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Letter

3-*N*-Acyl Uridines: Preparation and Properties of a New Class of Uracil Protecting Group

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It has been clearly shown by Reese and Ubasawa [1] that the protection of the aglycone in uridine is necessary to be able to carry out the chemical synthesis of oligoribonucleotides containing uridine moieties.

Thus, Reese and his co-workers have introduced a 4-*O*-aryl protective group [3] for the uracil moiety of the oligoribonucleotide synthesis. Similarly, Hata *et.al* [4] have proposed a 3-*N*-2,2,2-trichloro-*t*-butyloxycarbonyl group to protect the imide function during their seven-step synthesis of 2′-*O*-methyluridine. In the present work, we propose a more convenient set of N^3-acyl protecting groups, as in *2a* to *2e*, which may be prepared in high yields by a "one-pot" synthesis as outlined in Scheme 1. The general procedure for the "one-pot" synthesis of such N^3-protected acyl derivatives, (*2a*) to (*2e*), involved trimethylsilylation [5] of uridine in dry pyridine solution which is followed by the addition of acyl chlorides *in situ* and then hydrolysis. Standard work-up and purification by column chromatography on silinized silica gel (MeOH–H_2O mixture in the mobile phase) gave pure 3-*N*-protected acyl uridines *2a* to *2e* in 70, 55, 60, 50 and 50 % yields, respectively. To our knowledge, the present preparation of the above N^3-acyl uridines constitutes their first report in the literature.

It was then interesting to explore the relative stabilities of these N^3-acyl groups in 3-*N*-acyluridines, *2a* to *2e*, under a variety of basic conditions to evaluate their possible use in total chemical synthesis of tRNA molecules in conjuction with other base-labile groups on the pentose sugar and phosphotriester moieties. Table 1 describes the relative rates of removal of the acyl groups from the corresponding 3-*N*-acyl derivatives. Thus it becomes readily clear from experiments 2, 3 and 5 in Table 1 that most of these acyl groups may be used in conjunction with other sugar and phosphate protecting groups which are removable either by hydrazine hydrate (0.5 M) in pyridine–acetic acid 3:2 v/v (*e.g.* levulinyl [6]) or by fluoride ions (*e.g.* *t*-butyldimethyl silyl [7] and

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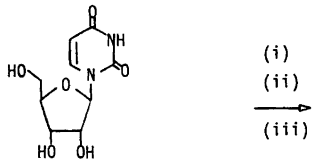

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2a Ar = Ph
2b Ar = p-tolyl
2c Ar = o-tolyl
2d Ar = p-anisyl
2e Ar = mesityl

Scheme 1. (i), Me_3SiCl; (ii), ArCOCl; (iii), H_2O.

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Table 1. The relative rates of hydrolyses of acyl groups from N^3-acyl uridines *2a–2e*.

Expt. No.	Reagents	$t_{1/2}$ (min) of hydrolyses; Ar=				
		Ph	*o*-Tolyl	Mesityl	*p*-Anisyl	*p*-Tolyl
1	Morpholine (5 eq.) THF–H_2O (98:2 v/v)	30	70	[a]	120	270
2	$N_2H_4 \cdot H_2O$ (0.5 M) in pyridine: AcOH (3:2 v/v)	[c]	[d]	[a]	[e]	[e]
3	n-$Bn_4N^+F^-$ (1 M) in dry THF	[a]	[a]	[a]	[a]	[a]
4	Aq. NH_3 (d.0.9) in dioxan (1:1 v/v)	9	20	720	30	13
5	Et_3N (20 eq.) in dry pyridine (10 ml/mmol)	[a]	[a]	[a]	[a]	[a]
6	2.5 M NH_3 in dry CH_3OH	6	25	[b]	37	15

[a] Stable for 8 h. [b] Stable for 1 h. [c] <*ca.* 10 % degradation in 1 h. [d] <*ca.* 5 % degradation in 1 h. [e] <*ca.* 1 % degradation in 1 h.

1,1,3,3-tetraisopropyldisiloxane-1,3-diyl [8]) or by a non-nucleophilic base like triethylamine (*e.g.* 2-phenylsulfonylethoxycarbonyl,[10] 2-(4-chlorophenyl)-sulfonylethoxycarbonyl,[11] fluoren-9-ylmethoxycarbonyl,[12] 2-phenylsulfonylethyl [13] and fluoren-9-methyl [14]).

However, it seems unlikely that the 3-*N*-mesityl uridine would find any useful synthetic application as a protecting group in view of its relatively high stability even under the condition of experiment 4 in Table 1.

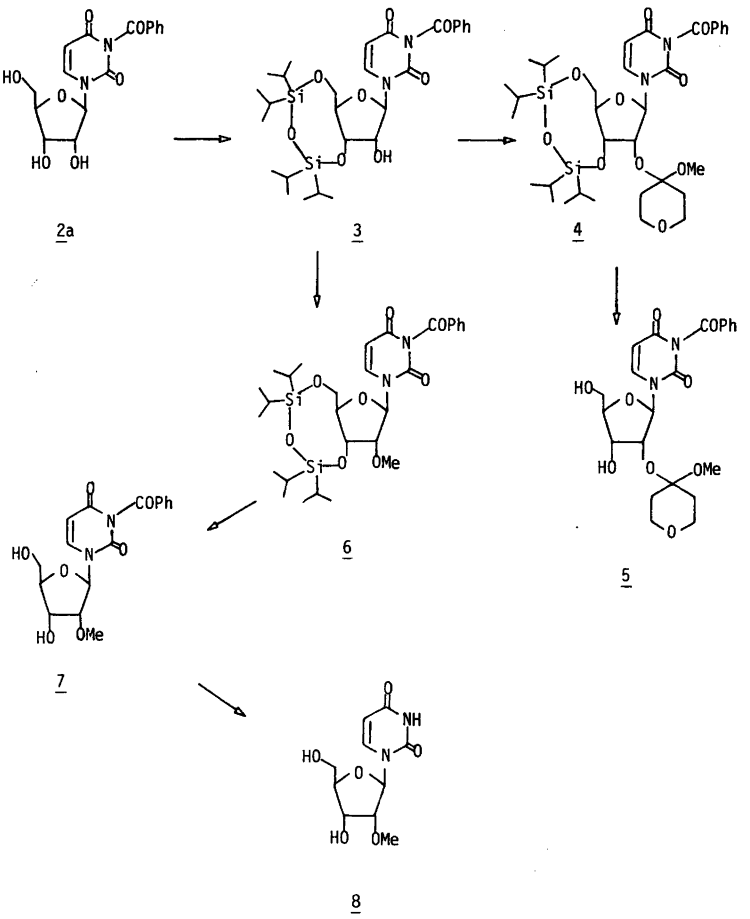
After carefully evaluating the stabilities of the above N^3-acyl groups under a variety of conditions, we are convinced that the 3-*N*-benzoyluridine itself would fulfill the requirements of a 3-*N*-protected building block of uridine [3] in an actual chemical synthesis. Thus, we have synthesized: (a) 3-*N*-benzoyl-2′-*O*-(4-methoxytetrahydropyranyl)-uridine (*5*), a crucial building block for oligoribonucleotide synthesis, and (b) 2′-*O*-methyl uridine (*8*) which has been prepared recently by a Japanese group [4] in seven steps in 29 % overall yield. The outline for the syntheses of *5* and *8* is shown in Scheme 2.

The common intermediate *3* for the synthesis of the above target compounds was prepared in 77 % yield by the treatment of *2a* with a slight excess of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSiCl_2) in dry pyridine solution following a procedure reported in the literature.[8] The 4-methoxytetrahydropyranyl group [9] was then introduced at 2′-position of *3*, by acid catalysis, to give *4*, which was isolated as a crude mixture (*ca.* 95 % purity by TLC; 10 % MeOH–$CHCl_3$ mixture). The crude mixture was subsequently treated with n-$Bu_4N^+F^-$ in dry tetrahydrofuran ([F^-]≈0.01 M; 2.2 equiv.) for 5 min at 20 °C, to remove the TIPDSi group, to obtain *5*, in 82 % overall yield from *2a*. The ^{1}H NMR absorptions confirmed the structure of *5*. This is currently in use in our laboratory for the synthesis of tRNA fragments.

The procedure for the preparation of *8* was as follows: *3* was methylated with methyl iodide (15 equiv.) in dry acetone solution (10 ml/mmol) in the presence of silver oxide (50 equiv.).[2a] Thus, *6* was obtained in 86 % yield as a white powder. The TIPDSi group was then removed from *6* with n-tetrabutylammonium fluoride, as described above, to give *7* in 90 % yield. Finally, the N^3-benzoyl group was removed by an excess of 5 M ammonia solution in dry methanol to give 2′-*O*-methyl uridine (*8*) in 92 % yield (overall yield from *2a* was 58 %). The identity of all new compounds has been established by ^{1}H NMR, UV and element analysis.

Thus, the present work offers a set of convenient 3-*N*-protected uridine derivatives which are easily accessible in high yields and useful for further chemical transformations.

Experimental. All new compounds have been characterized by ^{1}H NMR, UV and element analysis. ^{1}H NMR spectra were measured at 90 MHz with a Jeol FX 900 spectrometer. UV



Scheme 2.

absorption spectra were measured with a Cecil Ce 545 double beam scanning spectrometer.

Compound 2a. UV (water): λ_{max} 260 nm (pH 2); 260 nm (pH 7); 260 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.17 (*d*, 8.2 Hz, 1H), H-6; 8.0 (*m*, 1H), 1- and 5-H of 3-*N*-benzoyl group; 7.68 (*m*, 3H), 2-, 3- and 4-H of 3-*N*-benzoyl group; 5.93 (*d*, 8.2 Hz, 1H), H-5; 5.77 (*d*, 3.9 Hz, 1H), H-1′; 4.06 (*m*, 3H), H-2′, -3′ and -4′; 3.65 (*m*, 2H), H-5′.

Compound 2b. UV (water): λ_{max} 260 nm (pH 2); 260 nm (pH 7); 260 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.13 (*d*, 8.2 Hz, 1H), H-6; 7.86 (*d*, 9 Hz, 1H), *ortho* protons adjacent to >C=O of 3-*N*-4-tolyl group; 7.41 (*d*, 9 Hz, 1H), *meta* protons of 3-*N*-4-tolyl group; 5.93 (d, 8.2 Hz, 1H), H-5;5.77 (*d*, 4.3 Hz, 1H), H-1′; 4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), H-5′.

Compound 2c. UV (water): λ_{max} 270 nm (pH 2); 270 nm (pH 7); 270 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.14 (*d*, 8.2 Hz, 1H), H-6; 7.7–7.29 (*m*, 4H), *o*-tolyl group; 5.75 (*d*, 4.2 Hz, 1H), H-1′; 5.93 (*d*, 8.2 Hz, 1H), H-5; 4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.62 (*m*, 2H), H-5′.

Compound 2d. UV (water): λ_{max} 280 and 298 nm (pH 2); 282 and 298 nm (pH 7) 278 and 298 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.15 (*d*, 8.2 Hz, 1H), H-6; 7.92 (*d*, 8.1 Hz, 1H), *ortho* protons adjacent to >C=O of 3-*N*-*p*-anisyl group; 7.1 (*d*, 8.1 Hz, 1H), *meta* protons of 3-*N*-*p*-anisyl group; 5.75 (*d*, 4.3 Hz, 1H), H-1′; (*m*, 3H), 4.06 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), 5′-CH_2.

Compound 2e. UV (water): λ_{max} 274 nm (pH 2); 278 nm (pH 7); 280 nm (pH 13). ^{1}H NMR (DMSO-d_6+D_2O): δ 8.07 (*d*, 8.2 Hz, 1H), H-6;

6.97 (*s*, 2H), aromatic protons of 3-*N*-mesityl group; 5.85 (*d*, 8.2 Hz, 1H), H-5; 5.75 (*d*, 4.3 Hz, 1H), H-1′; 3.97 (*m*, 3H), H-2′, -3′ and -4′; 3.64 (*m*, 2H), H-5′.

Compound 3. ^{1}H NMR ($CDCl_3$+D_2O): δ 7.92 (*d*, 8.2 Hz, 1H), H-6; 7.84–7.4 (*m*, 5 H), 3-*N*-benzoyl group; 5.80 (*d*, 8.2 Hz, 1H), H-5; 5.69 (*d*, 5.0 Hz, 1H), H-1′; 4.28 (*m*, 1H), H-3′; 4.22 (*m*, 4 H), H-2′, -4′ and -5′; 1.1 (*m*, 28 H), tetraisopropyl groups.

Compound 5. ^{1}H NMR (DMSO-d_6+D_2O): δ 8.20 (*d*, 9 Hz, 1 H), H-6; 8.05–7.56 (*m*, 5H), 3-*N*-benzoyl group; 6.06 (*d*, 9 Hz, 1 H), H-5; 5.97 (*d*, 5 Hz, 1 H), H-1′; 4.40 (*m*, 1H), H-2′; 3.95 (*m*, 2H), H-3′ and -4′; 3.82 (*m*, 2H), 5′-CH_2; 3.54 (*m*, 4H), 2- and 6-protons of 4-methoxytetrahydropyranyl group (MTHP); 3.28 (*s*, 3H), –O*C*H_3 of 4-MTHP group; 1.68 (*m*, 4H), 3- and 5-protons of 4-MTHP group. This compound upon debenzoylation with 5*M* NH_3 in MeOH gave 2′-*O*-(4-methoxytetrahydropyranyl)uridine as the sole product.

Compound 6. ^{1}H NMR ($CDCl_3$); δ 8.0 (*d*, 8.5 Hz, 1 H), H-6; 7.93–7.4 (*m*, 5 H), 3-*N*-benzoyl group; 5.83 (*d*, 8.5 Hz, 1H), H-5; 5.79 (*d*, 4 Hz, 1 H) H-1′; 4.18–4.05 (*m*, 3H), H-2′, -3′ and -4′; 3.78 (*m*, 2H), H-5′; 3.60 (*s*, 3H), 2′-O-*C*H_3; 1.15 (*m*, 28H).

Compound 7. ^{1}H NMR (DMSO-d_6+D_2O): δ 8.20 (*d*, 9 Hz, 1 H), H-6; 8.02–7.85 (*m*, 2H) and 7.80–7.5 (*m*, 3H) are 3-*N*-benzoyl protons; 5.93 (*d*, 9 Hz, 1H), H-5; 5.87 (*d*, 4.6 Hz, 1 H), H-1′; 4.19 (*dd*, 4.6 andd 3.8 Hz, 1 H), H-2′; 3.87 (*m*, 2H), H-3′ and -4′; 3.64 (*m*, 2H), 5′-CH_2; 3.38 (*s*, 3H) 2′-O–*C*H_3.

Compound 8. ^{1}H NMR (DMSO-d_6+D_2O): δ 7.92 (*d*, 8.1 Hz, 1 H), H-6; 5.83 (*d*, 5 Hz, 1 H), H-1′; 5.63 (*d*, 8.1 Hz, 1 H), H-5; 4.09 (*m*, 1 H), H-2′; 3.82 ((*m*, 4H), H-3′, -4′ and -5′; 3.35 (*s*, 3H), 2′-O–CH_3.

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