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Synthesis of 2'(3')-O-(L-Phenylalanyl) and 2'(3')-O-(D-Phenylalanyl) Cytidylyl(3'-5')adenosine

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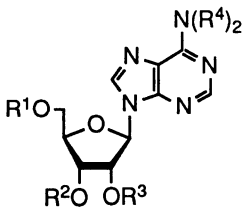
A general route to the synthesis of the 2'(3')-O-aminoacyl CpA using the 1-ethoxyethyl group for tentative protection of the 2'-hydroxyl group of adenosine, is described.

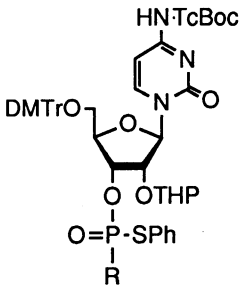
Recently, techniques for the design of proteins containing site-specific non-natural amino acid have been developed.[1,2] The chemistry involves the ligation reaction of the chemically synthesized 2'(3')-O-aminoacyl pCpA with tRNAs deleting the 3'-terminal cytidylyl(3'–5')adenosine (CpA) by use of RNA ligase.[3,4] More recently, we have reported the synthesis of 2'(3')-O-(L-leucyl)CpA by means of the phosphorothio-triester method. By this way, we could remove the whole protecting groups without significant cleavage of the leucyl ester bond.[5] However, the 2'-protective tetrahydropyranyl group (THP) neighboring to the 3'-leucyl ester considerably resisted to the hydrolysis under acidic conditions at pH 2.0. In order to overcome the problem, we have chosen 1-ethoxyethyl group (Ee)[6-8] in place of THP because the synthesis of 2'(3')-aminoacylated CpA requires more acid-labile protective group of the 2'-hydroxyl of the adenosine comparing the oligoribonucleotide synthesis.

In this paper, we examined the synthesis of 2'(3')-O-(L-phenylalanyl)CpA (**11a**) and 2'(3')-O-(D-phenylalanyl)CpA (**11b**).

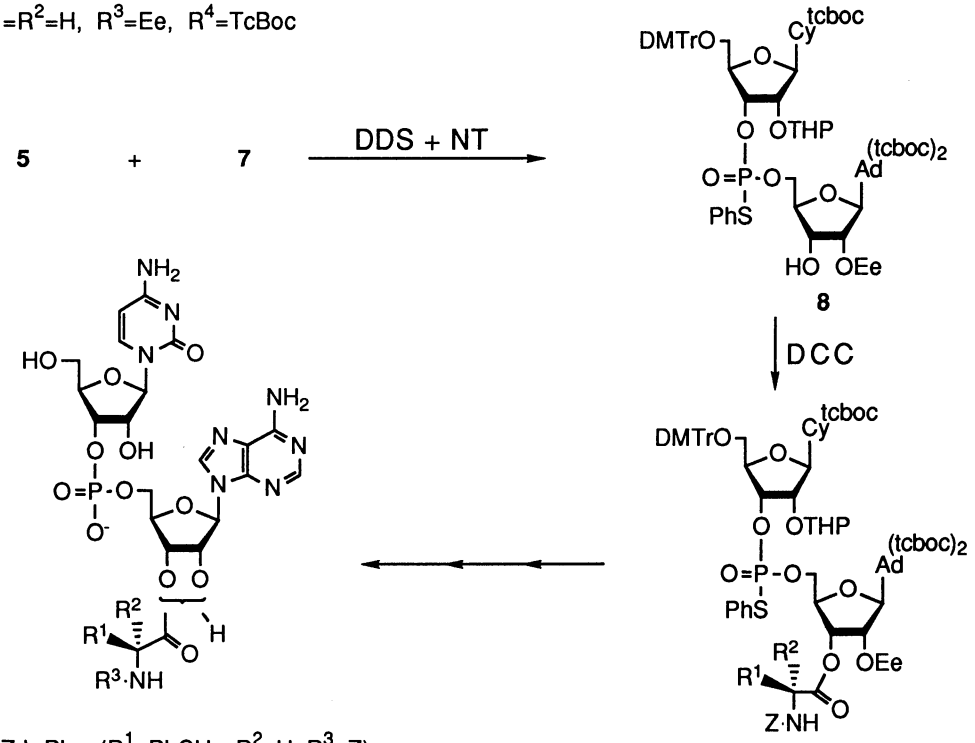
The amino groups of adenosine and cytidine were protected with trichloro-*tert*-butoxycarbonyl group (TcBoc) which could be removed by treatment with zinc-acetylacetone under neutral conditions.[9] Introduction of the TcBoc into the amino group of adenosine was performed by a modification of the procedure of Chattopadhyaya.[10] The hydroxyl groups of adenosine (**1**) (2.67 g; 10 mmol) were transiently trimethylsilylated and protected by the addition of TcBocCl (5.28 g; 22 mmol). After hydrolysis, the TcBoc derivative (**2**) was obtained. Treatment of the mixture with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS-Cl_2) (2.84 g; 9 mmol) followed by silica gel column chromatography gave the silylated compound (**3**) (7.49 g) in 81% yield from **1**. Compound **3** (1.43 g; 1.56 mmol) was treated with ethyl vinyl ether (2.88 g; 40 mmol)[6-8] in the presence of pyridinium p-toluenesulfonate(0.356 g; 4 mmol) in CH_2Cl_2 for 2 h to afford the Ee derivative (**4**).



1 $R^1=R^2=R^3=R^4=H$
2 $R^1=R^2=R^3=H$, R^4=TcBoc
3 R^1- R^2=TIPS, $R^3=H$, R^4=TcBoc
4 R^1- R^2=TIPS, R^3=Ee, R^4=TcBoc
5 $R^1=R^2=H$, R^3=Ee, R^4=TcBoc



6 R=PhS
7 $R=O^-(HN^+Et_3)$



10a Z-L-Phe ($R^1=PhCH_2$, $R^2=H$, R^3=Z)
10b Z-D-Phe ($R^1=H$, $R^2=PhCH_2$, R^3=Z)
11a L-Phe ($R^1=PhCH_2$ $R^2=H$, $R^3=H$)
11b D-Phe ($R^1=H$, $R^2=PhCH_2$, $R^3=H$)

9a Z-L-Phe ($R^1=PhCH_2$, $R^2=H$)
9b Z-D-Phe ($R^1=H$, $R^2=PhCH_2$)

The mixture was treated with KF-Et_4NBr (12 mmol) in wet CH_3CN at 50 °C for 1 h[12] to give the adenosine component (**5**) (0.82 g) in 70% yield from **3**.

On the other hand, the fully protected cytidine component (**6**) (1.43 g; 1.3 mmol)[5] was treated with 5 M triethylammonium phosphinate[11,12] in pyridine to give the corresponding phosphodiester (**7**) quantitatively. It was condensed with **5** (0.745 g; 1 ,mmol) in the presence of isodurenedisulfonyl dichloride (DDS)[13] (0.662 g; 2 mmol) and 3-nitro-1,2,4-triazole (NT)[14] (0.342 g; 3 mmol) in pyridine for 1 h to afford the CpA derivative (**8**) (1.26 g) in 73% yield. The 3'-hydroxyl group of **8** (0.59 g; 0.34 mmol) could be aminoacylated with N-benzyloxycarbonyl-(L)-phenylalanine (Z-L-Phe)[15] (0.24 g; 0.51 mmol) or N-benzyloxycarbonyl-D-phenylalanine (Z-D-Phe)[15] (0.24 g; 0.51 mmol) using N,N'-dicyclohexylcarbodiimide (DCC) (0.105 g; 0.51

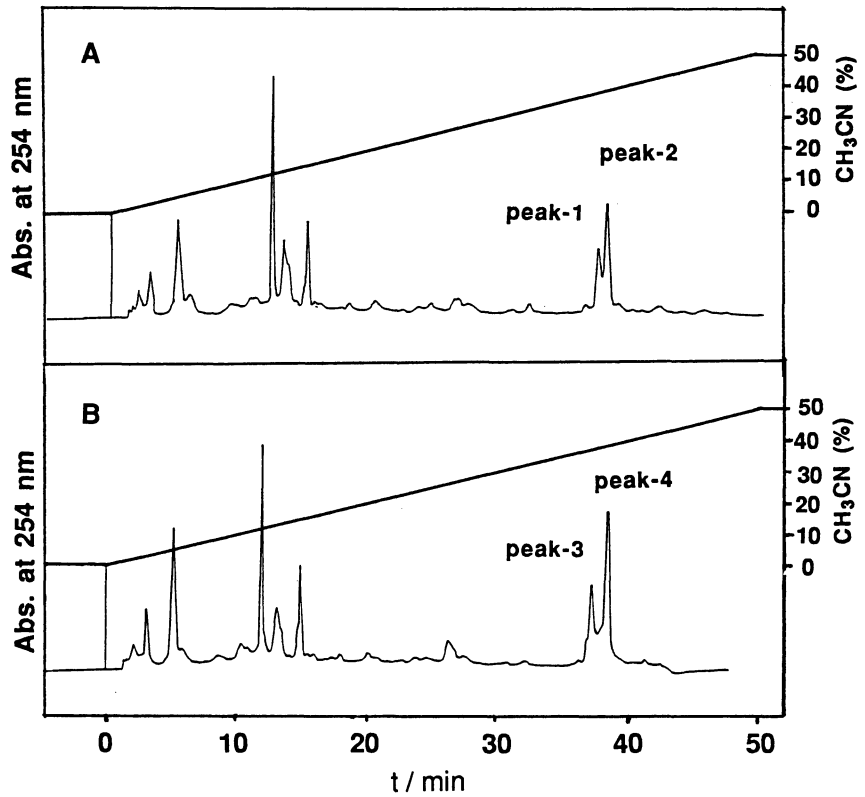


Fig. 1. Reversed-phase HPLC of the crude mixture.
(A) the profile of **10a**. (B) the profile of **10b**.

mmol) in the presence of 4-dimethylaminopyridine (DMAP) (9 mg; 0.07 mmol) in CH_2Cl_2 to give the fully protected 2'(3')-O-(L-phenylalanyl)CpA (**9a**) (0.480 g) or 2'(3')-O-(D-phenylalanyl)CpA (**9b**) (0.501 g) in 65% and 67%, respectively. Whole protecting groups were removed from **9a** (22 mg; 10 μmol) and **9b** (11 mg; 5 μmol) as follows: Treatment with (1.) 15 equiv. of $(n\text{-}Bu_3Sn)_2O$[16] in pyridine for 2 h to remove the phenylthio group, (2.) 45 equiv. of zinc-acetylacetone[9] in pyridine for 1 h to remove the TcBoc groups, (3.) 0.01 M HCl in aqueous dioxane (pH 2.0) for 16 h to remove the dimethoxytrityl (DMTr), THP, and Ee groups. After usual work-up, it was applied to reversed phase HPLC (0.01 M NH_4OAc, pH 4.5, 0-50% CH_3CN / 50 min). The two peaks corresponding to the 2'- and 3'-isomer of O-(Z-L-phenylalanyl)CpA (**10a**) were observed on HPLC (Fig. 1-A, peak 1 and peak 2). A similar phenomenon was observed as the case of L-leucine reported previously.[5] It was found that the THP and Ee were removed smoothly under the condition (pH 2.0, 16 h). The mixture of two peaks was treated with 0.1 M NaOH at room temperature for 5 min to afford Z-L-phenylalanine and CpA which was confirmed by nuclease P1 digestion. Similarly, in the case of the D-phenylalanyl ester, the two peaks corresponding to the 2'- and 3'-isomer of O-(Z-D-phenylalanyl)CpA (**10b**) were observed on HPLC (Fig. 1-B, peak 3 and peak 4). Compound **10a** and **10b** were isolated by repeated subjection to the HPLC in each 5% yield from **9a** and **9b**, respectively.

Finally, removal of the Z group was performed by hydrogenolysis according to the literature procedure of Khorana (H_2 / 5% Pd-$BaSO_4$ in 80% AcOH, 0 °C)[17] to give the 2'(3')-O-(L-phenylalanyl)CpA (**11a**) and 2'(3')-O-(D-phenylalanyl)CpA (**11b**) in 89% and 89% yield, respectively. The aminoacylated structure was confirmed by the dansylation of the released phenylalanine after hydrolysis of **11a** and **11b**, respectively. It is noted that the Z group had better be kept until the ligation because of the stability of the amino acid ester bond decreased by removal of the Z group.

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