65. Selective N^3- and 5′-*O*-Alkylation of 2′,3′-*O*-Isopropylideneuridine with Methyl Iodide

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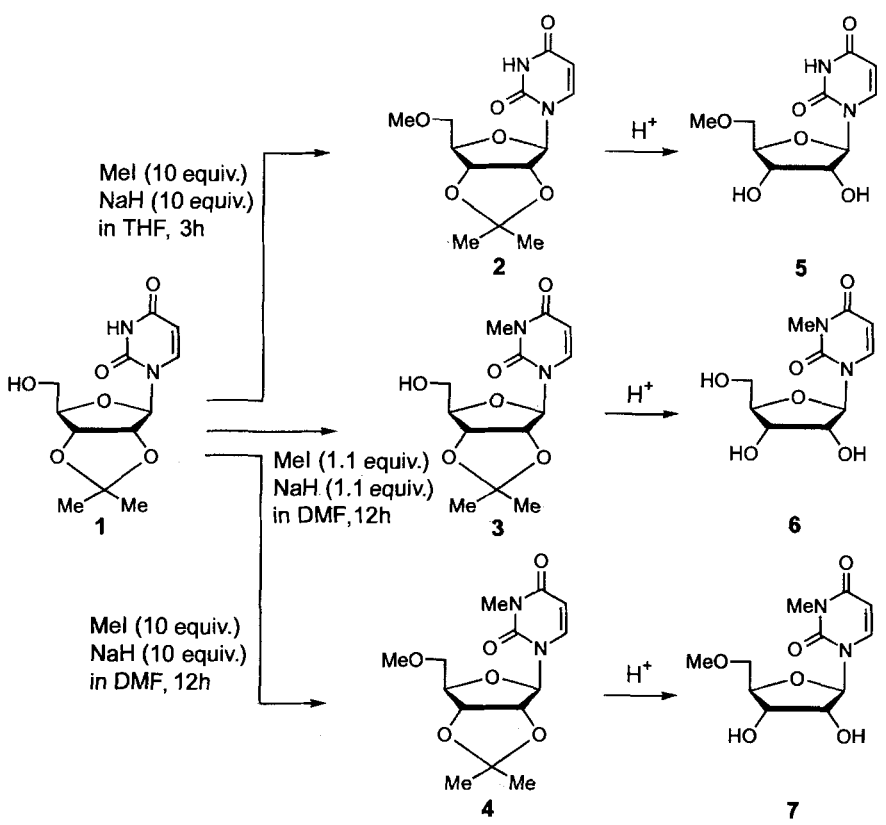
The 2′,3′-*O*-isopropylideneuridine (**1**) reacts with MeI in the presence of an excess of NaH in THF giving 2′,3′-*O*-isopropylidene-5′-*O*-methyluridine (**2**). Prolonged reaction time gives rise to 2′,3′-*O*-isopropylidene-3,5′-*O*-dimethyluridine (**4**). The use of an equimolar amount of base and alkylating agent results predominantly in methylation at N(3) (→ **3**).

Introduction. – It is well established that 'soft' alkylating agents react with nucleic acids in S_N2 fashion, the reaction taking place predominantly at N-sites [1]. In addition to classical alkylating agents such as dialkyl sulfates and alkyl halides, a large variety of better and more selective *N*-alkylating agents have been developed [2]. On the other hand, methods for alkylation of the carbohydrate moiety of nucleosides are finite. The widespread use of diazomethane for methylation of sugar OH groups is limited to the 2′(3′)-*O*-monomethyl derivatives of ribonucleosides [3]. Selective 2′-*O*-methylation of ribonucleosides can be performed by reaction of MeI with a 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)nucleoside in the presense of silver oxide [4] or by using 5′-*O*-trityl-2′,3′-*O*-(dibutylstannylene)nucleosides and diazomethane [5]. Naturally, these methods [3–5] call for *N*-protection of the base residues. The only method reported for the preparation of 5′-*O*-methylnucleosides involves alkylation of 2′,3′-*O*-isopropylidenecytidine at its 5′-OH group with dimethyl sulfate in 10M aqueous alkali [6]. The product, 5′-*O*-methyl-2′,3′-*O*-isopropylidenecytidine, can be further converted into 5′-*O*-methyluridine (**5**) by hydrogensulfite-ion-catalyzed deamination at the cytosine moiety and removal of the *cis*-diol protecting group. However, the overall yield of **5** is only 14%, and the use of the highly carcinogenic reagent dimethyl sulfate is needed. Here is presented a highly simplified synthesis of **5** using a considerably less carcinogenic reagent, methyl iodide. It is also shown that the site and level of alkylation can be controlled by reaction time, solvent, and the ratio of the reactants.

Results and Discussion. – *Blank* and *Pfleiderer* have shown in 1970, that 2′,3′-*O*-isopropylideneuridine (**1**) can be selectively benzylated at the 5′-OH group with benzyl chloride in dioxane/benzene under reflux in the presence of an excess of NaH [7]. However, when **1** was allowed to react with MeI under similar conditions, the corresponding 5′-*O*-methyl derivative **2** was obtained only in very low yield [8]; no *N*-methylated product **3** was detected. Since good site specifity was obtained, the reaction was further investigated as a function of reaction time and ratio of the reactants. Thus, **1** was

allowed to react with MeI in the presence of various amounts of NaH in dry THF at room temperature, and the progress of the reaction was followed by HPLC. When a 10-fold excess of base and alkylating agents was used for 3 h, the methylation occurred almost exclusively at the 5′-*O* position (→ **2**; see *Scheme* and *Table* (*Entry 1*)); Only traces of 2′,3′-*O*-isopropylidene-3-methyluridine (**3**) and 2′,3′-*O*-isopropylidene-3,5′-*O*-dimethyluridine (**4**) were formed (*Fig., a*). Prolonged reaction time produced more **4**. The amount of NaH and MeI could be reduced to 3 and 1 mol-equiv., respectively, without affecting dramatically the yield of **2** (*Table*, *Entry 2*). By contrast, when only an equimolar amount of base was used, the site of alkylation was N(3). Although the site specificity was excellent, the reaction was undesirably slow: after two days at room temperature, the reaction mixture contained the desired product **3** and an equimolar amount of unchanged starting material **1**. When the solvent was changed to DMF, the reaction was completed in 12 h at room temperature, the site specificity being still acceptable: according to HPLC analysis, the reaction mixture contained 80% of **3** and *ca.* 10% of **1** and **4** (*Fig., b*, *Table* (*Entry 3*)), both of which were easily removable by column chromatography (silica gel). When desired, the 3,5′-*O*-dimethylated product **4** was easily obtained by using an excess of base and alkylating agent in DMF [9] [10] (*Fig., c*, *Table* (*Entry 4*)).

Scheme



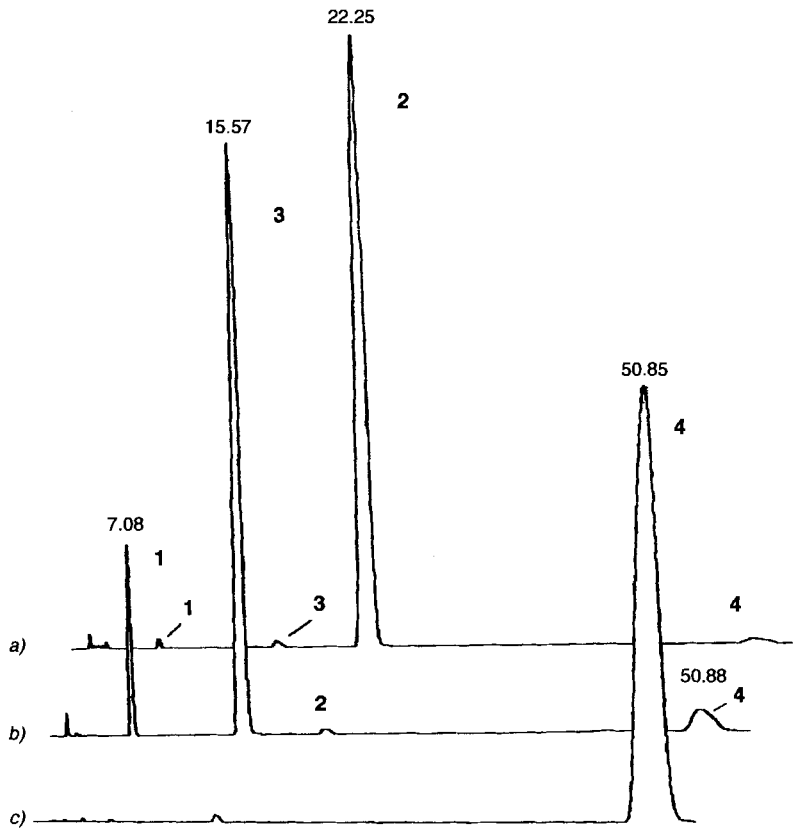


Figure. *HPLC Traces* (crude reaction mixtures) *of the reaction of 2',3'-O-isopropylideneuridine* (**1**) *with MeI in the presence of NaH at room temperature.* a) *10 equiv. of MeI and 10 equiv. of NaH in THF, after 3 h;* b) *1.1 equiv. of MeI and 1.1 equiv. of NaH in DMF, after 12 h;* c) *10 equiv. of MeI and 10 equiv. of NaH in DMF, after 12 h.* Column: *Hypersil C-18*, mobile phase 0.1M acetate buffer (pH 4.3) in 15% $MeCN/H_2O$, flow rate 1 $ml\,min^{-1}$, λ 260 nm.

Table. *Reaction Conditions for* N-, O- *and Bis-alkylation of 2',3'-O-Isopropylideneuridine* (**1**) *at Room Temperature*

Entry	Me_3I [equiv.]	NaH [equiv.]	Solvent	Reaction time [h]	Product	Yield [%][a])
1	10	10	THF	3	**2**	96
2	1.1	3	THF	12	**2**	88
3	1.5	1.1	DMF	12	**3**	80
4	10	10	DMF	12	**4**	97

[a]) According to HPLC analysis.

The change in the site of alkylation of **1** resembles alkylation of mono- and dicarbanions of ethyl acetoacetate [11]. When 1 equiv. of a strong base is used, the alkylation of **1** takes place at the most acidic position of the molecule, *i.e.*, at N(3) (pK_a 9.2 [12]).

When a 2nd equiv. of base is used, not only the most acidic proton is removed but also the second most acidic one (from the 5'-OH group; pK_a estimated to be *ca.* 15 [3d]). The 5'-alkoxide ion is considerably more reactive towards MeI in THF than the deprotonated N(3), since the nucleophilicity of the latter is reduced by conjugation to the C(2) and C(4) carbonyl groups. While 5'-*O*-alkylation is completed in few hours at room temperature, alkylation at N(3) takes considerably longer time. Hence, the alkylation at the 5'-*O* position can be driven into completion by using an excess of base and MeI without contamination of the product with the dimethylated derivative **4**.

In summary, a simple method for the preparation of **2**–**4** is described that is based on differences in the reactivities of the two nucleophilic sites and their conjugate bases. They can be further converted into the corresponding deprotected nucleosides **5**–**7** using standard literature procedures [13].

Experimental Part

General. Column chromatography (CC): silica gel *60* (*Merck*). Anal. TLC: silica gel *60* F_{254} plates (*Merck*), eluent CH_2Cl_2/MeOH 9:1 (*v/v*). HPLC: *Perkin-Elmer* instrument consisting of a series *400* gradient pump, *LC 290* UV/VIS detector, and *LCI-400* integrator; reversed-phase mode on *Hypersil C-18* 4.6 × 240 mm; *Merck*, 6 μm, isocratic elution with 0.1M acetate buffer (pH 4.3) containing 15% MeCN, flow rate 1 mlmin^{-1}; detection at λ 260 nm; samples were taken directly from reaction vessels and analyzed after dilution with eluent buffer, and attributions were confirmed by spiking with authentic materials. UV Spectra: *Perkin-Elmer Lamda-12* spectrophotometer; λ_{max} in nm. NMR Spectra: *Jeol-La-400* spectrometer, at 399.8 and 100.5 MHz for ^1H and ^{13}C, resp.; δ in ppm rel. to internal Me_4Si (= 0 ppm), coupling constants J in Hz; ^1H-signal attributions tentative. High resolution mass spectra (HR-MS): *VG ZabSpec-ao* TOF instrument, fast-atom bombardment (FAB) in the positive mode.

2',3'-O-Isopropylidene-5'-O-methyluridine (**2**). To a suspension of 2',3'-*O*-isopropylideneuridine (**1**) [14] (0.45 g, 1.5 mmol) in dry THF (5 ml), NaH (360 mg, 15 mmol) was added, and the mixture was stirred for 10 min at r.t. Then, MeI (934 μl, 15.0 mmol) was added in one portion and the mixture stirred for an additional 3 h. The reaction was quenched by dropwise addition of MeOH. The mixture was neutralized with AcOH and evaporated. The residue was suspended in CH_2Cl_2 and the org. phase washed with aq. $NaHSO_3$ soln., dried ($MgSO_4$), and evaporated: **2** as a glass which was chromatographically and spectroscopically identical with material synthesized according to [6]. R_f 0.56. UV (H_2O): 261; min. 231. UV (0.1M NaOH): 260; min. 242. ^1H-NMR ($CDCl_3$): 9.89 (br., H–N(3)); 7.54 (*d*, *J* = 8.3, H–C(6)); 5.87 (*d*, *J* = 1.9, H–C(1')); 5.73 (*d*, *J* = 8.0, H–C(5)); 4.77 (*m*, H–C(2'), H–C(3')); 4.37 (*m*, H–C(4')); 3.65 (*dd*, *J* = 3.2, 10.5, 1 H–C(5')); 3.59 (*dd*, *J* = 4.2, 10.4, 1 H–C(5')); 3.39 (*s*, MeO); 1.59 (*s*, 1 Me); 1.37 (*s*, 1 Me). ^{13}C-NMR ($CDCl_3$): 163.5, 150.2, 141.3, 114.2, 102.1, 93.3, 85.7, 85.0, 81.0, 72.7, 59.2, 27.2, 25.3. HR-MS: 299.1242 ($[M + H]^+$, $C_{13}H_{19}N_2O_6^+$; calc. 299.1243).

2',3'-O-Isopropylidene-3-methyluridine (**3**) was synthesized as described for **2**. For conditions, see *Table*. The product was purified by CC (silica gel, CH_2Cl_2/MeOH 95:5). R_f 0.49. UV (H_2O): 260; min. 244. UV (0.1M NaOH): 260; min. 234. ^1H-NMR: identical with the reported one [15]. ^{13}C-NMR ($CDCl_3$): 162.4, 150.8, 139.6, 113.8, 101.1, 94.6, 86.6, 84.4, 80.3, 62.1, 27.3, 26.9, 24.9. HR-MS: 299.1243 ($[M + H]^+$, $C_{13}H_{19}NO_6^+$; calc. 299.1243).

2',3'-O-Isopropylidene-3,5'-O-dimethyluridine (**4**) was synthesized as described for **2**. For conditions, see *Table*. R_f 0.82. ^1H-NMR: identical with the reported one [10]. UV (H_2O): 260; min. 231. UV (0.1M NaOH): 260; min. 231. ^{13}C-NMR ($CDCl_3$): 163.0, 151.0, 138.6, 114.0, 101.2, 94.2, 85.7, 85.4, 81.1, 72.7, 59.2, 27.5, 27.2, 25.3. HR-MS: 313.1408 ($[M + H]^+$, $C_{14}H_{21}N_2O_6^+$; cal. 313.1399).

General Method for Deprotection. To a soln. of **2**, **3** or **4** (1.0 mmol) in H_2O/MeCN 1:1 (50 ml), conc. HCl soln. (1 ml) was added and the mixture stirred overnight at r.t. All volatile material was evaporated and the residue co-evaporated twice from dry MeCN.

5'-O-*Methyluridine* (**5**): 0.23 g (87%). Chromatographically and spectroscopically identical with material synthesized according to [6]. UV (H_2O): 261; min. 231. UV (0.1M NaOH): 260; min. 242. ^1H-NMR ((D_6)DMSO): 11.32 (br., H–N(3)); 7.68 (*d*, *J* = 8.1, H–C(6)); 5.57 (*d*, *J* = 5.1, H–C(1')); 5.65 (*d*, *J* = 8.1, H–C(5)); 5.42 (*d*, *J* = 5.6, exchange with D_2O, 1 H); 5.19 (*d*, *J* = 5.1, exchange with D_2O, 1 H); 4.02 (*m*, H–C(2'));

3.90 (*m*, H–C(3'), H–C(4')); 3.55 (*dd*, *J* = 2.9, 11.0, 1H–C(5')); 3.46 (*dd*, *J* = 3.9, 11.0, 1H–C(5')); 3.30 (*s*, MeO). ^{13}C-NMR ((D$_6$)DMSO): 163.1, 150.7, 140.6, 102.0, 88.1, 82.7, 73.1, 72.0, 58.6. HR-MS: 259.0928 ([*M* + H]$^+$, $C_{10}H_{15}N_2O_6^+$; calc. 259.0932).

3-Methyluridine (**6**) 0.21 g, (83%). HPLC: identity confirmed by spiking with commercial product from *Sigma*.

3,5'-O-Dimethyluridine (**7**): Purified by CC (silica gel, CH_2Cl_2/MeOH 9:1). Yield 92%. UV (H_2O): 260; min. 231. UV (0.1M NaOH): 260; min. 231. ^{1}H-NMR ($CDCl_3$): 7.78 (*d*, *J* = 8.3, H–C(6)); 5.84 (*d*, *J* = 5.3, H–C(1')); 5.80 (*d*, *J* = 8.3, H–C(5)); 4.23 (*m*, H–C(2')); 4.20 (*m*, H–C(3')); H–C(4')); 3.69 (br., OH–C(2'), OH–C(3'); *dd*, *J* = 2.2, 10.7, 1H–C(5')); 3.59 (*dd*, *J* = 2.7, 10.7, 1H–C(5')); 3.41 (*s*, 1Me); 3.30 (*s*, 1Me). ^{13}C-NMR ($CDCl_3$): 163.1, 151.8, 138.0, 101.5, 90.9, 84.1, 75.6, 71.7, 70.7, 59.3, 27.7. HR-MS: 273.1086 ([*M* + H]$^+$, $C_{11}H_{17}N_2O_6^+$; calc. 273.1086).

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