# SYNTHESIS OF OLIGORIBONUCLEOTIDES CONTAINING PYRIMIDINE 2′-*O*-[(HYDROXYALKOXY)METHYL]RIBONUCLEOSIDES

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*This paper is dedicated to Professor Antonín Holý on the occasion of his 70th birthday.*

A simple and efficient method for the preparation of pyrimidine 2′-*O*-[(2-hydroxyethoxy)methyl]ribonucleosides and 2′-*O*-[(3-hydroxypropoxy)methyl]ribonucleosides has been developed. These modified nucleosides were incorporated into oligoribonucleotides, which were shown to form stable RNA/RNA duplexes.

**Keywords**: Ribonucleosides; Oligonucleotides; 2′-*O*-Modified nucleosides; Antisense agents; RNA duplexes; Thermal denaturation; siRNA.

Modified oligonucleotides are widely used in molecular biology and medicinal chemistry. Antisense agents are valuable tools to inhibit the expression of a target gene in a sequence specific manner, and may be used for functional genomics, target validation and theurapeutic purposes[1]. The impact of the research in this field increased due to the discovery of RNA interference[2,3].

Numerous attempts have been made to improve the properties of natural oligonucleotides[4,5]. It is believed that the 2′-modifications in sugar moiety are the most universal and promising[6–9]. A typical strategy for the preparation of such oligonucleotides is the synthesis of a modified nucleoside followed by conversion to the corresponding phosphoramidite suitable for the automated oligonucleotide synthesis. Most of these modifications were achieved via alkylation reactions of partially protected ribonucleosides. The heterocyclic bases should be protected in order to avoid their alkylation.

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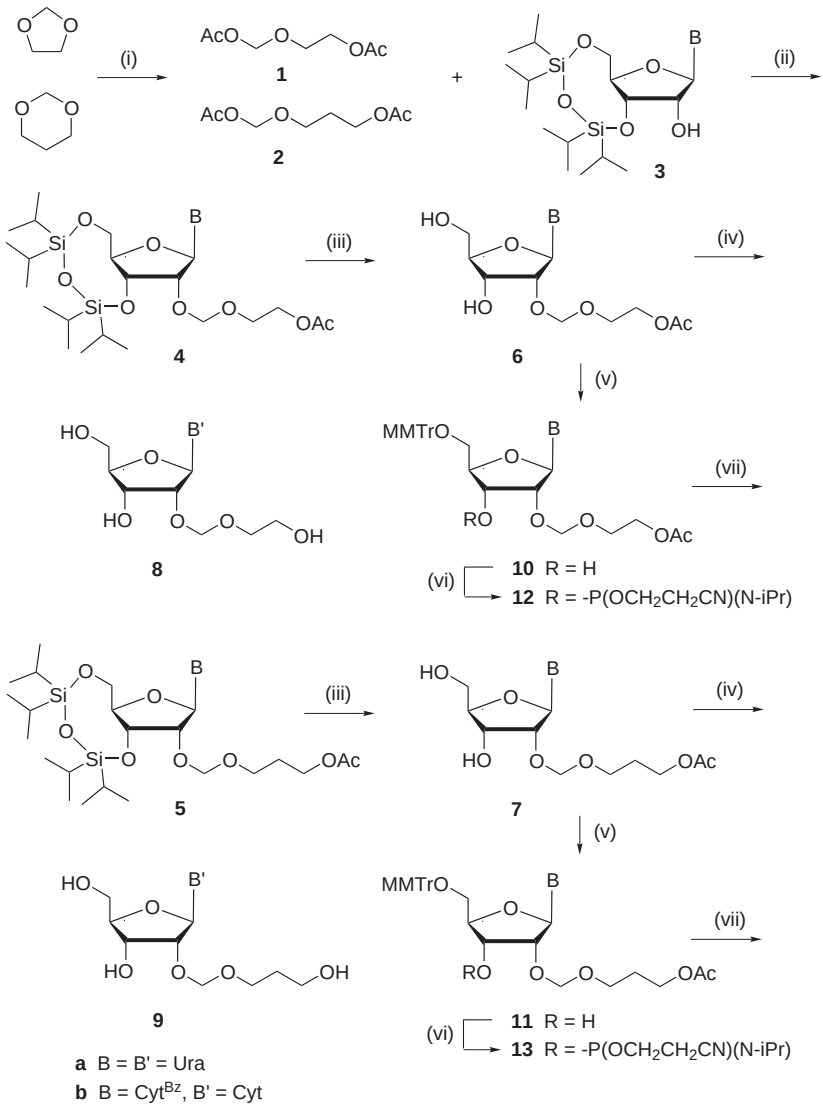
For every nucleoside, the specific blocking groups are used[10], which in most cases are not compatible with standard automated oligonucleotide synthesis. For the preparation of 2′-*O*-modified nucleosides we have recently developed another type of chemistry based on *O*-glycosylation reactions, which has several important advantages, such as increased yields and simplification[11,12]. A simple and effective method has been recently developed[13–16] for the preparation of 2′-*O*-(β-D-ribofuranosyl)nucleosides starting from readily available 3′,5′-*O*-blocked *N*-acylribonucleosides and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose preactivated with tin tetrachloride in 1,2-dichloroethane at 0 °C. The condensation proceeds by stereospecific formation of the β-D-glycosidic bond and gives a high yield. Several other fully acylated monosaccharide derivatives of D- and L-arabinofuranoses, D-erythrofuranose and D-ribopyranose have been successfully used in the same reaction[17].

## RESULTS AND DISCUSSION

### *Synthesis of the Monomeric Nucleosides*

Here we present our recent results on the preparation of 2′-*O*-hydroxyalkoxymethylribonucleosides and their incorporation into oligoribonucleotides. This scheme is nearly the same as for the preparation of disaccharide nucleosides and their incorporation into oligonucleotides[17]. The starting compounds **1** and **2** [18] were easily prepared from 1,3-dioxolane and 1,3-dioxane, respectively, and were condensed with 3′,5′-*O*-blocked ribonucleosides **3a**, **3b** [19] in the presence of tin tetrachloride in 1,2-dichloroethane at –12 °C for 20 min (Scheme 1). The yields (85–87%) of products **4a**, **4b** and **5a**, **5b** were even higher than in the case of preparation of the above mentioned disaccharide nucleosides[13]. The silyl group was deblocked to yield partially protected **6a**, **6b** and **7a**, **7b** and subsequent deblocking with ammonia in methanol gave free 2′-*O*-substituted nucleosides **8a**, **8b** and **9a**, **9b** in high overall yields. Nucleosides **6a**, **6b** and **7a**, **7b** were converted using standard procedures to the corresponding methoxytrityl derivatives **10a**, **10b** and **11a**, **11b** and their phosphoramidites **12a**, **12b** and **13a**, **13b**.

The structure of the compounds was supported by NMR spectroscopy and mass spectrometry. The chemical shifts were assigned using double resonance techniques and COSY experiments. Several conclusions were drawn from the $^1$H NMR spectral analysis. In nucleosides **3a**, **3b**–**5a**, **5b** with 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group the coupling constants




(i) $Ac_2O$, AcOH, $H_2SO_4$; (ii) $SnCl_2/ClCH_2CH_2Cl$, –12 °C; (iii) $Bu_4NF/THF$; (iv) $NH_3/MeOH$; (v) MMTrCl/Py; (vi) $iPrNPCl(OCH_2CH_2CN)$; (vii) oligonucleotide sunthesis

SCHEME 1

$J_{1',2'}$ of nucleoside moieties are lower than 0.5 Hz. Most of the chemical shifts and coupling constants may be calculated directly from NMR spectra. In some cases comparison with the published spectra of disaccharide nucleosides[13] and double resonance, $^{1}H$-$^{13}C$ correlation and COSY spectra were used for the assignment. It should be mentioned that some of the $^{1}H$ NMR spectra of the obtained compounds are rather complicated due to the overlap of signals of the ribofuranose residue and the diastereotopic protons of $CH_2OCH_2CH_2O$ and $CH_2OCH_2CH_2CH_2O$ groups. The acetal group appeared as two doublets around 5 ppm. As in the case of disaccharide nucleosides[13] and 2′-*O*-methylnucleosides[20] incorporation of the 2′-*O*-substituent results in a low-field shift (2.6–3.1 ppm) of C-2′ in $^{13}C$ NMR spectra.

*Incorporation Studies*

Countless modifications have been introduced into nucleosides in search for improved binding to complementary strands, but not many with formacetal sugar derivatized nucleoside analogues have been incorporated into oligonucleotides as a permanent modification[7,21]. The *O*-(alkoxymethyl) grouping has largely been used as an anchor for the development of sugar hydroxyl protecting groups like in (silylmethoxy)methyl[22] or 2-(silylethoxy)methyl ethers[23], or (2-methoxyethoxy)methyl ethers[24].

Fully protected analogues are mandatory for oligonucleotide synthesis, and standard reactions lead to the required amidites useful for incorporation into oligonucleotides. Hereto, phosphitylation was carried out on the monomethoxytritylated derivatives (**10a**, **10b**, **11a** and **11b**, respectively) in dichloromethane using freshly distilled ethyldiisopropylamine and 2-cyanoethyl diisopropylphosphoramidochloridite under argon to afford **12a**, **12b** and **13a**, **13b** in high yield. With these phosphoramidites, several oligoribonucleotides with one or more modifications were assembled, using commercial TOM amidites for the natural nucleosides. The 2′-*O*-[(triisopropylsilyl)oxy]methyl (TOM) protecting group is known for its high coupling efficiency along with fast, simple deprotection[25,26]. Trityl yields were excellent, for the shorter oligos reaching 100% for full-length assembly, even with multiple incorporations of the modified building blocks. All oligos described here were assembled on a LCAA-CPG support loaded with a dimethylformamidine-protected guanosine. For deprotection of the modified RNAs a methylamine cocktail in water–ethanol was used, followed by a 1 M TBAF treatment. All oligos were purified by ion exchange chromatography, desalted by gel filtration and analysed by mass spectrometry (Table I

and Fig. 1). Only the main part of the product containing peak was collected, affording in general 20 to 30 $OD_{260}$ starting from a 1 μmol loaded support. In addition several siRNAs comprising one or more of the new modifications have been prepared and are currently under evaluation.

As an example, the mass spectral analysis of modified oligonucleotide (entry 14 in Table I) carrying 5 modified nucleosides is shown in the accompanying Fig. 1, with the top panel showing the UV trace as well as the mass ion count (base peak intensity). In the UV trace a slight bump is noticed, but this is generated by the buffer, and the ion count is very low. The mass spectrum at the eluting time of the oligonucleotide is shown in panel b, with the deconvoluted spectrum with the expected mass as the inset. Altogether, it shows the identity and purity of the oligonucleotides obtained.

The effect of the new modifications was verified on nonamers by melting experiments studies, in analogy with our previous work on different hexitol

TABLE I

Molecular weight measurements and melting experiments for oligoribonucleotides containing 2′-*O*-[(hydroxyalkoxy)methyl]ribonucleosides in 0.1 M NaCl, 0.1 mM EDTA and 20 mM $KH_2PO_4$ at pH 7.5 ($T_m$, °C)

| No. | Starting synthon | Oligoribonucleotide | Complementary oligoribonucleotide 3′-CGACACAGC-5′ $T_m$, °C | Complementary oligoribonucleotide 3′-CGU GAG UGC-5′ $T_m$, °C | MS calcd. $M^+$ | MS found $M^+$ |
|---|---|---|---|---|---|---|
| 1 | | 5′-GCUGUGUCG-3′ | 55.4 | | | |
| 2 | | 5′-GCUGU$_{OMe}$GUCG-3′ | 57.2 | | | |
| 3 | **12a** | 5′-GCUGUGUCG-3′ | 54.8 | | 2920.4 | 2920.5 |
| 4 | **13a** | 5′-GCUGUGUCG-3′ | 54.7 | | 2934.4 | 2934.5 |
| 5 | | 5′-GCACUCACG-3′ | | 56.9 | | |
| 6 | | 5′-GCACU$_{OMe}$CACG-3′ | | 57.9 | | |
| 7 | **12b** | 5′-GCACUCACG-3′ | | 55.1 | 2886.5 | 2886.5 |
| 8 | **13b** | 5′-GCACUCACG-3′ | | 55.4 | 2900.5 | 2900.3 |
| 9 | **12a** | 5′-GCUGUGUCG-3′ | 54.6 | | 2994.5 | 2994.5 |
| 10 | **13a** | 5′-GCUGUGUCG-3′ | 54.2 | | 3022.5 | 3022.5 |
| 11 | **12b** | 5′-GCACUCACG-3′ | | 54.1 | 2960.5 | 2960.5 |
| 12 | **13b** | 5′-GCACUCACG-3′ | | 54.2 | 2988.5 | 2988.4 |
| 13 | **12a**/**12b** | 5′-GCUGUGUCG-3′ | 53.1 | | 3354.6 | 3354.7 |
| 14 | **13a**/**13b** | 5′-GCUGUGUCG-3′ | 51.4 | | 3424.7 | 3424.5 |

U: 2′-*O*-[(hydroxyalkoxy)methyl]uridine residue.
C: 2′-*O*-[(hydroxyalkoxy)methyl]cytidine residue.

nucleoside congeners[27]. In contrast for incorporation of a 2′-*O*-methylribonucleoside in such nonamers, which on average increased the melting temperature ($T_m$) by 1.5 °C per modification (from 1 to 2 °C depending on the sequence context; compare entries 1 and 2, 5 and 6 in Table I), the longer chains introduced with the new congeners **8a**, **8b** or **9a**, **9b** slightly destabilize the RNA duplex. While 2′-alkyl moieties (in *ribo* configuration) in general give considerable destabilisation of a duplex, most of the studied 2′-alkoxy substituents do stabilise the duplex[7]. Like in the present study, the only formacetal function incorporated into the duplex as a 2′-*O*-(ethoxymethyl)ribonucleoside[7], or a 2′-*O*-(methoxymethyl)ribonucleoside[21], however, proved disadvantageous. This largely contrasts with the well-known MOE modification (2-methoxyethyl)ribonucleosides[7,28]. It was reported that 2′-(2-methoxyethyl) substituents are conformationally preorganized for the duplex state in an A-type duplex as for anomeric and gauche effects and electrostatic interactions between the backbone and

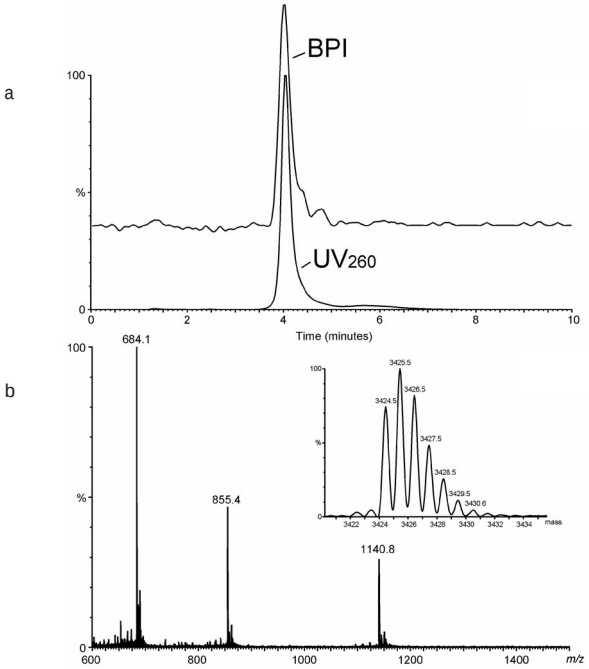



FIG. 1

HPLC-MS analysis of modified oligonucleotide (entry 14 in Table I). Panel a: Overlaid UV chromatogram at 260 nm ($UV_{260}$) and base peak intensity (BPI) chromatogram. Panel b: Mass spectrum taken at 4.0 min with deconvoluted spectrum in inset

substituent, and this contrasts with a 2′-*O*-(ethoxymethyl) group which leads to slightly reduced RNA affinity of the corresponding oligonucleotides[29]. The present results show that incorporation of the extra hydroxyl group in the side chain does not contribute to or compensate the overall effect. The destabilizing effect of the (3-hydroxypropoxymethyl) group is slightly larger in comparison with the (2-hydroxyethoxymethyl) group, which is noticed best after introducing five modifications in the nonamer sequence (entries 13 and 14 in Table I). It needs to be stressed, however, that stable duplexes are still obtained, with about 0.5 °C destabilisation per modification.

## CONCLUSIONS

The general method for the preparation of 2′-*O*-β-D-ribofuranosylnucleosides was found to be applicable to the synthesis of pyrimidine 2′-*O*-[(hydroxyalkoxy)methyl]ribonucleosides. The 2′-*O*-substituent was found to be stable during oligonucleotide synthesis. Additional work on the preparation of other 2′-*O*-functionalized nucleosides and oligonucleotides bearing amino groups is in progress and will be published shortly.

## EXPERIMENTAL

Column chromatography (CC) was performed on silica gel Kieselgel 60 (0.063–0.200 mm, Merck). TLC was carried out on Kieselgel 260 F (0.040–0.063 mm, Merck) with detection by UV and the following solvent systems (v/v): A $CH_2Cl_2$–EtOH, 95:5; B $CH_2Cl_2$–EtOH, 9:1; C iPrOH–concentrated $NH_4OH$–$H_2O$, 7:1:2; D hexane–acetone–$NEt_3$, 49:49:2. NMR spectra were recorded using Bruker AMX 400 and Varian Unity 500 spectrometers at 300 K. Chemical shifts δ in ppm were measured relative to the solvent signals ($^1H$ and $^{13}C$) or $H_3PO_4$ as relative external reference ($^{31}P$). The coupling constants (*J*) are given in Hz. The signals were assigned using double resonance techniques and COSY experiments. The UV spectra were recorded on a Cary-300 UV/VIS spectrophotometer (Varian). Mass spectrometry and exact mass measurements of nucleoside intermediates were performed on a quadrupole/orthogonal-acceleration time-of-flight tandem mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. ESI spectra for the modified oligonucleotides were obtained by coupling the Q-Tof-2 to a capillary HPLC (CapLC, Waters, Milford, MA, U.S.A.). Masses were obtained by deconvolution of the spectra using the MaxEnt 1 algorithm (MassLynx 3.4, Micromass, Manchester, U.K.). Chromatography: C18 column 0.5 mm × 15 mm (PepMap, LC Packings), mobile phase: 0.05 M triethylamine with 1,1,1,3,3,3-hexafluoropropan-2-ol (pH 7.5), acetonitrile as the organic phase. Flow rate: 12 μl/min. The gradient started at 2% of organic phase and increased 2% per minute over 15 min. Mass spectra were acquired every 2 s in negative ionization mode applying 2850 V on the electrospray capillary. Cone voltage 35 V, collision cell voltage 10 V. TOM-amidites and the phosphorylation reagent were purchased from Glen Research.

2-(Hydroxymethoxy)ethan-1-ol Diacetate (**1**)[18]

$^1$H NMR ($CDCl_3$): 5.25 s, 2 H ($OCH_2O$); 4.18 t, 2 H, *J* = 4.8 ($OCH_2C\mathbf{H}_2OAc$); 3.80 t, 2 H ($OC\mathbf{H}_2CH_2OAc$); 2.06 s, 3 H (Ac); 2.04 s, 3 H (Ac). $^{13}$C NMR ($CDCl_3$): 170.87, 170.46 (C=O); 89.16 ($OCH_2O$); 68.24 ($OCH_2CH_2OAc$); 63.25 ($OCH_2CH_2OAc$); 21.00, 20.86 (Me).

3-(Hydroxymethoxy)propan-1-ol Diacetate (**2**)[18]

$^1$H NMR ($CDCl_3$): 5.17 s, 2 H ($OCH_2O$); 4.07 t, 2 H, *J* = 6.4 ($OCH_2CH_2C\mathbf{H}_2OAc$); 3.63 t, 2 H, *J* = 6.4 ($OC\mathbf{H}_2CH_2CH_2OAc$); 2.01 s, 3 H (Ac); 1.97 s, 3 H (Ac); 1.83 p, 2 H ($OCH_2C\mathbf{H}_2CH_2OAc$). $^{13}$C NMR ($CDCl_3$): 170.98, 170.56 (C=O); 89.26 ($OCH_2O$); 66.99 ($OCH_2CH_2CH_2OAc$); 61.28 ($OCH_2CH_2CH_2OAc$); 28.97 ($OCH_2CH_2CH_2OAc$); 21.01, 20.91 (Me).

3′,5′-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)uridine (**3a**)[19]

$R_F$ 0.33 (A). $^1$H NMR ($CDCl_3$): 8.52 brs, 1 H (NH); 7.66 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.72 s, 1 H (H-1′), 5.67 dd, 1 H, *J*(5,NH) = 1.8 (H-5); 4.38 dd, 1 H, *J*(3′,2′) = 4.7, *J*(3′,4′) = 8.7 (H-3′); 4.19 dd, 1 H, *J*(5′a,4′) = 2.2, *J*(5′a,5′b) = –13.1 (H-5′a); 4.17 d, 1 H (H-2′); 4.09 ddd, 1 H, *J*(4′,5′b) = 2.8 (H-4′); 4.01 dd, 1 H (H-5′b); 1.09–1.02 m, 28 H (iPr). $^{13}$C NMR ($CDCl_3$): 163.43 (C-4); 150.21 (C-2); 140.15 (C-6); 102.13 (C-5); 91.24 (C-1′); 82.14 (C-4′); 75.32 (C-2′); 69.26 (C-3′); 60.58 (C-5′); 17.58, 17.47, 17.40, 17.24, 17.15, 16.97, 13.54, 13.11, 12.72 (iPr).

$N^4$-Benzoyl-3′,5′-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine (**3b**)[19]

$R_F$ 0.34 (A). $^1$H NMR ($CDCl_3$): 8.85 brs, 1 H (NH); 8.22 d, 1 H, *J*(6,5) = 7.5 (H-6); 7.92–7.50 m, 6 H (Bz, H-5); 5.84 s, 1 H (H-1′); 4.35 dd, 1 H, *J*(3′,2′) = 4.6, *J*(3′,4′) = 8.9 (H-3′); 4.28 dd, 1 H, *J*(5′a,4′) = 1.8, *J*(5′a,5′b) = –13.4 (H-5′a); 4.26 d, 1 H (H-2′); 4.21 ddd, 1 H, *J*(4′,5′b) = 2.8 (H-4′); 4.01 dd, 1 H (H-5′b); 1.11–1.00 m, 28 H (iPr). $^{13}$C NMR: 165.78 (C=O); 162.63 (C-4); 154.33 (C-2); 144.78 (C-6); 133.29, 129.13, 127.82 (Ph); 96.52 (C-5); 91.99 (C-1′); 82.25 (C-4′); 75.36 (C-2′); 68.89 (C-3′); 60.32 (C-5′); 17.57, 17.52, 17.42, 17.14, 16.97, 13.54, 13.09, 12.69 (iPr).

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}uracil (**4a**)

To a cool solution (–15 °C) under nitrogen of nucleoside **3a** (0.973 g, 2.0 mmol) and 2-(hydroxymethoxy)ethan-1-ol diacetate (**1**; 0.705 g, 4.0 mmol) in 1,2-dichloroethane (15 ml) tin tetrachloride (0.36 ml, 3 mmol) was added and the solution was kept at –12 °C for 20 min. A 10% aqueous solution of sodium hydrogencarbonate (10 ml) and methylene chloride (20 ml) were added and the suspension was stirred at 0 °C for 20 min. The suspension was filtered through Hyflo Super Cel, organic layer was separated, washed with water (20 ml), dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was purified by column chromatography on silica gel (30 g). The column was washed with methylene chloride (200 ml), methylene chloride–ethanol 99:1 (200 ml) and then eluted with methylene chloride–ethanol 98:2 to give **4a** as a foam. Yield 1.04 g (86%). $R_F$ 0.35 (A). $^1$H NMR ($CDCl_3$): 9.72 brs, 1 H (NH); 7.88 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.74 s, 1 H (H-1′), 5.65 d, 1 H (H-5); 5.02 d, 1 H, *J* = –6.8 ($OC\mathbf{H}HO$); 4.94 d, 1 H ($OCH\mathbf{H}O$); 4.22 d, 1 H, *J*(5′a,5′b) = –13.7 (H-5′a); 4.20–4.13 m, 4 H (H-2′, H-3′, $OCH_2C\mathbf{H}_2OAc$); 4.12 dd, 1 H, *J*(4′,3′) = 8.8, *J*(4′,5′b) = 1.8 (H-4′); 3.96 dd, 1 H (H-5′b); 3.88 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 2.05 s, 3 H (Ac); 1.05–0.99 m,

28 H (iPr). $^{13}$C NMR ($CDCl_3$): 171.08 (C=O, Ac); 163.70 (C-4); 150.14 (C-2); 139.34 (C-6); 101.77 (C-5); 94.60 ($OCH_2O$); 89.40 (C-1′); 81.92 (C-4′); 78.40 (C-2′); 68.19 (C-3′); 65.91 ($OCH_2CH_2OAc$); 63.60 ($OCH_2CH_2OAc$); 59.50 (C-5′); 20.99 (Me); 17.59, 17.50, 17.42, 17.33, 17.22, 17.07, 16.93, 13.51, 13.23, 13.00, 12.75 (iPr). LSI-MS: ($C_{26}H_{46}N_2O_{10}Si_2$ + $H^+$) 603.2764, calculated 603.2769.

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**4b**)

Analogous condensation of diacetate **1** (0.705 g, 4.0 mmol) in the presence of tin tetrachloride (0.36 ml, 3 mmol) with nucleoside **3b** (1.180 g, 2.0 mmol) in 1,2-dichloroethane (15 ml) at –12 °C for 20 min gave **4b** as a foam. Yield 1.235 g (87%). $R_F$ 0.37 (A). $^1$H NMR ($CDCl_3$): 8.75 brs, 1 H (NH), 8.34 d, 1 H, *J*(6,5) = 7.5 (H-6); 7.90–7.50 m, 6 H (Bz, H-5); 5.84 s, 1 H (H-1′), 5.14 d, 1 H, *J* = –6.7 (OC**H**HO); 4.99 d, 1 H (OCH**H**O), 4.29 d, 1 H, *J*(5′a,5′b) = –13.7 (H-5′a); 4.24–4.18 m, 5 H (H-2′, H-3′, H-4′, $OCH_2C\mathbf{H}_2OAc$); 4.01 d, 1 H (H-5′b); 3.92 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 2.07 s, 3 H (Ac); 1.11–0.92 m, 28 H (iPr). $^{13}$C NMR ($CDCl_3$): 171.13 (C=O, Ac); 167.40 (C=O, Bz); 162.55 (C-4); 154.30 (C-2); 144.36 (C-6); 133.30, 129.19, 127.67 (Ph); 96.22 (C-5); 94.74 ($OCH_2O$); 90.45 (C-1′); 82.07 (C-4′); 78.25 (C-2′); 67.96 (C-3′); 65.95 ($OCH_2OCH_2CH_2OAc$); 63.73 ($OCH_2OCH_2CH_2OAc$); 59.53 (C-5′); 21.06 (Me); 17.58, 17.42, 17.14, 16.98, 13.52, 13.04, 12.77 (iPr). LSI-MS: ($C_{33}H_{51}N_3O_{10}Si_2$ + $H^+$) 706.3186, calculated 706.3190.

1-{2-*O*-[(3-Acetoxypropoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}uracil (**5a**)

Analogous condensation of diacetate **2** (0.761 g, 4.0 mmol) in the presence of tin tetrachloride (0.36 ml, 3 mmol) with nucleoside **3a** (0.973 g, 2.0 mmol) in 1,2-dichloroethane (15 ml) at –12 °C for 20 min gave **5a** as a foam. Yield 1.065 g (86%). $R_F$ 0.35 (A). $^1$H NMR ($CDCl_3$): 9.68 brs, 1 H (NH); 7.87 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.74 s, 1 H (H-1′); 5.65 d, 1 H (H-5); 4.98 d, 1 H, *J* = –6.5 (OC**H**HO); 4.89 d, 1 H (OCH**H**O); 4.25 d, 1 H, *J*(5′a,5′b) = –13.4 (H-5′a); 4.30–4.10 m, 5 H (H-2′, H-3′, H-4′, $OCH_2CH_2C\mathbf{H}_2OAc$); 3.95 dd, 1 H, *J*(5′b,4′) = 2.2 (H-5′b); 3.71 t, 2 H, *J* = 6.4 ($OC\mathbf{H}_2CH_2CH_2OAc$); 2.02 s, 3 H (Ac); 1.90 p, 2 H, *J* = 6.4 ($OCH_2C\mathbf{H}_2CH_2OAc$); 1.08–0.99 m, 28 H (iPr). $^{13}$C NMR ($CDCl_3$): 171.16 (C=O); 163.71 (C-4); 150.06 (C-2); 139.41 (C-6); 101.70 (C-5); 94.64 ($OCH_2O$); 89.40 (C-1′); 81.92 (C-4′); 78.21 (C-2′); 68.17 (C-3′); 64.96 ($OCH_2CH_2CH_2OAc$); 61.87 ($OCH_2CH_2CH_2OAc$); 59.51 (C-5′); 28.89 ($OCH_2CH_2CH_2OAc$); 21.02 (Me); 17.58, 17.50, 17.41, 17.32, 17.2, 17.06, 16.93, 13.54, 13.22, 12.99, 12.71 (iPr). LSI-MS: ($C_{27}H_{48}N_2O_{10}Si_2$ + $H^+$) 617.2926, calculated 617.2925.

1-{2-*O*-[(2-Acetoxypropoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**5b**)

Analogous condensation of diacetate **2** (0.761 g, 4.0 mmol) in the presence of tin tetrachloride (0.36 ml, 3 mmol) with nucleoside **3b** (1.180 g, 2.0 mmol) in 1,2-dichloroethane (15 ml) at –12 °C for 20 min gave **5b** as a foam. $R_F$ 0.37 (A). Yield 1.220 g (85%). $^1$H NMR ($CDCl_3$): 9.15 brs, 1 H (NH); 8.39 d, 1 H, *J*(6,5) = 7.5 (H-6); 7.94–7.52 m, 6 H (Bz, H-5); 5.85 s, 1 H (H-1′); 5.09 d, 1 H, *J* = –6.5 (OC**H**HO); 4.96 d, 1 H (OCH**H**O); 4.31 d, 1 H, *J*(5′a,5′b) = –12.8 (H-5′a); 4.27–4.17 m, 5 H (H-2′, H-3′, H-4′, $OCH_2CH_2C\mathbf{H}_2OAc$); 4.02 d, 1 H (H-5′b); 3.77 m, 2 H ($OC\mathbf{H}_2CH_2CH_2OAc$); 2.07 s, 3 H (Ac); 1.94 p, 2 H, *J* = 6.3

($OCH_2C\mathbf{H}_2CH_2OAc$); 1.11–0.98 m, 28 H (iPr). $^{13}C$ NMR ($CDCl_3$): 171.19 (C=O, Ac); 166.80 (C=O, Bz); 162.48 (C-4); 154.50 (C-2); 144.50 (C-6); 133.31, 129.15, 127.75 (Ph); 96.23 (C-5); 94.77 ($OCH_2O$); 90.46 (C-1′); 82.08 (C-4′); 77.94 (C-2′); 67.92 (C-3′); 64.95 ($OCH_2CH_2CH_2OAc$); 61.95 ($OCH_2OCH_2CH_2CH_2OAc$); 59.54 (C-5′); 28.92 ($OCH_2OCH_2CH_2CH_2OAc$); 21.07 (Me); 17.60, 17.42, 17.14, 16.98, 13.52, 13.23, 13.02 (iPr). LSI-MS: ($C_{34}H_{53}N_3O_{10}Si_2 + H^+$) 720.3341, calculated 720.3347.

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-β-D-ribofuranosyl}uracil (**6a**)

Nucleoside **4a** (0.602 g, 1 mmol) was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (5 ml), kept at 20 °C for 10 min, evaporated to dryness, evaporated with chloroform (10 ml) and applied onto a column with silica gel (20 g). The column was washed with methylene chloride (100 ml), methylene chloride–ethanol 98:2 (200 ml), methylene chloride–ethanol 96:4 (200 ml), and then eluted with methylene chloride–ethanol 94:6 to give **6a** as a foam. Yield 0.330 g (92%). $R_F$ 0.22 (B). $^1H$ NMR ($CDCl_3$): 8.69 brs, 1 H (NH); 7.74 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.82 d, 1 H, *J*(1′,2′) = 4.5 (H-1′); 5.72 d, 1 H (H-5); 4.91 d, 1 H, *J* = –6.7 ($OC\mathbf{H}HO$); 4.87 d, 1 H ($OCH\mathbf{H}O$); 4.42 dd, 1 H, *J*(2′,3′) = 5.0 (H-2′); 4.37 t, 1 H, *J*(3′,4′) = 5.0 (H-3′); 4.23 m, 2 H ($OCH_2C\mathbf{H}_2OAc$); 4.10 dt, 1 H, *J*(4′,5′a) = 1.9, *J*(4′,5′b) = 1.9 (H-4′); 3.98 dd, 1 H, *J*(5′a,5′b) = –12.2 (H-5′a); 3.86 dd, 1 H (H-5′b); 3.82 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 2.08 s, 3 H (Ac). $^{13}C$ NMR ($CDCl_3$): 171.10 (C=O); 164.00 (C-4); 150.31 (C-2); 141.68 (C-6); 102.64 (C-5); 96.01 ($OCH_2O$); 90.29 (C-1′); 85.19 (C-4′); 80.30 (C-2′); 69.58 (C-3′); 66.98 ($OCH_2CH_2OAc$); 63.38 ($OCH_2CH_2OAc$); 61.62 (C-5′); 20.15 (Me). LSI-MS: ($C_{14}H_{20}N_2O_9 + H^+$) 361.1241, calculated 361.1246.

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**6b**)

Analogous desilylation of **4b** (706 mg, 1 mmol) yielded **6b** as a foam (407 mg, 88%). $R_F$ 0.25 (B). $^1H$ NMR ($CDCl_3$): 9.24 brs, 1 H (NH); 8.46 d, 1 H, *J*(6,5) = 7.5 (H-6); 7.91–7.47 m, 6 H (Bz, H-5); 5.86 d, 1 H, *J*(1′,2′) = 2.2 (H-1′); 5.03 d, 1 H, *J* = –6.4 ($OC\mathbf{H}HO$); 4.92 d, 1 H ($OCH\mathbf{H}O$); 4.44 dd, 1 H, *J*(2′,3′) = 4.5 (H-2′); 4.35 dd, 1 H, *J*(3′,4′) = 7.2 (H-3′); 4.19 m, 2 H ($OCH_2C\mathbf{H}_2OAc$); 4.13 ddd, 1 H, *J*(4′,5′a) = 1.9, *J*(4′,5′b) = 1.6 (H-4′); 4.06 dd, 1 H, *J*(5′a,5′b) = –12.5 (H-5′a); 3.90 dd, 1 H (H-5′b); 3.86–3.75 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 2.03 s, 3 H (Ac). $^{13}C$ NMR ($CDCl_3$): 171.21 (C=O, Ac); 167.62 (C=O, Bz); 162.61 (C-4); 154.53 (C-2); 146.44 (C-6); 133.25, 132.80, 128.95, 127.79 (Ph); 96.91 (C-5); 95.65 ($OCH_2O$); 91.54 (C-1′); 84.91 (C-4′); 80.13 (C-2′); 68.24 (C-3′); 66.66 ($OCH_2OCH_2CH_2OAc$); 63.35 ($OCH_2OCH_2CH_2OAc$); 60.48 (C-5′); 20.80 (Me). LSI-MS: ($C_{21}H_{25}N_3O_9 + H^+$) 464.1661, calculated 464.1668.

1-{2-*O*-[(3-Acetoxypropoxy)methyl]-β-D-ribofuranosyl}uracil (**7a**)

Analogous desilylation of **5a** (617 mg, 1 mmol) yielded **7a** as a foam (337 mg, 90%). $R_F$ 0.22 (B). $^1H$ NMR ($CDCl_3$): 9.34 brs, 1 H (NH); 7.89 d, 1 H, *J*(6,5) = 8.0 (H-6); 5.86 d, 1 H, *J*(1′,2′) = 3.1 (H-1′); 5.72 d, 1 H (H-5); 4.86 d, 1 H, *J* = –6.5 ($OC\mathbf{H}HO$); 4.82 d, 1 H ($OCH\mathbf{H}O$); 4.35 m, 2 H (H-2′, H-3′); 4.15–4.08 m, 3 H (H-4′, $OCH_2CH_2C\mathbf{H}_2OAc$); 3.95 dd, 1 H, *J*(5′a,4′) = 2.2, *J*(5′a,5′b) = –12.3 (H-5′a); 3.86 dd, 1 H, *J*(5′b,4′) = 2.1 (H-5′b); 3.69–3.58 m, 2 H ($OC\mathbf{H}_2CH_2CH_2OAc$); 2.08 s, 3 H (Ac); 1.86 p, 2 H, *J* = 6.3 ($OCH_2C\mathbf{H}_2CH_2OAc$). $^{13}C$ NMR ($CDCl_3$): 171.25 (C=O); 163.48 (C-4); 150.51 (C-2); 141.57 (C-6); 102.49 (C-5); 95.61 ($OCH_2O$); 89.67 (C-1′); 85.10 (C-4′); 79.78 (C-2′); 69.34 (C-3′); 65.50 ($OCH_2CH_2CH_2OAc$);

61.58 ($OCH_2CH_2CH_2OAc$); 59.29 (C-5′); 28.99 ($OCH_2CH_2CH_2OAc$); 21.04 (Me). LSI-MS: ($C_{15}H_{22}N_2O_9$ + $H^+$) 375.1406, calculated 375.1403.

1-{2-*O*-[(3-Acetoxypropoxy)methyl]-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**7b**)

Analogous desilylation of **5b** (720 mg, 1 mmol) yielded **7b** as a foam (423 mg, 89%). $R_F$ 0.28 (B). $^1$H NMR ($CDCl_3$): 9.17 brs, 1 H (NH); 8.45 d, 1 H, *J*(6,5) = 7.5 (H-6); 7.90–7.46 m, 6 H (Bz, H-5); 5.87 d, 1 H, *J*(1′,2′) = 2.2 (H-1′); 4.99 d, 1 H, *J* = –6.5 ($OCHHO$); 4.88 d, 1 H ($OCHHO$); 4.45 dd, 1 H, *J*(2′,3′) = 5.0 (H-2′); 4.36 dd, 1 H, *J*(3′,4′) = 6.5 (H-3′); 4.14–4.05 m, 4 H (H-4′, H-5′a, $OCH_2CH_2CH_2OAc$); 3.91 d, 1 H, *J*(5′a,5′b) = –12.1 (H-5′b); 3.71–3.56 m, 2 H ($OCH_2CH_2CH_2OAc$); 2.02 s, 3 H (Ac); 1.86 p, 2 H, *J* = 6.3 ($OCH_2CH_2CH_2OAc$). $^{13}$C NMR ($CDCl_3$): 171.50 (C=O, Ac); 167.40 (C=O, Bz); 162.65 (C-4); 154.70 (C-2); 146.60 (C-6); 133.41, 133.01, 129.13, 127.91 (Ph); 97.02 (C-5); 95.67 ($OCH_2O$); 91.69 (C-1′); 85.17 (C-4′); 79.99 (C-2′); 68.52 (C-3′); 65.52 ($OCH_2OCH_2CH_2CH_2OAc$); 61.53 ($OCH_2OCH_2CH_2CH_2OAc$); 60.79 (C-5′); 29.01 ($OCH_2OCH_2CH_2CH_2OAc$); 21.04 (Me). LSI-MS: ($C_{22}H_{27}N_3O_9$ + $H^+$) 478.1823, calculated 478.1825.

1-{2-*O*-[(2-Hydroxyethoxy)methyl]-β-D-ribofuranosyl}uracil (**8a**)

A solution of nucleoside **6a** (360 mg, 1.0 mmol) in 5 M ammonia in methanol (10 ml) was kept at 20 °C for 3 days and then concentrated to dryness in vacuo. The residue was partioned between methylene chloride (10 ml) and water (20 ml), and the water layer was washed with methylene chloride (2 × 10 ml). The aqueous layer was concentrated to dryness, the residue was dissolved in 5 ml of methanol, the solution evaporated to dryness in vacuo to give **8a** as a hygroscopic foam (300 mg, 94%). $R_F$ 0.70 (C). UV (pH 1–7): $\lambda_{max}$ 262 nm (ε 9600); (pH 13): $\lambda_{max}$ 261 nm (ε 6900). $^1$H NMR (DMSO-$d_6$): 11.38 brs, 1 H (NH); 7.91 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.89 d, 1 H, *J*(1′,2′) = 5.3 (H-1′); 5.64 d, 1 H (H-5); 5.25 brs, 2 H (OH-3′, OH-5′); 4.71 brs, 3 H ($OCH_2O$, OH); 4.16 t, 1 H, *J*(2′,3′) = 5.3 (H-2′); 4.11 dd, 1 H, *J*(3′,4′) = 3.3 (H-3′); 3.88 dt, 1 H, *J*(4′,5′a) = 3.0, *J*(4′,5′b) = 3.0 (H-4′); 3.63 dd, 1 H, *J*(5′a,5′b) = –12.2 (H-5′a); 3.56 dd, 1 H (H-5′b); 3.51–3.42 m, 4 H ($OCH_2CH_2OH$). $^{13}$C NMR (DMSO-$d_6$): 163.19 (C-4); 150.73 (C-2); 140.43 (C-6); 101.92 (C-5); 94.27 ($OCH_2O$); 86.11 (C-1′); 85.29 (C-4′); 78.24 (C-2′); 69.48 ($OCH_2CH_2OH$); 68.75 (C-3′); 60.72 (C-5′); 60.06 ($OCH_2CH_2OH$). LSI-MS: ($C_{12}H_{18}N_2O_8$ + $H^+$) 319.1147, calculated 319.1141.

1-{2-*O*-[(2-Hydroxyethoxy)methyl]-β-D-ribofuranosyl}cytosine (**8b**)

Analogous deacylation of **6b** (463 mg, 1.0 mmol), after evaporation with methanol yielded **8b** as a hygroscopic foam (295 mg, 93%). $R_F$ 0.60 (C). UV (pH 7–13): $\lambda_{max}$ 271 nm (ε 8400); (pH 1): $\lambda_{max}$ 279 nm (ε 13100). $^1$H NMR ($D_2O$): 7.96 d, 1 H, *J*(6,5) = 7.7 (H-6); 6.19 d, 1 H (H-5); 6.15 d, 1 H, *J*(1′,2′) = 4.9 (H-1′); 5.02 d, 1 H, *J* = –7.2 ($OCHHO$); 4.98 d, 1 H ($OCHHO$); 4.49 dd, 1 H, *J*(2′,3′) = 5.4 (H-2′); 4.43 t, 1 H, *J*(3′,4′) = 5.4 (H-3′); 4.25 ddd, 1 H, *J*(4′,5′a) = 2.8, *J*(4′,5′b) = 4.4 (H-4′); 4.02 dd, 1 H, *J*(5′a,5′b) = –12.8 (H-5′a); 3.93 dd, 1 H (H-5′b); 3.83–3.72 m, 4 H ($OCH_2CH_2OH$). $^{13}$C NMR (DMSO-$d_6$): 165.59 (C-4); 155.20 (C-2); 141.18 (C-6); 93.99 (C-5); 93.98 ($OCH_2O$); 87.52 (C-1′); 84.33 (C-4′); 78.50 (C-2′); 69.43 ($OCH_2OCH_2CH_2OH$); 68.31 (C-3′); 60.36 (C-5′); 60.13 ($OCH_2OCH_2CH_2OH$). LSI-MS: ($C_{12}H_{19}N_3O_7$ + $H^+$) 318.1304, calculated 318.1301.

1-{2-*O*-[(2-Hydroxypropoxy)methyl]-β-D-ribofuranosyl}uracil (**9a**)

A solution of nucleoside **7a** (374 mg, 1.0 mmol) in 5 M ammonia in methanol (10 ml) was kept at 20 °C for 3 days and then concentrated to dryness in vacuo. The residue was partioned between methylene chloride (10 ml) and water (20 ml), and the water layer was washed with methylene chloride (2 × 10 ml). The aqueous layer was concentrated to dryness, acetone (5 ml) was added to the residue and, after 20 h at 20 °C, the precipitate was filtered off, washed with ether and dried to give **9a** as a powder (267 mg, 80%). $R_F$ 0.70 (C). UV (pH 1–7): $\lambda_{max}$ 262 nm (ε 9700); (pH 13): $\lambda_{max}$ 261 nm (ε 6800). $^1$H NMR (DMSO-$d_6$): 11.21 brs, 1 H (NH); 7.89 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.91 d, 1 H, *J*(1′,2′) = 5.6 (H-1′); 5.64 d, 1 H (H-5); 5.06 d, 1 H, *J*(OH,3′) = 5.3 (OH-3′); 5.04 dd, 1 H, *J*(OH,5′a) = 4.9, *J*(OH,5′b) = 5.9 (OH-5′); 4.69 d, 1 H, *J* = –6.7 (OC**H**HO); 4.67 d, 1 H (OCH**H**O); 4.27 t, 1 H, *J*(OH,H) = 5.2 ($OCH_2CH_2CH_2O$**H**); 4.15 dd, 1 H, *J*(2′,3′) = 5.0 (H-2′); 4.12 ddd, 1 H, *J*(3′,4′) = 3.6 (H-3′); 3.89 ddd, 1 H, *J*(4′,5′a) = 3.4, *J*(4′,5′b) = 2.7 (H-4′); 3.64 ddd, 1 H, *J*(5′a,5′b) = –11.9 (H-5′a); 3.56 ddd, 1 H (H-5′b); 3.60–3.37 m, 4 H (OC**H**$_2$CH$_2$C**H**$_2$OH); 1.60 p, 2 H, *J* = 6.5 ($OCH_2$C**H**$_2$$CH_2$OH). $^{13}$C NMR (DMSO-$d_6$): 162.82 (C-4); 150.48 (C-2); 140.32 (C-6); 101.79 (C-5); 93.89 ($OCH_2O$); 86.07 (C-1′); 85.27 (C-4′); 77.78 (C-2′); 68.63 (C-3′); 64.63 ($OCH_2CH_2CH_2OH$); 60.70 (C-5′); 57.65 ($OCH_2CH_2CH_2OH$); 32.42 ($OCH_2CH_2CH_2OH$). LSI-MS: ($C_{13}H_{20}N_2O_8$ + H$^+$) 333.1291, calculated 333.1297.

1-{2-*O*-[(2-Hydroxypropoxy)methyl]-β-D-ribofuranosyl}cytosine (**9b**)

Analogous deacylation of **7b** (477 mg, 1 mmol) as in the case of **6a**, after evaporation with methanol, yielded **9b** as a hygroscopic foam (301 mg, 91%). $R_F$ 0.60 (C). UV (pH 7–13): $\lambda_{max}$ 271 nm (ε 8500); (pH 1): $\lambda_{max}$ 279 nm (ε 13200). $^1$H NMR ($D_2O$): 7.98 d, 1 H, *J*(6,5) = 7.7 (H-6); 6.24 d, 1 H (H-5); 6.20 d, 1 H, *J*(1′,2′) = 5.3 (H-1′); 5.00 d, 1 H, *J* = –7.0 (OC**H**HO); 4.97 d, 1 H (OCH**H**O); 4.51 dd, 1 H, *J*(2′,3′) = 5.6 (H-2′); 4.47 dd, 1 H, *J*(3′,4′) = 4.7 (H-3′); 4.30 ddd, 1 H, *J*(4′,5′a) = 3.1, *J*(4′,5′b) = 4.4 (H-4′); 4.04 dd, 1 H, *J*(5′a,5′b) = –12.6 (H-5′a); 3.96 dd, 1 H (H-5′b); 3.79–3.72 m, 4 H (OC**H**$_2$CH$_2$C**H**$_2$OH); 1.89 p, 2 H, *J* = 6.6 ($OCH_2$C**H**$_2$$CH_2$OH). $^{13}$C NMR (DMSO-$d_6$): 165.59 (C-4); 155.22 (C-2); 141.19 (C-6); 94.03 (C-5); 93.66 ($OCH_2O$); 87.50 (C-1′); 84.42 (C-4′); 78.04 (C-2′); 68.31 (C-3′); 64.44 ($OCH_2OCH_2CH_2CH_2OH$); 60.43 (C-5′); 57.72 ($OCH_2OCH_2CH_2CH_2OH$); 32.54 ($OCH_2OCH_2CH_2CH_2OH$). LSI-MS: ($C_{13}H_{21}N_3O_7$ + H$^+$) 332.1451, calculated 332.1457.

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}uracil (**10a**)

Nucleoside **6a** (0.676 g, 1.87 mmol) was dried by evaporation with pyridine (2 × 10 ml). The residue was dissolved in dry pyridine (15 ml), monomethoxytrityl chloride (0.648 g, 2.10 mmol) was added and the resulting solution was kept in the dark at 20 °C for 16 h. Then MeOH (0.5 ml) was added and after 30 min the mixture was concentrated almost to dryness in vacuo. The residue was dissolved in methylene chloride (50 ml), washed with 10% aqueous solution of sodium hydrogencarbonate (20 ml) and water (2 × 20 ml). The organic layer was dried over anhydrous $Na_2SO_4$, evaporated in vacuo, evaporated with toluene (2 × 10 ml) and purified by column chromatography on silica gel (30 g). The column was washed with methylene chloride (200 ml), methylene chloride–ethanol 99:1 (200 ml) and then eluted with methylene chloride–ethanol 97:3 to give **10a** as a foam. Yield 0.832 g (70%). $R_F$ 0.33 (A). $^1$H NMR (DMSO-$d_6$): 11.41 brs, 1 H (NH); 7.75 d, 1 H, *J*(6,5) = 8.1 (H-6); 7.40–7.22 m, 12 H (Ph); 6.91 d, 1 H, *J* = 8.8 (**Ph**OMe); 5.85 d, 1 H, *J*(1′,2′) = 3.4 (H-1′); 5.85 d, 1 H, *J*(OH,3′) =

5.9 (OH-3′); 5.31 dd, 1 H, *J*(5,NH) = 2.2 (H-5); 4.79 d, 1 H, *J* = –7.2 (OC**H**HO); 4.77 d, 1 H (OCH**H**O); 4.27–4.24 m, 2 H (H-2′, H-3′); 4.12–3.97 m, 2 H ($OCH_2C\mathbf{H}_2OAc$); 4.09 m, 1 H (H-4′); 3.74 s, 3 H (OMe); 3.69 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 3.29 dd, 1 H, *J*(5′a,4′) = 4.4, *J*(5′a,5′b) = –10.5 (H-5′a); 3.21 dd, 1 H, *J*(5′b,4′) = 2.2 (H-5′b); 1.97 s, 1 H (Ac). $^{13}$C NMR (DMSO-$d_6$): 170.17 (C=O); 162.76 (C-4); 158.22 (**Ph**OMe); 150.26 (C-2); 143.79, 143.53 (Ph); 140.36 (C-6); 134.35, 129.87, 127.71, 127.70, 126.97 (Ph); 113.31 (**Ph**OMe); 101.32 (C5); 94.10 ($OCH_2O$); 87.13 (C-1′); 86.10 ($Ph_3C$); 82.90 (C-4′); 77.43 (C-2′); 68.38 (C-3′); 65.18 ($OCH_2CH_2OAc$); 63.09 ($OCH_2CH_2OAc$); 61.58 (C-5′); 54.87 (OMe); 20.59 (Me, Ac).

1-{2-*O*-[(2-Acetoxyethoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**10b**)

Analogous tritylation of nucleoside **6b** (463 mg, 1 mmol) yielded **10b** as a foam (589 mg, 80%). $R_F$ 0.33 (A). $^1$H NMR (DMSO-$d_6$): 11.24 brs, 1 H (NH); 8.32 d, 1 H, *J*(6,5) = 7.5 (H-6); 8.02–7.28 m, 17 H (Bz, Tr); 7.16 brd, 1 H (H-5); 6.94 d, 2 H, *J* = 8.1 (**Ph**OMe); 5.89 d, 1 H, *J*(1′,2′) = 1.6 (H-1′); 5.35 brs, 1 H (OH); 4.97 d, 1 H, *J* = –6.7 (OC**H**HO); 4.87 d, 1 H (OCH**H**O); 4.36 dd, 1 H, *J*(3′,2′) = 5.0, *J*(3′,4′) = 8.1 (H-3′); 4.21 dd, 1 H (H-2′); 4.15 t, 2 H, *J* = 4.7 ($OCH_2C\mathbf{H}_2OAc$); 4.09 ddd, 1 H, *J*(4′,5a′) = 3.4, *J*(4′,5b′) = 2.7 (H-4′); 3.79 m, 2 H ($OC\mathbf{H}_2CH_2OAc$); 3.76 s, 3 H (OMe); 3.42 dd, 1 H, *J*(5′a,5′b) = –11.2 (H-5′a); 3.35 dd, 1 H (H-5′b); 1.99 s, 3 H (Ac). $^{13}$C NMR (DMSO-$d_6$): 170.24 (C=O, Ac); 167.65 (C=O, Bz); 163.11 (C-4); 158.27 (**Ph**OMe); 154.73 (C-2); 144.43 (C-6); 143.96, 143.68, 134.83, 132.65, 129.94, 128.37, 128.09, 127.79 (Ph); 113.30 (**Ph**OMe); 96.12 (C-5); 93.69 ($OCH_2O$); 89.30 (C-1′); 86.26 ($Ph_3C$); 82.02 (C-4′); 78.31 (C-2′); 67.86 (C-3′); 65.20 ($OCH_2OCH_2CH_2OAc$); 63.09 ($OCH_2OCH_2CH_2OAc$); 61.85 (C-5′); 54.98 (OMe); 20.57 (Me, Ac).

1-{2-*O*-[(3-Acetoxypropoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}uracil (**11a**)

Analogous tritylation of nucleoside **7a** (375 mg, 1 mmol) yielded **11a** as a foam (498 mg, 77%). $R_F$ 0.33 (A). $^1$H NMR ($CDCl_3$): 8.47 brs, 1 H (NH); 7.94 d, 1 H, *J*(6,5) = 8.1 (H-6); 7.52–7.34 m, 12 H (Ph); 6.86 d, 2 H, *J* = 8.7 (**Ph**OMe); 5.99 d, 1 H, *J*(1′,2′) = 2.8 (H-1′); 5.31 dd, 1 H, *J*(5,NH) = 1.9 (H-5); 4.98 d, 1 H, *J* = –6.7 (OC**H**HO); 4.87 d, 1 H (OCH**H**O); 4.48 dt, 1 H, *J*(3′,2′) = 5.6, *J*(3′,OH) = 7.5, *J*(3′,4′) = 5.6 (H-3′); 4.29 dd, 1 H (H-2′); 4.16 m, 2 H ($OCH_2CH_2C\mathbf{H}_2OAc$); 4.05 dt, 1 H, *J*(4′,5a′) = 2.2, *J*(4′,5b′) = 2.2 (H-4′); 3.80 s, 3 H (OMe); 3.72 m, 1 H ($OC\mathbf{H}HCH_2CH_2OAc$); 3.62 m, 1 H ($OCH\mathbf{H}CH_2CH_2OAc$); 3.54 d, 2 H (H-5′a, H-5′b); 2.72 d, 1 H (OH-3′); 2.04 d, 3 H (Ac); 1.92 p, 2 H, *J* = 6.2 ($OCH_2C\mathbf{H}_2CH_2OAc$). $^{13}$C NMR (DMSO-$d_6$): 170.17 (C=O); 162.76 (C-4); 158.22 (**Ph**OMe); 150.26 (C-2); 143.99, 143.73 (Ph); 140.11 (C-6); 134.55, 129.97, 127.91, 127.80, 126.87 (Ph); 113.21 (**Ph**OMe); 101.52 (C-5); 94.00 ($OCH_2O$); 87.03 (C-1′); 86.20 ($Ph_3C$); 82.80 (C-4′); 77.53 (C-2′); 68.58 (C-3′); 64.18 ($OCH_2CH_2CH_2OAc$); 62.88 (C-5′); 60.98 ($OCH_2CH_2CH_2OAc$); 54.97 (OMe); 28.40 ($OCH_2CH_2CH_2OAc$); 20.49 (Me, Ac).

1-{2-*O*-[(3-Acetoxypropoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-$N^4$-benzoylcytosine (**11b**)

Analogous tritylation of nucleoside **7b** (477 mg, 1 mmol) yielded **11b** as a foam (578 mg, 77%). $R_F$ 0.33 (A). $^1$H NMR (DMSO-$d_6$): 11.23 brs, 1 H (NH); 8.33 d, 1 H, *J*(6,5) = 7.6 (H-6); 8.02–7.28 m, 17 H (Bz, Tr); 7.15 brd, 1 H (H-5); 6.93 d, 2 H, *J* = 9.0 (**Ph**OMe); 5.90 s, 1 H (H-1′); 5.32 brs, 1 H (OH-3′); 4.93 d, 1 H, *J* = –6.5 (OC**H**HO); 4.83 d, 1 H (OCH**H**O); 4.35 dd,

1 H, *J*(3′,2′) = 5.6, *J*(3′,4′) = 6.8 (H-3′); 4.21 d, 1 H (H-2′); 4.10 ddd, 1 H, *J*(4′,5a′) = 3.3, *J*(4′,5b′) = 2.7 (H-4′); 4.05 t, 2 H, *J* = 6.5 ($OCH_2CH_2C\mathbf{H}_2OAc$); 3.76 s, 3 H (OMe); 3.69–3.57 m, 2 H ($OC\mathbf{H}_2CH_2CH_2OAc$); 3.41 dd, 1 H, *J*(5′a,5′b) = –10.9 (H-5′a); 3.35 dd, 1 H (H-5′b); 1.97 s, 3 H (Ac); 1.82 p, 2 H, *J* = 6.4 ($OCH_2C\mathbf{H}_2CH_2OAc$). $^{13}$C NMR (DMSO-$d_6$): 170.27 (C=O, Ac); 167.21 (C=O, Bz); 162.84 (C-4); 158.27 (**Ph**OMe); 154.81 (C-2); 144.54 (C-6); 143.96, 143.68, 134.82, 132.64, 129.95, 128.37, 128.07, 127.94, 127.00 (Ph); 113.30 (**Ph**OMe); 96.14 (C-5); 93.74 ($OCH_2O$); 89.16 (C-1′); 86.28 ($Ph_3\mathbf{C}$); 82.09 (C-4′); 78.25 (C-2′); 67.90 (C-3′); 64.07 ($OCH_2OCH_2CH_2CH_2OAc$); 61.90 (C-5′); 61.15 ($OCH_2OCH_2CH_2CH_2OAc$); 54.98 (OMe); 28.42 ($OCH_2OCH_2CH_2CH_2OAc$); 20.59 (Me, Ac).

2′-*O*-[(2-Acetoxyethoxy)methyl]-3′-*O*-[(2-cyanoethyl)(diisopropylamino)phosphanyl]-5′-*O*-(4-methoxytrityl)uridine (**12a**)

The methoxytritylated derivative **10a** (450 mg, 0.71 mmol) was dissolved in 6 ml dichloromethane under argon, and ethyl(diisopropyl)amine (371 µl, 2.13 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (285 µl, 1.28 mmol) were added and, after stirring the solution for 2 h, TLC indicated complete reaction. A 10% aqueous solution of sodium hydrogencarbonate (2 ml) was added, the solution was stirred for 10 min and partitioned between $CH_2Cl_2$ (50 ml) and aqueous $NaHCO_3$ (30 ml). The organic phase was washed with aqueous sodium chloride (2 × 30 ml) and the aqueous phases were back extracted with $CH_2Cl_2$ (20 ml). Evaporation of the organics left an oil which was flash-purified on 40 g of silica gel (hexane–acetone–TEA, 64:35:1) to afford the product as a foam after coevaporation with dichloromethane. The foam was dissolved in 2 ml of dichloromethane and precipitated in 150 ml cold (–70 °C) hexane–diisopropyl ether (9:1) to afford 373 mg (0.45 mmol, 63%) of the title product as a white powder. $R_F$ 0.37 (D). $^{31}$P NMR ($CDCl_3$): 151.21, 150.35. LSI-MS: ($C_{43}H_{53}N_4O_{11}P_1$ + $H^+$) 833.3517, calculated 833.3526.

Analogously to the previous procedure the following amidites were prepared:

*2′-O-[(3-Acetoxypropoxy)methyl]-3′-O-[(2-cyanoethyl)(diisopropylamino)phosphanyl]-5′-O-(4-methoxytrityl)uridine* (**13a**). Starting from compound **11a** (0.62 mmol, 398 mg), 377 mg of the required amidite was obtained (0.44 mmol, 72%). $R_F$ 0.41 (D). $^{31}$P NMR ($CDCl_3$): 150.43, 150.14. LSI-MS: ($C_{44}H_{55}N_4O_{11}P_1$ + $H^+$) 847.3697, calculated 847.3682.

*2′-O-[(2-Acetoxyethoxy)methyl]-N$^4$-benzoyl-3′-O-[(2-cyanoethyl)(diisopropylamino)-phosphanyl]-5′-O-(4-methoxytrityl)cytidine* (**12b**). Starting with compound **10b** (507 mg, 0.69 mmol), 523 mg of the required amidite was obtained (0.56 mmol, 81%). $R_F$ 0.43 (D). $^{31}$P NMR ($CDCl_3$): 151.40, 150.13. LSI-MS: ($C_{50}H_{58}N_5O_{11}P_1$ + $H^+$) 936.3932, calculated 936.3948.

*2′-O-[(3-Acetoxypropoxy)methyl]-N$^4$-benzoyl-3′-O-[(2-cyanoethyl)(diisopropylamino)-phosphanyl]-5′-O-(4-methoxytrityl)cytidine* (**13b**). Starting with compound **11b** (603 mg, 0.80 mmol), 458 mg of the required amidite was obtained (0.48 mmol, 60%). $R_F$ 0.40 (D). $^{31}$P NMR ($CDCl_3$): 151.40, 150.13. LSI-MS: ($C_{51}H_{60}N_5O_{11}P_1$ + $H^+$) 950.4117, calculated 950.4104.

Synthesis and Analysis of Oligonucleotides

Oligonucleotide assembly was performed on an Expedite™ DNA synthesizer (Applied Biosystems) using the phosphoramidite approach. The standard RNA assembly protocol was used with a 10 min coupling time, using 0.07 M of the newly synthesized unnatural amidites and 0.06 M of the TOM amidites with 0.25 M 5-(ethylsulfanyl)tetrazole (ETT) as the activator. The oligomers were deprotected and cleaved from the solid support by treatment with a 1:1 mixture of 40% aqueous methylamine and 8 M methylamine in ethanol at 35 °C

for 6 h. The supernatant was lyophilized and the residue was treated with 1 ml of a 1 M TBAF solution in THF at 55 °C for 10 min and at room temperature for 24 h. The mixture was neutralized with 1 ml of a 1 M solution of Tris-HCl, pH 7.4, slightly concentrated and desalted on a NAP-25® column (Sephadex G25-DNA grade; Pharmacia). The crude product was analyzed on a Mono-Q® HR 5/5 anion exchange column, then purified on a Mono-Q® HR 10/10 column (Pharmacia) with the following gradient system (A = 10 mM $NaClO_4$; B = 600 mM $NaClO_4$, both in aqueous 20 mM Tris-HCl, 15% $CH_3CN$, 0.1 mM EDTA). The low-pressure liquid chromatography system consisted of a Merck–Hitachi L 6200 A intelligent pump, a Uvicord SII 2138 UV detector (Pharmacia-LKB) and a recorder. The product-containing fraction was desalted on a NAP-25® column and lyophilized.

Oligonucleotides were characterized and their purity was checked by HPLC/MS on a capillary chromatograph (CapLC, Waters, Milford, MA, U.S.A.). Columns of 150 mm × 0.3 mm length (LCPackings, San Francisco, CA, U.S.A.) were used. Oligonucleotides were eluted with an acetonitrile gradient in 50 mM triethylammonium adjusted to pH 8.0 with 1,1,1,3,3,3-hexafluoropropan-2-ol. The flow rate was 5 μl/min. Electrospray spectra were acquired on an orthogonal acceleration/time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) in the negative ion mode. The scan time used was 2 s. The combined spectra from a chromatographic peak were deconvoluted using the MaxEnt algorithm of the software (Masslynx 3.4, Micromass, Manchester, U.K.). Theoretical oligonucleotide molecular weights were calculated using the monoisotopic atomic weights.

### Melting Temperatures

Oligomers were dissolved in 0.1 M NaCl, 0.02 M potassium phosphate, pH 7.5, 0.1 mM EDTA. The concentration was determined by measuring the absorbance in MilliQ water at 260 nm and 80 °C, and assuming the modified nucleoside analogues to have the same molar adsorption coefficients per base moiety in the denatured state as the natural nucleosides (C* ε = 7100; U* ε = 9600). The concentration in all experiments was 4 μM for each strand. Melting curves were determined with a Varian Cary 300 BIO spectrophotometer. Cuvettes were maintained at constant temperature by means of water circulation through the cuvette holder. The temperature of the solution was measured with a thermistor directly immersed in the cuvette. Temperature control and data acquisition were performed automatically with an IBM-compatible computer using Cary WinUV thermal application software. Following a fast heating and cooling cycle to allow proper annealing of both strands, the samples were heated at a rate of 0.2 °C/min starting at 10 °C up to 80 °C and cooling again at the same rate. Melting temperatures were determined by plotting the first derivative of the absorbance versus temperature curve and are the average of two runs. Heating and cooling curves were measured two times at two wavelengths 260 and 270 nm and in general showed the same $T_m$ values.

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