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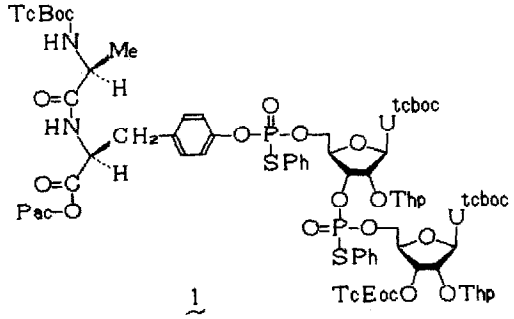
STUDIES ON THE SYNTHESIS OF NUCLEOTIDYL-PEPTIDE. I: A FACILE SYNTHESIS INVOLVING SELECTIVE P-S BOND CLEAVAGE

Hitoshi Hotoda, Yoshihito Ueno, Mitsuo Sekine, and Tsujiaki Hata*

Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku, Yokohama 227, Japan

Abstract: A dinucleotide dipeptide (H-Ala-Tyr(pUpU)-OH) was synthesized via a phosphorothioate triester intermediate (1). It was found that the P-S bond was selectively cleaved by the use of $(n\text{-}Bu_3Sn)_2O$ without undesired dephosphorylation of tyrosine.

To construct a phosphodiester bond between two different biopolymers is a challenging subject. Virion RNA of poliovirus type 1 is known to be covalently linked to a genome-protein VPg.[1] Synthesis of this unique linkage between the 5'-OH group of RNA and the phenolic OH group of tyrosine was first accomplished by van Boom et al.[2] The results with their following studies[3] suggested that base-lable protecting groups used in the usual oligonucleotide synthesis were not suitable for the synthesis of nucleotidyl-peptide (especially in the case that serine or threonine was present at the linkage point). In this paper, the application of our phosphorothioate chemistry[4] to the synthesis of dinucleotide dipeptide (H-Ala-Tyr(pUpU)-OH) via a phosphorothioate triester intermediate (1), which can be deprotected without basic treatment, is described.



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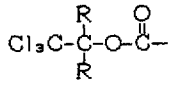
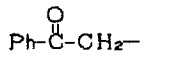


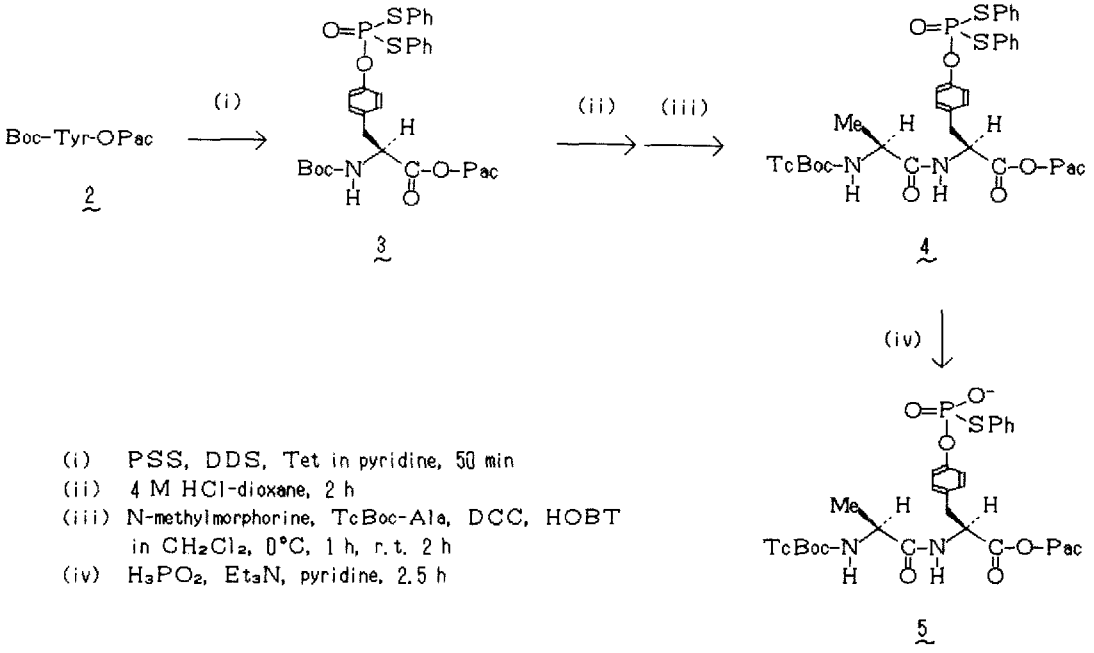
TcBoc (R=CH3)
TcEoc (R=H)



Pac

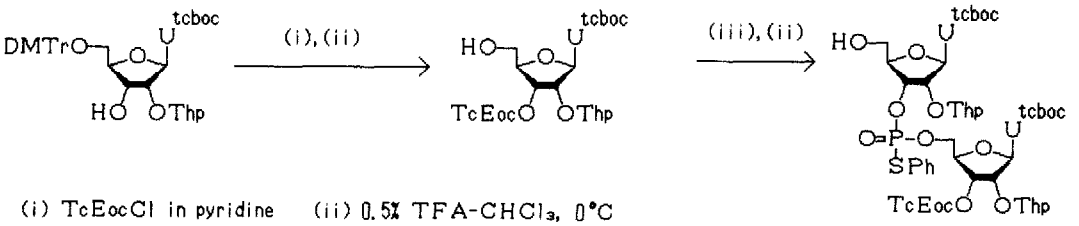
Van Boom's group used their bifunctional phosphorylating agent to combine a dinucleotide with a dipeptide.[2] In contrast to their procedure, we have chosen a synthetic route involving a phosphorylated monomer of tyrosine as a key intermediate. A partially protected tyrosine 2 was phosphorylated by using

cyclohexylammonium S,S-diphenyl phosphorodithioate (PSS), isodurenedisulfonyl chloride (DDS), and 1H-tetrazole (Tet) in 76% yield.[5] The Boc group of 3 was successfully removed by using 4 M HCl-dioxane and the resulting HCl-salt was condensed with TcBoc-Ala[6] (2 equiv) by the DCC-HOBT method to give the dipeptide 4[7] in 90% yield. When 1 equiv of TcBoc-Ala was used, 4 was obtained in 51% yield. This may be due to the steric hindrance of the PSS group. One of the two phenylthio groups could be selectively removed from 4 by an analogous method in the case of oligonucleotide synthesis[4] using a phosphinate reagent to give the corresponding phosphodiester moiety 5[8] in 80% yield.

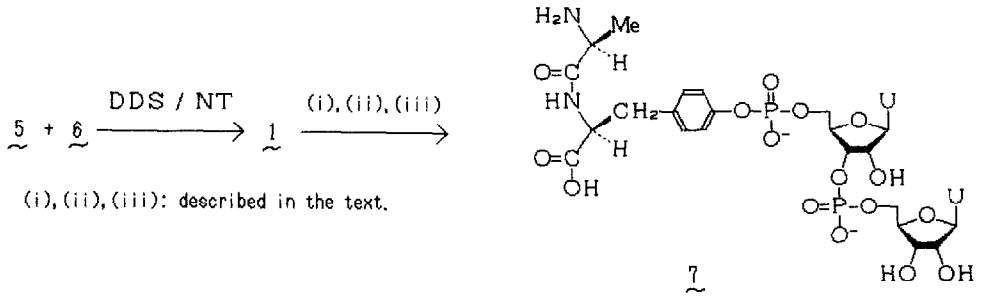


(i) PSS, DDS, Tet in pyridine, 50 min
(ii) 4 M HCl-dioxane, 2 h
(iii) N-methylmorphorine, TcBoc-Ala, DCC, HOBT in CH_2Cl_2, 0°C, 1 h, r.t. 2 h
(iv) H_3PO_2, Et_3N, pyridine, 2.5 h

On the other hand, the 5'-OH component of dinucleotide (6) was constructed as shown in the following scheme. The 3-N position of uridine was protected by the use of the TcBoc group,[9] which could be deprotected by using Zn-acetylacetone under neutral condition.



(i) TcEocCl in pyridine (ii) 0.5% TFA-$CHCl_3$, 0°C
(iii) condensation by DDS-NT method



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Condensation of 6 with 5 was performed by using DDS and 3-nitro-1,2,4-triazole (NT) to give the phosphorothioate triester intermediate 1 in 73% yield. Deprotection of 1 was carried out as follows; (i) 30 equiv of $(n\text{-}Bu_3Sn)_2O$ (TBT)[10] in pyridine for 90 min to remove phenylthio groups, (ii) 75 equiv of Zn-acetylacetone[11] in pyridine for 50 min to remove the TcBoc, TcEoc, and Pac groups, (iii) 0.01 N aqueous HCl (pH 2) for 2 days to remove the Thp groups. After workup, the resulting mixture was purified by reversed phase HPLC (0.01 M NH_4OAc, 0-30% CH_3CN/30 min). The main fraction (Rt=13 min) was collected and analyzed by 500 MHz ^1H-NMR.[12] Compound 7 (4.4 μmol, 80 OD) having the free carboxyl group[13] was obtained from 1 (20 μmol, 22% yield through the deprotection steps).

At the purification step, the peak corresponding to pUpU was not detected by HPLC. This finding implies that the TBT-mediated P-S bond activation effected the selective cleavage of the phosphorothioate ester of 1 without undesired dephosphorylation from tyrosine.[14]

In this study, the phosphorylated tyrosine 3 was used as a model case. When the peptide bond was constructed, the PSS group of 3 worked as a protecting group of the phenolic OH function of tyrosine. Then one of the two phenylthio groups could be selectively removed to make a new reaction site toward a nucleotide chain. In general, two different types of protecting groups (X,Y) are needed for the conversion of a phosphotriester (ROP(O)XY) into a phosphodiester (ROP(O)XOH). The phosphotriester ROP(O)XY has a chirality of phosphorus atom and it leads to a mixture of diastereoisomers, which make the isolation and identification of the triester intermediate difficult, when the R group has another chiral center. On the contrary the PSS group, which has no chiral center, does not form diastereomers.

In order to synthesize phospholylated long chain peptide which involves functional groups at the side chains, the post-phosphorylation of a certain functional group of the peptide is more difficult. Therefore, the strategy of peptide synthesis by use of pre-phosphorylated amino acid units[15] must become more important.

References and Notes

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7) ^{31}P-NMR (pyridine-$CDCl_3$, 3:1, v/v) δ 44.52; $[\alpha]_D$ -6.9°(c 1, $CHCl_3$)

8) ^{31}P-NMR (pyridine-$CDCl_3$, 3:1, v/v) δ 6.83

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12) ^{1}H-NMR (500 MHz, D_2O, DSS as an internal standard): δ 7.86 (d, J=8.1 Hz, 1H, 6-H); 7.72 (d, J=8.1 Hz, 1H, 6-H); 7.16 (d, J=8.4 Hz, 2H, phenyl of Tyr); 7.05 (d, J=8.4 Hz, 2H, phenyl of Tyr); 6.00 (d, J=6.2 Hz, 1H, 1'-H); 5.90 (d, J=4.0 Hz, 1H, 1'-H); 5.85 (d, J=8.1 Hz, 1H, 5-H); 5.73 (d, J=8.1 Hz, 1H, 5-H); 4.66-4.08 (m, 11H, 2',3',4',5'-H and CH of Tyr); 3.67 (q, J=7.0 Hz, 1H, CH of Ala); 3.15 (dd, J=4.6 Hz and J=14.1 Hz, 1H, CH_2 of Tyr); 2.88 (dd, J=8.6 Hz and J=14.1 Hz, 1H, CH_2 of Tyr); 1.29 (d, J=7.0 Hz, 3H, CH_3 of Ala).

13) The corresponding amide compound was synthesized by van Boom et al.[2]

14) A possibility of the dephosphorylation from tyrosine was discussed in the literature.[2]

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