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Synthesis of 5-Methylisocytidine Derivatives

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Summary. N^2-Substituted 5-methylisocytidine derivatives were synthesized from S^2-methyl-2-thiothymine either by direct substitution of the methylthio group by an amino group and subsequent condensation with 1,2,3,5-*tetra*-O-acetyl-β-D-ribofuranose to give the acetylated nucleoside or by the opposite sequence first preparing an acetylated 5,S^2-dimethyl-2-thiouridine followed by treatment with the appropriate amine.

Keywords. Herpes simplex virus; Human immunodeficiency virus; Isocytidines, N^2-alkyl-5-methyl; Nucleosides, convergent synthesis of; 2-Thiouridine, 5,S^2-dimethyl.

Synthese von 5-Methylisocytidin-Derivaten

Zusammenfassung. N^2-substituierte 5-Methylisocytidin-Derivate wurden ausgehend von S^2-Methyl-2-thiothymidin entweder durch direkte Substitution der Methylthiogruppe durch eine Aminogruppe und anschließende Kondensation mit 1,2,3,5-*tetra*-O-Acetyl-β-D-ribofuranose zum acetylierten Nucleosid oder umgekehrt durch Synthese eines acetylierten 5,S^2-Dimethyl-2-thiouridins und nachfolgende Behandlung mit dem entsprechenden Amin hergestellt.

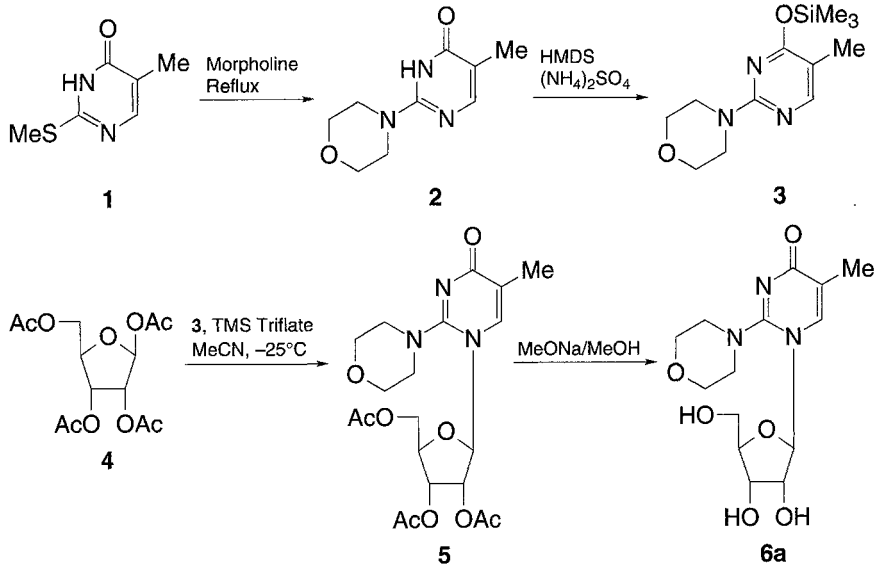
Introduction

Recently, there has been growing interest in isocytidines and their corresponding 2′-deoxy derivatives in order to extend the genetic alphabet of DNA and RNA [1–4]. In this context, we would also expect an interest in the corresponding N^2-substituted isocytidine derivatives. However, this type of cytidine derivatives is reported only sporadically in the literature. Treatment of 2,5′-anhydro-2′,3′-isopropylideneuridine with methylamine or liquid dimethylamine afforded the corresponding N^2-methyl and N^2,N^2-dimethyl-2′,3′-O-isopropylideneisocytidines [5]. N^2-(4-Methoxyphenyl)-isocytidine was similarly prepared by heating 2,5′-anhydrouridine with 4-methoxyaniline in *DMF* in a closed vessel [6]. In this paper, we describe other routes which may also be attractive for the synthesis of isocytidines with unnatural substituents in the 5-position of the nucleobase.

Results and Discussion

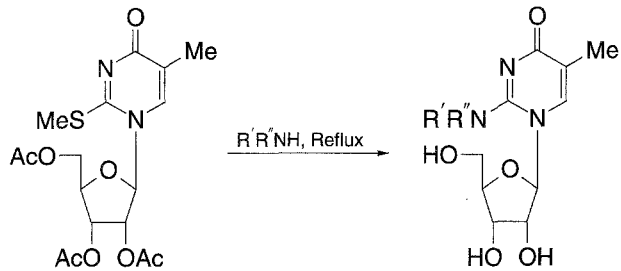
S^2-Methyl-2-thiothymine (**1**) was treated with excess morpholine at reflux temperature to give 5-methyl-2-morpholinopyrimidin-4-one (**2**) in 55% yield after crystalli-

zation from absolute ethanol. In the ^{13}C NMR spectrum, a line broadening of the C-6 resonance at 151.50 ppm indicated a tautomeric equilibrium between the N^1-H and N^3-H tautomers of **2**. The N^3-H tautomer is belived to be the predominant one because a considerable downfield shift was observed for C-6 in **2** when compared with the nucleosides **6** reported in the experimental part. Silylation of **2** with 1,1,1,3,3,3-hexamethyldisilazane (*HMDS*) was carried out according to *Vorbrüggen et al.* [7] by refluxing the nucleobase in *HMDS* in the presence of a catalytic amount of ammonium sulfate. The condensation of 1,2,3,5-*tetra*-O-acetyl-β-D-ribofuranose (**4**) with the silylated base **3** was carried out according to the *Vorbrüggen* conditions [8] in dry acetonitrile, using trimethylsilyl trifluoromethanesulfonate (*TMS* triflate) as the catalyst and **5** was produced in 53% yield. Treatment **5** with NaOMe/MeOH at room temperature afforded the corresponding deprotected product **6** in 69% yield.



Scheme 1

5-Methyl-2-methylthio-1-(2,3,5-*tri*-O-acetyl-β-D-ribofuranosyl)-pyrimidin-4(1*H*)-one (**7**) has previously been synthesized from the silylated nucleobase **1** and the acetylated ribose derivative **4** [9]. When **7** was treated with aliphatic amines at reflux temperature, we found concomitant deprotection of the hydroxy groups during the nucleophilic substitution reaction on the pyrimidine ring, and the corresponding isocytidine derivatives **6** were produced in 53–70% yields. In fact, we found it more easy to synthesize a larger number of isocytidine derivatives for the purposes of biological screening by first preparing the S^2-methyl nucleoside **7** followed by reaction with different amines. By treating **7** with conc. ammonia in a sealed tube at 55 °C overnight with stirring, we obtained 5-methyluridine and 2,5′-anhydro-5-methyluridine in 25 and 33% yields, respectively, but we did not observe any formation of 5-methylisocytidine. Consequently, although the conditions were different in the last reaction, one cannot exclude that the isocytosines **6** were formed *via* an intermediately formed 2,5′-anhydro nucleoside.



6	R'R"N
a	Morpholino
b	MeNH
c	EtNH
d	PrNH

6	R'R"N
e	BuNH
f	$PhCH_2NH$
g	Pyrrolidino

Scheme 2

The nucleosides **6a, b, d–f** did not show any significant activity at a concentration of 10 μ*M* against HIV-1 in MT-4 cells. Compound **6f** showed toxicity against MT-4 cells at 100 μ*M*, but no activity was observed against HIV-1 at a lower concentration. Expression of HIV in culture medium was quantified by the HIV antigen detection kit ELISA. The same compounds were also devoid of any activity at 100 μ*M* against herpes simplex virus, type 1 (HSV-1), strain *McIntyre* when tested in African green monkey kidney cell line *Vero*; no cell toxicity was observed.

Experimental

The NMR spectra were recorded on a Bruker AC 250 FT spectrometer. Chemical shifts are reported in ppm relative to internal *TMS*, and signals are described as a s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). EI mass spectra were recorded on a Varian MAT 311 A spectrometer. Silica gel TLC was performed on 60 F-254 precoated plates (Merck); column chromatography was performed on Merck silica gel (0.040–0.063). All solvents were distilled before use.

5-Methyl-2-morpholinopyrimidin-4-one (**2**)

A solution of S^2-methyl-2-thiothymine (**1**, 15.6 g, 0.1 mol) and an excess of morpholine was stirred at reflux temperature for 5 h (monitored by TLC analysis). The excess of morpholine was removed under reduced pressure and **2** was crystallized from ethanol.

Yield: 10.70 g (55%); m.p.: 163–164 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.80$ (3H, s, CH_3), 3.49 (4H, m, 2 × NCH_2), 3.61 (4H, m, 2 × OCH_2), 7.55 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 12.05$ (CH_3), 44.65 (2 × NCH_2), 65.56 (2 × OCH_2), 164.58 (C-4); MS: $m/z = 195$ (M^+); $C_9H_{13}N_3O_2$; calcd.: C 55.37, H 6.71, N 21.52; found: C 55.09, H 6.77, N 21.34.

5-Methyl-2-morpholino-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrimidin-4(1H)-one (**5**)

5-Methyl-2-morpholinopyrimidin-4-one (**2**, 1.56 g, 8 mmol) was treated with 1,1,1,3,3,3-hexamethyldisilazane (40 ml) and $(NH_4)_2SO_4$ (60 mg) at reflux for 1 h. The solvent was removed *in vacuo*, and the residue **3** was dissolved in dry MeCN (20 ml). 1,2,3,5-*tetra*-O-Acetyl-β-ribofuranose (**4**, 1.71 g, 5.4 mmol) was added and the reaction mixture was cooled to −30 °C. *TMS* triflate (1.3 ml, 6.5 mmol) in dry

MeCN (5 ml) was added dropwise with stirring. The mixture was stirred for 1 h, diluted with CH_2Cl_2 (200 ml), and extracted with ice-cold sat. aq. $NaHCO_3$ (300 ml). The organic phase was separated, washed with cold H_2O (300 ml), dried over Na_2SO_4, and evaporated to give the crude product which was chromatographed on a silica gel column with 0–0.5 % MeOH in $CHCl_3$.

Yield: 1.3 g (53%) as a white foam; ^{1}H NMR ($CDCl_3$): $\delta = 2.00$ (3H, s, CH_3), 2.07 (3H, s, Ac), 2.15 (3H, s, Ac), 2.19 (3H, s, Ac), 3.23 (2H, m, NCH_2), 3.46 (2H, m, NCH_2), 3.78 (4H, m, 2 × OCH_2), 4.33 (3H, m, 4′-H, 5′-H), 5.32 (2H, m, 2′-H, 3′-H), 5.85 (1H, d, $J = 6.6$ Hz, 1′-H), 7.17 (1H, s, 6-H); ^{13}C NMR ($CDCl_3$): $\delta = 13.83$ (CH_3), 20.08, 20.32, 20.58 (3 × Ac), 50.02 (2 × NCH_2), 63.04 (C-5′), 66.05 (2 × OCH_2), 70.10 (C-3′), 72.38 (C-2′), 79.80 (C-4′), 89.10 (C-1′), 120.37 (C-5), 132.34 (C-6), 158.21 (C-2), 168.79 (C-4), 169.25, 169.70, 170.64 (3 × Ac); FAB MS ($CDCl_3$ + 3-nitrobenzyl alcohol): $m/z = 454$ ($M + H^+$).

5-Methyl-2-morpholino-1-(D-ribofuranosyl)-pyrimidin-4(1H)-one (**6a**)

Compound **5** (1 g, 2.2 mmol) was treated with MeONa (50 mg, 2.2 mmol) in MeOH (40 ml) at 0 °C with stirring at room temperature for 1 h. After neutralization with NH_4Cl (120 mg, 2.3 mmol), the solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column with 6–8% MeOH in $CHCl_3$ to obtain **6a**.

Yield: 0.5 g (69%) as a white foam; ^{1}H NMR (*DMSO*-d_6): $\delta =$ (3H, s, CH_3), 3.17–3.21 (4H, m, 2 × NCH_2), 3.57 (2H, m, 5′-H), 3.67 (4H, m, 2 × OCH_2), 3.84 (1H, m, 4′-H), 4.00 (1H, m, 3′-H), 4.18 (1H, m, 2′-H), 5.59 (1H, d, $J = 6.1$ Hz, 1′-H), 7.66 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.24$ (CH_3), 49.90 (2 × NCH_2), 61.28 (C-5′), 65.38 (2 × OCH_2), 70.30 (C-3′), 73.33 (C-2′), 85.42 (C-4′), 91.16 (C-1′), 117.32 (C-5), 135.09 (C-9), 158.38 (C-2), 169.62 (C-4); FAB MS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 328$ ($M + H^+$).

Preparation of 5-methylisocytidines (**6**) *from 5,S²-dimethyl-2′,3′,5′-tri-O-acetyl-2-thiouridine* (**7**)

A solution of **7** (0.5 g, 1.2 mmol) and an excess of the appropriate amine was stirred under reflux for 3 h. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column with 6% MeOH in $CHCl_3$ to remove the impurities and then with 10–15% MeOH in $CHCl_3$ to obtain **6a** in 65% yield and **6b–g** in 53–70% yield.

5,N²-Dimethylisocytidine (**6b**)

Yield: 180 mg (55%); m.p.: 215–217 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.72$ (3H, s, CH_3), 2.71 (3H, d, $J = 4.0$ Hz, $NHCH_3$), 3.62 (2H, br s, 5′-H), 3.92 (1H, m, 4′-H), 3.99 (1H, m, 3′-H), 4.17 (1H, *t*, $J = 5.9$ Hz, 2′-H), 5.20 (1H, br s, OH), 5.38 (1H, d, $J = 6.7$ Hz, 1′-H), 5.42 (2H, br s, 2 × OH), 7.00 (1H, q, $J = 4.1$ Hz, NH), 7.48 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.28$ (CH_3), 27.94 ($NHCH_3$), 60.64 (C-5′), 69.78 (C-3′), 72.02 (C-2′), 85.63 (C-4′), 91.78 (C-1′), 112.68 (C-5), 135.45 (C-6), 153.13 (C-2), 169.91 (C-4); FAB MS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 272$ ($M + H^+$).

N²-Ethyl-5-methylisocytidine (**6c**)

Yield: 180 mg (53%) as a yellow gum; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.08$ (3H, t, $J = 7.1$ Hz, CH_3), 1.72 (3H, s, CH_3), 3.26 (2H, m, CH_2), 3.61 (2H, m, 5′-H), 3.91 (1H, m, 4′-H), 4.00 (1H, m, 3′-H), 4.15 (1H, m, 2′-H), 5.19 (1H, d, $J = 4.3$ Hz, 5′-OH), 5.37 (2H, m, 3′-OH, 1′-H), 5.49 (1H, d, $J = 6.6$ Hz, 2′-OH), 6.98 (1H, t, $J = 5.1$ Hz, NH), 7.47 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.32$ (CH_3), 14.36 (CH_3), 35.49 ($NHCH_2$), 60.61 (C-5′), 69.72 (C-3′), 72.08 (C-2′), 85.63 (C-4′), 91.88 (C-1′), 112.82 (C-5), 135.52 (C-6), 152.50 (C-2), 169.88 (C-4); FAB MS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 286$ ($M + H^+$).

5-Methyl-N^2-propylisocytidine (**6d**)

Yield: 225 mg (62%); m.p.: 133–135 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 0.88$ (3H, t, $J = 7.5$ Hz, CH_3), 1.51 (2H, hextet, $J = 7.4$ Hz, CH_2), 1.71 (3H, s, CH_3), 3.17 (2H, m, CH_2), 3.61 (2H, d, $J = 2.3$ Hz, 5′-H), 3.92 (1H, q, $J = 2.5$ Hz, 4′-H), 4.01 (1H, m, 3′-H), 4.18 (1H, t, $J = 6.3$ Hz, 2′-H), 5.38 (4H, d and br s, $J = 6.8$ Hz, 1′-H, 2′-OH, 3′-OH, 5′-OH), 6.97 (1H, t, $J = 5.1$ Hz, NH), 7.45 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 11.14$ (CH_3), 21.60 (CH_2), 42.37 ($NHCH_2$), 60.63 (C-5′), 69.78 (C-3′), 71.94 (C-2′), 85.73 (C-4′), 92.18 (C-1′), 112.73 (C-5), 135.75 (C-6), 152.59 (C-2), 169.86 (C-4); MS: $m/z = 299$ (M^+).

N^2-Butyl-5-methylisocytidine (**6e**)

Yield: 245 mg (65%); m.p.: 80–82 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 0.88$ (3H, t, $J = 7.2$ Hz, CH_3), 1.28 (2H, hextet, $J = 7.4$ Hz, CH_2), 1.46 (2H, m, CH_2), 1.71 (3H, s, CH_3), 3.22 (2H, m, CH_2), 3.61 (2H, m, 5′-H), 3.91 (1H, m, 4′-H), 4.00 (1H, br s, 3′-H), 4.17 (1H, m, 2′-H), 5.18 (1H, br s, 5′-OH), 5.36 (3H, m, 1′-H, 2′-OH, 3′-OH), 6.89 (1H, br s, NH), 7.42 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.28$ (CH_3), 13.60 (CH_3), 19.43 (CH_2), 30.58 (CH_2), 40.42 (CH_2), 60.66 (C-5′), 69.78 (C-3′), 71.88 (C-2′), 85.72 (C-4′), 92.20 (C-1′), 112.78 (C-5), 135.71 (C-6), 152.59 (C-2), 169.77 (C-4); MS: $m/z = 313$ (M^+).

N^2-Benzyl-5-methylisocytidine (**6f**)

Yield: 295 mg (70%); m.p.: 151–152 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.71$ (3H, s, CH_3), 3.61 (2H, br s, 5′-H), 4.94 (1H, m, 4′-H), 4.01 (1H, m, 3′-H), 4.22 (1H, m, 2′-H), 4.46 (2H, d, $J = 5.5$ Hz, CH_2), 5.19 (1H, br s, 5′-OH), 5.36–5.47 (3H, m, 1′-H, 2′-OH, 3′-OH), 7.19–7.53 (6H, m, H_{arom}, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.30$ (CH_3), 43.61 ($NHCH_2$), 60.79 (C-5′), 69.93 (C-3′), 72.24 (C-2′), 85.82 (C-4′), 91.75 (C-1′), 113.26 (C-5), 126.43, 126.91, 128.00, 139.53 (C_{arom}), 135.47 (C-6), 152.70 (C-2), 169.72 (C-4); MS: $m/z = 347$ (M^+).

5-Methyl-2-pyrrolidino-1-(D-ribofuranosyl)-pyrimidin-4(1H)-one (**6g**)

Yield: 255 mg (68%); m.p.: 85–86 °C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.76$–1.86 (7H, m, CH_3, 2 × CH_2), 3.40 (4H, m, 2 × CH_2), 3.58 (2H, m, 5′-H), 3.83 (1H, m, 4′-H), 3.97 (1H, m, 3′-H), 4.15 (1H, q, $J = 6.0$ Hz, 2′-H), 5.06 (1H, t, $J = 5.0$ Hz, 5′-OH), 5.12 (1H, d, $J = 4.7$ Hz, 3′-OH), 5.36 (1H, d, $J = 6.2$ Hz, 1′-H), 5.48 (1H, d, $J = 6.5$ Hz, 2′-OH), 7.50 (1H, s, 6-H); ^{13}C NMR (*DMSO*-d_6): $\delta = 13.27$ (CH_3), 24.87 (2 × CH_2), 61.28 (C-5′), 70.27 (C-3′), 73.17 (C-2′), 85.06 (C-4′), 90.61 (C-1′), 116.28 (C-5), 134.07 (C-6), 156.63 (C-2), 169.21 (C-4); MS: $m/z = 311$ (M^+).

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