CHEMISTRY LETTERS, pp. 117-120, 1987.

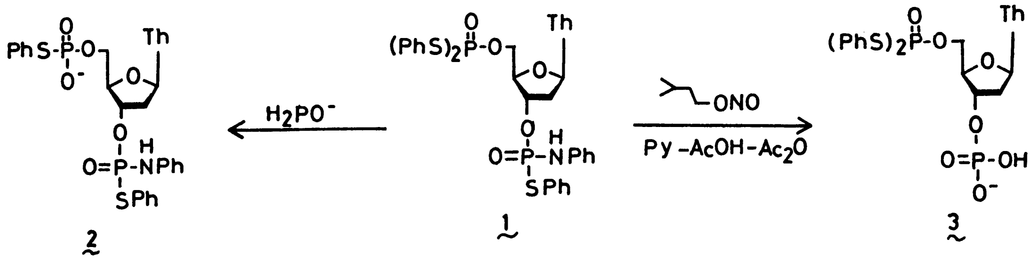
A New Method for the Synthesis of Oligodeoxyribonucleotides Bearing a 5'-Terminal Phosphate Group

Tsujiaki HATA,* Takashi KAMIMURA, Ken-ichi URAKAMI, Kyoko KOHNO, Mitsuo SEKINE,
Izumi KUMAGAI,+ Kazuko SHINOZAKI, and Kin-ichiro MIURA+
Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku, Yokohama 227
+Department of Industrial Chemistry, Faculty of Engineering,
The University of Tokyo, Bunkyo-ku, Tokyo 113

Two oligoribonucleotides, pAUG and pGUAUUAAUAAUG, which are present in the 5'-terminal region of brome mosaic virus mRNA 4, have been synthesized in the phosphotriester approach involving the use of the phenylthio and anilino (or anisidino) groups as the protecting groups for the 5'- terminal phosphate.

Oligoribonucleotides bearing a 5'-terminal phosphate group are of great importance as donors for joining RNA fragments with RNA ligase.1) However, only a few methods have been published for chemical synthesis of 5'-phosphorylated RNA species.2) In this paper, we wish to report a new method for the synthesis of oligoribonucleotides bearing a 5'-phosphate group by the use of a new phoshorytlating agent capable of chain elongation in the 3'-direction.

In the previous paper,3) we reported unique properties of S-phenyl 5'-O-bis-(phenylthio)phosphorylthymidine 3'-phosphoranilidothioate (1) toward isoamyl nitrite (IAN)4) and phosphinic acid.5) It was shown that the 3'-anilino(phenylthio)phosphoryl (ASP) group of 1 remained intact upon treatment with the latter reagent which allowed selective removal of one of two phenylthio groups from the 5'-bis(phenylthio)phosphoryl (SSP) group to give the 5'-free phosphate (2).

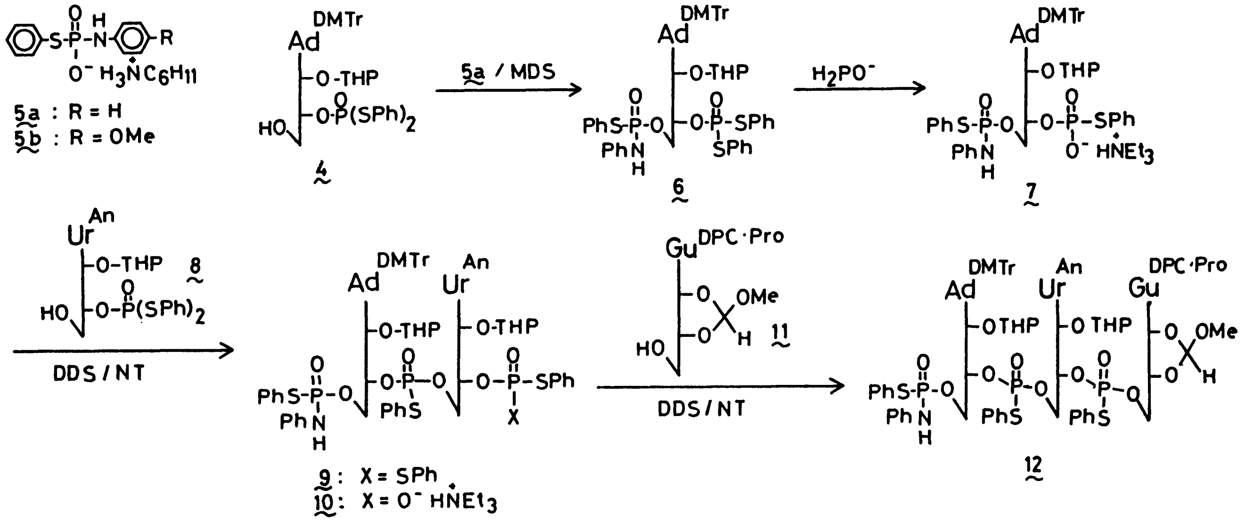


These facts suggest that the reverse use of the ASP and SSP groups as the 5'- and 3'-hydroxyl protecting groups, respectively, enables us to extend the oligoribonucleotide chain in the 3'-direction. Therefore, we first chose pAUG as a synthetic target which was a phosphorylated species of the initiation codon in the

---

Dedicated to Professor Teruaki Mukaiyama on the occasion of his 60th birthday.

peptide synthesis. Phosphorylation of 2'-O-(tetrahydropyran-2-yl)-N^6-(4,4'-dimethoxytrityl)adenosine[6] (4, 0.142 mmol) with cyclohexylammonium S-phenyl phosphoranilidothioate (5a, R=H, 0.213 mmol) in the presence of mesitylenedisulfonyl chloride[5] (MDS, 0.256 mmol) in pyridine (1 ml) for 17 h gave the 3',5'-diphosphorylated product (6) in 83% yield. Treatment of 6 (0.081 mmol) with a mixture of 4 M pyridinium phosphinate/pyridine[5] (0.61 ml, 2.45 mmol) and triethylamine (0.22 ml, 1.62 mmol) at 40 °C for 1 h gave the 3'-phosphodiester component (7) quantitatively. Under these conditions, the ASP group was confirmed to be stable. Condensation of 7 (0.081 mmol) with S,S-diphenyl 2'-O-(tetrahydropyran-2-yl)-N^3-anisoyluridine 3'-phosphorodithioate[6] (8, 0.062 mmol) in the presence of isodurenedisulfonyl chloride[5] (DDS, 0.186 mmol) and 3-nitro-1,2,4-triazole[7] (NT, 0.243 mmol) gave the dimer (9) in 88% yield. The dimer (0.055 mmol) was treated similarly with the phosphinate reagent to afford the 3'-terminal phosphodiester (10), which was in turn condensed with 2',3'-O-methoxymethylene-N^2-propionyl-O^6-diphenylcarbamoylguanosine[8] (11, 0.042 mmol) to give the fully protected trimer (12) in 87% yield.



Previously, we have reported that the ASP group at the 3'-oxygen of 1 allowed simultaneous deprotection of both the anilino (PhNH) and phenylthio (PhS) groups upon treatment with IAN[4] in pyridine-AcOH-Ac_2O[9] to give the 3'-free acid (3) as the main product. Based on this fact, we attempted to remove both the PhNH and PhS groups from 12 by the one-step procedure using IAN-Py-AcOH-Ac_2O. However, this system did not give the 5'-free phosphate. The 5'-terminal PhS group remained to an extent of 95%. A similar result was obtained in the case of S-phenyl 3'-O-acethylthymidine 5'-phosphoranilidothioate. Although it is not clear why there is a considerable difference in removal mode between the 3'- and 5'-ASP groups, the results obtained here are of interest especially in connection with the chemical synthesis of capped mRNAs since 5'-phosphorothioates of the diester type can be used as activatable sites for polyphosphate bond formation. Full deprotection of 12 was performed after the IAN treatment as follows: 1) 0.2 M NaOH-pyridine (1:1, v/v) at 0 °C for 40 min, 2) conc. ammonia 60 °C for 2 h, 3) AgOAc (30 equiv per one phosphate) in pyridine-H_2O (2:1, v/v) at r.t. for 24 h, and 4) 0.01 M HCl (pH 2.0) at r.t. for 40 h. Finally, pAUG was obtained in 35% yield by paper chromatography. This trimer was identified with the authentic sample.[10]

To demonstrate the utility of the present approach, a dodecaribonucleotide of pGUAUUAAUAAUG, i.e., the 5'-terminal sequence of filament 4 of brome mosaic virus mRNA,[11] was also synthesized. In this synthetic project, the anisidino group was employed in place of the PhNH group as one of the 5'-terminal phosphate protecting groups. The 5'-terminal guanosine unit (14) was similarly obtained 78% yield by phosphorylation of 2'-O-(tetrahydropyran-2-yl)-N^2-propionyl-O^6-diphenylcarbamoyl-guanosine[6] (13) with 5b (R=OMe).[12] The protecting groups for the base residues of U, A, and G were the same as used for the synthesis of pAUG. The dodecaribonucleotide was synthesized by fragment condensation as shown in Fig. 1. The 2',3'-cis diol of 3'-terminal guanosine was masked with the methoxymethylene group. It was found that the use of 0.5 M zinc bromide in CH_2Cl_2-iPrOH[13] (85:15, v/v) at 0 °C did not affect the methoxymethylene group. The 3'-terminal PhS and 5'-DMTr groups were selectively removed from fully protected oligomer building blocks by the action of the phosphinate reagent and $ZnBr_2$, respectively. The conditions and results of the fragment condensations are summarized in Table 1.

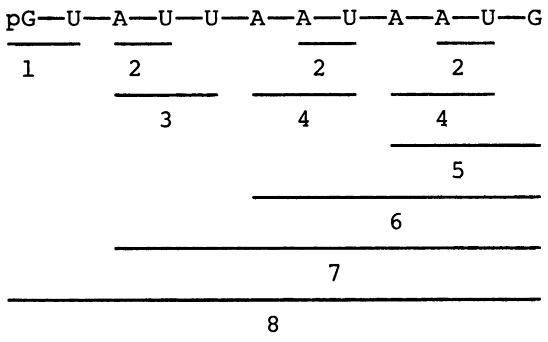


Fig. 1. Strategy for the synthesis of the dodecaribonucleotide.

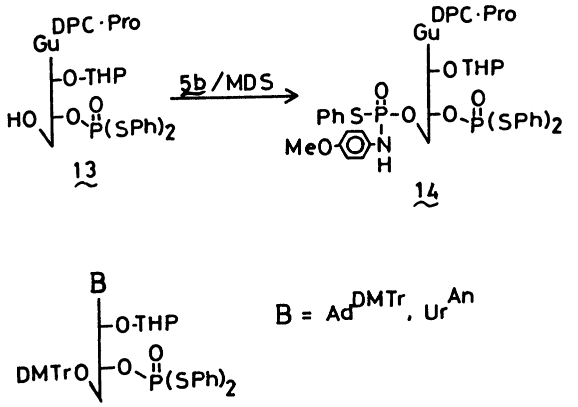


Fig. 2. Fully protected monomer units.

Table 1. The conditions and results of fragment condensations for the synthesis of the fully protected dodecaribonucleotide

3'-Diester compd (equiv.)	4 M H_2PO equiv.	Et_3N equiv.	Time min	5'-OH compd	DDS equiv.	NT equiv.	Time min	Frag. No.	Yield %	Removal of DMTr group with 0.5 M $ZnBr_2$ at -5 °C Time/min	Yield/%
pG (1.3)	30	20	60	U	2+1	3	40+20	1	89	10	90[a]
A (1.3)	30	20	60	U	2+1	3	40+20	2	96		
AU (1.3)	30	20	60	U	2+1	3	40+20	3	91		
A (1.3)	30	20	60	AU	2+1	3	40+20	4	93	40	93
AAU (1.3)	30	20	60	G	2+1	3	40+20	5	95	--	--
AAU (1.4)	30	20	60	AAUG	2+1	3	40+20	6	78	40	97
AUU (1.3)	30	20	60	AAUAAUG	2+1	3	45+45	7	92	60	59
pGU (1.6)	30	20	60	AUUAAUAAUG	2+1	3	60+180	8	72	60	87

[a] In this case, 0.5% trifluoroacetic acid in CH_2Cl_2 was used at 0 °C.

The coupling reactions proceeded very cleanly and a high yield of synthesis of the 12 mer was achieved. Removal of the DMTr group from the protected oligoribonucleotide building blocks became somewhat difficult since longer times were required with an increase of the oligomer chain. The Lewis acid treatment was stopped within 1 h to avoid loss of the methoxymethylene group.

Full deprotection of the protected 12 mer (10.3 mg, 1.20 µmol) was performed as described in the case of pAUG: 1) IAN (116 ul) in pyridine-AcOH-Ac_2O (3:2:2, v/v/v, 1.1 ml) at r.t. for 5 h, 2) 0.2 M NaOH-pyridine (1:1, v/v,2.6 ml) at 0 °C for 50 min, 3) AgOAc (60 µmol) in pyridine-H_2O (2:1, v/v, 0.12 ml) at r.t. for 14 h, and 4) 0.01 M HCl (pH 2.0) for 2 days. Finally, paper chromatography developed with nPrOH-concentrated ammonia-H_2O (55:10:35, v/v/v) gave the crude 12 mer (23 OD at 258 nm, ca. 20%). Final purification of the crude material was performed by 20% polyacrylamide gel electrophoresis in the presence of 7 M urea. The base sequence of the purified 12 mer was confirmed by both the Donis-Keller[14] and modified Maxam-Gilbert[15] methods.

References

1) E. Ohtsuka, S. Nishikawa, A. F. Markham, S. Tanaka, T. Miyake, T. Wakabayashi, M. Ikehara, and M. Sugimura, Biochemistry, 17, 4894 (1978) and references cited therein.
2) E. Ohtsuka, A. Yamane, and M. Ikehara, Chem. Pharm. Bull., 30, 376 (1982); Idem, 31, 1534 (1983); J. Imai and P. F. Torrence, J. Org. Chem., 46, 4015 (1982); H. Takaku, K. Kamaike, and K. Kagawa, ibid., 47, 4937 (1982); E. Ohtsuka, M. Ohkubo, A. Yamane, and M. Ikehara, Chem. Pharm. Bull., 31, 1910 (1983); W. Michels and E. Schlimme, Liebigs Ann. Chem., 1984, 867. For 5'-phosphoryled DNA fragments see a recent paper: T. Tanaka, Y. Yamada, and M. Ikehara, Tetrahedron Lett., 27, 3271 (1986) and references cited therein.
3) M. Sekine and T. Hata, Tetrahedron Lett., 24, 5741 (1983).
4) E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, J. Am. Chem. Soc., 92, 3441 (1970).
5) M. Sekine, J. Matsuzaki, and T. Hata, Tetrahedron Lett., 1981, 3209; Idem., Tetrahedron, 41, 5279 (1985).
6) T. Kamimura, M. Tsuchiya, K. Urakami, M. Sekine, K. Shinozaki, K. Miura, and T. Hata, J. Am. Chem. Soc., 106, 4552 (1984).
7) C. B. Reese, R. C. Titmas, and L. Yau, Tetrahedron Lett., 1978, 2727.
8) A. Kume, H. Tanimura, S. Nishiyama, M. Sekine, and T. Hata, Synthesis, 1985, 407.
9) M. Nishizawa, T. Kurihara, and T. Hata, Chem. Lett., 1984, 175.
10) M. Sekine, S. Nishiyama, T. Kamimura, Y. Ozaki, and T. Hata, Bull. Chem. Soc. Jpn., 58, 850 (1985).
11) R. Dasgupta, D. S. Shih, C. Saris, and P. Kaesberg, Nature, 256, 624 (1975).
12) Mp 176-177 °C (H_2O): This compound was prepared by a method similar to that described in the case of 5a.[3] Its satisfactory elemental analysis was obtained.
13) H. Ito, Y. Ike, S. Ikuta, and K. Itakura, Nucleic Acids Res., 10, 1755 (1982).
14) H. Donis-Keller, A. M. Maxam, and W. Gilbert, Nucleic Acids Res., 4, 2527 (1977).
15) D. A. Peattie, Proc. Natl. Acad. Sci. U.S.A., 76, 1760 (1979).

(Received September 30, 1986)