Communications to the Editor

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A NEW SYNTHETIC METHOD FOR 5'-TRIPHOSPHORYL-ADENYL- (2'-5')-ADENYL-
(2'-5')-ADENOSINE (2-5A) AND 2-5A CORE USING 3'-O-TETRAHYDROFURANYL-
ADENOSINE DERIVATIVES

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2' or 3'-O-Tetrahydrofuran derivatives of ribonucleosides have been synthesized by treating 2,3-dihydrofuran with bis *tert*-butyldimethylsilyl N-protected nucleosides. Using 3'-O-tetrahydrofuran-N-benzoyladenine as the starting material 5'-phosphoryladenyl-(2'-5')-adenyl-(2'-5')-adenine was synthesized in a good yield and converted to the triphosphoryl derivative (2-5A). The 2'-5' linked triadenylate (2-5A core) was also prepared by the same phosphotriester method.

KEYWORDS — 3'-O-tetrahydrofuran-N-benzoyladenine; 5'-O-phosphorylation; o-chlorophenyl phosphate; phosphoro-p-anisate; 2'-5' oligoadenylate

Protection of the 2'-hydroxyl group of ribonucleosides has been one of the most essential problems in the synthesis of ribooligonucleotides with 3'-5' internucleotide linkages.¹⁾ Discovery of 2'-5' linked oligoadenylates and their triphosphoryl derivatives (2-5A) as protein biosynthesis inhibitors²⁾ brought a new synthetic target which requires the protection of the 3'-hydroxyl function. We have developed a method for oligonucleotide synthesis using photo-labile o-nitrobenzyl ether as a protecting group for the 2'-hydroxyl group of ribonucleosides³⁾ and applied the method to the protection of the 3'-hydroxyl group of adenosine in the synthesis of 5'-triphosphoryladenyl-(2'-5')-adenyl-(2'-5')-adenine (2-5).⁴⁾ Recently we introduced an acid labile tetrahydrofuran group⁵⁾ to the secondary hydroxyl function of ribonucleosides and showed that it can be kept during syntheses of oligonucleotides and removed easily without migration of the internucleotide linkages.⁶⁾ In this communication we report the application of this protecting group to the synthesis of 2-5A by using 3'-O-tetrahydrofuran-N-benzoyladenine as the starting material. The 2'-5' linked triadenylate (2-5A core) was also synthesized via the common intermediates with mono- or bisphosphoryl group.

3'-O-Tetrahydrofuran-N-benzoyladenine (2) was prepared by treating 2',3'-bis-*tert*-butyldimethylsilyl-N-benzoyladenine⁷⁾ (1) with 2,3-dihydrofuran (1.5 eq) in the presence of p-toluenesulfonic acid (0.3 eq) followed by removal of the *tert*-butyldimethylsilyl group with tetra-n-butylammonium fluoride (3.5 eq) as shown in Chart 1. Diastereoisomers of 2 were separated by chromatography on silica gel. The combined yield was 63%.

For the synthesis of 2-5A, the 3'-protected nucleoside (2) was phosphorylated as shown in Chart 2. The 5'-terminal unit (3) was prepared by phosphorylation of 2 with dianilido phosphorochloridate⁸⁾ (6) and separated from the bis-phosphorylated

Chart 1

Si = tert-butyldimethylsilyl

Chart 2

compound in a yield of 72%. The internal unit (4) was synthesized with *o*-chlorophenyl *p*-anisidophosphorochloridate⁹⁾ (7) and 4 was converted to 2 by phosphorylation with *o*-chlorophenyl phosphoroditriazolidide¹⁰⁾ (8) followed by treatment with aqueous pyridine. The 3'-diesterified nucleotide (9) was then condensed with 2', 3'-ethoxymethylidene-*N*-benzoyladenine (5) by treatment with mesitylenesulfonyl tetrazolidide¹¹⁾ (MSTe) for 30 min to give the dinucleotide (10) which was isolated by chromatography on silica gel (89%). 10 was converted to the phosphodiester (11) by treatment with isoamyl nitrite in a mixture of pyridine-acetic acid (5:4) at 30°C for 2.5 h. 11 was isolated by extraction with solvents in a yield of 92%. The 5'-phosphorylated trimer (12) was synthesized by condensation of 11 with 3 using MSTe as the condensing reagent (76%) and deblocked to give 13. The deblocking of 12 included treatments with isoamyl nitrite in pyridine-acetic acid at 30°C for 12 h, with concentrated ammonia at 55°C for 5 h and with acetic acid-water (4:1) at 30°C for 2 h. The trinucleotide (13) was isolated by anion-exchange chromatography on DEAE-cellulose. The yield was 83% using ϵ value (31400). The conversion of 13 to 2-5A was performed by the imidazolidine method as described previously.⁴⁾ 2-5A thus obtained was characterized by reverse phase high pressure liquid chromatography (RPHPLC). The other approach involving phosphorylation of 3 with 8 was not successful presumably because of side reactions including phosphorylation on nitrogens of the dianilidate (3).

2-5A core (16) was synthesized by condensation of the dimer (11) with 5'-*O*-monomethoxytrityl-3'-*O*-tetrahydrofuran-*N*-benzoyladenine (14) as shown in Chart 2. The protected trimer (15) was isolated by silica gel chromatography in a yield of 62% and deblocked by the procedure described for 12. The product (16) was purified by anion-exchange chromatography on DEAE-cellulose and characterized by RPHPLC.

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