*Tetrahedron* Vol. 40, No. 1, pp. 33 to 38, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

# NUCLEOSIDES—126

## SELECTIVE METHYLATION OF THE C-NUCLEOSIDE, ψ-ISOCYTIDINE AND ITS 2′-DEOXY ANALOG. SYNTHESIS OF 1-METHYL, 3-METHYL AND 4-O-METHYL DERIVATIVES

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(*Received in UK* 22 *March* 1983)

**Abstract**—Methods were developed to prepare 1-methyl-, 3-methyl- and 4-O-methyl-ψ-isocytidine by selective methylation. 3′,5′-O-Tetraisopropyldisiloxanyl-ψ-isocytidine (**8**) was trimethylsilylated and then treated with MeI and, after deprotection, 1-methyl-ψ-isocytidine (**6**) was obtained. The 2′-deoxy analog (**7**) was also prepared in a similar manner from the 2′-deoxy analog (**10**) of **8**. Treatment of **8** with $CH_2N_2$ afforded the 3-methyl-ψ-isocytidine derivative (**19**) as the major product. Methylation with diazomethane also occurred mainly on N3 of the 2′-deoxy analog **10** to form **20**. Removal of the 3′,5′-O-protecting group from **19** and **20** afforded 3-methyl-ψ-isocytidine (**14**) and its 2′-deoxy analog (**15**), respectively. 2-N-Acetyl-3′,5′-O-tetraisopropyldisiloxanyl-ψ-isocytidine (**24**), on the other hand, gave the 4-O-methyl derivative (**25**) as the major product upon $CH_2N_2$ treatment. Subsequent deprotection of **25** afforded 4-O-methyl-ψ-isocytidine (**29**).

During the course of our search for a facile synthesis of ψ-isocytidine (**1**),[1] a synthetic antileukemic C-nucleoside,[2] we needed 1,3-dimethyl-ψ-uridine (**3**) as an intermediate in the ring transformation reaction[3] recently developed in our laboratory. Though treatment of a small amount of **2** with diazomethane to afford **3** was reported,[4] methylation of uracil or thymine with the same reagent is known[5] to occur not only on N but also on O to a significant extent (~30%). The diazomethane procedure, therefore, is not suitable for large scale preparation of **3**. Reaction of ψ-uridine (**2**) with dimethylsulfate in aqueous base gave a mixture of several methylated products.[6] We found,[3] however, that the desired product **3** was obtained in excellent yield by treatment with dimethoxymethyl(dimethyl)amine (DMF-dimethylacetal). Compound **3** was readily converted into ψ-isocytidine by treatment with guanidine which directly displaced the 1,3-dimethylurea portion of **3**.[3] 2′-Deoxy-ψ-isocytidine has also been prepared[7] from 2′-deoxy-1,3-dimethyl-ψ-uridine and guanidine, again by the pyrimidine-to-pyrimidine ring transformation reaction. This ring transformation reaction has been further extended to the conversion of 1,3-dimethyluracils into the corresponding pyridine[8] or benzene[9] systems by displacement of the $N_1$–$C_2$–$N_3$ fragment of the pyrimidines by the C–C–N or C–C–C fragment of various 1,3-ambident nucleophiles.

1-Methyl-ψ-uridine (**4**), a natural product elaborated by *Streptomyces platensis*,[10] synthesized chemically by methylation of 4,5′-anhydro-2′,3′-O-isopropylidene-ψ-uridine followed by deprotection and hydrolysis of the anhydro linkage[11] or by trimethylsilylation of **2** or its triacetate followed by methyl iodide treatment.[11,12] The latter methylation reaction apparently proceeded by a mechanism similar to the Hilbert–Johnson procedure.[13] 1-Methyl-2′-deoxy-ψ-uridine (**5**) was readily obtained from **4** by the Barton reduction of the 2′,3′-O-cyclic thiocarbonate[14] or 2′-O-imidazolylthiocarbonate.[15] 1-Methyl-ψ-isocytidine (**6**) was obtained from **1** by trimethylsilylation followed by methyl iodide treatment.[16] The fact that ψ-isocytidine (**1**) is an inhibitor of cytidine deaminase, whereas the 1-Me analog **6** is a substrate for this enzyme, led us to propose a new mechanism of enzymic deamination.[16] The 2′-deoxy analog **7**, however, could not be prepared from **6** because the intermediate, 3′,5′, - O - (1,1,3,3 tetraisopropyldisiloxanyl) - 1 - methyl - ψ - isocytidine (**9**), did not react with thiocarbonyldiimidazole. (Compound **9** was prepared from **6** by treatment with 1,3-dichloro-1,1,3,3,-tetraisopropyldisiloxane, or from **1** by 3′,5′-O-protection to **8** followed by methylation.) Therefore, compound **8** was converted into the 2′-deoxy analog **10**[15] which was then subjected to trimethylsilylation followed by methyl iodide treatment to form **11**. 1-Methyl-2′-deoxy-ψ-isocytidine (**7**) was obtained after deblocking of **11**.

The 3-Me analogs of 2′-deoxy-ψ-uridine (**12**) and 2′-deoxy-ψ-isocytidine (**14**) are of interest because the N-3 position of such nucleosides cannot participate in H-bonding. However, these C-nucleosides are still capable of interacting with adenine and guanine nucleosides, respectively, by H-bonding through the N-1 position, provided that the methylated C-nucleosides assume the *syn* conformation. 3-Methyl-ψ-uridine (**13**) was synthesized[17] from **2** by trimethylsilylation followed by treatment with acetyl chloride and subsequent methylation with DMF-dimethylacetal and saponification. 2′-Deoxy-3-methyl-ψ-uridine (**12**) was prepared[17] from **13** by conversion into the 3′,5′ - O - tetraisopropyldisiloxanyl 2′ - O - imidazolylthioca rbonyl derivative followed by n-$Bu_3SnH$ treatment. This method, however, is not applicable to the preparation of 3-methyl-ψ-isocytidine (**14**) and its 2′-deoxy analog (**15**). For example, treatment of 3′,5′ - O - tetraisopropyldisiloxanyl - ψ - isocytidine (**8**) with DMF-dimethylacetal gave, as expected from the work by Zemlicka *et al.*,[18] the N-2-dimethylaminomethylene nucleoside (**16**, Chart 1) in good

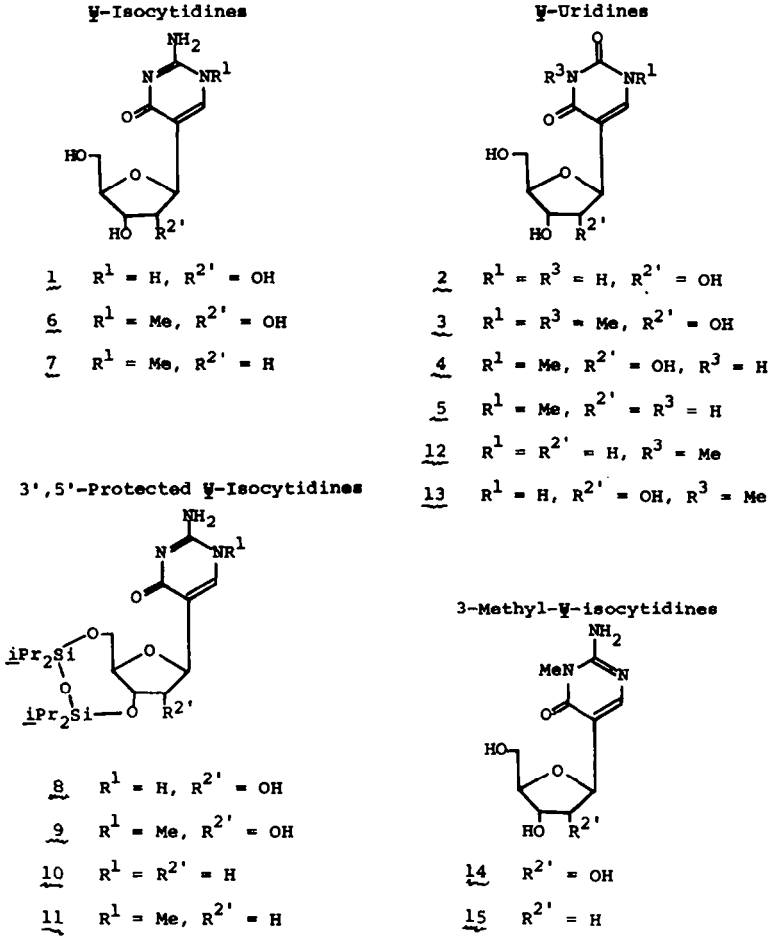


Fig. 1.

yield as a 6:1 mixture of the geometric isomers (around the exocyclic C–N bond) from which the major isomer was obtained in pure form after chromatography. No N-1 or N-3 methylated product (such as **21** or **22**) was obtained even under stringent conditions, although treatment of cytosine with the same reagent was reported to give the N1-methylated cytosine product.[19]

When a methanolic solution of **8** was treated with excess $CH_2N_2/Et_2O$, three products were formed, as judged by TLC and PMR spectroscopy [well separated signals for H-6 at $\delta$ 8.04 (27%), 7.76 (64%), 7.14 (9%)]. These products were separated by silica gel column chromatography. The first product eluted from the column ($CHCl_3$–EtOH 9:1) was the 4-methoxypyrimidine C-nucleoside (**17**, $\delta$ 8.04 H-6, 3.82 OMe). The second and major product ($\delta$ 7.76 H-6, 3.25 NMe) was the desired 3-methyl-$\psi$-isocytidine derivative (**19**) as shown by deamination of it with $NaNO_2$ in 80% HOAc to the known[15] 3 - methyl - 3',5' - O - tetraisopropyldisiloxanyl - $\psi$ - uridine. A minor component ($\delta$ 7.14 H-6, 3.29 NMe), eluted last with EtOH from the column was identical with the protected 1-Me analog of $\psi$-isocytidine prepared by an alternate procedure. It is interesting to note that the Me group on N-3 of $\psi$-isocytidines and $\psi$-uridines resonate at a higher magnetic field than does that on N-1. The marked differences in chemical shifts of H-6 for **9**, **17** and **19** reflect the cross conjugation in **9**, the fully conjucated character of **19** and the aromatic structure of **17**. Almost identical results were obtained when the 2'-deoxy analog **10** was treated with $CH_2N_2$ under similar conditions. The distribution of products was not markedly changed in other reaction solvents (e.g. MeCN, THF, dioxane, MeOH–$H_2O$). Removal of the 3',5'-O-disiloxanyl group in the 3-methylated C-nucleosides (**19** and **20**) by n-$Bu_4NF$ treatment was effected smoothly to afford the corresponding unblocked nucleosides (**14** and **15**) in ~65% yields. The fluoride treatment of the 4-methoxy derivatives (**17** and **18**), however, gave $\alpha,\beta$-isomeric mixtures of the corresponding 3',5'-deprotected C-nucleosides.

Methylation of the 2-N-dimethylaminomethylene derivative **16** (Fig. 1) with $CH_2N_2$ also afforded a mixture of three products which were separated on a silica gel column. The first two components eluted from the column were 3 - methyl - 2 - N - dimethylaminomethylene - 3',5' - O - tetraisopropyldisiloxanyl-$\psi$ - isocytidine (**21**, 66%) and its 1-methyl isomer (**22**, 2%). The structures of these isomers were established by the syntheses of **21** and **22** from **19** and **9**,

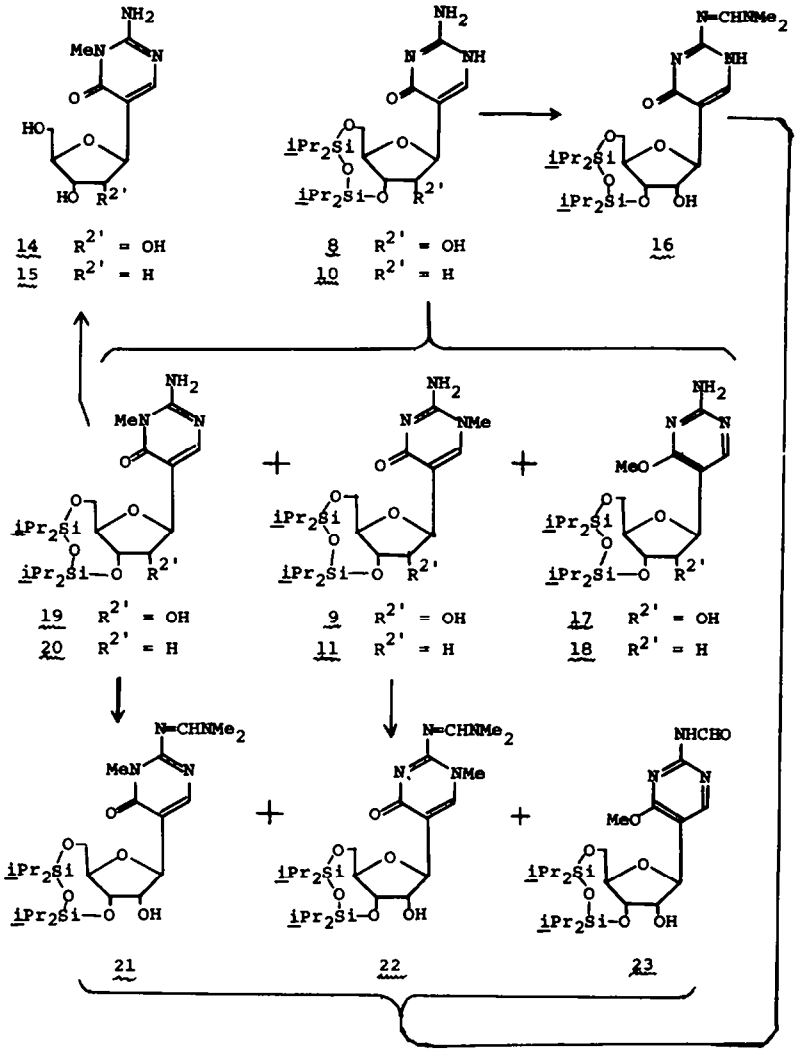


Chart 1.

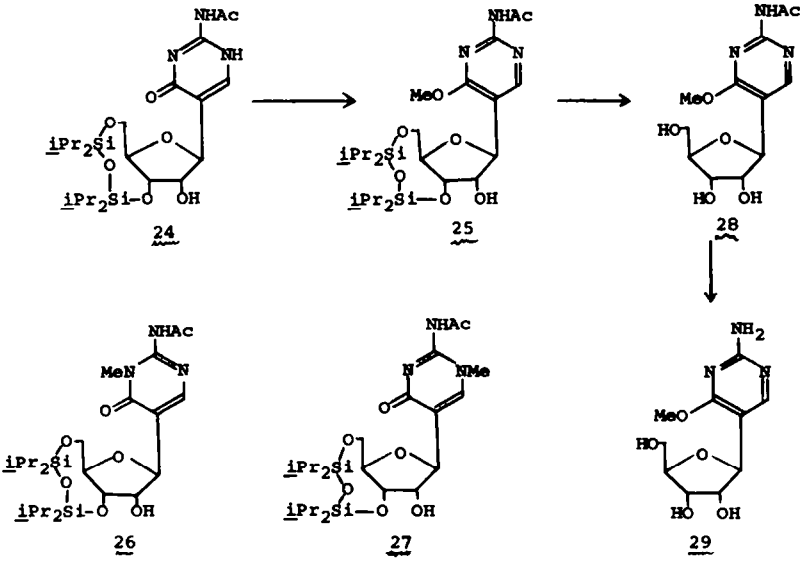


Chart 2.

respectively, by reaction with DMF-dimethylacetal (Fig. 1). The third product eluted from the column did not contain the dimethylaminomethylene group. The PMR spectrum showed signals for OMe (s) at $\delta$ 3.96, H-6 (s) at 8.38, NHCHO (d) at 9.38 (became singlet upon addition of $D_2O$) and NHCHO (d) at 10.9 (exchangeable) which is consistent with the N-2-formyl structure **23**. Apparently, 2-N-dimethylaminomethylene derivative initially formed hydrolyzed during silica gel chromatography.

An observation that the 4-methoxypyrimidine nucleosides (**17** and **18**) underwent isomerization during the fluoride ion treatment while N-methyl derivatives did not (*vide infra*), may be explained by the presence of an electron donating OMe group in **17** and **18**. An electron pair pushed toward C-1′ would bring about the lactol ring opening which would cause the $\alpha,\beta$-isomerization. If this account is correct, the introduction of an electron-withdrawing group in **17** (e.g. by N-acetylation) may prevent isomerization and an easy preparation of unprotected 4-methoxypyrimidine C-nucleosides may be achieved. Therefore, compound **8** was selectively N-acetylated with $Ac_2O$ in MeOH[20] to **24** (Chart 2). After methylation of **24** with diazomethane, the 4-methoxy derivative (**25**) was obtained as the major product. The 3-Me- and 1-Me analogs (**26** and **27**, respectively) were also present (<3%) in the mixture. The major product (**25**) was purified by column chromatography and treated with n-$Bu_4NF$ to afford 2-N-acetyl-4-O-methyl-$\psi$-isocytidine (**28**) in crystalline form. Deacetylation of **28** with methanolic $NH_3$ gave the free nucleoside **29**. No isomerization was observed (PMR) during the preparation of **29** from **24**.

It should be noted that 2-N-methyl-$\psi$-isocytidine has been prepared[5] by ring transformation reaction from 1,3-dimethyl-$\psi$-uridine (**3**) by treatment with methylguanidine. Thus, the syntheses of all pyrimidine ring N- and O-methylated analogs of $\psi$-isocytidine have been completed.

## EXPERIMENTAL

All m.p. values are uncorrected and were determined in open capillary tube using a Thomas–Hoover apparatus. PMR spectra were recorded on a JEOL-PFT-100 spectrometer using $Me_2SO$-$d_6$ as the solvent with TMS as the internal standard. Chemical shifts are reported in ppm ($\delta$) and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet) and dt (double triplet). Values given for coupling constants are first order. TLC was performed on 250 $\mu$ silica gel plates (Analtech, Inc., Newark, DE) and spots were visualized by UV light. Column chromatography was done using Woelm silica gel (70–230 mesh). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee and by M. H. W. Laboratories, Phoenix, Arizona.

3′,5′ - O - *Tetraisopropyldisiloxanyl* - $\psi$ - *isocytidine* (**8**). A mixture of $\psi$-isocytidine·HCl (**1**)[3] (2.8 g, 10 mmol) and 1,3 - dichloro - 1,1,3,3 - tetraisopropyldisiloxane[21] (3.48 g, 11 mmol) in pyridine (30 mL) was stirred overnight at room temp. The solvent was removed *in vacuo* and the residue partitioned between $CHCl_3$ (200 mL) and water (50 mL). The organic layer was separated, dried ($Na_2SO_4$) and concentrated to dryness. Traces of pyridine were removed by several coevaporations with toluene. The residue was chromatographed over a column of silica gel using $CHCl_3$–EtOH (12:1) as the eluent. The nucleoside fraction was concentrated to dryness *in vacuo* and the residue crystallized from MeOH to give 3.5 g (72%) of **8**, m.p. 167–170°. PMR $\delta$ 1.02 (28H, m, iPr), 3.89 (3H, m, H-4′,5′,5″), 4.11 (1H, m, H-3′), 4.55 (1H, s, H-1′), 4.96 (1H, d, 2′-OH, exchangeable), 6.88 (2H, s, $NH_2$, exchangeable), 7.64, (1H, s, H-6), 11.20 (1H, s, NH, exchangeable). (Found: C, 51.82; H, 8.16; N, 8.51. Calc for $C_{21}H_{39}N_3O_6Si_2$: C, 51.93; H, 8.09; N, 8.65%).

1 - *Methyl* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - $\psi$ - *isocytidine* (**9**). A suspension of **8** (200 mg, 0.41 mmol) and $(NH_4)_2SO_4$ (~10 mg) in hexamethyldisilazane (10 mL) was heated under reflux for 5 hr and then the solvent was removed *in vacuo*. The residue was dissolved in dry MeCN (10 mL) and MeI (2 mL) was added. The mixture was stirred for 3 days at room temp and then concentrated *in vacuo* to dryness. The residue was chromatographed on a silica gel column using $CHCl_3$–EtOH (9:1) as the eluent. The major fraction was evaporated *in vacuo* and the residue crystallized from $Me_2CO$ to give 130 mg (63%) of **9**, m.p. 199–200°. PMR $\delta$ 1.02 (28H, m, iPr), 3.29 (3H, s, NMe), 3.78–4.01 (5H, m, H-2′,3′,4′,5′,5″), 4.52 (1H, s, H-1′), 5.09 (1H, d, 2′-OH, exchangeable), 6.84 (2H, s, $NH_2$, exchangeable), 7.14 (1H, s, H-6). (Found: C, 51.05; H, 8.47; N, 8.22. Calc for $C_{22}H_{41}N_3O_6Si_2·H_2O$: C, 51.03; H, 8.37; N, 8.12%).

1 - *Methyl* - 2′ - *deoxy* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - $\psi$ - *isocytidine* (**11**). Methylation of **10**[15] (500 mg, 1.06 mmol) in a similar manner as described for **9** afforded **11** (310 mg, 60%) as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 1.96–2.23 (2H, m, H-2′,2″), 3.33 (3H, s, NMe), 3.66 (1H, m, H-4′), 3.87 (2H, m, H-5′,5″), 4.34 (1H, q, H-3′, $J_{2',3'} \cong J_{2',3'} \cong J_{3',4'} \cong 7.0$ Hz), 4.75 (1H, t, H-1′, $J_{1',2'} \cong J_{1',2''} \cong 7.0$ Hz), 6.81 (2H, s, $NH_2$), 7.14 (1H, s, H-6). (Found: C, 52.40; H, 8.62; N, 8.38. Calc for $C_{22}H_{41}N_3O_5Si_2·H_2O$: C, 52.66; H, 8.63; N, 8.37%).

*Reaction of* **8** *with diazomethane*. An ice-cooled soln of **8** (1.6 g, 3.3 mmol) in $Et_2O$–MeOH (1:1 v/v, 20 mL) was treated with $CH_2N_2$ (prepared from 2.94 g of N-methyl-N′-nitro-N-nitrosoguanidine and dissolved in 20 mL of ether). The mixture was kept at 0° until no **8** was detected by TLC. After concentration *in vacuo*, the residue (consisting of **9**, **19** and **17** in a ratio of 9:64:27 as determined by PMR) was chromatographed on a silica gel column using $CHCl_3$–EtOH (9:1 v/v) as the eluent. The 4-methoxy isomer **17** (390 mg, 24%) was eluted from the column first (obtained as a foam), followed by the 3-Me derivative **19** (975 mg, 59%, foam). The 1-Me product **9** (135 mg, 8%) was eluted from the column with EtOH (crystallized from $Me_2CO$), m.p. 199–200°, identical in all respects with **9** perpared earlier.

PMR data for **17**, $\delta$ 1.02 (28H, m, iPr), 3.82 (3H, s, OMe), 3.82–4.07 (5H, m, H-2′,3′,4′,5′,5″), 4.86 (1H, s, H-1′), 5.04 (1H, d, 2′-OH), 6.55 (2H, s, $NH_2$), 8.04 (1H, s, H-6). (Found: C, 52.74, H. 8.39; N, 8.18. Calc for $C_{22}H_{41}N_3O_6Si_2$: C, 52.87; H, 8.27; N, 8.41%). For **19**, $\delta$ 1.02 (28H, m, iPr), 3.25 (3H, s, NMe), 3.84–4.18 (5H, m, H-2′,3′,4′,5′,5″), 4.60 (1H, s, H-1′), 4.94 (1H, d, 2′-OH), 7.10 (2H, d, $NH_2$), 7.66 (1H, s, H-6). (Found: C, 52.64; H, 8.40; N, 8.25. Calc for $C_{22}H_{41}N_3O_6Si_2$: C, 52.87; H, 8.27; N, 8.41%).

3 - *Methyl* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - $\psi$ - *uridine from* **19**. Compound **19** (100 mg) was dissolved in 80% HOAc and treated with $NaNO_2$ (~50 mg). The progress of reaction was checked by TLC ($CHCl_3$–EtOH, 9:1 v/v). The mixture was concentrated *in vacuo* and traces of HOAc were removed by several co-evaporations with PhMe. The residue was purified by silica gel column chromatography using $CHCl_3$–EtOH (9:1 v/v) as the eluent. The product (40 mg, 40%) isolated as a foam had identical TLC and PMR characteristics with an authentic sample.[17]

*Reaction of* **10** *with diazomethane*. Compound **10** (1.5 g, 3.2 mmol) was treated with $CH_2N_2$ in a similar manner as described above. The mixture of methylated products (**11**, **20** and **18** in a ratio of 8:63:29) was chromatographed over a silica gel column using $CHCl_3$–EtOH (19:1 v/v). The 4-OMe derivative **18** (430 mg, 28%) was obtained as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 2.12 (2H, m, H-2′,2″), 3.66–3.90 (6H, m, H-4′,5′,5″ and OMe at 3.82), 4.43 (1H, m, H-3′), 4.95 (1H, t, H-1′, $J_{1',2'} = J_{1',2''} = 7.5$ Hz), 6.54 (2H, s, $NH_2$), 7.97 (1H, s, H-6). (Found: C, 54.59; H, 8.80; N, 8.42. Calc for $C_{22}H_{41}N_3O_5Si_2$: C, 54.62; H, 8.54; N, 8.68%). The 3-Me

isomer **20** (860 mg, 56%) was also a foam. PMR $\delta$ 1.02 (28H, m, iPr), 2.11 (2H, m, H-2′,2″), 3.25 (3H, s, NMe), 3.68 (1H, m, H-4′), 3.86 (2H, m, H-5′,5″), 4.42 (1H, m, H-3′), 4.85 (1H, t, H-1′, $J_{1',2'} = J_{1',2''} = 7.2$ Hz), 7.10 (2H, s, $NH_2$), 7.58 (1H, s, H-6). (Found: C, 54.65; H, 8.81; N, 8.60. Calc for $C_{22}H_{41}N_3O_5Si_2$: C, 54.62; H, 8.54; N, 8.68%). Compound **11** (120 mg, 8%) eluted with EtOH had TLC and PMR characteristics identical with those of an authentic sample of **11** (*vide supra*).

1-*Methyl-ψ-isocytidine* (**6**). Compound **9** (200 mg, 0.4 mmol) was dissolved in THF (2 mL) and treated with $nBu_4NF$ (0.5 M solution in THF prepared according to the procedure reported by Torrence and Imai[22]) (1.6 mL, 0.8 mmol). The course of reaction was monitored by TLC (iPrOH–EtOAc–$H_2O$, 10:10:1 v/v/v). When the reaction was over, the mixture was concentrated *in vacuo*. The residue was dissolved in a mixture of pyridine–MeOH–$H_2O$ (3:1:1 v/v/v) and evaporated. The residue, after several co-evaporations with PhMe, was chromatographed over a short column of silica gel using iPrOH–EtOAc–$H_2O$ (10:1:1 v/v/v) as the eluent to afford **6** which was crystallized from MeOH (52 mg, 50%), m.p. 239–241° which was not depressed on admixture with authentic sample.[16] PMR $\delta$ 3.35 (3H, s, NMe), 3.48 (1H, m, H-4′), 3.79–3.98 (4H, m, H-2′,3′,5′,5″), 4.37 (1H, d, H-1′, $J_{1',2'} = 5.0$ Hz), 4.61 (1H, d, OH), 5.18 (1H, t, 5′-OH), 5.44 (1H, d, OH), 6.96 (2H, s, $NH_2$), 7.41 (1H, s, H-6).

In a similar manner, compounds **11**, **19** and **20** were converted into **7**, **14** and **15**, respectively. 2′-Deoxy-1-methyl-ψ-isocytidine (**7**, 53%), m.p. 245–250° (crystallized from MeOH). PMR $\delta$ 1.81–1.99 (2H, m, H-2′,2″), 3.33 (3H, s, NMe), 3.40 (2H, m, H-5′,5″), 3.71 (1H, m, H-4′), 4.08 (1H, m, H-3′), 4.74 (1H, dd, H-1′, $J_{1',2'} \cong 5.5$ Hz $J_{1',2''} \cong 9.5$ Hz), 6.82 (2H, s, $NH_2$), 7.33 (1H, s, H-6). (Found: C, 49.70; H, 6.32; N, 17.20. Calc for $C_{10}H_{15}N_3O_4$: C, 49.78; H, 6.27; N, 17.42%). 3-Methyl-ψ-isocytidine (**14**, 69%) m.p. 197–200° (crystallized from MeOH). PMR ($D_2O$) $\delta$ 3.39 (3H, s, NMe), 3.77 (2H, m, H-5′,5″), 4.01 (1H, m, H-4′), 4.17 (1H, m, H-3′), 4.35 (1H, m, H-2′), 4.66 (1H, d, H-1′, $J_{1',2'} = 6.4$ Hz), 7.78 (1H, s, H-6). (Found: C, 46.56; H, 6.00; N, 16.14. Calc for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.32%). 2′-Deoxy-3-methyl-ψ-isocytidine (**15**, 64%), m.p. 165–170° (from MeOH–$Et_2O$). PMR ($D_2O$) $\delta$ 2.18 (2H, m, H-2′,2″), 3.40 (3H, s, NMe), 3.70 (2H, m, H-5′,5″), 4.01 (1H, m, H-4′), 4.38 (1H, m, H-3′), 5.08 (1H, dd, H-1′, $J_{1',2'} = 7.2$, $J_{1',2''} = 9.3$ Hz), 7.76 (1H, s, H-6). (Found: C, 49.65; H, 6.31; N, 17.16. Calc for $C_{10}H_{15}N_3O_4$: C, 49.78; H, 6.27; N, 17.42%).

2-N - (*Dimethylamino*)*methylene* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - ψ - *isocytidine* (**16**). A mixture of **8** (1.5 g, 3.1 mmol) and DMF-dimethylacetal (2 mL) in $ClCH_2CH_2Cl$ (30 mL) was heated under reflux for 15 min, and then concentrated *in vacuo*. The residue was chromatographed on a silica gel column using $CHCl_3$–EtOH (20:1 v/v) as the eluent. Compound **16** was obtained as a foam, 1.3 g (78%). PMR $\delta$ 1.02 (28H, m, iPr), 3.01 (3H, s, NMe), 3.13 (3H, s, NMe), 3.90–4.14 (5H, m, H-2′,3′,4′,5′,5″), 4.59 (1H, s, H-1′), 4.97 (1H, d, 2′-OH), 7.74 (1H, s, H-6), 8.56 (1H, s, = CH). (Found: C, 53.07; H, 8.39; N, 10.11. Calc for $C_{24}H_{44}N_4O_6Si_2$: C, 53.30; H, 8.20; N, 10.36%).

*Reaction of* **16** *with diazomethane*. As described for the methylation of **8**, compound **16** (1.5 g, 2.8 mmol) was treated with $CH_2N_2$. The PMR spectrum showed the mixture to contain **21** (78%), **22** (5%) and 2 - N - (dimethylamino)methylene - 3′,5′ - O - tetraisopropyldisiloxanyl - 4 - O - methyl - ψ - isocytidine (17%). The nucleosides were separated by silica gel column chromatography using $CHCl_3$–$Me_2CO$ (5:1 v/v) as the eluent. Compound **23** (140 mg, 9%) was eluted first from the column and obtained as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 3.90–4.19 (8H, m, H-2′,3′,4′,5′,5″ and OMe at 3.96), 4.80 (1H, s, H-1′), 5.25 (1H, d, 2′-OH), 8.36 (1H, s, H-6), 9.36 (1H, d, CHO), 10.90 (1H, d, NH). (Found: C, 52.49; H, 7.80; N, 7.98. Calc for $C_{21}H_{41}N_3O_7Si_2$: C, 52.34; H, 7.83; N, 7.96%). From the second fraction, **21** (1.01 g, 66%), was obtained as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 3.07 (3H, s, NMe), 3.18 (3H, s, NMe), 3.41 (3H, s, NMe), 3.91–4.11 (5H, m, H-2′,3′,4′,5′,5″), 4.64 (1H, s, H-1′), 4.99 (1H, d, 2′-OH), 7.76 (1H, s, H-6), 8.57 (1H, s, = CH–). (Found: C, 54.05; H, 8.50; N, 9.97. Calc for $C_{25}H_{46}N_4O_6Si_2$: C, 54.12; H, 8.36; N, 10.10%). The last fraction contained **22** which was isolated as a foam (30 mg, 2%). PMR $\delta$ 1.02 (28H, m, iPr), 3.06 (3H, s, NMe), 3.18 (3H, s, NMe), 3.44 (3H, s, NMe), 3.91–4.03 (5H, m, H-2′,3′,4′,5′,5″), 4.58 (1H, s, H-1′), 5.16 (1H, d, 2′-OH), 7.36 (1H, s, H-6), 8.59 (1H, s, = CH–). (Found: C, 54.00; H, 8.42; N, 9.95. Calc for $C_{25}H_{46}N_4O_6Si_2$: C, 54.12; H, 8.36; N, 10.10%).

3 - *Methyl* - 2 - N - (*dimethylamino*)*methylene* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - ψ - *isocytidine* (**21**). A mixture of **19** (200 mg, 0.4 mmol) and DMF-dimethylacetal (100 mg) in DMF (4 mL) was heated at 85° for 30 min, and then concentrated to dryness *in vacuo*. The residue was purified by column chromatography with $CHCl_3$–$Me_2CO$ (5:1 v/v) as the eluent. Compound **21** (200 mg, 92%) was obtained as a foam. The PMR spectrum of this sample was identical with that of **21** prepared by methylation of **16**.

In a similar manner, the 1-Me isomer (**22**) was obtained from **9** (200 mg, 0.38 mmol), and $CHCl_3$–EtOH (19:1) as the eluent for column chromatography. Compound **22** (140 mg, 65%) was obtained as a foam. The PMR spectrum of this sample was identical with **22** prepared by methylation of **16**.

2 - N - *Acetyl* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - ψ - *isocytidine* (**24**). A mixture of **8** (1.5 g, 3.1 mmol) and $Ac_2O$ (6 mL) in MeOH (50 mL) was refluxed for 30 min and then concentrated *in vacuo* to dryness. Traces of HOAc were azeotropically removed with PhMe. The residue was purified by silica gel column chromatography using $CHCl_3$–EtOH 19:1 v/v) as the eluent. Compound **24** was obtained as a foam, 1.25 g (78%). PMR $\delta$ 1.02 (28H, m, iPr), 2.13 (3H, s, Ac), 3.91–4.05 (5H, m, H-2′,3′,4′,5′,5″), 4.67 (1H, s, H-1′), 5.19 (1H, d, 2′-OH), 7.91 (1H, s, H-6), 11.65 (1H, s, NH). (Found: C, 52.28; H, 7.98; N, 7.91. Calc for $C_{23}H_{41}N_3O_7Si_2$: C, 52.34; H, 7.83; N, 7.96%).

*Reaction of* **24** *with diazomethane*. Compound **24** (1.5 g, 2.8 mmol) was treated with $CH_2N_2$ as described for methylation of **8**. The major product was separated by column chromatography ($CHCl_3$–EtOH, 5:3 v/v) to give the 4-OMe derivative (**25**) (800 mg, 52%) as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 2.22 (3H, s, Ac), 3.81–4.19 (8H, m, H-2′,3′,4′,5′,5″ and OMe at 3.94), 4.80 (1H, s, H-1′), 5.24 (1H, d, 2′-OH), 8.38 (1H, s, H-6), 10.41 (1H, s, NH). (Found: C, 53.02; H, 8.20; N, 7.63. Calc for $C_{24}H_{43}N_3O_7Si_2$: C, 53.20; H, 8.00; N, 7.75%).

2 - N - *Acetyl* - 1 - *methyl* - 3′,5′ - O - *tetraisopropyldisiloxanyl* - ψ - *isocytidine* (**27**). A mixture of **9** (200 mg, 0.38 mmol), $Ac_2O$ (300 mg) in MeOH (10 mL) was refluxed for 2 hr, and then concentrated to dryness *in vacuo*. Traces of HOAc were removed by several coevaporations with PhMe and the residue was purified by silica gel column chromatography using $CHCl_3$–EtOH (19:1 v/v) as the eluent. Compound **27** (161 mg, 77%) was obtained as a foam. PMR $\delta$ 1.02 (28H, m, iPr), 2.10 (3H, s, Ac), 3.40 (3H, s, NMe), 3.80–4.02 (5H, m, H-2′,3′,4′,5′,5″), 4.56 (1H, s, H-1′), 5.19 (1H, d, 2′-OH), 7.59 (1H, s, H-6). (Found: C, 53.15; H, 8.00; N, 7.66. Calc for $C_{24}H_{43}N_3O_7Si_2$: C, 53.20; H, 8.00; N, 7.75%).

In a similar manner, the 3-methyl isomer **26** (200 mg, 92%) was prepared from **19** (200 mg, 0.38 mmol). PMR $\delta$ 1.02 (28H, m, iPr), 2.10 (3H, s, Ac), 3.32 (3H, s, NMe), 3.91–4.03 (5H, m, H-2′,3′,4′,5′,5″), 4.68 (1H, s, H-1′), 5.20 (1H, d, 2′-OH), 7.90 (1H, s, H-6). Found: C, 53.39; H, 8.20; N, 7.65.

2 - N - *Acetyl* - 4 - O - *methyl* - ψ - *isocytidine* (**28**). Compound **25** (300 mg, 0.57 mmol) in THF (3 mL) was treated with 0.5 M n-$Bu_4NF$ in THF (2.3 mL, 1.15 mmol) as described for the preparation of **6** from **9**. The yield of **28** was 100 mg, 61%, m.p. 154–156° (crystallized from $Me_2CO$).

PMR $\delta$ 2.23 (3H, s, Ac), 3.33–3.92 (8H, m, H-2′,3′,4′,5′,5″ and OMe at 3.92), 4.73–4.81 (3H, m, H-1′, 2OH, $J_{1',2'} = 4.0$ Hz), 5.00 (1H, d, OH), 8.47 (1H, s, H-6), 10.37 (1H, s, NH). (Found: C, 48.31; H, 5.99; N, 13.79. Calc for $C_{12}H_{17}N_3O_6$: C, 48.16; H, 5.73; N, 14.04%).

4-O-*Methyl-ψ-isocytidine* (**29**). Compound **28** (50 mg, 0.1 mmol) was treated with $NH_3$/MeOH (~5 mL, saturated at 0°) for 3 days at room temperature. The solvent was removed *in vacuo* and the residue was purified by preparative TLC using an Analtech 1000 $\mu$ plate and EtOAc-iPrOH (1:1 v/v) as the solvent. From the UV absorbing band, 12 mg of **29** was obtained as a foam. PMR $\delta$ 3.50 (2H, m, H-5′,5″), 3.69–3.92 (5H, m, H-2′,3′ and OMe at 3.81), 4.34 (1H, d, OH), 4.61 (1H, d, H-1′, $J_{1',2'} \cong 5.1$ Hz), 4.71–4.86 (2H, m, OH), 6.51 (2H, s, $NH_2$), 8.05 (1H, s, H-6). (Found: C, 46.80; H, 5.90; N, 16.02. Calc for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33%).

*Acknowledgements*—We express our appreciation to Kyowa Hakko Kogyo Company, Tokyo, for the gift of $\psi$-uridine.

This investigation was supported, in part, by funds from the National Cancer Institute, D.H.H.S., Grants CA-08748 and CA-18601.

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