CHEMICAL SYNTHESIS OF 2′(3′)-O-AMINOACYLRIBO-OLIGONUCLEOTIDES : THE PREPARATION OF CYTIDYLYL-(3′→5′)-2′(3′)-O-GLYCYLADENOSINE

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In an important step of the biosynthesis of proteins amino acids are bound to t-RNA[+)] (Hoagland, Zamecnik, Stephenson, 1957) by an ester linkage to the 2′ or 3′hydroxyl groups of the vicinal glycol grouping in the terminal ("acceptor") CpCpA unit of t-RNA′s (Hecht, Stephenson, Zamecnik, 1958; 1959). The 2′(3′)-O-aminoacyl t-RNA′s can be degraded by pancreatic RNase to 2′(3′)-O-aminoacyladenosine (Zachau, Acs, Lipmann, 1958) or by T_1 RNase to ribo-oligonucleotides containing a terminal 2′(3′)-O-aminoacyl CpCpA unit. The properties of some fragments of the latter type have been investigated by paper chromatography and electrophoresis (Herbert, Smith, Wilson, 1964; Smith, Herbert, Wilson, 1964; Ishida, Miura, 1965). Takanami has studied the behaviour of such fragments in a cell-free protein-synthesising system and concluded that they can transfer the aminoacyl residue to the polypeptide chain (Takanami, 1964).

[+)]Abbreviations : t-RNA = transfer ribonucleic acid, CpCpA = cytidylyl-(3′→5′)-cytidylyl-(3′→5′)-adenosin, RNase = ribonuclease, CpA = cytidylyl-(3′→5′)-adenosin.

Although a number of chemical methods for the synthesis of the simplest compounds of this general type, i.e. 2′(3′)-O-aminoacylribonucleosides, has been described (Zachau, Feldmann, 1965), no procedure applicable to the synthesis of 2′(3′)-O-aminoacylribo-oligonucleotides has been reported so far. Recently, we have developed a new method for the preparation of 2′(3′)-O-aminoacylribonucleosides based on the acid catalyzed reesterification of N-protected amino acid ortho-esters with the 2′,3′glycol grouping of ribonucleosides (Žemlička, Chládek, 1965). The resulting ribonucleoside 2′,3′-cyclic orthoesters are readily hydrolysed in acid to give (after removal of protecting groups) a mixture of 2′- and 3′-O-aminoacylribonucleosides.

This method has been applied to the preparation of a 2′(3′)-O-aminoacylribo-oligonucleotide, cytidylyl-(3′→5′)-2′(3′)-O-glycyladenosine (V). CpA (I, the ammonium salt) treated with N-benzyloxycarbonylglycine ethylorthoester (II) (80 equivalents) (Žemlička, Chládek, 1965) in anhydrous dimethylformamide containing trifluoroacetic acid (80 equivalents) afforded the chromatographically homogeneous (A^{+}) orthoester III in 60 - 70 % yield (Scheme 1), which showed the expected UV spectrum. Degradation of III by pancreatic RNase afforded cytidine-3′-phosphate and the adenosine-2′,3′-cyclic ortho-ester (VI) in the ratio 0.9 : 1. No isomerisation of the 3′→5′internucleotidic linkage in III was detected when the reaction time was 30 min. at room temperature; however, after longer reaction periods, III was found to be partially isomerised (5 % of the 2′→5′isomer after 2 h). The ortho-

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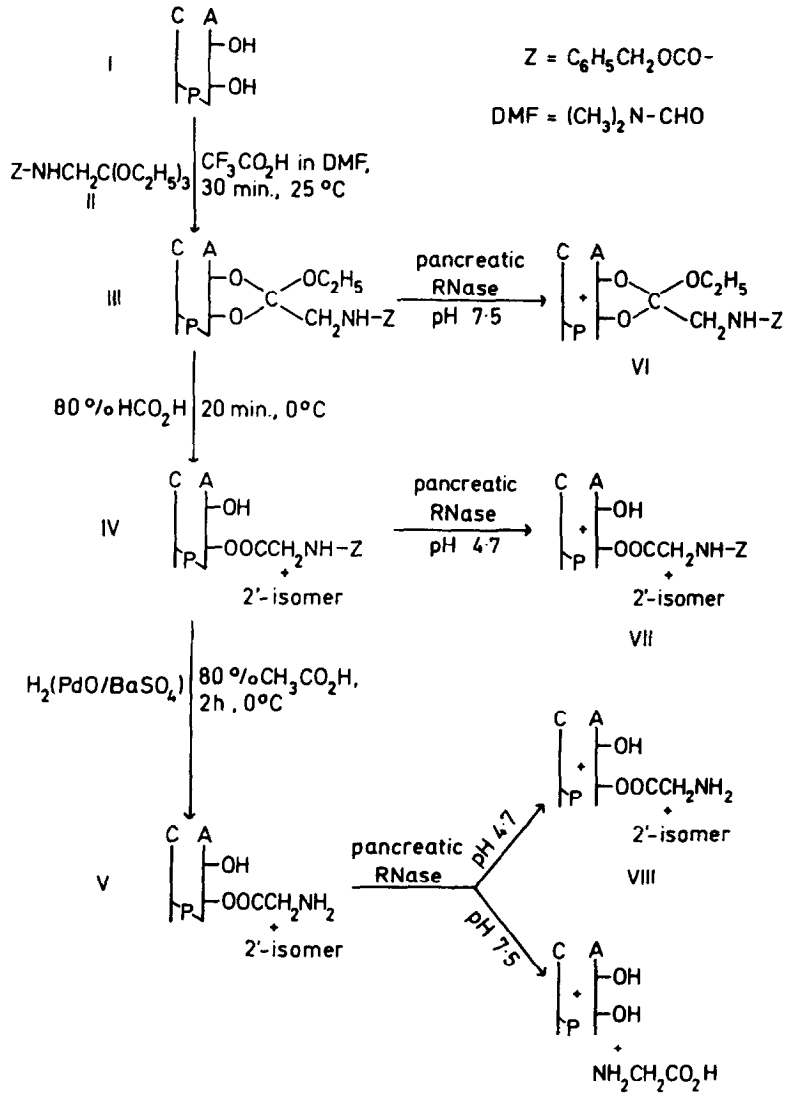
+) Solvent system A : 2-propanol - ammonia - water (7 : 1 : 2), B : 1-butanol - acetic acid - water (5 : 2 : 3).



ester III is stable to alkali; it was hydrolysed quantitatively in 80 % formic acid during 20 min. at 0°C (without isomerisation of 3′→5′linkage) to afford cytidylyl-(3′→5′)-2′(3′)-O-(N-benzyloxycarbonyl)glycyladenosine (IV) in 90 % yield. The UV spectrum of IV closely resembled that of CpA; the compound was chromatographically (system B) and electrophoretically (pH 2.4) homogeneous and showed one half of the

mobility of CpA in borate buffer (pH 6.8). Degradation by pancreatic RNase at pH 4.7 gave cytidine-3′-phosphate and 2′(3′)-O-(N-benzyloxycarbonyl)glycyladenosine (VII) in the ratio 1.1 : 1. The N-benzyloxycarbonyl group of IV was removed by hydrogenation in 80 % acetic acid over $PdO/BaSO_4$ (2 h at 0°C[+]) to give cytidylyl-(3′→5′)-2′(3′)-O-glycyl-adenosine (V)in quantitative yield. The aminoacylnucleotide V was characterised by a positive ninhydrin reaction, UV spectrum closely resembling that of CpA, paper chromatography (system B), electrophoresis at pH 2.4, hydrolysis with alkali to CpA and glycine and degradation with pancreatic RNase at pH 4.7 to cytidine-3′-phosphate and 2′(3′)-O-glycyladenosine (VIII) and at pH 7.5 to cytidine-3′-phosphate and adenosine in the ratio 1.07 : 1.

CpA can be regarded to some extent as a simple model of the polynucleotide chain of t-RNA. Our results indicate that the orthoester method could serve as a useful synthetic tool for the introduction of aminoacyl residues into ribo-oligonucleotides. The high selectivity and lack of side reactions observed in the reaction of CpA with the orthoester II make it particularly promising for this purpose. These possibilities, including attempts to introduce 2′(3′)-O-aminoacyl residues into t-RNA[++] by means of the orthoester method are under active investigation in our Laboratory.

[+] No hydrogenation of the cytosine residue (Furukawa et al.,1965) was observed under these conditions.

[++] Recently, the aminoacylation of t-RNA by N-triphenylmethylglycine in the presence of dicyclohexylcarbodiimide in pyridine in 1.5 % yield has been reported (Sluckij, Gottich, 1965).

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