Synthesis of 2',3'-Differentiated Ribonucleosides via Glycosylation Reactions with 2-*O*-Me or 2-*O*-TBDMS Ribofuranose Derivatives. 1. Pyrimidine Series

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The synthesis of 2',3' asymmetrically substituted pyrimidine ribonucleosides in 70–95% yields by using modified Vorbruggen conditions with "nonparticipating" 2-*O*-CH_3 and 2-*O*-TBDMS ribofuranoses is described. Such compounds are useful synthons for oligoribonucleotide synthesis, including incorporation of "rare" bases. New and practically useful conditions for placement (using 1,2,4-triazole) and removal (KF/crown ether) of the *tert*-butyldimethylsilyl (TBDMS) protecting group are also reported.

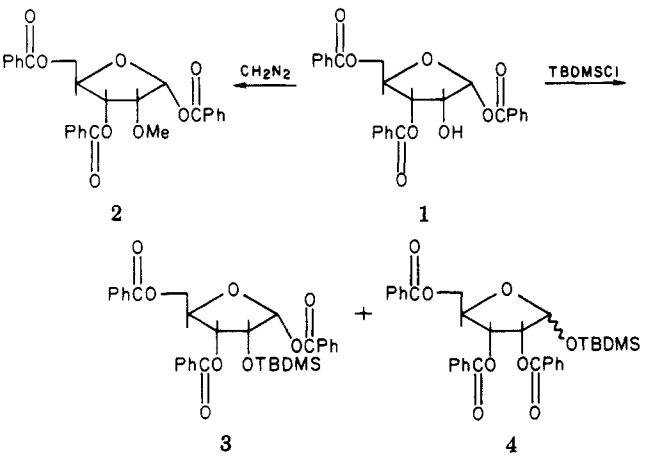
For the chemical synthesis of oligoribonucleotides it is necessary to have the monomeric nucleosidic units asymmetrically substituted.[1] Especially difficult is the differentiation of the secondary 2',3'-cis hydroxyl groups, and, in general, all such synthons have been prepared via a route that begins with the ribonucleoside. Thus, prior protection of the primary 5'-hydroxyl and primary amino function of the aglycon if necessary, is followed by a nonselective substitution of the 2'- and 3'-hydroxyls. This latter operation then necessitates separation of the isomeric mixture by chromatography or fractional crystallization; e.g., see ref 2–4. Most of the "building blocks" currently used in oligonucleotide synthesis have been obtained by some variation of this general procedure and include ribonucleosides blocked at the 2'-hydroxyl with tetrahydropyranyl,[5] methoxytetrahydropyranyl,[6] acyl,[7] *tert*-butyldimethylsilyl (TBDMS),[8] *o*-nitrobenzyl,[9] and methyl.[10] Enzymatic differentiation of a 2',3'-cyclic phosphate[11] or selective deacylation[12] have also been proposed as methods of obtaining 2',3'-differentiated ribonucleosides but not utilized to any great extent.

An attractive alternate route to such derivatives is the direct synthesis of an asymmetrically 2',3'-substituted ribonucleoside, i.e., *to achieve the differentiation at the carbohydrate stage*. To be practically useful, such a procedure not only must be reasonably short and of good overall yield for the 2-substituted ribofuranose derivatives but also must overcome the problems of anomerism/isomerism inherent in glycosylation reactions.

To date, the relatively few uses of sugars with "nonparticipating" groups at the 2-position, i.e., H, OH, *O*-alkyl, usually produce anomeric mixtures of nucleosides in glycosylation reactions.[13,14] Indeed, the two most pertinent examples using 2-*O*-methyl-[15] or 2-*O*-ethyl ribofuranose[16] derivatives report yields of the β-nucleosides not exceeding 35%. Nevertheless, some recent progress has been made in understanding how to direct the regioselectivity and stereospecificity of glycosylation reactions.[17] Additionally, a practical synthesis of 1,3,5-tri-*O*-acylribofuranoses variously substituted at the 2-hydroxyl is now available.[18]

In this paper we present a study which demonstrates for the first time that ribofuranoses **2** and **3** with "nonparticipating" groups, i.e., OMe or OTBDMS, at the 2-position can be used in glycosylation reactions to furnish

Scheme I



good to excellent yields of all the naturally occuring pyrimidine β-nucleosides **6** or **7** uncontaminated by α anom-

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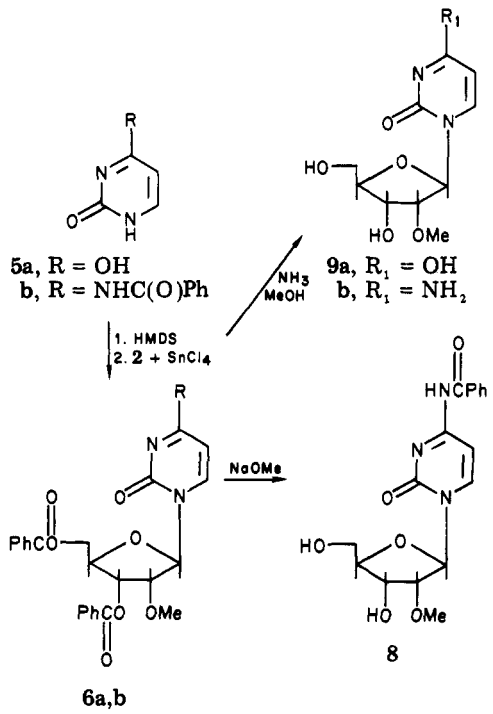
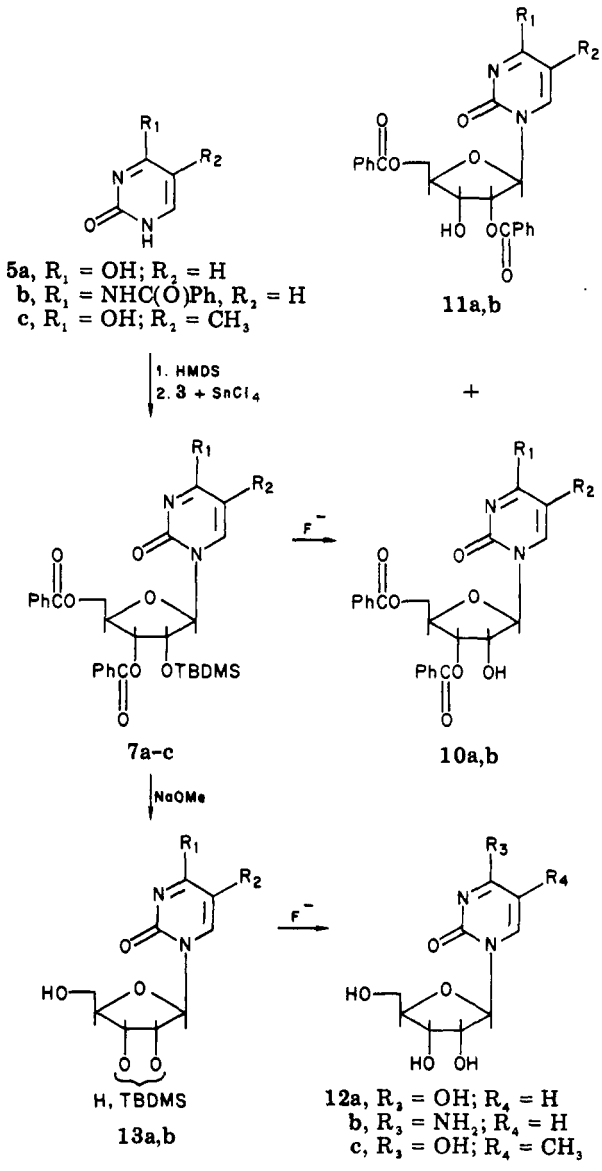
Scheme II



Scheme III



ers or others isomers. Furthermore, we believe this approach represents a potentially general and competitive synthesis not only for the "rare" bases[19] but also for some of the other disymmetrically substituted oligoribonucleotide building blocks. A preliminary account of this work has already appeared.[20]

Discussion

The required 2-substituted sugars **2** and **3** were prepared from the readily available 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (**1**)[18] (Scheme I). Reaction of **1** with diazomethane in the presence of BF_3 etherate to prevent acyl migration[21] produced **2** (~75%) always accompanied by some starting material **1** (~15%). Varying reaction conditions could never achieve complete conversion of the starting sugars.

Silylation of **1** under standard conditions[22] led to a mixture of the desired 2-*O*-TBDMS derivative, **3**, and the corresponding 1-*O*-TBDMS isomer, **4**; similar base-catalyzed isomerizations are now well documented.[8b,23] However, substitution of 1,2,4-triazole for imidazole as the activating agent[24] produced the desired 2-*O*-TBDMS derivative **3** in essentially quantitative yield. This useful result is presumably due to the reduced basicity of triazole compared to imidazole, and it would be of considerable interst to compare its effect on direct silylation of unprotected nucleosides.

These 2-ether derivatives, **2** or **3**, were submitted to glycosylation reactions with the silylated pyrimidines uracil (**5a**) and N^4-benzoylcytosine (**5b**) by using the $SnCl_4$ approach in dichloroethane as the solvent (Scheme II). However, "forcing" conditions, i.e., 2 equiv of $SnCl_4$ and heating, were employed to maximize the more stable and desired β-nucleosides **6** and **7**.[17] The yields (90% for the TBDMS derivatives **7a,b** and ~70% for the methylated nucleosides **6a,b**) amply demonstrate the effectiveness of this approach; only traces of the corresponding α isomers were ever detected by ^1H NMR. Thus, these syntheses illustrate for the first time that in glycosylation reactions requiring high stereoselectivity "nonparticipating" groups in the ribose 2-position can be as effectively employed as the much more common and "participating" acyl groups[25] provided correct conditions are used.

A further example of this approach is the direct synthesis of the 2'-*O*-TBDMS-substituted derivative of the "rare" base ribothymidine (**7c**) in 87% yield (Scheme III).

Unfortunately, similar sugar derivatives containing the methoxy THP group[18] were not stable to the reaction conditions, and no nucleosidic products containing this useful 2'-OH protecting group[26] could be isolated.

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(18) (a) C. Chavis, F. Dumont, and J. L. Imbach, *J. Carbohydr., Nucleosides, Nucleotides*, **5**, 133 (1978); (b) R. K. Ness and H. J. Fletcher, *J. Am. Chem. Soc.*, **78**, 4710 (1956).
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(24) For a related use of 1, 2, 4-triazole, see: N. Katagiri, K. Itakura, and S. A. Narang, *J. Am. Chem. Soc.*, **97**, 7332 (1975).
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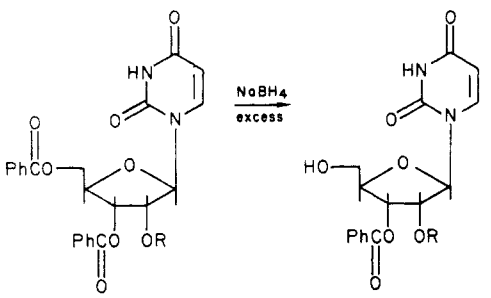
Scheme IV



7a, R = TBDMS
14, R = C(O)Ph

15, R = TBDMS
16, R = C(O)Ph

Deprotection of the 2′-*O*-CH_3 nucleosides **6a,b** was quite straightforward. Controlled treatment of **6b** with NaOMe produced the N-protected nucleoside, **8** ready for incorporation into oligoribonucleotide chains (e.g., see ref 10) in overall yields which make this method a useful alternate to methods involving methylation then N-protection of nucleosides (e.g., see ref 3). Removal of all protecting groups from **6a,b** by methanolic ammonia produced the "rare" 2′-O-methylated nucleosides **9a,b**.

Attempted removal of the 2′-*O*-TBDMS group invariably led to mixtures of the corresponding 3′,5′- (**10a,b**) and 2′,5′-di-*O*-benzoyl derivatives (**11a,b**) in ratios varying from 9:1 to 1:1 as could be ascertained by ^1H NMR. However, thanks to favorable equilibrium conditions the pure 3′,5′ isomers **10a,b** could be isolated in good yields (90%; see ref 1a also).[27] These derivatives have been shown to be very useful synthons for oligoribonucleotide synthesis). Best results were obtained by utilizing F^- for removal of the TBDMS group, and we have found that KF/crown ether is, practically, a much simpler reagent to prepare than TBAF.[22] In contrast, attempted removal of the 2′-*O*-TBDMS group by using 0.1 N methanolic HCl led to an unresolved mixture of products.

Elimination of the benzoyl groups from the model compound **7a** with sodium methylate produced isomeric mixtures of the 2′- and 3′-*O*-TBDMS derivatives **13a,b**, as shown by ^{13}C and ^1H NMR spectroscopy. Such base-catalyzed isomerization is well-known,[8a,28] but according to the literature, the isomeric derivatives can be separated by column chromatography and are useful synthons for oligoribonucleotide synthesis.[29a] Recently it has been shown that the more useful oligoribonucleotide synthon, the 2′-*O*-TBDMS isomer, can be isolated in very good yield by reequilibration of the mixture and recycling reverse-phase chromatography.[29b] Complete removal of all protecting groups from the sugar hydroxyls produced the ribonucleosides **12a** (uridine), **12b** (N^4-benzoylcytidine), and **12c** (ribothymidine).

Finally, initial results with a selective 5′-debenzoylation of some 3′,5′-di-*O*-benzoylated uridine derivatives (**7a, 14**) should be noted (Scheme IV). Reaction with excess $NaBH_4$ in anhydrous ethanol[30] produced directly the corresponding 5′-hydroxyl derivatives **15** and **16** in more than 50% yield. If this reaction proves to be of general applicability it will represent a very useful approach to the preparation of such oligonucleotide synthons which, at present, must be prepared by a lengthy sequence involving tritylation (protect 5′-OH), benzoylation (protect 3′(2′)-OH), and detritylation (deblock 5′-OH).

Conclusion

The use of the selectively protected ribofuranoses **2** and **3** plus the determination of optimal condensation conditions has permitted a new approach to the synthesis, stereospecifically and regioselectively, of two classes of 2′,3′ unsymmetrically substituted β-pyrimidine nucleosides.

The "rare" 2′-*O*-CH_3 nucleosides can be prepared in good overall yields and directly with protected aglycons ready for oligonucleotide synthesis. Of special importance, however, is the fact that this approach is amenable to large-scale syntheses, e.g., ~5–20 g. All previous work, involving direct methylation of nucleosides, leads to complex mixtures which are difficultly or incompletely separable in amounts exceeding ~1 g. A variety of alkylating agents have been reported, including diazomethane (in the presence or absence of $SnCl_2$),[31] methyl halide,[32a-c] or dimethyl sulfate[32d] in alkaline solution and trimethyl sulfoxonium hydroxide.[33]

In common with direct benzoylation or TBDM silylation of nucleosides, this method suffers from the facile 2′- to 3′-position migration of these groups, a problem which has not detracted from their extensive use in oligonucleotide synthesis. In contrast, however, this glycosylation approach will probably be the preferred procedure for preparation of the necessary amounts of protected nucleoside synthons containing unusual bases which will be necessary in any future tRNA synthesis.

A forthcoming paper will present the detailed results obtained in the purine series and thus confirm the generality of this approach.

Experimental Section

General Details. Melting points were obtained in capillaries on a Büchi 510 melting range apparatus and are uncorrected. UV spectra [λ_{max} in nanometers (ϵ)] were obtained on a Optica Model 10 spectrophotometer. Proton magnetic resonance (^1H NMR) spectra were recorded on a Varian 90 or HA-100 instrument in $CDCl_3$ or Me_2SO-d_6; all values are recorded in parts per million (δ) with reference to Me_4Si. Band shape is indicated by s (singlet), d (doublet), t (triplet), and m (multiplet). Thin-layer chromatography was performed by using Merck Kieselgel 60 F254 as 0.2-mm layers on aluminum foil. Merck silica (0.063–0.2 mm) was used for column chromatography; solvent ratios are expressed as volume per volume.

Mass spectra were obtained on a JEOL JMS D 100 instrument. Elemental analyses were performed by the Central microanalytical Service of CNRS, Montpellier division.

1,3,5-Tri-*O*-benzoyl-2-*O*-methyl-α-D-ribofuranose (2) was prepared as described in the literature for a mannose derivative[34] by slow addition of diazomethane in CH_2Cl_2 to a solution

(27) D. P. L. Green, T. Ravindranathan, C. B. Reese, and R. Saffhill, *Tetrahedron*, **26**, 1031 (1970).
(28) See ref 8b. See also K. K. Ogilvie, D. W. Entwistle, Carbohydr. Res. *89*, 203 (1981).
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(maintained at 0 °C) of the 2-hydroxy sugar **1** in CH_2Cl_2 with BF_3 etherate as a catalyst. Typically the sugar **1** (5.0 g) in CH_2Cl_2 (150 mL) was treated with diazomethane from *N*-methyl-*N*-nitrosourea (25 g) to give after the workup and chromatography ($CHCl_3$/ether 19:1) a clear oil, **2** (3.8 g, 74%). Also recovered was 15% of the starting sugar. These yields could not be altered by the addition of much more diazomethane, more catalyst, or alternating quantities of diazomethane and catalyst.

1,3,5-Tri-*O*-benzoyl-2-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (3). (a) Imidazole Method.[22] To a solution of the 2-hydroxy sugar **1** (3.46 g, 7.3 mmol) in ahydrous DMF (10 mL) were added *tert*-butyldimethylsilyl chloride (1.50 g, 10 mmol) and imidazole, (1.3 g, 20 mmol). After 20 h at room temperature, this solution was poured into ice–water and the resulting mixture extracted with hexane (4 × 15 mL). The combined organic phases were washed with water (1 × 75 mL), dried (Na_2SO_4), and filtered, and the solvent was removed in vacuo to yield an oil (5.10 g). Column chromatography ($CHCl_3$) yielded successively the 1-*O*-TBDMS sugar **4** (1.92 g, 45%) and the desired 2-*O*-TBDMS isomer **3**: 1.23 g (31%); ^1H NMR ($CDCl_3$) 8.30–7.20 (m, aromatics), 6.60 (H_1, $J_{1,2}$ = 4.2 Hz), 5.60 (dd, H_3, $J_{3,4}$ = 1.8 Hz), 4.88 (m, H_4), 4.60 (dd, H_2, $J_{2,3}$ = 6 Hz), 4.38 (m, H_5, $H_{5'}$), 0.7 [s, $(CH_3)_3C$], 0.03 [s, $(CH_3)_2$]. For **4**: ^1H NMR ($CDCl_3$) 8.40–7.10 (m, Ph), 6.00 (t, H_3, $J_{2,3}$ = 6 Hz), 5.70 (d, H_2, $J_{1,2}$ = 6 Hz), 5.60 (s, H_1), 5.00–4.50 (H_4, H_5, $H_{5'}$), 0.93 [s, $(CH_3)_3C$], 0.20 (s, CH_3Si), 0.16 (s, CH_3Si).

Anal. Calcd for $C_{32}H_{36}O_8Si$: C, 66.66; H, 6.25. Found for **3**: C, 66.37; H, 6.11. Found for **4**: C, 66.41; H, 6.08.

(b) Triazole Method. *tert*-Butyldimethylsilyl chloride (0.904 g, 6.0 mmol) and 1,2,4-triazole (0.828 g, 6.0 mmol) were allowed to dissolve in DMF (4 mL) during 30 min at room temperature at which time the sugar, **1** (0.924 g, 2.0 mmol), was added and the mixture stirred at room temperature with exclusion of moisture. After 48 h, petroleum ether (50 mL) and H_2O (50 mL) were added, the phases were separated, and the aqueous phase was extracted with petroleum ether (2 × 50 mL). The combined organic layers were washed with H_2O (1 × 75 mL), dried (Na_2SO_4), and evaporated to yield the silylated sugar **3**, 1.148 g (quantitative). HPLC indicated that this sugar contained 1–2% of the corresponding 1-*O*-TBDMS isomer **4** but was used "as is" in all subsequent glycosylation reactions.

Acylation of Heterocyclic Bases. N^4-Benzoylcytosine **5b** was obtained as described.[35]

Preparation of Pyrimidine Nucleosides 6a,b and 7a,b. General Silylation and Coupling Procedure.[14] When possible, all the following manipulations were performed in a glovebox with exclusion of moisture. To the heterocycle (**5a,b**, 1 g) were added HMDS (25 mL) and ammonium sulfate (50 mg). The mixture was refluxed overnight with exclusion of moisture. In the morning the homogenous solution was cooled to room temperature and evaporated in vacuo (oil pump); to the residue was added anhydrous dichloroethane (30 mL), and the solvent was again removed in vacuo to ensure complete removal of HMDS. The residue, assumed to be the completely silylated heterocycle, was used immediately for the next step. Thus, the silylated base (1 equiv) and the required sugar **2** or **3** (1 equiv) were dissolved in anhydrous dichloroethane (15 mL/g of sugar), and to this solution was added $SnCl_4$ (2 molar equiv). The homogenous solution was refluxed for 60 min, cooled, and poured into cold, saturated, aqueous $NaHCO_3$. After 10 min of agitation the Sn salts were removed by filtration through Celite and washed with dichloroethane. The filtrate was separated, the aqueous phase was washed with dichloroethane (1 × 50 mL), and the combined organics were washed with H_2O (2 × 75 mL) before drying (Na_2SO_4) and solvent removal. In this manner the following nucleosides were prepared.

3',5'-Di-*O*-benzoyl-2'-*O*-methyluridine (6a). Uracil (**5a**; 0.45 g, 4 mmol), the 2-*O*-methyl sugar **2** (1.90 g, 4 mmol), and $SnCl_4$ (2.08 g, 0.95 mL, 8 mmol) yielded, after chromatography ($CHCl_3$/ether, 3:1), a white foam (1.37 g, 74%) which was crystallized from ethanol as white needles: mp 132–133 °C (lit.[15] foam); ^1H NMR ($CDCl_3$) 8.20–7.30 (m, H_6 and aromatics), 6.04 (d, $H_{1'}$, $J_{1',2'}$ = 2.9 Hz), 5.54 (d, H_5, $J_{5,6}$ = 8 Hz), 5.38 (dd, $H_{3'}$, $J_{3',4'}$ = 5 Hz), 4.90–4.50 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4.23 (dd, $H_{2'}$, $J_{2',3'}$ = 5.9 Hz), 3.46 (s, OCH_3); UV (ethanol, 95%) λ_{max} 227 (30 000), 256 (13 000).

Anal. Calcd for $C_{24}H_{22}N_2O_8$: C, 61.80; H, 4.75; N, 6.00. Found: C, 61.71; H, 4.98; N, 5.91.

3',5'-Di-*O*-benzoyl-2'-*O*-(*tert*-butyldimethylsilyl)uridine (7a). Uracil (5a; 2.50 g, 22 mmol), the 2-*O*-TBDMS sugar **3** (12.0 g, 20 mmol), and $SnCl_4$ (4.7 mL, 40 mmol) yielded after the workup a white foam, **7a** (10.75 g, 95%; pure by TLC). Chromatography ($CHCl_3$/MeOH, 99:1) yielded an analytical sample (foam): ^1H NMR ($CDCl_3$) 8.78–7.23 (m, H_6 and aromatics), 5.99 (d, $H_{1'}$, $J_{1',2'}$ = 3.6 Hz), 5.56 (d, H_5, $J_{5,6}$ = 7.8 Hz), 5.40 (dd, $H_{3'}$, $J_{3',4'}$ = 4.5 Hz), 5.00–4.35 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4.09 (dd, $H_{2'}$, $J_{2',3'}$ = 4.8 Hz), 0.8 [s, $(CH_3)_3C$], 0.08 [s, $(CH_3)_2Si$]; mass spectrum, *m/e* 509 (M^+ − 57).

Anal. Calcd for $C_{29}H_{34}N_2O_8Si$: C, 61.48; H, 6.00; N, 4.95. Found: C, 60.97; H, 6.09; N, 4.85.

N^4-Benzoyl-3',5'-di-*O*-benzoyl-2'-*O*-methylcytidine (6b). N^4-Benzoylcytosine (**5b**; 1.35 g, 6.05 mmol), 2-*O*-methyl sugar **2** (2.95 g, 6.05 mmol), and $SnCl_4$ (3.22 g, 1.48 mL, 12.1 mmol) produced a beige foam (3.1 g) which could be crystallized directly from CH_2Cl_2/ether as white crystals of **6b**: 2.0 g; mp 169–171 °C. Chromatography (MeOH/$CHCl_3$, 1:19) of the mother liquors afforded additional product (0.5 g; total yield 70%). The product was crystallized from MeOH for analysis: mp (170–171 °C; ^1H NMR ($CDCl_3$) 8.40–7.40 (m, H_5 and aromatics), 8.17 (d, H_6, $J_{5,6}$ = 8 Hz), 6.09 (d, $H_{1'}$, $J_{1',2'}$ = 1.4 Hz), 5.25 (dd, $J_{3',4'}$ = 8.5 Hz), 4.98–4.58 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4.35 (dd, $H_{2'}$, $J_{2',3'}$ = 5 Hz), 3.58 (s, OCH_3); UV (ethanol, 95%) λ_{max} 228 (35 000), 263 (12 000); mass spectrum, *m/e* 570 (M^+).

Anal. Calcd for $C_{31}H_{27}N_3O_8$: C, 65.37; H, 4.78; N, 7.38. Found: C, 64.79; H, 4.72; N, 7.20.

N^4-Benzoyl-3',5'-*O*-dibenzoyl-2'-*O*-(*tert*-butyldimethylsilyl)cytidine (7b). N^4-Benzoylcytosine (**5b**; 0.72 g, 3 mmol), the 2-*O*-TBDMS sugar **3** (1.92 g, 3 mmol), and $SnCl_4$ (1.74 g, 0.8 mL, 6 mmol) produced a beige foam (2.0 g). Crystallization from ether gave white needles of **7b**: 1.70 g; mp 157–159 °C. Chromatography ($CHCl_3$/ether, 7:3) yielded additional product: 0.2 g (total yield 86%); ^1H NMR ($CDCl_3$) 8.20–7.20 (m, H_5, H_6, and aromatics), 5.98 (d, $J_{1',2'}$ = 1.8 Hz), 5.23 (s, $H_{3'}$), 5.21–4.70 (m, $H_{2'}$), 5.20–4.70 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 0.83 [s, $(CH_3)_3C$], 0.20 (s, CH_3Si), 0.01 (s, CH_3Si); UV (ethanol, 95%) λ_{max} 228 (38 000), 263 (14 000); mass spectrum, *m/e* 670 (M^+).

Anal. Calcd for $C_{36}H_{39}O_8N_3Si$: C, 64.55; H, 5.87; N, 6.27. Found: C, 64.48; H, 5.77; N, 6.37.

3',5'-Di-*O*-benzoyl-2'-*O*-(*tert*-butyldimethylsilyl)-5-methyluridine (7c). This derivative of a "rare base" was prepared by using the "one-pot" procedure of Vorbrüggen.[36] Thymine (0.252 g, 2 mmol), the 2-O TBDMS sugar, **3** (1.15 g, 2 mmol), HMDS (0.34 mL, 1.6 mmol), trimethylsilyl chloride (0.20 mL, 1.6 mmol), and $SnCl_4$ (0.28 mL, 2.4 mmol) were added to dry CH_3CN (30 mL). The solution was refluxed for 20 min until TLC indicated the disappearance of all the starting sugar and was worked up as described (vide supra). The oily residue (1.10 g) was chromatographed ($CHCl_3$/MeOH, 99:1) to yield an oil: 0.99 g (87%); ^1H NMR ($CDCl_3$) 8.21–7.20 (m, H_6 and aromatics), 6.07 (d, $H_{1'}$, $J_{1',2'}$ = 4.8 Hz), 5.51 (dd, $H_{3'}$, $J_{3',4'}$ = 6 Hz), 5.02–4.50 (m, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4.57 (dd, $H_{3'}$, $J_{3',4'}$ = 6 Hz), 1.63 (s, CH_3), 0.80 [s, $(CH_3)_3C$], 0.04 [s, $(CH_3)_2Si$]; UV (ethanol, 95%) λ_{max} 228 (25 500), 264 (9300); mass spectrum, *m/e* 565 (M^+ − 15).

Anal. Calcd for $C_{30}H_{26}N_2O_8Si$: C, 62.06; H, 6.20; N, 4.82. Found: C, 61.75; H, 6.15; N, 4.80.

Deprotecton of 2'-*O*-Methyl Nucleoside. (1) Partial: *N*-Acyl-2'-*O*-methyl Nucleoside 8. As commonly carried out,[28] the completely protected nucleoside **6b** (1 equiv) in THF was treated with 1 N methanolic NaOMe (3 equiv) for 12 min at room temperature. Sodium ions were removed with Amberlite IR 120 resin (pyridinium form), and after filtration and complete removal of solvent in vacuo, the residue was chromatographed ($CHCl_3$/MeOH, 9:1) or crystallized directly. In this manner there was obtained **N^4-benzoyl-2'-*O*-methylcytidine (8)**: yield ≥80%; mp 180–181 °C (acetone or CH_2Cl_2) (lit. mp 181–182,[10] 83–84 °C[37]; ^1H NMR (Me_2SO-d_6) 8.56 (d, H_6, $J_{5,6}$ = 7.5 Hz), 8.30–7.18 (m, H_5 and aromatics), 5.90 (d, $H_{1'}$, $J_{1',2'}$ = 2 Hz), 4.34–3.30 (m, 5 H), 3.50

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(2) Complete: Preparation of 2′-*O*-Methyl Nucleosides 9a,b. The fully protected nucleosides **6a,b** were dissolved in anhydrous methanol previously saturated at 0 °C with NH_3; the flask was stoppered and stored in the refrigerator (4 °C). After 2–4 days when the reaction was complete, as monitored by TLC, the solvent was removed at water-pump pressure and the residue subjected to column chromatography. Benzamide was removed with $CHCl_3$/MeOH (19:1), and the desired product was obtained with $CHCl_3$/MeOH (4:1). The following compounds were obtained in yields ≥80%.

2′-*O*-Methyluridine (9a): mp 159–161 °C (acetone/ether) (lit.[3] mp 159–161 °C); mass spectrum, m/e 244 (M^+) and all other peaks as reported in ref 3.

2′-*O*-Methylcytidine (9b): mp 251–252 °C (methanol/ether) (lit.[3] mp 255 °C); mass spectrum, m/e 257 (M^+) and all other peaks as reported in ref 3.

Removal of TBDMS Protecting Groups. (1) TBAF in THF.[22] To the nucleoside **7a–c** (1 equiv) was added TBAF (2.5 equiv) in THF. After 2 h at room temperature the reaction was judged complete as monitored by TLC. The reaction mixture was evaporated to dryness and the residue purified by chromatography or crystallized directly.

(2) KF/Crown Ether.[38] The flame-dried KF (15 equiv) were added dibenzo-18-crown-6 (1 equiv) and dry benzene (300 mL/9% KF). Most of the benzene (75%) was distilled to dry the reaction mixture, which was then cooled to room temperature and treated with the nucleoside (1 equiv) dissolved in CH_3CN (30 mL/g of nucleoside) plus acetic acid (0.1 mL/3 mL of CH_3CN). The reaction was refluxed for 2 h, cooled, treated with ethanol (25 mL), and evaporated to dryness. The residue was purified by chromatography and then crystallization to give the following nucleosides.

3′,5′-Di-*O*-benzoyluridine (10a). Method a. 2′-*O*-TBDMS-substituted uridine (**7a**; 2.0 g, 3.5 mmol) after treatment with TBAF (8.5 mmole) in THF (13 mL) and chromatography ($CHCl_3$/MeOH, 99:1) yielded an oil (1.43 g, 90%). Crystallization from methanol in several crops yielded the deblocked nucleoside **10a**: 1.29 g (82%); mp 185–187 °C (lit.[39] mp 187–189 °C); ^1H NMR (Me_2SO-d_6) 8.33–7.33 (m, H_5 and aromatics), 5.90 (d, $H_{1'}$, $J_{1',2'}$ = 5 Hz), 5.86 (d, OH, J = 6 Hz), 5.63 (d, H_6, $J_{5,6}$ = 7.8 Hz), 5.50 (m, $H_{3'}$), 4.93–4.33 (m, $H_{2'}$, $H_{4'}$, $H_{5'}$, $H_{5''}$).

Method b. The same 2′-*O*-TBDMS nucleoside (**7a**; 0.1 g, 0.175 mmol) on treatment with KF (0.15 g, 2.6 mmol) and crown ether (0.064 g, 0.175 mmol) in dry benzene (10 mL), acetonitrile, and acetic acid yielded the deblocked nucleoside **10a** (0.059 g, 74%) after chromatography ($CHCl_3$/MeOH, 99:1).

N^4-Benzoyl-3′,5′-di-*O*-benzoylcytidine (10b). The 2′-*O*-TBDMS nucleoside **7b** (0.5 g, 0.74 mmol) after treatment with TBAF (2.2 mmol) in THF (3.5 mL) yielded an oil which was directly crystallized from anhydrous ethanol to give **10b**: 0.405 g (97%); mp 205–206 °C (lit.[40] mp 198–202 °C); ^1H NMR (Me_2SO-d_6) 8.23 (d, H_6, $J_{5,6}$ = 7, 5 Hz), 8.16–7.15 (m, H_5 and aromatics), 5.96 (d, $H_{1'}$, $J_{1',2'}$ = 4.5 Hz), 5.46 (m, $H_{2'}$), 4.83–4.50 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{5''}$).

Selective Removal of the 5′-*O*-Benzoate. The following general procedure was employed. To the 3′,5′-di-*O*-benzoyl nucleoside (1 mmol) in anhydrous ethanol (15 mL/g of nucleoside) was added $NaBH_4$ (4 mmol). The reaction was stirred, allowed to proceed at room temperature, and followed by TLC. After a few minutes the solution became homogeneous, and after 3 h the reaction was judged to be complete. The reaction mixture was then chilled to 0 °C and treated simultaneously with acetone and 1 N aqueous acetic acid (16 mmol, i.e., stoichiometric equivalents). The mixture was agitated for 15 min, the organic solvents were evaporated, and the remaining aqueous phase was extracted with CH_2Cl_2 3 times. The combined organic phases were dried (Na_2SO_4) and evaporated to yield a product which was usually purified by chromatography. The following products were thus prepared.

(a) 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-benzoyluridine (15). The fully protected nucleoside **7a** (0.306 g, 0.53 mmol) and $NaBH_4$ (0.100 g) in anhydrous ethanol (2.5 mL) produced, after the workup and direct precipitation from ether, a solid (0.135 g, 55%) which could be crystallized from CH_2Cl_2/hexane to give **13a**: mp 217–219 °C; ^1H NMR (Me_2SO-d_6) 11.43 (NH), 8.46–7.46 (m, H_5 and aromatics), 6.10 (d, H_1, $J_{1',2'}$ = 6.3 Hz), 5.86 (d, H_6, $J_{5,6}$ = 7.4 Hz), 5.60 (dd, $H_{3'}$, $J_{3',4'}$ = 2.1 Hz), 4.63 (dd, $H_{3'}$, $J_{2',3'}$ = 5.1 Hz), 4.53–4.28 (m, $H_{4'}$), 4.3–5.8 (m, $H_{5'}$, $H_{5''}$); UV (ethanol, 95%) λ_{max} 230 (12 400), 258 (9500); mass spectrum, m/e 452 (M^+).

Anal. Calcd for $C_{22}H_{30}N_2O_7Si$: C, 57.14; H, 6.49; N, 6.06. Found: C, 57.05; H, 6.72; N, 6.07.

(b) 2′,3′-*O*-Benzoyluridine (16). 2′,3′,5′-Tri-*O*-benzoyluridine (**14**; 0.93 g, 1.7 mmol) and $NaBH_4$ (0.254 g) in anhydrous ethanol (10 mL) produced an oily residue (1.07 g) which yielded after chromatography some starting material (0.275 g) with ether as eluent and the desired product **16** [0.431 g, (56%); mp 194–196 °C (lit.[41] mp 195–197 °C)] with ether/ethyl acetate (98:2) as eluent.

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