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STRUCTURAL ASSIGNMENT OF N^3-ACYLATED URIDINE DERIVATIVES BY MEANS OF ^{13}C NMR SPECTROSCOPY

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Diisopropylethylamine was effective as a base for acylation of 2',3',5'-tri-O-acetyluridine with various acid chlorides. The ^{13}C NMR spectra of the products and related compounds showed clearly that the acyl groups intoduced to the uracil moiety are attached to the N^3-nitrogen.

Recent studies on the phosphotriester approach to oligoribonucleotide synthesis revealed that the O^4-modification of the uracil residue actually occured at the condensation stage.[1] In order to overcome this problem, a new protection mode for the imide group has been required. Originally, Reese reported 2,4-dimethylphenyl as the O^4 masking group.[2] In this masking mode, however, C^4-ammonolysis occurs upon treatment with ammonia, which is used for deprotection of N-acyl groups. Accordingly, specific conditions have been used only for removal of this type of protective group prior to unblocking of the N-acyl groups.[2]

On the other hand, we have independently reported 2,2,2-trichloro-1,1-dimethylethoxycarbonyl and anisoyl as the imide blockers of uridine for the synthesis of 2'-O-methyluridine[3] and uridylyl(3'-5')uridine 3'-phosphate,[4] respectively. Especially, the latter has proved to be very useful for the synthesis of a relatively long oligoribonucleotide, GUAUCAAUAAUG.[5] Recently, Chattopadhyaya has similarly proposed aroyl groups as the possible N^3-blockers.[6] However, it is not yet clear whether these acyl groups are attached to the N^3-nitrogen or the O^4-oxygen. Only a little has been known about the structure of base-acylated uridine derivatives. Reese[1a,b] and Sung[1c-e] reported that sulfonation and phosphorylation of 2',3',5'-O-protected uridine derivatives resulted in O^4-modified products in high yields. In the case of guanosine having a similar imide function, modifications involving acylations are known to occur on the O^6-oxygen.[7-9] In contrast to these facts, Pfleiderer suggested on the basis of infrared spectrum analysis that tetrabenzoyluridine, prepared by the full benzoylation of uridine with benzoyl chloride, has a N^3-benzoyl structure.[10]

In this paper, we wish to report a more clear-cut conclusion, on the basis

of ^{13}C NMR, that all the acyl groups studied here are bonded to the N^3-nitrogen.

Introduction of several kinds of acyl groups into the uracil moiety of 2',3',5'-tri-O-acetyluridine (1) was carried out in pyridine in the presence of Hünig base,[4,11] which served as a powerful catalyst.

These conditions and results are summarized in Table I. In all cases, chromatographically stable acylated products (2a-g) were obtained.

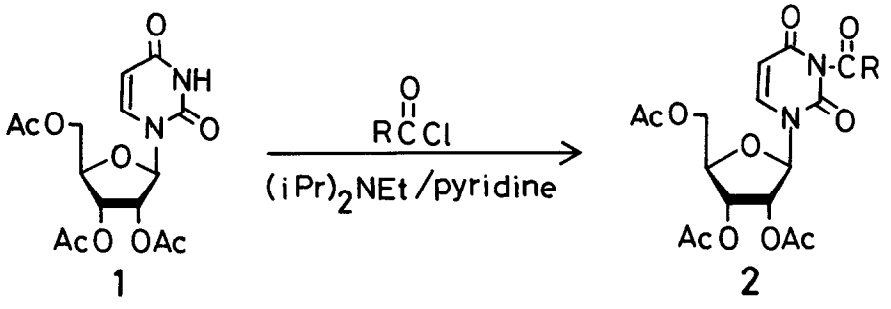

Table I. Acylation of 1 with various acid chlorides.[a]

RCCl	Equivs of RC(O)Cl and $(iPr)_2NEt$	Time (h)	Product	Yield (%)
CH_3CH_2OCCl	3	1	2a	19
$(CH_3)_2CHCH_2OCCl$	3	3	2b	68
Cl_3CCH_2OCCl	1.5	1	2c	98
$Cl_3C(CH_3)_2COCCl$	1.5	5	2d	99
C_6H_5OCCl	3	1	2e	96
C_6H_5CCl	5	1.5	2f	95
$p\text{-}CH_3O\text{-}C_6H_4CCl$	5	2	2g	96

[a]All the products were isolated by silica gel column chromatography and gave satisfactory elemental analyses.

In order to determine the stucture of 2a-g, 2',3',5'-tri-O-acetyl-3-N-benzyluridine (3) was prepared as reference material according to the literature method.[10] Reese reported that azole functions were introduced to the C^4-carbon of the uracil residue by treatment with the corresponding arenesulfonyl azolides. Sung has recently applied this reaction to conversion of uridine into cytidine at the oligomer level.[1d,e] Therefore, we prepared 2',3',5'-tri-O-acetyl-4-O-benzyluridine (6) in 30% overall yield via 2',3',5'-tri-O-acetyluridine (5) from 2',3',5'-tri-O-acetyl-4-O-(2,4,6-triisopropylbenzenesulfonyl)-uridine (4).

Initial attempts to convert 4 into 6 under various conditions were unsuccessful. A similar difficulty has also been reported in the case of

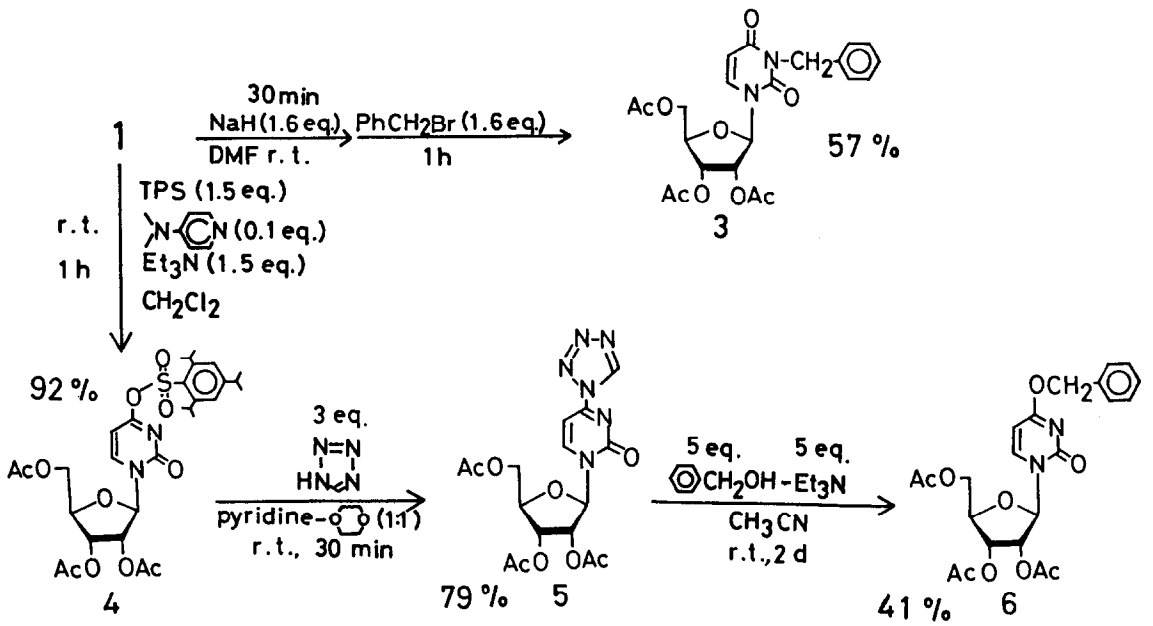

Table II. ^{13}C NMR spectra of acylated uridine derivatives 2a-g and related compounds.

Compound	Substituent	Position	^{13}C NMR ($CDCl_3$), δ			
			C^2	C^4	C^5	C^6
1	H	N^3	150.5	163.3	103.4	139.5
3	CH_2-Ph	N^3	150.9	162.1	102.8	137.2
6	CH_2-Ph	O^4	155.4	171.3	96.4	142.2
4		O^4	154.6	167.2	95.5	130.3
5		C^4	153.8	157.7	95.5	140.9
2a	$COCH_2CH_3$		149.4	159.8	102.7	139.0
2b	$COCH_2CH(CH_3)_2$		149.5	159.8	102.8	138.9
2c	$COCH_2CCl_3$		148.0	159.5	102.6	139.3
2d	$COC(CH_3)CCl_3$		146.8	159.5	103.0	139.8
2e	COC_6H_5		150.6	159.7	102.7	139.4
2f	CC_6H_5		149.2	161.7	102.8	139.9
2g	$CC_6H_4OCH_3$(p-)		149.3	161.6	103.2	139.1

guanosine by Jones[12] who synthesized O^6-alkyl guanosine derivatives from O^6-sulfonated derivatives via C^6-trimethylammonium sulfonate intermediates. Therefore, compound 4 was converted to 5 by treatment with tetrazole (3 equiv) in pyridine-dioxane (1:1, v/v) for 30 min.

Triethylamine was effective for the conversion of 5 to 6. From a mechanistic point of view, it is plausible that the benzyl group of 6 is attached to the oxygen at the 4-position. In fact, the compound 6 was clearly distinguished from its regioisomer 3 in their ^{13}C NMR spectra as shown in Table II. The chemical shifts of C^4 of compounds 3-6 varied significantly owing to the inherent electronic effect of the substituents bound to the C^4-carbon, while the chemical shifts of C^5 in the intermediates 4 and 5 were very similar to that of 6. Contrary to these facts, the C^5 signal of 3 is shifted considerably to a low field compared with those of 4-6 and very near that of uridine. The chemical shifts of C^2 and C^6 of all the compounds 1-6 did not change so much except in the case of 4. The ^{13}C NMR spectra of the acylated products 2a-g are very similar to each other, suggesting that they have an uniform structure. By comparison with the chemical shifts of C^5, we concluded that the acylation (alkoxycarbonylation and benzoylation) of 1 gave exclusively the N^3-substituted products.

The ^{13}C NMR spectroscopy will be a powerful tool for determining the structure of other base-substituted uridine derivatives.

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