Synthesis of $O^{2'}$-Methyluridine, $O^{2'}$-Methylcytidine, N^4,$O^{2'}$-Dimethylcytidine and N^4,N^4,$O^{2'}$-Trimethylcytidine from a Common Intermediate

A. Nyilas and J. Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

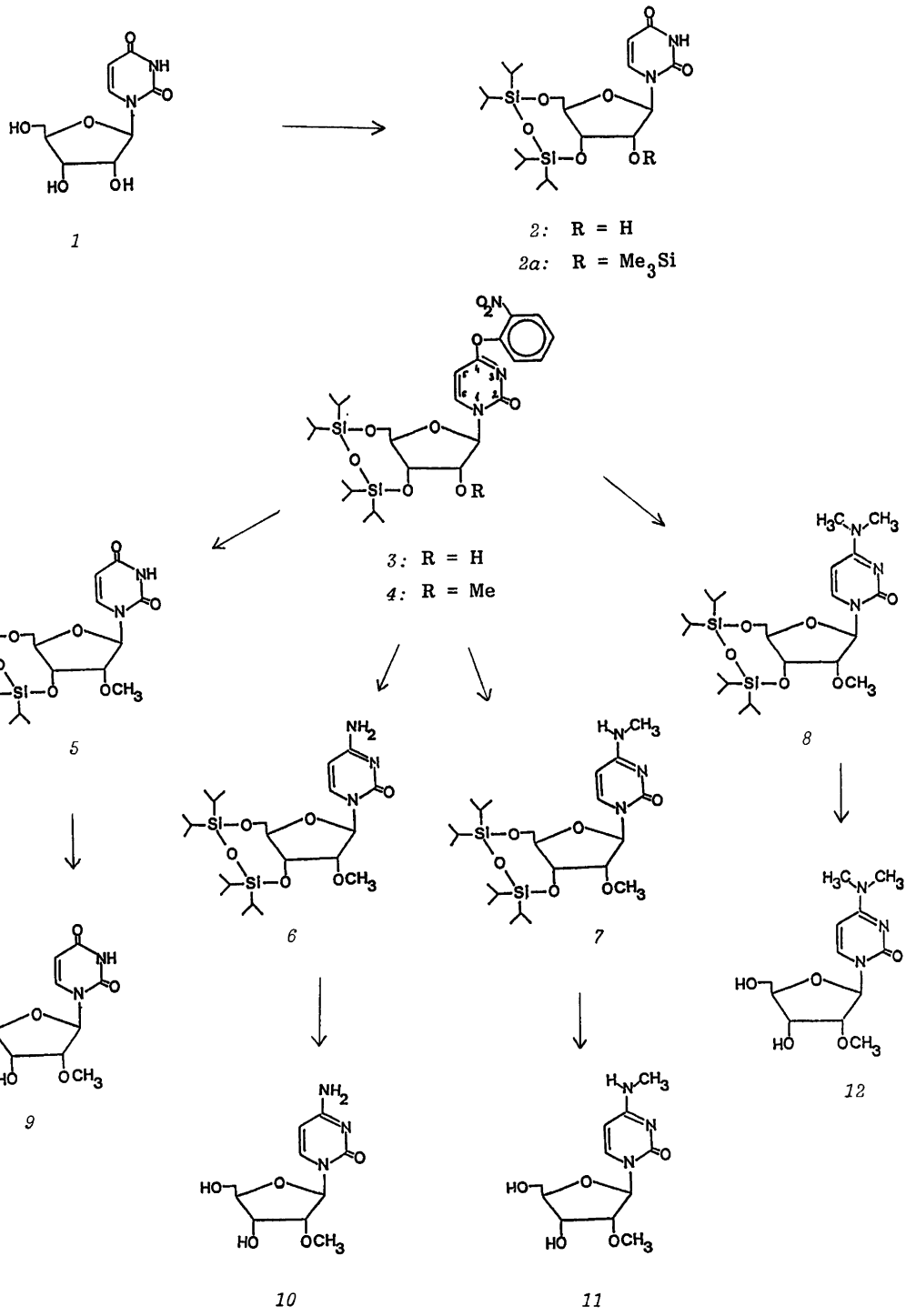
Nyilas, A. and Chattopadhyaya, J., 1986. Synthesis of $O^{2'}$-Methyluridine, $O^{2'}$-Methylcytidine, N^4,$O^{2'}$-Dimethylcytidine and N^4,N^4,$O^{2'}$-Trimethylcytidine from a Common Intermediate. – Acta Chem. Scand. B 40: 826–830.

A facile synthesis of the title compounds from a readily accessible precursor, 3′,5′-*O*-bis-protected O^4-(2-nitrophenyl)uridine, is described.

Subtle modification of nucleic acid structure is critical in controlling the mechanism of protein biosynthesis and for the effective functioning of nucleic acid molecules in a complex biochemical process.[1] In nature, these modifications are largely regulated by methylation of the base and/or the 2′-hydroxyl function of the pentofuranose moiety of nucleic acids.[2–5] Chemical modifications of nucleic acids have therefore attracted increasing attention in nucleic acid research[1–6] and in chemical mutagenesis[7] and carcinogenesis studies.[8] We herein report a facile synthesis of $O^{2'}$-methyluridine (*9*), $O^{2'}$-methylcytidine (*10*), N^4,$O^{2'}$-dimethylcytidine (*11*) and N^4,N^4,$O^{2'}$-trimethylcytidine (*11*) from a common precursor *3*. These compounds have been prepared previously using multistep synthetic routes[1,9–15] with poor overall yields. The main problem in the synthesis of methylated nucleosides is the side reaction of the pyrimidine base during the methylation of the 2′-hydroxyl function. We reasoned that a protected intermediate *3*, with an appropriate 0–4 aryl group, should be able to perform a dual role in the synthesis of the target molecules *9–12*. Clearly, the 0–4 protecting group of compound *3* should not only protect the heterocyclic base during the methylation of the sugar hydroxyl function but it should also constitute a good leaving group for the displacement reaction with a nitrogen or oxygen nucleophile.

The precursor *2* of the key intermediate *3* was easily accessible in a large quantity (10 g) by a standard reaction of uridine (*1*) with 1,1,3,3-tetraisopropyl-1,3-dichlorodisiloxane[16] in dry pyridine at 20 °C. Compound *2* could be easily converted to the key intermediate *3* in 78 % overall yield by a one-pot four-step procedure involving (i) trimethylsilylation (TMS)[17] of the 2′-hydroxyl function, (ii) its conversion to the *C*-4-(2-mesitylenesulfonate), (iii) nucleophilic displacement at the C-4 by the conjugate base of 2-nitrophenol and (iv) the removal of the TMS group from 2′-hydroxyl function by a brief acid treatment. The 2′-hydroxyl function of *3* was then methylated using methyl iodide and silver oxide[20] to give *4* in 81 % yield. Compound *4* was then subjected to four different nucleophilic substitution reactions under the following conditions to give the desired target compounds: (a) treatment with 4-nitrobenzaldoximate ion[21] gave *5* which, upon deblocking with fluoride ion, gave $O^{2'}$-methyluridine (*9;* 66.5 %); (b) treatment with liquid ammonia for 16 h gave *6* which, upon fluoride-promoted deblocking, gave $O^{2'}$-methylcytidine (*10;* 72 %); (c) treatment with methylamine in absolute ethanol for 72 h gave *7* which, upon deblocking with fluoride ion, gave N^4,$O^{2'}$-dimethylcytidine (*11;* 76 %); (d) finally, treatment with dimethylamine in absolute ethanol for ca. 16 h gave *8* which, upon brief treatment with fluoride ion, gave N^4, N^4, $O^{2'}$-trimethylcytidine (*12;* 94 %). ^{1}H and ^{13}C NMR data for compounds *3–12* which

*To whom correspondence should be addressed.



Scheme 1.

corroborate the structures are shown in Tables 1 and 2.

The present work demonstrates that a judicious choice of an O-4 aryl protecting group for uridine[22] can protect the pyrimidine moiety against electrophilic attack, and yet it can be used as a good leaving group for further modifications of nucleosides.

Experimental

A Jeol FX 90Q spectrometer was used at 89.5 and 23.5 MHz to measure ^1H and ^{13}C NMR, respectively; tetramethylsilane or sodium 3-trimethylsilylpropanesulfonate were used as internal standards in $CDCl_3$ and D_2O solutions, respectively (δ scale). UV absorption spectra were recorded with a Varian-Cary 2200 spectrometer.

1-(3',5'-O-1,1,3,3-Tetraisopropyl-1,3-disilyl)-β-D-ribofuranosyl-4-(2-nitrophenyl)-2-pyrimidinone (3). Compound *2* (0.97 g, 2 mmol) was dissolved in dichloromethane (20 ml) and triethylamine (1.39 ml, 10 mmol) and trimethylsilyl chloride (0.76 ml, 6 mmol) added at 20 °C and stirred for 15 min. The reaction mixture was poured into a saturated solution of sodium hydrogen carbonate and extracted with dichloromethane (3×50 ml). The organic layers were pooled, dried over anhydrous magnesium sulfate and evaporated *in vacuo*. The residue was dissolved in dichloromethane (20 ml) and triethylamine (2.8 ml, 20 mmol), 2-mesitylenesulfonyl chloride (1.32 g, 6 mmol) and *N,N*-dimethylaminopyridine (61 mg, 0.5 mmol) were added. After 30 min, 2-nitrophenol (1.3 g, 10 mmol and 1,4-diazabicyclo[2,2,2]octane (44 mg, 0.4 mmol) were added and stirred for 60 min. The reaction mixture was partitioned between a saturated solution of sodium hydrogen carbonate (100 ml) and dichloromethane (100 ml). The aqueous phase was further washed with more dichloromethane (3 × 70 ml). The organic layers were pooled, dried over magnesium sulfate and evap-

Table 1. ^1H NMR of substituted nucleosides.

Compound	H-1'	H-2'	H-3'	H-4'	H-5'	H-5	H-6	Others
3[a]	5.75 (*s*)	←	4.2 (*m*, 5H)	→		6.2 (*d*, 7.3)	8.21 (*d*)	1.1 (m, 28H), 8.09 (*d*, 1H), 7.7–7.2 (*m*, 3H)
4[a]	5.78 (*s*)	←	4.38–3.77 (*m*, 5H)	→		6.19 (*d*, 7.3)	8.38 (*d*)	3.68 (*s*, 3H), 1.09(*m*, 28H), 8.12 (*s*, 1H), 7.67–7.29 (*m*, 3H)
5[a]	5.77 (*s*)	←	4.15 (m, 5H)	→		5.69 (*d*, 8.1)	7.93 (*d*)	3.67 (s, 3H), 1.03 (*m*, 28H)
6[a]	5.8 (*s*)	←	4.12 (m, 5H)	→		5.67 (*d*, 7.6)	7.99 (*d*)	3.7 (*s*, 3H), 1.03 (*m*, 28H)
7[a]	5.83 (*s*)	←	4.14 (m, 5H)	→		5.6 (*d*, 7.1)	7.86 (*d*)	3.7 (*s*, 3H), 2.98 (*s*, 3H)
8[a]	5.82 (*s*)	←	4.1 (m, 5H)	→		5.73 (*d*, 7.6)	7.96 (*d*)	3.71 (*s*, 3H), 3.13(*s*, 6H)
9[c]	5.97 (*d*, 3.9)	4.03 (*m*)	4.33 (*m*)	4.09 (*m*)	3.85 (*m*)	5.88 (*d*, 8.3)	7.99 (*d*)	3.5 (*s*, 3H)
10[c]	5.96 (*d*, 3.1)	4.0 (*m*)	4.3 (*m*)	4.1 (*m*)	3.9 (*m*)	6.05 (*d*, 7.6)	7.9 (*d*, 7.3)	3.52 (*s*, 3H)
11[c]	5.99 (*d*, 3.9)	4.0 (*m*)	4.3 (*m*, 1H)	4.0 (*m*)	3.9 (*m*)	5.99 (*d*, 7.32)	7.82 (*d*, 8.3)	3.51 (*s*, 3H), 2.9 (*s*, 3H)
12[b]	5.8 (*d*, 3.7)	3.62 (*m*)	4.07 (*m*)	3.8 (*m*)	5.8 (*m*)	6.05 (*d*, 8.1)	8.0 (*d*)	3.38 (*s*, 3H), 3.03 (*s*, 6H)

[a] In $CDCl_3$; [b] in DMSO–d_6; [c] in D_2O.

Table 2. ^{13}C NMR of substituted nucleosides.[d]

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-5	C-6	Others
3[a]	92.0 (174.6)	74.9 (148.3)	68.7 (130.2)	81.9 (152.8)	60.2 (144.4)	94.6 (179.7)	114.9 (178.6)	
4[a]	89.5 (178.6)	81.7 (148.3)	67.85 (138.2)	83.3 (153.9)	59.5 (141.6)	94.3 (171.8)	–	59.1 (142.65)
5[a]	88.3 (177.3)	81.6 (156.1)	68.1 (140.4)	83.9 (147.2)	59.4 (145.5)	126.1 (178.6)	139.6 (182.0)	59.1 (142.7)
6[a]	88.7 (176.3)	81.35 (151.6)	68.0 (140.4)	83.8 (155.0)	59.6 (140.9)	94.4 (170.7)	140.3 (185.3)	59.0 (144.9)
7[a]	88.9 (176.3)	81.3 (147.1)	69.0 (141.5)	83.85 (152.2)	59.7 (146.0)	95.15 (170.7)	138.3 (181.7)	59.0, 27.8 (147.1) (138.0)
8[a]	88.3 (174.1)	80.6 (158.4)	67.4 (134.8)	83.0 (148.3)	59.0 (143.8)	89.8 (170.7)	139.5 (183.1)	58.4 (140.4), 37.6 (138.7)
9[c]	87.85 (170.7)	83.0 (152.8)	68.4 (150.5)	84.5 (150.5)	60.7 (144.3)	102.5 (178.5)	141.9 (184.2)	58.5 (143.5)
10[c]	91.1 (171.8)	85.7 (157.2)	70.8 (148.3)	86.4 (150.5)	63.1 (142.7)	98.8 (176.4)	144.2 (183.1)	60.9 (141.5)
11[c]	87.9 (175.3)	83.35 (150.5)	68.2 (150.5)	84.3 (148.3)	60.4 (139.3)	94.8 (171.9)	139.7 (182.0)	57.5 (137.6, 26.8 (137.0)
12[b]	86.9 (169.6)	83.5 (150.5)	67.9 (148.3)	84.2 (147.2)	60.0 (139.3)	91.4 (176.3)	141.0 (179.8)	57.5 (141.5), 36.9 (138.7)

[a]In $CDCl_3$; [b]in DMSO–d_6; [c]in D_2O; [d]the values in parenthesis signify $^1J_{CH}$.

orated to dryness. The residue was dissolved in dichloromethane (20 ml) and 2% *p*-toluenesulfonic acid monohydrate in dichloromethane (20 ml) added and stirred for 2 min at 20 °C. The solution was then poured into saturated sodium hydrogen carbonate (100 ml) which was extracted with dichloromethane (3×80 ml). The organic layers were pooled, dried and evaporated *in vacuo* to give a glass which was purified on a silica gel column to give *3* (1.12 g; 78%).

1-(3′,5′-O-1,3-Tetraisopropyl-1,3-disilyl)-β-D-ribofuranosyl-2′-O-methyl-4-(2-nitrophenyl)-2-pyrimidinone (4). Compound *3* (3.23 g, 5.3 mmol) was coevaporated with dry acetone and then dissolved in dry acetone (40 ml). Silver oxide (12.3 g, 53 mmol) and methyl iodide (6.6 ml, 106 mmol) were added in two portions and the mixture stirred at 20 °C for 72 h. The reaction mixture was filtered through Celite under reduced pressure, the filtrate coevaporated with toluene and purified on a silica gel column to give *4* (2.68 g; 81%).

3′,5′.O-(1,3-Tetraisopropyl-1,3-disilyl)-2′-O-methyluridine (5). Compound *4* (1.0 g, 1.6 mmol) was dissolved in aqueous dioxane (1:1, v/v, 10 ml) and *syn*-4-nitrobenzaldoxime (2.6 g) and N^1, N^1, N^3,N^3-tetramethylguanidine (1.9 ml) were added at 20 °C. After 16 h, the reaction mixture was evaporated, dissolved in dichloromethane (30 ml) and extracted with water (5×20 ml). The organic layer was concentrated and the residue purified on a silica gel column to give *5* (0.56 g; 70%).

2′-O-Methyluridine (9). Compound *5* (0.56 g, 1.1 mmol) was dissolved in dry tetrahydrofuran (5 ml) and tetrabutylammonium fluoride in tetrahydrofuran (1 M, 1 eq.) added at 20 °C. After 2 min, the reaction mixture was concentrated. The residue was dissolved in distilled water (20 ml)

and extracted with dichloromethane (5×20 ml). The aqueous layer was washed with diethyl ether (3×20 ml) and concentrated. The oily residue was purified on a silica gel column to give *9* (0.27 g; 95 %).

3',5'-O-(1,3-Tetraisopropyl-1,3-disilyl)-2'-O-methylcytidine (6). Compound *4* (0.31 g, 0.5 mmol) was dissolved in tetrahydrofuran (20 ml) and treated with liquid ammonia overnight. The reaction mixture was evaporated and purified on a silica gel column go give *6* (188 mg, 75 %).

2'-O-Methylcytidine (10). Compound *6* (188 mg, 0.38 mmol) was deprotected using conditions analogous to the preparation of *9* to give *10* (94 mg; 96 %).

4-N-Methyl-3',5'-(1,3-tetraisopropyl-1,3-disilyl)-2'-O-methylcytidine (7) *and* N^4, $O^{2'}$*-Dimethylcytidine* (11). To a solution of compound *4* (1.0 g, 1.6 mmol) in absolute ethanol (30 ml), methylamine hydrochloride (0.216 g, 3.2 mmol) and triethylamine (0.446 ml, 3.2 mmol) were added and the reaction mixture stirred for 72 h at 20 °C. Volatile material was removed and the residue dissolved in dichloromethane (50 ml) and washed with distilled water (3×25 ml). The organic phase was concentrated and purified on a silica gel column to give *7* (0.65 g, 80 %) which was deprotected[16] to give *11* (0.33 g; 95 %).

4,4-N,N-Dimethyl-3',5'-(1,3-tetraisopropyl-1,3-disilyl)-2'-O-methylcytidine (8) *and* N^4,N^4,$O^{2'}$*-Trimethylcytidine* (12). Compound 4 (0.31 g, 0.5 mmol) was dissolved in absolute ethanol (10 ml) Dimethylamine hydrochloride (81 mg, 1 mmol) and triethylamine (0.14 g, 1 mmol) were added and the reaction mixture stirred overnight at 20 °C. The reaction mixture was worked up using a procedure analogous to the preparation of *7* to give *8* (0.26 g, 98 %) *8* which was deprotected[16] to give *12* (134 mg; 96 %).

Acknowledgements. The authors gratefully acknowledge financial assistance from the Swedish Board for Technical Development and the Swedish Natural Science Research Council. The authors also thank Ingegärd Schiller for excellent secretarial assistance.

References

1. Hall, R. H. *The Modified Nucleosides in Nucleic Acids*, Columbia Univ. Press, New York 1971.
2. Furnichi, Y. and Miura, K. *Nature (London) 253* (1975) 374.
3. Shatkin, A. J. *Cell 9* (1976) 645.
4. Bonloy, M., Plotch, S. J. and Krug, R. *Proc. Natl. Acad. Sci. U.S.A. 77* (1980) 3952.
5. Lothorp, C. D. and Uziel, M. *J. Cell. Phys. 114* (1983) 111 and references therein.
6. Brown, D. M. In: Ts'O, P. O. P., Ed., *Basic Principles in Nucleic Acid Chemistry*, Academic Press, New York 1974, Vol. II, p. 1.
7. Hollaender, A. *Chemical Mutagens*, Vol. VII, Plenum Press, New York 1982.
8. Graver, P. L. *Chemical Carcinogenesis and DNA*, Chemical Rubber Publ. Co., Boca Raton, FL 1979.
9. Kamimura, T., Masegi, T. and Hata, T. *Chem. Lett.* (1982) 965.
10. Furukawa, Y., Kobayashi, K., Kanai, Y. and Honjo, M. *Chem. Pharm. Bull. 13* (1965) 1273.
11. Robins, M. J. and Naik, S. R. *Biochem. 10* (1971) 3591.
12. Yamauchi, K., Nakamura, K. and Kinoshita, M. *J. Org. Chem. 43* (1978) 1593.
13. Yamuchi, K., Nakamura, K. and Kinoshita, M. *J. Org. Chem. 45* (1980) 3865.
14. Robins, M. J., Naik, S. R. and Lee, A. S. K. *J. Org. Chem. 39* (1974) 1891.
15. Martin, D. M. G., Reese, C. B. and Stephenson, G. F. *Biochem. 7* (1968) 1406.
16. Markiewicz, W. T. *J. Chem. Res. (S)* (1979) 24.
17. Welch, C. J., Bazin, H., Heikkilä, J. and Chattopadhyaya, J. *Acta Chem. Scand. B39* (1985) 203.
18. Gaffney, B. L. and Jones, R. A. *Tetrahedron Lett. 23* (1982) 2253.
19. Kamimura, T., Masegi, T., Sekine, M. and Hata, T. *Tetrahedron Lett. 25* (1984) 4241.
20. Furukawa, Y., Kobayashi, K., Kanai, Y. and Honjo, M. *Chem. Pharm. Bull. 13* (1965) 1273.
21. Reese, C. B., Titmus, R. C. and Yan, L. *Tetrahedron Lett.* (1978) 2727.
22. Jones, S. S., Reese, C. B., Sibanda, S. and Ubasawa, A. *Tetrahedron Lett. 22* (1981) 4755.

Received June 3, 1986.