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SYNTHESIS OF 2'(3')-O-AMINOACYL TRIRIBONUCLEOSIDE DIPHOSPHATES WITH THE SEQUENCE OF THE ACCEPTOR TERMINUS OF AA-tRNA

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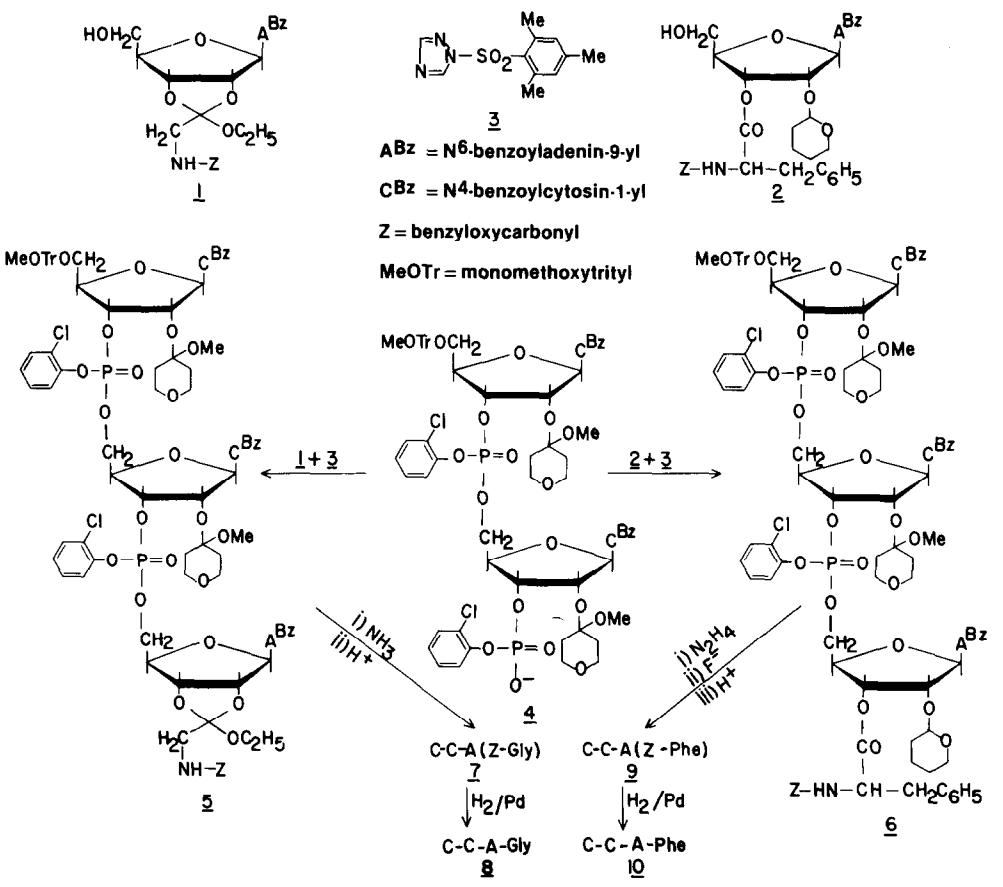
ABSTRACT: The specific synthesis of the 2'(3')-O-aminoacyl triribonucleoside diphosphates, analogs of the 3'-terminus of AA-tRNA is described *via* phosphotriester intermediates.

The 2'(3')-O-aminoacyl oligoribonucleotides, which enter the 3'-terminal sequence of AA-tRNA or of an analogous sequence, may participate in various subreactions of protein biosynthesis, functioning, in fact, as simple analogs of the 3'-terminus of AA-tRNA[1]. This observation provides the principal rationale for the chemical synthesis of 2'(3')-O-aminoacyl oligoribonucleotides. Although the chemical synthesis of 2'(3')-O-aminoacyl dinucleoside phosphates has been extensively studied[2], the desired extension to the synthesis of higher 2'(3')-O-aminoacyl oligoribonucleotides has met with considerable difficulty[3]. Significant progress has, however, been achieved recently in the synthesis of oligoribonucleotides *via* the triester method[4]. The latter approach would appear to provide a convenient synthesis of 2'(3')-O-aminoacyl oligoribonucleotides as well, if all protecting groups of triester intermediates could be removed in the final stages of synthesis with simultaneous preservation of integrity of the extremely unstable aminoacyl bond.

In this contribution, we describe a specific synthesis of two 2'(3')-O-aminoacyl triribonucleoside diphosphates, namely C-C-A-Gly and C-C-A-Phe using the triester methodology. Moreover, we have developed a protection system for the 2'(3')-O-aminoacyl oligoribonucleotide molecules which allows the selective deblocking of fully protected intermediates with the preservation of the integrity of the aminoacyl bond. The synthetic design (Scheme 1) utilizes the specifically protected nucleoside components 1 and 2, which incorporate the aminoacyl moieties in the molecule[5]. Component 1 includes an alkali-stable orthoester derivative of N-benzyloxycarbonylglycine which makes it possible to remove the N-benzoyl groups by treatment with ammonia from oligonucleotides incorporating component 1. Since no orthoesters of optically active amino acids are available at present and the ester linkage of

SCHEME I



N-benzyloxycarbonyl-L-phenylalanine in component 2 is labile in alkaline medium, a different method had to be developed for the removal of N-benzoyl groups from oligonucleotide containing component 2. It was found that the N-benzoyl group of 2 could be removed quantitatively with hydrazine acetate[8] (at pH 5.1) without cleavage of the N-benzyloxycarbonylphenylalanyl residue, if the 2'-hydroxyl group of 2 is blocked by a hydrazine-stable tetrahydropyranyl group. It is important to note that in the absence of such blockage the 3'-O-aminoacyl group is removed by hydrazine acetate, apparently due to the participation of the free neighboring hydroxyl group. The other key intermediate for the synthesis of the target compounds is dinucleotide 4, which was prepared by the stepwise triester procedure[9]. The 2-chlorophenyl group was chosen for blocking the phosphodiester linkages, since it may be removed with ease from the triester intermediates, without cleavage of the aminoacyl bond, by the displacement reaction with fluoride ion[12]. The other protecting groups in the intermediate 4 are readily removed in a mildly acidic medium[9,10]. The condensation of building blocks 1 or 2 with dinucleotide 4 was effected by reagent 3 in pyridine solution[11] and the protected C-C-A

derivatives 5 and 6 were isolated in 30-40% yield using chromatography on silica gel. The spectral (UV and pmr) properties of these derivatives are in agreement with the proposed structures. Intermediate 5 was deprotected by successive treatment with aqueous ammonium hydroxide at 50° (24 hrs) and 0.05N HCl in dioxane at 20° (16 hrs) to form C-C-A(Z-Gly) (7), which was isolated (in 40% yield) by TLC on cellulose[2]. Treatment of 6 with hydrazine acetate (excess 0.5M N_2H_4 in pyridine-acetic acid; 6:4; v/v; 20°; 16 hrs) removed the N-benzoyl groups from aglycons. The reaction mixture was quenched with acetylacetone and chromatographed on silica gel. Cleavage of 2-chlorophenyl groups from phosphorus was achieved by fluoride ion (0.05M $Bu_4N^+F^-$ in tetrahydrofuran-pyridine-water, 8:1:1; v/v/v; 20°; 12 hrs). Hydrolysis of the resulting product with 0.05N HCl in dioxane (20°, 16 hrs) furnished C-C-A(Z-Phe) (9), which was isolated as described above for 7 in 20% total yield. The desired compounds 8 and 10 were obtained in quantitative yields by hydrogenolysis ($Pd/BaSO_4$; 0°; 2.5 hrs; 80% acetic acid) of compounds 7 and 9 respectively, followed by preparative paper electrophoresis in 1M acetic acid[2]. The structures of C-C-A-Gly (8) and C-C-A-Phe (10) were firmly established by: i) hydrolysis in alkaline medium to C-C-A and parent amino acid; ii) electrophoretic and chromatographic mobilities; iii) positive reaction with ninhydrin; iv) spectral (UV) properties; and v) quantitative digestion with pancreatic RNAse and snake venom phosphodiesterase to expected products in correct ratios.

The methods described herein are capable of providing various 2'(3')-O-aminoacyl triribonucleoside diphosphates that are of considerable biochemical interest but which, to date, have not been readily accessible. Indeed, it appears possible to extend the nucleotide chain length of such compounds to furnish even more complicated models.

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amino groups of cytidine and adenosine moieties, as well as both hydroxyl groups on the 3'-terminus of C-C-A were left uprotected, the aminoacylation reaction has resulted in formation of several products. This fact, together with relatively harsh deblocking conditions were responsible for the very low yield of the final compounds.

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5. The compound 1 was prepared via benzoylation and selective N-debenzoylation (affected by NaOH) procedure of the known 2',3'-O-(N-benzyloxycarbonylaminomethylene)ethoxymethylene adenosine[6] in 71% yield. The compound 2 was synthesized starting from N^6-benzoyl-2'-O-tetrahydropyranyl adenosine[7] via 5'-dimethoxytritylation, followed by the DCC mediated acylation of the 3'-hydroxylgroup with N-benzyloxycarbonyl-L-phenylalanine in pyridine solution. The final selective deprotection of the 5'-hydroxyl group was achieved by by treatment with 80% acetic acid (15 min, 20°) and the compound 2 was isolated by silica gel chromatography in 51% yield. The structure of compounds 1 and 2, as well as that of intermediates, were confirmed by pmr and UV spectra, as well as by satisfactory elemental analysis data. The details of these procedures will be published elsewhere.

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