might be increased by introduction of hydroxylic functions into the alkyl group serving to protect the internucleotidic bond. The known lability of ribonucleoside 3′-phosphate diesters[2] over a wide pH range does not permit to form a phosphotriester on a ribointernucleotidic bond; the synthesis of the suitable triesters is thus limited to compounds containing a deoxyribo-internucleotidic bond.

In the present paper, we wish to report the synthesis of some model substances of the above type, namely, deoxyuridylyl-(3′→5′)-ribonucleoside P-(2-hydroxyethyl) esters *Va*–*Vc*. The first step consisted in condensation of the free $C_{(3')}$-hydroxylic function of 5′-O-dimethoxytrityldeoxyuridine[3] (*I*) with 5′-phosphates *IIa*–*IIc* of the corresponding peracylribonucleosides[4] by the action of 2,3,5-triisopropylbenzenesulfonyl chloride. The thus-formed phosphodiester bond is transformed into a triester by reaction with ethylene glycol monomethoxytrityl ether. After the

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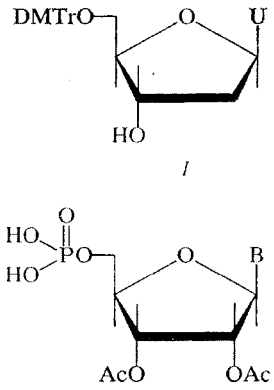
isolation of the crude, completely protected triesters *IIIa–IIIc* on loose layers of silica gel, the acetyl groups are removed by the action of methanolic ammonia. The resulting 5'-O-dimethoxytrityldeoxyuridylyl-(3'→5')-ribonucleoside [P-(2-monomethoxytrityloxyethyl) esters] *IVa–IVc* were separated from the accompanying compound *I* and the bis(2-monomethoxytrityloxyethyl) esters of the starting phosphates *II* by thin-layer chromatography on Merck preparative silica gel plates. After the spray with dilute aqueous perchloric acid, the spots turned yellow-orange as an indication of the presence of both the dimethoxytrityl and monomethoxytrityl group. These groups were removed by the action of 90% aqueous acetic acid. One hour was required for removal of the dimethoxytrityl group and 5 h in the case of the monomethoxytrityl group.

Products *Va–Vc* were characterised by a zero mobility on electrophoresis. The unspecific degradation with conc. aqueous ammonia affords deoxyuridine, the corresponding ribonucleoside and a mixture of deoxyuridine 3'-phosphate and ribonucleoside 5'-phosphate 2-hydroxyethyl esters. In view of the free 2-hydroxylic function, compounds *Va–Vc* may be regarded as analogues of ribonucleoside 3'-phosphate diesters. The alkaline degradation proceeds under participation of the β-hydroxylic group and is stopped in the phosphodiester stage.

Towards a weakly acidic medium (90% aqueous acetic acid), compounds *Va–Vc*



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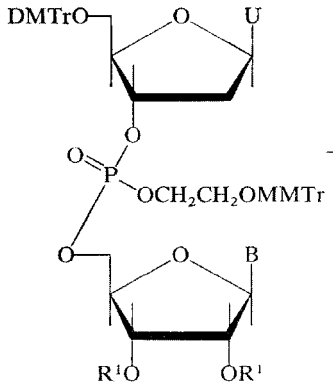

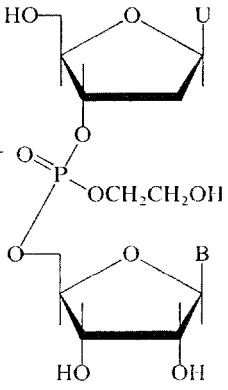

IIa, B = uracil
IIb, B = N^6-acetyladenine
IIc, B = N^2-acetylguanine

IIIa, R^1 = Ac, B = uracil
IIIb, R^1 = Ac, B = N^6-acetyladenine
IIIc, R^1 = Ac, B = N^2-acetylguanine
IVa, R^1 = H, B = uracil
IVb, R^1 = H, B = adenine
IVc, R^1 = H, B = guanine

Va, B = uracil
Vb, B = adenine
Vc, B = guanine

MMTr = monomethoxytrityl
DMTr = dimethoxytrityl

are stable as shown by the successful deblocking in the last step of the whole synthesis. The products are stable in water at pH 7·5 and 40°C. Their solubility in water makes possible to synthesize greater oligonucleotidic systems of this type.

EXPERIMENTAL

Unless stated otherwise, thin-layer chromatography, paper chromatography, and electrophoresis were performed analogously to ref.[5] in the following solvent systems: S_1, chloroform; S_2, chloroform–methanol (95 : 5); S_3, chloroform–methanol (9 : 1); S_4, chloroform–methanol (8 : 2); S_5, chloroform–methanol (1 : 1), S_6, chloroform–methanol–pyridine (90 : 5 : 5); S_7, 1-butanol–water (85 : 15); and S_8, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2).

Ethylene Glycol Monomethoxytrityl Ether

A solution of monomethoxytrityl chloride (0·1 mol) and ethylene glycol (1 mol) in pyridine (100 ml) is kept at room temperature for 2 days, poured into water, and extracted with two 300 ml portions of chloroform. The extracts are washed with water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure to afford 32 g (96%) of an oil, R_F 0·59 (on Silufol, in S_1). The oil is stored in the form of an 1M pyridine solution.

5′-O-Dimethoxytrityldeoxyuridylyl-(3′→5′)-adenosine [P-(2-Monomethoxytrityloxyethyl) Ester] (*IVb*)

A solution of 5′-O-dimethoxytrityldeoxyuridine[3] (*I*; 0·25 mmol) and N,$O^{2'}$,$O^{3'}$-triacetyladenosine 5′-phosphate pyridinium sali[4] (0·35 mmol) in pyridine (10 ml) is taken down and the residue is coevaporated with pyridine. The final residue is dissolved in pyridine (5 ml) and the solution shaken with 2,3,5-triisopropylbenzenesulfonyl chloride (0·7 mmol) for several minutes. The whole mixture is then concentrated to an incipient crystallisation, the concentrate kept at 20°C for 20 h, treated with additional 2,3,5-triisopropylbenzenesulfonyl chloride (1 mmol) and 1M pyridine solution (3·5 ml) of ethylene glycol monomethoxytrityl ether, and evaporated under diminished pressure to the consistence of a sirup. The sirup is kept at 20°C for 3 days, diluted with chloroform (2 ml), and chromatographed on a 20 × 40 × 0·6 cm layer of loose silica gel in the solvent system S_6. The dimethoxytrityl-positive band (R_F 0·8—0·9) is eluted with the solvent system S_5, the eluate evaporated, and the residue coevaporated with two portions of toluene. The final residue is dissolved in chloroform (2 ml) and the solution is chromatographed on one layer of loose silica gel in S_2. The dimethoxytrityl-positive band (R_F 0·4—0·5) is eluted with S_5 and the eluate evaporated to afford crude compound *IIIb* (R_F 0·35 in S_2). The crude product is dissolved in 5M methanolic ammonia (10 ml), the solution kept at room temperature for 40 h, and evaporated under diminished pressure. The residue is dissolved in chloroform (2 ml) and chromatographed on the Merck preparative silica gel plate (20 × 20 cm) in the solvent system S_3. After the identification of the UV-absorbing bands, the side of the plate was sprayed with 10% aqueous perchloric acid to make visible bands containing exclusively the monomethoxytrityl group (yellow), bands containing exclusively the dimethoxytrityl group (orange), and finally, a band containing both the monomethoxytrityl and the dimethoxytrityl group (yellow-orange; R_F 0·40). The R_F 0·40 band is eluted with the solvent system S_5, the eluate evaporated, and the residue dried under diminished pressure. Yield, 50 mg (17%) of the ester *IVb*, R_F 0·34 (on Silufol, in S_3).

Deoxyuridylyl-(3′→5′)-adenosine [P-(2-Hydroxyethyl) Ester] (*Vb*)

Compound *IVb* (40 mg) is dissolved in 90% aqueous acetic acid and the removal of methoxytrityl groups is checked by thin-layer chromatography in the solvent system S_4 for 5 h. The solution is then evaporated, the residue coevaporated with two portions of 1-butanol, and the final residue triturated with ether (50 ml). The solid is collected with suction, washed with ether, and dried under diminished pressure. Yield. 23 mg (90%) of the ester *Vb*, R_F 0·20 (on Silufol, in S_5) and 0·13 (on paper, in S_7), E_{Up} 0·0. UV spectrum (water): λ_{max} 261 nm, ε 22600), λ_{min} 229 nm (ε 6000).

Deoxyuridylyl-(3′→5′)-uridine [P-(2-Hydroxyethyl) Ester] (*Va*)

Compound *Va* is prepared analogously to compound *Vb* except for the removal of acyl groups which requires 3 h only. R_F values: 0·30 (on Silufol, in S_5) and 0·11 (on paper, in S_7). UV spectrum (water): λ_{max} 261 nm (ε 19000), λ_{min} 231 nm (ε 5400).

Deoxyuridylyl-(3′→5′)-guanosine [P-(2-Hydroxyethyl) Ester] (*Vc*)

Compound *Vc* is prepared in 9% yield analogously to compound *Vb*. R_F values: 0·18 (on Silufol, in S_5) and 0·08 (on paper, in S_7). UV spectrum (water): λ_{max} 255 nm (ε 19000), λ_{min} 226 nm (ε 5200).

Alkaline Hydrolysis of Compounds *Va*—*Vc*

A solution of compounds *Va*—*Vc* (0·3—0·5 mg each) in conc. aqueous ammonia (0·1 ml) is maintained at 20°C for 4 h and then subjected to analysis by paper chromatography (solvent systems S_7 and S_8) and electrophoresis. As indicated by paper chromatography, the starting triesters are quantitatively unspecifically degraded into mixtures of two nucleosides and two corresponding phosphodiesters. As shown by electrophoresis, the ratio of the nucleoside to the phosphodiester components is 1 : 1.

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