Communications to the Editor

Chem. Pharm. Bull. 30(1) 376—378 (1982)

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

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2' or 3'-O-Tetrahydrofuranyl derivatives of ribonucleosides have been synthesized by treating 2,3-dihydrofurane with bis tert-butyldimethylsilyl N-protected nucleosides. Using 3'-O-tetrahydrofuranyl-N-benzoyladenosine as the starting material 5'-phosphoryladenylyl-(2'-5')-adenylyl-(2'-5')-adenosine 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-tetrahydrofuranyl-N-benzoyladenosine; 5'-O-phosphorylation; o-chlorophenyl phosphate; phosphoro-p-anisidate; 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 inhibitors[2] 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 ribonucleosides[3] and applied the method to the protection of the 3'-hydroxyl group of adenosine in the synthesis of 5'-triphosphoryladenylyl-(2'-5')-adenylyl-(2'-5')-adenosine (2-5).[4] Recently we introduced an acid labile tetrahydrofuranyl group[5] 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-tetrahydrofuranyl-N-benzoyladenosine 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-Tetrahydrofuranyl-N-benzoyladenosine (2) was prepared by treating 2',3'-bis-tert- butyldimethylsilyl-N-benzoyladenosine[7] (1) with 2,3-dihydrofurane (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 phosphorochloridate[8] (6) and separated from the bis-phosphorylated

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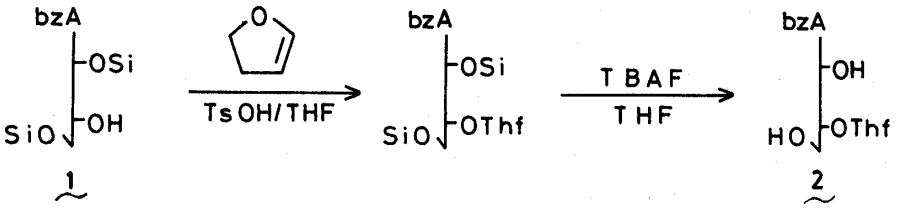
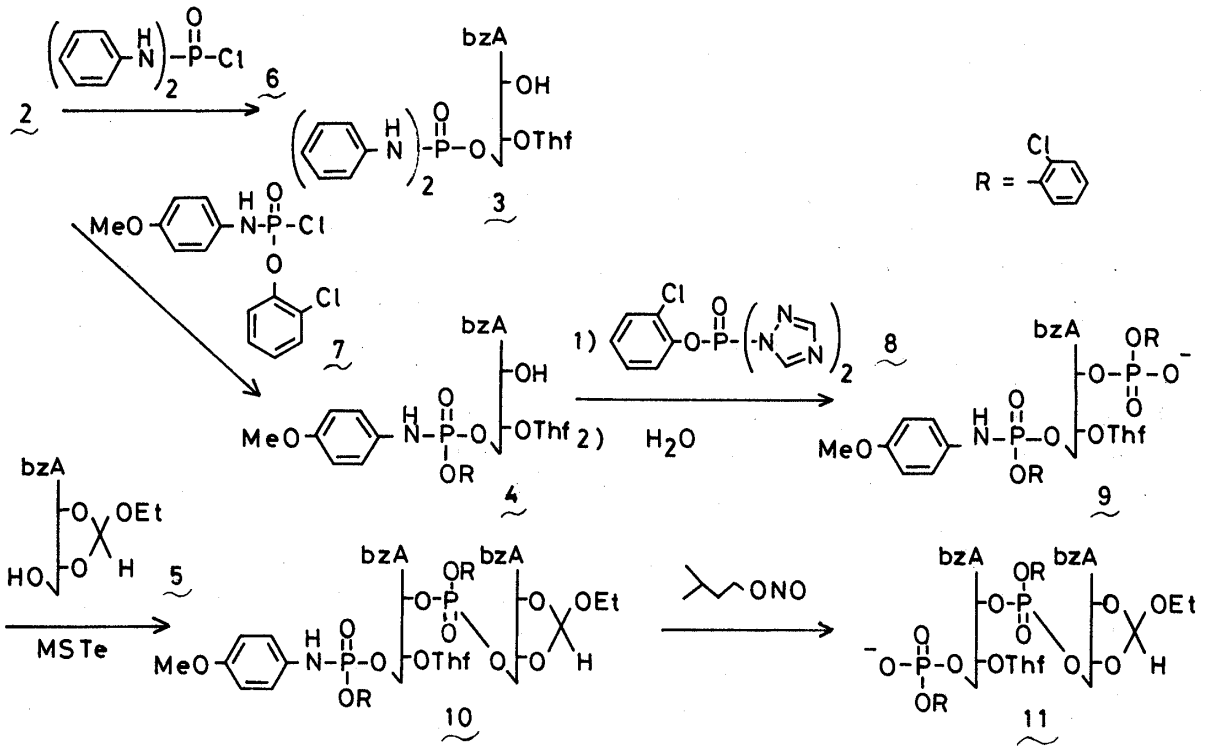


Chart 1

Si = tert-butyldimethylsilyl



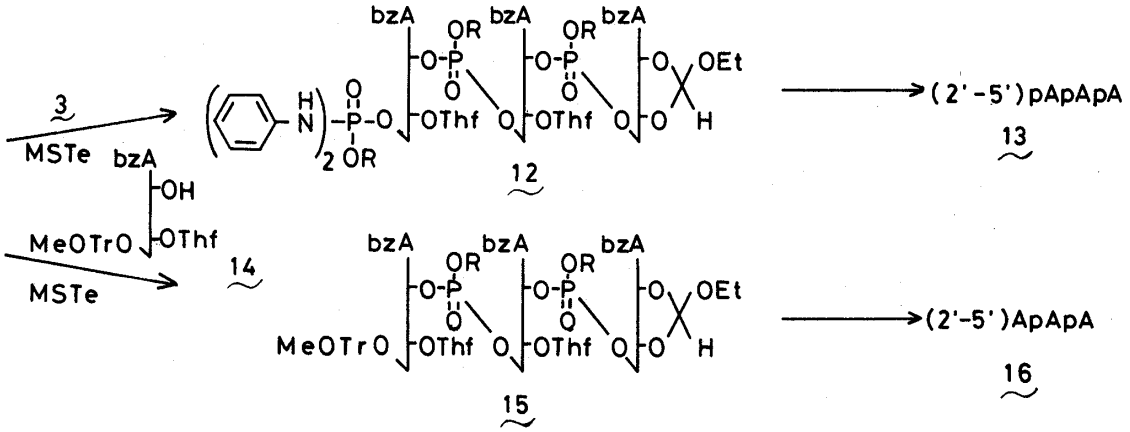


Chart 2

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compound in a yield of 72%. The internal unit (4) was synthesized with o-chlorophenyl p-anisidophosphorochloridate[9] (7) and 4 was converted to 9 by phosphorylation with o-chlorophenyl phosphoroditriazolide[10] (8) followed by treatment with aqueous pyridine. The 3'-diesterified nucleotide (9) was then condensed with 2', 3'-ethoxymethylidene-N-benzoyladenosine (5) by treatment with mesitylenesulfonyl tetrazolide[11] (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 aicd (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 trinucleoitde (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 imidazolide 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 phopshorylation on nitrogens of the dianilidate (3).

2-5A core (16) was synthesized by condensation of the dimer (11) with 5'-O-monomethoxytrityl-3'-O-tetrahydrofuranyl-N-benzoyladenosine (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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(Received November 20, 1981)

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