CHEMISTRY LETTERS, pp. 1051-1054, 1983.

A NEW PROTECTING TACTICS FOR THE URACIL RESIDUE IN OLIGORIBONUCLEOTIDE SYNTHESIS

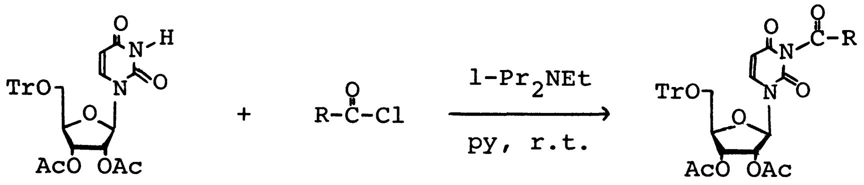
Takashi KAMIMURA, Tsukio MASEGI, Ken-ichi URAKAMI, Shinkichi HONDA,
Mitsuo SEKINE, and Tsujiaki HATA*
Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku, Yokohama 227

Four kinds of acyl groups as protecting group for the uracil imide functionhave been examined. Among them, the anisoyl group was found to be most promissing and used to demonstrate the synthesis of the dimer, UpUp.

Recent progress in oligonucleotide synthesis by the phosphotriester approach is due to the efficient combinations[1)] of condensing agents and internucleotidic protecting groups. However, there appeared the inevitable side reactions of the uracil residue in generating inter-ribonucleotide linkage by use of arenesulfonyl azoles[2)] and aryl phosphoroditriazolides.[3)] Although the side reactions have been utilized favorably in the chemical conversions of uridine into biologically important pyrimidine derivatives,[2f,3a,c)] these side reactions should be eliminated by developing a new protecting tactics for the uracil residue for the synthesis of oligonucleotide.[3c,4)]

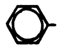
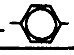
In the preceding paper,[5)] we have demonstrated the utility of the 2,2,2-trichloro-1,1-dimethylethoxycarbonyl (TCBOC) group for the protection of the uracil residue in the synthesis of 2'-O-methyluridine. We have studied the TCBOC group and 4-substituted benzoyl groups[6)] in utilizing as the protecting group in oligoribonucleotide synthesis.

First, we tried to introduce four kinds of acyl groups onto the 3-N-position of the uracil moiety.[7)] 2',3'-Di-O-acetyl-5'-O-trityluridine (1) was treated with



(1) (2a-d) (3)

Table 1. Reaction of (1) and acyl chlorides (2a-d)

R (equiv.)		1-Pr$_2$NEt (equiv.)	time (h)	yield (%)
a) Cl$_3$C(CH$_3$)$_2$CO-	(1.5)	1.5	2	96
b) CH$_3$O- 	(2.0)	2.0	4	99
c) 	(2.0)	2.0	4	98
d) Cl- 	(2.0)	2.0	4	99

TCBOC, anisoyl, benzoyl and 4-chlorobenzoyl chlorides (2a-d) in the presence of ethyldiisopropylamine in dry pyridine at room temperature to give the corresponding 3-N-acylated derivatives (3)8) as shown in Table 1. In order to examine whether these acyl groups can be used in oligonucleotide synthesis, the correspoding 3-N-acylated derivatives 3a-d were treated under the conditions (I-IV) as described below and the degrees or rates of elimination of the acyl groups from 3a-d were estimated by thin layer chromatography.

I) 0.5% trifluoroacetic acid (TFA) in dry CHCl$_3$ (50 ml/mmol) at 0°C for 4 h9)

2a-d : stable

II) 4 M Et$_3$NH$^+$ $^-$HPO$_2$ (20 equiv.) in dry pyridine at 45°C for 5 h10)

2a : 5%, 2b-d : stable

III) 1 M 1H-tetrazole (10 equiv.) in dry pyridine at room temperature

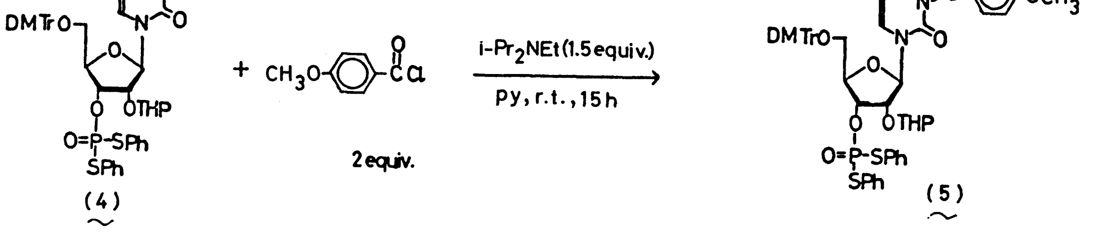
	11 h	41 h	92 h
2a	< 5%	< 5%	5%
2b	< 5%	< 5%	< 5%
2c	< 5%	5%	15%
2d	5%	15%	35%

IV) 0.04 M n-butylamine (10 equiv.) in methanol at room temperature

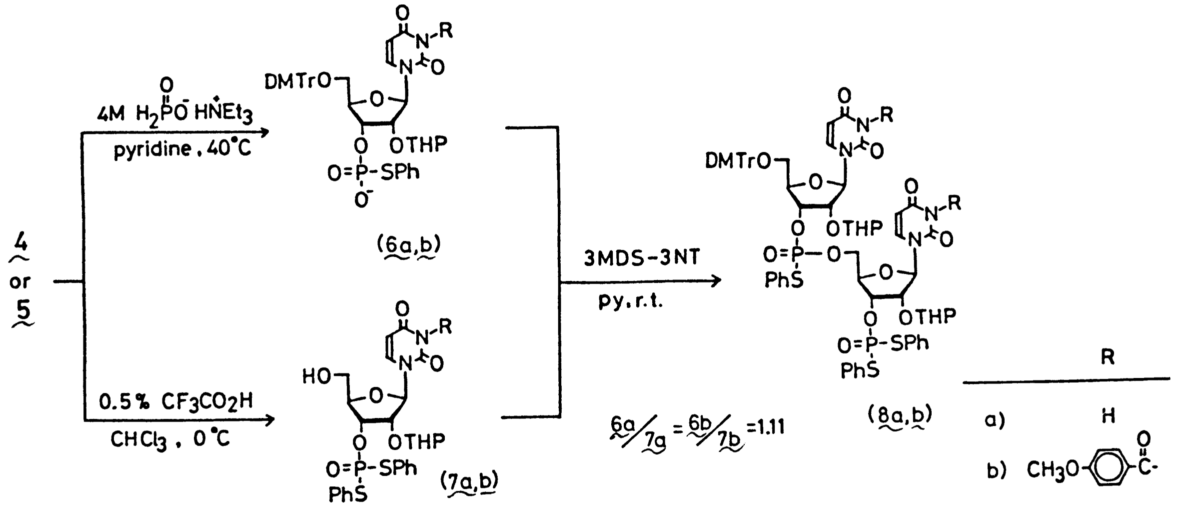
	$t_{1/2}$(min)	t_{∞}(h)
2a	60	6
2b	15	2
2c	10	1.5
2d	10	1.5

These results suggest that the anisoyl group is superior to the TCBOC group5) especially in terms of the stability under the conditions of II). Compound 2b was treated with a mixture of conc. ammonia and methanol (1:3, v/v) at room temperature for 3 h to afford 5'-O-trityluridine in 91% yield. It was confirmed that 5'-O-tritylcytidine was not detected at all under the above conditions where the common N-acyl protecting groups of the nucleotide bases were removed.11)

On the basis of the above facts, the availability of the new tactics can be demonstrated by the synthesis of UpUp. The anisoyl group was introduced onto 3-N-position of uridylic acid unit (4)12) by a procedure similar to that described in the case of 3. The new "fully"-protected uridylic acid unit (5)8) was obtained in 92% yield.



According to our previous works,[12,13)] treatment of 4 and 5 with 4 M phosphinic acid (PSA) (20 equiv.)—triethylamine (20 equiv.) in dry pyridine at 40°C for 16 min and 10 min gave the phosphodiester components (6a) and (6b) respectively, as triethylammonium salts which were used in the next coupling reactions without further purification. Removal of the dimethoxytrityl group from 4 and 5 was performed by treatment with 0.5% trifuluoroacetic acid (TFA) in $CHCl_3$ at 0°C for 30 min and 10 min to give the hydroxyl components (7a) and (7b) in 83 and 90% yield, respectively.[8)]



The condensations of 7a with 6a and 7b with 6b, were carried out by using mesitylenedisulfonyl chloride (MDS)[13a)] (3 equiv.) and 3-nitro-1H-1,2,4-triazole (NT) (3 equiv.).[14)] The reactions were completed in 30 min and gave the desired dimers 8a and 8b in 84 and 94% yields, respectively. The by-product that has a higher Rf-value than 8a was detected on tlc in the coupling reaction from 4, whereas the only desired 8b was detected from 5 and the anisoyl group remained intact during the above treatments.

Deprotection of all the protecting groups from 8b was performed as follows: 1) 0.2 M NaOH—pyridine (1:1, v/v) for 30 min at room temperature to remove the internucleotidic PhS group and one of the two PhS groups at the 3'-terminal phosphate ; 2) concd. ammonia for 24 h at room temperature to remove the anisoyl group; 3) 30 equiv. of silver acetate in pyridine—water (2:1, v/v) for 24 h at room temperature to remove the remaining PhS group at the 3'-terminal phosphate; 4) 0.01 M HCl (pH 2.0) for 40 h at room temperature to remove the 5'-O-dimethoxytrityl and 2'-O-THP groups. Thus, UpUp was isolated in 89% yield after chromatographic separation using Whatman 3MM paper with i-PrOH—concd. ammonia—water (6:1:3, v/v/v). The obtained dimer was degraded by spleen phosphodiesterase to give a single spot of Up.

We thank DOJINDO Laboratories for the gift of 3-nitro-1H-1,2,4-triazole. This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

References

1) a) A. Kraszewski, J. Stawiński, and M. Wiewiórowski, Nucleic Acids Res., 8, 2302 (1980); b) M. J. Gait and S. G. Popov, Tetrahedron Lett., 21, 2841 (1980).
2) a) C. B. Reese and A. Ubasawa, Tetrahedron Lett., 21, 2265 (1980); b) C. B. Reese and A. Ubasawa, Nucleic Acids Res., Symposium Series No. 7, s5 (1980); c) J. A. J. den Hartog, G. Wille, R. A. Scheublin, and J. H. van Boom, Biochemistry, 21, 1009 (1982); d) B. Rayner, C. B. Reese, and A. Ubasawa, J. Chem. Soc., Chem. Commun., 1980, 972; e) E. Ohtsuka, T. Wakabayashi, and Ikehara, Chem. Pharm. Bull., 29, 759 (1981); f) K. J. Divaker and C. B. Reese, J. Chem. Soc., Perkin Trans. 1, 1982, 1171.
3) a) W. L. Sung, Nucleic Acids Res., 9, 6139 (1981); b) W. L. Sung and S. A. Narang, Can. J. Chem., 60, 111 (1982); c) W. L. Sung, J. Org. Chem. 47, 3623 (1982).
4) S. S. Jones, C. B. Reese, S. Sibanda, and A. Ubasawa, Tetrahedron Lett., 22, 4755 (1981).
5) T. Kamimura, T. Masegi, and T. Hata, Chem. Lett., 1982, 965.
6) J. A. Montgomery and H. J. Thomas, J. Org. Chem., 46, 594 (1981).
7) The structures were confirmed by ^{13}C-NMR spectra compared with standard compounds. Further details will be reported elsewhere. F. E. Hruska and W. J. D. Blonski, Can J. Chem., 60, 3026 (1982).
8) Satisfactory microanalytic and spectroscopic data was obtained for all new compounds described here.
9) A modification of the method reported Wiewiórowski was used. See W. T. Markiewicz, E. Biała, R. W. Adamiak, K. Grześkowiak, R. Kierzek, A. Kraszewski, J. Stawiński, and M. Wiewiórowski, Nucleic Acids Res., Symposium Series No. 7, s115 (1980).
10) M. Sekine, K. Hamaoki, and T. Hata, Bull. Chem. Soc. Jpn., 54, 3851 (1981).
11) The structure was confirmed by ^{1}H-NMR and UV spectra.
12) S. Honda, K. Terada, Y. Sato, M. Sekine, and T. Hata, Chem. Lett., 1982, 15.
13) a) M. Sekine, J. Matsuzaki, and T. Hata, Tetrahedron Lett., 22, 3209 (1981); b) A. Kume, M. Sekine, and T. Hata, ibid., 23, 4365 (1982); c) M. Sekine, J. Matsuzaki, and T. Hata, ibid., 23, 5287 (1982).
14) A. K. Seth and E. Jay, Nucleic Acids Res., 8, 5445 (1980).

(Received April 26, 1983)